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THE PATHOLOGICAL STUDY OF PARATUBERCULOSIS IN GOATS, CENTERED AROUND THE FORMATION OF REMOTE LESIONS*1

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INTRODUCTION

Paratuberculosis (Johne's disease) is a specific infectious disease of domestic animals caused by an acid-fast bacillus (*Mycobacterium johnei*) and characterized by a chronic hypertrophic catarrhal enteritis. This disease is known to infect cattle, sheep and goats.

Until the last decade, there had been only one report of a natural case of paratuberculosis in Japan. This exception was a report by TAKEHARA (1930) concerning an imported cow. In 1960, a case of paratuberculosis in an imported cow was reported by HATAKEYAMA et al.[9,10] At the 50th Meeting of the Japanese Veterinary Science, in 1960, the present authors reported the first naturally occurring paratuberculosis in Japan, in goats[30]. At the same meeting, UEDA & ONO reported several natural cases in sheep, from the Tokachi Livestock Breeding Station, Hokkaido. Since the 1960 meeting many cases of paratuberculosis in goats, sheep and cattle have been reported[11,12,29,31,38]. From these reports it is suggested that paratuberculosis has been widely distributed throughout Japan, in goats, sheep and cattle for sometime without being noticed. Therefore, the occurrence of the disease is very important from the economical point of view.

The present study was undertaken to thoroughly investigate the systemic pathological changes seen with paratuberculosis in goats. It is common knowledge that the characteristic lesions seen with this disease are located mainly in the intestines and regional lymph nodes. There are, however, very few descriptions of lesions in other parts of the body. The authors had emphasized the formation of remote lesions, which has been neglected until this time, as an important

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phenomenon and they have attempted to elucidate the mode of development of these lesions. The authors have tried to elucidate fully the pathological character of the disease and to some extent facilitate understanding of the immunological attitude of the disease.

MATERIALS AND METHODS

The materials investigated, as listed in table 1, consisted of 45 naturally infected cases of paratuberculosis in goats which were collected from July 1959 to January 1961 at the Takikawa Experimental Station of Sheep Breeding. These 45 cases were divided into 4 groups for the convenience of description, according to the degree of the characteristic lesions of paratuberculosis in the intestines and the mesenteric lymph nodes, and in some cases according to the bacteriological results: Group I (severe)-18 cases; Group II (moderate)-10 cases; Group III (mild)-8 cases; and Group IV-9 cases (these cases lacked the characteristic lesions of paratuberculosis as described in the text books, but they showed positive results for paratubercle bacilli in both bacterial culture and direct smear preparations of the intestines and the mesenteric lymph nodes). The results of the bacteriological examination and johnin reactions seen in all investigated cases were compared as often as possible. To further elucidate the disease the 36 cases in groups I, II and III were the chief object of our investigation, while the 9 cases in group IV were studied only for reference. Six cases (Case Nos. 5, 8, 30, 33, 39 & 45) were used for a satiation experiment by our Department of Veterinary Internal Medicine during the 3 months immediately following the first positive johnin test. The second johnin test run 3 months later showed negative results. All materials for investigation were fixed in a 10% formalin solution following macroscopic observation. These materials were obtained from as many parts of the various organs as possible. The only exceptions being the 9 cases (Case Nos. 7, 11, 12, 13, 15, 17, 18, 20 & 21) sent for diagnosis. The fixed materials were embedded in paraffin and the tissue sections were stained mainly with hematoxylin and eosin. Most sections of the liver, kidneys, intestines and regional lymph nodes were stained with ZIEHL-NEELSEN's carbol-fuchsin-hematoxylin in order to detect any acid-fast bacilli that might be present. We also used GÖMÖRI's method of BIELSCHOWSKI's silver impregnation for argyrophile fibers, HEIDENHEIN's method of MALLORY's azan stain, VAN GIESON's stain for collagen fibers (accompanied by WEIGERT's stain for elastic fibers in some sections), MCMANUS's periodic acid-Schiff (PAS) reaction and a toluidine blue stain. To detect amyloid substances MAYER's method of methyl violet stain, jod reaction and a Congo red stain were used. The isolation of *M. johnei* was conducted from affected intestines and mesenteric lymph nodes by the Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University.

RESULTS

A Clinical findings

Generally speaking the onset of symptoms was characterized by anorexia and a marked reduction in milk production. In severe cases, the animal usually showed a watery diarrhea, anemia and edema as the main symptoms and then finally died in a severe emaciated and

TABLE 1 *Classification by the grade of lesions*

GROUP	CASE NO	PROTOCOL NO	SEX	AGE (Yr)	ISOLATION OF *M. johnei*	JOHNIN TEST	TERMINATION
I	1	E 3438	♀	4	·	+	⚰
	2	E 3433	〃	3	+	+	〃
	3	E 3437	〃	2	+	+	〃
	4	E 3442	〃	3	+	+	〃
	5	Pr 3925	〃	〃	+	+*	〃
	6	E 3440	〃	〃	+	·	†
	7	E 3590	〃	2	·	+	〃
	8	Pr 3918	〃	6	+	+*	⚰
	9	E 3441	〃	3	+	+	〃
	10	E 3439	〃	〃	+	+	〃
	11	E 3270	〃	〃	·	·	〃
	12	E 3171	〃	8	·	·	〃
	13	E 3273	♂	4	·	·	†
	14	E 3430	♀	6	·	+	⚰
	15	E 3271	〃	〃	·	·	†
	16	E 3436	〃	2	·	+	⚰
	17	E 3290	〃	6	·	+	†
	18	E 3274	〃	7	·	+	〃
II	19	E 3444	〃	2	·	+	⚰
	20	E 3399	〃	5	·	·	†
	21	E 3168	〃	4	·	·	⚰
	22	E 3454	〃	〃	+	+	〃
	23	E 3446	〃	2	·	+	〃
	24	E 3432	〃	3	+	±	〃
	25	E 3435	〃	〃	+	+	〃
	26	E 3428	〃	〃	·	+	〃
	27	E 3445	⚲	2	·	+	〃
	28	E 3427	♀	6	·	+	〃
III	29	E 3452	♂	2	+	+	〃
	30	Pr 3923	♀	3	+	+*	〃
	31	E 3429	〃	2	·	+	〃
	32	E 3457	⚲	〃	+	·	〃
	33	Pr 3927	♀	1	+	+*	〃
	34	E 3443	〃	3	+	+	〃
	35	E 3455	♂	〃	·	·	〃
	36	E 3447	〃	1	·	±	〃
IV	37	E 3461	〃	9	+	·	〃
	38	E 3451	♀	3	+	+*	〃
	39	Pr 3926	〃	1	+	+	†
	40	E 3459	⚥	〃	+	—	⚰
	41	E 3460	〃	〃	+	—	〃
	42	E 3434	♀	6	+	+	〃
	43	Pr 3906	〃	4	+	·	〃
	44	E 3458	⚥	1	+	—	〃
	45	Pr 3920	♀	7	+	+*	〃

N. B. I: Severe cases II: Moderate cases III: Mild cases IV: Cases showed no characteristic lesions ⚥: Intersex ⚲: Castrated male *: Cases showed negative result in the second johnin test on the 3 months after the first johnin test was conducted †: dead ⚰: killed ·: non examined

cachexia condition. The course of the disease was usually very chronic and pyrexia was not noticed. As the disease developed the total serum protein decreased and positive results were seen with the Gross reaction. A number of apparently normal animals showed a positive

johnin test. When these animals were euthanized, most of them were diagnosed as paratuberculosis following pathological and bacteriological examination. However some animals with extensive lesions, clinically appeared normal. On the other hand, some animals showing severe clinical signs did not have comparable lesions. Some animals showing severe lesions retained a normal appetite. From these facts, it may be suggested that many cases of paratuberculosis in goats were regarded as clinically normal.

B Necropsy findings

The results of our investigations are summarized as follows:

1) Chronic hyperplastic entero-colitis (characteristic lesions were located mainly in the ileum, the jejunum, a part of the caecum and the anterior part of the colon) (figs, 1 & 2)

2) Enlargement and edema of the intestinal and mesenteric lymph nodes (in severe and moderate cases)

3) Chronic lymphangitis (only in severe cases)

4) Slight enlargement of the spleen and hyperplasia of the Malpighian bodies of the spleen

5) Anemia, edema and slight enlargement of the kidneys

6) Slight edematous swelling of the general lymph nodes

7) Slight enlargement of the liver (sometimes scattered pin-head-sized, whitish-grey foci were seen)

8) Generalized anemia and emaciation

9) Focal verminous pneumonia (pulmonary nematodiasis)

10) Dilatation of the right ventricle of the heart

11) Twisted stomach worms in the abomasum, tape worms in the small intestine and nodular worms in the large intestine

C Histopathological findings

1) Digestive tract

The most characteristic and typical lesions of the disease were easily found in the intestines, especially in cases with well developed gross lesions. These lesions were usually located in the lamina propria of the mucosa of the small and large intestines and they sometimes extended into the submucosal and subserous tissues. These lesions were characterized by both a diffuse and focal proliferation of epithelioid cells.

Mucosal lamina propria: Focal accumulations of histiocytic cells, which may have originated from reticulum cells of the mucosa, were found in mild cases. At this stage very few epithelioid cells could be seen. In the more advanced cases the epithelioid cells increased in number, and at the same time, the granulomatous lesions increased in number as well as size in a step by step manner. Finally a diffuse distribution of epithelioid cells was seen in the lesions (figs. 3 & 4). At this stage the epithelioid cells were well rounded and enlarged, and the cytoplasm was packed with numerous acid-fast bacilli (fig. 5). Sometimes, the central lacteals of the villi were dilated and contained epithelioid cells. Giant cells of Langhans type were occasionally observed in the granulomatous lesions. Furthermore, at the peripheral

zone of the focal granulomatous lesions, we noted transitional cells between the histiocytic cells and epithelioid cells. A loose infiltration of lymphocytes, plasma cells, and occasional eosinophil and neutrophil was noted in the lamina propria mucosa. Some of the plasma cells has Russell's bodies in their cytoplasm. The neutrophils occasionally contained phagocytized acid-fast bacilli. The lymphoid nodules in the lamina propria mucosa were hyperplastic and some of them compressed the adjacent intestinal glands. Occasionally small foci of epithelioid cells were observed in the lymphoid nodules. In severe cases, similar lesions were observed in the large intestine and in some cases ulceration of the caecum was seen. Usually the lesions seen in the large intestine were not as severe as those in the small intestine.

Submucosa: An edema and loosening of the connective tissue, and active reticulo-endothelial cells were observed in the small intestine. The proliferation of epithelioid cells was marked in some parts. Both a focal and diffuse infiltration of lymphocytes and plasma cells was seen. Furthermore, a granulomatous and obstructive endolymphangitis was noted in some cases. A caseous lymphatic intimagranuloma and perilymphangitis were detected in some cases (figs. 6~8.).

Muscularis mucosa: An edema and loosening of the intermuscular connective tissue, and proliferation of the histiocytic cells were also observed.

Serosa: The subserosa was markedly edematous and proliferation of histiocytic cells was marked. In some parts, a focal aggregation of lymphocytes was recognized. Sometimes a granulomatous obstructive endolymphangitis and perilymphangitis were present (figs. 9 ~11).

2) Lymphatic vessels related to the digestive tracts

Considering the mode of development of the lesions in this disease, lymphatic spread was much more conspicuous than hematogenous spread. A marked granulomatous and proliferative inflammation in the lamina propria of the intestinal mucosa starting at the central lacteals and extending into the lymphatic plexus of the submucosa and subserosa was seen. Subsequently the inflammatory process extended into the pericapsular lymphatic vessels of the regional lymph nodes (mainly the mesenteric lymph nodes) by way of the first prenodal lymphatic vessels.

Intimagranuloma (figs. 9 & 12): The intima of the lymphatic vessels showed an edematous or fibrinoid swelling. In the more advanced cases showed intimagranuloma of the lymphatic vessels which was initiated by a focal proliferation of histiocytic cells. The histiocytic cells were then replaced by epithelioid cells. This inflammatory process extended into the area around the lymphatic vessels and resulted in perilymphangitis. In some cases, the integrity of the wall of the lymphatic vessel was almost completely lost. These intimagranulomatous lesions were first observed in the submucosa of the intestine where the proliferation of argyrophil and collagen fibers was marked. These lesions were also observed in the anterior part of the lymphatic capillaries and fine lymphatic vessels of the submucosa and subserosa. Furthermore in the pre- and post-nodal, small and medium sized, lymphatic vessels, the intimagranulomatous lesions consisted of large pale and small dark nuclear histiocytic cells with a small number of lymphocytes. The granulomatous lesions projected into the lumens of the vessels and giant cells of Langhans type or foreign body type were

also found in the lumens. Such lesions of the lymphatic vessels were especially marked in the animals of group I.

Endolymphangitis obliterans: Some of the above mentioned granulomatous lesions gradually obstructed the lymphatic vessels, while others resulted in a necrobiosis or caseous degeneration in their central areas (figs. 6 & 10). Occasionlly an active infiltration of neutrophils was noted. Proliferation of argyrophil and collagen fibers was also found in the granulomatous lesions.

Perilymphangitis (figs. 6, 7 & 11): Infiltration of lymphocytes, proliferation of histiocytic cells, and furthermore granulomatous lesions were found around the lymphatic vessels. These granulomatous lesions were sometimes accompanied by newly formed blood capillaries. In severe lesions the original structure of the walls of the lymphatic vessels was indistinguishable, because of the highly proliferative changes in the intimal and adventitial sides of the vessels. In some of the lymphatic vessels the structure of the wall was still recognizable only by the argyrophil fibers.

Acid-fast bacilli were observed in the granuloma, primarily in the necrotic foci, but also in the epithelioid cells and giant cells of Langhans type.

3) Mesenteric lymph nodes

Changes in the capsule and pericapsular area: An edema, thickening of the capsule, proliferation of reticulo-endothelial cells and the connective tissue fibers, and infiltration of lymphocytes and eosinophils were observed, primarily in the areas of the afferent lymphatic vessels. A granulomatous endolymphangitis was observed in the afferent lymphatic vessels of severe cases (fig. 12). Also endolymphangitis obliterans and caseation of the granulomatous lesions were observed in some of the cases (fig. 13).

Changes in the cortex (figs. 14~16): The formation of characteristic epithelioid cell nodules (granuloma) and active reticulo-endothelial cells were observed in mild cases. The epithelioid cells showed a diffuse proliferation which resulted in conglomerated nodules in the severe cases. These granulomatous lesions were accompanied by a small number of lymphocytes and sometimes giant cells of the Langhans type. These granulomatous lesions also frequently showed necrosis and occasionally caseous degeneration in the center. Although the boundary between a granulomatous lesion and the adjacent area was generally well defined, sometimes transitional cells between epithelioid cells and swelled activated histiocytic cells were noted in the adjacent area. Some of the granulomatous lesions showed calcification and fibrosis. Dilatation of the subcapsular and medullary sinuses, lymph stagnation, and swelling and activation of the sinus endothelial cells were observed. Epithelioid cells and giant cells of Langhans type proliferated in the sinuses of some cases. Sometimes lymphocytes and eosinophils infiltrated in the cortex, and there was a marked infiltration of plasma cells in the medullary cords.

Changes in the lymphoid nodules: In mild cases, the lymphoid nodules featured a reactive hyperplasia. Enlargement of the germinal center with a slight swelling, paleness and proliferation of reticulo-endothelial cells were seen in some cases (fig. 19) Furthermore, a moderate edema of the reticular networks and the central capillary walls was found. Only a few cases showed karyorrhexis in the lymphoid nodules. The boundaries of the lymphoid nodules were relatively well defined (type I lesion in the lymphoid nodules). In

advanced cases, conspicuous edema was seen in the reticular networks and the walls of the blood capillaries of the enlarged germinal centers. Sometimes fibrinoid swelling or deposits of amyloid-like substances were seen on the walls of the capillaries (fig. 18). In some cases, there was an increase of nuclear debris and the boundaries of the lymphoid nodules, were

TABLE 2 *Relationship between lesions and detection of paratubercle bacilli*

GROUP	CASE NO	LIVER		KIDNEYS		INTESTINES		MESENT. LYMPH NODES	
		Granuloma	Bacilli	Glomerular lesions	Bacilli	Lesions	Bacilli	Lesions	Bacilli
I	1	###	—	##	—	###	##	###	+
	2	###	—	+	—	##	##	###	+
	3	##	—	##	—	###	##	###	±
	4	###	+	##	—	###	###	###	###
	5	###	+	##	—	###	###	###	+
	6	###	—	##	—	###	###	###	##
	7	•	•	•	•	###	##	###	+
	8	###	—	+	—	###	##	###	+
	9	###	—	###	—	###	##	###	+
	10	###	+	+	—	###	###	##	+
	11	###	+	###	—	###	###	##	##
	12	###	—	##	—	###	##	##	+
	13	##	+	###	—	###	###	##	##
	14	##	—	##	—	###	##	##	+
	15	##	+	##	—	###	###	•	•
	16	+	—	##	—	###	+	###	—
	17	+	—	###	—	###	###	+	+
	18	+	+	###	—	###	###	•	•
II	19	###	—	###	—	##	+	##	+
	20	###	+	###	—	*	±	##	##
	21	###	—	###	—	##	##	•	•
	22	##	—	+	—	+	+	##	+
	23	##	—	+	—	+	—	##	+
	24	+	—	+	—	###	—	##	—
	25	+	—	+	—	##	+	##	+
	26	+	—	+	—	+	+	##	+
	27	+	—	###	—	+	—	##	—
	28	—	—	+	—	+	+	##	+
III	29	+	—	###	—	—	—	+	—
	30	—	—	###	—	+	—	##	—
	31	+	—	##	—	+	—	+	—
	32	+	—	##	—	+	—	+	—
	33	+	+	##	—	+	—	+	—
	34	±	—	##	—	+	—	+	+
	35	±	—	##	—	—	—	+	+
	36	##	—	+	—	—	—	±	+
IV	37	—	—	###	—	—	—	—	—
	38	—	—	##	—	—	—	—	—
	39	—	—	##	—	—	—	—	—
	40	—	—	##	—	—	—	—	—
	41	—	—	##	—	—	—	—	—
	42	—	•	+	—	—	—	—	—
	43	—	•	+	—	—	—	—	—
	44	—	—	+	—	—	—	—	—
	45	—	—	+	—	—	—	—	—

N. B.: * Post mortem changes were severe.

well defined. Around the area of the lymphoid nodules, infiltration of lymphocytes and proliferation of histiocytic cells were observed (type II lesion in the lymphoid nodules). In another type of the lesion of the lymphoid nodules in advanced cases, desolation of the lymphoid nodules was conspicuous. The cells composing the germinal center decreased in number and showed loosening (type III lesion in the lymphoid nodules) (fig. 17). The boundaries of the lymphoid nodules were ill defined. This type of lesion of the lymphoid nodules was frequently observed in the severe fatal cases. Granulomatous foci were not usually observed in the germinal centers, except in some of the severe cases.

Changes in the medulla: The changes in the medulla were not as severe as those seen in the capsule or the cortex. In advanced cases, granulomatous lesions were observed and the lymphoid nodules were hardly recognizable. The medullary sinuses were dilated and sometimes showed a marked lymph stagnation and a large amount of lymphocytes were observed in the sinuses. The sinus endothelial cells were desquamated, and in the neighboring areas of the cortex, large desquamated reticulum cells were found. Eosinophils and neutrophils were observed, infiltrating the medullary sinus.

Acid-fast bacilli were observed in the granulomatous lesions of the capsule, cortex, and medullary cords. These bacilli were found primarily in the epithelioid cells and giant cells, and less frequently in the reticulo-endothelial cells and degenerative foci. However, free acid-fast bacilli were observed in the subcapsular and intermediate sinuses, but they were not found in the medullary sinus. These bacilli were not observed in the walls of the blood vessels or their endothelial cells, but many bacilli were seen in the endothelial cells of the intermediate sinus. In severe cases, the bacilli were sometimes found in the germinal centers. Numerous epithelioid cells containing bacilli were seen around the germinal centers of the severe cases. The number of bacilli which were observed in the tissue sections did not always correspond to the severity of pathological changes (tab. 2).

4) Liver lesions detected as remote lesions of paratuberculosis

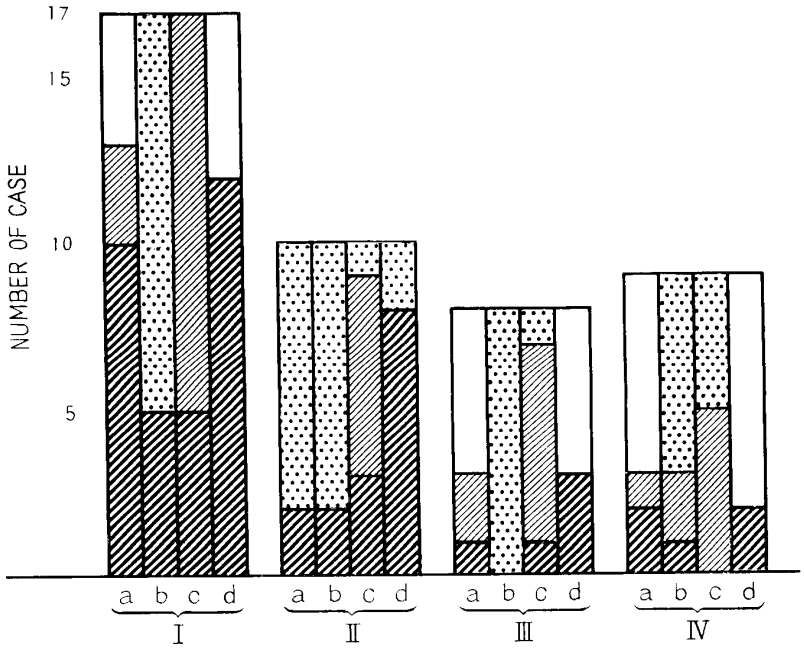
The characteristic liver lesions of paratuberculosis were granuloma formations. In advanced cases, enlarged and active sinusoidal endothelial cells were observed. Furthermore, small cellular nodules originating from the endothelial cells were seen and these nodules applied pressure to the peripheral hepatic cells. As these lesions developed, these nodules gradually invaded the peripheral hepatic parenchyma and formed granulomatous lesions with the epithelioid cells. Sometimes the granulomatous lesions were formed adjacent to the interlobular connective tissues. The granulomatous lesions consisted of large pale histiocytic cells originating from endothelial cells and some of the granulomatous lesions were replaced by epithelioid cells (fig. 21). Some granulomatous lesions were accompanied by small dark nuclear histiocytic cells and occasionally by a small number of lymphocytes and a blood capillary loop (fig. 22). A ZIEHL-NEELSEN stain was conducted in all cases examined, but only 1 or 2 bacilli were detected in one or two granulomas of a few cases (tab. 2).

5) Renal glomerular changes detected as remote lesions of paratuberculosis

Characteristic lesions were observed in the glomeruli of all cases. Generally the kidneys were anemic, but in some cases they showed a slight congestion. Almost all of the glomeruli had an edematous appearance. In advanced cases, there was an inflammatory exudate in

the capsular lumen and edema in the interstitial tissue or mesangium, and swelling of the endothelium of the capillary loops (fig. 23). There was also a greatly increased cellularity of the tuft due to the proliferation of the vascular endothelium and intercapillary connective tissue cells (figs. 24 & 25). These cellularity was especially marked at the vascular pole. In severe cases, dissolution and fibrosis of the glomeruli were observed. Furthermore, fibrinoid swelling or deposits of amyloid-like substances and thickening of the walls of the glomerular capsules were recognized (figs. 26~28). These relatively marked glomerular lesions were distributed diffusely in the kidneys. Furthermore, fibrinoid swelling or deposits of amyloid-like substances in the walls of the small blood vessels were also observed in the kidneys (fig. 29). A slight degree of nephrosis and sometimes calcium deposits were observed in the renal tubuli of many cases. In some cases the interstitial connective tissues showed a focal proliferation and a marked edematous swelling of the connective tissue fibers in the severe cases, primarily around the blood vessels. The relationship between the degree and type of glomerular lesions, in each group, is shown in table 3. The degree and distribution

TABLE 3 *Relationship between degree and type of glomerular lesions in each group*



N. B. a: Thickening of the glomerular capsules
b: Cellular proliferation in the glomerular loops
c: Edematous or fibrinoid swelling of the glomerular loops
d: Exsudation in the lumen of the glomerular capsules

Severe cases

Mild cases

Slight cases

Cases showed no characteristic lesions

of the glomerular lesions was calculated by the examination of 100 glomeruli in one section. From table 3, we may suggest that the glomerular lesions seen in group I were severe than those in the other groups. A ZIEHL-NEELSEN stain showed negative results in all the glomeruli examined (tab. 3).

6) Changes of the blood vessels and the connective tissues detected as remote lesions of paratuberculosis

Blood capillaries in the lymphoid nodules of the lymph nodes: A fibrinoid swelling or deposits of amyloid-like substances were frequently observed in the walls of the capillaries. These lesions were observed in the mesenteric and other lymph nodes of most of the cases examined. Lesions were found in the mesenteric lymph nodes of 34 out of 42 cases (we did not collect the mesenteric lymph nodes from 3 cases) and sometimes they were observed in several places of the same section. Other lymph nodes, in which lesions were observed were the hepatic lymph nodes (13/29) (fig. 30), bronchial lymph nodes (10/20), mediastinal lymph nodes (8/22), gastric lymph nodes (9/15), internal iliac lymph nodes (7/14), subiliac lymph nodes (4/4), superficial cervical lymph nodes (4/9), submandibular lymph nodes (6/13), superficial and profundal parotic lymph nodes (2/14), submaxillary lymph nodes (1/3), retropharyngeal lymph nodes (1/1), and inguinal lymph nodes (0/3). Lesions were observed in other lymph nodes in 34 cases. Consequently, these lesions were observed in 142 lymph nodes out of the 276 examined.

Interlobular artery of the kidneys: Fibrinoid swelling of the interlobular arterial walls of the kidneys was also observed and sometimes deposits of amyloid-like substances were noted.

Aorta: Hyalinous swelling of the smooth muscles fibers was seen in the walls of the aortae of 16 out of 22 cases investigated and in other cases, the walls were sometimes edematous. In the swollen muscle fibers, sometimes there were acidophilic, refractive and granular crystalized substances seen. Calcium deposits were also observed in the aortic intima of one case (Case No. 29).

Udder: Fibrinoid swellings or deposits of amyloid-like substances were observed, in 3 out of 22 cases, in the interstitium of the udder (fig. 32).

Adrenal glands: Fibrinoid swelling or deposits of amyloid-like substances were observed, in 30 cases out of 41, in the connective tissues of the boundary of the cortex and the medulla (fig. 31).

A specific staining technique was performed on the tissues just described and on the lymph nodes and kidneys of some cases containing amyloid-like substances. These substances showed positive results for the methyl violet stain and exhibited a reddish purple color. They also showed an intensely positive results to the PAS reaction, slight positive results to the Congo red stain and negative results to the toluidine blue metachromasia. They showed an orange yellowish color to the VAN GIESON stain and negative to the jod reaction.

7) Lesions in the other organs and tissues

Granulomatous lesions were observed in the spleen (3/44; Case Nos. 2, 5 & 16) (fig. 20), thymus (1/14; Case No. 41) and hepatic lymph nodes (3/29; Case Nos. 4, 5 & 26), except the intestines, mesenteric lymph nodes and liver.

DISCUSSION

The characteristic lesions of paratuberculosis in goats found in the present investigation were located mainly in the intestines and mesenteric lymph nodes. These findings coincide with the reports of previous workers[7,8,10,~12,15,25,36,40,42]. However, other lesions which were attributed to *M. johnei* were pointed out in various parts of the other organs and tissues of the body.

From the results of our present investigation, it seems reasonable to assume that *M. johnei* infection spread mainly by way of the lymphatic stream from the intestines, but in some cases it spread by way of the blood stream. On the basis of experimental studies in goats, HARDING stated that first there is a progressive lymphatic spread with organisms eventually reaching the blood stream through the thoracic duct; and second there is a direct invasion of the portal venous circulation. He pointed out the fact that the latter can occur has been shown by the demonstration of an infected macrophage in an intra-hepatic branch of the portal vein: the distribution of lesions within the liver lobules also suggests this route of spread. The presence of lesions in the central hepatic veins indicated that the infection probability spreads to the systemic circulation through the liver. RAJYA & SINGH stated that in most cases in sheep that they studied, the infection spreads through the lymphatics, but when the lesions become extensive, infected macrophages and organisms may enter the portal circulation and might also be distributed to other organs of the body. HALLMAN & WITTER's findings indicated the lymphatic route of spread in cattle. HOUTHUIS pointed out the hematogenous spread in addition to the lymphatic spread in cattle. This study revealed that paratubercle bacilli primarily invaded the intestinal mucosa and produced a proliferative granulomatous inflammation. Acid-fast bacilli were phagocytized by the epithelioid cells (macrophages) and neutrophils. These cells containing bacilli suggested infection of the lymphatic vessels and produced characteristic lesions in the submucosa and subserosa. From the findings of intimagranuloma, endolymphangitis and perilymphangitis, it clearly pointed towards the lymphatic spread. The paratubercle bacilli may reach the lymph node and finally to the lymphatic stream via vasa afferentia. Furthermore, they may reach the blood stream through the thoracic duct. On the other hand, the bacilli may reach the liver from the intestines through the portal venous circulation. This route of spread is suggested by the presence of bacilli in the liver, even though very few were found in this study. A systemic bacteremia is also suggested by the bacteriological investigation of goats by LEVI[20,21], HIRCH & LAWRENCE and HARDING, and cattle by ALEXEJEFF-GOLOFF, and TAYLOR. However, the author knew that it is not sufficient to study the pathology of the disease by only looking at the bacteremic events.

The histopathology of paratuberculosis seems to be somewhat different between animal breeds. M'FADYEAN and HALLMAN & WITTER considered the absence of necrosis or caseation to be a feature distinguishing paratuberculosis from tuberculosis in cattle. However the present authors, RAJYA & SINGH (sheep), STAMP & WATT (sheep), LEVI (goats)[20] and HARDING (goats) all found caseation, calcification and even fibrous tissue encapsulation, though HATAKEYAMA et al. did not find these changes in their sheep cases. RAJYA & SINGH stated that these changes did occur in the lymph nodes of sheep and the degenerative changes may result from symplasm. STAMP & WATT attributed the possible cause of these various degenerative changes to be due to variations in the strains of the organisms.

On the other hand, one manifestation of the tissue response of the living body, seen on dead cases of groups I and II was a large number of bacilli in sections of the intestines and mesenteric lymph nodes. In such cases, the lymphoid nodules of the lymph nodes showed a tendency toward loosening, desolation and collapsed features (type III lesion in the lymphoid nodules). The lymphoid nodules of those cases having a small bacilli in their tissue sections by comparison with the severity of lesions in groups I and II showed a tendency to have fibrinoid swelling or deposits of amyloid-like substances in the walls of the capillaries and reticular net works (type II lesion in the lymphoid nodules). The cases from groups III and IV had almost no bacilli in their tissue sections. The lymphoid nodules of such cases showed a tendency to have a reactive hyperplasia (type I lesion in the lymphoid nodules).

Some of the discrepancies between the presence of active lesions and the detection of *M. johnei* in sections posed a question. This fact has already been pointed out by many workers[7,8,24,36]. However, these discrepancies were especially marked in the remote lesions found in our study. MCEWEN suggested that resistance or immunity may sometimes occur and that the bacilli are effectively disposed of and the epithelioid cell accumulations remaining serve as witness to the former active infection. HARDING suggested that in some instances the organisms had probability just reached the organ and had not yet had time to give rise to histologically recognizable changes; or on the other hand that lesions persisted for sometime after the organisms had been killed by the body defences. HALLMAN & WITTER suggested two possibilities: First in the early lesions of the first stage, a few rods in the macrophages stimulated an extensive increase of macrophages or else the macrophages destroy the engulfed bacteria, a property which, if possessed at this time, is apparently lost as the disease progress. In this study, granulomas, which were found in the liver, spleen, thymus and hepatic lymph nodes, detected as remote lesions were negative for the acid-fast bacilli,

except for the liver which had only a few bacilli in one or two granulomas. The authors would like to emphasize the existence of remote effects due to hypersensitivity as an additional modified lesion of the disease, even though the meaning of the primary bacteremia of the disease is significant.

We must use caution in judging whether or not the fibrinoid swelling is a true indicator of the allergic reaction and there is much controversy as to the nature of this fibrinoid materials. However the authors considered the material which we described as fibrinoid swelling in this study, as fibrinoid in a broad sense from the results of our histochemical investigations. Because this material was intensely positive to the PAS reaction (altered mucopolysaccharide) and showed an orange-yellowish color to the VAN GIESON stain, and negative results to the toluidine blue metachromasia. On the other hand, some of the materials seemed to have a nature somewhat near amyloid substances, because they showed a weak positive Congo red stain, a positive methyl violet stain and a negative jod reaction. However as these results are inconclusive, we tentatively called these materials fibrinoid or amyloid-like substances. It is a well known fact that fibrinoid swelling is observed in chronic diseases. But many workers[33] consider that fibrinoid swelling may occur due to an allergic mechanism. OKABAYASHI[33] stated that fibrinoid swelling represents the morphological basis of the so-called "vasculo-mesenchymal tissue reaction" as allergic changes of an infection. OKABAYASHI[34], on the basis of experimental studies on collagen disease, primarily in systemic lupus erythematosus, stated that in chronic dys-gamma-globulinemia, a series of metabolic disturbances of degeneration, such as fibrinoid, amyloid, para-amyloid and hyaline degenerations appear in the systemic connective tissues. He also stated that if a dysoria like aggravation takes place it may result in severe serous exudation during these processes, then the histological findings will become more complicated by the piling up of fibrinoid materials during degenerative changes. KASUGA also stated that fibrinoid, amyloid and hyaline materials are histochemically and morphologically interchangeable. From the above findings, it might be said that the so-called fibrinoid, hyaline and amyloid materials may be related to each other.

The most noteworthy lesions seen in the disease are the renal glomerular changes. There has been some description on the renal lesions of the disease, by HARDING, who pointed out an interstitial nephritis and discussed its relationship between the acid-fast bacilli and the lesions. But there has been almost no description of glomerular changes in the past. The detection of acid-fast bacilli in kidney sections was negative in all cases examined in this study (tab. 2). Distribution of glomerular lesions was rather diffuse and these renal changes seemed to be categorized as an allergic glomerulonephritis. Although there are

many reports on allergic glomerulonephritis in experimental animals[23,26~28,32,35]. The incidence of the disease in the domestic animals is very low. But this is not strange. EBERBECK considered cases of diffuse glomerulonephritis in horses which appeared after the infection of strangles or infectious bronchitis, as an allergic nature. If we consider this change as an allergic nature, it may be a delayed reaction (bacterial type). It occurs most frequently in conjunction with living organisms. As the primary factor of its occurrence, the tissues need repeated contact with the organisms. In our cases, the occurrence of the disease has been continued for many years on the same farm. Repeated infection was thus possible, because this farm was severely infected with paratubercle bacilli. We should like to state here that the glomerular changes were a vasculo-mesenchymal tissue reaction. JONES[16~18] applicated the same interpretation to the glomerulonephritis by regarding the site of inflammation as extracapillary connective tissues. FUJIMOTO stated that the glomerular structure consists of capillary loops, intercapillary connective tissues and epithelium, and the pathological changes occurred in the capillary loops and the intercapillary connective tissues. OKABAYASHI[33,34] regarded the changes of diffuse glomerulonephritis as a vasculo-mesenchymal tissue reaction of the capillary loops and the mesangium. He also stated that histological changes of MASUGI's allergic nephritis are characterized by fibrinoid swelling of the capillary loops. Therefore the glomerular changes observed in this study are regarded as a vasculo-mesenchymal tissue reaction by an allergic mechanism.

The origin of epithelioid cells or giant cells which compose granulomas of paratuberculosis poses some varied opinions[2~4,22,25,36]. Generally speaking the granulomatous inflammation is a highly specific reaction of the reticulo-endothelial system. These productive processes show the transitional features between histiocytes belonging to the reticulo-endothelial system and to the epithelioid cells. Epithelioid cells have a tendency to form giant cells of Langhans or foreign body types. Therefore we considered the epithelioid cells or giant cells of paratuberculosis as reticulo-endothelial in origin.

Many workers attach importance to the direct action of the living organisms for the formation of granulomatous lesions. The authors do not deny this fact, but some questions arise from the discrepancies between the presence of active lesions and detection of bacilli. The granulomatous lesions of paratuberculosis also take place due to killed paratubercle bacilli like in tuberculosis[37]. Therefore, in regarding the mechanism of the formation of the epithelioid granulomas as the remote lesions, it may be postulated that the formation of the granulomatous lesions is not due only to the action of living paratubercle bacilli themselves, but rather due to a remote reaction. This remote reaction is considered of a tissue

reaction in the living body against the repeated infection of paratubercle bacilli in the intestinal tracts and that the formation of the granuloma is founded on the immunological tissue response due to an allergic mechanism. In order to understand this disease, we must consider the glomerular lesions and the changes of the general blood vessels and connective tissues, except the direct lesions due to living paratubercle bacilli. For that reason the disease in the living body is the basis for allergic mechanism.

SUMMARY

A histopathological study was carried out on 45 cases of naturally infected paratuberculosis in goats and following results were obtained:

1) The characteristic lesions of the disease were located mainly in the intestines and the regional lymph nodes. The authors considered the mode of development of the lesions to be spread mainly by way of the lymphatic stream from the intestines and by way of the blood stream.

2) In regard to the remote lesions, characteristic glomerulonephritis, fibrinoid swelling or deposits of amyloid-like substances in the walls of the capillaries and connective tissues, and granulomas in the various parts of the organs and tissues, except the intestines and the mesenteric lymph nodes, were found.

3) Special attention was paid to the pathogenesis of the remote lesions and discussed as the pathological character of the disease. The author eventually emphasized that the disease is not only due to the direct reaction of the *M. johnei*, but also due to vasculo-mesenchymal tissue reaction (allergic nature) as a systemic disease.

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EXPLANATION OF PLATES

PLATE I

Fig. 1 Case No. 9 Thickening and folding of the small intestine mucosa × 1.6

Fig. 2 Case No. 9 Thickening of the colon mucosa × 1.6

Fig. 3 Case No. 4 Diffuse proliferation of epithelioid cells in the mucosal lamina propria of the small intestine
Hematoxylin-eosin stain × 81

Fig. 4 Case No. 3 Diffuse proliferation of epithelioid cells and a few giant cells of Langhans type in the mucosal lamina propria of the small intestine
H.-E. × 325

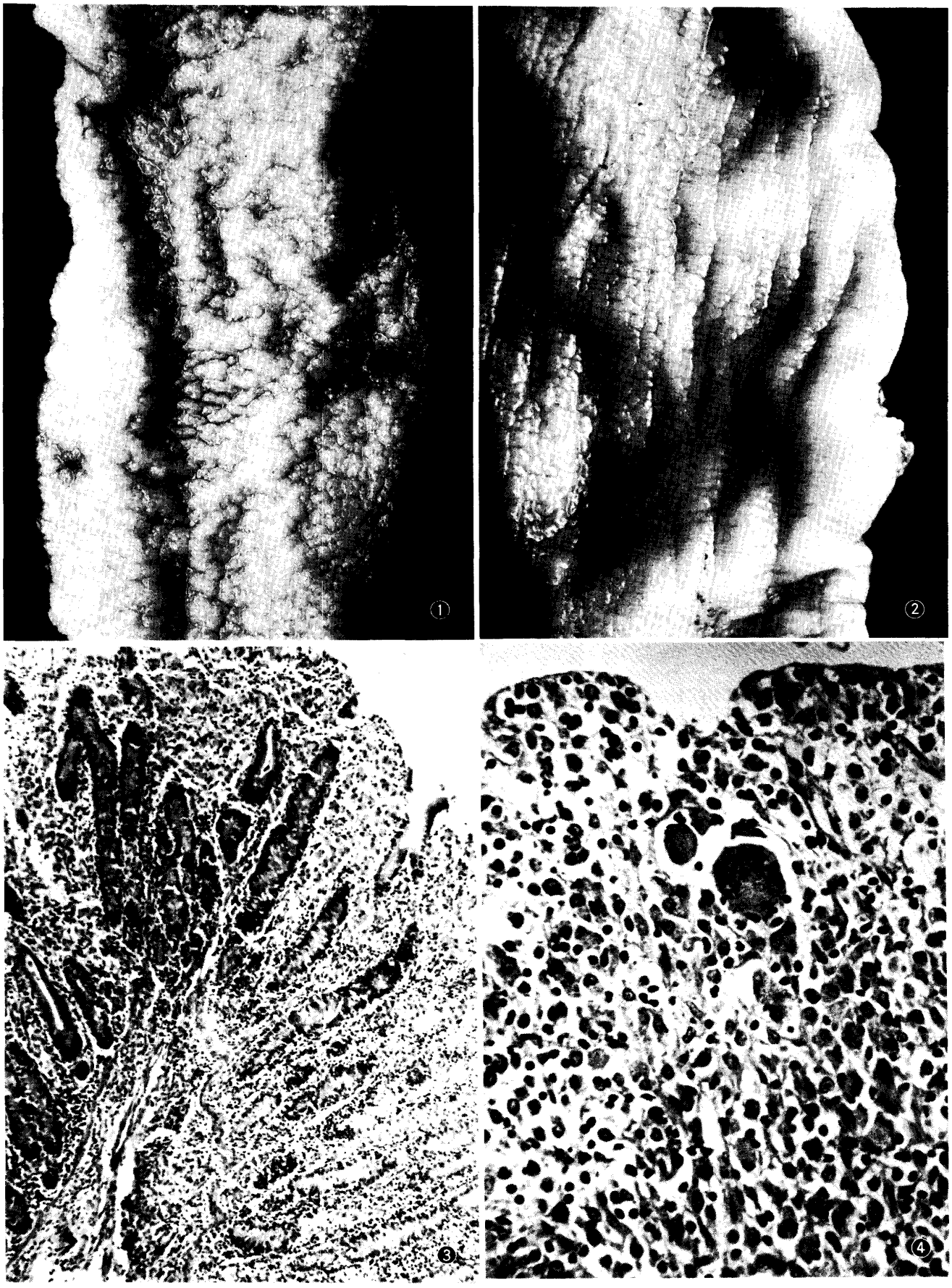


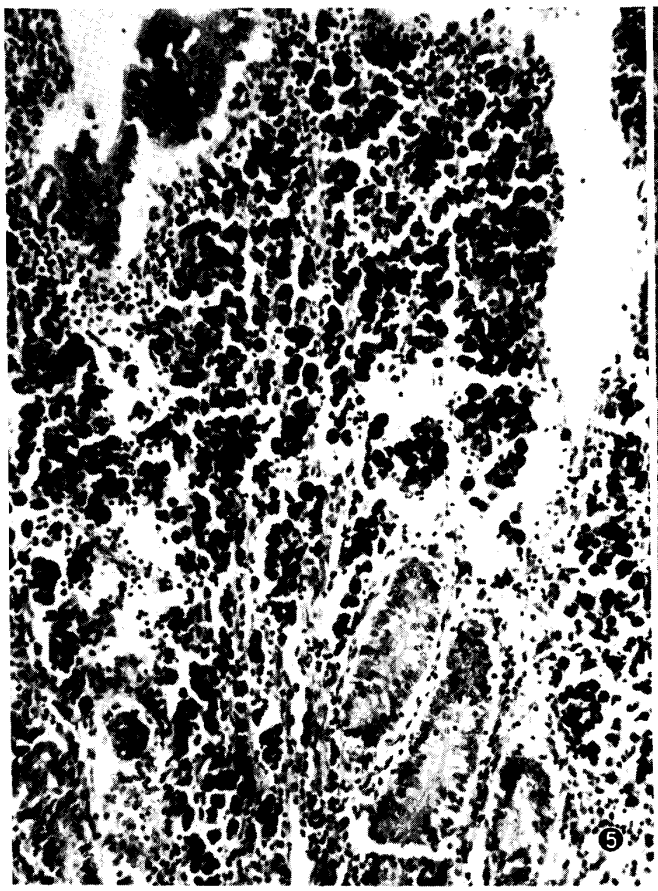
PLATE II

Fig. 5 Case No. 4 A large number of epithelioid cells with phagocytized acid-fast bacilli, in the mucosal lamina propria of the small intestine
ZIEHL-NEELSEN stain × 132

Fig. 6 Case No. 14 Endolymphangitis, lymphocytic infiltration, proliferation of histiocytic cells and edema in the submucosa of the small intestine
H.-E. × 81

Fig. 7 Case No. 10 Nodular endolymphangitis and perilymphangitis in the submucosa of the small intestine
H.-E. and ZIEHL-NEELSEN stain × 81

Fig. 8 Case No. 10 Phagocytized acid-fast bacilli in the epithelioid cells and a giant cell in the lumen of the lymphatic vessel of Fig. 7
H.-E. and ZIEHL-NEELSEN stain × 510



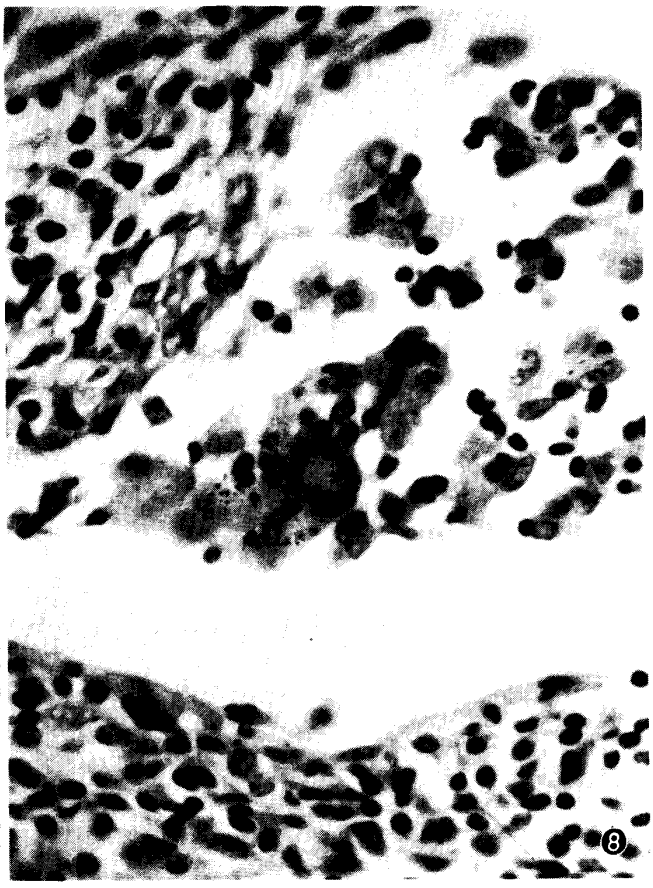


PLATE III

Fig. 9 Case No. 10 Endolymphangitis (intimagranuloma) in the subserosa of the small intestine
H.-E. × 325

Fig. 10 Case No. 16 Focal caseous degeneration (endolymphangitis obliterans and perilymphangitis) in the subserosa of the small intestine
H.-E. × 81

Fig. 11 Case No. 1 Perilymphangitis in the subserosa of the small intestine
H.-E. × 132

Fig. 12 Case No. 2 Granulomatous endolymphangitis accompanied by epithelioid cells and giant cells in the pericapsular tissue of the mesenteric lymph node
H.-E. × 132

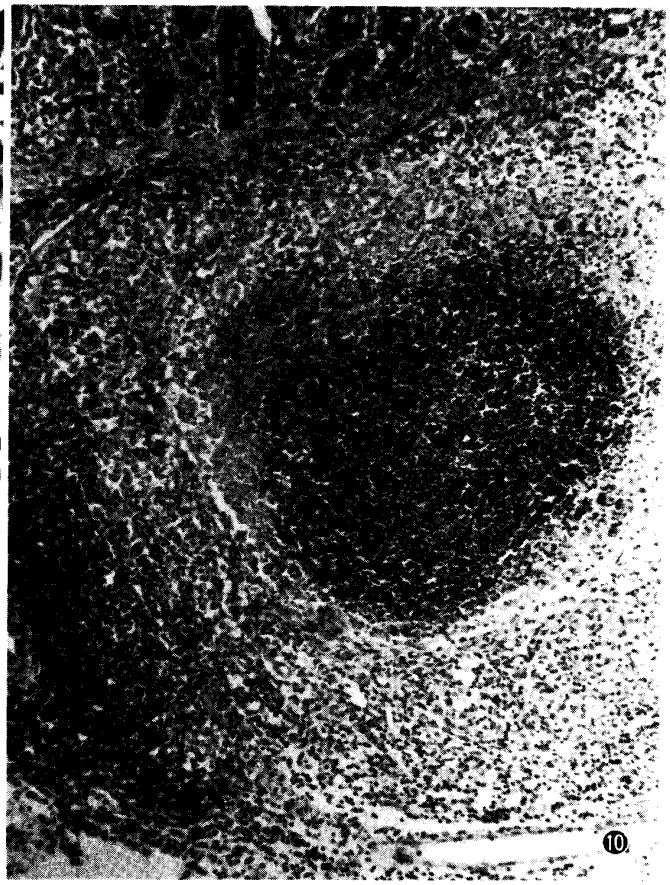


PLATE IV

Fig. 13 Case No. 1 Granulomatous and obliterative endolymphangitis with caseous degeneration in the capsule of the mesenteric lymph node
H.-E. × 81

Fig. 14 Case No. 10 Focal caseous degeneration of the cortical area of the mesenteric lymph node
H.-E. × 81

Fig. 15 Case No. 2 Proliferation of epithelioid cells and giant cells of Langhans type in the peripheral sinus of the mesenteric lymph node
H.-E. × 81

Fig. 16 Case No. 1 Multiple epithelioid cell granulomas in the mesenteric lymph node
H.-E. × 81

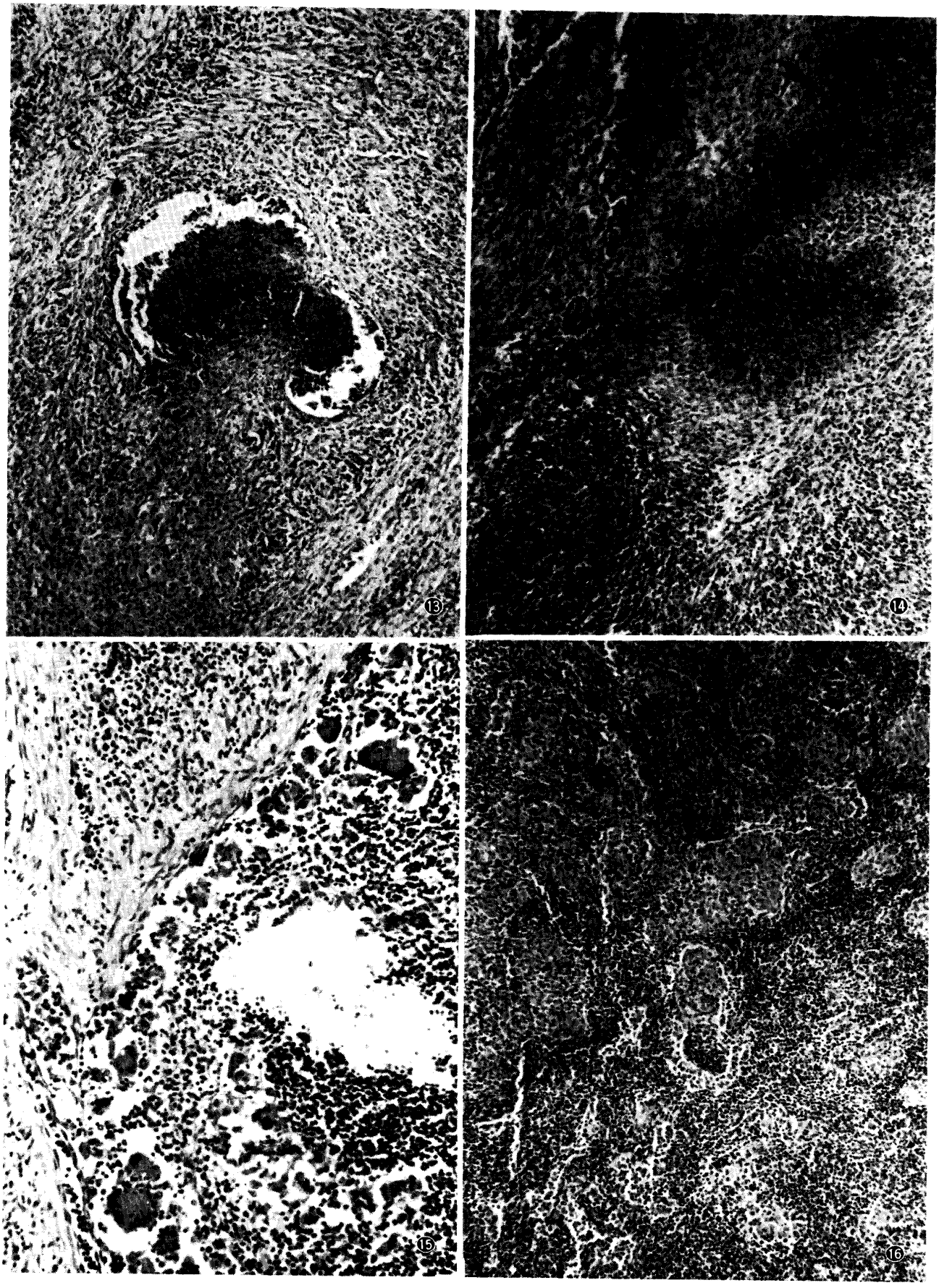

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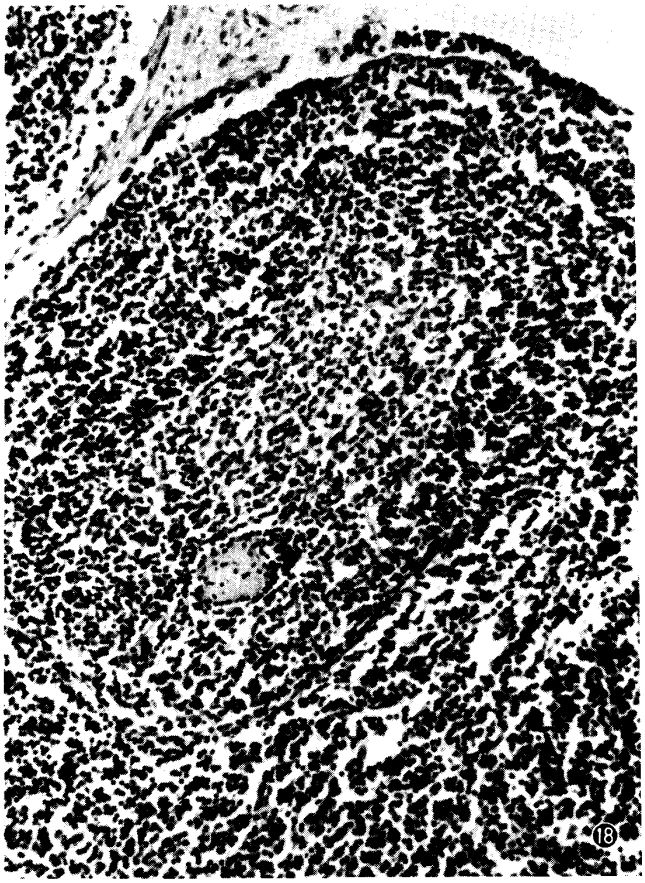
PLATE V

Fig. 17 Case No. 17 Lymphoid nodules of the mesenteric lymph node having a loosen and collapsed appearance (type III)
H.-E. × 81

Fig. 18 Case No. 26 A lymphoid nodule of the mesenteric lymph node showing fibrinoid swelling or deposits of amyloid-like substances in the wall of a capillary (type II)
H.-E. × 132

Fig. 19 Case No. 32 Lymphoid nodules of the mesenteric lymph node showing active hyperplasia
H.-E. × 81

Fig. 20 Case No. 16 A granuloma in the splenic red pulp
H.-E. × 325



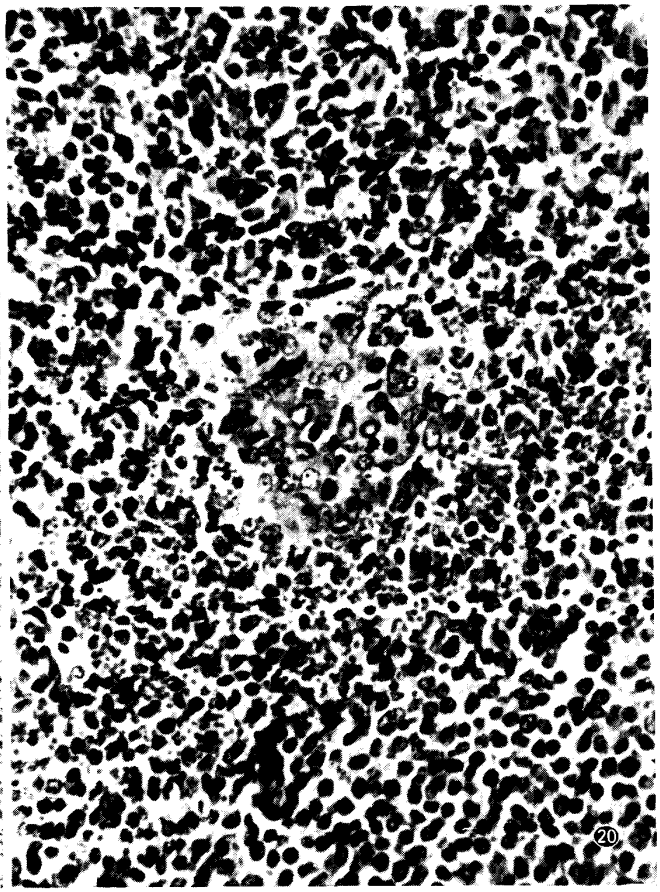


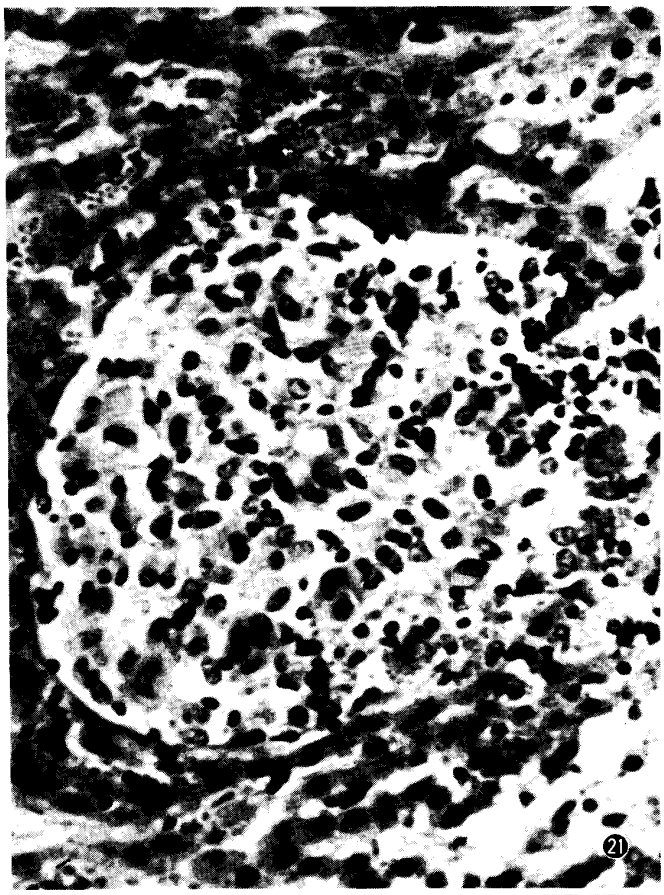
PLATE VI

Fig. 21 Case No. 12 An epithelioid cell granuloma in the liver
H.-E. × 325

Fig. 22 Case No. 19 A hepatic granuloma consisting of epithelioid cells in the center and with small dark nuclear histiocytic cells and a small number of lymphocytes on the periphery. A capillary loop is present in the center of the granuloma.
H.-E. × 325

Fig. 23 Case No. 19 Glomerular lesions in the kidney: Precipitation of proteinous substances in the capsular space. Swelling and proliferation of epithelial and endothelial cells in the glomerulus. This proliferation is especially marked at the vascular pole.
H.-E. × 325

Fig. 24 Case No. 40 Glomerular lesions in the kidney: Exudation in the capsular space, enlargement of the glomerulus, and proliferation of epithelial and endothelial cells in the glomerulus
H.-E. × 325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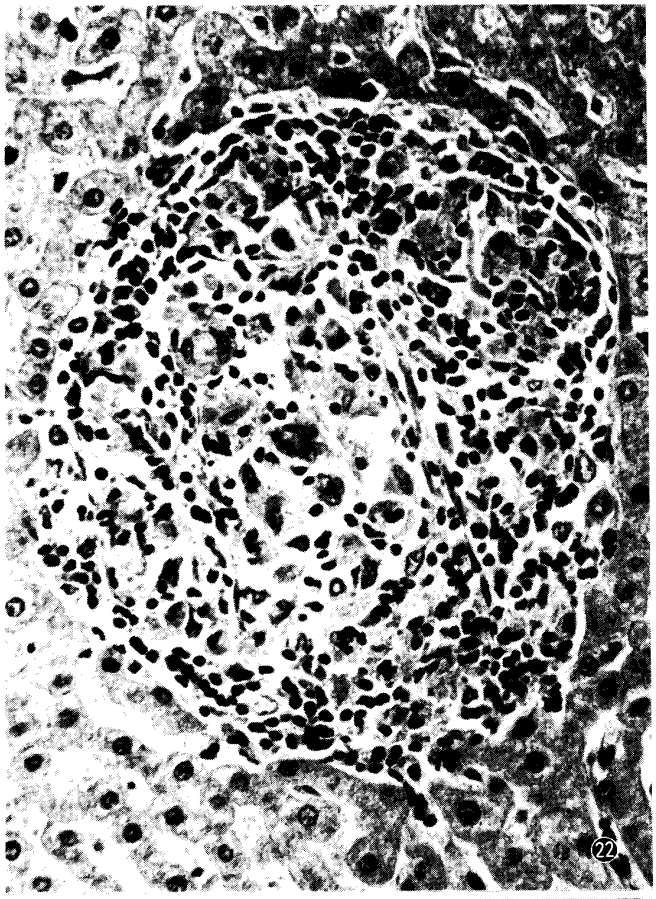
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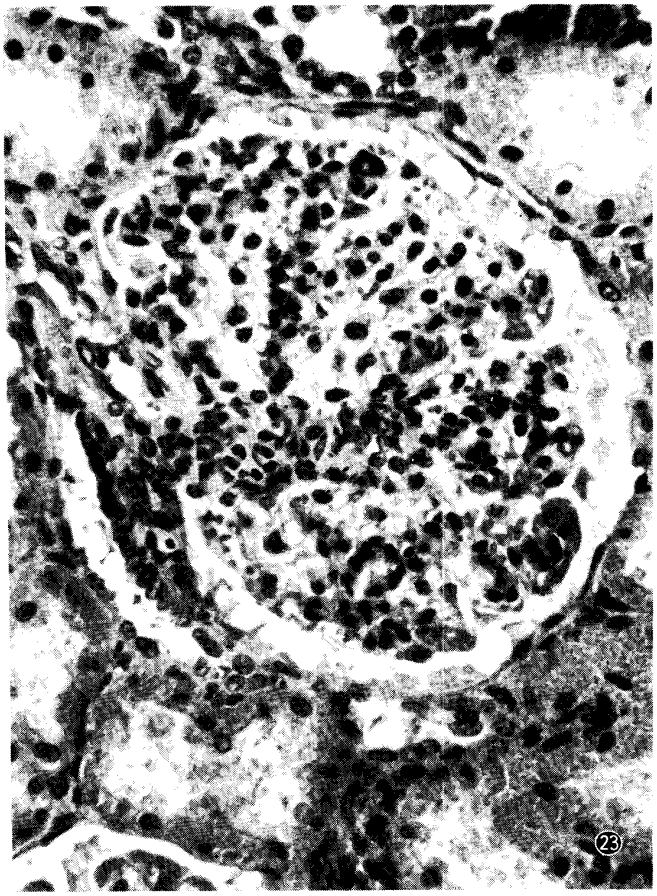
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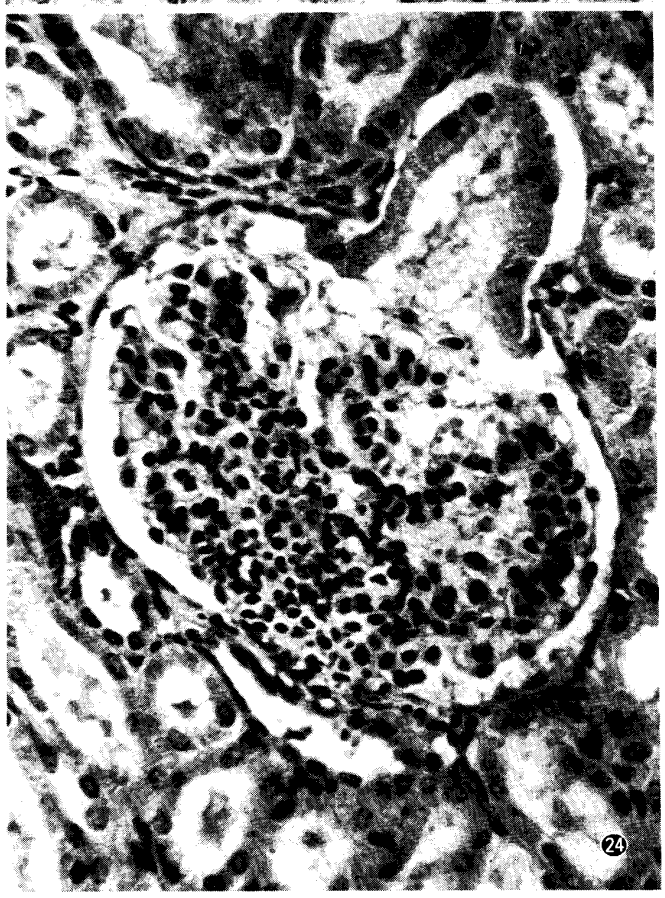
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PLATE VII

Fig. 25 Case No. 13 Glomerular lesions in the kidney: Marked enlargement of the glomerulus, edema and swelling of the intercapillary connective tissue, proliferation of epithelial and endothelial cells and fibrin thrombus (arrow)
H.-E. × 325

Fig. 26 Case No. 37 Glomerular lesions in the kidney: Marked enlargement of the glomerulus, fibrinoid swelling of the intercapillary connective tissue and proliferation of epithelial and endothelial cells
H.-E. × 325

Fig. 27 Case No. 17 Glomerular lesions in the kidney: A marked fibrinoid swelling is present in the intercapillary connective tissue of the glomerulus and Bowman's capsule.
H.-E. × 325

Fig. 28 Case No. 18 Deposits of amyloid-like substances in the glomerulus of the kidney
H.-E. × 325

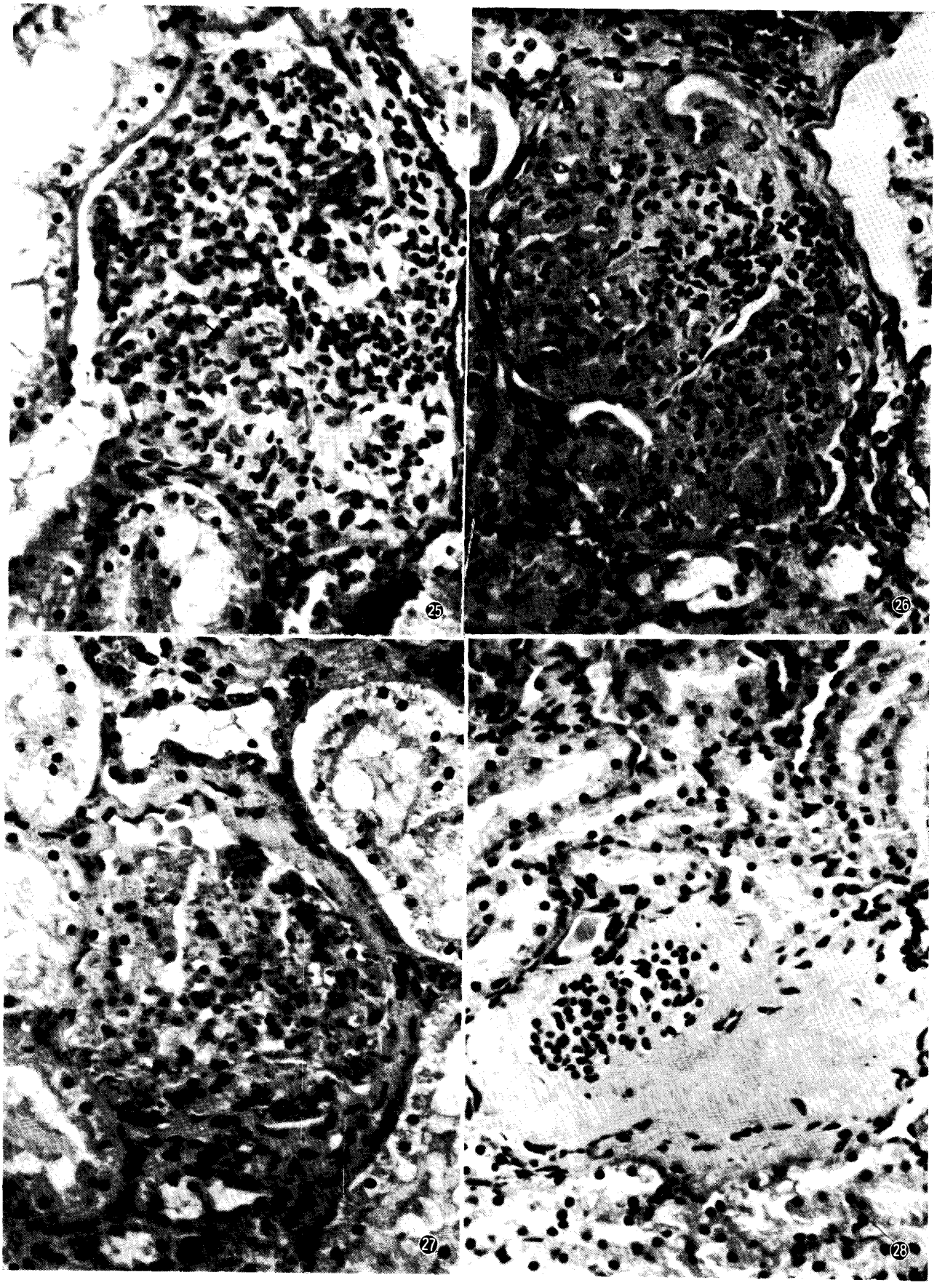

PLATE VIII

Fig. 29 Case No. 18 Deposits of amyloid-like substances in the wall of the *Arteria interlobularis*
H.-E. × 325

Fig. 30 Case No. 14 Hepatic lymph node: Deposits of amyloid-like substances in the wall of the capillary of the lymphoid nodule
H.-E. × 132

Fig. 31 Case No. 2 Fibrinoid swelling or deposits of amyloid-like substances in the connective tissue of the adrenal cortico-medullary junction
H.-E. × 132
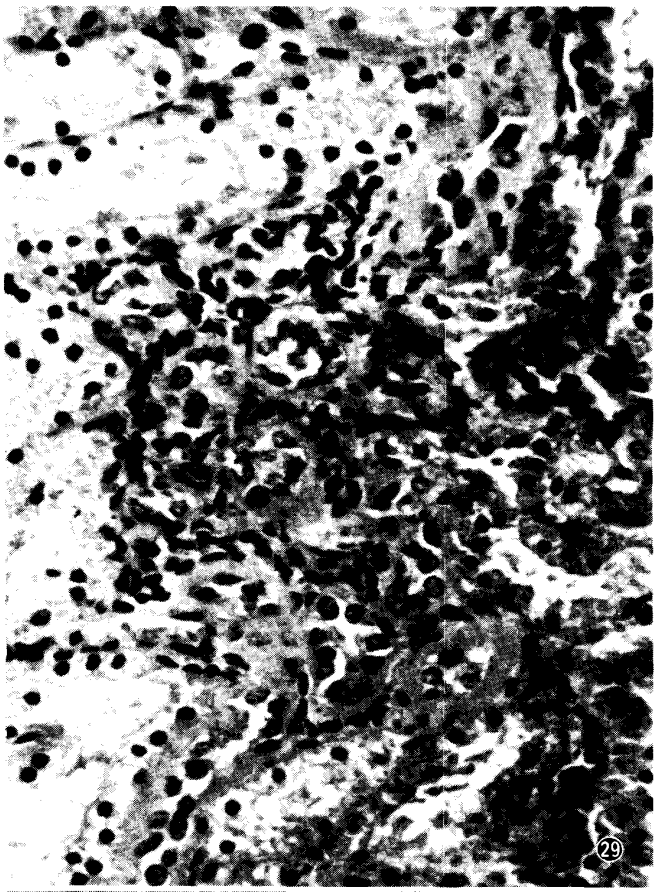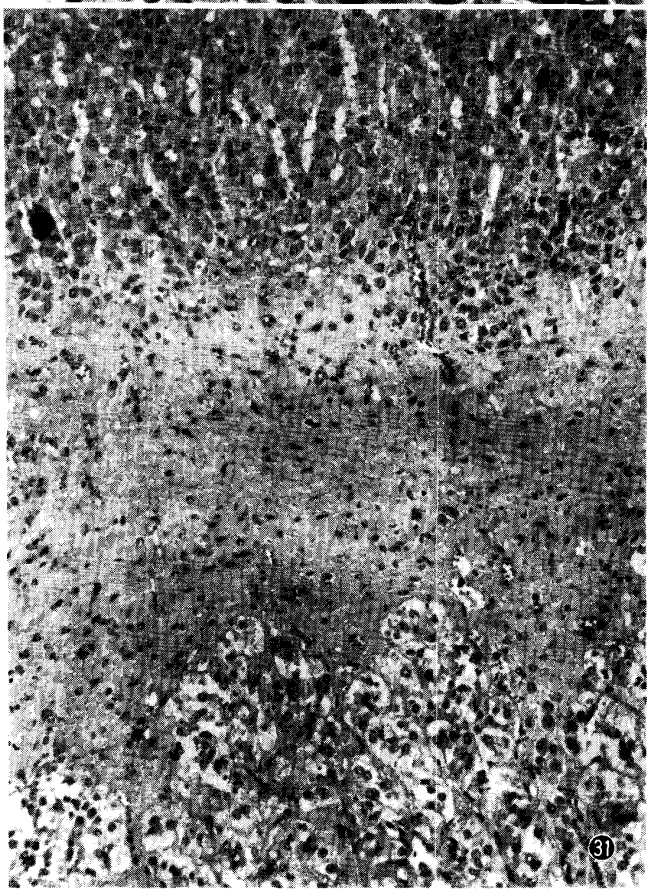
Fig. 32 Case No. 33 Fibrinoid swelling or deposits of amyloid-like substances in the connective tissue around the glandular epithelial cells of the mammary gland
H.-E. × 325