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Citation	Japanese Journal of Veterinary Research, 23(4), 119-124
Issue Date	1975-10
DOI	https://doi.org/10.14943/jjvr.23.4.119
Doc URL	https://hdl.handle.net/2115/2069
Type	departmental bulletin paper
File Information	KJ00002371229.pdf



PATHOLOGICAL STUDIES OF MAREK'S DISEASE IN JAPANESE QUAIL

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(Received for publication, June 18, 1975)

Pathological studies of Japanese quail, which were inoculated intraperitoneally with JM strain of Marek's disease herpesvirus (MDHV) at one day of age or infected by contact with inoculated-quails, were described. A group of quails accidentally infected with Marek's disease (MD) and other uninfected normal quails were also included in the present experiments. The quails were examined for various periods from 30 to 360 days. Some birds showed slightly enlarged livers, spleens and gonads, and the walls of the duodenum were thickened. The histopathological lesions characterized by lymphoreticular cell proliferation, however, were found in various organs or tissues of these infected quails, and the lesions were similar to those of chickens infected with MDHV. Immunofluorescent antigen existed in the epidermal layer of the skin and the superficial epithelial cells of the feather follicles.

INTRODUCTION

Marek's disease herpesvirus (MDHV) is a member of the herpes group which can cause lymphoid tumors in various organs or tissues of chickens. There are many reports on histopathological studies of Marek's disease (MD) in chickens; however, the reports on Japanese quails are limited in number^{1,14}).

The purpose of the present studies is to examine the pathological lesions in organs and tissues of Japanese quails infected with JM strain of MDHV.

MATERIALS AND METHODS

Virus Either infectious blood (virus titer; 80 plaque forming units (PFU)/0.1 ml) from chickens inoculated with the JM strain of MDHV³) or trypsinized cells (virus titer; 100 PFU/0.1 ml, third passage) from chick kidney cultures inoculated with the MDHV⁵) were used as inocula. The source of the MDHV was described previously⁷).

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Japanese quail A total of 14 quails was selected for histopathological examination from 75 quails (60 infected and 15 normal quails) used in a previous experiment⁸. Six quails (Case Nos. 1~6) were injected intraperitoneally with MD blood and one quail (Case No. 7) was injected with the trypsinized cells at one day of age. One quail (Case No. 8) was infected by contact with quails inoculated with the MD blood. Three quails (Case Nos. 9~11), which have been raised in our laboratory as a source for hatching eggs, were accidentally infected with MD. Uninfected normal control quails (Case Nos. 12~14) were reared in isolation. All quails, except one (Case No. 5), which died without any apparent gross tumors, were killed for the examination at 30 to 360 days. Most of the 10 infected quails (Case Nos. 2~11) showed depression and diarrhea, but no nervous symptoms at the time of autopsy. The source of normal quail was described previously⁷.

Histopathology After post-mortem examination, tissues from all quails were fixed in 10% formalin solution. Many blocks of tissues were collected from various parts of the organs and tissues. Paraffin sections were stained with hematoxylin-eosin.

Agar gel precipitation test The agar gel precipitation (AGP) test and preparation of the AGP antigen from feather tips of individual quails were performed as described^{3,6}. The feather tip preparations were tested against MD-specific chicken serum using the AGP test⁸. The sera were tested against antigens prepared from skins of MD-infected chickens^{8,9}. The feather tips and sera were collected from quails at the time of autopsy; however, the feather tips were occasionally collected during the observation period.

Immunofluorescence The skins taken from 4 quails and kidneys, lungs, intestines, and gonads from 2 quails were examined for the presence of immunofluorescent antigens as described³.

RESULTS

Gross changes

Many quails showed no visible lesions in any of the visceral organs or tissues at autopsy. In some quails (Case Nos. 2~7, 11), however, the livers were slightly enlarged and sometimes had scattered or diffuse foci on their surface and in their parenchyma, but no solid, large tumors. The spleens and the gonads were also slightly enlarged (Case Nos. 2~7). The walls of the small intestines, especially the duodenum, were thickened (Case Nos. 2~7, 11).

Microscopic findings

The severity of microscopic lesions and serologic observations of 14 quails

TABLE 1 *Severity of lesions and serological observation of quails infected with Marek's disease virus (JM strain)*

GROUP*1	CASE*2 NO.	AGE ON AUTOPSY	TISSUE EXAMINED*3														AGP TEST*4		
			Li	Sp	K	H	Lu	Pr	I	T	B	G	A	PN	CN	Sk	Anti- gen	Anti- body	
I	1	30 d	-	-	-	-	-	-	-	-	-	-	-	ND	-	-	-	P	N
	2	70	#	#	-	±	#	#	#	ND	#	#	#	+	-	-	-	P	N
	3	90	#	#	-	#	#	+	#	#	ND	#	ND	±	±	+(P)	-	P	N
	4	100	#	#	-	-	-	-	#	-	ND	+	-	-	-	-	-	P	N
	5	116	#	#	+	-	-	#	#	-	ND	#	ND	-	-	-	-	P	ND
	6	150	#	+	##(P)	+	-(P)	+	##(P)	-	ND	##(P)	+	-	±	-(P)	-	N*5	N
	7	280	#	#	#	-	-	ND	#	ND	ND	-	+	-	-	#	-	P	P
	8	30	-	ND	#	-	-	-	-	-	-	ND	ND	+	-	-(P)	-	P	N
	9	360	-	-	-	ND	ND	ND	-	ND	ND	ND	-	-	-	+	-	N*6	N
	10	360	-	-	-	ND	ND	ND	-	ND	ND	ND	ND	-	-	-	-	N*6	N
	11	360	#	+	+	ND	ND	ND	#	ND	-	-	-	-	-	-	-	N*6	P
NC	12	45	-	-	-(N)	-	-(N)	-	-(N)	-	ND	ND(N)	ND	-	-	-(N)	-	N	N
	13	300	-	-	-	-	-	-	ND	ND	ND	-	-	-	-	-	-	N	N
	14	300	-	-	-	-	-	-	-	ND	-	-	ND	-	-	-	-	N	N

*1 I: infected, NC: normal control

*2 All quails, except a dead quail (Case No. 5), were killed.

*3 Li: liver, Sp: spleen, K: kidneys, H: heart, Lu: lungs, Pr: proventriculus, I: intestines, T: thymus, B: bursa, G: gonads, A: adrenals, PN: peripheral nerves, CN: central nerves, Sk: skin. Severity of microscopic lesions; # to + reactions denote degrees of lymphoreticular cell proliferation in tissues, ranging from severe (#) to mild proliferations; ±: suspected reaction, -: negative reaction, ND: not done. The results of fluorescent antibody staining on frozen section shown in parenthesis and expressed by positive (P) or negative (N).

*4 Parts of these results were described previously (MIKAMI et al., 1975); the AGP test results were expressed by positive (P) or negative (N).

*5 AGP antigen was positive 100 days before autopsy.

*6 AGP antigen was positive 6 months before autopsy.

from various groups are summarized in table 1. The livers had small, diffuse foci or extensive invading masses of lymphoreticular cells (Case Nos. 2~7, 11) (fig. 1). Intimagramulomatous proliferation of lymphoreticular cells was also found in the veins of the Glisson's capsules. Cytologically, the neoplastic masses consisted of a mixture of small and medium lymphoid cells, lymphoblastic cells, and reticulum cells (fig. 2). In the spleen, lymphoreticular cell proliferation was marked and loss of architecture could be seen in the inoculated group (Case Nos. 2~7). Nodular foci or diffuse invasions of lymphoreticular cells were often found in the kidneys (Case Nos. 5~8), lungs (Case Nos. 2, 3) (fig. 3), heart (Case No. 3) (fig. 4), and adrenals. The intestines, especially the duodenum, had extensive masses of lymphoreticular cells in the lamina propria, submucosa, muscularis, and serosa (Case Nos. 2~7, 11) (fig. 5). The pancreas (fig. 6) was also invaded by the neoplastic cells (Case No. 3). In the proventriculus (Case Nos. 2, 5), lymphoreticular cells accumulated in the lamina propria in diffuse or focal distributions. The gonad tissues were occupied by proliferated lymphoreticular cells (Case Nos. 2, 3, 5, 6). In the coeliac plexus, lymphoreticular cell proliferation was marked in the interstitial tissues (Case Nos. 2, 8) (fig. 7), but not in the nerve fibers and ganglia themselves. The peripheral nerves (plexus lumbosacralis) were also affected in only a small area, and the extent of the lesions was very slight. In most cases the skin was almost normal, except Case No. 7, which showed tumorous invasions of lymphoreticular cells in the muscular layers (fig. 8). Immunofluorescent antigen for MDHV was found in the epidermis (fig. 9) and the superficial epithelial cells (fig. 10) of the skins of all infected quails examined. In addition, immunofluorescent antigen was found in the kidney, lung, intestine, and gonad of the infected case examined.

The AGP antigen in the feather tip was found in many infected quails, whereas the antibody was detected in only 2 quails. The details of the serological observations in the quails was previously described⁸⁾.

DISCUSSION

Microscopical lesions similar to those chickens with MD have been observed in Japanese quails^{1,14)}. WIGHT (1963) reported gross and microscopic pathology of naturally occurring lymphoid leukosis (4 cases) and fowl paralysis (1 case) in the Japanese quails obtained from his laboratory flock. The peripheral nerve lesions of fowl paralysis corresponded to that designated as Type I by WIGHT (1962). In experimentally induced MD in Japanese quails, DUTTON et al. (1973) also reported similar lesions in quails which had been infected with the CR 64 strain of acute MDHV by contact exposure. They described the gross and microscopic lesions of MD which appeared in quail at 75 days of age (68 days

post exposure). The MD lesions were generally present in the liver, spleen, kidney, and small intestines (especially in the duodenum), and characterized by the presence of heterogenous lymphoid cells and, occasionally, plasma cells. Lesions were most prevalent in the liver. The liver masses would vary from a diffuse to an extensive invading mass of a uniform cell type. Most neural lesions were very slight. KENZY & CHO (1969) reported that a quail with the ocular form of MD was found to have MDHV in the blood and to have transmitted MD to monitor chicks by contact. Recently, MIKAMI et al. (1975) demonstrated the existence of MDHV-specific antigen and antibody and reisolated virus from Japanese quails experimentally infected with the JM strain of MDHV.

From the present studies and other MD transmission experiments in quail (MIKAMI et al., 1975 and unpublished data), the clinical manifestation of MD in both inoculated and contact-exposed quails seems to be limited. A few quails, which were selected for the present experiment, in both groups showed depression and diarrhea; however, we did not observe the usual clinical signs of MD, especially nervous symptoms, as seen in chickens infected with the JM strain of MDHV¹¹). Three out of 60 infected quails died without apparent gross tumors during the experiment⁸). In contrast with the observations made by DUTTON et al. (1973), these results indicate that death losses due to MD were few and gross lesions were not so extensive and limited in number.

Microscopical lesions of quails exposed to the virus in the present experiment were similar to the findings of the others^{1,14}) and corresponded to those of the T-type lesion in chickens found by FUJIMOTO et al. (1971). Proliferation of lymphoid cells in the peripheral nerves was also slight, similar to that described by DUTTON et al. (1973) and it was less severe than that described by WIGHT (1963). These different pathological responses may be due to the different strains of MDHV, as observed in tissues of chickens inoculated with the different strains^{10,12}).

Although we reported the presence of immunofluorescent antigen in the feather follicular epithelium of MD-infected quails⁸), the antigen was also found in the cutaneous epithelium, kidneys, lungs, intestine, and gonad in the present experiment. To our knowledge, there is no evidence of the immunofluorescent antigen in the cutaneous epithelium of chickens or quails infected with MD.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. S. KATO (The Research Institute for Microbial Diseases, Osaka University, Osaka, Japan) for supplying Japanese quail and

the conjugated anti-MDHV chicken serum, and T. T. A. HAYASHI (Department of Microbiology, Sapporo Medical College, Sapporo, Japan) for helpful suggestions.

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EXPLANATION OF PLATES

PLATE I

- Fig. 1 Extensive invading masses of lymphoreticular cells found in the liver of inoculated quails
Case No. 2, hematoxylin-eosin (HE) stain × 110
- Fig. 2 Enlarged figure of the same liver of figure 1
Proliferated foci consisted of a mixture of a few small and medium lymphoid cells, a large number of lymphoreticular cells, and some reticulum cells
HE stain × 660
- Fig. 3 Lung parenchyma without the bronchioli was almost occupied by proliferated tumor cells.
Case No. 3, HE stain × 165
- Fig. 4 Lymphoreticular cell proliferation was remarkable in the intestinal tissues of the myocardium.
Case No. 3, HE stain × 270

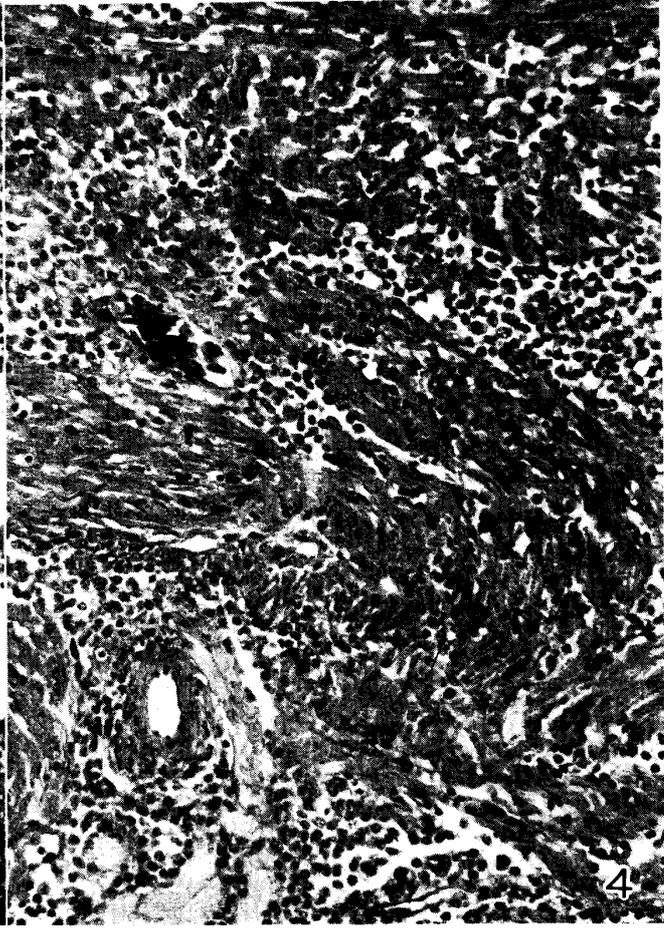
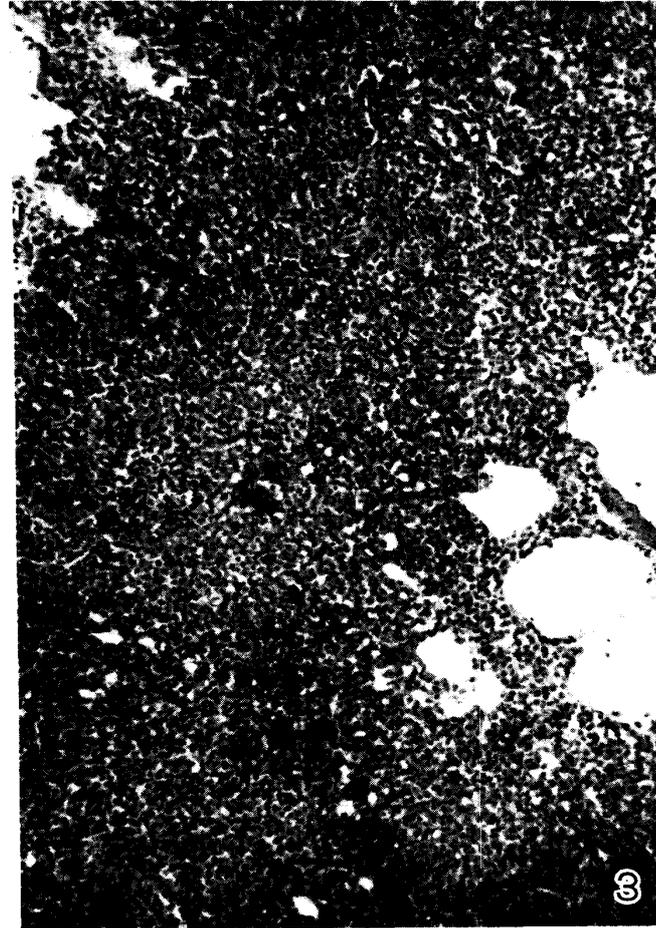
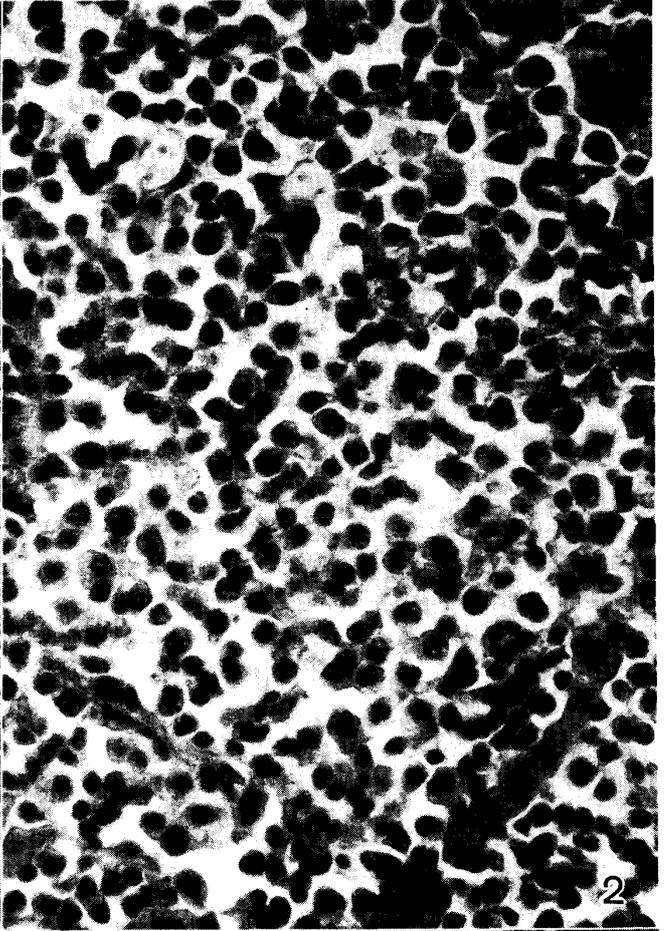
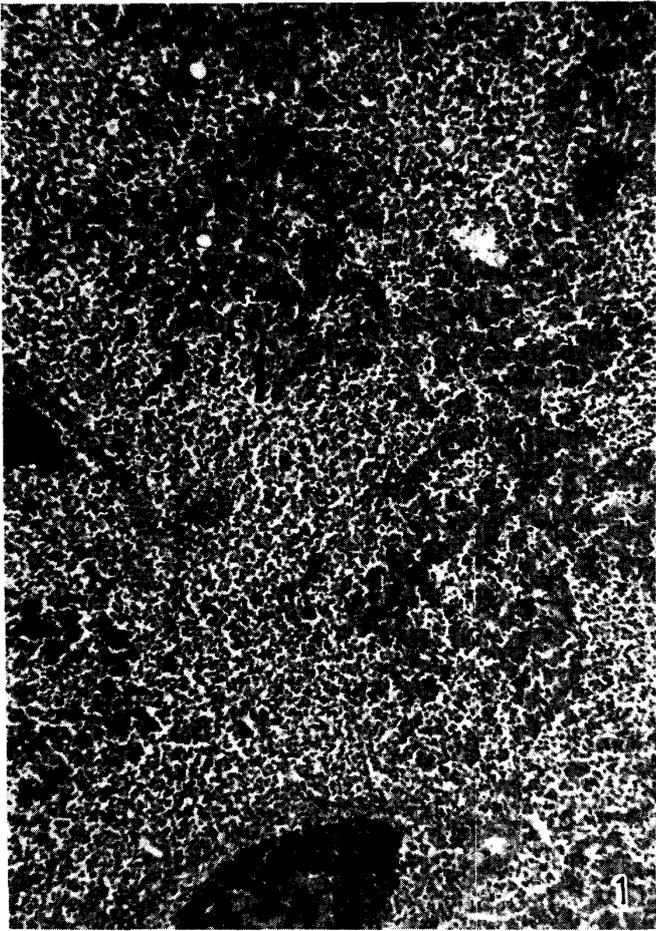
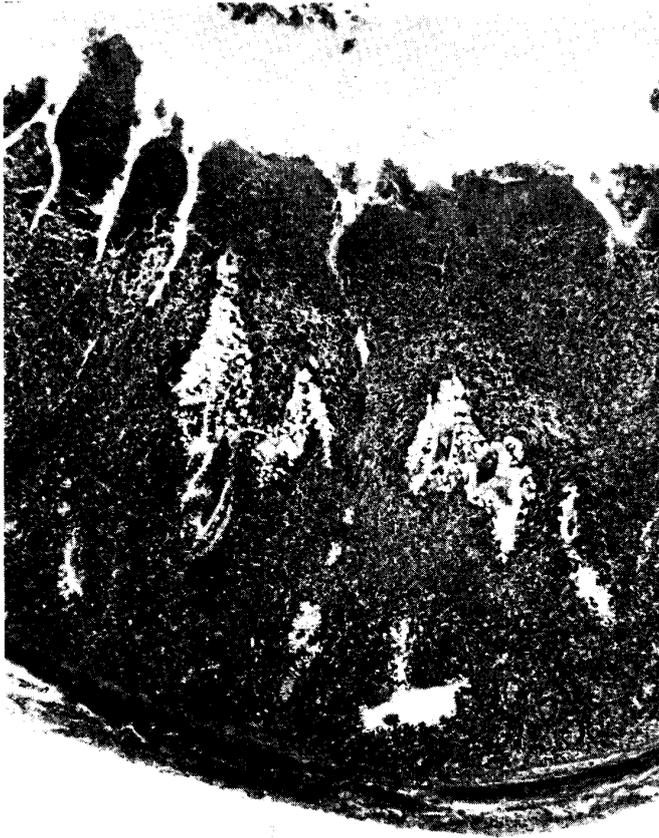
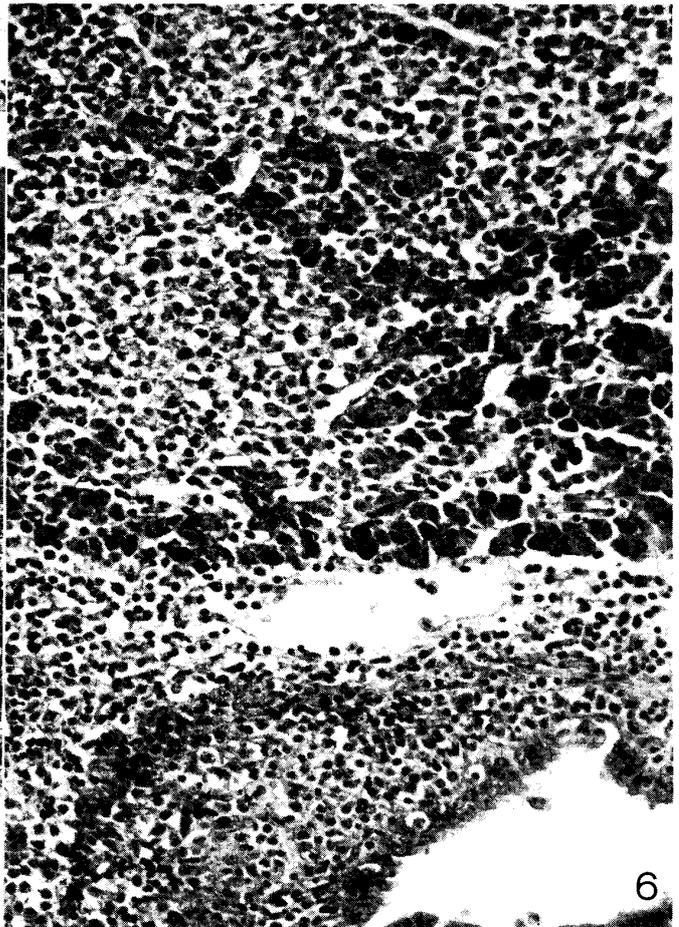


PLATE II

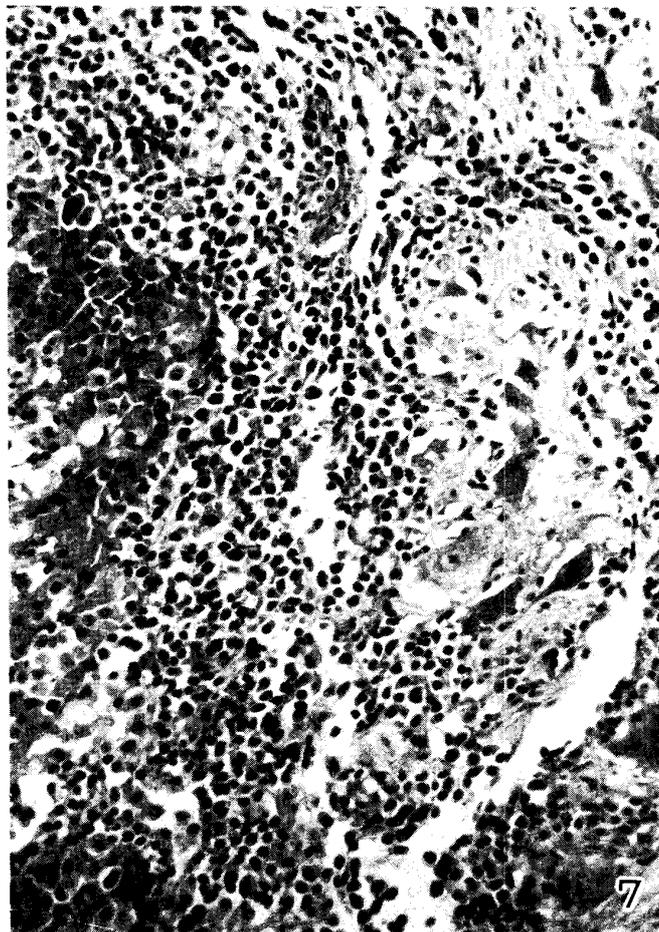
- Fig. 5 Extensive masses of lymphoreticular cells found in the lamina propria, submucosa, muscularis, and serosa of the duodenum
Case No. 2, HE stain × 47
- Fig. 6 Proliferated lymphoreticular cells occupied most of the pancreatic parenchyma
Case No. 3, HE stain × 270
- Fig. 7 Distinct lymphoreticular cell proliferation found in the intestinal tissues around the nerve fibers and ganglia in the coeliac plexus and the adrenal gland
Case No. 2, HE stain × 270
- Fig. 8 Tumorous invasion of lymphoreticular cells was marked in the muscular layer of the skin.
Case No. 7, HE stain × 110



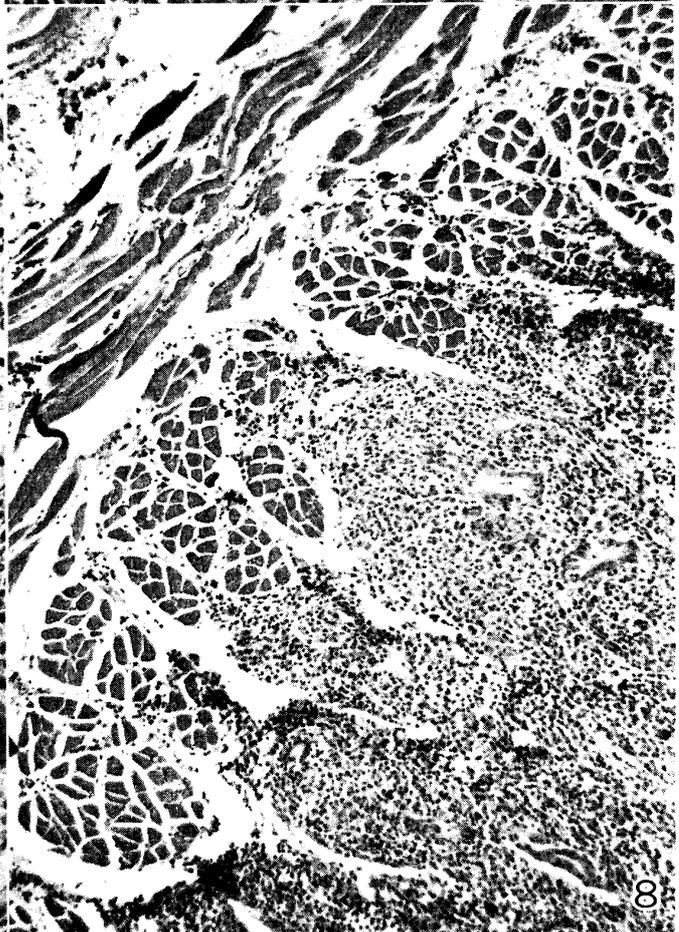
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6



7



8

PLATE III

Fig. 9 Immunofluorescent antigen existed in the epidermal layer of the skin

Case No. 8, Fluorescent antibody × 240

Fig. 10 Immunofluorescent antigen existed in the superficial epithelial cells of the feather follicles of the skin

Fluorescent antibody × 210

