



Title	INITIAL LESIONS IN CHICKENS INFECTED WITH JM STRAIN OF MAREK'S DISEASE VIRUS
Author(s)	FUJIMOTO, Yutaka; OKADA, Kosuke; KAKIHATA, Koji; MATSUI, Takane; NARITA, Minoru; ONUMA, Misao; MIKAMI, Takeshi
Citation	Japanese Journal of Veterinary Research, 22(3), 80-94
Issue Date	1974-07
DOI	10.14943/jjvr.22.3.80
Doc URL	http://hdl.handle.net/2115/2045
Type	bulletin (article)
File Information	KJ00002371160.pdf



[Instructions for use](#)

INITIAL LESIONS IN CHICKENS INFECTED WITH JM STRAIN OF MAREK'S DISEASE VIRUS*¹

Yutaka FUJIMOTO, Kōsuke OKADA, Kōji KAKIHATA, Takane MATSUI,
Minoru NARITA*², Misao ONUMA*^{3,4} and Takeshi MIKAMI*^{3,5}

*Department of Comparative Pathology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo, Japan*

(Received for publication, February 26, 1974)

Severe lymphoid degeneration and hyperplasia of the reticular and epithelial components were present in the lymphoid tissues on the 6~8th day after inoculation of JM strain of Marek's disease virus (MDV). At the same time, intranuclear and cytoplasmic inclusions with many virus particles were frequently found in these components of the lymphoid tissues (the bursa of Fabricius and thymus). Inclusions were also observed in the epithelial cells of the pancreas, adrenal glands, kidneys and feather follicles. Two types of enveloped virus particles were found in adrenal cells and the feather follicle epithelium (FFE). Inoculated chickens were free from precipitating antibodies to MDV through a 48 day observation period. Immunofluorescent (IF) antigen was first detected in the thymus and lungs (8th day). MDV was demonstrated to exist by kidney cell cultures from the 8th day postinoculation. Microscopic lesions characteristic for MD were seen on and after the 12th day postinoculation.

In contact-exposed chickens, there were no remarkable changes in the lymphoid tissues at the early stage of the disease. Thereafter inclusions could not be found in any tissues, except the skin and lungs. Precipitating antibodies to MDV were detected from the 60th day postexposure. IF antigen was detected in the thymus and FFE (23rd day). MDV was demonstrated to exist and microscopic lesions were seen on and after the 32nd day postexposure.

INTRODUCTION

Marek's disease (MD) of the fowl is an infectious disease which is caused by a highly cell-associated herpesvirus^{9,26,41)} and is characterized by the proliferation of lymphoreticular cells in the neural, ocular and cutaneous tissues, and in the visceral organs^{2,13,19)}.

*¹ Supported in part by a grant from the Ministry of the Agriculture and Forestry, Japan

*² National Institute of Animal Health, Hokkaido Branch, Sapporo, Japan

*³ Department of Microbiology, Sapporo Medical College, Sapporo, Japan

*⁴ Present address: Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin, 53706, U. S. A.

*⁵ Present address: Institut für Geflügelkrankheiten, Tierärztliche Hochschule Hannover, 3 Hannover, West Germany

By extensive electron microscopic studies and immunofluorescent (IF) antibody techniques, virus particles^{4,6,12,25,33,37,44)} or specific IF antigen^{3,5,6,20,33,42)} were found in various tissues of infected birds. Although virus particles were more frequently found in the feather follicle epithelium (FFE) of the skin^{6,25,27)}, the particles were less frequently observed in the other tissues^{6,25,37,44)}. Especially in the FFE of the skin, intranuclear^{5,6,20,27,33)} and cytoplasmic inclusions^{25,27,43)}, and infectious enveloped virions^{4,6,27,43)} were demonstrated to exist.

The purpose of the present study was mainly to examine the initial lesions of chickens inoculated with MD virus (MDV) and those of chickens infected by contact-exposure. We also investigated the presence of herpesvirus particles, virus-associated antigen and intranuclear and cytoplasmic inclusions in the various tissues of these chickens.

MATERIALS AND METHODS

Source of the virus Infectious blood from birds inoculated with JM strain of MDV was used as the source of the virus. Donor birds showed clinical signs and histopathological lesions of MD. The source of the JM strain of MD was described previously²⁹⁾.

Chickens Chicks were obtained from a resistance-inducing-factor free and specific-pathogen free flock of White Leghorn (Line M), Nippon Institute for Biological Science, Tokyo. Twenty one-day-old chicks (Case Nos. 1~20) were inoculated intra-abdominally with 0.2 ml of the infected blood (titer; 80 p.f.u./0.1 ml, 2nd passage in Line M chicken) (Inoculated group). Twenty chicks (Case Nos. 21~40) of the same hatch were placed in the same room as the inoculated birds at one-day-old (Contact-exposed group). Twenty chicks (Case Nos. 41~60) were housed in another building in an isolated condition (Control group). The chicks used in the present experiment were all free from maternal antibody to MDV. Chicks were then killed chronologically for histopathological, virological and serological examinations, except dead chickens (tabs. 1 & 2).

Agar gel precipitation The agar gel precipitation (AGP) test was performed as previously described²²⁾. For determination of the presence of AGP antigen in individual chickens, the feather tips were removed at necropsy from chickens of each group for antigen preparations. A small number (10~20) of feather tips was suspended into 0.1~0.2 ml of phosphate buffered saline (PBS) (pH 7.2), disrupted by a glass homogenizer and then the mixture was used as antigen. For detection of MDV-specific antibody in individual chickens, sera were collected at necropsy from inoculated and uninoculated chickens.

Virus isolation Virus isolation was performed by direct kidney culture assay. Kidney tissues were aseptically removed at necropsy from individual

TABLE 1 Summary of virological, immunological and microscopical findings in inoculated group³

CASE NO.	DAYS POST-INOCULATION	IF STAINING ¹				VIRUS ISOLATION ¹	AGP-TEST ¹		INCLUSION ¹		MICROSCOPIC LESIONS ²	
		Skin	Bursa	Thymus	Others		Antigen	Antibody	Skin	Others	Visceral Organs	Peripheral nerves
1	1	-	-	-	-	.	.	.	-	-	-	-
2	3	-	-	-	-	-	-	-	-	-	-	-
3	6*	-	+T	-	-
4	8*	-	+T#, B, P	-	-
5	8	-	-	+	+L	+	-	-	-	+T#, B	+	-
6	12	-	-	-	+K	+	-	-	+	-	+	+
7	13*	-	+	-	-	.	.	.	-	-	-	-
8	14	-	.	-	-	.	.	.	+	-	-	-
9	16	+	+	-	-	+	+	-	+	-	#	#
10	19	+	+	+	-	.	.	.	+#	-	+	+
11	20*	+	-	-	-
12	20*	+	+B	-	+
13	20*	+	-	-	+
14	23	+	+	+	+O	+	+	-	+	+B#	#	#
15	24*	+	+	+	+L	.	.	.	+	+B, A#	+	+
16	28*	+	+B, A, K	+	+
17	29	+	+	-	-	.	.	.	+	+B#	#	#
18	32	+	+	+	-	+	+	-	+	-	#	#
19	42	+	+	+	-	.	.	.	+	-	#	#
20	48	+	+	-	-	+	+	-	+	-	#	#

- 1 Results of IF staining, virus isolation and AGP test and findings of inclusion body in skin or other tissues were expressed by +: positive or -: negative and .: not done
- 2 Severity of microscopic lesions: # to - reactions denote degrees of lymphoreticular cell proliferation or infiltration in tissues, ranging from severe (#) to mild (+) proliferations, - = negative reaction
- 3 * = dead case L = lung K = kidney T = thymus O = ovary B = bursa of Fabricius A = adrenal gland P = pancreas # = presence of virus particles by electron microscopy

TABLE 2 *Summary of virological, immunological and microscopical findings in contact-exposed group³*

CASE NO.	DAYS POST-EXPOSURE	IF STAINING ¹				VIRUS ISOLATION ¹	AGP-TEST ¹		INCLUSION		MICROSCOPIC LESIONS ²	
		Skin	Bursa	Thymus	Others		Antigen	Antibody	Skin	Others	Visceral organs	Peripheral nerves
21	1	•	•	•	•	•	•	•	-	-	-	-
22	3	-	-	-	-	-	-	-	-	-	-	-
23	8	-	-	-	-	-	-	-	-	-	-	-
24	12	-	-	-	-	-	-	-	-	-	-	-
25	16	-	-	-	-	-	-	-	-	-	-	-
26	23	+	-	+	-	-	+	-	-	-	-	-
27	32	+	-	-	-	+	+	-	-	-	+	+
28	48	+	-	-	-	+	+	-	+	-	+	+
29	60	-	-	•	+Br	•	+	+	+	-	-	#
30	62*	+	-	•	+O	•	+	•	+	-	+	+
31	95	+	+	•	+L	+	+	+	-	-	+	+
32	95	+	-	-	-	+	+	+	+	-	#	#
33	98	+	-	-	-	•	+	+	+	-	#	+
34	116	+	-	-	-	•	+	+	-	+L	#	+
35	118*	•	•	•	•	•	+	•	-	-	#	+
36	126*	+	+	-	-	•	+	+	-	-	+	+
37	156	+	-	-	-	+	+	+	-	-	+	#
38	156	+	-	+	-	+	+	+	+	-	-	+
39	163	•	•	•	•	•	•	•	+	-	+	+
40	163	•	•	•	•	•	•	•	-	-	+	-

1 Results of IF staining, virus isolation and AGP test and findings of inclusion body in skin or other tissues were expressed by +: positive, -: negative and •: not done

2 Severity of microscopic lesions: # to - reactions denote degrees of lymphoreticular cell proliferation or infiltration in tissues, ranging from severe (#) to mild (+) proliferations, - = negative reaction

3 * = dead case O = ovary L = lung Br = bronchia

Control chickens (Case Nos. 41~60) showed all negative results of virological, immunological and microscopical findings.

chickens and prepared for cultures as described previously²¹). Eagle's minimal essential medium supplemented by 10% tryptose phosphate broth and 5% calf serum was used for the growth medium. The same medium, except containing 1% calf serum, was used for the maintenance medium. Kidney culture were maintained for 10 to 14 days in order to examine them for appearance of MDV-specific microplaques. The criterion for virus isolation is based on the presence of microplaques in the cultures²¹).

Microscopy Tissues from all chickens fixed in formalin were processed through paraffin and stained with hematoxylin and eosin (H-E). Specimens of bursa, thymus, lung, peripheral nerves (cervical spinal ganglia) and skin for electron microscopy were fixed in glutaraldehyde, washed in buffer, and post-fixed in OsO₄²⁹). Sometimes, specimens which were taken from the paraffin blocks corresponding to the lesions of cytoplasmic and intranuclear inclusions seen in the H-E sections, were removed from the paraffin, re-fixed in 1% OsO₄ (1 hour), and then rinsed in buffer for electron microscopical examination. After dehydration through ethanols, these specimens were embedded in epoxy resin (Epon 812). Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a JEM-7 electron microscope.

Immunofluorescence The tissues which were examined by IF microscopy were from the skin, bursa, thymus, gonad, kidney and lung. Excised tissues were immediately frozen in a tube containing n-hexane immersed in a dry ice-ethanol bath. Six-micron thick sections were cut in a cryostat and mounted on the slides. After being air dried and fixed for 15 minutes in chilled acetone, the sections were rinsed in PBS and stained with fluorescein isothiocyanate-labeled antiserum to MDV²⁴) for 1 hour at 37°C or for over night at 4°C in a moist chamber. After the sections were rinsed with PBS and mounted in a buffered glycerol medium, they were examined by a Nikkon fluorescent microscope FT type with UV-exciter filters.

RESULTS

Inoculated group

As shown in table 1, all together 8 chickens died and other 12 chickens were chronologically killed, during 48 days of observation period.

MDV was recovered from chickens after the 8th day postinoculation (PI). Precipitating antigen was detected on and after the 16th day PI, but precipitating antibody was not found at any time throughout observation period. Specific IF antigen was found for the first time in the thymus and lung on the 8th day PI and thereafter found in the kidney, the bursa of Fabricius, the FFE (fig. 1) and other organs (tab. 1).

Microscopic lesions As initial lesions, severe lymphoid degeneration and necrosis were observed in the lymphoid tissues, preceding the appearance of proliferation of lymphoreticular cells, characteristic for MD, in the tissues of chickens inoculated with MDV.

Acute thymic involution was noted on the 6~8th day PI. The first detectable cellular changes in the thymus consisted of depletion and pyknosis of individual lymphocytes. Focal areas of necrosis were frequently observed. The thymic parenchyma was replaced by proliferated reticulum cells, vacuolated macrophages and multinucleate syncytia. In these reticulum cells and multinucleate syncytia, basophilic intranuclear inclusions were frequently observed, but granular acidophilic cytoplasmic inclusions were only occasionally seen (fig. 2). The thymuses in most cases of aged chickens showed involuted changes, but those in some cases showed regenerative changes.

Destruction of the medullary areas of the bursal follicles and appearance of inclusions were prominent on the 6~8th day PI. In general, the medullary areas of the bursal follicles often showed necrosis and depletion of lymphocytes, and this was accompanied by heterophil reaction. Reticulum cell and epithelial cell hyperplasia was noted following lymphocyte necrosis. Large pale vacuolated phagocytes (lipid laden foamy cells) developed at the same time, and contained remnants of pyknotic lymphocyte nuclei and debris. Then large or small cysts developed in the bursal follicles. These cysts were often lined by columnar or pseudo-stratified epithelial cells and occasionally contained many globules of mucin. Proliferation of the bursal epithelial layer had produced a glandular structure. Many of these glandular or cystic structures appeared to re-establish continuity with the lumen of the bursa and eventually to disappear. Proliferated reticulum cells and epithelial cells in the medullary areas of the follicles often had basophilic intranuclear inclusions and occasionally had granular acidophilic inclusions (fig. 3). Degeneration and necrosis of the bursal follicles were generally severe in the inoculated group, but in only one case (28th day), was remarkable lymphoreticular cell proliferation observed in the interfollicular connective tissues.

In the spleen and cecal tonsils on the 8th day PI, generalized depletion of lymphocytes was associated with marked phagocytosis of lymphocyte debris by large pale vacuolated macrophages.

In the pancreas on the 8th day PI, glandular epithelial cells underwent degeneration, and these pale, loose degenerated areas were replaced by macrophages. These glandular epithelial cells often had intranuclear (fig. 4) and cytoplasmic inclusions.

In the adrenal glands on the 24 and 28th day PI, regressive changes and

focal lymphoid cell proliferation were observed at the same time. Adrenal glandular epithelial cells and multinucleate syncytia in these areas had both intranuclear and cytoplasmic inclusions (fig. 5).

In the kidneys on the 28th day PI, basophilic intranuclear inclusions were located in the renal epithelial cells independently of focal lymphoid cell proliferation. Lymphoid tumors were found on the 16th day PI.

In the FFE of the skin on the 12th, and on and after the 14th day PI, intranuclear (fig. 6) and cytoplasmic inclusions were constantly observed (tab. 1).

In the peripheral nerves on the 12th day PI, slight infiltration of lymphoreticular cells appeared. Thereafter, apparent tumorous lymphoid lesions were found in 6 cases (fig. 7). Eventually accumulation of lymphoreticular cells in the peripheral nerves appeared in 12 out of 20 cases. In the central nervous system on the 8th day PI, perivascular cuffing of lymphoid cells initially appeared and eventually cuffing was found in 9 out of 20 cases. In the visceral organs, lymphoid tumors were found in 6 cases corresponded with tumorous lymphoid lesions in the peripheral nerves. Lymphoid tumors were also found in the heart, ovary, lungs (fig. 8), thyroid glands and intestines. In the liver, there were no macroscopical tumor nodules. However, microscopically, focal accumulations of lymphoid cells in the liver lobules and intimagranulomatous proliferation in the veins of the interlobular septa were observed.

From these results, it seems that severe destruction of the lymphoid tissues occurred in the early stages of the disease and proliferation of lymphoreticular cells appeared in later.

Electron microscopic findings Large amorphous accumulations of electron opaque granular materials were observed in some of the nuclei with the intranuclear inclusions seen in H-E sections. Most of the nuclei were accompanied by margination of nuclear chromatin. Numerous naked particles of herpesvirus-type in various developmental stages and small nuclear particles (ca. $30\text{ m}\mu$) were seen in the nuclei (figs. 9~11, 13~15). Two morphologically distinct inclusions which appeared to correspond to the cytoplasmic inclusions described above in size, shape and intracellular location were seen. The inclusions of one type consisted of aggregates of electron opaque materials in the cytoplasm and contained a large number of virus particles (fig. 12). The inclusions of the other type consisted of electron opaque granules, in various sizes, surrounded by a limiting membrane and some enveloped particles and many of them were degenerated (fig. 13). The inclusions in the latter appeared to be lysosomes or phagosomes. Virus particles of herpesvirus-type were found in the FFE of the skin (figs. 14 & 15), the reticulum cells and multinucleate syncytia in both the bursa of Fabricius (fig. 9) and the thymus (fig. 13), and adrenal epithelial cells (figs. 10~12) in 6

examined cases. Most of the virus particles consisted of unenveloped particles, although some were enveloped particles. Two different types of enveloped particles could be seen. Enveloped particles of the one type were found in the intranuclear vesicles and they measured ca. 140~170 m μ in diameter. Enveloped particles of the other type were found in the cytoplasm (fig. 12) or extracellular spaces in the adrenal glands and the FFE and they measured ca. 190~230 m μ in diameter. They were coated by electron opaque materials and had numerous spikes on the outer surface of the virions. Other rare features in the nucleus were the presence of filamentous or tubular structures (long microtubules) (fig. 10) and "tyre-track" structures (fig. 11).

Contact-exposed group

Except for 3 chickens, 17 chickens from the 1st to 163rd day postexposure (PE) were chronologically killed and examined (tab. 2). MDV was recovered from chickens after the 32nd day PE. Precipitating antigen was detected on and after the 23rd day PE, but precipitating antibody was found late on and after the 60th day PE. IF antigen was detected in the thymus and FFE for the first time on the 23rd day PE and it appeared constantly in the FFE on and after the 32nd day PE, except on the 60th day PE. IF antigen was found in the bronchi, ovary, bursa of Fabricius, lung and thymus (tab. 2).

Microscopic lesions The bursa of Fabricius and the thymus showed no remarkable changes at least until the 31st day PE. On and after the 32nd day PE, although extent of the lesions of follicles varied, atrophy and disappearance of the bursal follicles due to lymphoid degeneration were seen. But the lesions themselves were not so severe as those seen in the inoculated group. Marked bursal atrophy was found in 5 cases, in comparison with those of the control group. Lymphoreticular cell proliferation in the interfollicular connective tissues (fig. 16) was prominent in 4 cases. Severe thymic involution was observed on the 116th day PE; the cortex showed considerable decrease in thickness or was replaced by the medulla. Distribution of atypical epithelial cell groups and myoid cells became very prominent. Intranuclear and cytoplasmic inclusions were found in the FFE of the skin with high frequency on and after the 48th day PE (tab. 2). Intranuclear inclusions were also found in reticulum cells of the lungs on the 116th day PE. No evidence of inclusions could be seen in the other organs and tissues.

Focal accumulations characterized by lymphoreticular cell proliferation, were found in the peripheral nerves on the 32nd day PE. But thereafter most of the lesions found in the peripheral nerves consisted of infiltration of lymphocytes and plasma cells and proliferation of Schwann cells, and accompanied by somewhat edema (fig. 17, 12 cases). Only one case (on the 156th day PE) showed tumorous

lymphoid lesions in the peripheral nerves. In the visceral organs, lymphoreticular cell proliferation was prominent in 6 cases. Most of these lesions consisted of perivascular proliferation of these cells. Lymphoid tumors were found in 4 cases from the 95th to 118th day PE. Lymphoid tumors were especially found in the kidneys (on the 95th day), liver (on the 118th and 126th day) and heart (on the 98th and 126th day)

Control group

Control chickens were killed and examined at various intervals according to the schedule indicated in table 2. No control chickens had any characteristic MD lesions. AGP-tests, virus isolation and IF antigen for MDV gave negative results. Virus particles could not be found in any tissue examined by electron microscopy.

DISCUSSION

Although the lesions characteristic of MD in chickens infected with various strains of MDV were reported by many investigators^{1,10,38~40,45}). However, the reports on the lesions in the early stages of infections were limited in number^{10,16,17,34}).

As initial lesions (on the 6~8th day PI), severe lymphoid degeneration and necrosis with reticular and epithelial cell hyperplasia were observed in the lymphoid organs and tissues (the thymus, bursa of Fabricius, spleen and cecal tonsils) in the inoculated group. Intranuclear and cytoplasmic inclusions were often present in these reticular and epithelial cells and contained numerous virus particles. Thereafter, lymphoreticular cell proliferation appeared in the peripheral nerves on the 12th day PI. Lymphoid tumors were found in the peripheral nerves and the visceral organs in 6 cases on and after the 16th day PI. Such lesions corresponded to T-type lesions (tumorous proliferation of lymphoreticular cells) as named by FUJIMOTO et al.¹³) and were also similar to those of A-type as named by PAYNE & BIGGS and Type III as named by WIGHT. Degenerative changes observed in the lymphoid tissues in inoculated birds were similar to those described by others^{16,17,34}). JAKOWSKI et al.¹⁶) considered that hematopoietic destructive lesions might occur in overwhelming infections in chickens lacking maternal antibody. Incidentally, chickens used in the present experiment were also lacking maternal antibody. Co-existence of degenerative lesions, and viral antigen and particles in the lymphoid tissues was frequently observed in the inoculated group.

On the other hand, in contact-exposed chickens, there were neither severe destructive changes in the lymphoid tissues nor inclusion bodies in the early

stages of the disease. These findings were similar to those of FLETCHER et al. Thereafter except one case with T-type lesions, 12 cases showed R-type lesions (reactive infiltration of lymphoreticular cells) as named by FUJIMOTO et al.¹³⁾ in the peripheral nerve lesions. In the visceral organs, only 4 cases showed T-type lesions. R-type lesions in the peripheral nerves were similar to those of B- and C-types as named by PAYNE & BIGGS and Type I as named by WIGHT. Such lesions seen in the peripheral nerves resemble to those of experimental allergic neuritis^{14,15,31,48)}. Treatment with immunosuppressive drugs alters the pathogenesis of MD^{11,35)}, which supports the idea of an autoimmune element in the disease. From these reasons, participation of some immunological effect might be suggested for the development of the lesions. Except R-type lesions seen in the peripheral nerves, lymphoreticular cell proliferation consisted of T-type lesions which are the most characteristic for MD lesions.

In our present experiment, there were at least about 3 weeks difference in the appearance of MD lesions between the inoculated and contact-exposed groups. One to 2 weeks difference in the appearance of MD lesions between the two groups were also reported by others^{18,34)}.

In spite of existing IF antigen in various tissues and AGP antigen in the skin of inoculated and contact-exposed chickens in early stages of infection, there was no antibody response during 48 day observation period in the inoculated chickens and the delayed response (60 day after exposure to inoculated chickens) in contact-exposed chickens. Although the exact reaction is not clear, this phenomenon might be due to the genetic resistance of the chickens or the low titer of inoculum used in the present experiment. A similar observation was made by others using the same strain of virus and line of chickens²⁸⁾. In preliminary experiment, we found an early antibody response in Line M chickens when inoculated with infectious heparinized blood obtained from MD chickens after 4th or 5th serial passages in the same lines of chickens. Therefore, an adaptation of the virus in a particular line of chicken may have some influence on enhancement of the pathogenesis.

It is very interesting to note the appearance and distribution of the viral antigen or virus in vivo in the early stages after inoculation of MDV. In the inoculated birds of the experiment, IF antigen was detected in the thymus and lung, and then it appeared in the FFE. In contact-exposed birds, IF antigen was first detected in the thymus and FFE. Therefore, it seems that IF antigen in the lymphoid tissues appears from the early stages of infection and with high incidence. PHILLIPS & BIGGS suggested that the viruses may localize first in the lymphoreticular tissues and then disseminate via blood to all other tissues. Many investigators^{3,5,6,33,42)} recognized the production of IF antigen in the early

stage of MD, particularly in the bursa, thymus and FFE, to be associated with a cytolytic process in cells. In our contact-exposed birds, in contrast to the inoculated ones, there were no remarkable changes in the thymic and bursal systems in the early stages of the disease. Although CALNEK & HITCHNER considered that MDV had a direct cytocidal effect, still questions remain concerning the direct cytocidal effect by MDV.

In regard to the existence of the MDV in *in vivo* systems, virus particles were frequently observed in the FFE^{6,25,27}), whereas the particles were only occasionally found in other tissues: Particles were detected in lymphoid cells in the bursa of Fabricius⁶) and gonad tumors^{6,25}), in epithelial cells of the kidney³⁷) with lymphoid tumors, and in Schwann cell nuclei and lymphoid cells^{25,44}) in peripheral nerves. Recently FRAZIER & BIGGS reported the findings of herpesvirus particles in tissues from birds free from precipitating antibodies to MDV antigens. Herpesvirus particles were shown to exist in immature lymphoid cells and primitive reticulum cells in the spleen (4~7 days after infection), in the thymus and bursa of Fabricius (7 days), and in the sciatic plexus and bursa of Fabricius (28 days). Most of the particles were unenveloped. Intranuclear inclusions have been reported by many researchers, but reports on cytoplasmic inclusions are comparatively few. Hitherto, cytoplasmic inclusions were reported only in the FFE and they contained enveloped particles^{25,27,43}). FRAZIER & BIGGS also reported the existence of cytoplasmic inclusions in the cytoplasm of cells which closely resembled lymphoid cells in the thymus. Except for the cells in the FFE and thymus, the authors observed both intranuclear and cytoplasmic inclusions in reticulum cells and epithelial cells in the bursal follicles, glandular epithelial cells and multinucleate syncytia in the adrenal glands, and glandular epithelial cells in the pancreas. Although polykaryocytosis was a general phenomenon observed in cell cultures infected with MDV, hitherto this phenomenon was not encountered either in epithelial cells or lymphoid cells of diseased chickens. Nevertheless, polykaryocytosis (multinucleate syncytia) was often observed in the thymus and the adrenal glands in our inoculated birds. Electron-microscopically, herpesvirus-type particles were found in large aggregates apparently corresponding to the cytoplasmic and intranuclear inclusions observed with a light microscope. Immature unenveloped particles were either within intranuclear inclusions or randomly distributed in the nucleoplasm. Enveloped particles were found in large quantities in the FFE and were mainly seen in cytoplasmic inclusions. Two different types of enveloped particles could be seen. Enveloped particles were found not only in the FFE, but also in the cytoplasm of the adrenal glandular epithelial cells. Electron-microscopically two kinds of cytoplasmic inclusions were seen; one consisted of amorphous aggre-

gates of electron opaque materials and contained some enveloped or unenveloped particles, and the other consisted of electron opaque granules of various sizes, surrounded by a limiting membrane and containing some enveloped and degenerated particles. The inclusions of the latter appeared to be lysosomes or phagosomes. In the nuclei, various types of immature unenveloped particles and small nuclear particles were present which were similar to those described by OKADA et al. in cell cultures inoculated with MDV and herpesvirus of turkey. Sometimes filamentous or tubular structures were contained in the nuclei of reticulum cells in the bursal follicles. "Tyre-tracks" structures which were similar to those described by CAMPBELL & WOODE and tubular structures were also found in the adrenal cortical cells. Filamentous or tubular structures seen in the nuclei of cells infected with herpesvirus have been reported by many researchers^{8,23,36,46}). Some researchers⁸) consider that these filaments are an assembly of viral subunits and some⁷) are thought to represent the product of aberrant viral replication. But the nature of these structures is still unknown.

ACKNOWLEDGEMENTS

The authors wish to thank Drs. S. KATO and M. NAITO (The Research Institute for Microbial Diseases, Osaka University, Osaka, Japan) for supplying the fluoresein-conjugated antibody to MDV and Dr. T. T. A. HAYASHI (Department of Microbiology, Sapporo Medical College, Sapporo, Japan) for helpful suggestions.

REFERENCES

- 1) BANKOWSKI, R. A., MOULTON, J. E. & MIKAMI, T. (1969): *Am. J. vet. Res.*, **30**, 1667
- 2) BIGGS, P. M. (1967): *Vet. Res.*, **81**, 583
- 3) v. BÜLOW, V. & PAYNE, L. N. (1970): *Zentbl. VetMed.*, **17**, 460
- 4) CALNEK, B. W., ADLDINGER, H. K. & KAHN, D. E. (1970): *Avian Dis.*, **14**, 219
- 5) CALNEK, B. W. & HITCHNER, S. B. (1969): *J. natn. Cancer Inst.*, **43**, 935
- 6) CALNEK, B. W., UBERTINI, T. & ADLDINGER, H. D. (1970): *Ibid.*, **45**, 341
- 7) CAMPBELL, J. G. & WOODE, G. N. (1970): *J. med. Microbiology*, **3**, 463
- 8) CHITWOOD, L. A. & BRACKEN, E. C. (1964): *Virology*, **24**, 116
- 9) CHURCHILL, A. E. & BIGGS, P. M. (1967): *Nature, Lond.*, **215**, 528
- 10) FLETCHER, O. J., EIDSON, C. S. & PAGE, R. K. (1971): *Am. J. vet. Res.*, **32**, 1407
- 11) FOSTER, A. G. & MOLL, T. (1968): *Ibid.*, **29**, 1831
- 12) FRAZIER, J. A. & BIGGS, P. M. (1972): *J. natn. Cancer Inst.*, **48**, 1519
- 13) FUJIMOTO, Y., NAKAGAWA, M., OKADA, K., OKADA, M. & MATSUKAWA, K. (1971): *Jap. J. vet. Res.*, **19**, 7
- 14) FUJIMOTO, Y. & OKADA, K. (1972): Proceeding of the 74th Meeting of the Japanese Society of Veterinary Science, *Jap. J. vet. Sci.*, **34**, Suppl., 226 (summary in Japanese)

- 15) FUJIMOTO, Y., OKADA, K., MATSUI, T., KAKIHATA, K. & NARITA, M. (1971): Proceeding of the 72nd Meeting of the Japanese Society of Veterinary Science, *Jap. J. vet. Sci.*, **33**, Suppl., 236 (summary in Japanese)
- 16) JAKOWSKI, R. M., FREDRICKSON, T. N., CHOMIAK, T. W. & LUGINBUHL, R. E. (1970): *Avian Dis.*, **14**, 374
- 17) JAKOWSKI, R. M., FREDRICKSON, T. N., LUGINBUHL, R. E. & HELMBOLDT, C. F. (1969): *Ibid.*, **13**, 215
- 18) KENZY, S. G. & BIGGS, P. M. (1967): *Vet. Rec.*, **80**, 565
- 19) KOTTARDIS, S. D. (1969): *World's Poultry Sci. J.*, **25**, 35
- 20) LAPEN, R. F., PIPER, R. C. & KENZY, S. G. (1970): *J. natn. Cancer Inst.*, **45**, 941
- 21) MIKAMI, T. & BANKOWSKI, R. A. (1970): *Ibid.*, **45**, 319
- 22) MIKAMI, T. & BANKOWSKI, R. A. (1971): *Am. J. vet. Res.*, **32**, 303
- 23) MURPHY, F. A., HARRISON, A. K. & WHITFIELD, S. G. (1967): *Arch. ges. Virusforsch.*, **21**, 463
- 24) NAITO, M., ONO, K., TANABE, S. & KATO, S. (1969): *Biken J.*, **12**, 257
- 25) NAZERIAN, K. (1971): *J. natn. Cancer Inst.*, **47**, 207
- 26) NAZERIAN, K., SOLOMON, J. J., WITTER, R. L. & BURMESTER, B. R. (1968): *Proc. Soc. exp. Biol. Med.*, **127**, 177
- 27) NAZERIAN, K. & WITTER, R. L. (1970): *J. Virol.*, **5**, 388
- 28) NISHIMURA, Y., SASAKI, E., ABE, N., KOEDA, T., YAMAMOTO, T., NAKAGAWA, H., OGAWA, T. & SAZAWA, K. (1971): Proceeding of the 72nd Meeting of the Japanese Society of Veterinary Science, *Jap. J. vet. Sci.*, **33**, Suppl., 276 (summary in Japanese)
- 29) OKADA, K., FUJIMOTO, Y., MIKAMI, T. & YONEHARA, K. (1972): *Jap. J. vet. Res.*, **20**, 57
- 30) PAYNE, L. N. & BIGGS, P. M. (1967): *J. natn. Cancer Inst.*, **39**, 281
- 31) PETEK, M. & QUAGLIO, G. L. (1967): *Path. vet.*, **4**, 464
- 32) PHILLIPS, P. A. & BIGGS, P. M. (1972): *J. natn. Cancer Inst.*, **49**, 1367
- 33) PURCHASE, H. G. (1970): *Cancer Res.*, **30**, 1898
- 34) PURCHASE, H. G. & BIGGS, P. M. (1967): *Res. vet. Sci.*, **8**, 440
- 35) RINGEN, L. M. & AKHTAR, A. S. (1968): *Avian Dis.*, **12**, 4
- 36) RUEBNER, B. H., HIRANO, T., SLUSSER, R. J., OSBORN, J. & MEDEARIS, D. N. (1966): *Am. J. Path.*, **48**, 971
- 37) SCHDLOVSKY, G., AHMED, M. & JENSEN, K. E. (1969): *Science*, N. Y. **164**, 959
- 38) SEVOIAN, M. & CHAMBERLAIN, D. M. (1964): *Avian Dis.*, **8**, 281
- 39) SEVOIAN, M., CHAMBERLAIN, D. M. & COUNTER, F. (1962): *Vet. Med.*, **57**, 500
- 40) SEVOIAN, M., CHAMBERLAIN, D. M. & LAROSE, R. N. (1963): *Avian Dis.*, **7**, 102
- 41) SOLOMON, J. J., WITTER, R. L., NAZERIAN, K. & BURMESTER, B. R. (1968): *Proc. Soc. exp. Biol. Med.*, **127**, 173
- 42) SPENCER, J. L. & CALNEK, B. W. (1970): *Am. J. vet. Res.*, **31**, 345
- 43) TAJIMA, M., ITABASHI, M. & KITANI, S. (1973): *J. Jap. vet. med. Ass.*, **26**, 128 (in Japanese with English summary)
- 44) UBERTINI, T. & CALNEK, B. W. (1970): *J. natn. Cancer Res.*, **45**, 507
- 45) VICKERS, J. H., HELMBOLDT, C. F. & LUGINBUHL, R. E. (1967): *Avian Dis.*, **11**, 531

- 46) WATRACH, A. M. (1962): *Virology*, **18**, 324
- 47) WIGHT, P. A. L. (1962): *J. comp. Path.*, **72**, 40
- 48) WIGHT, P. A. L. & SILLER, W. G. (1965): *Res. vet. Sci.*, **6**, 324

EXPLANATION OF PLATES

PLATE I

- Fig. 1 Immunofluorescent antigen (arrow) in the superficial layers of the feather follicle epithelium (FFE) on the 24th day postinoculation
Case No. 15 Fluorescent antibody stain $\times 290$
- Fig. 2 Many basophilic intranuclear and eosinophilic granular cytoplasmic inclusions (arrow) in the multinucleate syncytia of the thymus on the 8th day postinoculation
Case No. 4 Hematoxylin-eosin stain (H-E) $\times 1,500$
- Fig. 3 The medullary area of the bursal follicle is replaced by epithelial cells. Two cysts containing desquamated epithelial cells and cell debris, and focal necrobiosis could be seen in the follicle. Many intranuclear (arrow) and cytoplasmic (double arrow) inclusions are seen in these epithelial cells. Bursa of Fabricius on the 28th day postinoculation
Case No. 16 H-E $\times 1,000$
- Fig. 4 Intranuclear inclusions (arrow) of the epithelial cells in the necrotic area of the pancreas on the 8th day postinoculation
Case No. 4 H-E $\times 1,500$

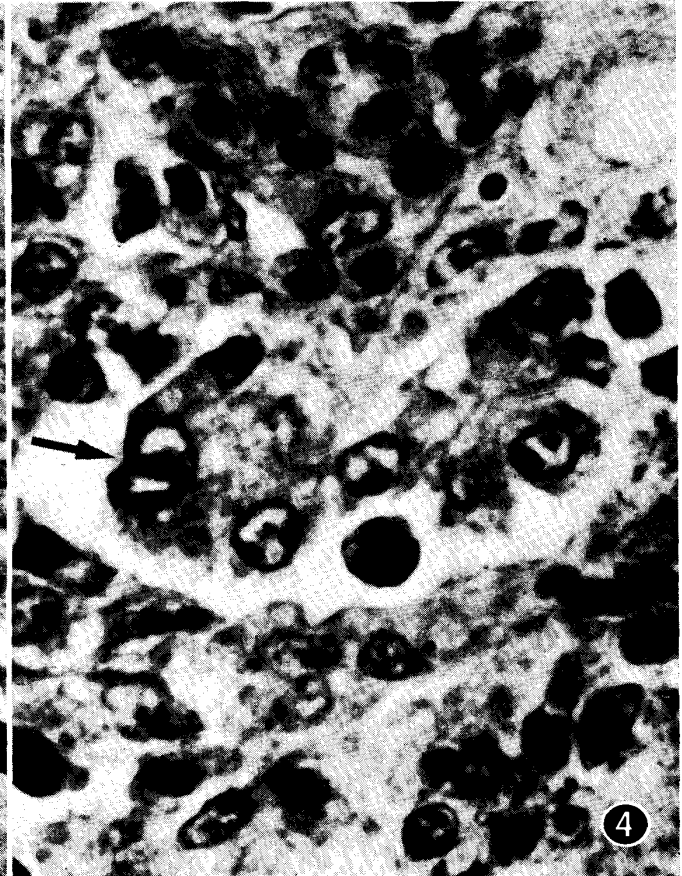
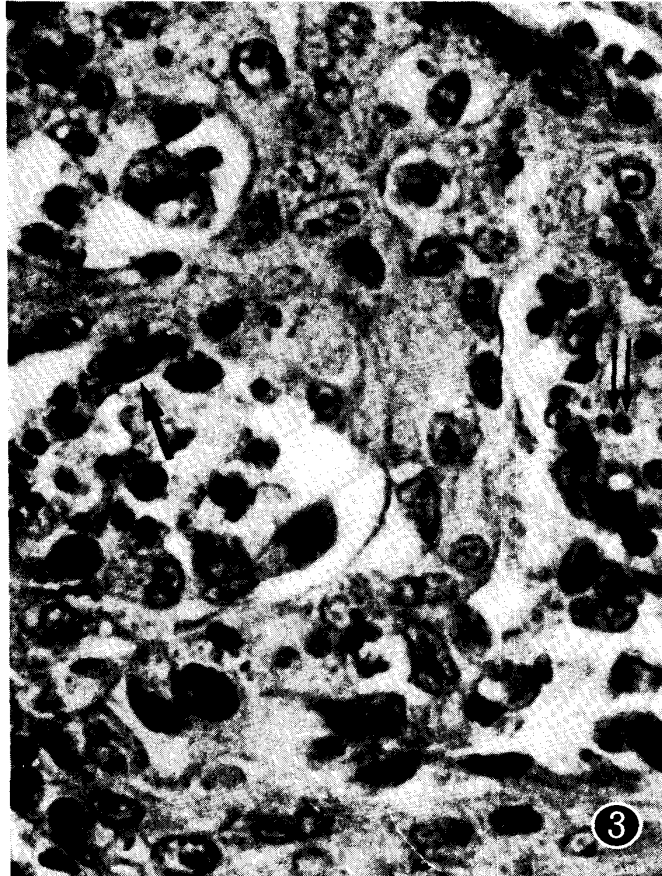


PLATE II

- Eig. 5 Intranuclear (arrow) and cytoplasmic (double arrow) inclusions of the epithelial cells in the necrobiotic area of the adrenal gland on the 24th day postinoculation
Case No. 15 H-E \times 1,300
- Fig. 6 Intranuclear inclusion (arrow) in the superficial layer of the FFE on the 19th day postinoculation
Case No. 10 H-E \times 900
- Fig. 7 Severe T-type nerve lesion, showing lymphoreticular cell proliferation between the neurites
Plexus lumbosacralis on the 16th day postinoculation
Case No. 9 H-E \times 520
- Fig. 8 Focal proliferation of lymphoreticular cells in the lung on the 32nd day postinoculation
Case No. 18 H-E \times 270

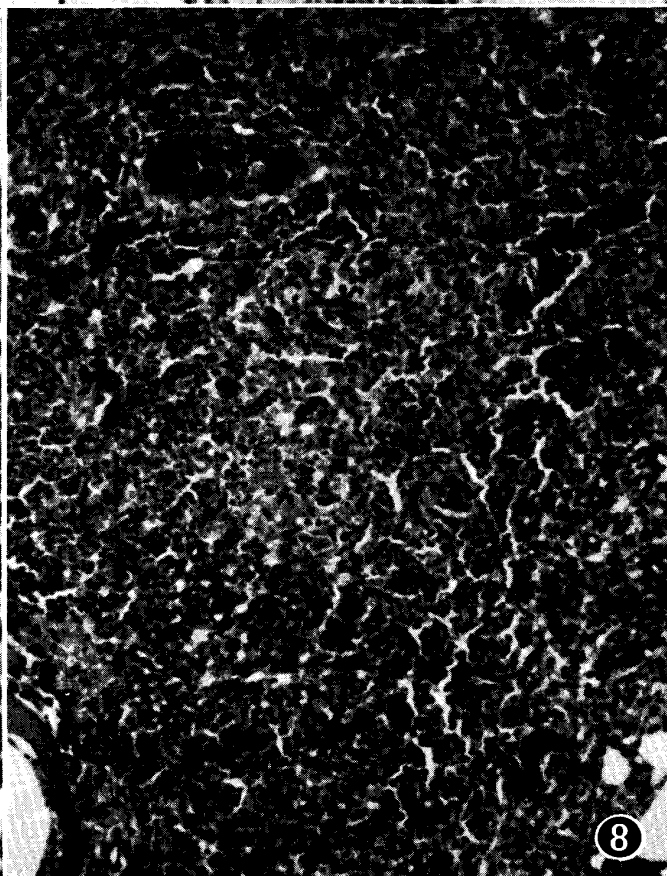
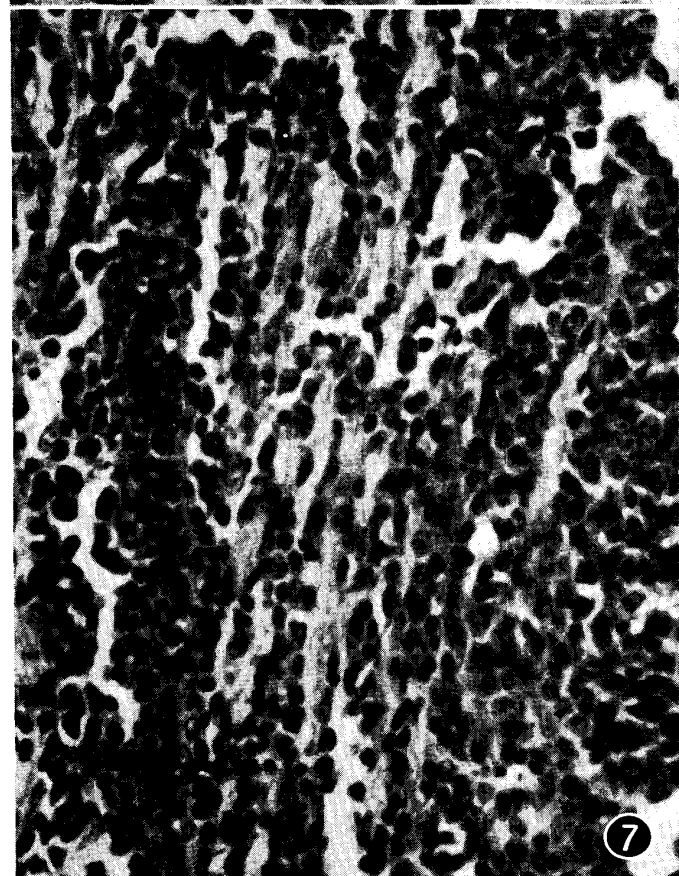
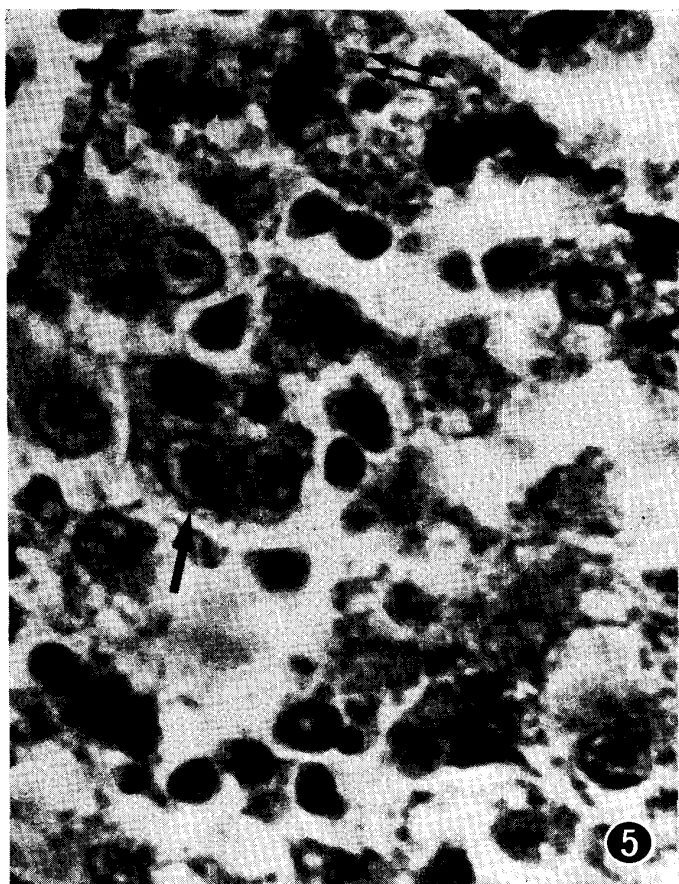


PLATE III

Fig. 9 Intranuclear inclusion in the epithelial cell of the bursal follicle on the 23rd day postinoculation

Small nuclear particles (double arrows) and several unenveloped particles (arrows) are seen in the nucleus. Specimen for electron microscopy was taken from a paraffin block.

Case No. 14 \times 34,000

Figs. 10~12 Electron micrograph of intranuclear (figs. 10 & 11) and cytoplasmic inclusion (fig. 12) in the adrenal epithelial cells on the 24th day postinoculation

Specimen for electron microscopy was taken from the paraffin block of the same tissue in figure 5. Amorphous accumulation of electron opaque materials (fig. 10), unenveloped particles (figs. 10 & 11), cross-sections of microtubular structure (fig. 10, a), "tyre-track" structure (fig. 11, b), aggregates of electron opaque materials (fig. 12, c) and enveloped particles (fig. 12, d) can be seen.

Case No. 15 \times 58,000

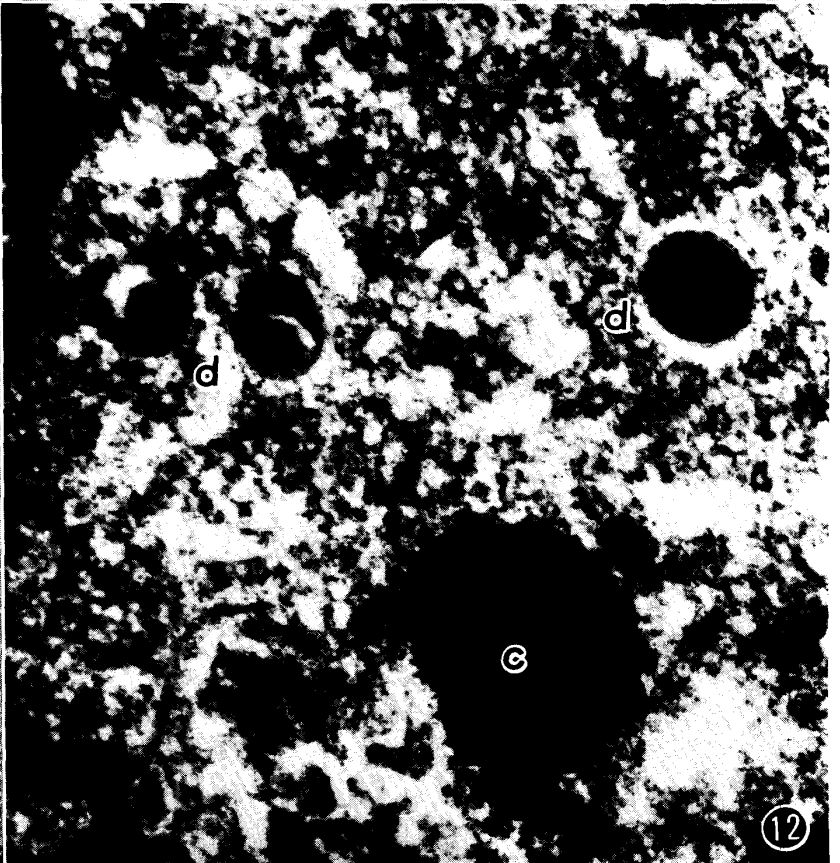
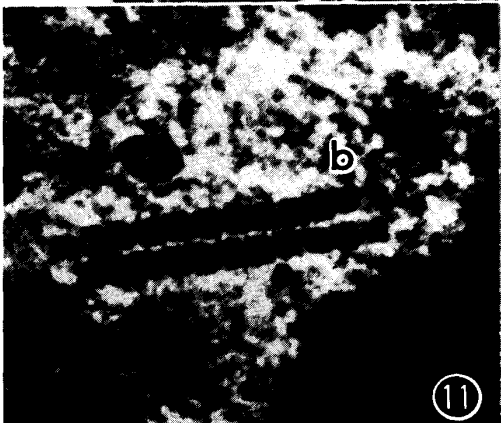
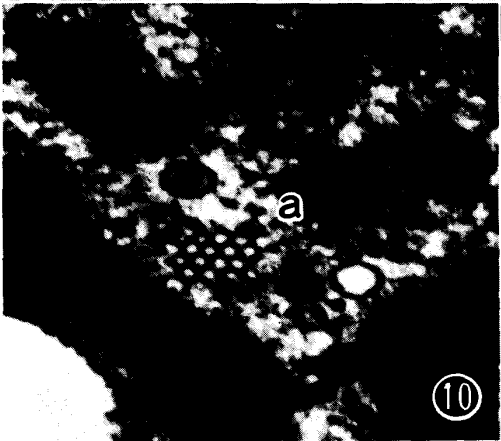
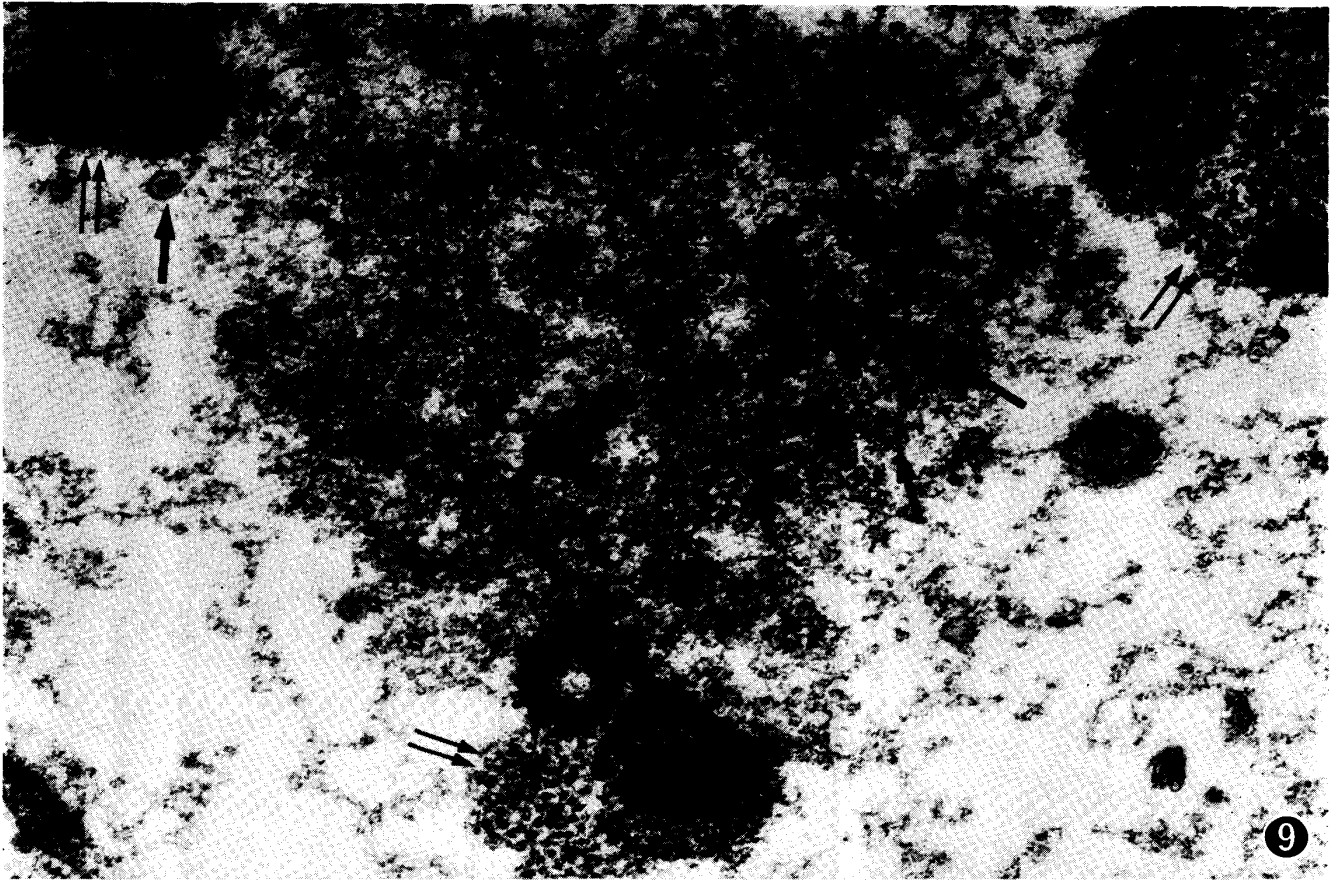


PLATE IV

Fig. 13 Multinucleate syncytia in the thymus on the 8th day postinoculation

Many unenveloped particles (arrows) can be seen in the several nuclei with irregular indentations. Cytoplasmic inclusions (double arrows) are also visible

Case No. 5 $\times 21,000$

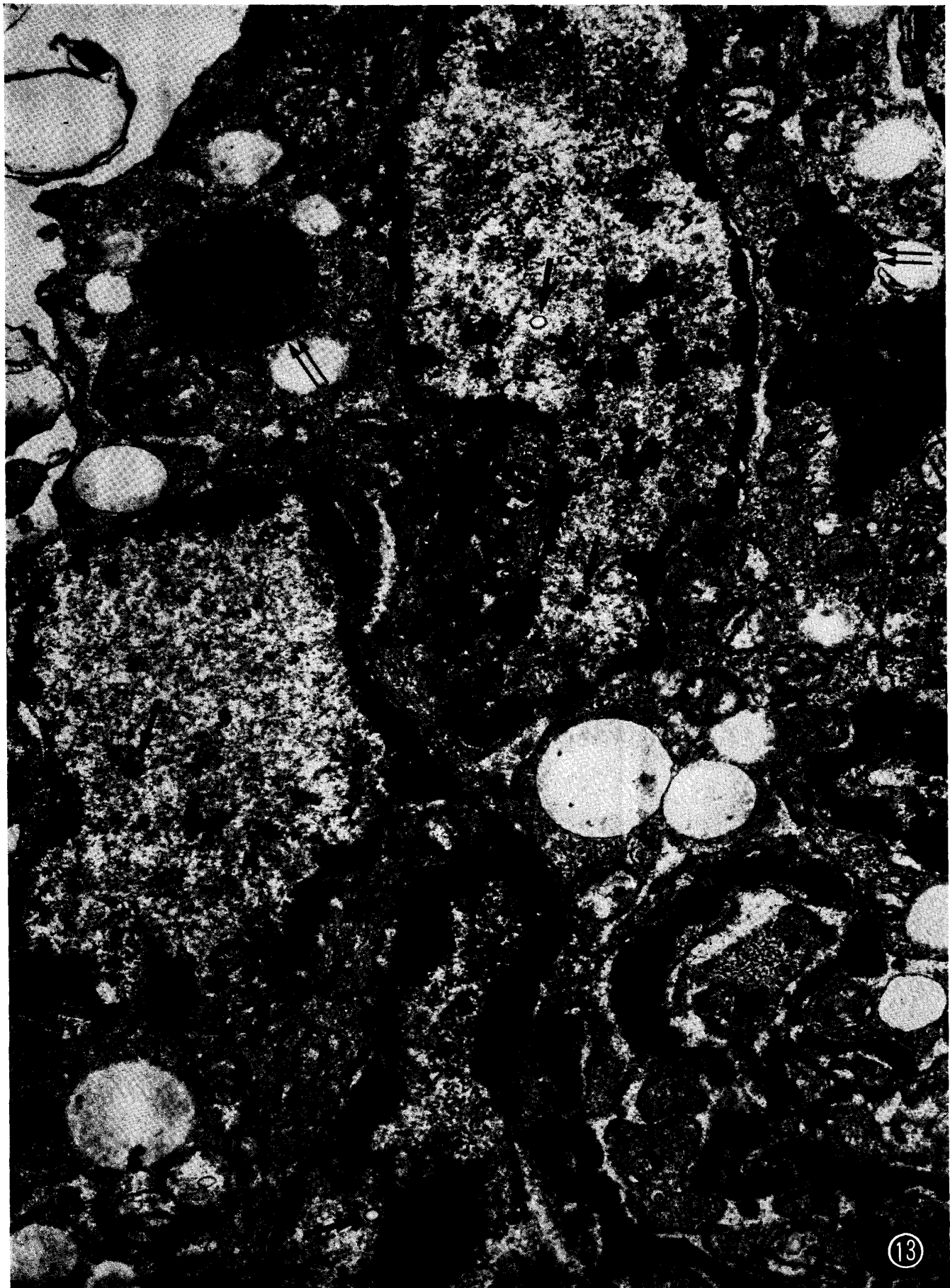


PLATE V

Figs. 14 & 15 Electron micrograph of a portion of the FFE from a chicken on the 19th day postinoculation

Intranuclear inclusions containing electron opaque granular materials and a large number of unveloped immature particles (arrows)

Specimen for electron microscopy was taken from the paraffin block of the same tissue in figure 6.

Case No. 10 Fig. 14 $\times 17,400$, Fig. 19 $\times 58,000$

Fig. 16 Lymphoreticular cell proliferation in the interfollicular connective tissue in the bursa of Fabricius on the 98th day postexposure

Case No. 33 H-E. $\times 90$

Fig. 17 R-type nerve lesion, showing lymphocytic and plasma cellular infiltration between the neurites with some edema

Plexus lumbosacralis on the 32nd day postexposure

Case No. 27 H-E. $\times 520$

