ELECTRON MICROSCOPY OF THE BURSA OF FABRICIUS OF THE EMBRYONIC CHICK WITH PARTICULAR REFERENCE TO THE LYMPHO-EPITHELIAL NODULES

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ABSTRACT

Electron microscopic studies of the bursa of Fabricius during the 15th and 16th day of embryonic development in the chick have shown the following findings in the submicroscopic structure of the cellular elements of the lympho-epithelial follicles. In the medulla, basal endodermal epithelial cells undergo mitosis and differentiation into lymphoblasts. During this transformation, there is a reduction in the amount of rough endoplasmic reticulum, an increase in the number or ribosomes, and frequently an enlargement of the Golgi complex. As lymphoblasts differentiate into medium lymphocytes there is a loss of endoplasmic reticulum, a reduction in the number of ribosomes and in the size of the Golgi complex, as well as a decrease in the number and size of mitochondria and in the size of the cell and nucleus. Cytoplasmic processes of reticular-epithelial cells extend between proliferating lymphocytic cells. Desmosomes connect stellate reticular-epithelial and basal epithelial cells but are not present in lymphocytic cells. Nuclear blebbing and vesiculation are frequently observed in the various cell forms of the developing lympho-epithelial nodules. Although lymphocytes and lymphocytopoietic activities in the cortex are sparse during this stage of embryonic development of the bursa, transitional forms between mesenchymal cells and lymphoblasts have been encountered. In addition, lymphoblasts and/or undifferentiated epithelial cells occasionally may pass through the basement membrane from the medulla into the cortical region of the developing nodule. That lymphocytes in the bursa of Fabricius originate from both endodermal and mesodermal derivatives during embryonic development appears to be consistent with both light and electron microscopic observations.

INTRODUCTION

Interest in the bursa of Fabricius has developed recently because of morphologic evidence indicating that the lymphocytes of this lympho-epithelial organ of birds have a dual origin in the embryo: they arise from undifferentiated endodermal epithelial cells and from mesenchymal cells (1, 3, 8, 30). Light microscopic studies (3) of the development of the bursa of Fabricius in the chick embryo indicate that the medullary portion of the lymphoid follicle arises as an epithelial bud from the surface endodermal epithelium. This bud projects into the tunica propria and gradually enlarges. The epithelial bud is composed of undifferentiated epithelial cells which undergo mitosis and/or appear to transform directly into lymphoblasts and developing lymphoctyes. Lymphocytic cells of the cortical portion of the follicle are derived from two sources: from mesenchymal cells of the tunica propria surrounding the epithelial bud or medulla and from undifferentiated epi-

thelial cells or lymphoblasts which migrate through the basement membrane from the medulla into the tunica propria. Both the cortex and medulla appear as sites of lymphocytopoiesis although this activity is most striking in the medullary portion of the nodule. The basement membrane separating the cortical and medullary portions of the lymphoid nodule is continuous with the basement membrane underlying the surface epithelium, and the continuity of the basement membrane shows disruption only when lymphocytic progenitors are seen passing through this membrane.

It is the purpose of this study to examine the fine structure of the cellular elements in the lympho-epithelial nodules of the bursa of Fabricius of the chick embryo and to characterize the morphological changes occurring in these cells during differentiation. 15- and 16-day-old chick embryos were chosen for our initial studies since during this stage of embryonic development the bursa shows not only all stages of lymphocytopoiesis in the medullary portion of the nodule but also the early formation of lymphocytic cells in the cortical region of the nodule. The passage of cells through the basement membrane also could be expected to be observed during this time.

MATERIALS AND METHODS

The bursa of Fabricius was rapidly excised from 15- and 16-day-old white Leghorn chick embryos, cut into small pieces, and fixed for 1 hour in cold 2 per cent osmium tetroxide buffered to pH 7.8 with veronal acetate. Tissues were dehydrated in graded ethanol and embedded in a 1:5 mixture of methyl and butyl methacrylate. Sections cut with glass knives on a Porter-Blum microtome were mounted

FIGURE 1

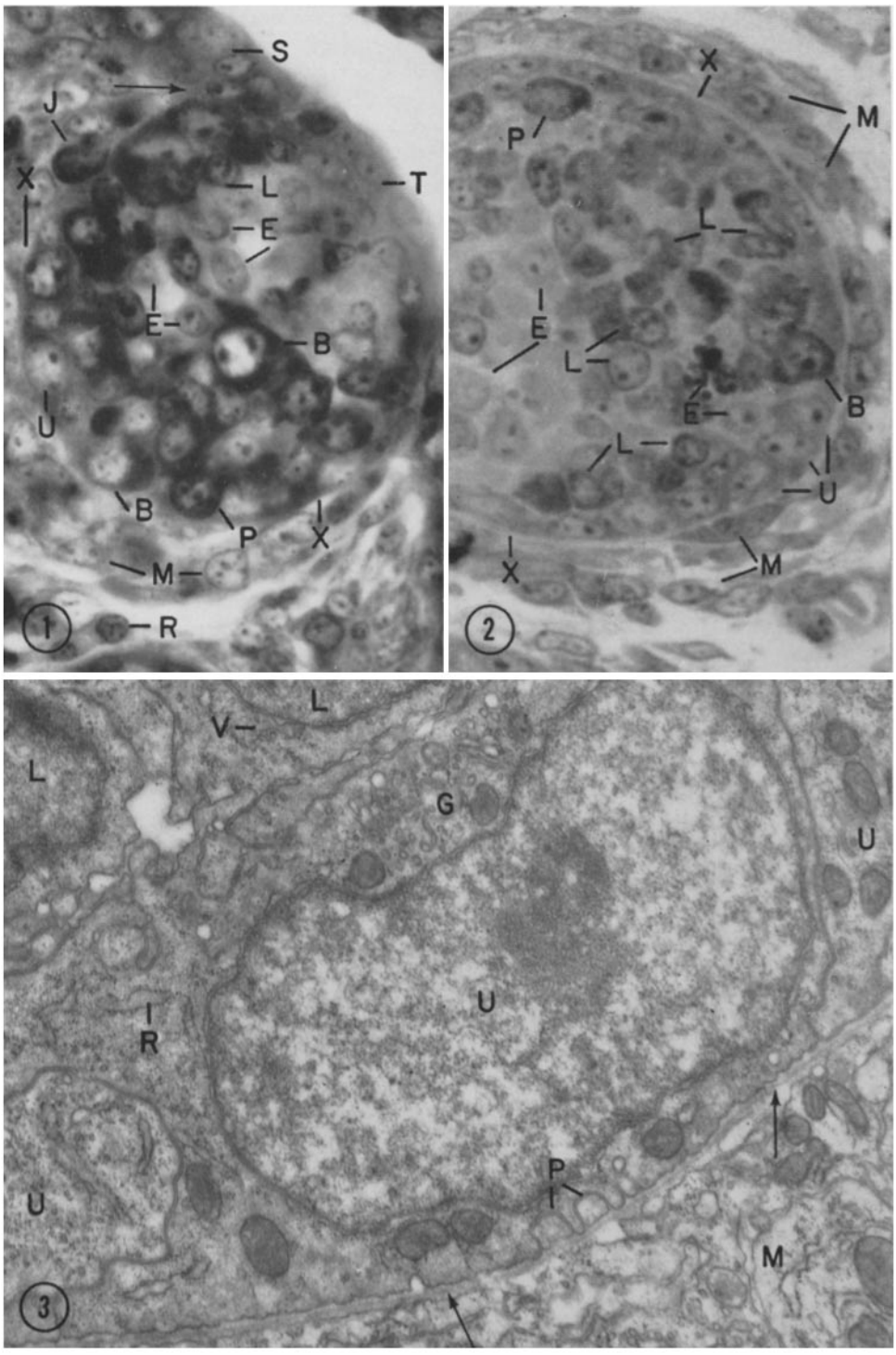
A lympho-epithelial nodule showing the surface endodermal epithelium (*S*), the epithelial-nodular junction (arrow), and basement membrane (*X*) separating the medullary and cortical portions of the developing follicle. Various cells comprising the follicle may be recognized: undifferentiated basal epithelial cells (*U*), lymphoblasts (*B*), large lymphocytes (*P*), medium lymphocytes (*L*), reticular-epithelial cells (*E*), modified epithelial cells of the epithelial tuft of the nodule (*T*), mesenchymal cells (*M*), and an erythrocyte (*R*) in a capillary in the cortex. A primitive lymphocytic cell (*J*) is located in the cortex in contact with the basement membrane. 16-day-old chick embryo. Bursa fixed in Zenker's formol, embedded in paraffin, and stained with Giemsa. Light micrograph. × 1450.

FIGURE 2

Similar to Fig. 1, except tissue was fixed in buffered 2 per cent osmium tetroxide, embedded in methacrylate, and stained with 0.2 per cent azure A. The basement membrane separating the so-called cortex and medulla of the follicle is well defined. Note the paucity of lymphocytes in the cortical zone as compared to the marked lymphocytopoietic activity of the medulla during this stage of embryonic development. Phagocytized nuclear material may be seen in the cytoplasm of one of the reticular-epithelial cells (*E*). 16-day-old chick embryo. Light micrograph. × 1450.

FIGURE 3

Undifferentiated basal endodermal epithelial cells (*U*), medium lymphocytes (*L*), and a mesenchymal cell (*M*) are illustrated. The basement membrane (arrows) composed of a thin central membrane and associated on either side with an amorphous material separates the cortical and meduallary zones of the follicle. The undifferentiated epithelial cell shows a prominent Golgi complex (*G*) near the nuclear hof, strands of rough endoplasmic reticulum (*R*), infoldings of the plasma membrane (*P*), and interdigitation with adjacent processes of undfifferentiated basal cells. Small spherical vesicles (*V*) arising from the outer nuclear membrane may be distinguished in the cytoplasm of one of the lymphocytes. 16-day-old embryonic chick. Bursa fixed in buffered 2 per cent osmium tetroxide, embedded in methacrylate, stained with lead hydroxide. × 21,000.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on Formvar grids and stained for 30 to 45 minutes with a saturated aqueous solution of lead hydroxide (37). Stained sections were examined with the RCA EMU-3f electron microscope.

OBSERVATIONS

General histology and relationships of the various cellular elements of the lymphocytic nodules of the bursa of Fabricius during the 16th day of embryonic development in the chick are indicated in Figs. 1 and 2. Further histologic and cellular detail concerning the bursa of Fabricius and its development may be obtained from our earlier studies (3) with the light microscope. The prime consideration of this report will be the electron microscopic examination of the cellular elements directly concerned with lymphocytopoiesis, their fine structure, relationships, and the changes that occur in them during cytodifferentiation.

Electron microscopic evidence derived from the examination of the bursa of Fabricius from the 15 and 16-day embryonic chick is in agreement with our light microscopic observation on this organ (3). The endodermal surface epithelium is separated from the underlying mesenchyme by a delicate basement membrane which extends entirely around the nodular invaginations (developing medullary regions of the lymphocytic nodules) (Figs. 1 to 6) and is not disrupted at the surface epithelial-nodular junction. The basement membrane is continuous although interruptions do occur along its extent through which protoplasmic processes of undifferentiated epithelial cells and lymphoblasts extend into the surrounding mesenchyme. Examples of cells passing through the basement membrane have been observed infrequently with the electron microscope but correlative light microscopy and selective staining suggests that the cellular elements, usually lymphoblasts, most probably are passing from the medullary portion of the nodule into the developing cortical region (3). This view seems further substantiated since lymphocytopoietic activity in the cortical area is sparse in comparison with that in the medullary portion of the developing nodule (Figs. 1, 2). The appearance of interruptions in the basement membrane as seen with the electron microscope suggests localized areas of dissolution and apparently the basement membrane is reconstructed following the passage of the cell or cells through this zone. The basement membrane is approximately 100 to 140 mμ in thickness and consists of a thin, moderately dense membrane (about 15 mμ wide) associated on either side with a layer of amorphous (glyco

FIGURES 4 THROUGH 23

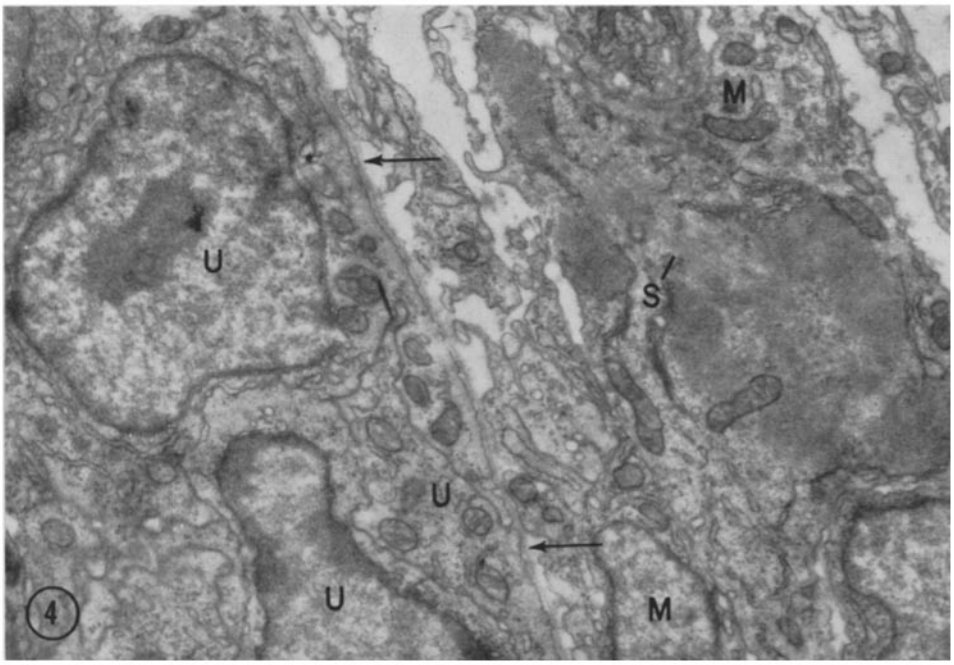
16-day-old chick embryo. Bursas fixed in buffered 2 per cent osmium tetroxide, embedded in methacrylate, and stained with lead hydroxide.

FIGURE 4

The thin basement membrane (arrows) separates the mesenchymal cells (*M*) of the cortex from the undifferentiated basal epithelial cells (*U*) of the medulla. The interdigitation of cytoplasmic processes of the basal cells and the intimate association of these cells with the basement membrane can be seen. Mesenchymal cells reside near the basement membrane but are not always in direct apposition with this structure. One mesenchymal cell is in the process of mitosis and spindle fibers (*S*) may be distinguished. × 11,000.

FIGURE 5

Clusters of ribosomes, strands of rough endoplasmic reticulum, rod and oval-shaped mitochondria may be distinguished in the cytoplasmic processes of the undifferentiated basal epithelial cells (*U*). A lymphoblast (*B*) exhibits numerous mitochondria, a few strands of rough endoplasmic reticulum, and many ribosomes. Processes of mesenchymal cells (*M*) are situated in the cortex near the basement membrane (arrows). Oval and rod-like mitochondria, smooth and rough endoplasmic reticulum are conspicuous in these cells. A portion of a medium-size lymphocyte (*L*) exhibits a local region of nuclear vesiculation and associated small spherical cytoplasmic vesicles (*V*). × 17,000.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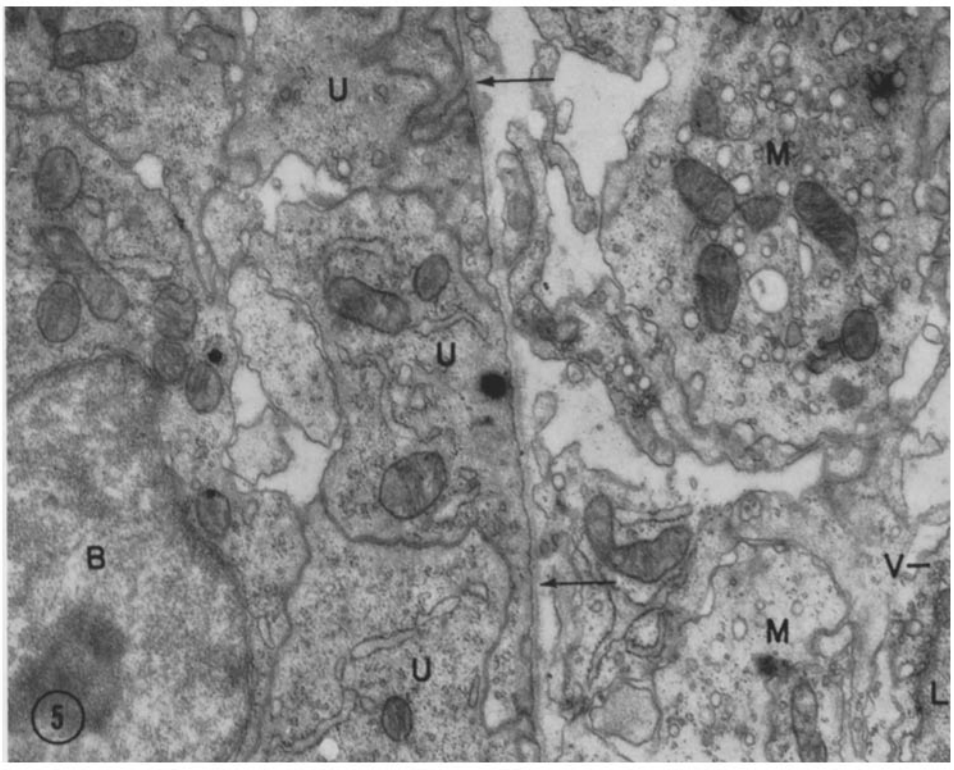
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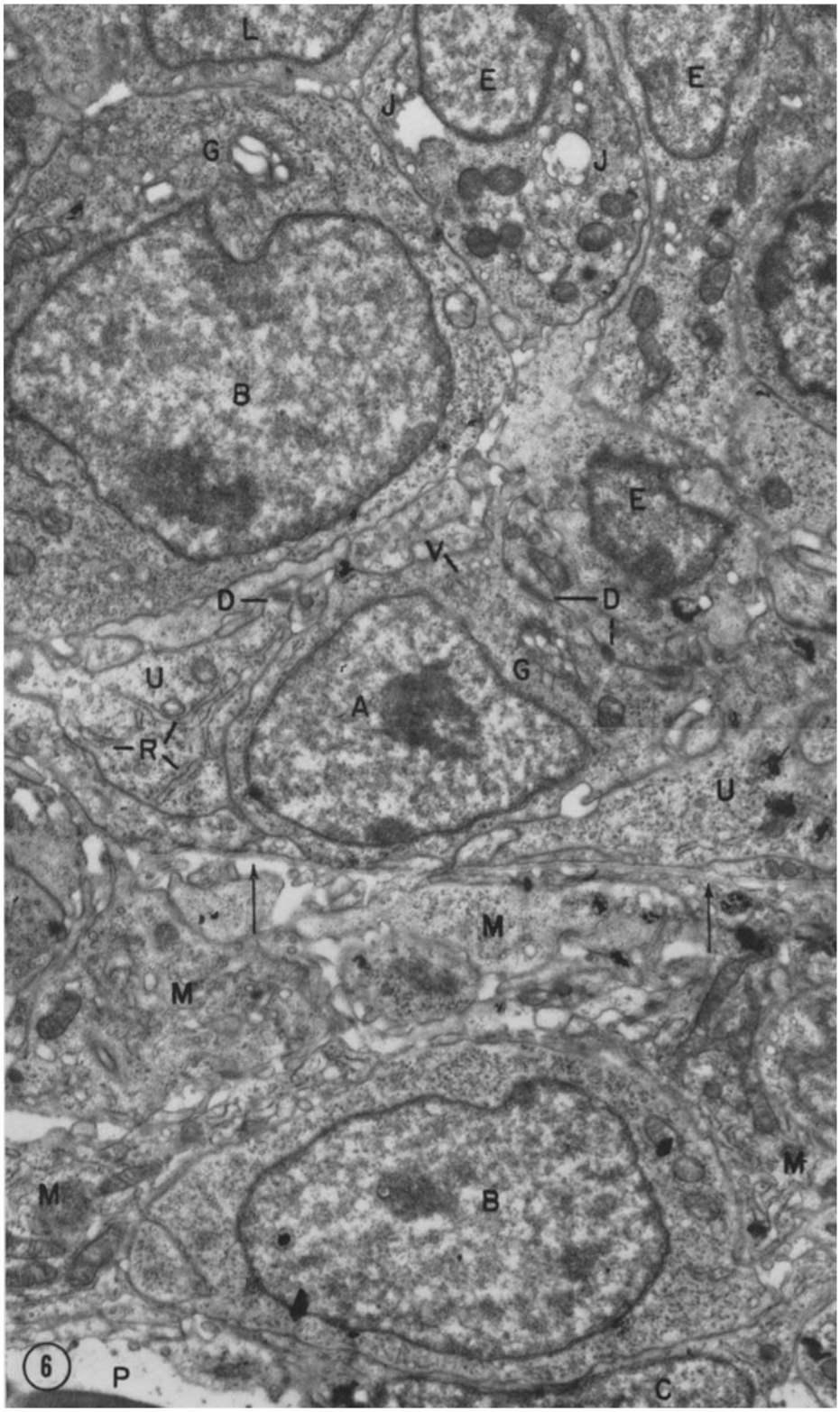
protein) substance (Figs. 3 to 6). The basement membrane is in direct contact with the basal epithelial cells of the medulla while the cytoplasmic extensions of the adjacent mesenchymal cells are in close proximity but not necessarily in direct apposition with this membrane (Figs. 3 to 6).

The basal epithelial cells of the medullary portion of the follicle lie in contact with the basement membrane and exhibit considerable variation in contour (Figs. 1 to 6). The more undifferentiated cells tend to be elongate and irregular in shape, gradually assuming a more rounded contour prior to mitosis and cytodifferentiation. The elongate cells have numerous protoplasmic processes which interdigitate with adjacent cells; infolding of the plasma membrane of the basal epithelial cells is relatively common (Figs. 3, 5). Desmosomes connecting basal cells (Fig. 6) and adjacent undifferentiated epithelial cells of the developing medulla are frequent. Desmosomes also connect the protoplasmic processes of the modified reticular-epithelial cells within the nodule (Fig. 9) but have not been noted in association with lymphoblasts or developing lymphocytic cells. The nuclei of the undifferentiated epithelial cells tend to be rounded and contain one or two nucleoli. The nuclear envelope consists of a double membrane with nuclear pores and may show evidence of vesiculation and/or blebbing. The nuclei of the basal cells lie close to the basement membrane and the cells exhibit polarity, with the Golgi apparatus, centrosome, and centrioles situated in the proximal region of the cells, *i.e.*, towards the center of the medulla (Fig. 3). The cytoplasm of the basal cells contains numerous free ribosomes and moderate amounts of elongate sacs of rough endoplasmic reticulum within which may be seen a homogeneous substance of slightly greater density than the cytoplasmic matrix (Figs. 3 to 6, 19). Smooth endoplasmic reticulum is sparse in these cells. Small spherical vesicles about 40 to 90 mμ have been observed in the cytoplasm of the basal cells and may differ from the smooth endoplasmic reticulum *per se* as will be discussed later. Compound vesicles (300 to 400 mμ) composed of smaller round vesicles (40 to 60 mμ) surrounded by a single membrane also are observed occasionally in the cytoplasm of the basal cells (Fig. 6), as well as in the other cellular elements of the bursa, *i.e.*, developing lymphocytic, reticular-epithelial (Figs. 20, 23) and mesenchymal cells. Oval and rod-shaped mitochondria are present in moderate numbers and are scattered throughout the cytoplasm of the basal cells.

FIGURE 6

The relationship of the various cells of the lymphocytic nodules may be readily ascertained in this illustration. The basment membrane (arrows) delineates the cortical-medullary boundary. Processes of undifferentiated basal epithelial cells (*U*) lie along the basement membrane in the medulla and contain numerous strands of rough endoplasmic reticulum (*R*). These cells are connected by desmosomes (*D*) with the protoplasmic processes of adjacent undifferentiated epithelial and/or reticular-epithelial cells (*E*). Reticular-epithelial cells (*E*) have a more central location in the medulla and the cytoplasmic processes interdigitate with one another and tend to surround the developing lymphocytes (*L*) and lymphoblasts (*B*). Unusual cytoplasmic vacuoles (*J*) are present in the cytoplasm of one of the reticular-epithelial cells. The more rounded cell (*A*) along the basement membrane contains more ribosomes than the undifferentiated epithelial cells, has scant rough endoplasmic reticulum and a very prominent Golgi complex (*G*); a compound vesicle (multivesicular body) also is present within the cytoplasm. Desmosomes (*D*) connect this cell with adjacent undifferentiated epithelial or reticular-epithelial cells. This cell represents a transitional stage between an undifferentiated epithelial cell and a lymphoblast. The lymphoblast (*B*) in the medulla is large, has a very prominent Golgi complex, few sacs of rough endoplasmic reticulum, and numerous ribosomes. A similar cell (*B*) is present in the cortex and represents either a lymphoblast or a young large lymphocyte. Protoplasmic processes of the mesenchymal cells (*M*) have an irregular contour, contain elongate mitochondria, strands of rough endoplasmic reticulum, and scattered groups of ribosomes. A capillary (*P*) containing an erythrocyte may be seen with the lining endothelial cell (*C*) at the peripheral region of the cortex. × 13,000.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Golgi membranes and vesicles are located near the nuclear indentation (hof) of the basal cells and tend to encompass the centrioles and centrosome. These structures reveal no unusual morphologic features. The more rounded basal cells tend to have less extensive rough endoplasmic reticulum and more numerous free ribosomes than the less rounded basal cells. Variations in cytoplasmic basophilia of the basal cells are seen with light microscopy and selective staining (Figs. 1, 2) and correspond to the variations in ribosomal content of these cells. The more rounded and more basophilic cells along the basal region of the nodule are considered to be undergoing cytodifferentiation since they possess certain morphologic features in common with lymphoblasts.

Lymphoblasts (Figs. 1, 2, 5, 6) forming in the medulla arise from the mitosis and/or differentiation of the basal and adjacent undifferentiated epithelial cells and also possibly from the reticular-epithelial cells scattered throughout the medulla. Transitions between the undifferentiated basal epithelial cells and lymphoblasts have been observed frequently with both light and electron microscopy (Fig. 6). Lymphoblasts tend to be slightly larger than the undifferentiated basal cells and are rounded in contour with deeply basophilic cytoplasm (Figs. 1, 2), abundant free ribosomes and scant rough endoplasmic reticulum (Fig. 6). The rounded nuclei appear slightly more dense than the nuclei of the undifferentiated cells and one or two prominent nucleoli are present. There is a tendency for both the lymphoblasts and developing lymphocytes to have a greater concentration of chromatin material along the inner nuclear membrane than is seen in the more undifferentiated cells. The outer nuclear membrane is generally devoid of ribosomes (Figs. 6, 15) and may have localized regions of vesiculation (Fig. 15) or blebbing. Electron microscopy indicates little difference in the size, number, or location of the mitochondria between the lymphoblasts and undifferentiated epithelial cells. The Golgi membranes of these two cell forms are very similar although in some instances there seems to be some dilation of the Golgi apparatus during the transition from the undifferentiated cells into lymphoblasts (Fig. 6). Centrioles are present in the lymphoblasts and developing lymphocytic cells and are surrounded by the modified cytoplasmic matrix of the centrosome and reflections of the Golgi membranes and vesicles.

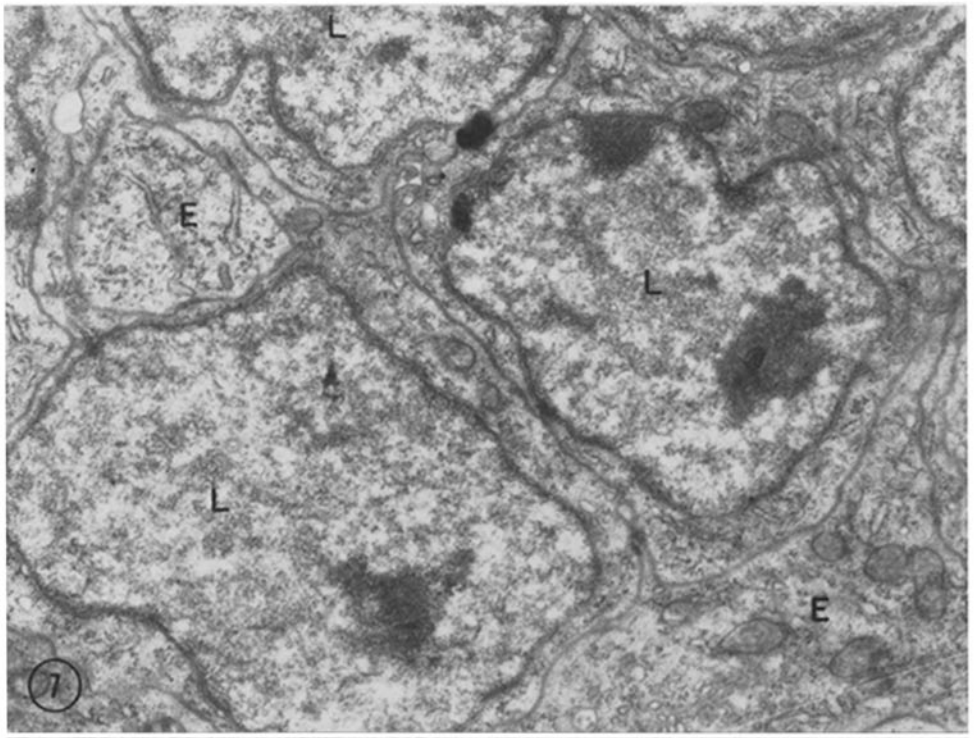
Both undifferentiated epithelial cells and lymphoblasts have been observed, in several instances, passing through the basement membrane of the nodule into the developing cortex. Lymphoblasts in the cortical region most probably arise from these cells as well as from mesenchymal cells present in this zone (Figs. 1, 6). Electron microscopic evidence of these early transitions between mesenchymal cells and lymphoblasts, as might be expected, are relatively uncommon. Fig. 10 shows a mesenchymal-like cell which seems to be in the process of rounding up; it

FIGURE 7

Several medium-size lymphocytes (*L*) crowded together in the central portion of the medulla. Note the narrow rim of cytoplasm and the few organoids present in these cells. Protoplasmic processes of reticular-epithelial cells (*E*) also may be seen between the developing lymphocytes. Intercellular spaces of small size can be distinguished occasionally between these cells. × 14,000.

FIGURE 8

Two medium-size lymphocytes (*L*) exhibit scant cytoplasm and few ribosomal clusters. The nuclei of the lymphocytes are somewhat irregular in contour, show some chromatin condensation along the inner nuclear membrane; nuclear pores (*P*) are relatively few and have no uniformity in their distribution along the nuclear envelope. Areas of vesiculation of the outer nuclear membrane and associated cytoplasmic vesicles (*V*) may be readily seen. An unusual cytoplasmic inclusion (*I*) is surrounded by a double membrane and is composed of dense matrix and fine dense granules and possesses a portion of an internal double membrane. This inclusion is present in the cytoplasm of one of the reticular-epithelial cells (*E*) illustrated. A discrete cluster of ribosomes (*R*) is present in the cytoplasm of another of the reticular-epithelial cells. × 22,000.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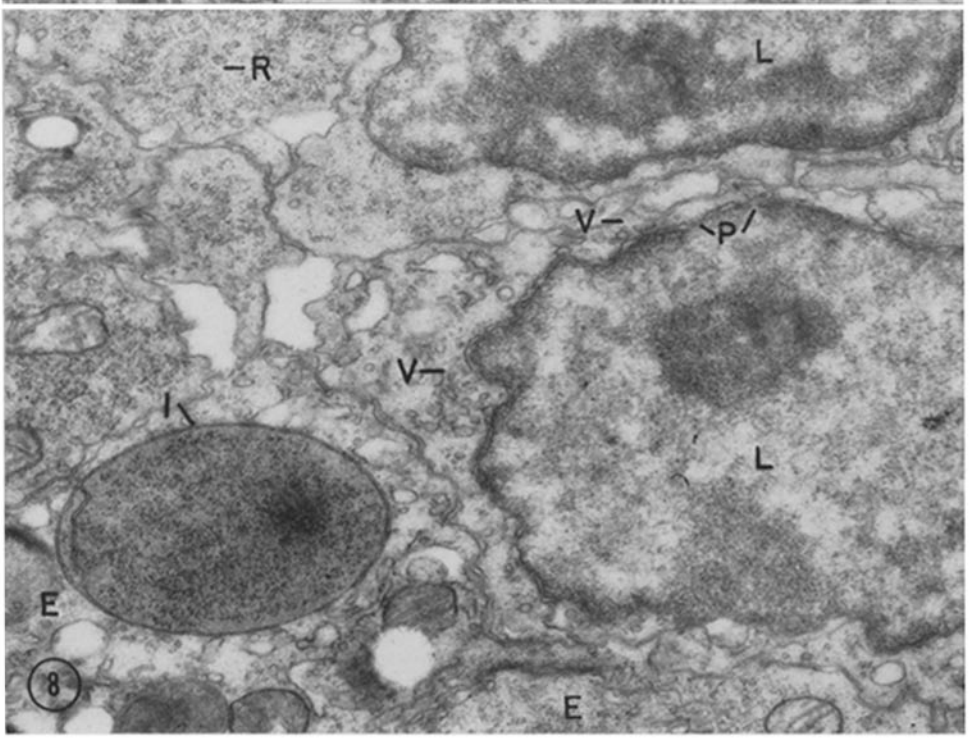
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contains a greater number of free ribosomes and less rough endoplasmic reticulum than adjacent mesenchymal and fibroblastic cells. This cell is considered to represent an early transition between a mesenchymal cell and a lymphoblast.

Lymphoblasts and large lymphocytes exhibit only minor morphological differences as visualized with either light or electron microscopy, *i.e.*, a slight decrease in cell size and a greater condensation of chromatin in the large lymphocytes as compared with the lymphoblast. Medium-size lymphocytes (Figs. 1, 2, 6–8) are considerably smaller, possess less cytoplasm and fewer mitochondria, and have a less developed Golgi apparatus than the lymphoblasts and large lymphocytes. The intercellular region of the medulla is difficult to delineate since the various cell forms are so tightly packed that only a thin film of extracellular fluid separates these cells (Figs. 3 to 9). Medium lymphocytes are rounded but actually exhibit a somewhat irregular outline due to cellular compression within the nodule (Figs. 7, 8). The outer nuclear membrane of these cells usually is devoid of ribosomes, and regions of blebbing and vesiculation are frequently observed along the nuclear membrane (Figs. 8, 12 to 14, 16). The nuclei of the medium-size lymphocytes appear more condensed than the nuclei of the large lymphocytes and contain one or two small nucleoli. Nuclear pores seem less numerous and occur more irregularly as the lymphocytic cells mature; the lymphocytes have fewer nuclear pores in comparison with most other cellular elements in the bursa. The medium lymphocytes exhibit a deep basophilia but it is less intense than in the lymphoblasts following selective staining and light microscopy (Figs. 1, 2). Ribosomes are abundant in the lymphocytes (Figs. 7, 8) but are less numerous than in the lymphoblasts and large lymphocytes. Small round vesicles (Figs. 7, 8, 16) and, rarely, compound vesicles are observed in the cytoplasm of the lymphocytes; however, the lymphocytic cells are generally devoid of rough endoplasmic reticulum and possess very few profiles of smooth endoplasmic reticulum (Figs. 7, 8). Mitochondria are sparse and appear spherical to oval in contour. Mitosis of the lymphocytic cells in both the cortex and medulla is quite common. Medium-size lymphocytes are rare in the cortical region of the nodule at this stage of embryonic development of the bursa (Figs. 1, 2); when present they are usually seen with a small foci of developing lymphocytic cells.

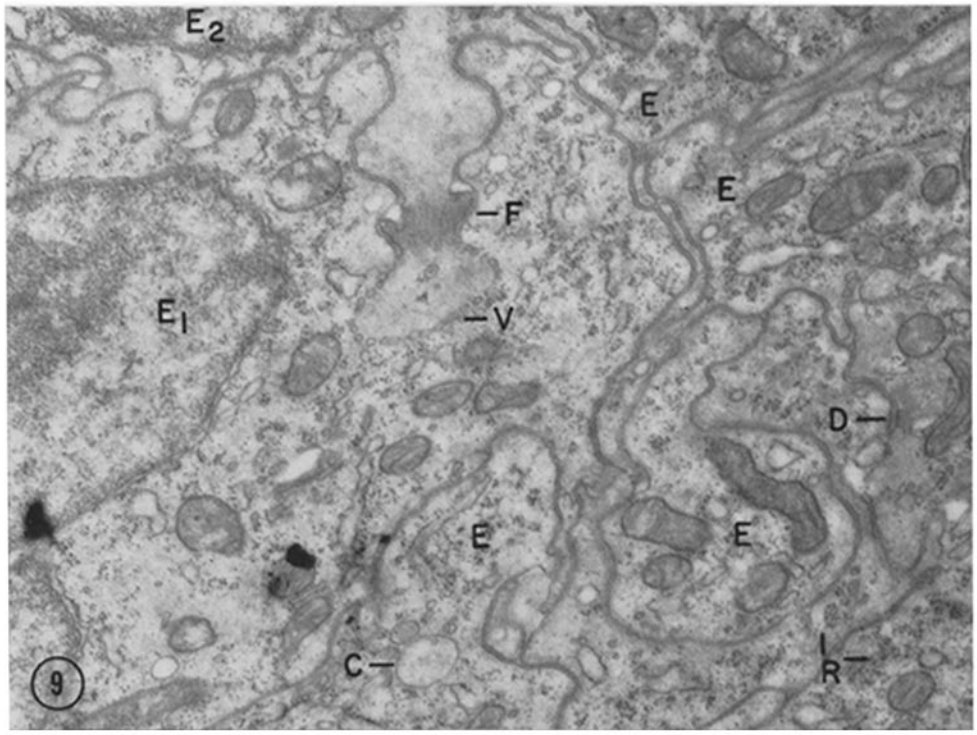
Reticular-epithelial cells (Figs. 1, 2, 6, 9) in the medulla represent modified undifferentiated epithelial cells which develop in the interior region of the medulla. These cells are characterized by a stellate contour, their markedly irregular cytoplasmic outline resulting from compression and crowding produced by the developing lymphocytes and by lymphopoietic activity in the medulla (Figs. 6, 7, 9). The cytoplasmic processes of the reticular-epithelial cells extend between the proliferating lymphocytic cells (Fig. 7); these processes tend to interdigitate and may be joined to one another by desmosomes (Fig. 9). Reticular-

FIGURE 9

A group of reticular-epithelial cells showing the marked interdigitation of the cytoplasmic processes, desmosomes (*D*), ribosomal clusters (*R*), elongate mitochondria, rough endoplasmic reticulum and compound vesicles (*C*). Reticular-epithelial cell nuclei E_1 and E_2 represent daughter cells. The cytoplasmic extensions of these two cells connect at the body of Flemming (*F*). Daughter cell E_1 has tended to fold or telescope back over the connection between these two cells. A group of vesicles (*V*) are situated at the junction of the body of Flemming and the cell cytoplasm. × 23,000.

FIGURE 10

A transitional cell at a stage between a mesenchymal cell and a lymphoblast is shown (*B*). The ribosomal content, mitochondrial contour, and the extent of development of the endoplasmic reticulum may be compared in this developing blast cell and the surrounding mesenchymal (*M*) and fibroblast-like (*F*) cells. The Golgi complex (*G*) may be seen in the fibroblast along with prominent rough and smooth endoplasmic reticulum. A few cross-sections of reticular fibers (*R*) are present in the intercellular space. × 22,000.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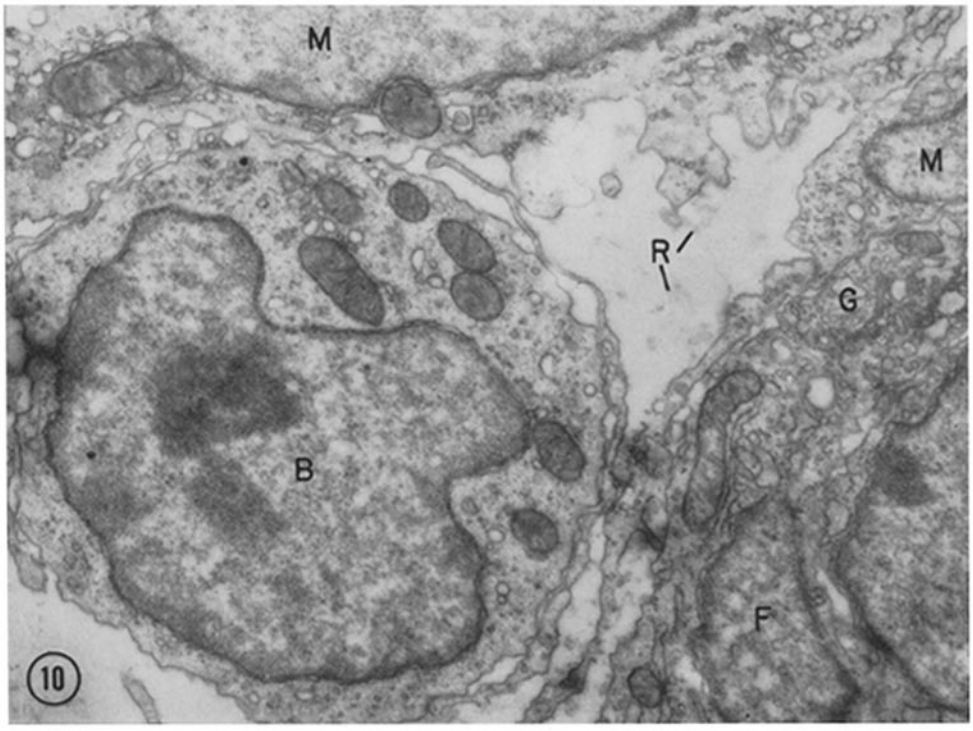
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epithelial cells have oval nuclei which usually contain one or two small nucleoli; regions of nuclear vesiculation are frequently observed. The cytoplasm exhibits rather poorly developed rough endoplasmic reticulum; the free ribosomes, while variable in number, are fewer than they are in the undifferentiated basal cells or lymphocytic elements (Figs. 6, 9). Light microscopy indicates these cells to be faintly basophilic (Figs. 1, 2), thus showing a positive correlation with the relative number of ribosomes observed in these cells. Mitochondria are more rod-shaped (Fig. 9) and appear to be more numerous in the reticular epithelial cells than in other cells of the medulla. The Golgi apparatus is more extensive than in the developing lymphocytic cells and the centrioles and centrosomes are surrounded by well developed Golgi membranes and vesicles. The cytoplasm exhibits numerous small round vesicles as well as sacs of smooth endoplasmic reticulum. Compound vesicles (Figs. 20, 23), small lipid droplets, vacuoles (Fig. 6) and granules (Fig. 21) may be observed in the cytoplasm of the reticular-epithelial cells; however, other cytoplasmic inclusions (Fig. 8)

FIGURE 11

Several fibroblastic cells (*F*) in the cortex which contain prominent sacs of rough endoplasmic reticulum, oval to elongate mitochondria, and scattered ribosomes and vesicles. Cross-sections of reticular fibers (*R*) tend to be associated along the plasma membrane of these cells and areas of cytoplasmic fragmentation (arrows) and fiber clusters are shown; small vesicles also may be present in these areas, as well as in the peripheral cytoplasm of the fibroblastic cells. A portion of a developing blast cell (*B*) is also shown. × 14,000.

FIGURE 12

A large bleb involving both the inner (arrow) and outer nuclear membrane of the nucleus (*N*) of a medium-size lymphocyte shows the presence of a finely granular substance in the interior portion of the bleb bounded by the inner membrane. × 29,000.

FIGURE 13

Nuclear bleb of a medium lymphocyte illustrating the loss of density of the nuclear envelope of the bleb, dispersed intranuclear substance within the bleb, and perhaps the formation of vesicles along the nuclear membrane of this outpocketing (arrow). *N*, nucleus. × 29,000.

FIGURE 14

A small nuclear bleb in a medium lymphocyte contains three spherical vesicles (arrow) and an ill defined vesicle within the chromatin clump immediately below these vesicles. *N*, nucleus. × 29,000.

FIGURE 15

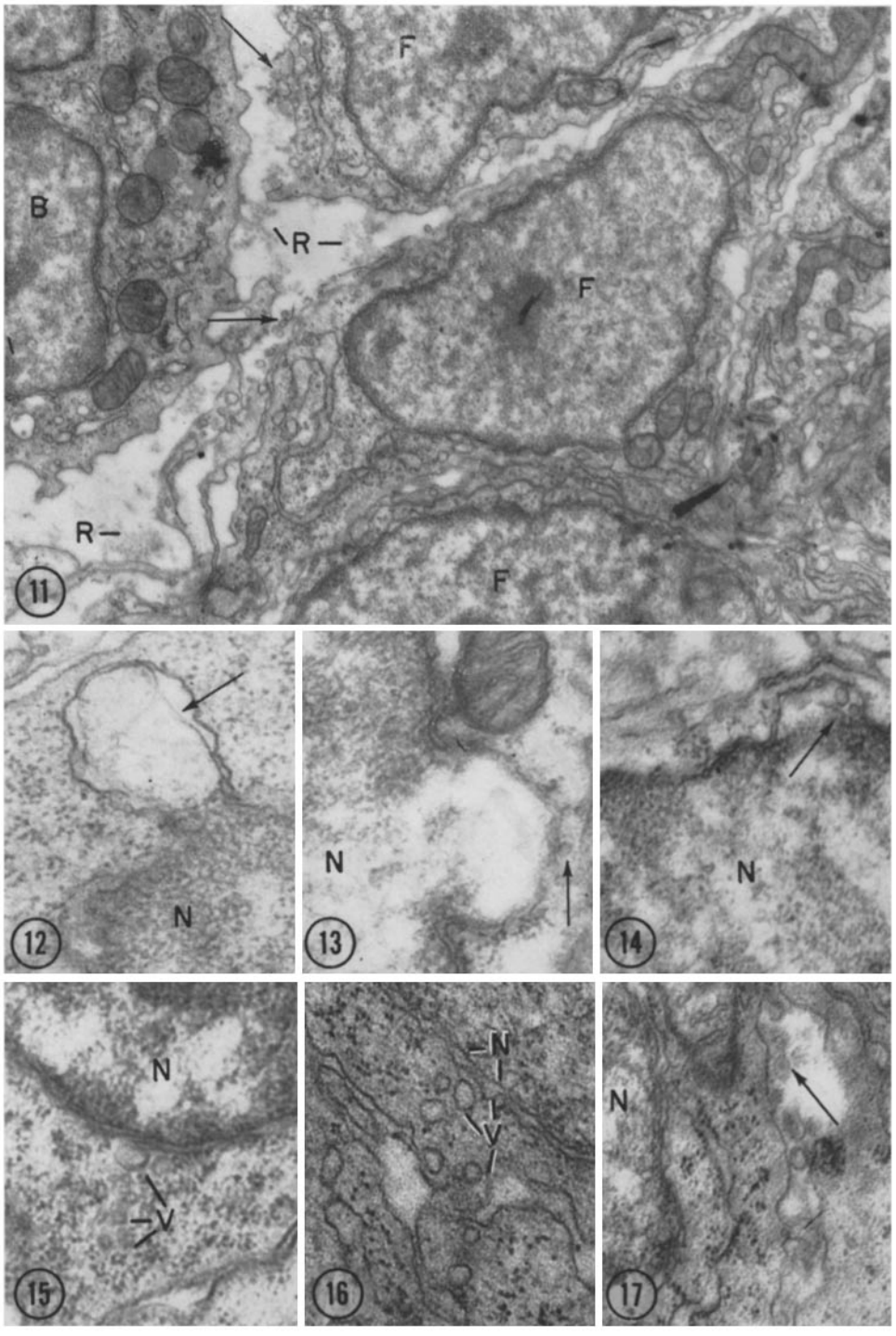
A lymphoblast showing a column of rounded vesicles (*V*) extending from the perinuclear region toward the plasma membrane. *N*, nucleus. × 47,000.

FIGURE 16

Medium lymphocyte with nuclear vesiculation (*N*) and columns of vesicles (*V*) extending toward the plasma membrane. × 47,000.

FIGURE 17

A number of vesicle-like structures appear to be located in the intercellular space between adjacent reticular-epithelial cells. Note the central granule present (arrow) in one of these structures. *N*, nucleus. × 40,000.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are only rarely seen. The possibility exists that lymphocytic cells arise from these stellate reticular-epithelial cells although a complete series of transitional forms has not been found as yet in our electron microscopic studies.

The developing cortical portion of the follicle is composed primarily of mesenchymal cells and contains very few and scattered lymphocytic elements during the 15- and 16-day stage of embryonic development (Figs. 1, 2). Mesenchymal cells (Figs. 1, 2, 4 to 6, 10) are stellate in contour with many long cytoplasmic processes. They contain abundant smooth and rough endoplasmic reticulum, scattered free ribosomes, elongate mitochondria, and numerous Golgi vesicles which surround the centrosome and centrioles. Many of the mesenchymal-like cells exhibit markedly developed, dilated rough endoplasmic reticulum (Figs. 10, 11) which contains a moderately dense material. Small fibrils (Fig. 11), which occasionally show indications of periodicity, tend to localize in groups along or near the cytoplasmic extensions of these cells; cytoplasmic fragmentation is frequently observed (Fig. 11) (28). Those cells which contain dilated sacs of endoplasmic reticulum and are associated with "reticular" fibrils are considered to represent early developmental stages of fibroblastic differentiation. It seems likely that mesenchymal cells are capable of transformation into either fibroblast-like cells or lymphocytic progenitors during this period of embryonic development. Mesenchymal cells transforming into lymphoblasts may be distinguished primarily by the amount and extent of development of the rough endoplasmic reticulum, the relative ribosomal content of the cytoplasm, and the contour of the cell (Fig. 10).

Particular comment concerning nuclear vesicu-

FIGURE 18

Medium lymphocyte exhibiting nuclear vesiculation and cytoplasmic vesicles (*V*). At one point three vesicles appear to be extruded into the intercellular fluid. Vesicles with poorly defined limiting membranes (arrow) are present in the intercellular space. *N* indicates the inner nuclear membrane and *O* the outer nuclear membrane. × 40,000.

FIGURE 19

Undifferentiated basal epithelial cell containing numerous elongate sacs of endoplasmic reticulum. Areas of the endoplasmic reticulum are indicated by arrows and have no apparent or distinct limiting membrane. × 16,00.

FIGURE 20

The cytoplasm of a reticular-epithelial cell showing two compound vesicles (*C*), several mitochondria (*M*), and two dense granules or vacuoles (*V*). The upper granule appears to contain a segment of an inner membrane. A possible series of mitochondrial modifications between the typical mitochondria and these granules is suggested. × 26,000.

FIGURE 21

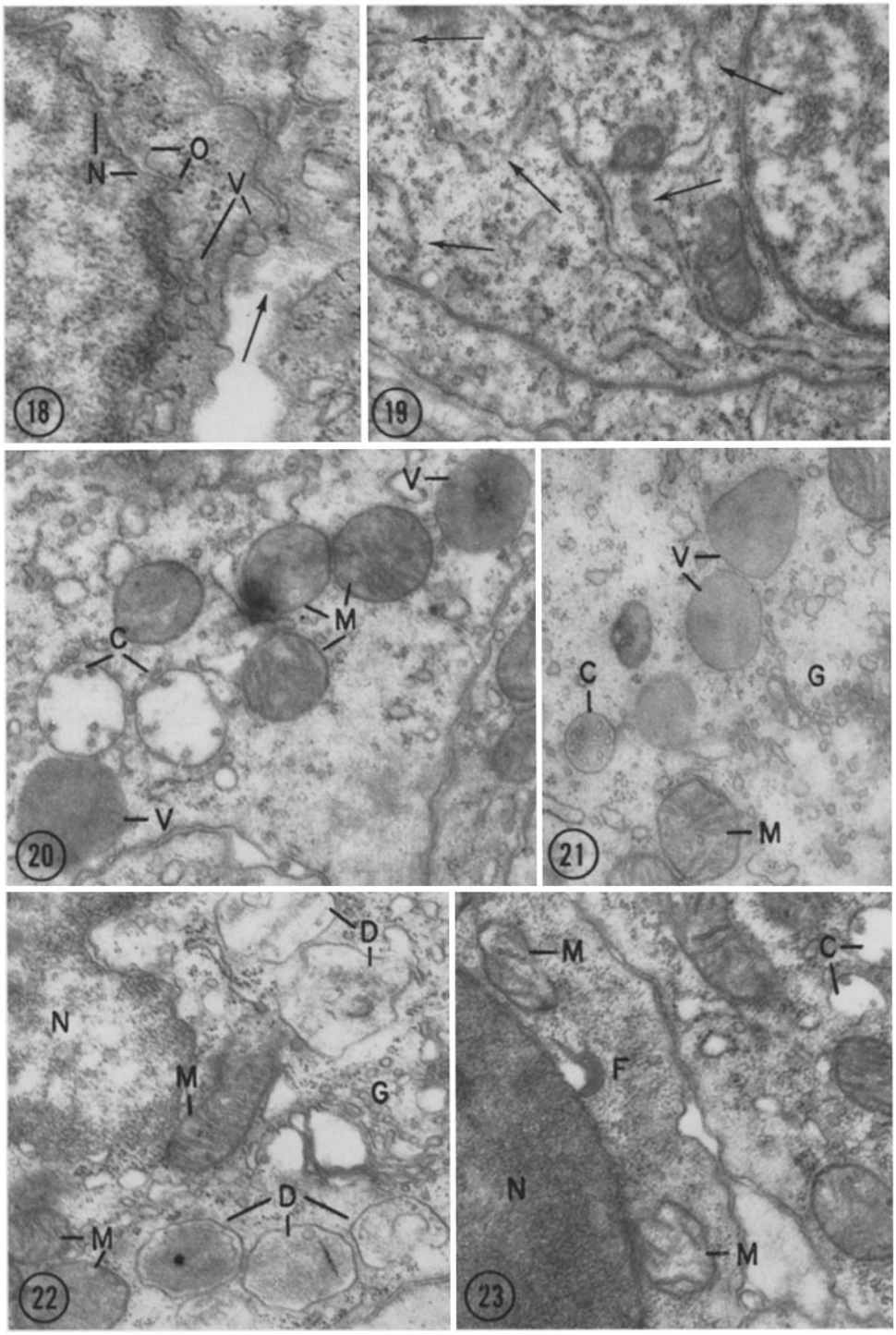
A reticular-epithelial cell containing a compound vesicle (*C*) with a moderately dense matrix. The vacuoles or granules (*V*) have a similar density. Normal mitochondria (*M*) and a portion of the Golgi complex (*G*) also may be distinguished. × 25,000.

FIGURE 22

Reticular-epithelial cell showing cell nucleus (*N*), prominent Golgi complex (*G*) and several normal mitochondria (*M*). Structures at (*D*) may possibly represent a series of transitional stages in the degeneration of the mitochondria. × 26,000.

FIGURE 23

Medium lymphocyte on the left containing a fat droplet (*F*) and two wrinked (degenerating?) mitochondria (*M*). It lies adjacent to a reticular-epithelial cell on the right containing normal mitochondria and two compound vesicles (*C*). *N*, nucleus. × 26,000.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lation and blebbing seems desirable because of the frequency of their occurrence in the various cells of the developing follicle. The term vesiculation is employed herein to indicate the formation of small spherical or slightly irregular vesicles (approximately 40 to 90 mμ) from the outer nuclear membrane (Figs. 5, 15, 16, 18). Vesicles are frequently seen in columns perpendicular to the nuclear envelope and extending toward the plasma membrane, as if the vesicles were passing from the perinuclear location to the peripheral region of the cytoplasm (Figs. 15, 16). In rare instances, vesicles of similar size and density have been observed in the extracellular spaces (Figs. 17, 18), usually adjacent to cells exhibiting active nuclear vesiculation. Rarely, a moderately dense granule may be distinguished within some of the vesicles either in the cytoplasm or intercellular spaces (Fig. 17). The uniform size of these vesicles, their rounded contour, and their arrangement in rows or groups radiating from the nuclear envelope to the peripheral cytoplasm suggest distinguishing morphological and perhaps functional differences between these vesicles and the typical smooth surfaced endoplasmic reticulum and/or pinocytotic vesicles. Similar vesicles arising from the nuclear membrane in the region of the nuclear hof appear to contribute to the Golgi complex.

Nuclear blebbing differs from nuclear vesiculation in that blebbing is considered to be the formation of large sac-like pouches from the nucleus and involves both the inner and outer nuclear membranes and associated intranuclear material (Figs. 12 to 14). Such blebs form along the nuclear membrane in areas associated with chromatin aggregations and, occasionally, the larger blebs appear to have an association with the nucleolus. Usually the inner and outer nuclear membranes of the bleb are less dense than the regular nuclear envelope. Vesicle-like structures within the nuclear blebs have been observed in several instances (Fig. 14). Structures resembling nuclear blebs have not been seen free in the cytoplasm of the cells studied in the bursa of Fabricius.

Mitochondrial alterations and various stages in mitochondrial dissolution have been observed occasionally in both developing lymphocytes and reticular-epithelial cells (Figs. 20, 22, 23). One form of mitochondrial alteration is recognized by the apparent wrinkling of the mitochondrial wall and resulting disorientation of internal structure (Fig. 23). In other instances, mitochondria may undergo internal dissolution with loss and/or fragmentation of the internal membranes as the mitochrondrial matrix assumes a rather amorphous, moderately dense appearance (Fig. 22). Subsequent alterations may consist of a loss of density of the internal material, mitochondrial swelling, and wrinkling and fragmentation of the outer membrane (Fig. 22). The formation of dense vacuoles or granules (Fig. 20) also has been suggested in several electron micrographs.

DISCUSSION

Electron microscopic observations of the bursa of Fabricius support the view (3) that lymphocytes arise in the medullary portion of the nodules by mitosis and differentiation of endodermal epithelial cells, while lymphocytes present in the cortical zones have a dual origin, arising either from lymphocytic progenitors passing from the medulla into the cortex or by the transformation of mesenchymal cells into lymphocytic elements. All stages in the transformation and differentiation of lymphocytic cells have been identified and the submicroscopic changes occurring during cellular differentiation reported herein.

Studies concerning the submicroscopic structure of the lymphocytic cells and their development usually have included only those stages of maturation from the lymphoblasts to the mature lymphocytes (5, 13, 19, 20, 32). During these stages of lymphocytopoiesis the decrease in the size of the cell, nucleus, and nucleoli, and in the size and number of mitochondria, in the size of the Golgi complex, and in the number of ribosomes has been indicated. Such changes are consistent with the submicroscopic appearance of the lymphocytic elements in the bursa of Fabricius during early embryonic development. The fact that only a few profiles of endoplasmic reticulum are present in both young and mature lymphocytic cells is well recognized (5, 13, 19, 20, 25) and is found to be true of the lymphocytes of the bursa. It is of interest that in the bursa of Fabricius during the differentiation of lymphoblasts from undifferentiated basal epithelial cells (and from mesenchymal cells) there is a decrease in the amount of rough endolasmic reticulum. In addition, during subsequent cytodifferentiation of the lymphocytic cells these sacs essentially disappear. A similar loss of endoplasmic reticulum has been observed during the differentiation of erythrocytes in erythropoiesis (2, 15–17, 26). Such changes in the

endoplasmic reticulum, however, differ from those observed in most cells during their differentiation in that there is usually an increase in amount and complexity of the endoplasmic reticulum as the cells differentiate and mature (27).

Although the amount of rough endoplasmic reticulum is not extensive in the undifferentiated basal epithelial cells, the diminution in the number of these sacs in the developing lymphoblasts indicates that these membranes may undergo dissolution during the differentiation process. This conjecture may have some fragmentary support in the fact that in localized regions of the rough endoplasmic reticulum the sacs are not infrequently devoid of intimately associated ribosomes and have no apparent or distinct limiting membrane (Fig. 19); occasionally the moderately dense substance present in the sacs of endoplasmic reticulum seems to expand slightly into the surrounding cytoplasmic matrix (Fig. 19). Occasionally amorphous, irregular elongate structures having a density and contour similar to that of the material noted in the rough endoplasmic reticulum may be observed in the cytoplasm although no limiting membrane or associated ribosomes can be discerned (Fig. 19). These structures might represent remants of the matrix of the endoplasmic reticulum following dissolution of the membranes. If the formation and dissolution of the endoplasmic reticulum is considered to be a dynamic process continually taking place in the cytoplasm, then the alteration of such a process during cellular differentiation also may be possible. A justifiable criticism of the interpretation of the loss of the membranes of the endoplasmic reticulum may be that instead of actually being regions of dissolution such areas may represent only tangential sections through these sacs. It seems unlikely, however, that this is the case in all instances, since the internal matrix is sharply delineated although the limiting membrane is not apparent but may be recognized in the adjacent wall of the sac. More extensive studies of the endoplasmic reticulum during differentiation of these cells is needed to confirm or refute the conjectures and interpretations proposed at this time.

The presence of abundant free ribosomes in lymphoblasts and other primitive cells of the hemopoietic system is well recognized (2, 5, 13, 15–17, 26, 32). There is a marked increase in the number of free ribosomes during the differentiation of lymphoblasts in the bursa of Fabricius. As the lymphoblasts mature the ribosomal content of the cytoplasm decreases and tends to parallel the gradual decrease in the size of the cell and nucleus. Ribosomes are necessary for protein synthesis within the cell (14, 24, 25, 27), and with particular regard to the hemopoietic system there seems to be nearly an inverse relationship between the ribosomal content (and cytoplasmic basophilia) and the hemoglobin content of developing erythrocytic cells (2, 15–18, 35). In the erythrocytic cells, ribosomes disappear while the density of the cytoplasmic matrix and the amount of amorphous material of the cytoplasmic matrix increase, corresponding to the formation and accumulation of hemoglobin in these developing cells (2, 15–17). Protein synthesis in developing granulocytes is related to the formation of specific granules, and in plasma cells to the presence of large amounts of dilated rough endoplasmic reticulum (6, 13, 20, 26, 34). Thus far, no similar increase in the density of the cytoplasm, in the amount of endoplasmic reticulum, or in the elaboration of granules has been observed in lymphocytic cells during their differentiation and maturation. It might be expected that, even though developing lymphocytic cells undergo frequent mitosis with possible dilution of cytoplasmic substance, the diminution in cell size and cytoplasmic volume particularly would be expected to produce some increases in the general density of the cytoplasmic matrix if protein synthesis is actively taking place within the lymphocytes. The possibility that the intracytoplasmic protein material has only a minimal density is likely, although the elaboration of a readily soluble protein substance also must be considered. Neither the function of the lymphocyte nor the substance or substances elaborated by these cells is known, although lymphocytes have been considered to play a possible role in antibody formation (21). The bursa of Fabricius has been shown from immunological studies to be implicated in antibody production (9, 22, 30). The morphological changes in the lymphocytes as they reach maturity suggest that the mature lymphocytes are metabolically less active than the immature forms: they have fewer mitochondria, smaller Golgi complexes, less cytoplasm, and less morphological evidence of active protein synthesis (fewer ribosomes and sacs of endoplasmic reticulum). Increase in cellular activity and function may depend upon specific stimulatory or environmental (stress) conditions affecting the

more mature lymphocytic cells. Lymphocytes may perform their functional role when they undergo degeneration and dissolution with the liberation or release of some unknown substance. However, the possibility that the immature lymphocytes and lymphoblasts represent the more active cell forms also must be considered when one is attempting to understand the functional activity and significance of the lymphocyte.

The nature and functional significance of nuclear vesiculation in rapidly proliferating and differentiating cells of the lymphocytic follicles of the bursa of Fabricius is not known. The synthesis of some material within the nucleus and its transport to the cytoplasm by vesicles which appear to be formed at the outer nuclear envelope seems possible. Following their detachment from the outer nuclear envelope, the vesicles tend to move toward the plasma membrane and, in some instances, may be extruded into the extracellular space. Such findings not only suggest nucleocytoplasmic transfer but also the transfer of material to the intercellular fluid. The possibility that such substances have a role in the formation of ground substance, are nutritive material, or perhaps act as a differentiating factor for other cells in the nodule is suggested. Cell-to-cell transfer of vesicles has been considered although such vesicles have been seen only infrequently near the plasma membrane of adjacent cells.

Nuclear blebbing, although not so frequently observed as vesiculation, differs from vesiculation in that the blebs vary in size and are larger than the nuclear vesicles; and, in addition, blebbing involves both the inner and outer nuclear membranes and associated intranuclear material (nuclear fluid, chromatin, and nucleolar substance). Similar structures have been reported in other cell types (10). Occasionally larger blebs have been observed to have an association with the nucleolus and chromatin aggregations as if nucleolar material were being transfered into the bleb along with nuclear fluid and chromatin. The relationship of nuclear pores, formation of sacs of endoplasmic reticulum from the nuclear membrane, as well as blebbing have been considered in recent studies to be factors in nucleocytoplasmic interchange and transfer (4, 7, 10, 12, 24, 25, 36, 38).

Compound vesicles (multivesicular bodies) are frequently observed in small numbers in all of the cell forms observed in the developing lymphocytic follicles of the bursa. The internal vesicles are usually completely surrounded by the thin outer membrane to form the compound vesicular body, although this outer membrane has been observed frequently to be incomplete (23, 29, 33). Similar vesicles have been described in many cell types by other workers (5, 11, 19, 20, 23, 25, 29, 31, 33). The nature of these structures has been variously attributed to the accumulation of pinocytotic vesicles (11, 23), acting as a mechanism for the transfer of water from the cell to the extracellular space (5) or perhaps having some differentiating effect upon the cell during early stages of cleavage (29). Although these multivesicular bodies may well represent the aggregation of pinocytotic vesicles, the similarities in size and structure between the component internal vesicles and the spherical vesicles that arise from the nuclear membrane may point to their similar origin. The presence of such structures in rapidly growing and differentiating cells is of interest. On several occasions multivesicular bodies within the same cell have shown the dissolution of their internal vesicles and the accumulation of a moderately dense material (Fig. 21).

Much of this discussion has been quite speculative and serves mainly to indicate lines for further specialized study of the fine structural changes occurring during the process of cellular differentiation, particularly as related to the bursa of Fabricius and hemopoiesis. The problem of whether lymphocytes of different germ-layer origins possess submicroscopic morphological differences as well as different physiological activities requires further experimental work. The examination of other stages of the formation and development of the lymphocytic nodules of this lympho-epithelial organ can be expected to clarify and provide insight concerning the submicroscopic structure and alteration in lymphocytic cells during their differentiation and development.

The electron microscopic observations presented confirm the pathways of lymphocyte origin and differentiation that have been proposed from light microscopic studies of the bursa of Fabricius during the development of the lymphocytic nodules in the embryo (3). Lymphocytes of this lympho-epithelial organ arise from *both* endodermal and mesodermal derivatives, a view

that is contrary to the general concepts concerning the origin of hemopoietic cells in the various blood-forming organs of birds and other animals.

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