COMPARATIVE RESPONSE OF LINE 7x7 CHICKENS TO JM AND GA MAREK'S DISEASE AGENTS

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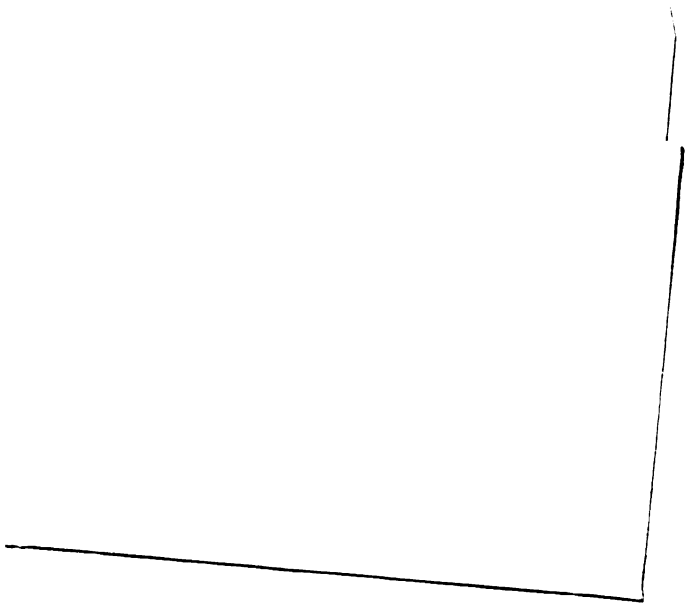

ABSTRACT

COMPARATIVE RESPONSE OF LINE 7x7 CHICKENS

TO JM AND GA MAREK'S DISEASE AGENTS

By

Ralph Lawrence Muhm

Field investigations indicate that there may be considerable variation of the disease process in different outbreaks of Marek's disease (MD). A study of this condition in a single line of chickens produced by 2 different strains of virus was intended to provide some insight into clinical, gross and histopathologic differences that have been seen in infected flocks.

Pathogenesis of MD in Line 7x7 chickens, caused by exposure to JM and GA viral agents, was investigated by clinical, necropsy and histopathologic examinations. Typical, well developed lesions in the average case were described and compared rather than trying to enumerate all variations in response.

Baby chicks inoculated with material from JM- and GA-infected donors were placed in separate quarters. Thirty days later, 50 1-day-old Line 7x7 chicks were placed in each pen in contact with the surviving infected birds. Fifty Line 7x7 chicks were placed in another building to be used as controls. When birds became ill an attempt was made to select moribund individuals from each pen so that comparative variations in response could be made. Randomly selected control birds were taken at the same time.

Differences in clinical response were observed. All dead birds were necropsied as were the selected moribund individuals and controls. Sections of the brain and 3 nerves, the brachial, vagus and sciatic, were taken for microscopic examination. Sections of the bursa, gonad, heart, kidney, liver, lung, proventriculus, spleen and thymus were also taken. Wet smears were prepared from selected gross visceral tumors and lymphoid organs. The average nuclear diameter and percentage of immature cells were determined on smears prepared from tissues of typical infected and control birds. Each tissue was examined microscopically for the purpose of describing comparative pathologic changes in the 2 pens of chickens infected with JM and GA MD agents.

The first sickness due to MD was observed in the JM pen on Day 19 and the first death occurred on Day 28. Similar responses in the GA pen occurred about a week later. This seemed to be true for the entire trial with the GA lot sickening slightly later than the JM birds. However, about the same percentage of birds from each lot was eventually affected. Sick birds became emaciated. Considerably more JM birds became paralyzed than did GA-infected birds.

Necropsy revealed markedly enlarged nerves in most JM-infected birds. This response was not common in birds from the GA pen. However, gross visceral lesions, especially in the liver and spleen, occurred most frequently in GA birds. Other lesions, such as those of intestine, kidney, and subcutis, were found in the GA lot but not in the JM group.

Histopathologic examination of the brain, nerves and viscera revealed marked lesions in both lots of birds. Few differences in cellular response, other than incidence of lymphocytic aggregations in particular organs, could be detected. Both lots had about the same percentage of brain and nerve lesions. The gonad was most commonly affected

in JM birds while other visceral organs were most commonly involved in the GA group.

Excellent cellular detail was observed in impression preparations. It appeared that lymphocytes from neoplasms were somewhat larger and more immature than those of normal tissues. However, these changes were not prominent enough to differentiate normal lymphocytes from those seen in Marek's disease.

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By

Ralph Lawrence Muhm

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INTRODUCTION

Marek's disease (MD) may be the most serious problem ever faced by our rapidly expanding poultry industry. Economic losses have been severe and thus far, despite intensive research, no practical way of controlling the disease has been found. Field and laboratory investigations indicate that there may be considerable variation in the pathogenesis of different outbreaks of MD. A study of this condition in a single line of chicken produced by 2 different strains of virus was intended to provide some insight into clinical, gross and histopathologic differences that have been seen in infected flocks. Descriptions were limited to the characterization of typical, well developed lesions rather than trying to evaluate all variations that might be found.

Marek (1907) originally described a lymphocytic disease of chickens which he called "polyneuritis". Early investigators occasionally called the condition "Marek's disease" or "Marek's paralysis". Eventually, more descriptive terms such as "fowl paralysis", "range paralysis" and "neural leukosis" were used. When poultry husbandry methods changed from farm flock to large integrated operations, a disease commonly called "acute neural leukosis" became a serious problem. British workers proposed the term "acute Marek's disease" for this condition. The name "Marek's disease" has now been generally accepted and will probably have a permanent place in scientific literature.

Marek's disease (MD) is one of 2 common proliferative lymphocytic diseases of the domestic chicken, the other being lymphoid leukosis (LL).

Lesions of these 2 conditions are difficult to differentiate grossly and microscopically and have often been confused. However, it has recently been determined that MD and LL are caused by separate viral agents with distinctly different serologic and cultural characteristics.

MD usually affects chickens at 3 to 4 months of age. It occasionally occurs in older birds and has been seen in those 3 weeks of age. Paralysis and other less definite signs associated with involvement of the visceral nerves are not uncommon. Nerves from these animals may be edematous and contain infiltrations of lymphocytes. Lymphocytic visceral lesions may occur.

MD can be experimentally produced by exposing chickens to the etiologic agent. The strain of virus, dosage, route of inoculation, age and genetic constitution of the chicken are all factors which influence the results.

JM virus is a highly virulent isolate which was originally described by Sevoian, Chamberlain and Counter (1962). A viral isolate designated GA, which apparently produced a different MD response, was described by Edison and Schmittle (1968). These viruses have been used in transmission studies of MD, and were used in this experiment.

REVIEW OF THE LITERATURE

A. Marek's Disease

The term "Marek's disease" has been suggested as a more appropriate name for the neural form of the avian leukosis complex than "fowl paralysis" or "neural lymphomatosis" (Gordon, 1960; Campbell, 1961; Biggs, 1961a; Biggs, Purchase, Bee, and Dalton, 1965). The condition was first described by Marek (1907). The first successful transmission of MD was reported by Van der Walle and Winkler-Junius (1924). Pappenheimer, Dunn and Cone (1926) described it as an aleukemic transmissible disease and considered it to be etiologically related to lymphoid leukosis. Pappenheimer, Dunn and Seidlen (1929) were unable to reproduce the disease with a cell-free filtrate of a brain-cord suspension from a paralyzed chicken.

B. Etiologic Agent

JM virus was the designation given a highly virulent isolate which has been used in transmission studies of MD (Sevoian, Chamberlain and Counter, 1962). Another MD agent has been called GA (Edison and Schmittle, 1968a).

Studies of the different avian leukosis viruses have been hampered by the lack of a convenient assay system. Jungherr and Hughes (1965) stated that the only way of detecting JM virus was by inoculating susceptible chickens. Recently, however, MD virus has been propagated in duck-embryo fibroblast and chicken-kidney cell cultures in which it produced a cytopathic effect. Electron microscopic studies, plus the

fact that the cytopathic agent was highly cell associated, indicated that a group B herpesvirus was involved in the etiology of MD (Churchill and Biggs, 1967; Solomon, Witter, Nazerian and Burmester, 1968; Nazerian, Solomon, Witter and Burmester, 1968). Virus isolation studies provided further evidence that the etiologic agent of MD was a herpesvirus (Witter, Burgoyne and Solomon, 1969). An agar gel double diffusion technique (Chubb and Churchill, 1968) has been used to detect a precipitating antigen of MD in suspected sera. A direct fluorescent antibody test has been used to locate viral associated antigen in frozen tissue sections from MD-infected birds (Spencer, 1969).

MD has been transmitted in cell-free preparations obtained from lymphomatous gonads taken from JM infected chickens. However, cell suspensions of the same tissues produced a much higher disease incidence (Sevoian and Chamberlain, 1963a). Since cell-free preparations do not consistently produce a high virus titer, inoculum containing intact cells is ordinarily used (Biggs and Payne, 1964). The infective agent will pass through a filter with a pore diameter of 0.3 μ (Sevoian, 1962).

An assay system for chick-embryo tissue cultures infected with Rous sarcoma virus was reported by Rubin (1960, 1961). Chickens infected with some leukosis viruses, especially those that cause LL, produce a resistance-inducing factor (RIF) which inhibits the replication of the Rous sarcoma virus in tissue culture. This factor is measured in the RIF test.

The JM and GA agents do not produce this inhibitory effect and appear to be immunologically and biologically distinct from RIF-positive strains of leukosis viruses (Witter, 1964; Calnek, 1965).

C. Epizootiology

The epizootiology of MD is not well understood. A survey indicated that such factors as mixing chicks from different parental flocks, incubating eggs from different sources, rearing chickens in houses with a previous history of MD and exposure to wild birds and rodents might influence the incidence of the disease (Muhm and Burmester, 1969). Under field conditions MD affects birds from 7 to 20 weeks of age, is involved with genetic factors, but is not sex related (Rich, 1968). The disease spreads readily by contact and airborne transmission has been proven (Hutt and Cole, 1954; Sevoian, Chamberlain and Larose, 1962; Biggs and Payne, 1967). Egg transmission has not been proven. The response by the chicken after natural exposure or inoculation is inconsistent due to variations in exposure, passive immunity transmitted by the dam and other factors (Burmester, Fontes, Waters, Bryan and Groupé, 1960).

Fresh droppings and litter from chickens inoculated with the JM or GA strain of MD were collected, and after 16 weeks of storage in plastic bags at room temperature, were infective to chicks by contact (Witter, Burgoyne and Burmester, 1968). Nasal washings containing the GA agent were infective to chicks by contact, but feces from infected birds did not transmit the disease either by contact or inoculation (Edison and Schmittle, 1968b).

D. Pathology

Smith and Jones (1966) said that microscopic examination constitutes the most precise method available for the diagnosis of neural-lymphomatosis (MD). Biggs (1961b) stated that the proliferative lesions of MD cannot be differentiated from the lesions of LL on histopathologic grounds alone.

Campbell (1956) considered fowl paralysis (MD) to be an inflammatory process. He described a "lymphogranuloma" which he believed to be the typical lesion of MD. Burmester (1967) said that in MD lesions usually occur in the nerves, rarely occur in the bursa and often occur in the gonad and other viscera; while in LL, lesions never occur in the nerves, almost always occur in the bursa, usually occur in the liver and spleen and often in the other viscera.

Payne and Biggs (1967) stated that bursectomy, combined with X-irradiation, may reduce the incidence of MD. Atrophy of the follicles of the bursa of Fabricius in MD-infected birds has been described (Purchase and Biggs, 1967). Reduction of bursal size, necrosis and absence of follicles was reported by Jakowski, Fredrickson, Luginbuhl and Helmboldt (1969). These changes were more apparent in birds inoculated with whole blood from donors inoculated with Conn-A and FF-1 strains of MD agent and not as prominent in birds inoculated with JM virus.

Fluorescent antibody studies indicated that MD virus has a predilection for medullary cells of the bursa and for epithelial cells of the kidney tubules and feather follicles (Calnek and Hitchner, 1969). Fluorescence was seen in only a small percentage of cells from lymphoid lesions. However, it was felt that the viral genome was present in these cells, as they were nearly always infectious.

Intranuclear and cytoplasmic viral inclusion bodies in epithelial cells from feather follicles of MD infected birds have been described (Nazerian and Witter, 1970).

1. Nervous tissues

Pappenheimer, Dunn and Cone (1926), Pappenheimer, Dunn and Seidlen (1929, and Jungherr (1934) reported that lymphocytes were present in

nervous tissues of a high percentage of normal chickens. However, Oakberg (1950) suggested that any extravascular aggregation of lymphocytes in nerves of chickens be considered abnormal.

Wight (1962a, 1962b) considered fowl paralysis (MD) to be essentially a disease of the peripheral nerves and stated that the central nervous system involvement was usually minimal. There was little difference in the central nervous systemic involvement in birds killed after varying periods of clinical disease. The intensity of lesions was extremely variable. Affected nerves were described as slightly to greatly enlarged, yellowish in color, with occasional loss of cross striations. Nerves were classified as having type I, II or III lesions depending upon the amount of edema, degree of cellular infiltration and amount of myelin sheath degeneration.

Pappenheimer, Dunn and Cone (1929) said that, in avian leukosis, striking alterations were found in the brain, spinal cord, dorsal root ganglia, spinal nerve roots and peripheral nerves. Nerve lesions consisted of either follicular or diffuse infiltration with mononuclear cells indistinguishable from lymphocytes. Most cells were well differentiated. Others observed were large mononuclear cells, histiocytes and some resembling plasma cells. Some lesions were associated with edema and myelin degeneration. Brain lesions consisted of compact perivascular rings of small lymphoid cells or submiliary nodules of lymphoid cells and paler staining elements.

Biggs (1961b) said that in severe lesions of MD lymphoid cells sometimes extended beyond the normal boundaries of the nerve.

2. Viscera

Because of occurrence of focal aggregations of normal mononuclear cells in visceral organs, it may be difficult to draw a sharp line between the normal and the abnormal (Pappenheimer, Dunn and Cone, 1929). In MD, enlarged foci of hyperplastic mononuclear cells surround the small arterioles and, as the lesion progresses, crowd out the normal parenchyma (Sevoian and Chamberlain, 1963b). Any or all of the visceral organs may be affected; however, the gonad is most commonly involved (Biggs and Payne, 1964).

E. Genetic Studies

The ability of chickens to resist infection by such organisms as viruses, bacteria, fungi and protozoa is partly determined by inherited genetic factors. The susceptibility of breeds, strains of the same breed and even individual families may differ (Waters and Burmester, 1963; Hutt, 1958). The incidence of experimentally induced MD varies with the strain of the chicken (Biggs, 1963). The mode of inheritance for resistance to Rous sarcoma virus is controlled by a single gene (Waters and Burmester, 1961; Crittenden, Okazaki and Reamer, 1963). Regional Poultry Research Laboratory line 7x7 is homozygous for resistance to Bryan's strain of the Rous sarcoma virus (Crittenden and Okazaki, 1966; Kenzy, Conrad and Fluharty, 1958; Kenzy, McClary and Zander, 1961). Line 7x7 is resistant to RIF-positive viruses (Crittenden, Okazaki and Reamer, 1964; Crittenden and Okazaki, 1965), while it is susceptible to the RIF-negative JM virus (Purchase, 1963). The resistance of line 7x7 to RIF-positive viruses occurs in cell culture, embryos and hatched chickens and is, therefore, a cellular phenomenon (Crittenden, Okazaki and Reamer, 1963; Vogt, 1965). Because of this resistance, only low levels of neutralizing antibody are produced against RIF-

positive viruses (Burmester, 1962; Crittenden and Okazaki, 1966). Line 7x7 has had a relatively high degree of naturally occurring neural leukosis through the years and is not resistant to JM virus (Burmester and Fredrickson, 1965).

MATERIALS AND METHODS

A. Inoculum

The inoculum used in this experiment was heparainized, whole blood taken at the Regional Poultry Research Laboratory (RPRL), East Lansing, Michigan, from donors inoculated at 1 day of age with JM or GA virus.* (The donors were necropsied after exsanguination and most had gross lesions of MD.)

Several viral agents may be associated with the etiology of avian leukotic tumors. Therefore, the presence of a single, pure viral strain in a whole blood preparation is debatable. MD isolate JM (Sevoian, Chamberlain and Counter, 1962) is a virulent, RIF-free virus which affects chicks at an early age. MD virus GA (Edison and Schmittle, 1968) is also highly virulent for young chickens. Most LL viruses do not produce clinical leukosis until chickens reach 4 months of age (Burmester, 1967). For these reasons, passage through young, RIF-free chickens tends to preserve MD agents in a relatively pure form.

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*The infective materials were obtained through the courtesy of Dr. Richard Witter, who had passaged the agents numerous times at the RPRL.

The original JM material was obtained by the RPRL from Dr. Martin Sevoian, Amherst, Massachusetts.

The original GA material was obtained by the RPRL from Dr. Samuel Schmittle, Athens, Georgia.

B. Chickens

Line 7x7 chickens (RPRL)* were from a highly inbred strain of Single Comb White Leghorns. Line 7x7 has been kept in semi-isolation and has a low natural incidence of LL and MD. Previous trials have indicated that this strain of chicken is genetically resistant to LL but is extremely susceptible to MD.

Two randomly selected lots of 15 newly hatched chicks were placed in separate isolated pens on corn-cob litter and fed a commercial chick starter ration. These were the infected birds used for direct exposure of the 2 experimental groups by contact. One lot was inoculated intraperitoneally with .2 ml. each of heparainized, whole blood from JM infected donors. The other lot was given a similar dosage taken from GA infected donors. Three weeks after inoculation both lots were clinically ill and at 4 weeks several birds in each lot had died. Gross necropsy and microscopic examination of tissues from dead birds from both pens revealed nerve and visceral lesions characteristic of Marek's disease.

Four weeks after the original 2 lots of chicks were inoculated, 50 randomly selected day-old chicks were placed in each of the 2 infected pens. The original litter containing the droppings from the inoculated birds was not disturbed. The surviving 4-week-old birds were left in the pens but were restrained in a wire cage so that the baby chickens could not be injured.

Fifty 1-day-old control chickens were placed in isolation in other quarters.

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*These chickens were received through the courtesy of Dr. Richard Witter of the Regional Poultry Research Laboratory (RPRL), East Lansing, Michigan.

After 10 days' exposure of the chickens the remaining inoculated birds were removed from the experimental pens and killed.

The first 2 lots of 15 chickens each were inoculated on 6-14-68. The lots of 50 birds each were placed in the infected pens or in isolation for controls on 7-12-68. The experiment was terminated on 10-7-68.

C. Isolation Procedure

The 3 lots of chickens (2 infected and 1 control) were kept in different rooms, each of which had an individual forced air supply. Each lot was cared for by a different caretaker. Visitors to the pens were required to change clothing when entering and to take a shower when leaving. Dead birds, or those to be killed, were taken to the necropsy room in plastic bags. Necropsy procedures were performed in a hood.

D. Necropsy and Histopathologic Procedures

Each pen was visited at least once a day. Sick birds were selected from each lot for necropsy at the same time intervals so that a comparison of lesions could be made as the disease progressed. Controls were killed each time infected birds were necropsied. The number of controls taken usually corresponded to the least number of GA- or JM-infected birds examined.

All birds that died or were killed were examined for gross lesions of MD at necropsy. Sections of the brain and three nerves, the brachial, sciatic and vagus, were taken for histopathologic examination. Also taken were sections of the bursa, gonad, heart, kidney, liver, lung, proventriculus, spleen and thymus. Tissues for histopathologic examination were fixed in 10% formalin, embedded in paraffin, cut at 6 μ and stained with hematoxylin and eosin.

Wet smears were prepared from selected visceral lesions by incising tissues with a scalpel and drawing a slide across the exposed surface. Smears were also prepared from spleen, thymus and bursa of control and infected birds killed at that time even though no lesions were visible in the infected birds. These smears were fixed in Bouin's solution and stained with Schorr's or methyl green pyronine (MGP) stains. Average nuclear diameters were measured using a Leitz micrometer eyepiece on the smears stained with Schorr's preparation. The percentage of immature or "blast" cells was determined using the MGP stain for nucleic acids. One hundred cells from each smear were counted or measured and the average taken as the final determination.

E. Immunologic Procedures

Serum samples were collected from 32 survivors at the termination of the experiment. Survivors included 11 controls, 12 birds from the JM infected pen and 9 birds from the GA infected pen.

The following tests were run on these serum samples:

1. A hemagglutination-inhibition test for Newcastle disease antibodies.*

2. A virus neutralization test for avian encephalomyelitis antibodies.*

3. An agar gel double diffusion technique for a precipitating antibody of MD.**

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*These tests are described in *Methods for the Examination of Poultry Biologics*, 2nd Ed. (Revised), Publication 1038, Nat'l. Acad. of Sci., Nat'l. Res. Council, Washington, D.C., 1963.

I am indebted to Mr. Larry Lee and Dr. E. A. Carbrey of the Virology Section, Diagnostic Services, NADL, Ames, Iowa, for doing these tests.

**I am indebted to Dr. Richard Witter for the results of the agar gel double diffusion tests. The procedure used followed that described by Chubb and Churchill, 1968.

RESULTS

A. Clinical Remarks

1. JM-infected chickens

Chicks with ataxia, lameness and paralysis were observed on Day 19. The first death due to MD occurred on Day 25, with a peak in morbidity and mortality at Day 45. These birds were almost always ataxic prior to death and usually assumed a typical MD posture (Figure 1). Feathers surrounding the vent of sick or dead birds were often soiled with fecal material.

2. GA-infected chickens

Sick birds were first observed in this pen on Day 28. The first death occurred on Day 30, and a peak in morbidity and mortality was reached on Day 50. These birds occasionally became paralyzed but more often only lost weight, became pale, had ruffled up feathers and died (Figure 2). Some birds appeared to have central nervous system involvement prior to death. Feathers around the vent were usually soiled.

3. Control chickens

Control birds remained healthy during the entire study (Figure 3).

B. Gross Pathology

1. JM-infected chickens

At necropsy some nerves were markedly enlarged (Figure 4), especially the sciatic and vagus. Birds with the vagus nerve involved often had

Figure 1. Line 7x7 chicken with paralyzed legs because of infection with JM virus by contact in typical MD posture (Day 43).

Figure 2. Line 7x7 chicken infected with GA virus by contact. Notice twisting of head indicating CNS involvement (Day 43).

Figure 1

Figure 2

Figure 3. Control (center) has grown much faster than JM-infected bird (right) and GA-infected bird (left) (Day 43).

Figure 4. Edematous, discolored sciatic nerve (bottom) from a JM-infected chicken compared with normal sciatic nerve (top) (Day 57).

Figure 3

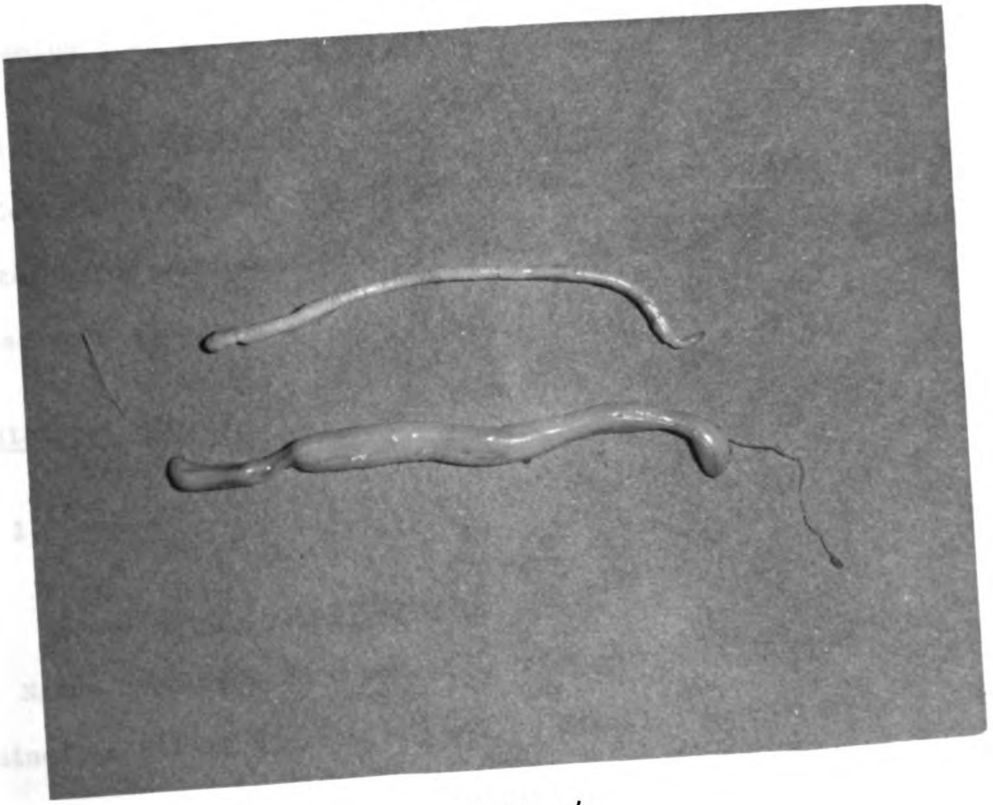

Figure 4

atony of the proventriculus and cloaca. Chicks which died early (Day 25 through Day 33) usually had an atrophic spleen (Figure 5) and lesions of the gonad (Figure 6). Gross lesions were sometimes observed in other visceral organs, especially in those birds which died later in the experiment.

2. GA-infected chickens

Gross nerve enlargements were infrequent. However, atony of the proventriculus (Figure 7) and cloaca was found in some birds. Nearly all GA-infected birds that died had characteristic gross changes of the liver and spleen. The spleen was usually enlarged, dark in color (Figure 8) and contained numerous light-colored lymphocytic foci. The liver usually appeared swollen and had a characteristic reddish-bronze color. Large intestinal and kidney lesions (Figures 9 and 10) were observed in some cases, and a few birds had subcutaneous swellings on the cranium just above and behind the external ear.

3. Control chickens

Control birds were killed and necropsied each time JM- or GA-infected birds were examined. No gross lesions of MD or any other disease were observed.

C. Histopathology

1. Nerves

a. JM-infected chickens

Nerve lesions were observed in sections of 1 or more of the 3 nerves examined in 84% of these birds (42). Usually, when one nerve was involved, others were also. Lesions consisted of infiltration and

Figure 5. Small, shrunken spleen from a JM-infected bird (right) compared with a normal spleen (center) and a grossly enlarged spleen from a GA-infected bird (left) (Day 43).

Figure 6. Enlarged and deformed testicles from a JM-infected bird (Day 57).

Figure 5

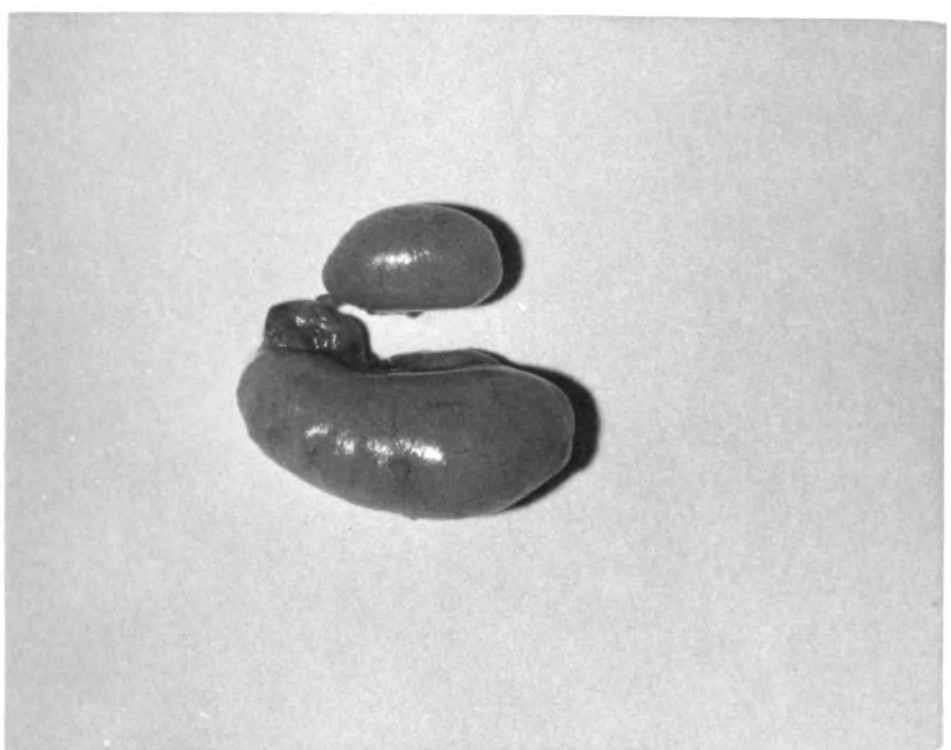

Figure 6

Figure 7. Impacted proventriculus (a) in chicken infected with a Marek's disease agent (Day 43).

Figure 8. Enlarged spleen (b) in GA-infected bird (Day 43).

Figure 7

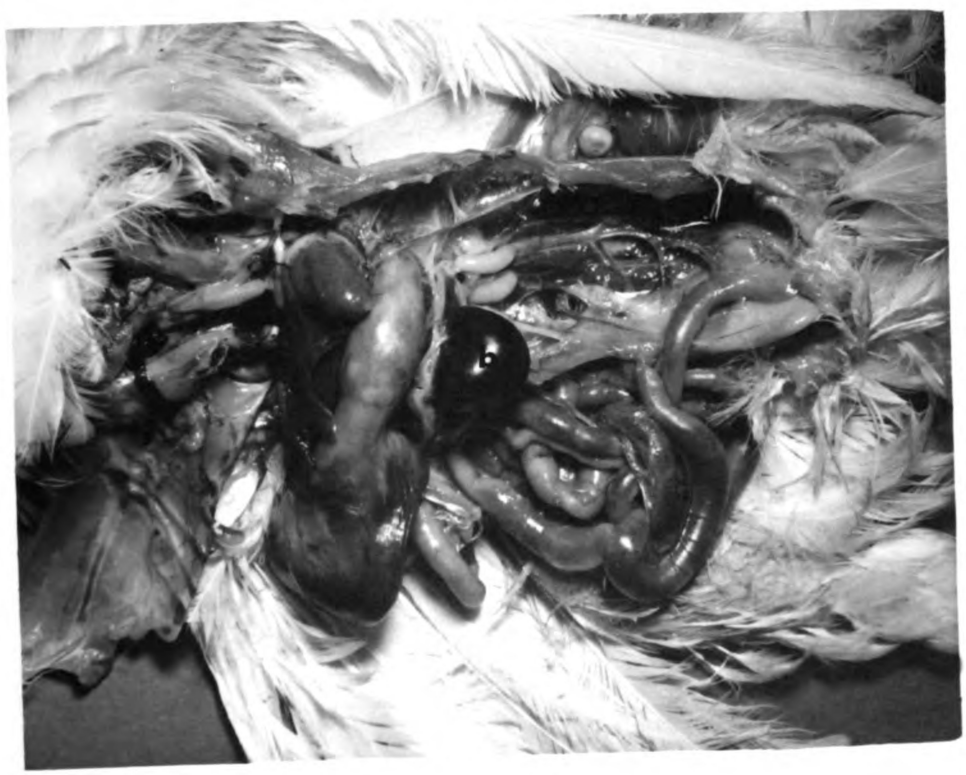

Figure 8

Figure 9. Intestinal lesions (1 and 2) in GA-infected bird (Day 50).

Figure 10. GA-infected bird which died with gross lesions of the kidneys (3), spleen (4), and liver (5) (Day 50).

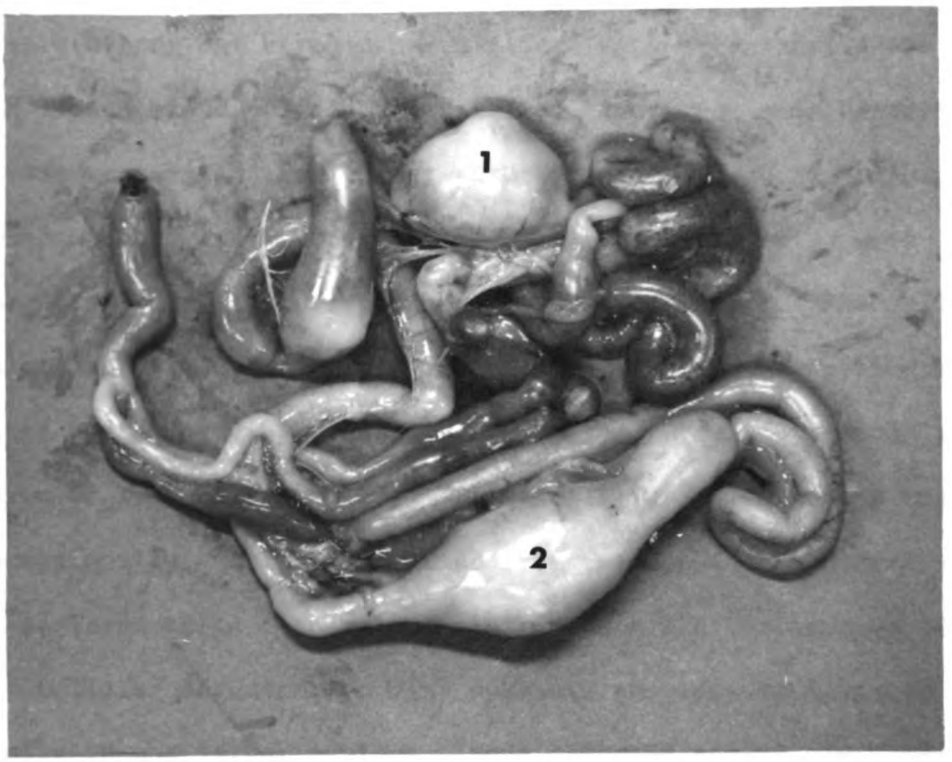

Figure 9

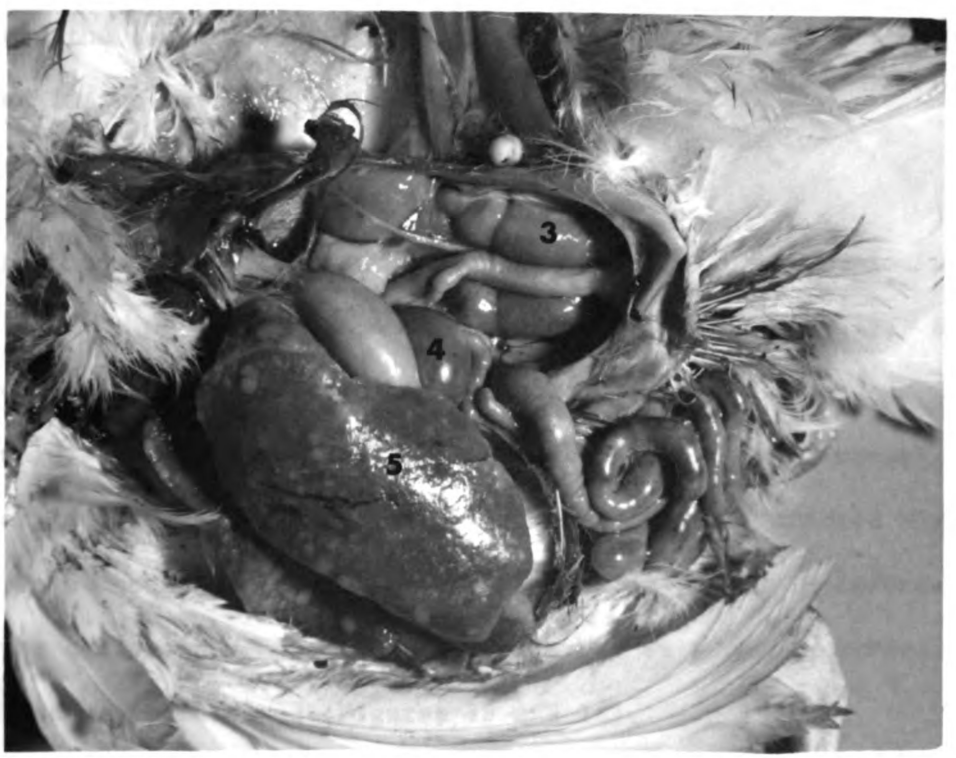

Figure 10

proliferation of mononuclear cells (Figure 11). These cells were mostly of the lymphocytic series, but an occasional plasma cell was observed. Cells were strung out between nerve fibers with occasional dense accumulations around blood vessels (Figure 12). There was some hemorrhage from these vessels and few intact endothelial cells could be identified. In advanced cases large accumulations of lymphocytic cells were observed, and the normal architecture of the nerve was disrupted. The cellular accumulations were definitely invasive, and adjacent nerve fibers were displaced and damaged by pressure. The vagus nerve most commonly had this type of lesion (Figure 13). Blast cells and mitotic figures were seen occasionally. Cell size varied from small cells with dark staining nuclei to large cells with pale nuclei and a higher proportion of cytoplasm. Cellular degeneration with pyknosis and karyorrhexis was observed, especially in the larger lesions. Some affected nerves had a few mononuclear cells in prominent areas of edema which extended between and separated the nerve fibers. This type of lesion was most commonly observed in the brachial nerve (Figure 14).

b. GA-infected chickens

In spite of the fact that few gross nerve lesions were found, microscopic lesions were present in 82% of the birds examined (41). Slight differences in characteristic histopathology between GA- and JM-infected birds were observed. These variations were not prominent enough to be used for identification purposes. Lesions in the GA-infected birds tended to be somewhat less edematous than those in the JM-infected birds. Also, the cells often appeared to be arranged in a dense pattern with the entire nerve involved (Figures 15 and 16).

Figure 11. Infiltration and proliferation of mononuclear cells in sciatic nerve of JM-infected bird (Day 32). H & E. x 250.

Figure 12. Lymphoid cells strung out between nerve fibers in sciatic nerve of JM-infected bird (Day 38). H & E. x 100.

Figure 11

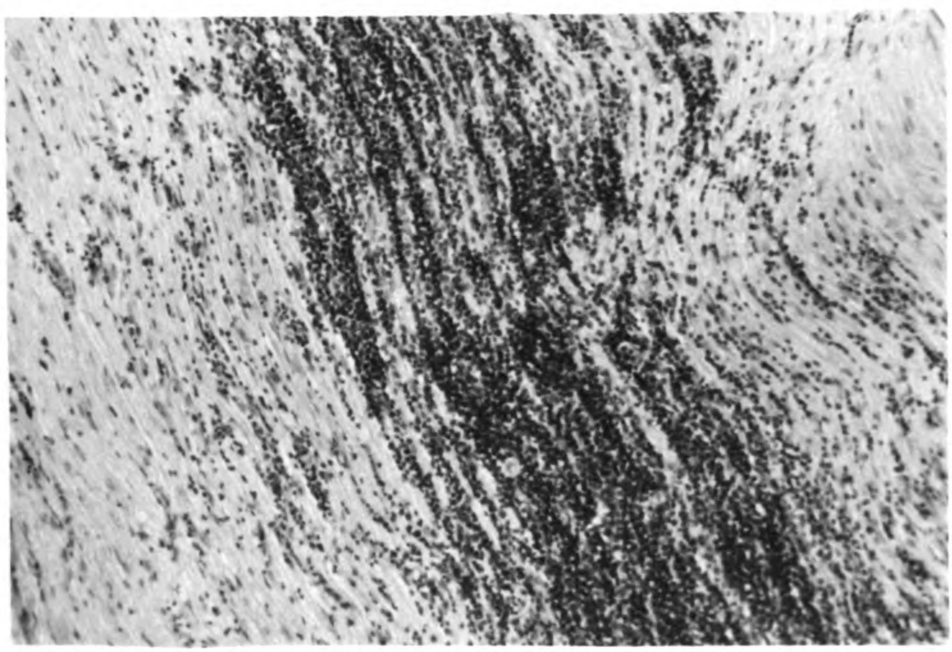

Figure 12

Figure 13. Disruption of normal architecture because of lymphocytic lesion in vagus nerve from JM-infected bird (Day 35). H & E. x 250.

Figure 14. Edema and lymphoid cells in brachial nerve from JM-infected bird (Day 38). H & E. x 100.

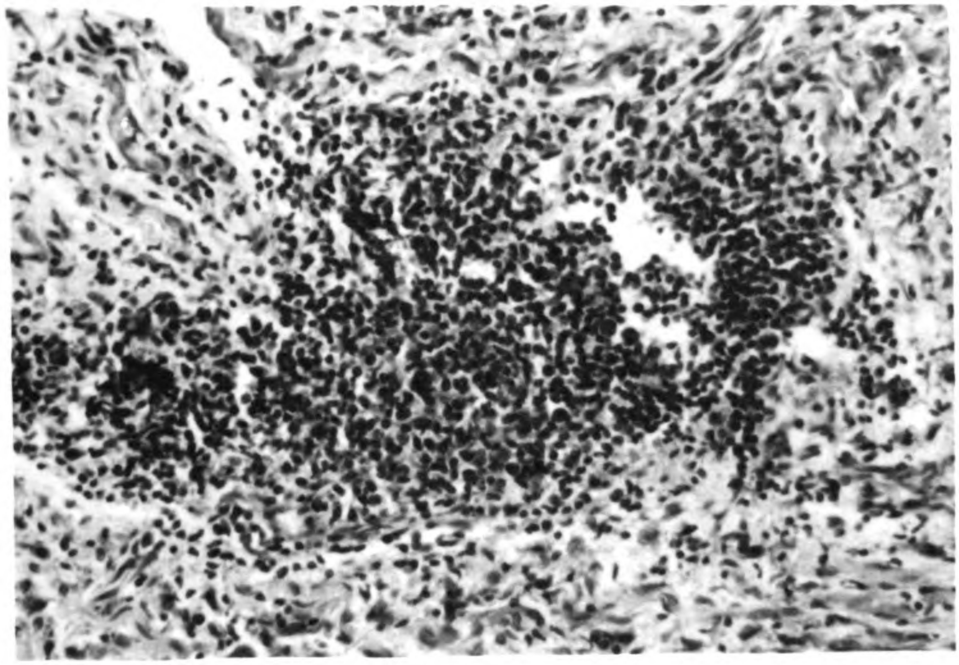

Figure 13

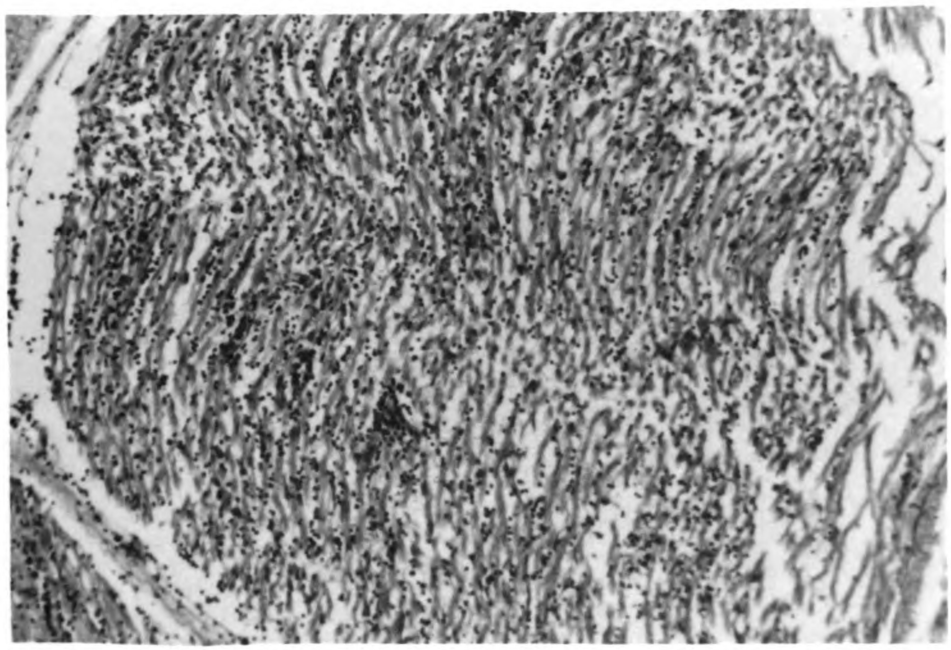

Figure 14

Figure 15. Diffuse lymphocytic involvement of vagus nerve from GA-infected bird (Day 35). H & E. x 250.

Figure 16. Lymphocytes strung out between nerve fibers in sciatic nerve from GA-infected bird (Day 32). H & E. x 100.

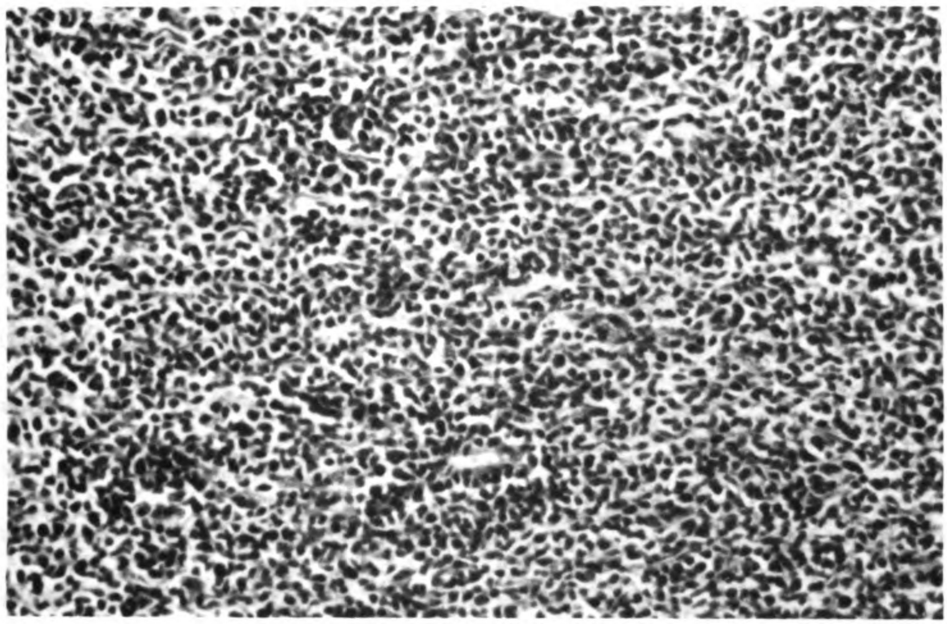

Figure 15

Figure 16

c. Control chickens

No microscopic lesions were observed in nerves taken from control birds.

2. Brain

a. JM-infected chickens

Brain lesions were found in 78% of birds from the JM pen (39). These lesions were vascular in character and varied in location from subdural to deep in the brain parenchyma (Figures 17 and 18). They were seen in the cerebrum, cerebellum and medulla (Figure 19) and were especially prominent in birds which died or were killed early in the experiment. They consisted of dense accumulations of mononuclear cells with dark-staining nuclei which were arranged around blood vessels in which little normal endothelium could be identified (Figures 20 and 21). These cells were indistinguishable from those of the lymphocytic series seen in cellular infiltration of other tissues. However, they did appear somewhat more uniform in size and staining characteristics, resembling small to medium-sized lymphocytes in this respect. An occasional mitotic figure was observed.

Brain lesions occurred in birds killed during the entire trial period and were even observed in survivors examined at the end of the experiment. However, after the 50th day lesions were usually less prominent and there was not as much hemorrhage. The cells involved were paler staining and some appeared to be degenerating. A few connective tissue cells were present, suggesting that there may have been an attempt of resolution and healing of the lesions.

Figure 17. Multiple vascular lesions containing mononuclear cells in cerebrum from JM-infected bird (Day 27). H & E. x 100.

Figure 18. Higher magnification of single vascular lesion in cerebrum from JM-infected bird (Day 27). H & E. x 250.

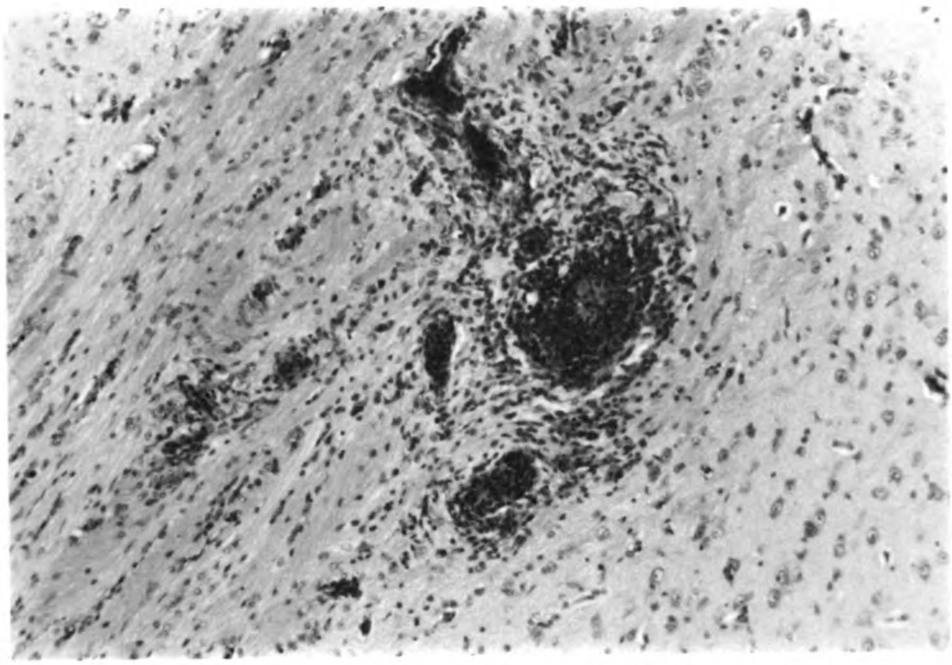

Figure 17

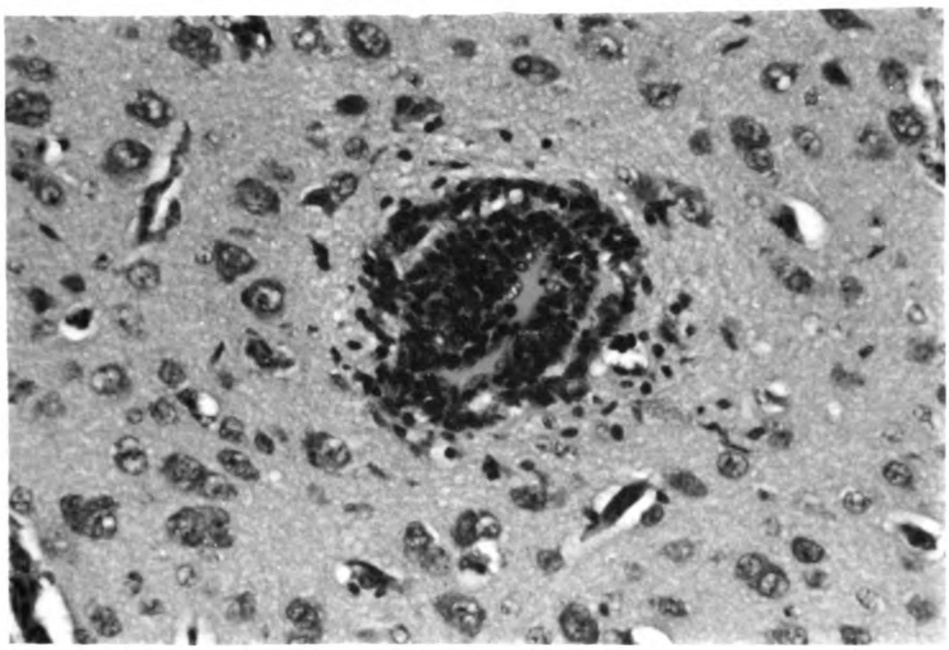

Figure 18

Figure 19. Vascular lesion in cerebellum from JM-infected bird (Day 43). H & E. x 100.

Figure 20. Hemorrhage in cerebrum from JM-infected bird (Day 27). H & E. x 100.

Figure 19

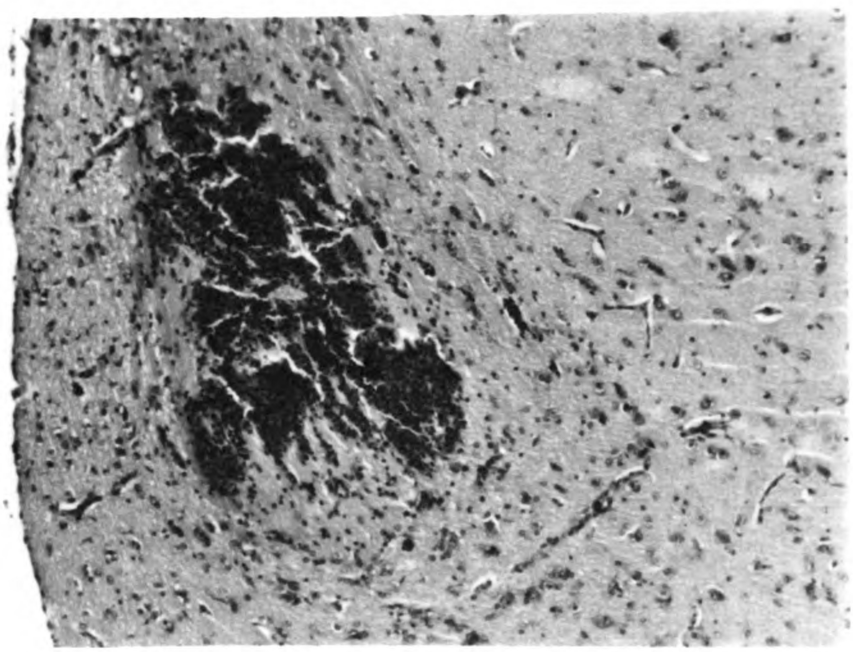

Figure 20

Figure 21. Mononuclear cells and erythrocytes surrounding disrupted blood vessel in cerebrum from JM-infected bird (Day 43). H & E. x 250.

Figure 22. Multiple vascular lesions in cerebrum from GA-infected bird (Day 36). H & E. x 100.

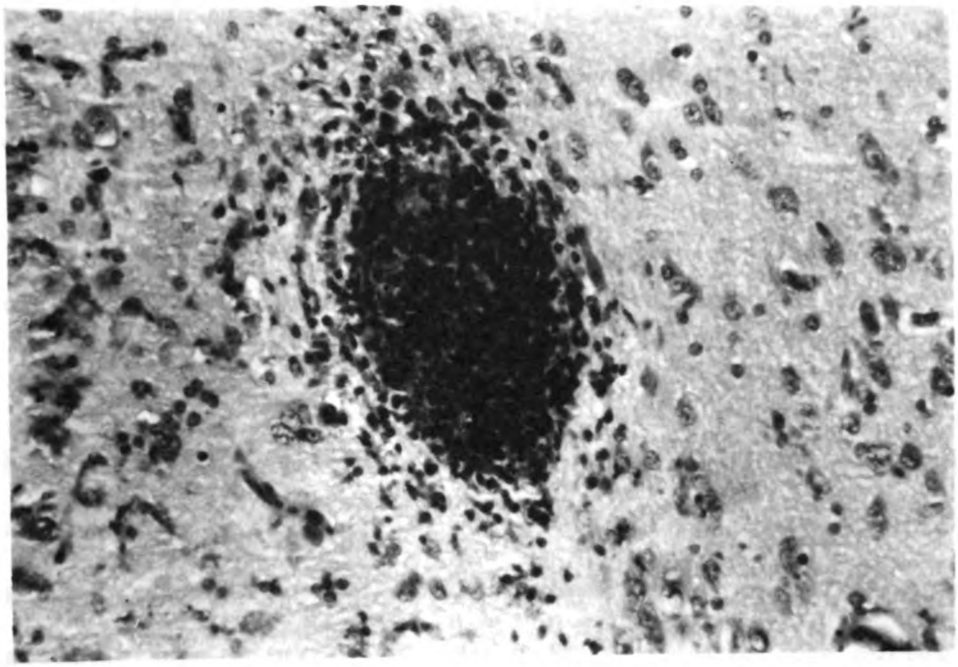

Figure 21

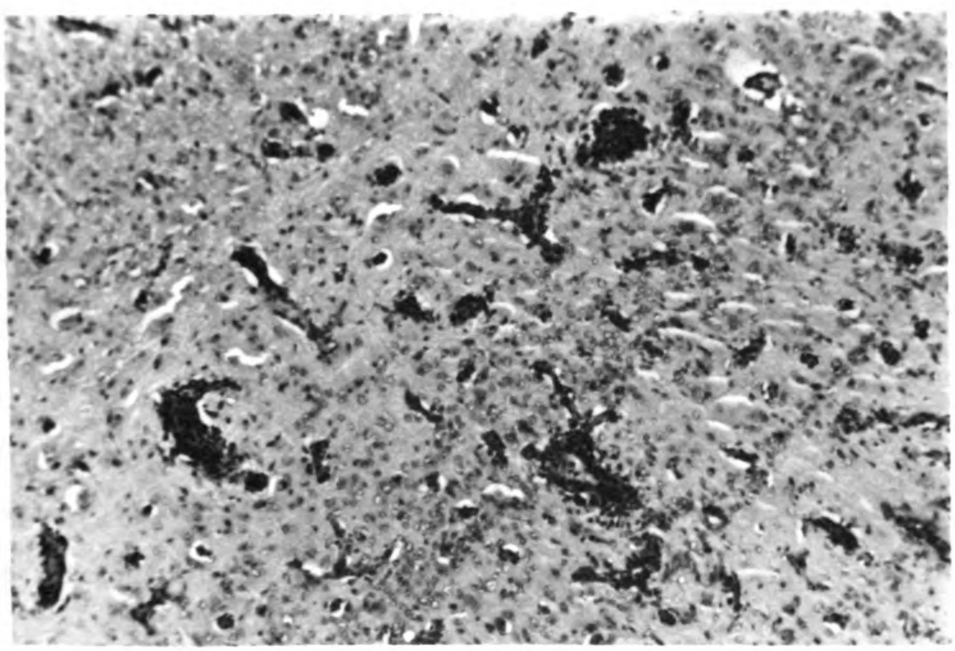

Figure 22

b. GA-infected chickens

Brain lesions were observed in 76% of birds from this pen. These lesions did not appear to differ from those seen in JM-infected birds (Figures 22, 23, and 24).

c. Control chickens

No lesions were seen in brain sections prepared from control birds.

3. Viscera and other tissues

The occurrence of visceral lesions in various organs differed greatly between pens and among individual chickens. Lesions consisted of infiltrating and proliferating masses of lymphoid cells and varied from small, focal accumulations to extensive aggregations in which the normal architecture of the organ was disrupted (Figures 25 through 42). The cell type varied from small lymphocytes to large, epithelioid cells. The largest percentage of these cells could be classified as medium lymphocytes. An occasional mitotic figure was observed. In some large lesions beginning cellular degeneration and necrosis were observed. Some degeneration and small areas of necrosis were seen in bursal follicles of a small percentage of infected birds. Intestinal and subcutaneous lymphocytic lesions on the cranium were seen in GA-infected birds but were not seen in the JM-infected groups (Figures 43 through 46). No visceral lesions were seen in control chickens.

Table 1 illustrates differences in lesion incidence between the pens of chickens.

4. Smear preparations

a. Schorr's stain

Microscopic examination of this preparation revealed excellent nuclear and cytoplasmic detail (Figures 48 through 53), but no inclusion

Figure 23. Vascular lesions in cerebellum from GA-infected bird (Day 36). H & E. x 100.

Figure 24. Higher magnification of cerebellar lesion from GA-infected bird (Day 36). H & E. x 250.

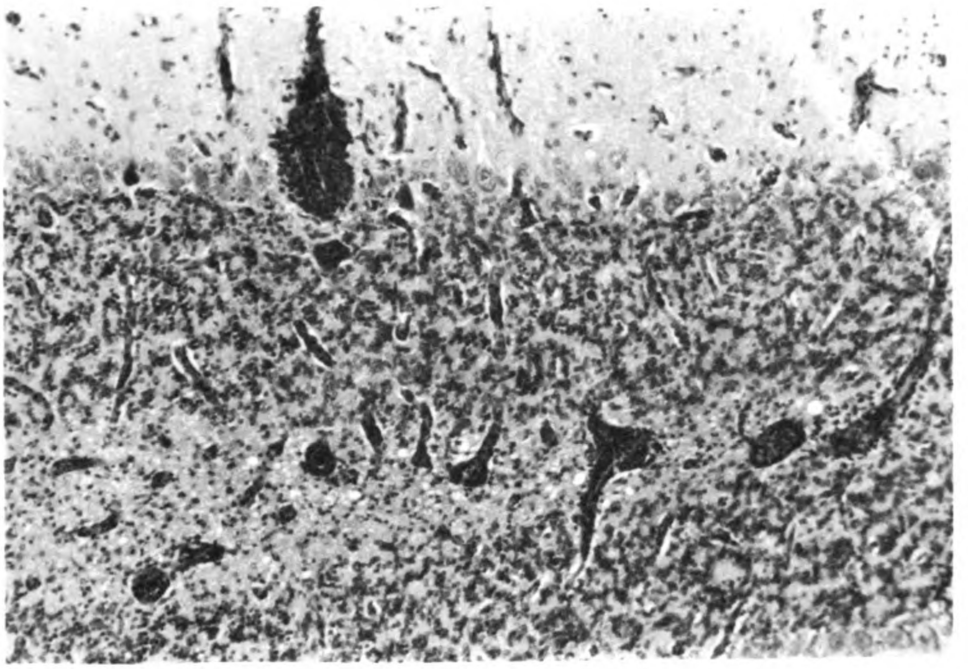

Figure 23

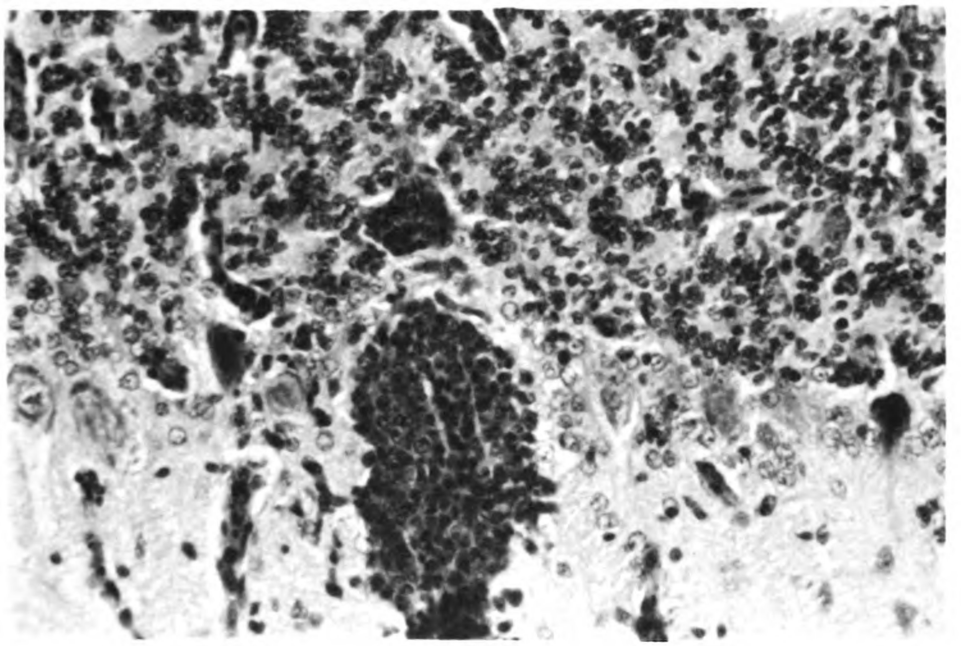

Figure 24

Figure 25. Lymphocytic proliferation and infiltration in testicle from JM-infected bird (Day 27). H & E. x 100.

Figure 26. Lesion in testicle from GA-infected bird (Day 36). Note similarity to Figure 25. H & E. x 100.

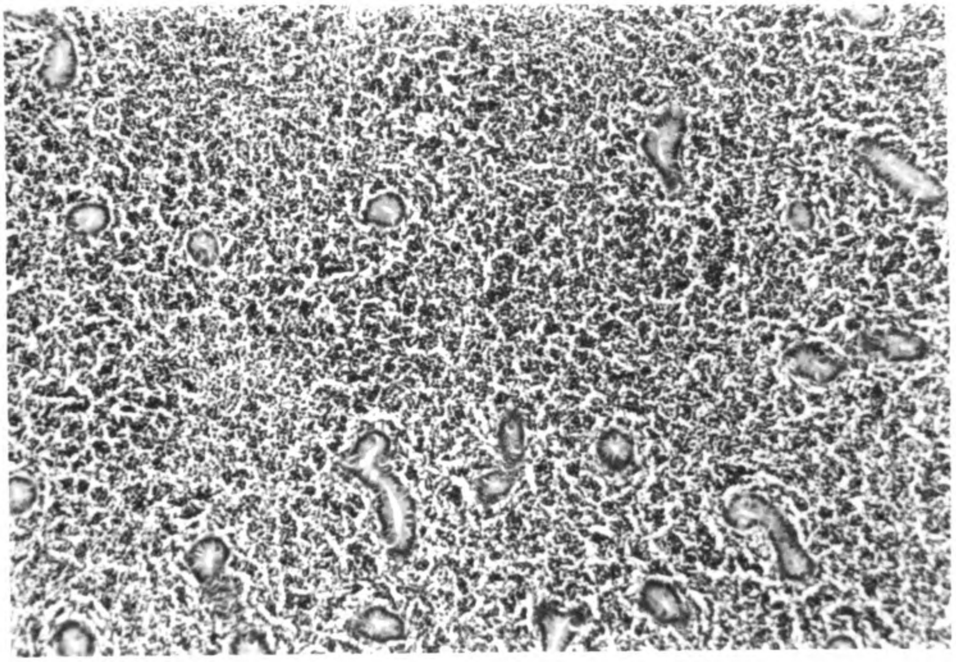

Figure 25

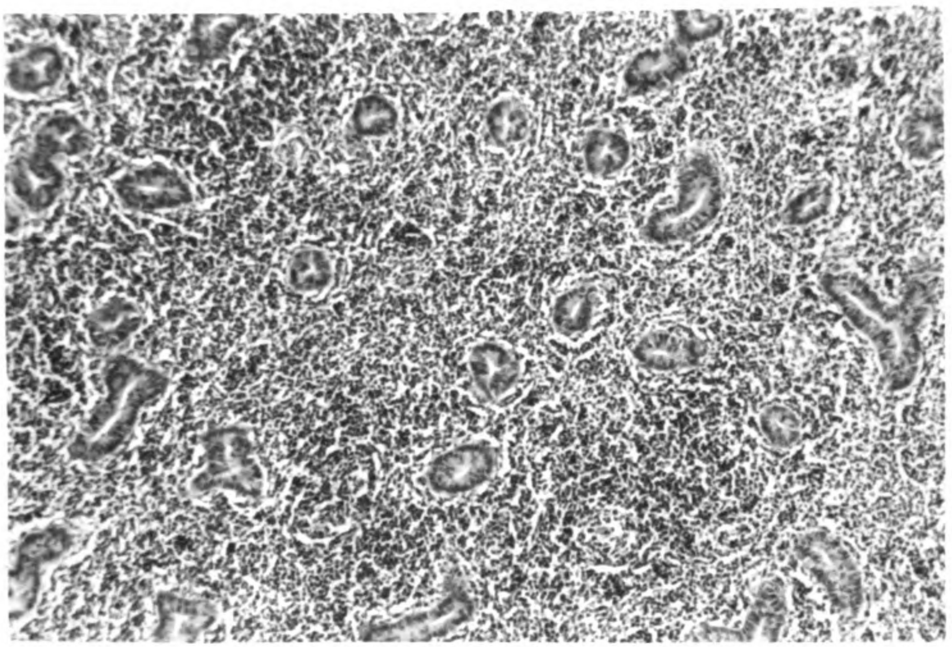

Figure 26

Figure 27. Extensive lymphocytic lesion in ovary from JM-infected bird (Day 32). H & E. x 100.

Figure 28. Small lymphocytic lesions in ovary from GA-infected bird (Day 88). H & E. x 100.

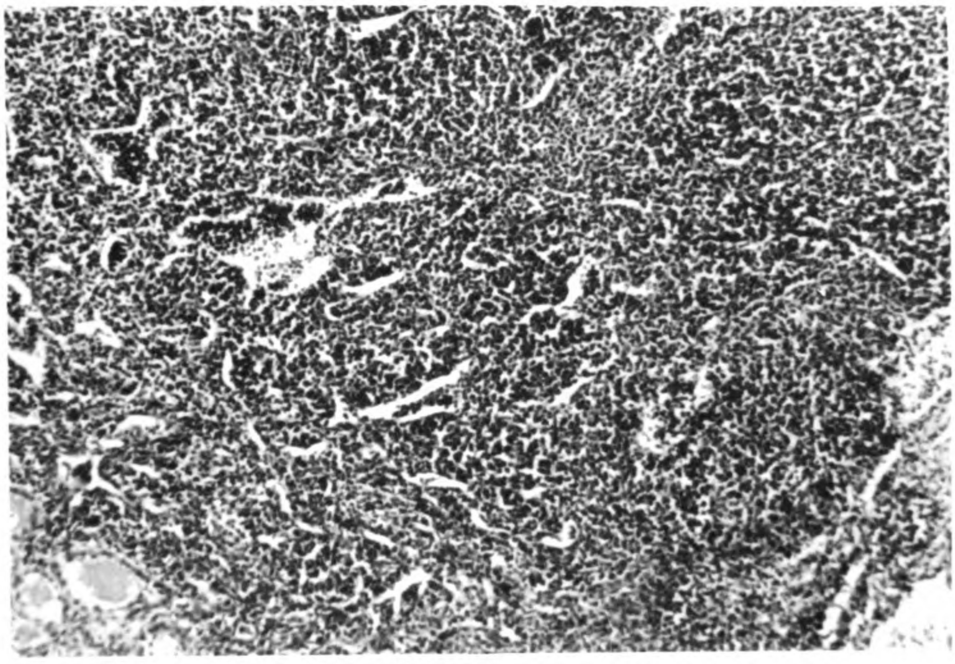

Figure 27

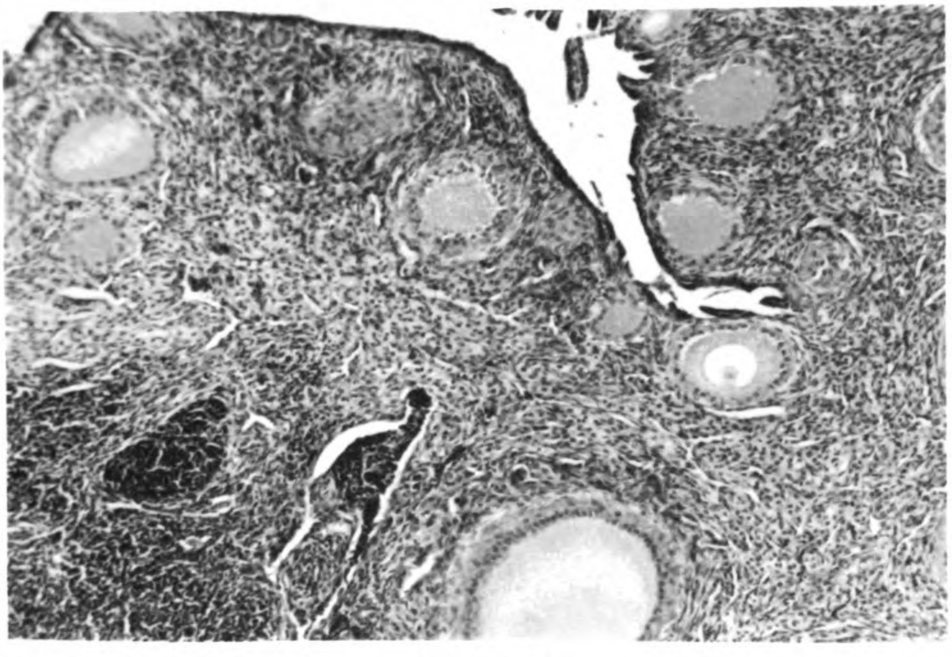

Figure 28

Figure 29. Higher magnification of small lesions in ovary from GA-infected bird (Day 88). H & E. x 250.

Figure 30. High magnification of lymphocytic lesion in kidney from GA-infected bird (Day 36). H & E. x 250.

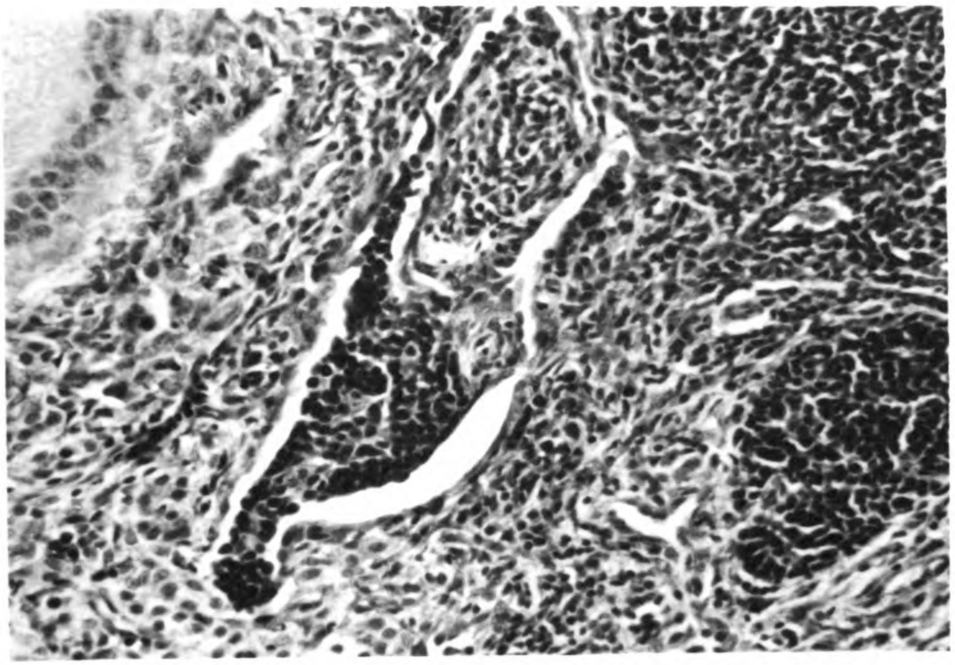

Figure 29

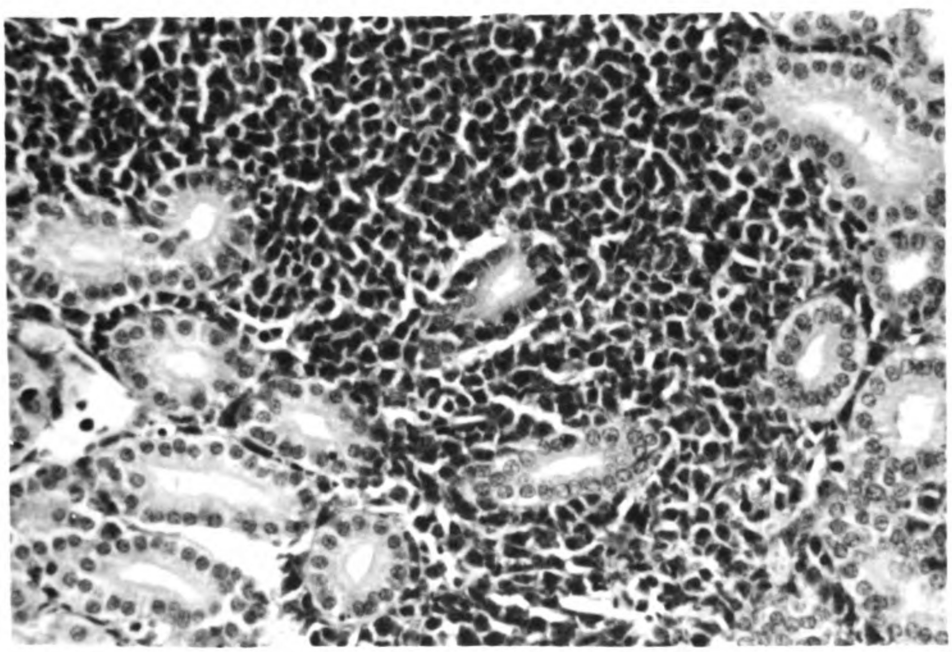

Figure 30

Figure 31. Lymphocytic lesion in kidney from JM-infected bird (Day 57). H & E. x 100.

Figure 32. Lymphocytic lesion in kidney from GA-infected bird (Day 36). Note similarity to Figure 31. H & E. x 100.

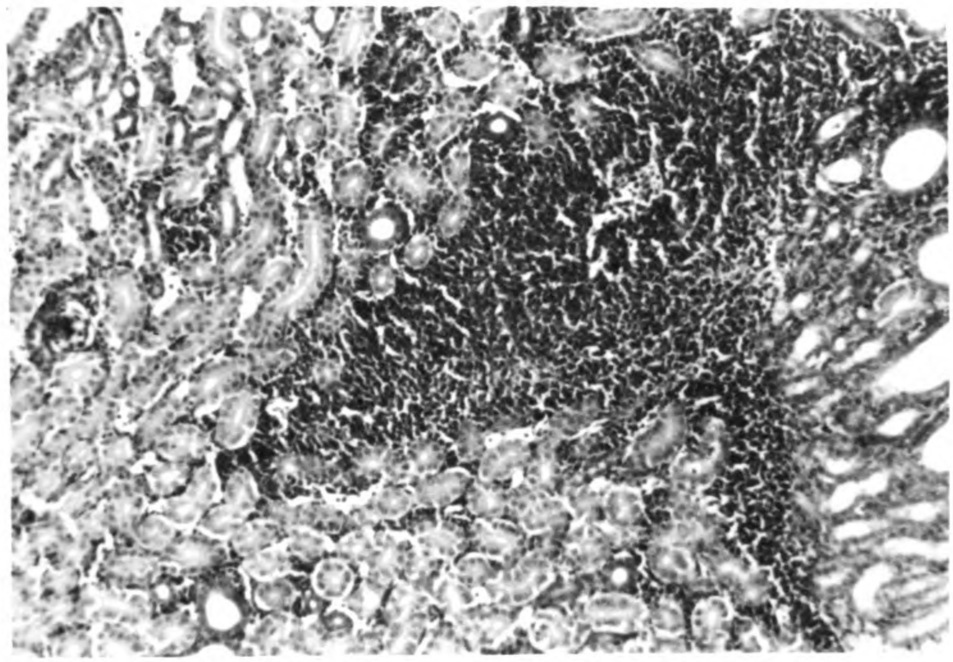

Figure 31

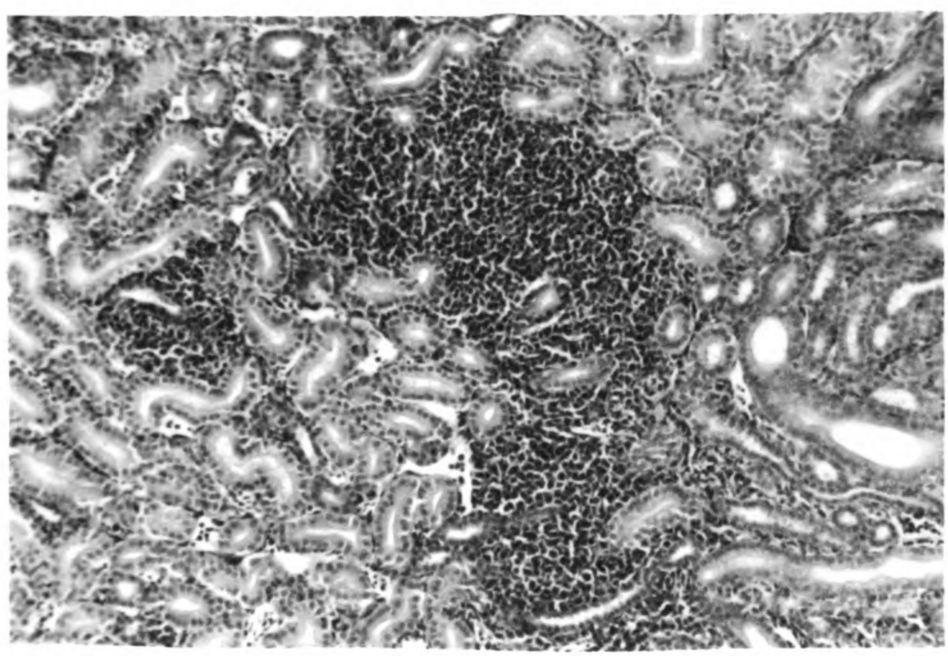

Figure 32

Figure 33. Lymphocytic foci as observed in swollen, bronze-colored livers from GA-infected birds (Day 43). H & E. x 100.

Figure 34. Higher magnification of liver lesion shown in Figure 33 (Day 43). H & E. x 250.

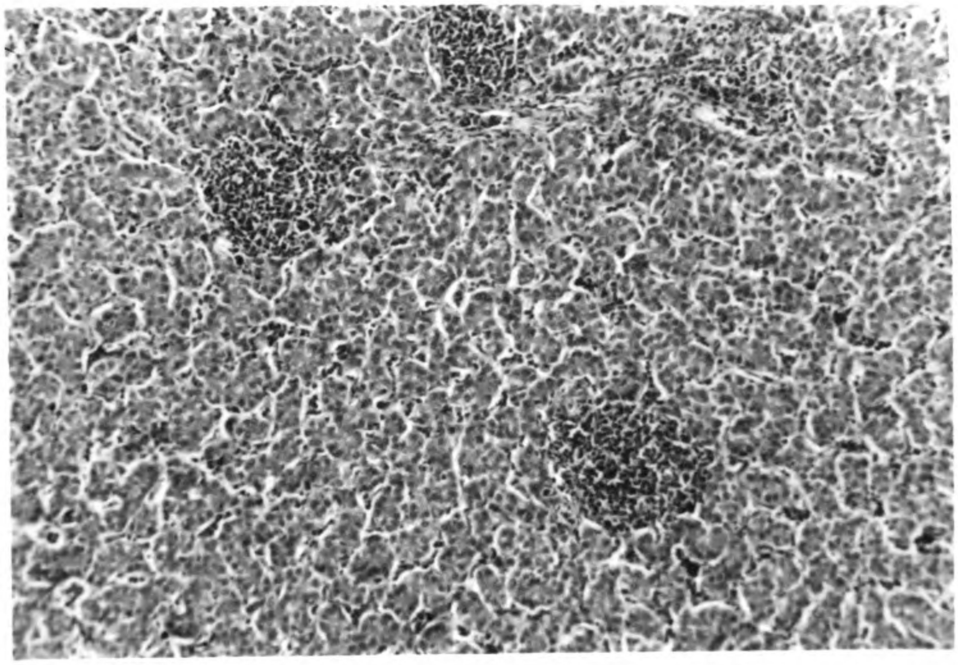

Figure 33

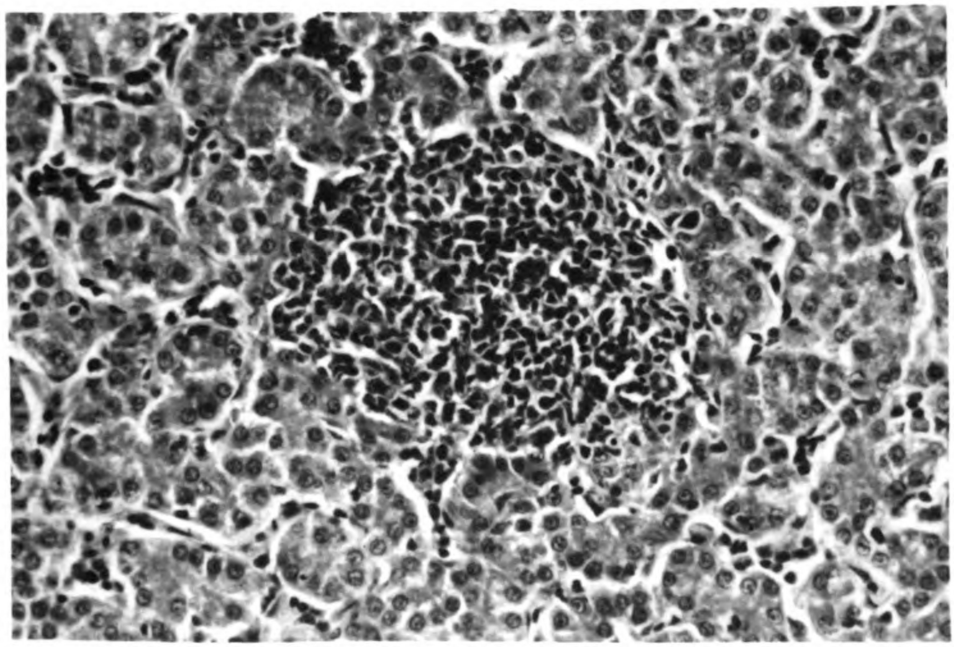

Figure 34

Figure 35. Extensive lymphocytic lesion in liver from JM-infected bird (Day 57). H & E. x 100.

Figure 36. Lesion in proventriculus from GA-infected bird (Day 36). H & E. x 250.

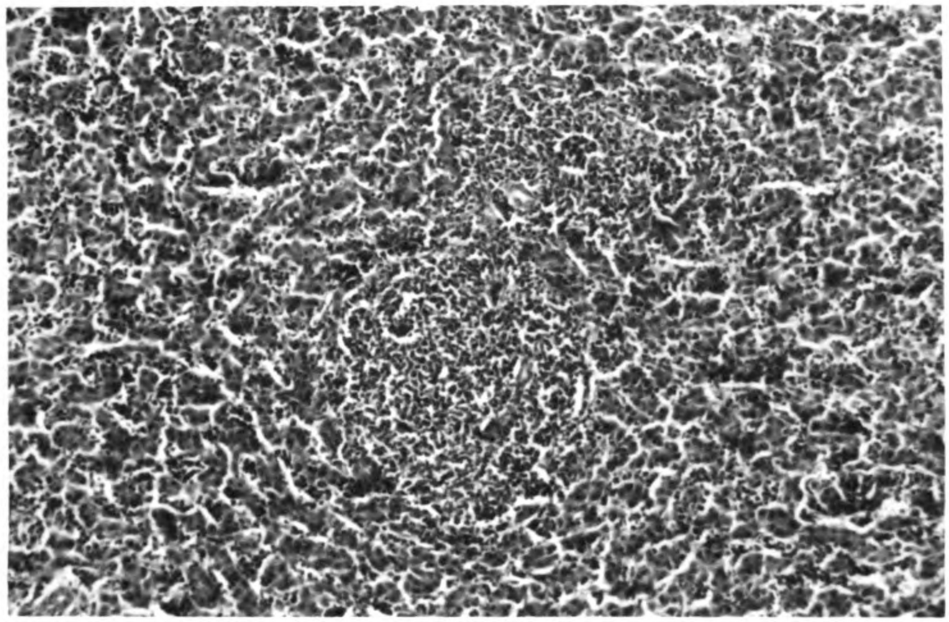

Figure 35

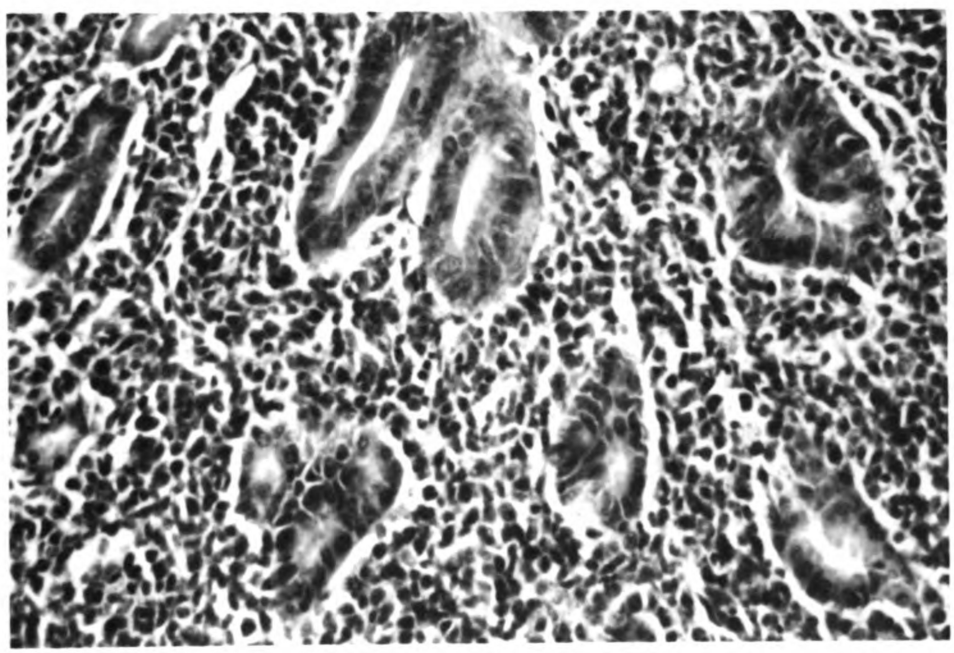

Figure 36

Figure 37. Lymphocytic lesion in lung from JM-infected bird (Day 57). H & E. x 100.

Figure 38. Lymphocytic lesion in lung from GA-infected bird (Day 36). H & E. x 100.

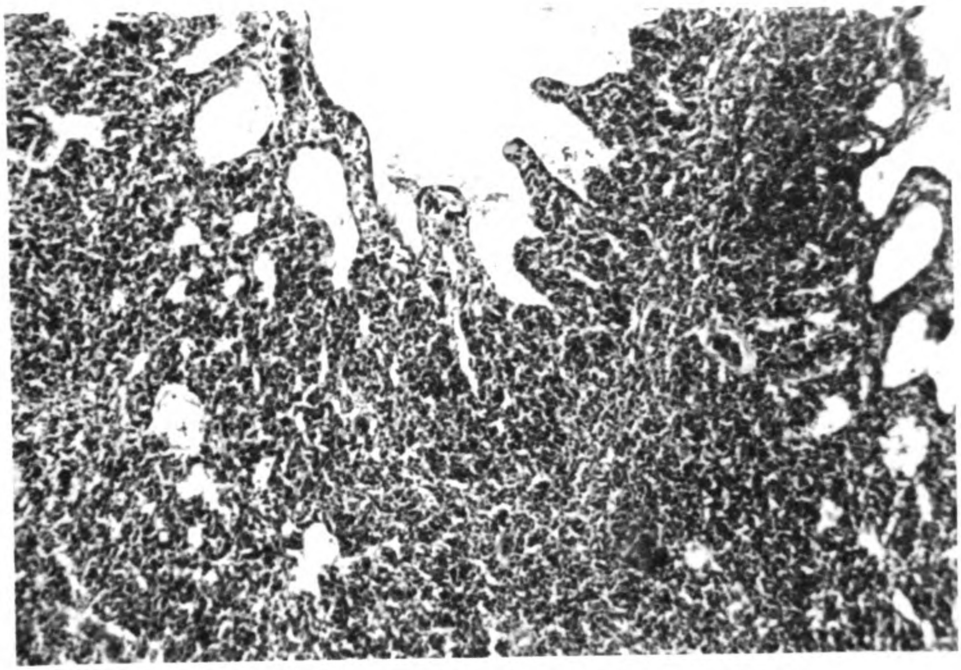

Figure 37

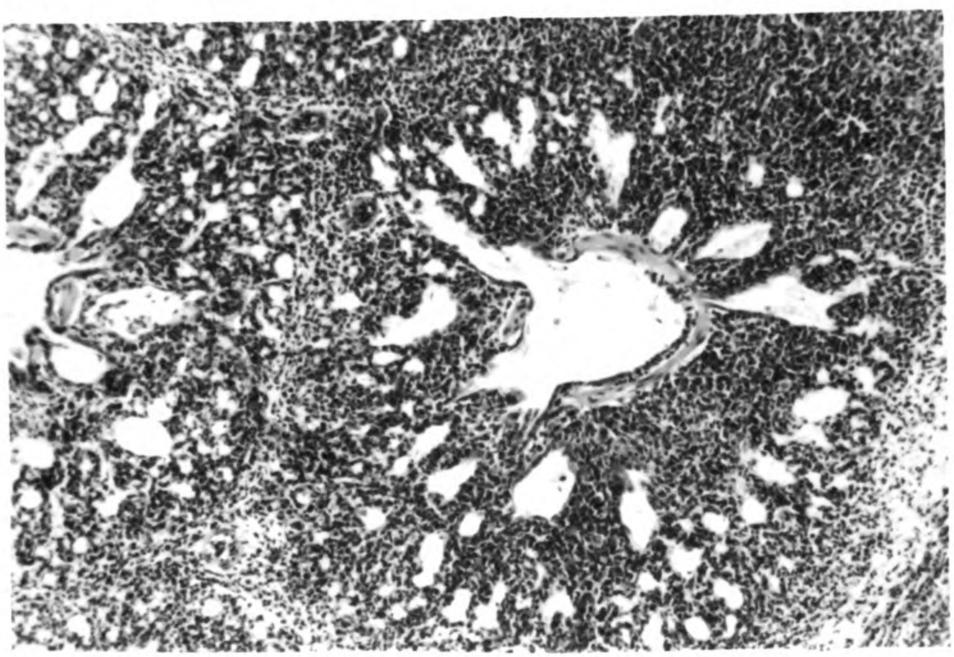

Figure 38

Figure 39. Spleen from JM-infected bird with some lymphocytic depletion (Day 32). H & E. x 100.

Figure 40. Lesion in spleen from GA-infected bird (Day 36). H & E. x 250.

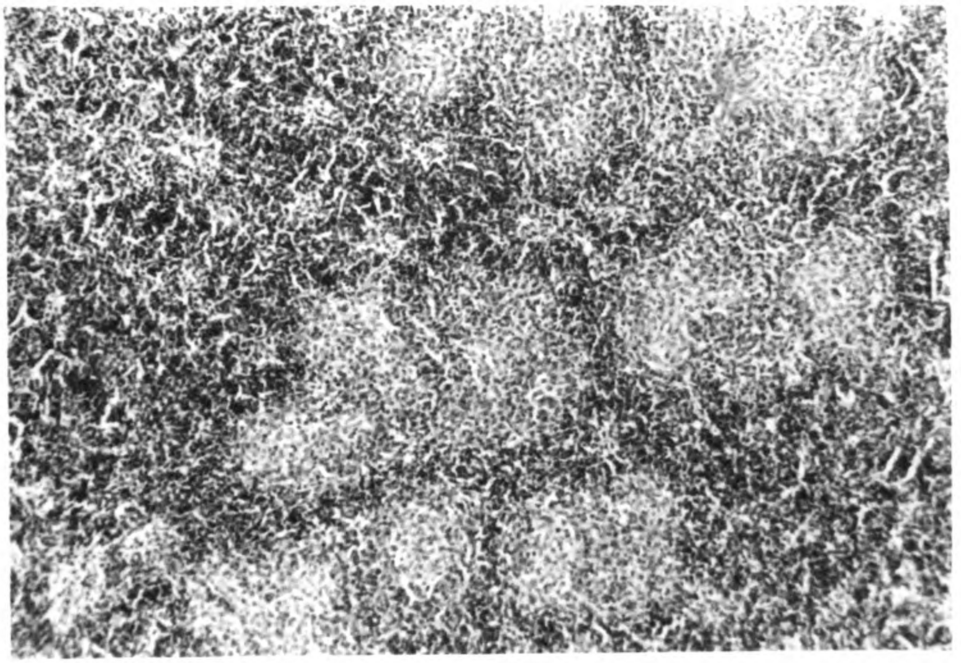

Figure 39

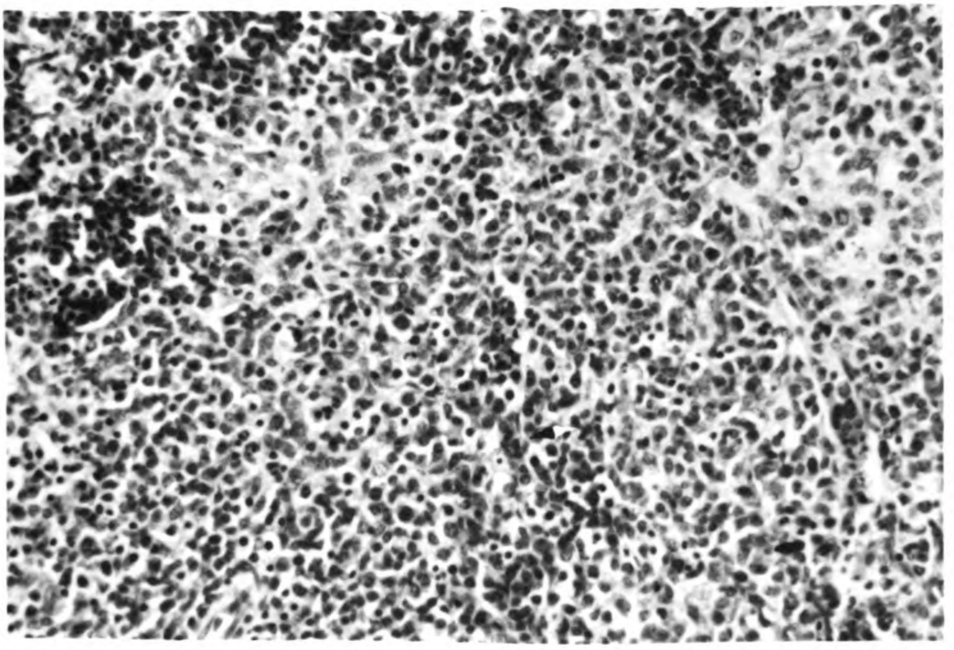

Figure 40

Figure 41. Lymphocytic infiltration and proliferation in myocardium from JM-infected bird (Day 57). H & E. x 100.

Figure 42. Lymphocytic infiltration and proliferation in myocardium from GA-infected bird (Day 36). H & E. x 250.

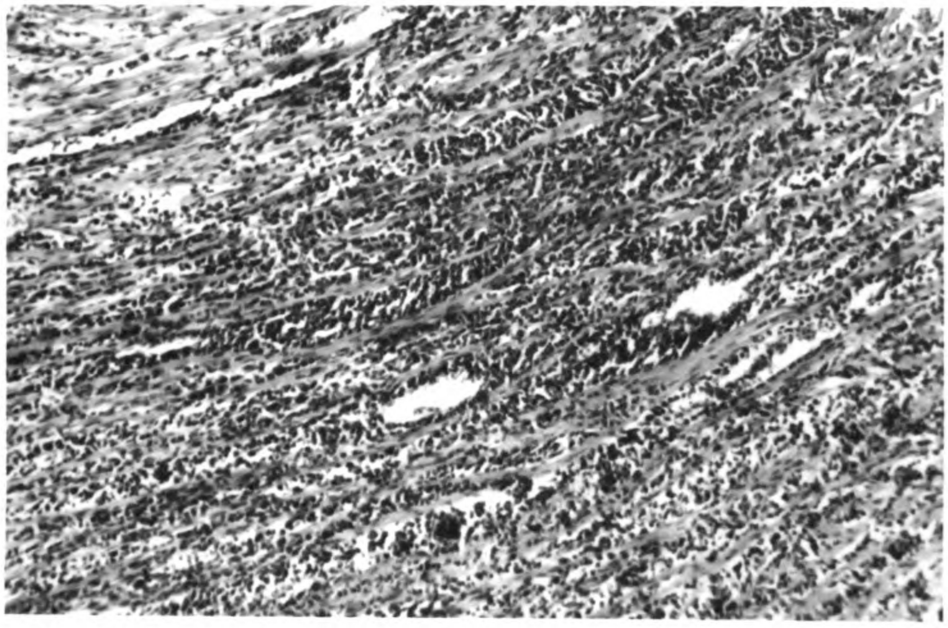

Figure 41

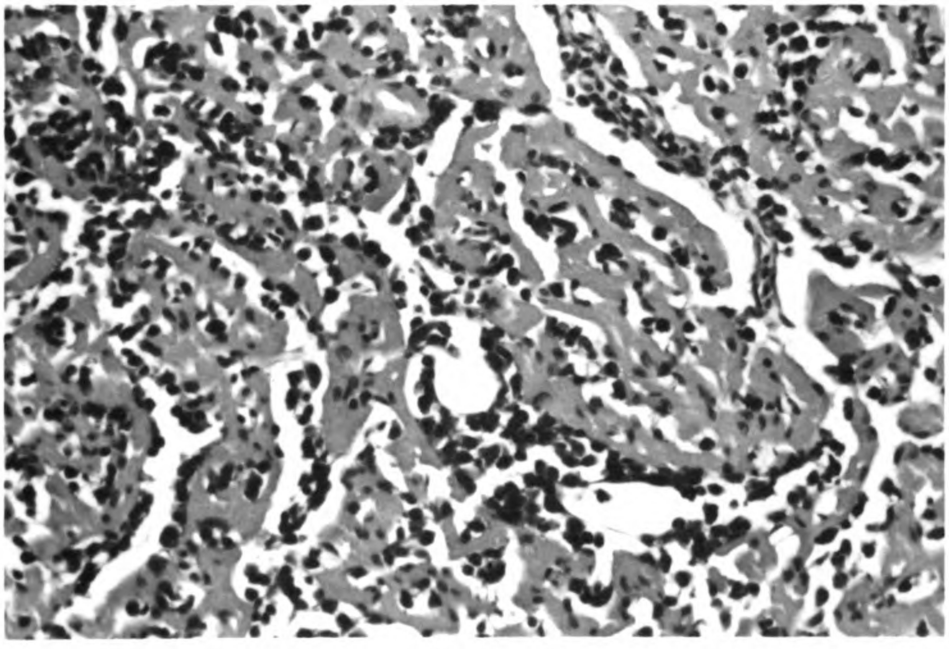

Figure 42

Figure 43. Lymphocytic lesion of intestine from GA-infected bird (Day 50). H & E. x 100.

Figure 44. Higher magnification of intestinal lesion (Day 50). H & E. x 250.

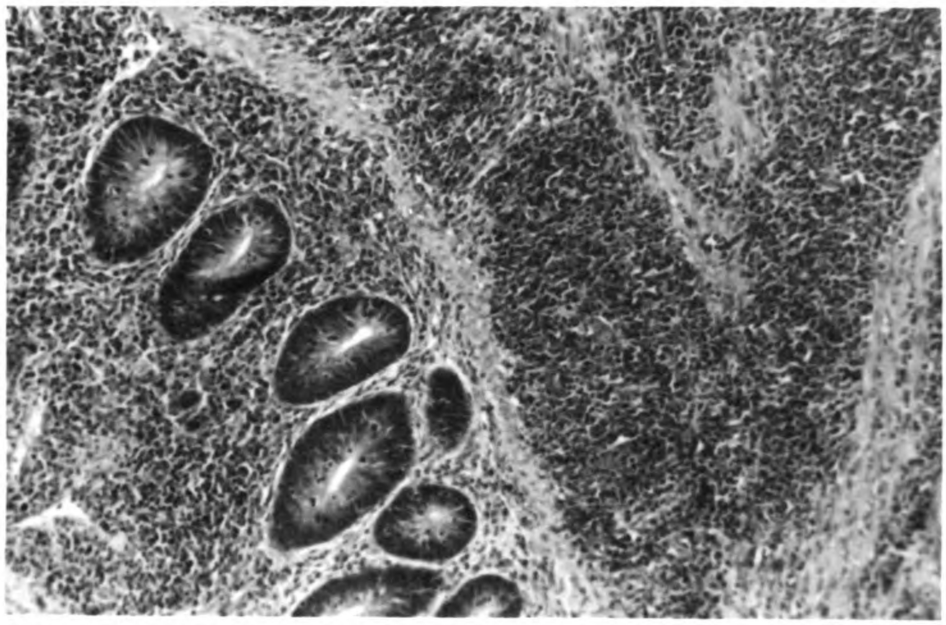

Figure 43

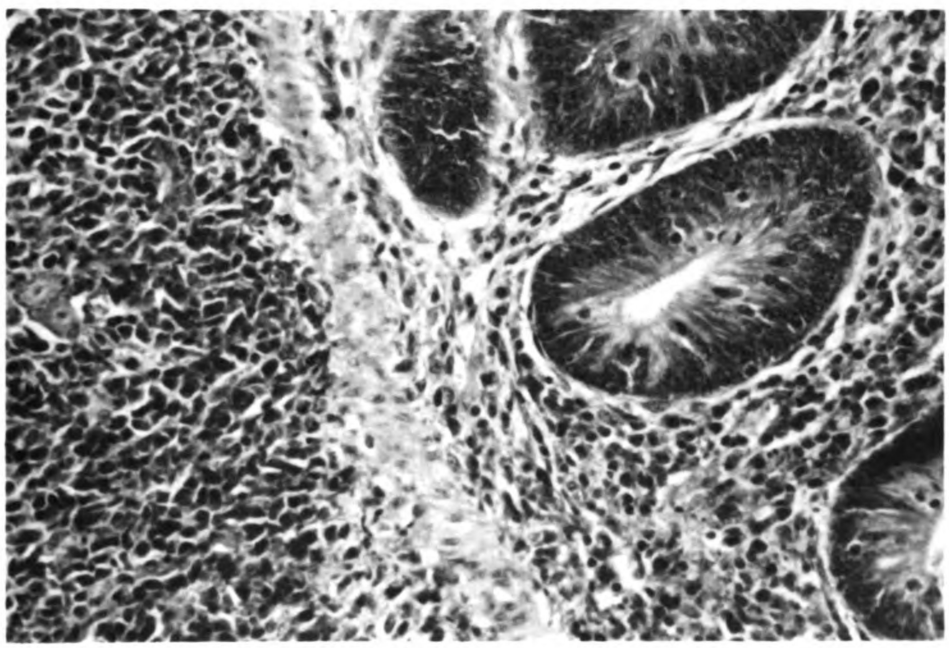

Figure 44

Figure 45. Subcutaneous lesion from cranium of GA-infected bird (Day 57). H & E. x 100.

Figure 46. Higher magnification of subcutaneous lesion (Day 57). H & E. x 250.

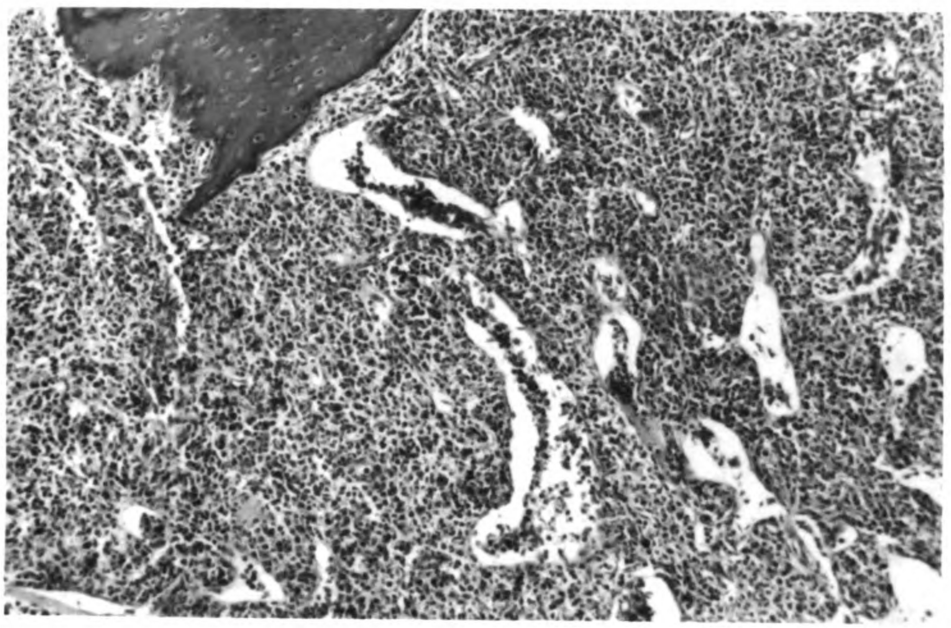

Figure 45

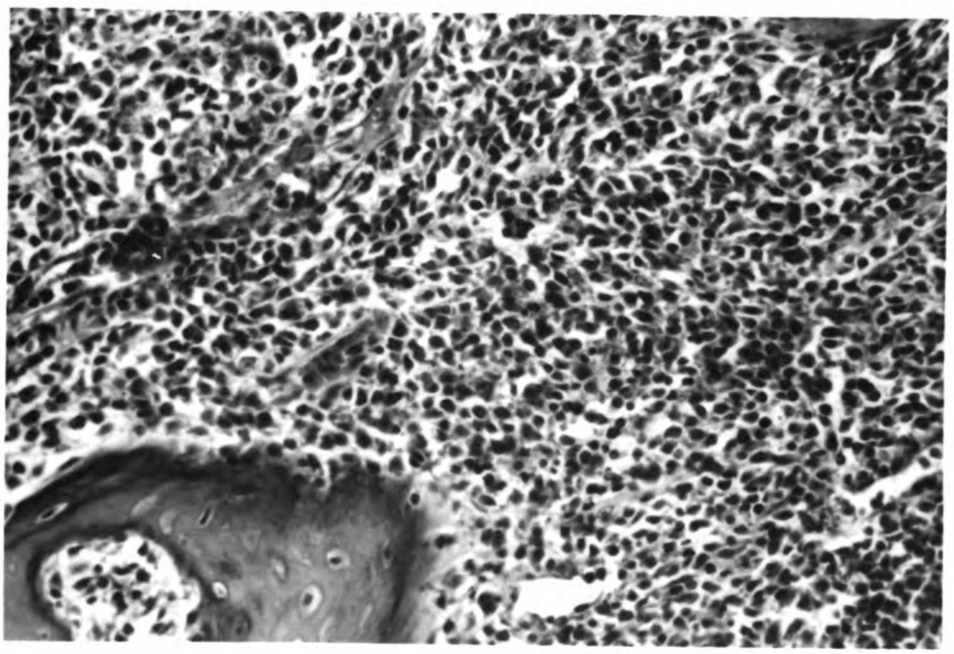

Figure 46

Table 1. Lymphocytic lesions in Line 7x7 chickens infected with JM and GA agents (number of birds with organs positive on microscopic examination)

	JM Pen (50 birds)	GA Pen (50 birds)
Nerves	42	41
Brain	39	38
Gonad	29	16
Heart	6	13
Kidney	5	14
Liver	7	16
Lung	4	14
Proventriculus	3	14
Spleen	3	16
Thymus	0	2
Bursa	0	1
Other	0	2

bodies were seen. Prominent nucleoli were observed in most cells and many mitotic figures were observed in both infected and control tissues. Average nuclear measurements are given in Table 2.

b. Methyl Green Pyronine stain (Figures 47 and 54)

The percentage of cells positive with the MGP stain for nucleic acids is given in Table 3.

D. Serologic Tests

The results of serologic tests are given in Table 4.

Figure 47. Smear preparation of spleen from GA-infected bird. Notice MGP-positive lymphoblast (1) (Day 50). MGP. x 750.

Figure 48. Smear preparation of spleen from GA-infected bird. Notice cellular detail with large nucleoli and mitotic figures (Day 50). Schorr's. x 750.

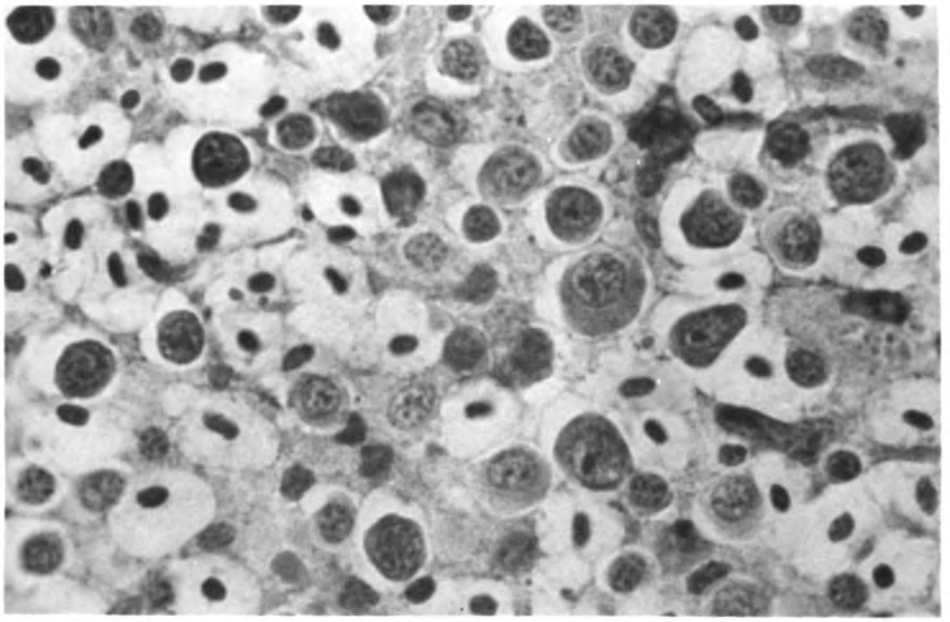

Figure 47

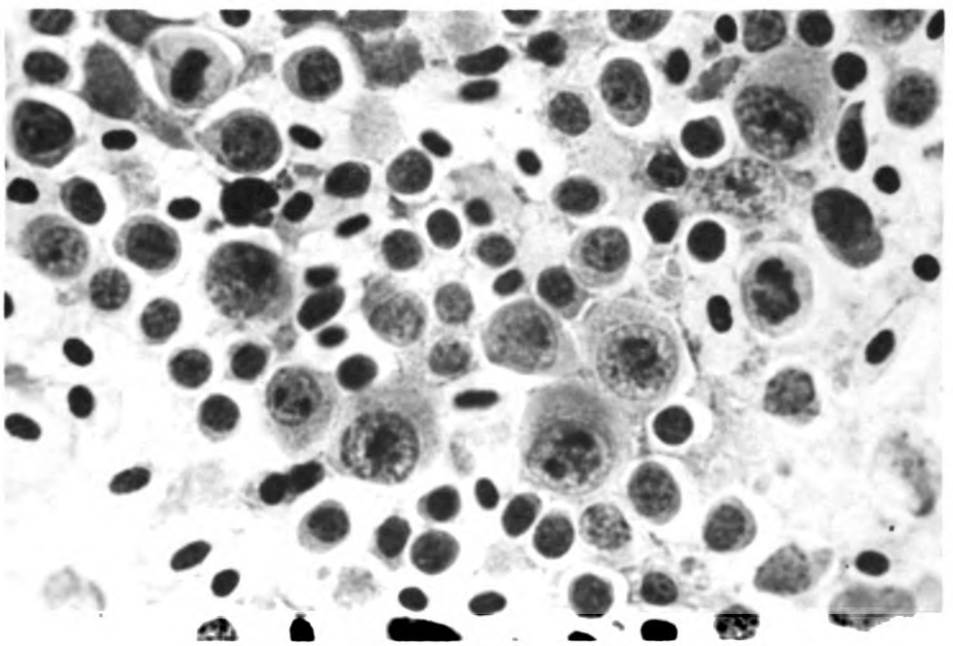

Figure 48

Figure 49. Smear preparation of spleen from JM-infected bird (Day 50). Schorr's. x 750.

Figure 50. Smear preparation of spleen from control (Day 50). Notice similarity to Figure 49. Schorr's. x 750.

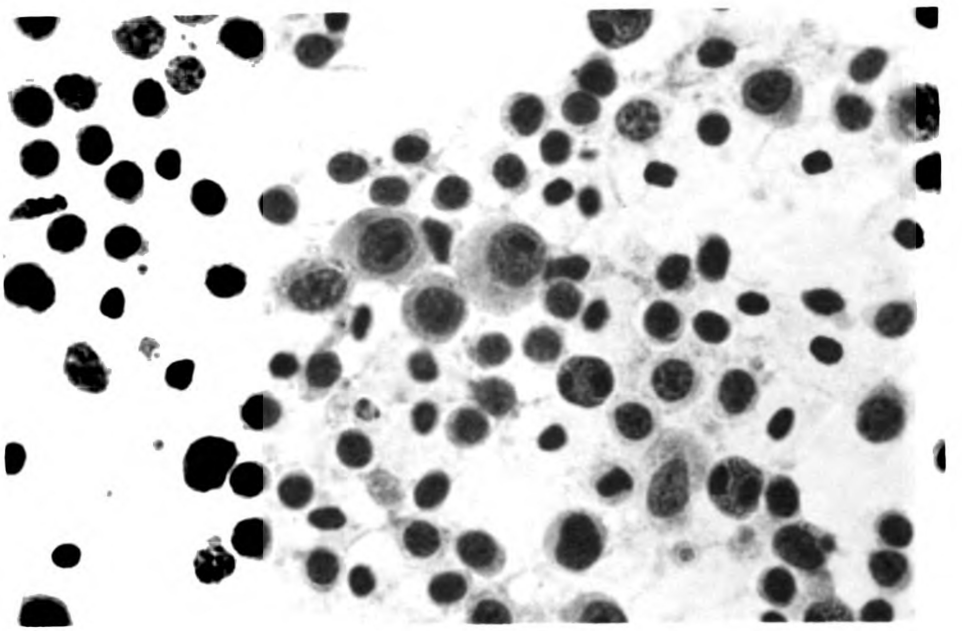

Figure 49

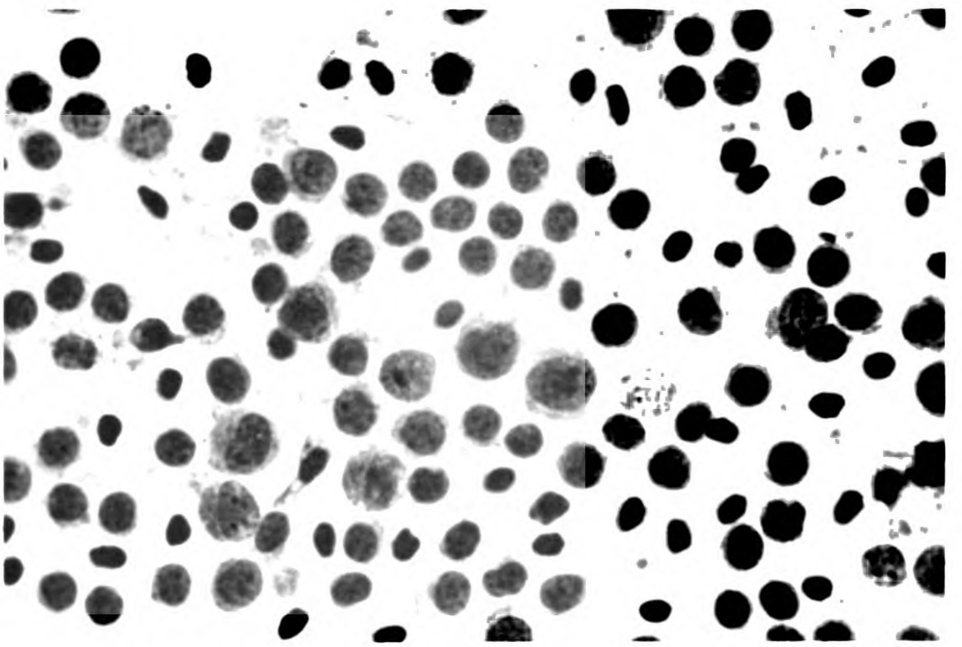

Figure 50

Figure 51. Smear preparation of bursa from GA-infected bird (Day 50). Cells cannot be differentiated from splenic lymphocytes in Figures 49 and 50. Schorr's. x 750.

Figure 52. Smear preparation of thymus from GA-infected bird (Day 50). Lymphocytes look much like those pictured in Figures 49, 50 and 51. Schorr's. x 750.

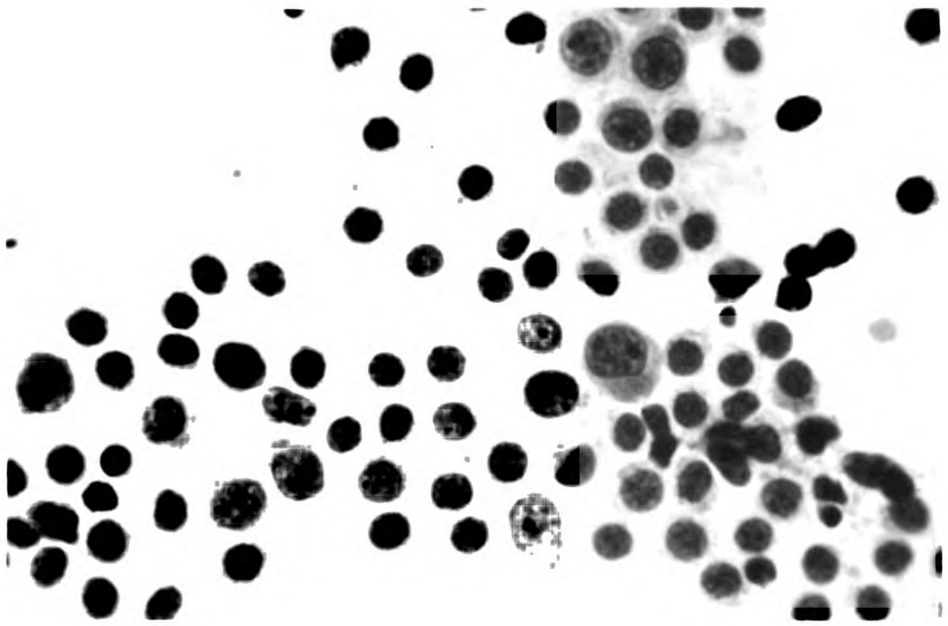

Figure 51

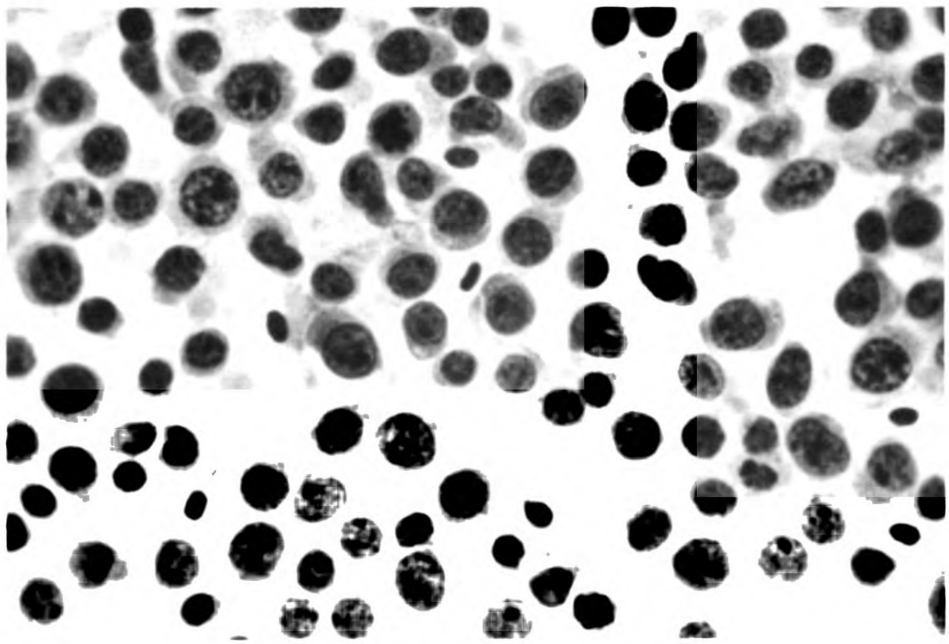

Figure 52

Figure 53. Smear preparation of kidney lesion from GA-infected bird (Day 50). Good cellular detail with many immature cells and mitotic figures. Schorr's. x 750.

Figure 54. Smear preparation of kidney lesion from GA-infected bird (Day 50). Some lymphoblasts positive to MGP stain (1,2). MGP. x 750.

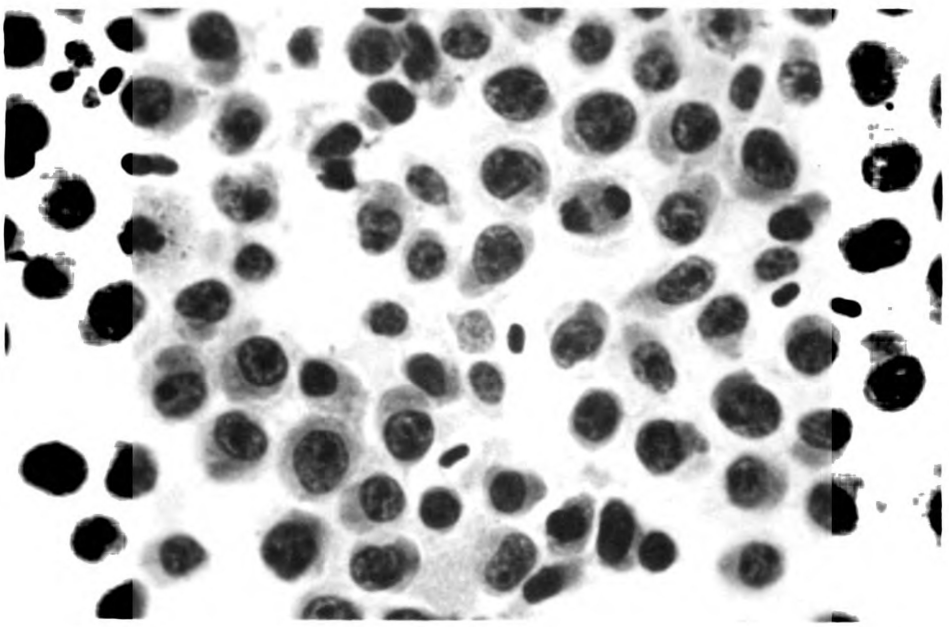

Figure 53

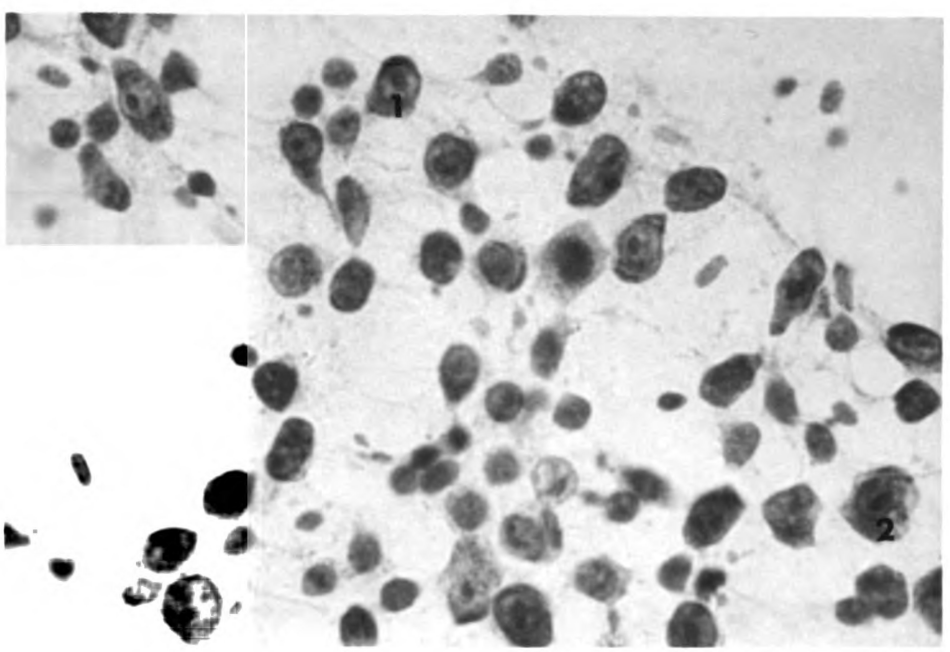

Figure 54

Table 2. Average nuclear diameter in microns of 100 cells each from JM, GA and control tissue smears

	JM	GA	Control
Spleen	5.6	6.36	5.4
Thymus	5.2	6.0	5.0
Bursa	5.2	5.0	6.5
Kidney tumor		6.2	
Subcutaneous tumor		6.0	
Gonad tumor	6.5		

Table 3. Percent cells positive for nucleic acids by Methyl Green Pyronine stain (100 cells counted on each smear)

	JM	GA	Control
Spleen	28	31	16
Thymus	21	39	16
Bursa	19	30	33
Kidney tumor		34	
Subcutaneous tumor		33	
Gonad tumor	21		

Table 4. Results of tests of sera drawn at termination from 32 survivors

	JM		GA		Control	
	+	-	+	-	+	-
1. Hemagglutination test for Newcastle	0	12	0	9	0	11
2. Neutralization test for Avian Encephalomyelitis	0	12	0	9	0	11
3. Agar gel double diffusion test for Marek's disease	12	0	9	0	0	11

DISCUSSION

A. Response to Marek's Disease Agents

Marek's disease, experimentally caused by JM or GA virus, was characterized by excessive lymphocytic infiltration and proliferation in affected tissues of young susceptible chickens. The following discussion deals with differences in response between the JM and GA lymphomatosis agents in Line 7x7 chickens as observed by clinical, gross and histopathologic examination.

1. Clinical observations

Sick birds were seen in the JM pen 9 days before any were noted in the GA group. Additionally, during the entire trial period, signs and mortality occurred, on an average, about 1 week later in GA birds than in the JM group. However, in the final summation, just as many birds became clinically ill in the GA lot as in the JM group (over 80%).

Birds in the JM group usually became lame, paralyzed and emaciated prior to death, while those in the GA pen usually became emaciated, with occasional CNS involvement and died.

Clinical differences were noted between JM and GA lots of chickens in the laboratory. However, it seems apparent that differentiation between the 2 agents in the average field case in which the infection might be mixed, and the chickens more resistant, would be very difficult.

2. Gross and histopathologic findings

Significant differences were observed between JM- and GA-infected birds at necropsy, but few histopathologic differences could be detected in affected organs from JM or GA birds.

a. Nerves

Enlargement of nerves, especially the sciatic and vagus, was almost always seen in JM infected birds, while nerves from the GA-infected lot had few gross changes. However, microscopic examination revealed an almost equal amount of nerve involvement in both lots. There were no apparent differences in arrangement of lymphocytes between JM- and GA-infected birds. The only microscopic difference between the 2 lots was the occurrence of more edema in nerves from JM-infected birds.

It seems significant from a field standpoint that Marek's disease could be diagnosed in both lots by microscopic examination but only in JM-infected birds by gross examination.

Few, if any, lymphocytes were observed in nerves collected from controls. This supports Oakberg's (1950) suggestion that the presence of these cells in the nerves of chickens is abnormal. It must be remembered that the controls in this experiment were in strict isolation and that lymphocytes do occur in the nerves of chickens under average field conditions. In this study nerve lesions were usually quite extensive. However, from a practical standpoint, any extravascular lymphocytic focus, especially if it were invasive, could be called evidence of MD.

b. Brain

No gross lesions were seen in the brain of any of the birds examined. Birds from both lots had marked microscopic vascular lesions, especially

those which died early. This type of lesion was much less marked in birds which died later in the experiment, and some regression may have occurred. However, brain and nerve lesions were found in some survivors which appeared clinically healthy and were killed at termination of the experiment. It seems significant that the occurrence of brain lesions was almost as common as peripheral nerve lesions in both lots of birds.

c. Gonad

Lesions of the gonad were much more common and usually more extensive in the JM lot than they were in the GA lot (58% and 32%, respectively). This is significant because GA-infected birds had a higher percentage of lesion incidence in all other visceral organs examined. Gonad lesions were frequently seen upon gross examination, especially in the JM infected birds.

d. Heart

Heart lesions were seen upon microscopic examination in 11%(6) of the JM infected birds and 26%(6) of the GA infected birds. These lesions were occasionally found by gross examination.

e. Kidney

Extensive lesions of the kidney were occasionally seen in GA-infected birds upon gross examination. These were not seen in JM-infected birds. However, microscopic lesions were found in a small percentage of JM-infected birds.

f. Liver

Gross examination of the liver revealed marked differences between JM-and GA-infected birds, especially in those that died early. Livers from GA-infected birds appeared swollen and were a characteristic

reddish-bronze color, but microscopic examination usually revealed only small focal lymphocytic aggregations. Livers from JM-infected birds had few gross changes, other than being somewhat shrunken and dark in color, as might be seen in any emaciated individual. A few birds from both lots which died later in the trial had gross liver lesions.

g. Lung

The percentage of microscopic lesions of the lung was much higher in GA-infected birds than in JM infected birds. A few of these lesions could be detected grossly, especially in GA-infected birds.

h. Proventriculus

Some lesions of the proventriculus were quite large, particularly in GA-infected birds. This organ became greatly dilated and impacted when there was a severe vagus nerve involvement which probably was an important factor in the death of the birds.

i. Spleen

In the spleen, as in all lymphocytic organs, identification of MD lesions may be difficult, as it is hard to say just where normal tissue stops and pathologic changes begin. In this paper lymphocytic tissues were only called positive when gross lesions were observed at necropsy, or distortion of normal architecture of the organ was seen upon microscopic examination.

Lesions of MD were observed in the spleen in 33%$^{(16)}$ of GA-infected and in 6%$^{(3)}$ of JM-infected birds. In GA infected birds which died early the spleen was often greatly enlarged, dark in color, with light-colored focal lesions. In contrast, spleens of JM-infected birds were often small and shrunken, probably because of lymphocytic depletion in that organ.

j. Thymus

No MD lesions were found in the thymus of JM-infected birds and in only 4%(2) of GA-infected birds.

k. Bursa of Fabricius

No proliferative MD lesions were found in the bursa of JM-infected birds and in only 2%(1) of GA-infected birds. This is in contrast to visceral lymphomatosis, in which proliferative lymphocytic lesions are seen in virtually 100% of affected birds.

Some follicular degeneration was observed in a small percentage of GA- and JM-infected birds. These lesions were not extensive, and did not compare with the bursal atrophy described in birds inoculated with other strains of MD virus (Biggs, Purchase, Bee and Dalton, 1965; Jakowski, Fredrickson, Luginbuhl and Helmboldt, 1969).

l. Other tissues

Intestinal, adrenal, pancreatic and subcutaneous lesions were found in a small percentage of GA-infected birds. No such lesions were found in JM-infected birds.

A subcutaneous lymphocytic lesion located dorsal and posterior to the external ear was found in a GA-infected bird necropsied at 7 weeks. It is suspected that this type of lesion occurred in GA birds which died earlier and was missed at necropsy.

m. Smear preparations

The smear preparations under discussion were from tissues of selected, comparable GA- and JM-infected birds and controls at the peak of the disease or about the 50th day. Gross lesions were observed before and after that time but were not found in both infected lots simultaneously.

While excellent nuclear and cytoplasmic detail was observed with the Schorr's stain, cells from normal and affected tissues appeared very similar and could not be differentiated.

An intensive search for inclusion bodies was made but none was found.

Measurements of average nuclear diameters of infected and normal cells indicated the former were probably somewhat larger. Infected cells appeared to be about the size of those from the bursa of control birds.

The percentage of cells positive by MGP staining for nucleic acids was somewhat higher in infected birds than in controls.

Results of smear examination indicated slight differences between different tissues, normal cells and infected cells. However, it is probable that variations exist between individuals, lines of chicken and strains of infective agent. It is apparent that much more work would need to be done before an evaluation of smear preparations as a diagnostic tool can be made.

3. Serologic tests

The results of tests for Newcastle disease and avian encephalomyelitis indicated that these diseases were not present. This would indicate that the lesions observed in the brains of infected birds were due to MD.

The results of the agar gel double diffusion test indicated that birds from the infected pens had been exposed to the MD agent and that the control birds had not.

SUMMARY

Field investigations indicate that there may be considerable variation of the disease process in different outbreaks of Marek's disease (MD). A study of this condition in a single line of chickens produced by different strains of virus was intended to provide some insight into clinical, gross and histopathologic differences that have been seen in infected flocks.

Pathogenesis of MD in Line 7x7 chickens, caused by exposure to JM and GA viral agents, was described, as revealed by clinical, gross necropsy and histopathologic examinations. An attempt was made to describe typical, well developed lesions in the average case, rather than trying to enumerate all variations in response.

Two lots of 50 day-old Line 7x7 chicks were exposed by pen contact to older chickens that were shedding either JM or GA MD virus. Another lot of 50 chicks were held in a separate building to be used as controls. When experimental birds sickened, an attempt was made to select moribund individuals from each pen so that comparative variations in response could be made. Randomly selected control birds were taken at the same time.

Differences in clinical response were observed. Sections of the brain and 3 nerves, the brachial, vagus and sciatic, plus bursa, gonad, heart, kidney, liver, lung, proventriculus, spleen and thymus, were taken from experimental and control birds for microscopic examination. Wet smears were prepared from selected gross visceral lesions and lymphocytic organs.

The average nuclear diameter and percentage of immature cells on smears prepared from tissues of typical infected and control birds were recorded. Comparative histopathologic changes in Line 7x7 chickens infected with JM or GA MD virus were described.

The first illness due to MD was seen in the JM pen on Day 19 and the first death occurred on Day 28. Similar responses in the GA pen occurred about a week later. This seemed to be true for the entire trial with the GA lot becoming ill slightly later than the JM birds. However, about the same percentage of birds from each lot was eventually affected. Sick birds became emaciated. Considerably more JM-infected birds became paralyzed than did GA-infected birds.

Necropsy revealed markedly enlarged nerves in most JM-infected birds. This response was not usual in birds from the GA pen. However, gross visceral lesions, especially of the liver and spleen, occurred most frequently in GA-infected birds. Other lesions, such as those of the intestine, kidney and subcutis, were observed in the GA lot but were not seen in the JM group.

Histopathologic examination of the brain, nerves and viscera revealed marked lesions in both lots of birds. Few differences in cellular response, other than incidence of lymphocytic aggregations in particular organs, could be detected. Both lots had about the same percentage of brain and nerve lesions. The gonad was most commonly affected in JM-infected birds, while other visceral organs were most commonly involved in the GA-infected group.

Excellent cellular detail was seen in the smear preparations. It appeared that lymphocytes from MD were somewhat larger and more immature than those of normal tissues. However, these changes were not prominent enough to differentiate normal lymphocytes from those seen in MD.

These results indicate that there were certain differences produced in Line 7x7 chickens by the 2 strains of virus. These consisted mainly of the clinical and necropsy variations. Microscopic examination revealed few histopathologic differences. Cells from MD lesions seemed somewhat larger and more immature than normal cells. However, large, immature cells also occurred in normal tissues. For this reason MD cells could not be positively identified.

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