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Ibaraki Virus, an Agent of Epizootic Disease of Cattle Resembling Bluetongue

I. Epidemiologic, Clinical and Pathologic Observations and Experimental Transmission to Calves

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ABSTRACT

Outbreaks of an acute febrile disease of cattle occurred in Japan in 1959 and 1960. Its occurrence was limited in late summer and autumn, and in Kyushu, Shikoku and Honshu roughly south of 37 degrees north latitude, suggesting a close correlation of the incidence with the climatic conditions, hence a possibility of the presence of arthropod vector. The disease was characterized by fever and lesions affecting the mucous membrane and skin, musculature and vascular system. Degeneration of striated muscles was observed in the esophagus, larynx, pharynx, tongue and skeletal muscular system. Edema and hemorrhage were marked in the mouth, lips, abomasum, coronets etc., occasionally followed by degeneration of the epithelium leaving erosions or ulcerations. Severe lesions affecting the esophageal and laryngopharyngeal musculature caused deglutitive difficulty which in turn resulted in dehydration and emaciation, and occasionally in aspiration pneumonia, constituting the major causes of death of the affected animals. These findings indicate that the disease resembles bluetongue in sheep and cattle. The clinical materials obtained from natural cases induced a clinical illness when inoculated into calves, and the disease was transmitted serially in calves by intravenous inoculation of the blood obtained at the height of febrile reaction. The experimentally produced disease was clinically and pathologically indistinguishable from the natural disease.

An outbreak of an acute febrile disease of cattle swept over the central and western parts of Japan during the months from August through December in 1959. A more limited outbreak of the disease occurred in the central Japan from September to December in 1960. The disease was characterized by fever, stomatitis and deglutitive difficulty. The clinical, and pathologic picture was very reminiscent of bluetongue in sheep and cattle. The disease was readily transmitted to calves by inoculation with the blood or tissues from naturally affected cattle. A cytopathogenic virus was recovered in bovine cell cultures from naturally and experimentally infected cattle. The isolated virus, designated as "Ibaraki virus", was found to resemble bluetongue

virus in its biological and physiochemical properties, and evidence was obtained for the virus to be the causative agent of the disease.

The disease was once confused with another epizootic disease of cattle, "bovine epizootic fever" or "bovine influenza" [8, 9, 20], which is similar to ephemeral disease, recognized in Australia and other countries, in the clinical and pathological features. The causative agent of bovine epizootic fever has been identified and is distinct from Ibaraki virus [12, 13, 19].

Preliminary reports on these studies have been published [11, 17]. The present communication describes in more detail the epidemiologic, clinical and pathologic observations of the disease, and the results of its experimental transmission to calves.

Epidemiologic Observations

The disease was first recognized in the southern parts of Japan in August of 1959, and swept over the southern and central parts of Japan during the months from August through December. As shown in Table 1, reported cases amounted to 39,076 with 4,023 deaths. The morbidity reached 1.96% of the total cattle population in the prefectures involved in the outbreak. The mortality was 0.20% and the case fatality was 10.3%.

The morbidity varied among the prefectures (Table 1, Fig. 1). Aichi and Mie Prefectures were most severely attacked, the morbidity reaching 12.6% and 10.3%, respectively. In Tohoku District, the northern part of the main island Honshu, and Hokkaido Prefecture, the northernmost island, no cases were reported. The disease was prevalent in Kanto District except Tokyo Prefecture, and in Chubu and Kinki Districts except the prefectures bordering Sea of Japan and the mountainous Yamanashi and Nagano Prefectures. The disease was conspicuously absent in Hyogo, Tottori and Okayama Prefectures and reappeared in Hiroshima and other prefectures located in the western end of the main island Honshu. In Shikoku and Kyushu most prefectures were involved in the outbreak.

The 1960 outbreak involved only parts of Chubu and Kinki Districts (Table 1, Fig. 1). A total of 4,717 cases was reported with a morbidity of 1.16%, a mortality of 0.06% and a case fatality of 5.8%.

As shown in Table 2, in 1959 the disease was first recognized in August in Miyazaki and Kagoshima Prefectures of Kyushu and Kochi Prefecture of Shikoku, and spread to the adjoining prefectures. About one month later in September the disease began to occur in Ibaraki Prefecture of Kanto. Cases were initially reported from the rice paddy field areas of the Tone River basin and the disease spread to the adjoining areas in Ibaraki, Chiba and Saitama Prefectures. Some weeks later the outbreak was reported in other districts, i.e. Chubu, Kinki and Chugoku. The epidemic lasted for 2 to 3 months in each prefecture where a substantial number of cases occurred, and terminated by the end of November in most prefectures and in December in some. The peak of the outbreak was reached in October or November in most prefectures and in September in a few southern prefectures. The 1960 outbreak occurred from the latter part of September through December with a peak in October, although a few cases were recognized early in September in Aichi Prefecture.

The seasonal incidence and geographical distribution of cases suggest a close correlation of the incidence with the climatic conditions, hence a possibility of the presence of arthropod vector.

The disease tended to occur rather sporadically. In Mie and Aichi Prefectures

Table 1. Reported cases and deaths in the 1959 and 1960 epizootics[1]

District	Prefecture	Population of cattle	No. of cases	No. of deaths	Morbidity %	Mortality %	Case fatality %
1959							
Kanto	Ibaraki	96,520	4,330	593	4.49	0.61	13.7
	Tochigi	59,604	1,664	291	2.79	0.49	17.5
	Gumma	87,630	820	79	0.94	0.09	9.6
	Chiba	101,940	2,508	294	2.46	0.29	11.7
	Saitama	72,570	1,528	164	2.11	0.23	10.7
	Tokyo	18,520	4	2	0.02	0.01	50.0
	Kanagawa	50,010	481	63	0.96	0.13	13.1
Chubu	Nagano	98,470	6	1	0.01	0.00	16.7
	Shizuoka	61,460	3,001	342	4.88	0.55	11.4
	Aichi	66,500	8,364	519	12.58	0.78	6.2
	Gifu	50,650	2,724	265	5.38	0.52	9.7
	Mie	62,500	6,455	717	10.33	1.15	11.1
Kinki	Shiga	39,280	302	61	0.77	0.16	20.2
	Kyoto	45,660	204	7	0.45	0.02	3.4
	Nara	24,980	821	92	3.29	0.37	11.2
	Osaka	39,170	92	18	0.23	0.05	19.6
	Wakayama	38,390	224	40	0.58	0.10	17.9
Chugoku	Hiroshima	118,920	1,390	124	1.17	0.10	8.9
	Shimane	71,960	63	5	0.09	0.07	7.9
	Yamaguchi	81,030	211	19	0.26	0.02	9.0
Shikoku	Tokushima	57,170	104	11	0.18	0.02	10.6
	Kochi	40,620	210	34	0.52	0.08	16.2
	Kagawa	57,150	69	2	0.12	0.00	2.9
	Ehime	67,670	799	119	1.18	0.18	14.9
Kyushu	Fukuoka	81,330	395	6	0.49	0.01	1.5
	Oita	87,100	158	11	0.18	0.01	7.0
	Kumamoto	105,020	727	66	0.69	0.06	9.2
	Miyazaki	78,910	1,149	65	1.46	0.08	5.7
	Kagoshima	129,420	273	31	0.21	0.02	11.4
	Total	1,990,154	39,076	4,023	1.96	0.20	10.3
1960							
Chubu	Nagano	98,980	6	2	0.01	0.00	33.3
	Shizuoka	62,430	707	73	1.13	0.11	11.5
	Aichi	65,170	2,085	27	3.20	0.04	1.3
	Gifu	52,250	826	91	1.58	0.17	11.0
	Mie	63,180	1,013	76	1.60	0.12	8.2
Kinki	Shiga	39,140	38	3	0.10	0.00	26.3
	Nara	25,740	42	3	0.16	0.01	7.1
	Total	406,890	4,717	275	1.16	0.06	5.8

[1] Cited from Statistics of Animal Hygiene, 1960, Bureau of Animal Industry, Ministry of Agriculture and Forestry, Japan.

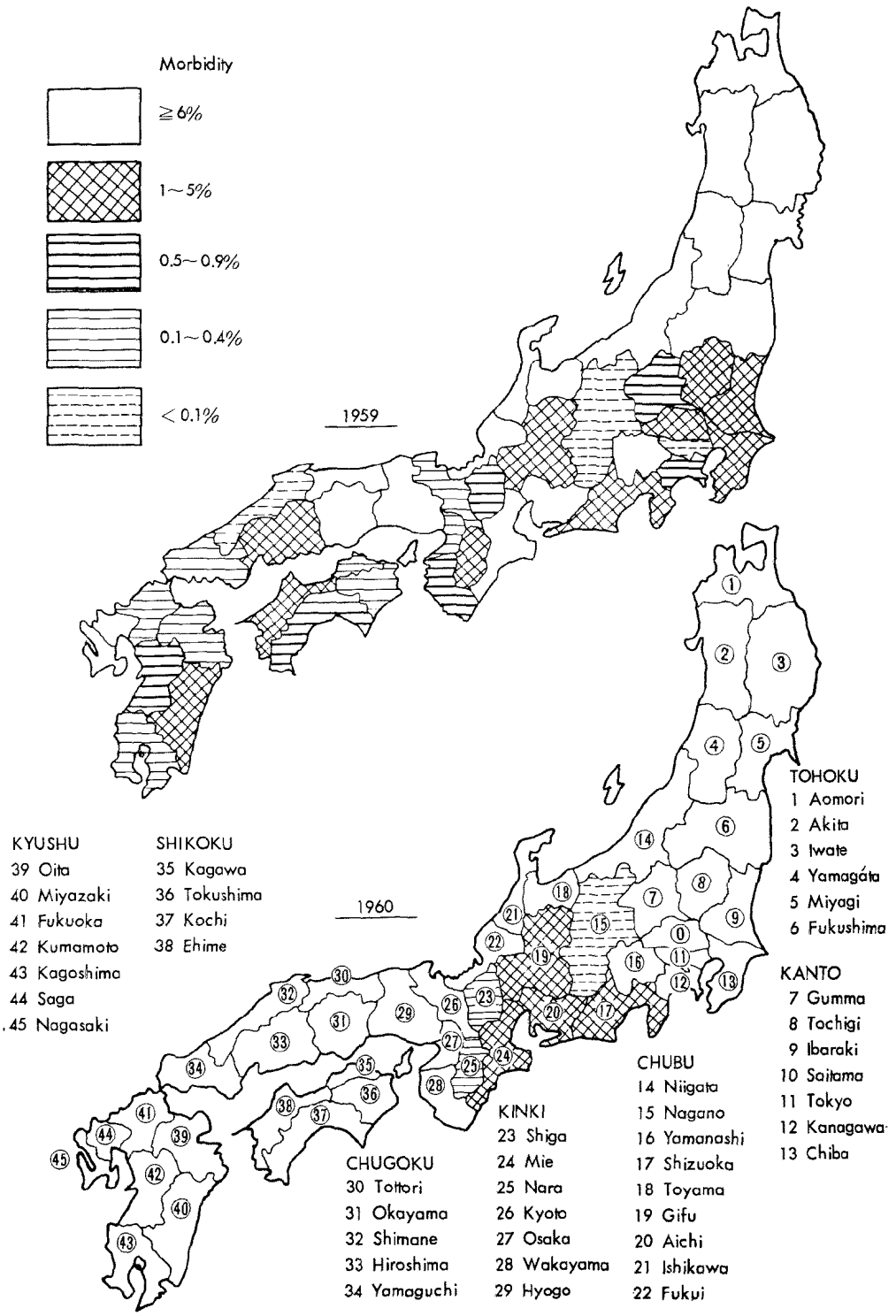

Fig. 1. Morbidity distribution by prefectures in the 1959 and 1960 epizootics.

Table 2. Reported cases by months and prefectures in the 1959 and 1960 epizootics[1]

1959

District	Prefecture	Number of cases in						Total
		July	August	September	October	November	December	
Kanto	Ibaraki	0	0	540	3,439	351	0	4,330
	Tochigi	0	0	83	1,347	234	0	1,664
	Gumma	0	0	26	589	205	0	820
	Chiba	0	0	41	1,871	596	0	2,508
	Saitama	0	0	74	1,192	262	0	1,528
	Tokyo	0	0	0	3	1	0	4
	Kanagawa	0	0	0	475	6	0	481
Chubu	Nagano	0	0	0	5	1	0	6
	Shizuoka	0	0	0	177	2,676	148	3,001
	Aichi	0	0	2,277	6,087	0	0	8,364
	Gifu	0	0	88	1,416	1,204	16	2,724
	Mie	0	0	345	6,110	0	0	6,455
Kinki	Shiga	0	0	0	232	70	0	302
	Kyoto	0	0	0	185	19	0	204
	Nara	0	0	0	334	457	30	821
	Osaka	0	0	0	41	51	0	92
	Wakayama	0	0	0	116	102	6	224
Chugoku	Hiroshima	0	0	32	538	820	0	1,390
	Shimane	0	0	0	0	63	0	63
	Yamaguchi	0	0	29	182	0	0	211
Shikoku	Tokushima	0	0	0	24	80	0	104
	Kochi	0	4	67	135	4	0	210
	Kagawa	0	0	0	0	59	10	69
	Ehime	0	0	66	124	267	342	799
Kyushu	Fukuoka	0	0	0	0	331	64	395
	Oita	0	0	0	158	0	0	158
	Kumamoto	0	0	162	565	0	0	727
	Miyazaki	0	228	643	278	0	0	1,149
	Kagoshima	0	85	188	0	0	0	273
	Total	0	317	4,661	25,623	7,859	616	39,076

1960

Chubu	Nagano	0	0	0	0	6	0	6
	Shizuoka	0	0	52	559	92	4	707
	Aichi	0	0	399	1,502	184	0	2,085
	Gifu	0	0	0	279	380	167	826
	Mie	0	0	73	745	195	0	1,013
Kinki	Shiga	0	0	0	13	25	0	38
	Nara	0	0	0	21	21	0	42
	Total	0	0	524	3,119	903	171	4,717

[1] Cited from Statistics of Animal Hygiene 1960, Bureau of Animal Industry, Ministry of Agriculture and Forestry, Japan.

where the outbreak occurred in both 1959 and 1960, it was noticed that the animals attacked in 1959 escaped from the disease in 1960, indicating the development of immunity after recovery. In these prefectures the beef production was high; a large number of young cattle were introduced from other areas for beef production. Despite of the immunity acquired in the 1959 outbreak, this high rate of turnover appears to have provided a favorable condition for the occurrence of the 1960 outbreak by introduction of a considerable number of young susceptible animals.

Animals of the Japanese breed for beef production showed a higher morbidity than those of the Holstein breed, and tended to be more severely ill when attacked, indicating different susceptibilities for the disease among different breeds of cattle. Cases were found generally in young animals less than 5 or 6 years of age, although the age distribution of cases varied from place to place. Animals under one year of age tended to escape the disease, but in Miyazaki and Kagoshima Prefectures some cases were reported.

Clinical Observations

The onset of the disease was generally abrupt with fever up to about 40 C, which lasted for 2 or 3 days but a few cases lasted as long as 7 to 10 days and even longer. The pyrexia was accompanied by general malaise, anorexia, lachrymation, foamy salivation, and suspension of rumination. At that time or somewhat later congestion and edema of the conjunctivae (Plate-Fig. 1), oral and nasal mucous membranes and nasal pad might occur. Leukopenia was common. But the disease was usually mild and the animals recovered completely in 2 or 3 days.

In some cases, congested oral and nasal mucous membranes, nasal pad or lips became cyanotic and erosive or ulcerative lesions eventually developed; the lesions became covered with crusts, which sloughed off leaving erosions or shallow ulcerations which bled easily (Plate-Fig. 2), but which healed soon afterwards. Painful swelling of the leg joints was common; in some cases erosions or ulcerations developed at the coronets crippling the animal. Similar lesions were also observed on the udder and/or vulva.

The most conspicuous symptom was deglutitive difficulty which was observed in 20 to 30% of the affected animals. The symptom usually developed 7 to 10 days after the onset of the disease when the pyrexia and other initial symptoms had subsided, but the degenerative process in the naso-oral regions might be aggravated. In some cases it was observed without any prodromal symptoms. The symptom was due to severe lesions in the esophagus, larynx, pharynx or tongue. Swollen stiff tongue protruded and continued to do so in some cases (Plate-Fig. 3). In animals with severe esophageal lesions, water or liquid foods poured into the mouth readily went down the esophagus when the head was kept high, but as soon as the head was lowered the contents of the esophagus, mixed with some contents of the rumen in some occasions, freely flowed out of the mouth and nose (Plate-Fig. 4). In mild cases vomiting was the only symptom suggestive of this condition. The difficulty in swallowing led to dehydration and emaciation and occasionally to aspiration pneumonia which constituted the major causes of death of the affected animals. Thirty to 40% of the animals with deglutitive difficulty died or were slaughtered with no hope of their recovery.

Experimental Transmission of the Disease to Calves

The disease was readily transmitted to

Table 3. Clinical findings in calves experimentally infected with the Kyushu-1 and Ibaraki-5 strains

Strain	Passage	Calf No.	Fever			Conjunctival congestion, lachrymation	Oro-nasal congestion, salivation	Deglutitive difficulty	Leukopenia	Other findings
			Incubation (days)	Duration ≧39.5C (days)	Maximum (C)					
Kyushu-1	I	106	5	3	40.0	−	−	−	+	
	II	109	4	2	40.8	+	+	−	+	Mucohemorrhagic stool
		100	4	7	41.0	+	+	+	+	Soft stool
	III	111	9	1	40.0	+	−	−	+	
		99	6	3	40.1	−	−	−	+	
		117	5	2	40.7	−	−	−	+	
		101	4	2	39.7	−	−	−	+	
	IV	115	6	4	40.3	−	−	−	+	
	V	160	6	3	41.0	−	−	−	+	
	VI	158	7	2	40.8	+	−	−	+	
	VII	151	11	1	39.9	−	−	−	−	
Ibaraki-5	I	107	6	2	39.8	−	−	−	+	
	II	102	4	9	40.8	+	+	+	+	Diarrhea
	III	116	4	3	40.4	−	−	+	+	
		98	4	3	40.2	−	−	−	+	
	IV	114	9	6	40.4	−	−	−	+	
	V	138	4	3	39.9	−	−	−	+	
		139	10	1	39.7	−	−	−	+	
	VI	150	7	2	40.0	−	−	−	erratic	
		140	4	2	40.0	−	−	−	erratic	
		142	9	4	41.2	−	−	−	nt	
		133	12	1	39.8	−	−	−	nt	
	VII	156	2	1	40.5	−	−	−	−	Diarrhea
	VIII	163	8	1	39.9	−	−	−	+	Diarrhea
		188	−	−	−	−	−	−	+	

calves by inoculation with clinical materials from naturally affected cattle, and was maintained serially in calves by intravenous inoculation with the blood obtained at the acute stages of the disease. The calves employed were mostly of Japanese black breed, a few of Holstein breed, and all one year of age except 2 two year old calves. Clinical materials from nine natural cases, four in 1959 and five in 1960, were used and all induced clinical illness. We selected two of those for serial passage in calves and could establish strains of pathogenic agent, Kyushu-1 and Ibaraki-5.

Strain Kyushu-1. A specimen of defibrinated blood was obtained from a natural case in Kagoshima Prefecture, Kyushu. The specimen was obtained on the second day of illness, September 10, 1959, and was shipped by air, frozen with dry ice, to our laboratory in Tokyo. Upon arrival 10 ml of the specimen diluted tenfold with Hanks' solution was injected intravenously into calf 106 (Table 3, Fig. 2).

After 5 days of incubation the calf developed a fever of 40.2 C and remained febrile for 3 days. Transient leukopenia was observed but no other symptoms.

Defibrinated blood was obtained on the second day of fever from the calf, and inoculated intravenously into two calves 100 and 109 in 5 ml amounts. Four days later calf 109 developed fever which lasted for 2 days, accompanied by leukopenia, mild foamy salivation and eye secretion (Table 3).

The other calf, No. 100, had a fever after 3 days of incubation (Table 3, Fig. 2). Fever continued for 3 days and subsided. Ten days after inoculation the animal had again fever accompanied by conjunctival congestion, profuse foamy salivation, slight rhinorrhea, diarrhea and reduced appetite. The next day deglutitive difficulty was ap-

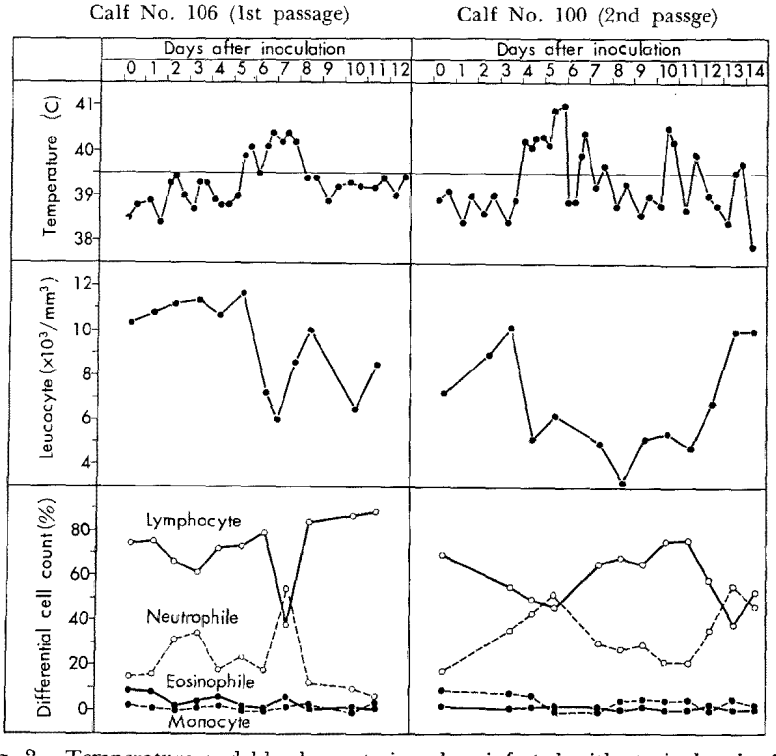

Fig. 2. Temperature and blood counts in calves infected with strain kyushu-1.

parent; water flowed out freely from the mouth and nose. The rumen was full of gas and feces was mucohemorrhagic. The calf rapidly developed dehydration with hemoconcentration as high as 1.68×10^7 erythrocytes per mm^3. The calf was sacrificed 14 days after inoculation, and showed pathologic changes as observed in natural cases, which will be discussed later.

Further passages were readily accomplished through the 7th passage by intravenous inoculation of calves with defibrinated blood drawn at the height of febrile reaction. Clinical findings are summarized in Table 3. All the animals developed fever after 4 to 11 days of incubation and remained febrile for 2 to 4 days. Calf 100 had recurrence of fever after an initial fever for 3 days and developed deglutitive difficulty. Inflammation was observed in the conjunctivae and oro-nasal mucous membranes in 4 calves, and the remaining 7 calves showed no symptoms other than fever. Leukopenia was common.

Strain Ibaraki-5. The clinical material employed was obtained from a natural case, Ibaraki No. 5, in a 7-year-old cow occurring in Ibaraki Prefecture during the 1959 outbreak. The cow had fever, lachrymation, complete inappetence, stomatitis and deglutitive difficulty, and died on the fifth day of illness, September 20. A pooled 10% emulsion was prepared with the lungs, liver, spleen, kidneys and mesenteric and submandibular lymph nodes, and inoculated into calf 107 by ocular instillation and rubbing on scarified mucous membranes of the mouth and nose with the undiluted material, and by intravenous injection with 30 ml of the material diluted tenfold.

After 6 days of incubation the calf developed a fever of 39.8 C, lasting for a few days. Transient leukopenia was observed but no other symptoms (Table 3, Fig. 3). Ten ml of defibrinated blood obtained

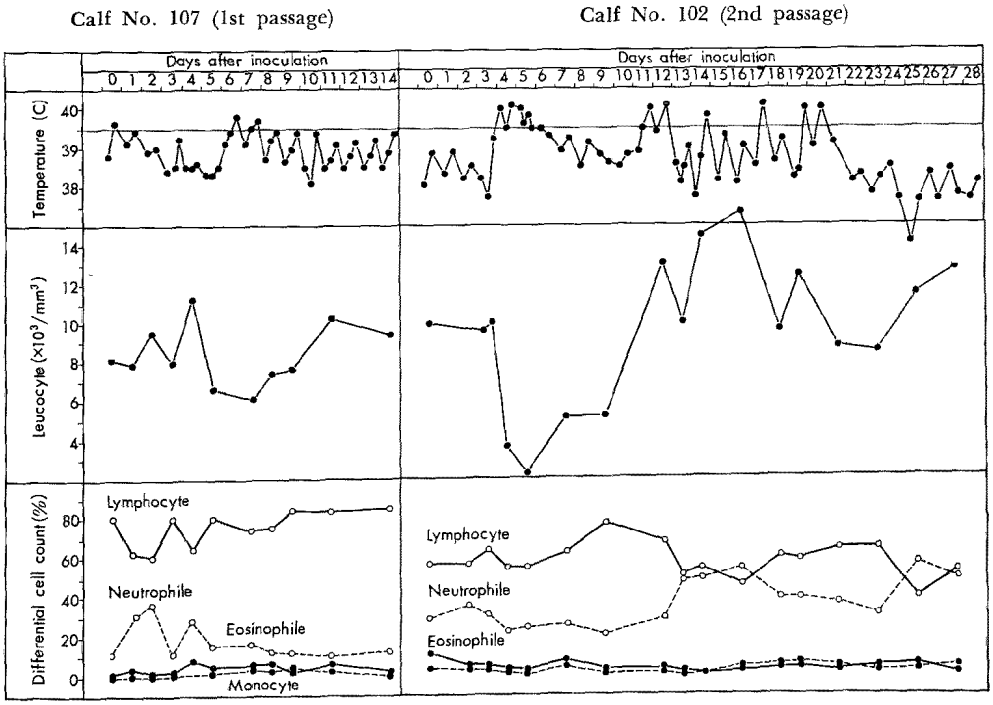

Fig. 3. Temperature and blood counts in calves infected with strain Ibaraki-5.

from the calf at the height of febrile reaction was inoculated intravenously into calf 102.

As shown in Fig. 3, the calf had a fever after 4 days of incubation. Fever remained at about 40 C for 2 days and gradually declined. Leukopenia was observed but no other symptoms. Eleven days after inoculation fever reappeared and two days later deglutitive difficulty developed. The calf also had congestion of the conjunctival and nasal mucous membranes, foamy salivation, cough, profuse nasal secretion, complete inappetence and bowels full of gas. The calf lay down on the floor 16 days after inoculation. Irregular fever continued, the leukocyte count turned to leukocytosis, and the calf was severely ill. Animal was treated for 13 days from 13 days after inoculation by intraperitoneal injection of large volumes of Ringer's solution supplemented with glucose to prevent dehydration and by administration of oxytetracycline hydrochloride (Terramycin) to prevent aspiration pneumonia. The calf had irregular fever until 21 days after inoculation, and by that time the leukocyte count returned to normal. The deglutitive difficulty was present until 20 days after inoculation, the other symptoms and signs disappeared and the animal recovered completely 23 days after inoculation.

Further passages were readily performed through the 8th passage. The clinical observations are summarized in Table 3. Fever appeared 4 to 12 days after inoculation and lasted for one to 6 days except calf 102 which had recurrence of fever and calf 188 which had no fever and nor other symptoms. Mild deglutitive difficulty was observed in calf 116. Leukopenia was common, although two calves showed erratic leukocyte counts and tended to show leukocytosis.

Other experiments. In addition to Kyushu-1 and Ibaraki-5, the disease was successfully transmitted to calves from two additional cases in 1959 and five cases in 1960. In each case defibrinated blood was inoculated intravenously into a calf. All the inoculated calves developed fever and transient leukopenia. The calf inoculated with the specimen from a 1960 case, Imaizumi, in Aichi Prefecture was affected more severely than the other calves and showed purulent rhinorrhea, foamy salivation, and anorexia, but recovered without developing deglutitive difficulty. When sacrificed 24 days after inoculation, however, the calf had necrotic lesions in the mucous membranes of the mouth, esophagus and abomasum, as presented later.

Pathological Observations

Natural cases. A total of 18 cases, 17 in 1959 and one in 1960, were examined. Those were all fatal or severe cases killed with no hope for recovery.

As described earlier, the disease began with abrupt fever accompanied by malaise, anorexia and salivation. At the same time or somewhat later, changes in the mucous membranes and skin developed. Congestion of the conjunctivae, swelling of the eyelids and lacrimation were marked; the mucous membranes of the mouth, lips, tongue and nose, and the skin of the muzzle and coronets were congested and occasionally erosions or ulcerations developed. In these lesions epithelial cells showed degenerative changes such as vacuolization and nuclear disintegration, necrotic masses formed crusts and sloughed off leaving erosions or ulcerations (Plate-Fig. 5). In severe cases necrotic areas were extensive, and infiltration of cells, mostly neutrophils, and proliferation of fibroblasts were observed in the ulcerative areas. But the necrotic changes were generally superficial and readily repaired. In some cases almost re-

paired epithelia were observed under crusts, and congestion, hemorrhage and edema, and rarely perivascular cell infiltration were observed in the underneath connective tissues (Plate-Fig. 6). No inclusion bodies were detected in the epithelial cells.

Similar changes of the mucous membrane were frequent in the abomasum, but less frequent in the esophagus, rumen, reticulum and omasum. Erosions and ulcerations in the abomasum were sometimes associated with marked congestion, hemorrhage and edema.

The cases examined were all severely affected ones and had characteristic lesions in the esophagus. Similar lesions were also observed in the larynx, pharynx or tongue in some cases. The most conspicuous change in these organs was degeneration and necrosis of the striated muscles (Plate-Fig. 7–9). The process began with loss of striation, followed by swelling, hyaline degeneration, fragmentation, and dissolution of the myofibrils. Perimysial cells were swollen and hyperplastic, and infiltration of neutrophils, lymphocytes and histiocytes and proliferation of fibroblasts were present. Older lesions tended to show marked proliferation of fibroblasts and infiltration of lymphocytes and histiocytes (Plate-Fig. 10), but both newer and older lesions were present in some individual cases. In these lesions hemorrhages were frequent; in some cases marked hemorrhage was observed with marked edema. Blood vessels of small and intermediate sizes in the hemorrhagic lesions showed edematous looseness of the wall and hyaline thrombosis (Plate-Fig. 11). These changes were also observed in the laryngo-pharyngeal and lingual musculatures.

The lesions in muscles impaired the function of those organs, resulting in deglutitive difficulty which in turn caused marked dehydration and emaciation. The subcutaneous tissue was severely dry; the contents of the rumen, reticulum and omasum lost their water and looked like feces in the rectum. Such conditions were more or less apparent in all the cases.

Systematic injuries of the circulatory system were observed in some fatal cases. Marked hemorrhages were found in the spleen, endo-, myo- and pericardium, liver, bladder, uterus, intestines, lymph nodes, etc. The spleen had hemorrhages, severe and extensive in some cases, and activation of the reticulo-endothelial system. The liver showed hemorrhage and focal necrosis, and activation of the reticulo-endothelial system was slight or absent (Plate-Fig. 12). In a few cases, focal interstitial nephritis and hyaline degeneration of epithelial cells of the proximal convoluted tubules were observed. Bronchopneumonia or aspiration pneumonia, probably due to secondary bacterial invasion, was common; some cases without pneumonia had hemorrhagic spots or interstitial and lobular emphysema. In the central nervous system, congestion, slight hemorrhage and perivascular edema were observed.

Experimental cases. A total of eight experimentally infected calves were examined. Three calves, Nos. 100, 111, and 202 had pathologic lesions indistinguishable from those observed in natural cases. The remaining five calves showed little pathological change.

Calf 100, the calf at the second passage of strain Kyushu-1, developed a severe disease with typical deglutitive difficulty (Table 3, Fig. 2), and was sacrificed 14 days after inoculation. Macroscopic hemorrhage and congestion were present in the esophagus. Histologic examination revealed marked degeneration of muscles and cell proliferation throughout the esophagus, larynx and pharynx. The abomasum had congestion, erosions and slight ulcerations.

Calf 111 was also one of the calves employed for serial passage of strain Kyushu-1 (Table 3). The calf developed fever, anorexia, lacrimation and soft stool, but recovered soon. Nineteen days after inoculation the calf was killed. Marked cell proliferation with muscular degeneration was detected in the esophagus, larynx and pharynx. The abomasum had congestion and slight erosions.

Calf 202 which was inoculated with blood from a 1960 natural cases, Imaizumi, developed a rather severe illness but recovered. The calf was killed 24 days after inoculation. The larynx, pharynx and the adjoining parts of the esophagus had hemorrhages and erosions with marked necrotic changes of epithelial cells but no muscular changes (Plate-Fig. 13).

DISCUSSION

In the present study clinical materials obtained from natural cases of the disease induced a clinical illness when inoculated into calves, and the disease was transmitted in series to calves by intravenous inoculation of the blood obtained at the height of febrile reaction. The disease experimentally induced in calves was clinically and pathologically indistinguishable from the natural disease. Thus, the artificially inoculated calves developed fever and leukopenia. Many of the inoculated calves developed no other clinical manifestations and readily recovered. But some calves developed conjunctival congestion with lacrimation or congestion of the oro-nasal regions with excessive salivation or both, as observed in natural cases.

The deglutitive difficulty which was the most outstanding clinical feature of the natural disease was observed in three of the artificial cases, Nos. 100, 102 and 116. Calf 100 was autopsied and shown to have extensive degeneration of muscles and cell proliferation throughout the esophagus, larynx and pharynx. Congestion, erosions and shallow ulcerations were found in the abomasum. These pathological changes are similar to those in natural cases. In addition, similar pathological changes, though mild, were observed in the esophagus, larynx, pharynx and abomasum of calf 111, and hemorrhages and erosions accompanied by marked degeneration of epithelial cells were found in calf 202, although these two calves had no deglutitive difficulty.

These findings strongly suggest that the etiologic agent of the disease under study was maintained in series in calves. In subsequent studies to be reported elsewhere, a virus was isolated in bovine cell cultures from the experimentally infected calves as well as from cattle with the natural disease, and the virus, designated as "Ibaraki virus", was eventually identified as the causative agent of the disease.

One notices that the disease resembles bluetongue in its epidemiologic, clinical and pathologic features. The pathologic picture of bluetongue in sheep can be confined to the mucous membranes of the digestive tract, skeletal musculature and vascular system, and lesions essentially consist of hyperemia, edema, hemorrhage, and degeneration of the epithelium and musculature [4, 10, 15]. The picture of bluetongue in cattle is quite similar except that cattle generally suffer only mild infections. Sometimes there may be frank symptoms and well developed lesions [1–5, 14, 15]. These descriptions essentially agree with our findings. In our cases muscular degeneration was principally observed in the esophagus, larynx, pharynx or tongue, which was also reported by Sugano [20], Goto [8, 9] reported degeneration in skeletal musculature as well. Bluetongue is strictly a seasonal disease, as is the disease under study. Evidence leaves little doubt

that bluetongue is transmitted by one or more insect species, in fact *Culicoides* species were found to be able to transmit bluetongue [6, 7, 16, 18]. As presented elsewhere, the causative agent of the disease under study was also found to resemble bluetongue in its biological and physicochemical properties.

Mucosal disease of cattle is similar to the disease under study in producing erosions or ulcerations in the muzzle, mouth, tongue, esophagus, rumen, abomasum and small intestines, but differs in producing no muscular degeneration. Erosions and ulcerations in the Peyer's patches as observed in mucosal disease of cattle was not found in our disease. Bovine malignant catarrh fever, of which no confirmed cases have been reported in Japan, produces necrotic stomatitis, pharyngitis, and conjunctivitis, but differs from our disease in producing nonpurulent encephalitis and round-cell infiltration in the Glisson's capsule of the liver. Our disease is somewhat similar to foot-and-mouth disease and vesicular stomatitis in cattle in producing lesions affecting mucous membranes and skin, but the lesions of the latter diseases consist of vesicles which never appeared in our disease.

The disease was once confused with another epizootic disease of cattle which has been known as "bovine epizootic fever" or "bovine influenza" [8, 9, 20] in Japan since 1949 and resembles bovine ephemeral fever in Australia and other countries. In the 1950 and 1951 outbreaks of bovine epizootic fever some cases with deglutitive difficulty were observed, and some workers thought that this might be a sequela of the disease. Controversy whether this condition was caused by bovine epizootic fever or had a different etiology continued until the studies since 1959 demonstrated clearly the etiological distinctness of the two diseases [11–13, 17, 19]. It seems most probable that the outbreaks of the two diseases were partially overlapped in time and area in 1950 and 1951. Serological evidence was obtained to support this explanation as reported elsewhere. This interpretation is also in agreement with the fact that outbreaks of bovine epizootic fever also occurred in 1949, 1955, 1956 and 1958, but no cases of deglutitive difficulty were reported.

Bovine epizootic fever, as we know now, is clinically and pathologically quite different from the disease under study, although the differentiation of these two diseases may be difficult in the early stages of the disease or in mild cases. Bovine epizootic fever is characterized by fever of short duration, respiratory distress, nasopharyngeal secretion and lacrimation. Anorexia, decreased lactation, joint pain and muscle tremor are common and the case fatality is low. The constant pathological change is catarrhal pneumonitis. Lesions in the esophagus, larynx, pharynx and tongue, and in the mucous membrane of the mouth, adjacent skin, nose, abomasum etc. as observed in the disease under study, are absent in bovine epizootic fever. But the seasonal and geographical incidences are not much different from those of the disease under study.

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EXPLANATION OF PLATES

Plate-Fig. 1. Congestion and swelling of the conjunctiva and lacrimation (Natural case).

Plate-Fig. 2. Erosions of the muzzle and nasal mucosa (Natural case).

Plate-Fig. 3. Protruding tongue and foamy salivation (Natural case).

Plate-Fig. 4. Deglutitive difficulty. Water went down the esophagus when the animal's head was kept high, but, as soon as the head was lowered, the water flowed out of the mouth and nose (Natural case).

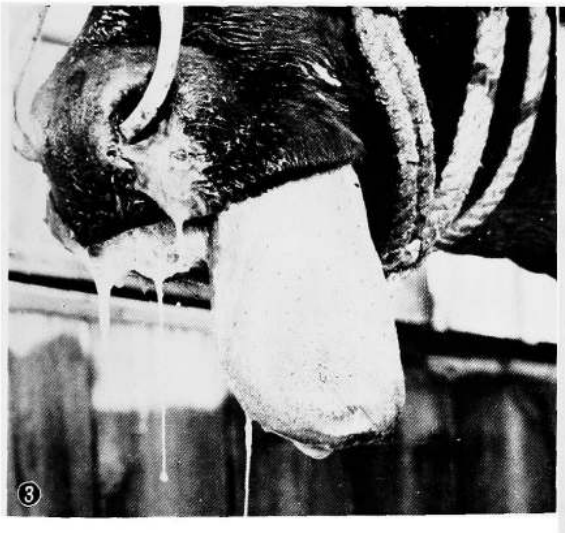

Plate-Fig. 5. Erosion in the mouth. Necrosis of epithelial cells and edema and cell infiltration of the underneath connective tissue. ×120 (Natural case).

Plate-Fig. 6. Lesion in the coronet. Crust covering the epithelium and hemorrhages in papillae. ×120 (Natural case).

Plate-Fig. 7. Degeneration of striated muscles and hemorrhage in the esophageal muscle tissue. ×120 (Natural case).

Plate-Fig. 8. Fresh lesion in the esophageal muscle tissue. Degenerated fasciculi are seen in edematous interstitium with mild neutrophil infiltration. ×120 (Natural case).

Plate-Fig. 9. Hyaline degeneration of esophageal muscle. ×320 (Natural case).

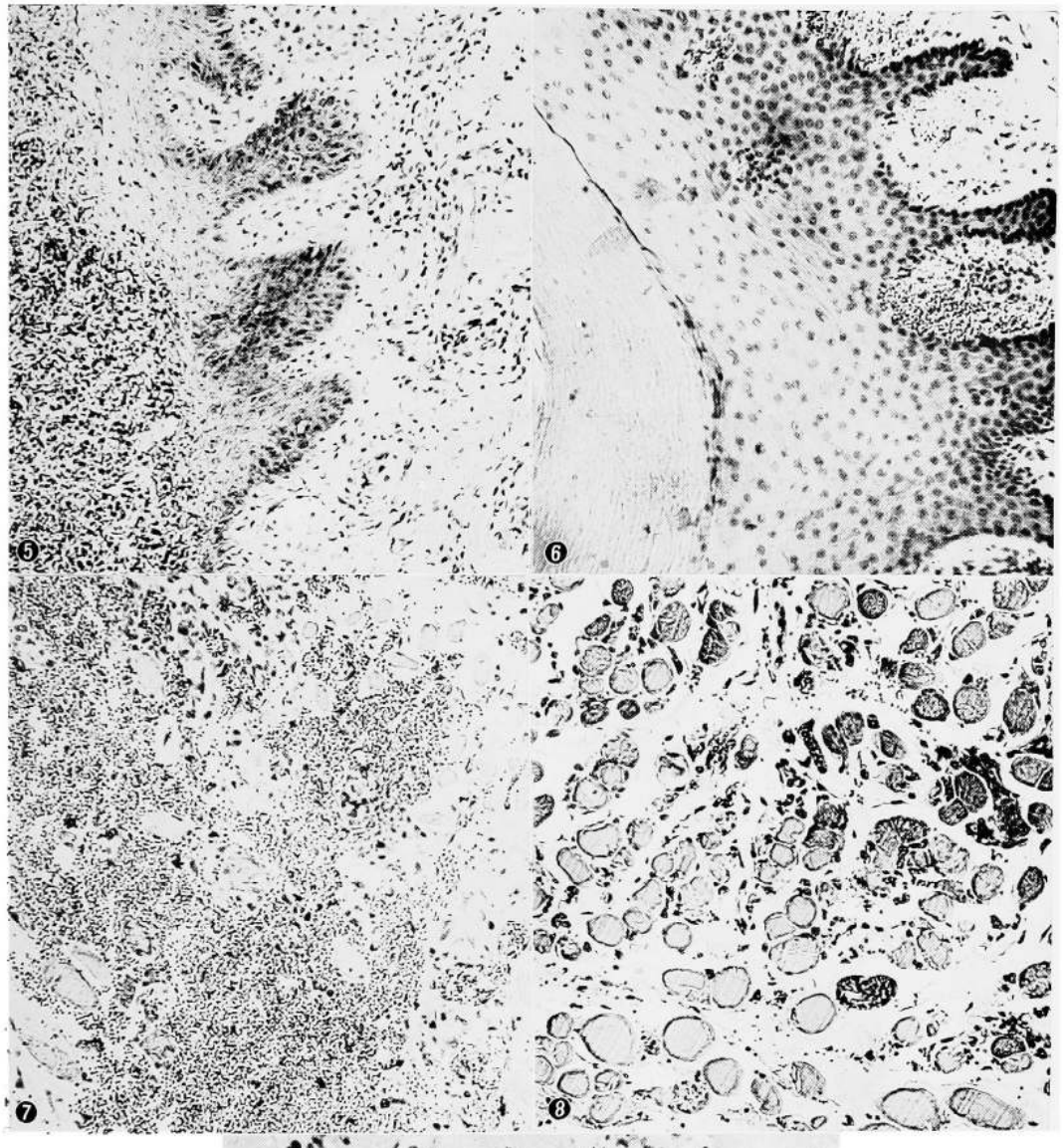
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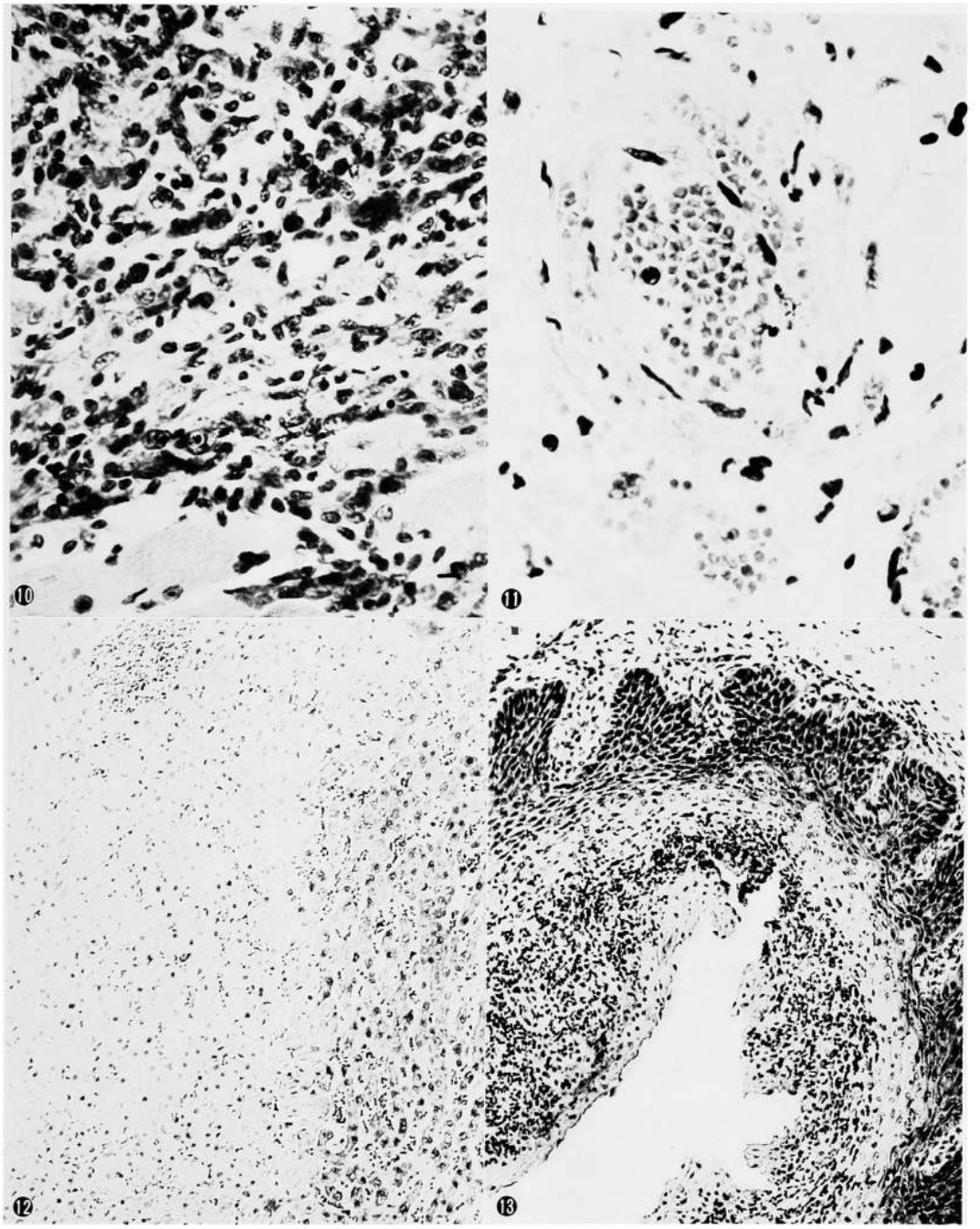
Plate-Fig. 10. Infiltration of lymphocytes and histiocytes and proliferation of fibroblasts in an old lesion of the esophageal muscle tissue. ×540 (Natural case).

Plate-Fig. 11. Edematous alteration of arterial wall in the esophagus. ×540 (Natural case).

Plate-Fig. 12. Necrosis in the liver. ×120 (Natural case).

Plate-Fig. 13. Erosion in the esophagus. Necrosis of epithelial cells and infiltration of neutrophils. ×120 (Artificial case No. 202).

Plate-Fig. 1, 2 were provided through the courtesy of Dr. T. Nozaki, Chiba Serum Institute, Japan.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