STUDIES ON THE PATHOGENESIS OF GUMBORO DISEASE IN THE BURSA OF FABRICIUS, SPLEEN, AND THYMUS OF THE CHICKEN

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A viral disease of chickens causing necrosis of lymphocytes has recently been described.[1-4] The syndrome, termed Gumboro disease, is transmitted via the intestinal tract and primarily affects lymphocytes of the bursa of Fabricius, although the spleen, thymus, and cecal lymphoid tonsils are also damaged. It does not clinically affect neonatal or adult birds, but is limited to the age range wherein the bursa of Fabricius is at its greatest development.

The chicken has two primary or "central" lymphoid organs: the thymus and the bursa of Fabricius.[5] These organs resemble one another in respect to their endodermal origin, embryonic development, lymphoid content, and postnatal involution. Although immune reactions do not normally occur in these structures, their presence in neonatal life is essential if normal populations of immunologically competent lymphoid cells are to develop.[6] The bursa contains lymphoid follicles whose medulla and cortex is separated by a single layer of undifferentiated endodermal epithelial cells (Text-fig. 1) which give rise to blast cells of the lymphocyte and plasmacyte series.[8,9] Neonatal bursectomy is effective in inhibiting the production of circulating antibody, whereas the similar procedure in young adulthood has little effect.[10,11] Cooper *et al.*,[12] using the techniques of bursectomy, thymectomy, and irradiation, have presented evidence that the bursa and thymus may develop independently of each other. They propose that small lymphocytes in the peripheral lymphoid tissue may be thymus-dependent whereas larger blast cells and plasmacytes are bursa-dependent. This implies a distinct cellular dissociation of the formation of circulating antibody and those immunologic reactions collectively termed "cellular immunity."

The potential significance of the reaction of lymphocytes, possibly of two functional types, to viral infection only during a specific phase of lymphoid development prompted the following study. The experiment was designed to examine the effect of the lymphocidal Gumboro disease virus on lymphocytes and reticular cells of the bursa of Fabricius,

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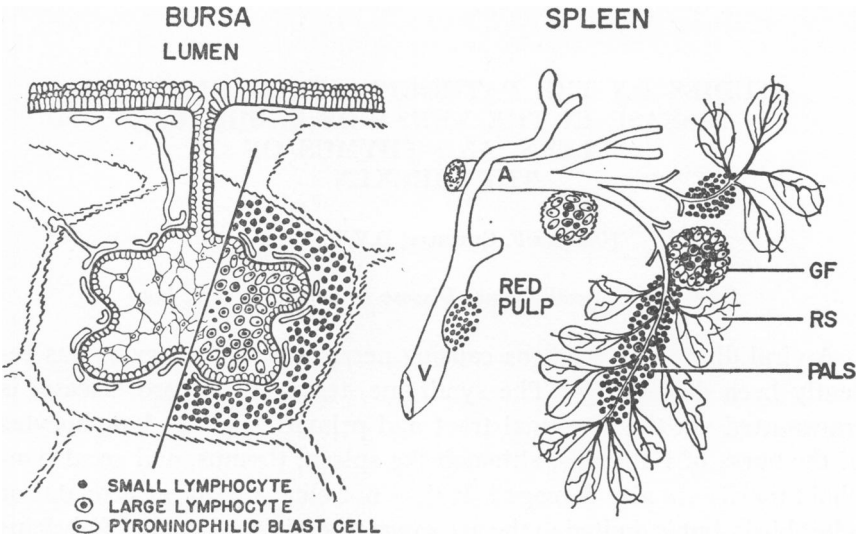


TEXT-FIG. 1. Diagram of anatomical arrangement of lymphoid and reticular tissues of bursa of Fabricius and spleen of chicken. Epithelium separating bursal medulla and cortex is continuous with epithelium of bursal lumen. It gives rise to medullary lymphocytes. In spleen, terminal arterioles are surrounded by lymphocytes, periarteriolar lymphoid sheath (*PALS*). These vessels then branch, becoming sheathed with reactive reticular cells (*RS*) and finally dilate forming splenic sinuses of red pulp. Germinal follicles (*GF*) which consist of pyroninophilic blast cells, lymphoblasts, and large lymphocytes are assoicated with splenic arterioles.

thymus, and spleen. Particular attention was paid to the possible predilection of the virus for lymphoid and reticular areas of the bursa (medulla and cortex) and spleen (germinal follicles, periarteriolar lymphoid sheaths, and reticular sheathed arteries) which have been implicated in the functional dichotomy of the lymphocyte.

MATERIALS AND METHODS

Virus

The viral inoculum (supplied by Dr. E. A. Edgar, Auburn, Ala.), was prepared as a 10% tissue suspension. Chickens were inoculated and infective bursae were collected, ground in a TenBroeck grinder with saline, and centrifuged at low speed. The supernatant fluid was used as the stock inoculum.

Chickens

Four-week-old white Leghorn chickens from a closed flock allegedly free of Rous sarcoma virus and leukosis agents were used in the study. The bursa of Fabricius reaches its peak of development (4 weeks) earlier in the Leghorn breed than in others.[13] Preliminary studies revealed the dose of 0.05 ml. of stock inoculum to induce clinical appearance of Gumboro disease on the third day after inoculation, with all birds recovering. Larger doses caused severe symptoms and death. Thirty-two birds were inoculated by dropping 0.05 ml. of stock inoculum under the conjunctiva. Two birds were killed at each of the following times after inoculation: 16, 20, 36, and 48 hr., and at 24-hr. intervals up to 14 days. Blood was collected for serum, hematocrit

determination, and differential white blood cell counts.[14] Serum was examined for total protein by ultraviolet spectrophotometry [15] for electrophoretic pattern using the Model R electrophoresis apparatus (Spinco Company), and for viremia by intravenous injection and oral inoculation of two 4-week-old susceptible chickens with 0.3 ml. of serum. White and red cells were collected by centrifugation and were washed 3 times in phosphate-buffered saline. Red cells were resuspended in saline to 6 million cells per cubic millimeter and white cells to 6000/cu.mm. Two drops were given orally to susceptible chickens to determine blood-cell-associated virus.

Small portions of the bursa of Fabricius, spleen, thymus, and bone marrow were fixed in 10% formalin, 2.5% glutaraldehyde, and Zenker's fixative. Bursa, spleen, and thymus tissues were quenched in liquid nitrogen and cut on a cryostat for histochemical reactions.

Microscopy

Tissues fixed in formalin and Zenker solution were processed by conventional paraffin embedding. The Feulgen reaction,[16] and methyl green-pyronine,[17] and acridine orange stains [18] were used to detect nucleic acids. Acid phosphatase was detected using β-glycerophosphate with lead precipitation,[16] neutral lipids with oil red O, and glycogen with periodic acid-Schiff (PAS), using diastase controls. Preliminary studies revealed the oil red O stain to be as accurate as antiviral immunofluorescence in detecting early lysosomal accumulations in affected cells. Specimens of bursa, spleen, and thymus for electron microscopy were fixed in glutaraldehyde, washed in buffer, postfixed in osmium, dehydrated in ethanols, and embedded in Epon 812 (Shell Chemical Company). Ultrathin sections were stained with lead citrate. The mitotic index (number of mitotic figures per 1000 cells) was determined by counting the number of mitotic figures and of total cells in 20 grid areas on a 200-mesh copper grid under low power (3000 ×) of the electron microscope. The average number or total cells in such grid areas was 210.

Immunofluorescence

Two young adult rabbits and 4 adult male chickens (recovered from Gumboro disease) were hyperimmunized with intramuscular injections of 1.0 ml. of partially purified virus suspension emulsified in an equal volume of Freund's complete adjuvant (Difco, Inc., Detroit). Forty milliliters of a 10% suspension of infected bursae in saline had been centrifuged for clarification (20 min. at 800 × g), removal of bacteria (20 min. at 2500 × g), and sedimentation of virus (60 min. at 104,000 × g). The resulting pellet was resuspended in 1.0 cc. of saline. Each animal was given 2 injections, at 2-week intervals. Two weeks following the last injection, the serum was harvested and the gamma globulin precipitated with ammonium sulfate.[19] Gamma globulin was conjugated with fluorescein isothiocyanate, dialyzed, and stored in 1.0-ml. aliquots at −65° C. Before use, serums were absorbed with acetone-extracted canine liver powder. Frozen sections were stained, air dried, and examined microscopically for intracellular viral antigen, using a mercury-vapor fluorescence microscope (Leitz).

Results

The data on bursa weight, body temperature, and viremia are summarized in Table I and Text-fig. 2. At approximately 48 hr., the bursa of infected birds began to increase markedly in size and weight owing to edema and hyperemia. Viremia and body temperature elevation coincided with the inflammatory response in the bursa. Splenomegaly corresponding to enlargement of the bursae was moderate to severe and

TABLE I

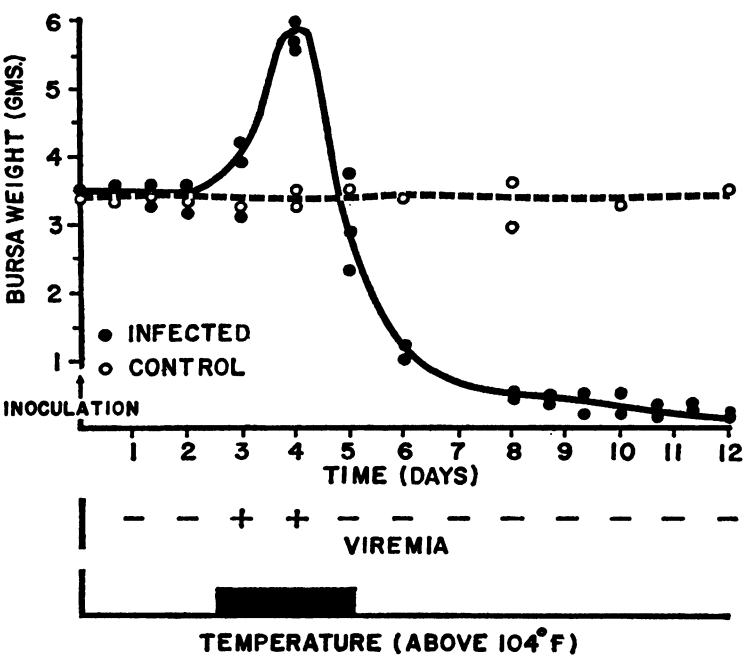
EXAMINATION OF THE BLOOD FOR VIREMIA, RED CELL VOLUME, AND TOTAL SERUM PROTEINS

Time after inoculation	*Viremia*							*Total protein (Gm./100 ml.)*
	Serum	*Serum 1:10*	*Serum 1:100*	*WBC*	*WBC 1:10*	*RBC*	*Hct*	
10 hr.	—	—	—	—	—	—	32	2.55
20 hr.	—	—	—	—	—	—	28	2.47
36 hr.	—	—	—	—	—	—	31	3.79
48 hr.	—	—	—	—	—	—	15	3.05
72 hr.	+	+	—	+	—	—	19	3.33
	+	+	—	+	—	—	24	3.46
96 hr.	+	+	+	*		—	28	3.36
5 days	—	—	—	—	—	—	26	3.22
6 days	—	—	—	—	—	—	28	4.03

* Severe panleukopenia at 96 hr. after inoculation prevented the harvest of white blood cells.

occurred at 72 hr. after inoculation. Gross thymic changes were less severe and appeared slightly later than those in the spleen.

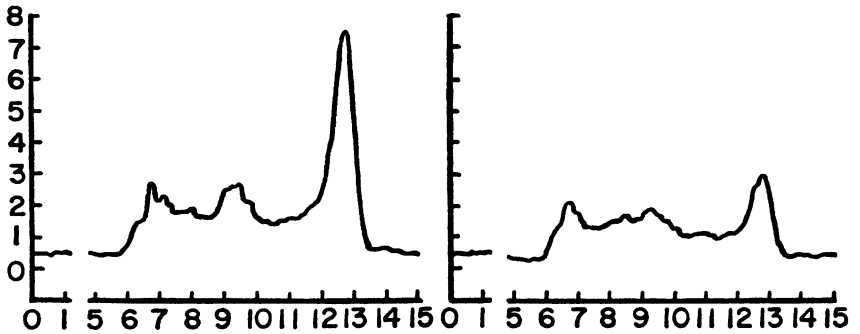
Examination of stained blood smears at 2 and 3 days after inoculation revealed lymphocytopenia. Smears during the severe inflammatory stages of the disease indicated severe panleukopenia. Blood smears after



TEXT-FIG. 2. Correlation of weight of bursa of Fabricius with body temperature and viremia. Marked increase in bursal weight was due to severe edema.

this period were less severely affected, and by 9 days after inoculation, the white blood cell types appeared to be normal.

The total serum proteins and serum protein electrophoretic patterns were normal except for those chickens examined from 4 to 8 days after inoculation. Serum albumin was markedly decreased in serums collected 4 and 5 days after inoculation. Slight decreases in serum albumin were present 6 days after inoculation (Text-fig. 3). The serum globulins did



TEXT-FIG. 3. Tracing of serum protein samples at 1 (*left*) and 4 (*right*) days after inoculation. Samples were processed by separation by paper electrophoresis and staining of paper strip.

not appear to be affected during the disease according to the methods used for examination.

Bursae

Cytopathologic Findings. The first detectable cellular change was seen in the medullary area of the bursa 36 hr. after inoculation and consisted of degeneration and necrosis of individual lymphocytes. Degeneration, characterized by nuclear pyknosis and accumulations of lipid droplets in the cytoplasm, was present in scattered lymphocytes in only a few lymphoid follicles. Large, pale, phagocytic reticulum cells (macrophages) had developed adjacent to necrotic lymphocytes. The cytoplasm of the macrophages contained remnants of pyknotic lymphocyte nuclei and debris. These small focal dense areas did not stain with the Feulgen reaction, were bright yellow with acridine orange, and reacted strongly with the acid phosphatase and PAS stains. The mitotic index in affected follicles 36 hr. after inoculation was 4.71 as compared with the mitotic index of 2.90 in normal bursae.

By 48 hr., the medullary areas of affected follicles were devoid of small lymphocytes. The lymphoid follicles affected early in the disease were scattered randomly throughout the bursa. As these follicles were obliterated by reticuloendothelial hyperplasia, adjacent follicles began to

show early viral damage (Fig. 1). The cortex contained foci of lymphocyte necrosis and phagocytosis. The medulla was intensely positive for neutral lipid, and small positive foci appeared in the cortex (Fig. 2). Mitotic activity was increased in the corticomedullary epithelial layer, which contained pyroninophilic plasmablasts (Fig. 3), and in later stages, plasma cells.

By the third and fourth days after inoculation, the changes had spread to all lymphoid follicles in the bursa. Severe edema, hyperemia, and marked accumulations of heterophils were responsible for the increase in weight of affected bursae. Large globules of neutral lipids (fatty degeneration) were present in all cell types within the bursa, including the interfollicular connective tissue cells. Plasma cells were commonly associated with the large numbers of phagocytic reticular cells which contained large, dense globules of PAS-positive material.

As the inflammatory reaction declined in intensity, cystic cavities developed in the medullary areas of the lymphoid follicles. Necrosis and phagocytosis of heterophils and plasma cells were present not only within the follicle but in the interfollicular connective tissue. Fibroplasia was responsible for interfollicular connective tissue formation. Proliferation of the bursal epithelial layer had produced a glandular structure, the columnar epithelial cells of which contained many globules of mucin (Fig. 4 and 5). This material was present in the cytoplasm of the epithelial cells and in the lumen, as evidenced by the strong reactivity to alcian blue.

Immunofluorescent Findings. The detection of viral antigen due to conjugated antiviral globulin, as revealed by specific fluorescence, was first seen in the cytoplasm of a few scattered cells in the medulla of a few bursal lymphoid follicles 48 hr. after inoculation. By 72 hr., many fluorescing cells were present throughout affected follicles. Bursal tissue examined 4 and 5 days after inoculation had widespread cytoplasmic fluorescence believed to be in macrophages (Fig. 6). Scattered fluorescing cells were present 6 days after inoculation, and fluorescence was absent in tissue after 8 days.

Specific fluorescence was not observed in uninfected bursae (controls), bursal tissue blocked with unconjugated specific antiglobulin, or with infected tissue taken prior to 48 hr. after inoculation. Nonspecific, yellow fluorescence was emitted by lipid globules, fat cells, and some lipid-containing cytoplasmic globules. Buff-orange fluorescence was emitted by eosinophils.

Electron Microscopic Findings. Degenerating lymphocytes were shrunken and contained lipid vacuoles and a general disorganization of cytoplasmic organelles. The nuclear membrane was distorted and

chromatin was aggregated, prominent, and occasionally filled the nucleus. Necrotic lymphocytes appeared as masses of electron-dense material and were usually present within the cytoplasm of large macrophages. No particles resembling virus were seen within affected lymphocytes.

The large, pale reticular cells (macrophages) were filled with membrane-bound aggregates of electron-dense debris (Fig. 7). These were presumed to be lysosomes since similar structures in frozen sections stained for the acid phosphatase reaction. The lysosomal debris consisted of remnants of necrotic lymphocytes, lipid vacuoles, and myelin figures (Fig. 8). Approximately 3% of the macrophages examined contained particles, 58–65 mμ in diameter, which were considered to be virus. Macrophages were surrounded in the early stages of the disease by degenerating lymphocytes, and in later stages by large pyroninophilic blast cells which had a high content of free ribosomal material (Fig. 9). Aggregates of viral particles were always within macrophages, heterophils, or endothelial cells, and were most often associated with electron-dense myelin figures in lysosomal debris (Fig. 10–12).

Large pyroninophilic blast cells, proplasma cells, and plasma cells did not contain viral particles (Fig. 13). The mitotic apparatus of blast cells examined in anaphase, metaphase, and telophase was not associated with viral particles even though evidence of cellular degeneration was present (Fig. 14). The presence or absence of particles in necrotic lymphocytic and plasmacytic cells which had been phagocytized could not be determined since such cells were massive intracellular accumulations of electron-dense material (Fig. 15).

In the atrophic bursae following the disease, no evidence of viral particles was seen in the basal epithelial cells or in mucin-containing (goblet) cells near the lumen (Fig. 16 and 17) by either fluorescent or electron microscopy.

Spleen

Lymphoid necrosis occurred simultaneously in the germinal follicles and the periarteriolar lymphoid sheath (PALS) (Text-fig. 1) at 3 days after inoculation and was confirmed with lipid stains (Fig. 18). By 4 days, when specific immunofluorescence was first seen, germinal follicles were necrotic, and reticular cell hyperplasia of the sheathed arteries was severe (Fig. 19). Fluorescing cells were few in number and were scattered randomly throughout the spleen. Pyroninophilic blast cells developed within the PALS (Fig. 20) which apparently led to "lymphoid recovery" as many new germinal follicles were present in these areas at 9 days after inoculation. The sheathed arteries stained intensely with lipid stains and, in the later phases of the disease, contained many

macrophages and large areas of PAS-positive necrotic debris (Fig. 21). Large pale macrophages were present in the center of the germinal follicle (Fig. 19).

Thymus

Moderate to severe lymphoid necrosis and hyperplasia of the reticular and epithelial components were present in the medullary area 4 days after inoculation. Heavy lipid deposits were present in the epithelial cells and some had a strong affinity for alcian blue (for acid mucopolysaccharides). The reaction of the thymic cortex did not proceed beyond scattered foci of lymphoid necrosis with reticular cell phagocytosis—i.e., the lymphoid population remained intact. In 2 of the 6 cases examined 6–8 days after inoculation however, severe changes were present throughout the thymus. Regeneration appeared to occur, since all thymuses examined after postinoculation Day 12 had cortical lymphocyte populations which approached normal.

Only random specimens of spleen and thymus were examined by electron microscopy. Tissue changes appeared to parallel those in the bursa and a thorough study was not done.

Discussion

Lymphocyte necrosis, the earliest lesion of Gumboro disease virus, is associated with phagocytosis of lymphocyte debris by reticulum cells. The cellular changes in this reaction are strikingly similar to those in acute irradiation necrosis[20] and are probably related to the rapidity of the degenerative process in the lymphocyte. The macrophagocytosis of malignant lymphoma (Burkitt) cells by nonmalignant reticulum cells[22] —e.g., the "starry-sky" appearance—appears to be a similar process. Failure to find viral particles in degenerating lymphocytes in Gumboro disease does not preclude their presence, for they may be dispersed throughout the cell and remain unrecognized due to their small size. A plausible explanation is simply that the methods used for detection of virus within the lymphocyte were insufficiently critical. The absence of specific immunofluorescence, however, also suggests that virus may not multiply in lymphocytes.

Reticular cell hyperplasia is severe following lymphocyte necrosis. Evidence of viral replication is present in lysosomal debris of reticular macrophages while it is lacking in degenerate lymphocytes. Bowers and de Duve[21] have demonstrated the possible existence of two types of lysosomes—L15 and L19—according to density distribution patterns in isopycnic centrifugation in sucrose. The former lack hydrolases and are believed to be present in lymphocytes; the latter contain a full comple-

ment of enzymes and are present in macrophages. It may be that the additional enzymatic components within the macrophage are required for the formation of complete virus.

Viral damage was most severe in the bursal medulla and, following viremia, in the thymic medulla and splenic reticular sheath. Although lymphocytes are rapidly destroyed, the responsiveness of the reticular cells in these anatomical sites appears to be essential for viral replication.

RE virus of chickens, which also induces reticuloendothelial hyperplasia,[23] does not affect the lymphoid organs as does Gumboro disease virus; nor are the viral particles similar in thin sections. Damage to the reticuloendothelial system (RES) due to cell destruction and to such agents as the lactic dehydrogenase (LDH) virus of mice induces elevated serum enzyme levels.[24] Although enzyme levels were not determined in this study, it is doubtful if Gumboro disease parallels the chronic viremia and rapid, high serum virus titers of LDH virus.

Formation of pyroninophilic blast cells and plasma cells following lymphoid necrosis may theoretically be due to: (1) a response to Gumboro disease virus or other unknown antigens formed during viral-induced lymphocyte necrosis; (2) a direct stimulating effect of the virus; or (3) a response to correct the lymphocyte deficit.

Since blast cell development was coexistent with cell necrosis, it is doubtful if lymphocyte deficit is a reasonable explanation. The fact that viral particles are not associated with the mitotic apparatus of blast cells suggests that there is no direct influence on mitosis. Gumboro disease, however, may be analogous to other examples of viral-induced mitotic activity where intracellular viral particles are not seen—e.g., the papilloma viruses.[25,26]

The occurrence of blast cells adjacent to the bursal epithelium and in the splenic periarteriolar lymphoid sheath is similar to findings in the primary antibody response to protein antigens.[27–29] Whether the pyroninophilic blast cells in this study contained anti-Gumboro viral antibody was not determined. Plasmacytosis follows lymphoid necrosis in such diverse entities as murine viral hepatitis[30] and malignant (Burkitt) lymphoma.[22] Here again, it is unknown whether such plasmacytes contain specific antibody. Whatever the function of such cells, it appears that the reactivity of lymphopoietic tissue to viral infection includes the following sequence: blast cell mitosis, lymphocyte degeneration and necrosis, macrophagocytosis, and plasmacytosis.

Lymphoid necrosis with reticular cell hyperplasia is characteristic of many other viral infections—none of which have been studied sufficiently regarding the virus-lymphocyte association. The viruses of yel-

low fever,[31] bovine viral diarrhea,[32] rinderpest,[33] African swine fever,[34] hog cholera,[35] and feline panleukopenia [36] all produce severe and selective damage to lymphoid tissue. The relationship of the cellular reaction of these viral diseases to other mechanisms of lymphoid tissue injury—the runting syndrome,[37] adrenal cortical excess,[38] and irradiation [20]—is unknown.

Failure of the bursa to repopulate with lymphocytes and the development of mucin-secreting glandular acini cannot be explained by present studies. Obviously the humoral influences which normally maintain the lymphoid population of the bursa [39-41] are incapable of inducing lymphoid repopulation either by regeneration of lymphoid cells within the bursa or by migration of cells from other lymphoid centers. It may be that the bursal epithelial layer, which differentiates to give origin to bursal lymphoid tissue during embryonic development,[8] is so altered in the course of viral infection that it reverts to the type of endodermic epithelium from which it originated. Intense alcian blue-staining foci in the degenerating thymus may be analogous to the similar reaction in the bursae. In both organs, it appears to be regression to the less differentiated epithelium of the gut endoderm from which both the thymus and bursa arise.[8,42] Continued presence of virus is doubtful since it was not detected by fluorescent or electron microscopy in the bursae. Furthermore, lymphocyte necrosis which occurs in the spleen is followed by marked reparative increases both of germinal centers and foci of small lymphocytes.

The transient hypoalbuminemia in Gumboro disease occurred only during the severe exudative phase of the disease. This may have been due to loss of serum albumin during the inflammatory exudation into the bursa and subsequent excretion with fecal material. Hypoalbuminemia has been seen in the exudative process which occurs in Vitamin E deficiency (exudative diathesis) in chicks [43] and is known to accompany gastrointestinal diseases in mammals.[44]

Summary

The cytopathologic features of the chickens' bursa of Fabricius, spleen, and thymus during Gumboro disease have been investigated by light, fluorescent, and electron microscopy. Necrosis of lymphocytes was the initial lesion observed and resulted in destruction of all lymphoid tissue in the bursa. Phagocytosis of necrotic lymphoid cells by large pale reticulum cells progressed to severe reticular cell hyperplasia. Viral particles were seen in lysosomal debris within macrophages and endothelial cells but not within lymphocytes. Plasma cells and pyroninophilic blast cells developed in areas of reticular cell hyperplasia and subsequently became necrotic.

In the bursa, a severe inflammatory reaction with exudation of fluid and heterophils accompanied the lymphoid damage. Atrophy followed with proliferation of the corticomedullary epithelium, and the formation of mucus-secreting glands. Lymphocyte repopulation did not occur in the bursa but did in the spleen and thymus.

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[*Illustrations follow*]

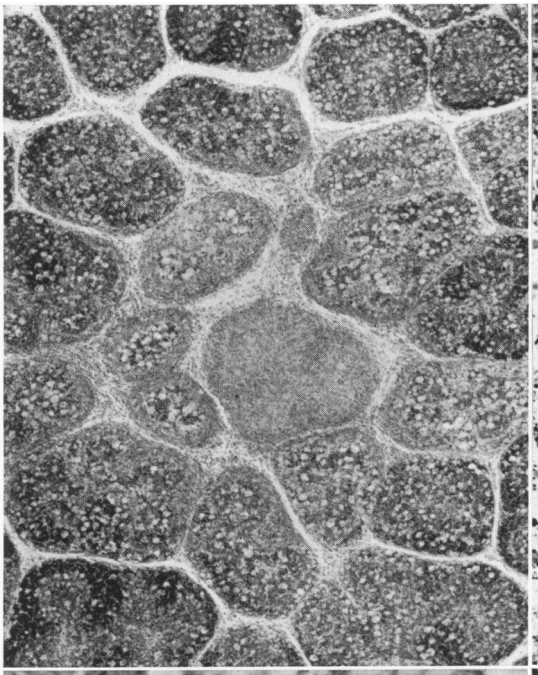
Legends for Figures

Fig. 1. Lymphoid follicles from bursa at 48 hr. after innoculation, illustrating focal nature of damage early during infection. One follicle (center) is obliterated by severe reticular cell hyperplasia. Surrounding follicles show various degrees of damage—those furthest from center follicle showing only early changes. Marked lymphoid phagocytosis ("starry sky" appearance) is present. Hematoxylin and eosin stain. × 25.

Fig. 2. Stain for neutral lipids from bursa 48 hr. after inoculation. Bursal epithelium separates medulla and cortex (arrow). Fatty degeneration appears first and is most severe in medullary areas. Lipid globules are also present in cortex and interfollicular connective tissue. Oil red O stains. × 350.

Fig. 3. Plasmablasts present in cortex of lymphoid follicle 72 hr. after innoculation. Medullary area (upper right) is filled with macrophages. Methyl green-pyronine stain. × 900.

Fig. 4. Bursa from chicken killed 8 days after inoculation. Proliferation of bursal epithelium has replaced lymphoid follicles with glandular structures. Epithelial cells contain acid mucopolysaccharide which also stains intensely with alcian blue. This material is also seen in lumen of glands. Interstitial connective tissue is prominent. Hematoxylin and eosin stain. × 280.



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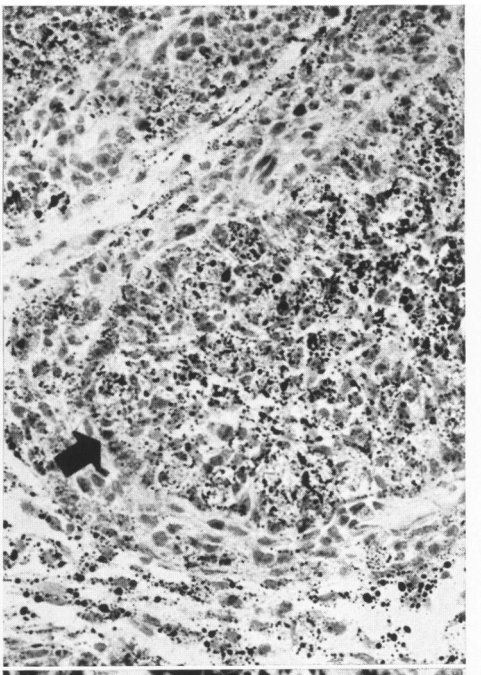

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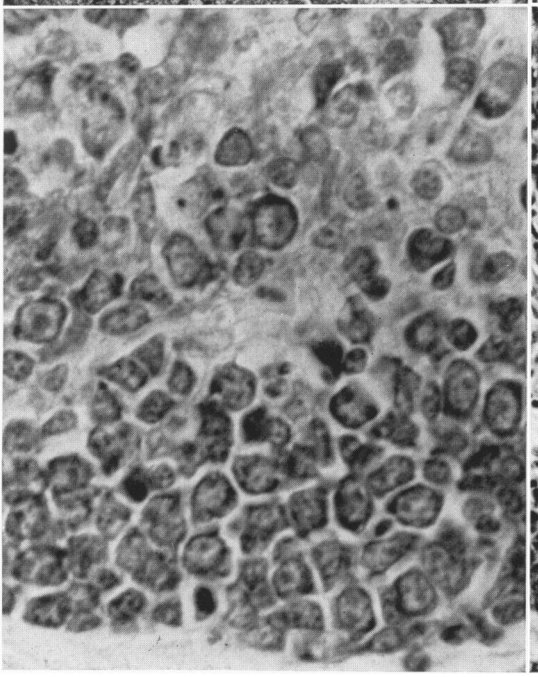

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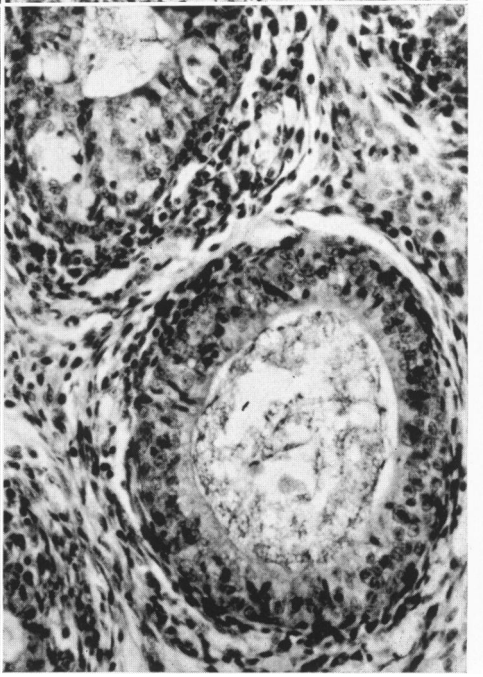

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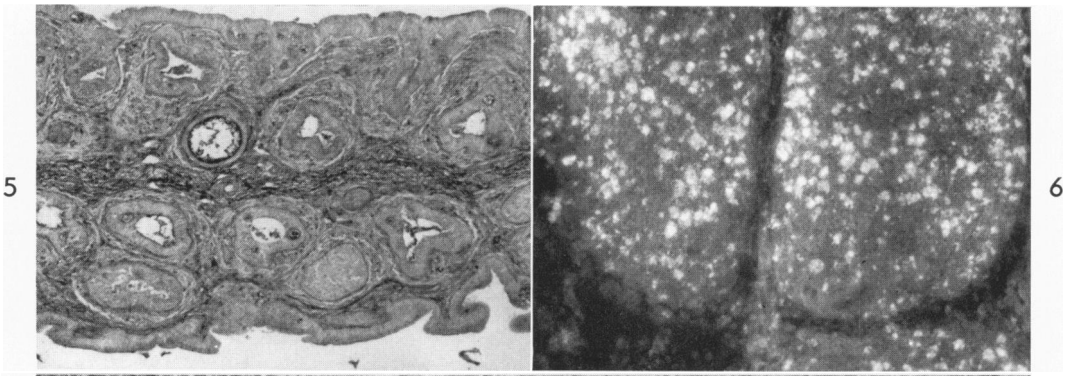


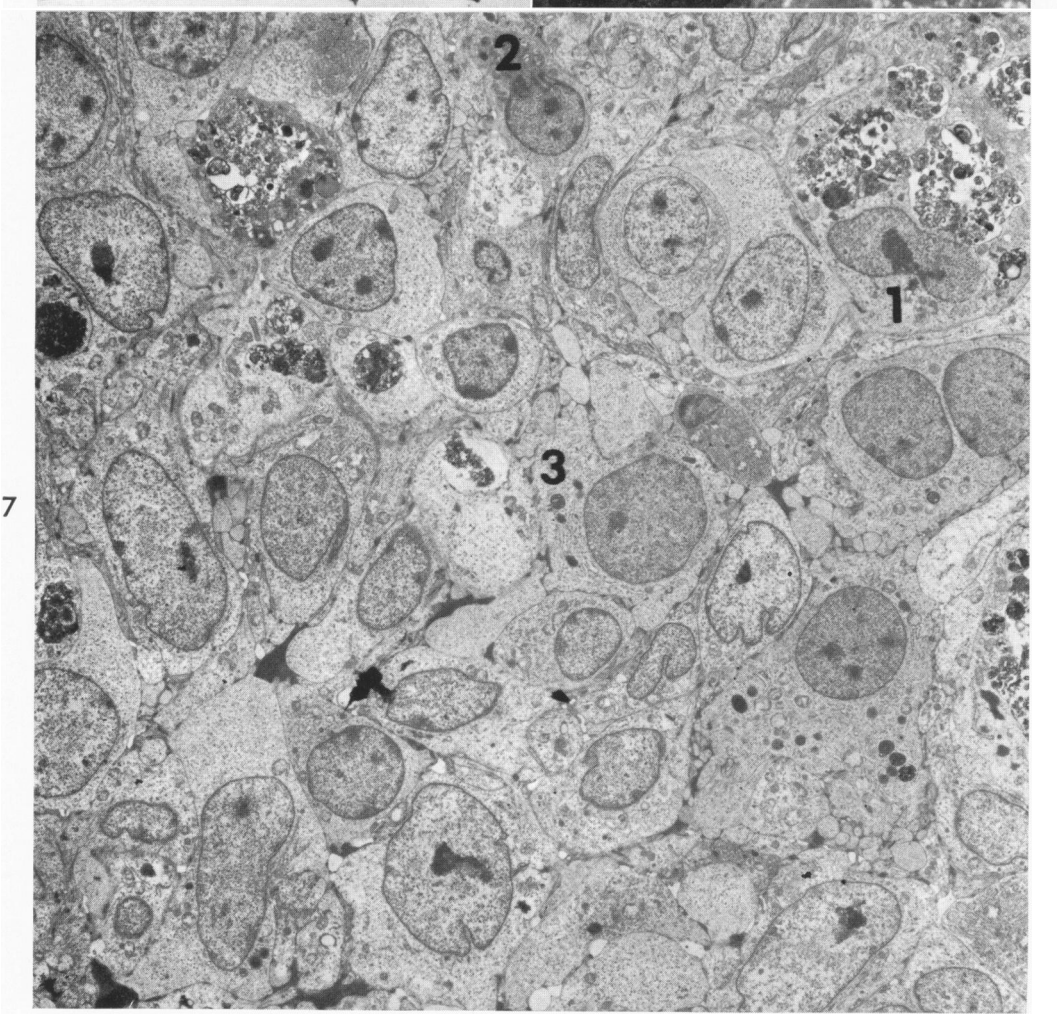


FIG. 5. Lower power from same section as Fig. 4. Alcian blue-periodic acid-Schiff stain. × 25.

FIG. 6. Bursa of chick killed 72 hr. after inoculation and stained with specific conjugated anti-Gumboro serum. Viral antigen as detected by fluorescence is present in cytoplasm of many macrophages throughout lymphoid follicle. Smaller lymphocytes do not fluoresce. × 150.

FIG. 7. Cortical area of bursa of chicken 72 hr. after inoculation. Macrophages (*1*) containing membrane-bound masses of debris are present throughout section. Degenerating lymphocytes (*2*), large pyroninophilic cells (*3*), and plasma cells are prominent. × 4200.

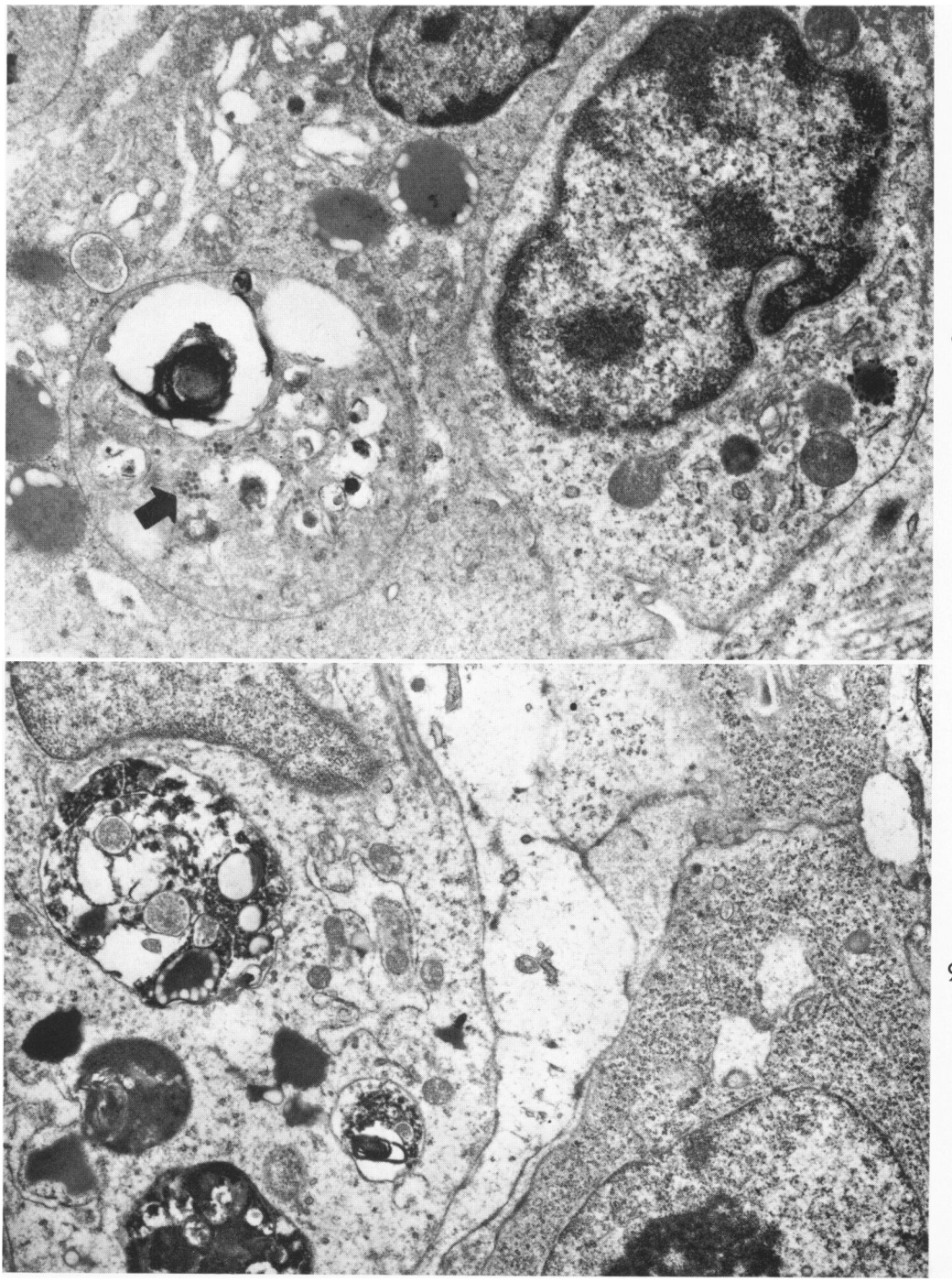


FIG. 8. Degenerating lymphocyte (right) adjacent to macrophage. Membrane-bound cytoplasmic structure in macrophage contains lipid, myelin figures, and viral particles (arrow). × 18,500.

FIG. 9. Pyroninophilic blast cell (right) adjacent to macrophage. Macrophage contains several large lysosomes. Cytoplasm of blast cell contains chiefly free ribosomal particles. × 16,750.

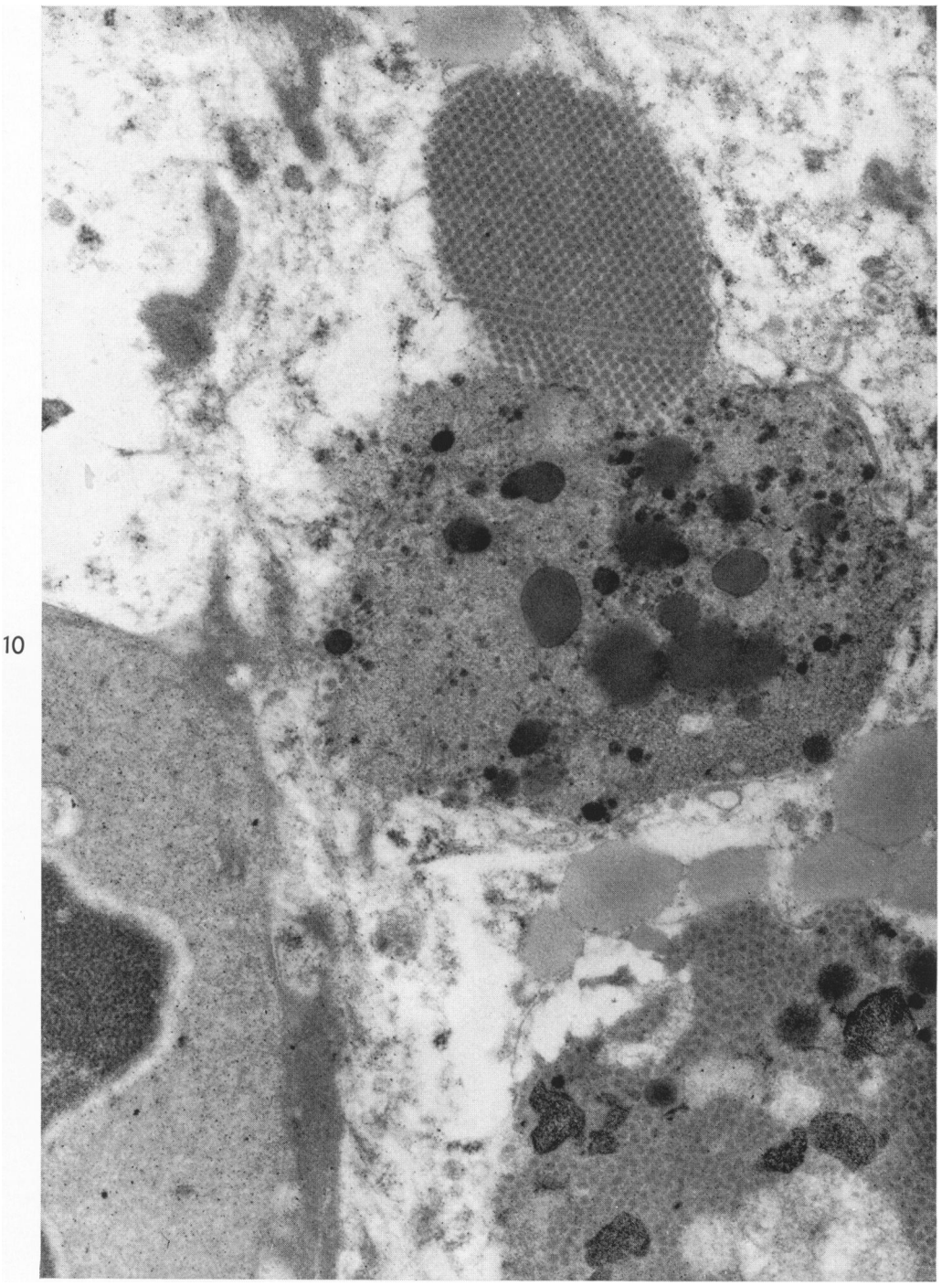


FIG. 10. Endothelial cell with viral particles forming at border of area of lysosomal debris. In lower right, viral particles are scattered diffusely throughout lysosome. Erythrocyte is present in lower left.

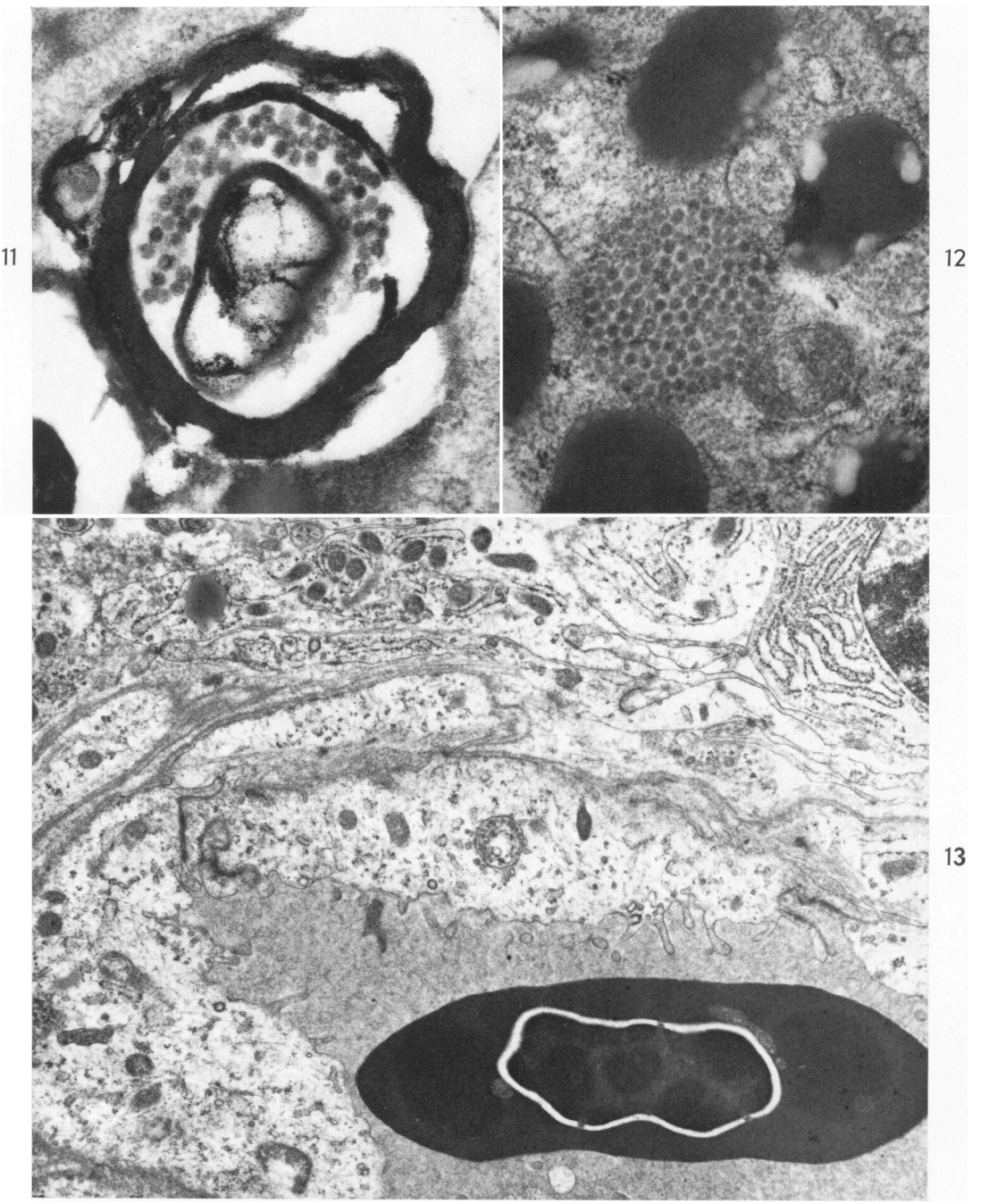


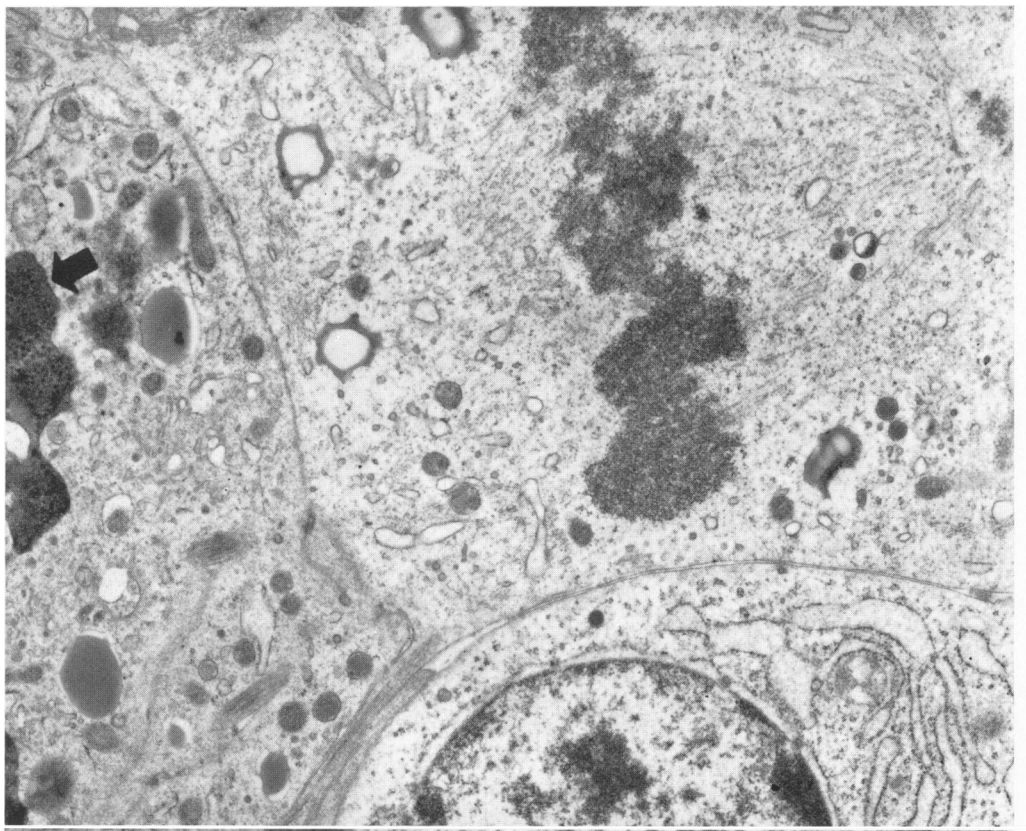
Fig. 11. Viral particles between lipid membranes from structure similar to that of Fig. 10. × 37,000.

Fig. 12. Aggregate of viral particles surrounded by lipid globules free in cytoplasm of macrophage. × 35,500.

Fig. 13. Small arteriole containing erythrocyte and lymphocyte. Macrophage and Plasma cell are present in upper part of micrograph. Erythrocytes did not undergo degeneration and intracellular evidence of virus was not detected. × 8250.

FIG. 14. Degenerating blast cell, probably of plasmacytic series, at metaphase of mitosis. No viral particles were seen associated with spindle fibers or heterochromatin. Plasma cell (bottom) and heterophil (left) are adjacent to cell in mitosis. Lipid structure of heterophil contains many small electron-dense particles (arrow). × 18,500.

FIG. 15. Macrophage which has phagocytized necrotic plasma cell, 5 days after inoculation. × 18,000.



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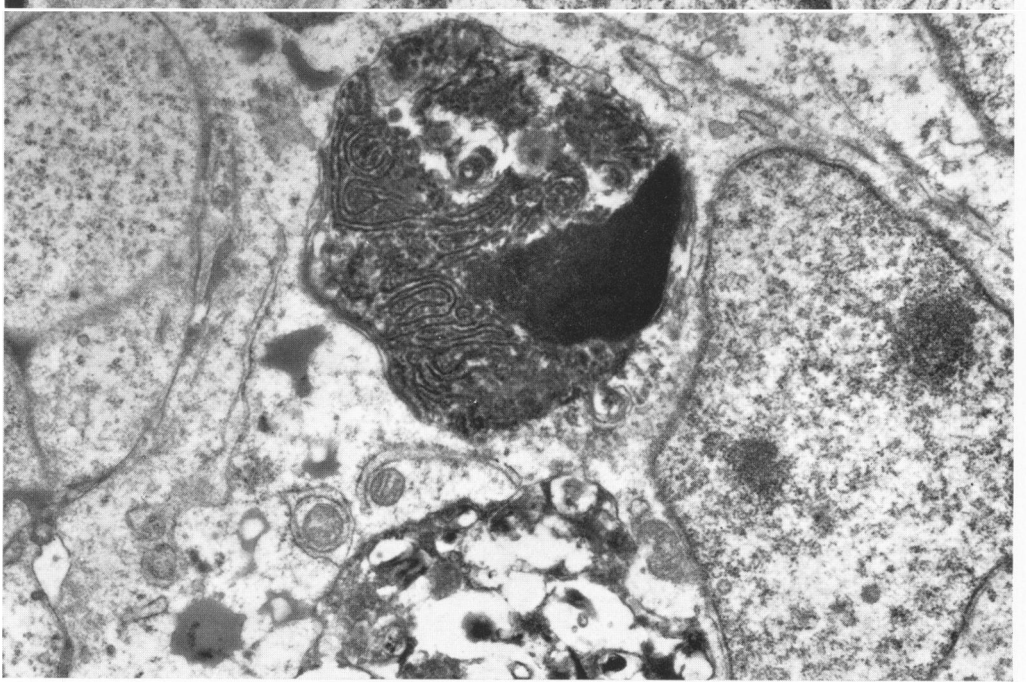

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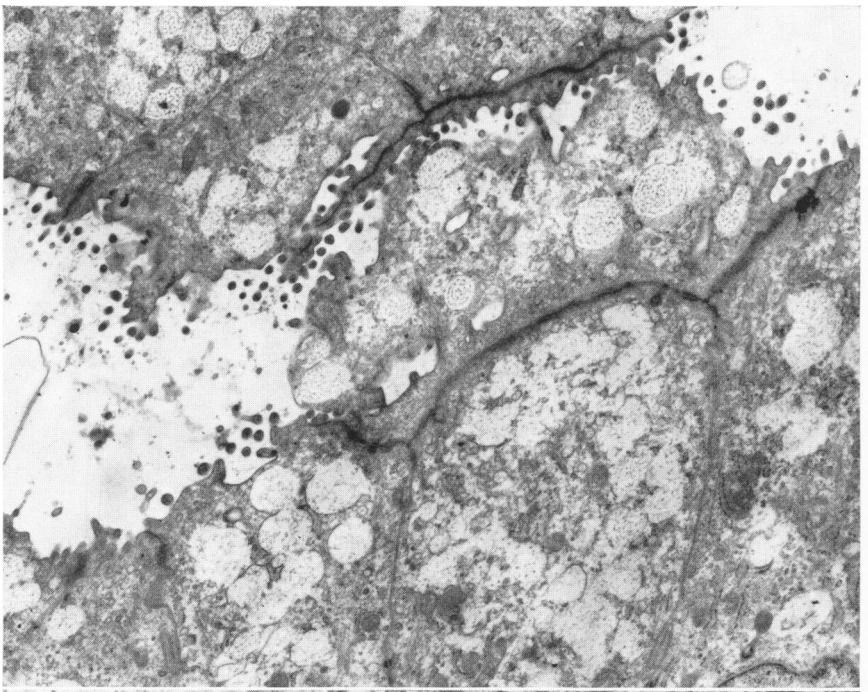
FIG. 16. Epithelial cells adjacent to lumen of newly formed gland structures. Large cytoplasmic vacuoles contain mucin. No evidence of virus was seen; 10 days after inoculation. × 12,500.

FIG. 17. Epithelial cells from epithelial layer adjacent to basement membrane (arrow), 8 days after inoculation. Scattered lipid globules occur but no evidence of virus was seen. × 14,000.



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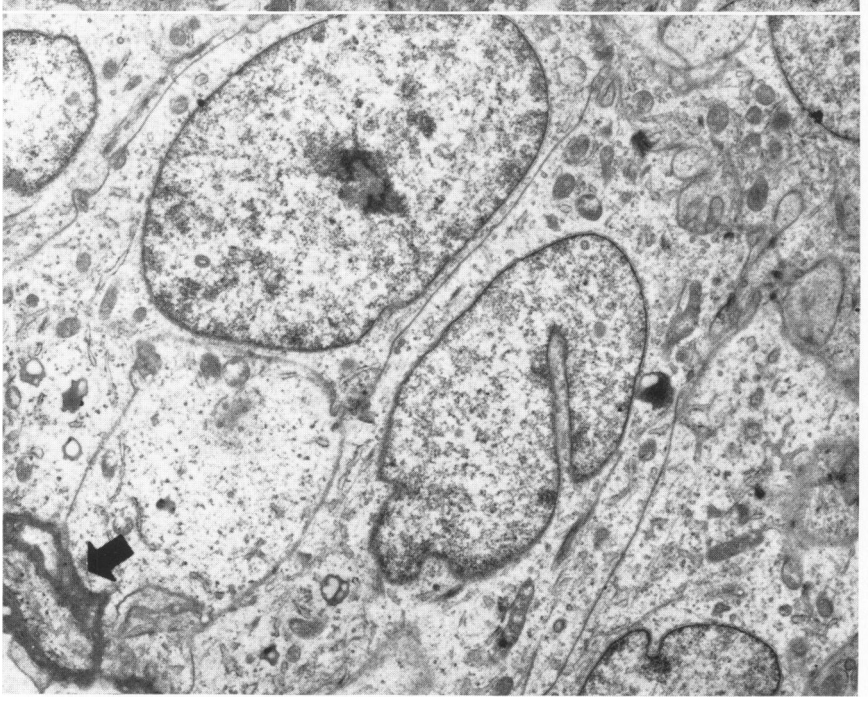

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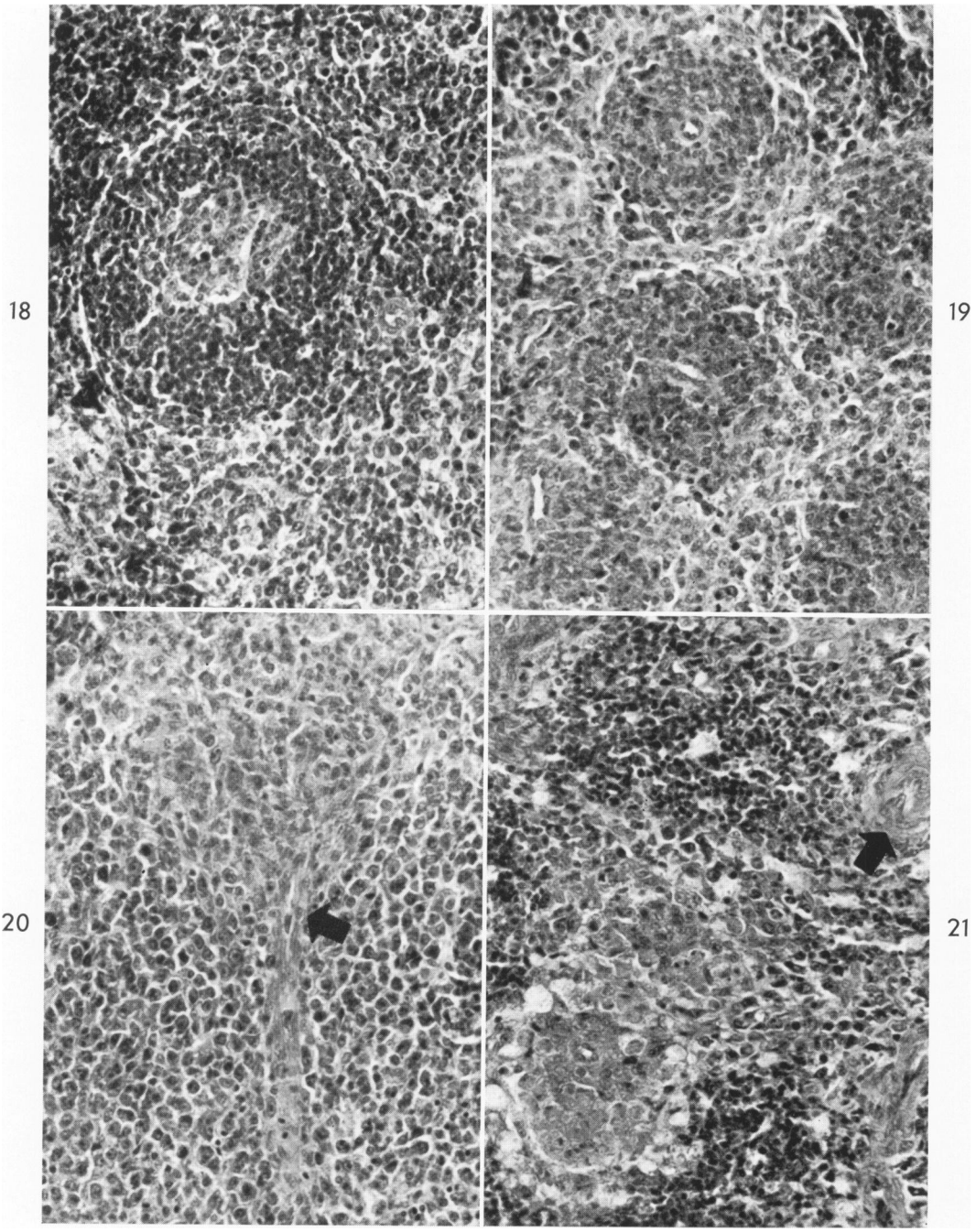
FIG. 18. Germinal follicle in spleen of chicken killed 3 days after inoculation. Degenerating lymphocytes are present within follicle, and center is filled with hyperplastic reticular cells. Hematoxylin and eosin stain. × 250.

FIG. 19. Marked reticular cell hyperplasia in sheathed arteries 4 days after inoculation. No necrotic cells are present. Hematoxylin and eosin stain. × 100.

FIG. 20. Arteriole of periarteriolar lymphoid sheath which has been replaced by large pyroninophilic blast cells and plasma cells. Arteriole (arrow) passes into reticular cell sheath, which is disorganized and contains cellular and lipid debris. Hematoxylin and eosin stain. × 250.

FIG. 21. Area of spleen 9 days after inoculation. Periarteriolar lymphoid sheath (arrow on arteriole) contains small lymphocytes. Sheathed arteriole contains large macrophages filled with necrotic material and debris. Hematoxylin and eosin stain. × 250.



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