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STUDIES ON INFECTIOUS CANINE HEPATITIS II. HISTOPATHOLOGICAL STUDIES ON EXPERIMENTAL CASES

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INTRODUCTION

Experiments by GREEN and SHILLINGER¹²⁾, in which infectious canine hepatitis (H. c. c.) including enzootic fox encephalitis has been transmitted to dogs have been reported by RUBARTH and many other authors. The virus of H. c. c. is infectious to the dog, the silver fox, the coyote and the racoon¹⁴⁾. The black bear³²⁾ and the gray fox¹³⁾ have been found non-susceptible or only slightly susceptible to this virus. Some authors have reported that the cat^{19,20)}, the mink¹⁰⁾ and the ferret⁶⁻⁹⁾ are susceptible, but in general, many workers consider that the ferret and other laboratory animals are non-susceptible.

In Japan, in 1955, OSAMURA et al. and OCHI et al. reported experiments on transmission to dogs. Although it has already been reported that pathological findings of experimental cases are identical to those of spontaneous cases, detailed pathological studies on experimental cases are few except for RUBARTH's reports.

The present author had opportunity to make histopathological studies on the serial transmission experimental cases of OSAMURA et al. through the kindness of Prof. HIRATO (Chief of the Department of Veterinary Hygiene and Microbiology of this University).

In this presentation the author will describe the pathological findings of experimental cases; cases in which the author furthered his knowledge of the relation between initial lesions of H. c. c. and the development of inclusion bodies, and chronic lesions of the disease.

MATERIALS AND METHODS

Materials for the investigations consisted of 38 cases in the serial transmission experiments on H. c. c. in the Department of Veterinary Hygiene and Microbiology of the University. These transmission experiments were started from 2 strains ("MATSUDA" and "YAMAGUCHI") of H. c. c. virus which were isolated from the author's spontaneous cases. The dogs employed for the experiments were all healthy mongrel puppies, 14 to 90 days

old, without any previous sign of the presence of complement fixing antibody. These dogs were inoculated in various manners such as intraperitoneally, subcutaneously, intravenously and orally with 10~20% saline suspension of the liver. Four consecutive passages were positively made with the "MATSUDA" strain.

According to clinical symptoms, pathological findings and results of the complement fixation test 3 forms of the disease were demonstrated: 1) fatal severe form (8 cases), 2) mild and inapparent form (19 cases), 3) negative form (11 cases).

After post mortem examinations, materials were fixed with Carnoy's fluid, 10% formalin and Zenker's fluid. Paraffin sections were stained by various methods, using mainly hematoxylin-eosin, PAP's modifications of BIELSCHOWSKY-MARESCH's silver impregnation, Feulgen technique, methylgreen-pyronin stain, ribonuclease test, thionine stain, MCMANUS's PAS method, phloxin-tartrazine stain and Alloxan-SCHIFF reaction.

RESULTS

1. Fatal Severe Form

Cases of this form were found dead or were killed in a highly moribund condition with 2~6 days rapid course after inoculation. These cases were diagnosed as H.c.c. clinico-pathologically and numerous nuclear inclusions were detected in all parts of the body. Materials investigated consists of a total 8 cases as indicated in table 1.

1) Cases 2nd day after inoculation

Case 1, Dog No. 45, E 1558, ♀

On 24/IX 1954, at 5:00 p.m. the bitch was given 3 ml of 10% liver suspension and had a temperature of 39.1°C. On the following day the dog was depressed and the temperature was between 39.25°C and 40.8°C; on 26/IX it was 39.6°C to 36.6°C, and the dog was found dead.

Anatomical diagnosis: 27/IX 1954, 1) *Hepatitis parenchymatosa acuta* and *perihepatitis fibrinosa acuta*, 2) Subserous edema in the gall-bladder, 3) Enlargement of the lymph nodes and the spleen, 4) Edema of the thymus, 5) General anemia, 6) Edema in the lungs, 7) Ascariasis.

Histological findings: In the liver, the portal veins and the sinusoids were markedly dilated and sanguineous. The DISSE's spaces were also dilated, periportal areas were edematous, and dissociation of the liver cells was conspicuous. The liver cells were swollen and contained numerous vacuoles in the cytoplasm. "Eosinophilic necrosis" (MALLORY bodies)²⁰⁾ was distributed in the liver lobules. KUPFFER cells and sinusoidal endothelia were activated and nuclear inclusions were represented in the endothelia and hepatic cells. Cells containing such inclusions were rich in chromatin in the wall of the nucleus and these granular-appearing bodies were surrounded by light haloes. In the sinusoids, fibrin deposits were found and a small number of mononuclears and lymphocytes, mixed with a large number of polymorphonuclears were present. Subserous edema of the gall-bladder was strongly noted. The blood content of the spleen was somewhat increased. Reticulo-endothelial cells (R.E.S. cells) were proliferated, especially around the wall of the

TABLE 1. *Summary of Materials Investigated in Fatal Severe Cases*

CASE NO.	DOG NO.	PROTOCOL NO.	DAYS AFTER BIRTH	SEX	STRAIN OF VIRUS	PASSAGE	MATERIAL FOR INOCULATION	ROUTE	DOSE (ml)	TERMINATION (in days)
1	45	E 1558	41	♀	MATSUDA	3	10% liver susp. of case 2	i. v.	3	2 †
2	31	E 1511	?	♂	"	2	10% liver susp. of cases 5 & 7	"	"	3 †
3	46	E 1562	45	♀	"	3	10% liver susp. of case 2	"	"	" †
4	43	E 1560	48	♂	"	"	"	"	"	4 "
5	1	E 1403	45	"	"	1	20% liver susp. of spont. case	i. p.	15	" "
6	44	E 1561	?	♀	"	3	10% liver susp. of case 2	i. v.	3	" "
7	2	3557	90	"	"	1	20% liver susp. of spont. case	i. p., s. c.	20, 5	" "
8	48	E 1563	30	♂	"	3	10% liver susp. of case 2	i. v.	3	6 †

arteries. Nuclear inclusions were found in the reticulum cells. The kidneys were congested and endothelial cells with nuclear inclusions were detected in the glomeruli. In the lungs, alveolar pneumonia and focal pneumonia were found. Peribronchial tissues were edematous. Nuclear inclusions were also found in the endothelia. In the lymph nodes, catarrhal changes and edema were conspicuous and often accompanied with necrobiotic changes. R.E.S. cells were mobilized and often contained nuclear inclusions. In the adrenal glands, interstitial cell infiltration, especially of polymorphonuclears, was markedly observed. In the tonsils, necrobiotic changes and R.E.S. cell proliferation were found in the mucosa epithelia. The thymus and the pancreas showed interstitial edema. In the skeletal musculature, nuclear inclusions were found in the endothelia and histiocytes. The bone marrow showed hyperemia and was rich in cells. Nuclear inclusions were found in reticulum cells. In every part of the brain, hyperemia and hemorrhages were noted, and nuclear inclusions were also noted in vascular endothelia. Other organs showed no remarkable changes.

2) Cases 3rd day after inoculation

Case 2, Dog No. 31, E 1511, ♂

On 22/VI 1954, 3 ml of 10% liver suspension (Cases 5 & 7) was given intravenously. The dog had a temperature of 38.7°C; on 23/VI it was 39.2°C, 40.2°C; on 24/VI, 39.5°C, 39.8°C; on 25/VI, 39.6°C, 35.7°C. The conjunctiva was pale and the cornea became turbid. The dog could hardly stand and was killed at 4 p.m., in a moribund condition on 25/VI.

Anatomical diagnosis: 25/VI 1954, 1) *Hepatitis parenchymatosa acuta*, 2) Intensive subserous edema in the gall-bladder, 3) Slight enlargement of the spleen, 4) *Perihepatitis sero-fibrinosa acuta*, 5) Edema in the pancreas.

Histological findings: The liver was markedly sanguineous, especially in the central parts of the lobules, where the sinusoids were dilated and frequent hemorrhages were noted. Dissociation of liver cells and a small number of MALLORY bodies were observed. The liver cells and the endothelial cells contained numerous nuclear inclusions with granular appearance and various figures. KUPFFER cells and sinusoidal endothelial cells were activated and often showed regressive changes. In the sinusoids, a large number of polymorphonuclears and a small number of mononuclears and lymphocytes were found. Many of the endothelia in larger vessels had nuclear inclusions. Subserous edema of the gall-bladder was intensive. The spleen was markedly sanguineous. Lienal sinuses were markedly dilated and the Malpighian bodies were atrophic. In the red pulp, R.E.S. cells were proliferated often with nuclear inclusions. In the subepithelial tissue in the *papilla renalis*, a large number of polymorphonuclears with karyorrhectic changes were observed. Lymphocytic cell infiltration was found in the subepithelial layer in the pelvis. Lymphadenitis and tonsillitis were found. Nuclear inclusions were present in various organ as listed in table 3. In the brain, congestion and hemorrhages were conspicuous, especially in the subpial region. Adventitial cells and endothelial cells were both proliferated.

Case 3, Dog No. 46, E 1562, ♀

On 24/IX 1954, the bitch was inoculated with 3 ml of 10% liver suspension (Case 2) and had a temperature of 38.5°C. On 25/IX, the temperature was 38.0°C, 38.3°C; on 26/IX it

was 39.6°C; on 27/IX, 38.7°C, 39.3°C. The dog was found dead.

Anatomical diagnosis: 28/IX 1954, 1) *Hepatitis parenchymatosa acuta*, 2) *Perihepatitis fibrinosa acuta*, 3) Increased fluids of the body cavities, 4) Marked subserous edema in the gall-bladder, 5) Marked general anemia, 6) Edema in the pancreas, the thymus and the lungs, 7) Dilatation and stagnation in the portal lymph vessels, 8) Enlargement of the spleen and the lymph nodes, 9) *Tonsillitis acuta*, 10) *Gastro-entero-colitis catarrhalis chronica*, 11) Ascariasis.

Histological findings: The blood content of the liver was markedly increased with intensive dilatation of *Vv. interlobulares*. Lacunose dilatations of the sinusoids and slight dilatation of DISSE's spaces were observed. Hemorrhages were found in the parenchyma and the edematous GLISSON's capsules. Liver cells, as a rule, were swollen, but some of them were atrophic due to the blood content. Endothelial cells were proliferated and in the sinusoids, polymorphonuclear, mononuclear and lymphocytic cell infiltrations were observed. MALLORY bodies were also found. Characteristic nuclear inclusions were noted in the liver cells, endothelia and histiocytes in the GLISSON's capsule. Fatty infiltration of the liver cells was also found. The wall of the gall-bladder was edematous with hemorrhages. In the spleen, the follicles were atrophic and the red pulp was rich in cells. In the myocardium, histiocytic cell proliferation was detected in the subendocardium and interstitium. The wall of the alveoli in the lungs was thickened. The lymph nodes were hyperemic and the medullary sinus as well as marginal sinus were rich in blood. R.E.S. cells were proliferated and showed a marked erythrophagy. Nuclear inclusions were found in adventitia cells, histiocytes, reticulum cells and endothelium. Focal necrobioses were found. In the adrenal glands, endothelial cells were activated and polymorphonuclear infiltration was noted in the interstitium. Some of the adrenal cortex cells and endothelia contained nuclear inclusions. The tonsils were hyperemic and regressive changes were conspicuous. R.E.S. cells were proliferated and inclusions were found in the epithelial cells. In the thymus, interstitial edema, regressive changes, congestion and hemorrhages were also noted. In the brain, congestion and hemorrhages were found. Adventitia cells in the *pia mater* were proliferated and endothelia in the blood capillaries were also activated often with nuclear inclusions. In the striatum, the thalamus, the mid-brain, the pons, the *medulla oblongata* and the *medulla spinalis*, slight perivascular cuffs were found. Composite cells were chiefly mononuclear cells. In the mid-brain and the pons, slight glia cell foci were distributed. Nuclear inclusions were also found in the vascular endothelia.

3) Cases 4th day after inoculation

Case 4, Dog No. 43, E 1560, ♂

On 24/IX 1954, the dog was inoculated with 3 ml of 10% liver suspension (Case 2) and had a temperature of 39.1°C; on 25/IX, it was 39.1°C, 38.7°C; on 26/IX, 39.9°C, 40.6°C; on 27/IX, the dog was sluggish and had no appetite, and had a temperature of 39.6°C, 39.7°C, 38.1°C. On the following morning the dog was found dead.

Anatomical diagnosis: 28/IX 1954, 1) *Hepatitis parenchymatosa acuta*, 2) *Perihepatitis fibrinosa acuta*, 3) Enlargement of the spleen and the lymph nodes, 4) Subserous edema in the gall-bladder, 5) Edema in the lungs and the thymus, 6) General anemia, 7) *Gastro-enteritis catarrhalis acuta*.

Histological findings: In the liver, small focal necroses were distributed often with hemorrhages. MALLORY bodies were found in comparatively large number (PAS positive). Lacunal dilatation of the sinusoids was noted due to the increased blood content. DISSE's spaces was dilated and the GLISSON's capsules were edematous. A large number of nuclear inclusions with granular appearance were found in the liver cells and endothelia. In the sinusoids, swollen, rounded endothelial cells with karyorrhexis, polymorphonuclears, mononuclears, lymphocytes and fibrin deposits were present. The wall of the gall-bladder was markedly edematous. The spleen was sanguineous and focal necrobioses were distributed in the Malpighian bodies and the red pulp. Nuclear inclusions were found in the endothelium and reticulum cells. *Lymphadenitis catarrhalis acuta* and *tonsillitis acuta* were also found. The thymus was markedly edematous. The bone marrow was congested and megakaryocytes were degenerative. Nuclear inclusions were found in the skeletal muscles, the tongue, the stomach, the bone marrow and the brain. In the brain, congestion and hemorrhages were found. Endothelial cells with nuclear inclusion were activated and glia cell foci were found in the pons.

Case 5, Dog No. 1, E 1403, ♂

On 18/III 1954, the dog was given 15 ml of 20% liver suspension (MATSUDA strain) intraperitoneally and the temperature was 38.5°C; on 19/III, 39.3°C; on 20/III, 38.5°C, 40.1°C; on 21/III, 39.8°C, 39.1°C; on 22/III, 37.9°C. Bloody excrements were found. He became worse and died.

Anatomical diagnosis: 22/III 1954, 1) General anemia, 2) Increased fluids of the body cavities, 3) Enlargement of the lymph nodes, 4) Marked edema in the thymus, 5) Left side subendocardiac hemorrhages, 6) Ascariasis.

Histological findings: Undemarcated focal necroses were found. A large number of MALLORY bodies were observed in the liver. The sinusoids were dilated and polymorphonuclear infiltration was detected in them. R.E.S. cells were swollen and rounded often containing nuclear inclusions. In the gall-bladder, subserous edema and nuclear inclusions in the vascular endothelia were observed. The kidneys were anemic. In the myocardium, focal histiocytic cell proliferation was observed in the interstitium. In the lungs, alveolitis with polymorphonuclear infiltration and perivascular edema were also noted. In the lymph nodes, blood resorption and catarrhal changes in the sinuses were conspicuous. The follicles showed necrobiotic changes. Reticulum cells and endothelia contained nuclear inclusions. In the tonsils, the follicles were hyperplastic and accompanied by necrobiosis. Polymorphonuclear infiltration, defect of the epithelia in some parts and nuclear inclusions in the epithelia were found. Vascular endothelial cells and serosa epithelial cells often contained nuclear inclusions. In the urinary bladder, *peritonitis purulenta* and nuclear inclusions in the serosa epithelia were noted. In the brain, adventitia cells in the *pia mater* were proliferated. Congestion and hemorrhages, were also found in every part of the parenchyma. Perivascular cuffs were observed in the thalamus and *L. frontalis*. A large number of nuclear inclusions were found in the endothelia.

Case 6, Dog No. 44, E 1561, ♀

On 24/IX 1954, the bitch was inoculated with 3 ml of 10% liver suspension (Case 2)

and had a temperature of 39.2°C, 39.5°C: on 25/IX, it was 38.8°C, 39.0°C; on 26/IX, 40.3°C. On 27/IX, the dog was depressed and the temperature was 40.1°C, 39.7°C, 39.3°C; on 28/IX, it was 35.0°C, the dog was in a moribund state and soon died.

Anatomical diagnosis: 28/IX 1954, 1) *Hepatitis parenchymatosa acuta* 2) Marked subserous edema in the gall-bladder, 3) Enlargement of the spleen and the lymph nodes, 4) Edema in the pancreas, the thymus and the lungs, 5) *Tonsillitis simplex acuta*.

Histological findings: Undemarcated focal necrobioses were much distributed and MALLORY bodies were also scattered in the liver lobules. Dilatation of *Vv. interlobulares* and the sinusoids were marked due to increased blood contents. R.E.S. cells were swollen and rounded, and often exfoliated. In the sinusoids, polymorphonuclear infiltration was observed. Nuclear inclusions were found in the endothelia, liver cells and numerous in the venous endothelia in the GLISSON's capsules. The GLISSON's capsules were edematous and accompanied by cell infiltration. Subserosa in the gall-bladder was markedly edematous with cellular infiltration. The blood content in the spleen was increased and the follicles were clearly found with necrobiosis. The red pulp showed a large number of macrophages and nuclear inclusions were found in endothelia and reticulum cells. In every part of the brain, congestion and hemorrhages were observed. Nuclear inclusions were also found in the vascular endothelia. Glia cell foci were noted in the mid-brain, the pons and the cerebellum. Slight degeneration of nerve cells due to hemorrhages was also noted. Changes of the other organs were similar to those of case 5.

Case 7, Dog No. 2, 3557, ♀

On 18/III 1954, the bitch was given 20 ml of 20% liver suspension (MATSUDA strain) intraperitoneally. At the same time, 5 ml were injected subcutaneously. The temperature was 38.9°C; on 20/III, it was 39.5°C, 39.5°C; on 21/III, 39.5°C, 38.8°C. On 22/III, the temperature was 39.0°C and then it fell rapidly. The dog became worse and died.

Anatomical diagnosis: 22/III 1954, 1) *Peritonitis sero-fibrinosa acuta*, 2) Enlargement of the liver and spleen, 3) General anemia, 4) Increased fluids of the thoracic cavities, 5) Edema and swelling of the lymph nodes, 6) Ascariasis.

Histological findings: In the liver, focal necroses were scattered irregularly, but the size was small. The liver cells of these areas were swollen, pyknotic or karyorrhectic and acidophilic. These cells were positive to PAS method and showed loss of basophilia (PNA). A large number of MALLORY bodies were noted. *Vv. interlobulares* and the sinusoids were dilated and congested. Fibrin deposits were found in the sinusoids. Vacuolar degeneration of liver cells was markedly observed. In the vacuoles, round and acidophilic bodies which were PAS positive and Alloxan-SCHIFF positive (Protein) were observed in great number (Hyaline droplet degeneration). R.E.S. cells were mobilized and polymorphonuclear and lymphocytic cell infiltration was also seen. Sometimes a granulomatous proliferation of endothelial cells was found. The GLISSON's capsules were edematous and accompanied by cell infiltration. Nuclear inclusions were found in the liver cells, sinusoidal endothelium, venous endothelium and histiocytes in the GLISSON's capsules. Reticular fibers of the liver were found to be intact. *Perihepatitis fibrinosa acuta* was noted. The wall of the gall-bladder was markedly edematous. The spleen was rich in blood and the follicles were atrophic. Focal necrobioses were detected in the follicles

and the red pulp. R.E.S. cells were proliferated and sometimes giant cells were found. Nuclear inclusions were found in the small and medium-sized vessels in the trabeculae. The kidneys were congested. Nuclear inclusions were found in the glomerular endothelia and the endothelia in the small or medium-sized vessels in the pelvis and *papilla renalis*. The myocardium was congested and nuclear inclusions were found in vascular and endocardial endothelia. The lungs showed congestion and alveolitis. *Lymphadenitis catarrhalis acuta* and *tonsillitis simplex acuta* were also found. Nuclear inclusions were found in various organs as listed in table 3. Changes in other organs were similar to those in above described cases.

4) Case 6th day after inoculation

Case 8, Dog No. 48, E 1563, ♂

On 24/IX 1954, the dog was inoculated with 3 ml of 10% liver suspension (Case 2) intravenously. The temperature was 39.3°C; on 25/IX, it was 36.7°C, 36.1°C; on 26/IX, 39.5°C; on 27/IX, 40.1°C, 40.3°C; on 28/IX, 39.3°C, 38.3°C. Loss of appetite and diarrhea were observed. On 30/IX, the temperature fell to 35.0°C and the dog became worse. The dog was killed in a moribund condition.

Anatomical diagnosis: 30/IX 1954, 1) Subserous edema in the gall-bladder, 2) Increased fluids of the thoracic and abdominal cavities, 3) Edema in the pancreas, the thymus and the lymph nodes, 4) General anemia, 5) *Gastro-entero-colitis catarrhalis chronica*, 6) Parasite: *Toxocara canis*, *Dipylidium caninum*.

Histological findings: Granulomatous proliferations of endothelial cells were scattered in the liver lobules. MALLORY bodies were also distributed. Especially small focal necrobioses were occasionally observed. R.E.S. cells were also proliferated and inflammatory cell reaction was similar to that of the other cases. The blood content was increased as in the other cases. Nuclear inclusions were found in the liver cells, endothelia, histiocytes and especially in the bile duct epithelia. (The latter finding is very rarely noted in literature on this subject). Subserous edema in the gall-bladder was conspicuous. In the spleen, numerous hyalinized changes were observed around the marginal areas in the Malpighian bodies. Changes in other organs were similar to those of the above-described cases.

2. Mild and Inapparent Form

Cases of this form manifested mild symptoms or no clinical signs of illness, but positive results to complement fixation test (C.F.T.) after inoculation of this virus. These cases did not show characteristic pathological changes as H.c.c. The findings were as follows.

1) Post mortem findings

Enlargement of the lymph nodes, general anemia, hyperplasia of lienal follicles, *nephritis interstitialis*, slight enlargement of the liver and general edema, etc., were observed. Especially, in one case (Dog No. 37, E 1635, 197 days old) which was observed for a long period, post mortem findings were similar to those of cases of a fatal severe infection. That is, enlargement of the liver, *perihepatitis fibrinosa acuta*, increase of

abdominal fluids, right side cardiac dilatation, marked subserous edema in the gall-bladder, edema and swelling of the lymph nodes, enlargement of the tonsils and *nephritis interstitialis chronica* were noted.

2) Histopathological findings

Liver: Except in one case which was observed for a long period of time, R.E.S. cells were, as a rule, comparatively inactive. Double nuclei in the liver cells were often observed and arrangement of the liver cell cord was irregular. Scattered small cell foci in the liver lobules were often indicated. Especially in the cases of 50~62 days course (Dogs Nos. 56, 57 & 58), a granulomatous proliferation of endothelial cells was noted. Frequently polymorphonuclear infiltration was found in the sinusoids. In some cases the walls of the *V. centralis* were fibrous and a small number of cells were accumulated around them. In one case which was observed for a long period of time (Dog No. 37), the sinusoids and DISSE's spaces were both dilated and edematous. The liver was congested and R.E.S. cells were swollen. Polymorphonuclear infiltration in the sinusoids and vacuolar degeneration of parenchymatous cells were conspicuous. But characteristic nuclear inclusions could not be found in the liver cells and endothelium. Subserous edema in the gall-bladder and dilatation of the lymph vessels were both extensively observed.

Spleen: In the earlier stages of the cases, as a rule, the Malpighian bodies were not enlarged and were often accompanied by necrosis. But in the advanced stages of the disease, the follicles and the red pulp were hyperplastic and R.E.S. cells were slightly activated. In one case (Dog No. 37) of comparatively longer duration, the blood content was increased and sinus was dilated. The follicles were atrophic and the red pulp was hyperplastic.

Kidneys: In the earlier stages of the diseases, the glomeruli were enlarged. In general, lymphocytic cell infiltration with histiocytes in the subepithelial layer in the pelvis and *pipilla renalis* were frequently observed. Sometimes lymphocytic nodular cell foci were found. These changes were observed in all cases except two (Dog Nos. 8 & 58). *Nephritis interstitialis chronica* was often found.

Lungs: The walls of the alveoli were all thickened and swollen. Proliferation of R.E.S. cells, such as endothelia and histiocytes, was also observed in almost all cases.

Lymph nodes: In the earlier stage of the cases, necrobiosis of the follicles was often observed. In general, the sinus was dilated and edematous. Mobilization of R.E.S. cells, especially macrophages was conspicuous.

Brain: Congestion and hemorrhages were commonly observed. Swelling and proliferation of vascular endothelia and adventitia cells often accompanied. But activity of reticulo-endothelial system was of a lesser extent than in fatal severe cases. Glia cell foci and nuclear inclusions were not found in any cases of this type.

3. Negative Form

Cases of this form showed no signs of illness, and no characteristic pathological findings and C.F.T. was negative. It is of interest that *pyelitis chronica* or *nephritis interstitialis chronica* as in mild and inapparent cases were not observed.

TABLE 2. *Histological Features of the Fatal Severe Form Found in the Liver*

DESCRIPTION OF CHANGES		CASE NO.							
		1	2	3	4	5	6	7	8
Parenchymal Changes	Eosinophilic necrosis	+	+	+	++	#	#	#	++
	Focal necrosis	-	-	-	+	+	+	++	+
	Dissociation	++	+	+	++	+	++	+	+
	Swelling of cells	++	+	+	++	+	++	++	+
	Hyaline droplet degeneration	-	-	-	-	-	-	#	-
	Fat infiltration	++	+	++	++	-	+	+	+
Interstitial Changes	R. E. S. cell stimulation	+	+	++	+	+	+	++	++
	Pigment phagocytes	+	-	-	-	-	-	-	-
	Mononuclear cells	+	+	++	+	+	+	+	++
	Polymorphonuclears	+	+	++	+	++	++	+	++
	Lymphocytes	±	±	±	±	±	±	±	±
	Bile thrombi	-	-	-	-	-	-	-	-
	Reticulin disturbance	-	-	-	-	-	-	-	-
	Cell infiltration in G. capsule	-	-	-	-	+	+	+	+
Circulatory Disturbances	Hemorrhages	-	+	++	+	-	+	++	-
	Edema	+	+	++	++	+	++	+	-
	Dilatation of sinusoids	++	++	#	#	++	#	++	+
	Dilatation of DISSE's spaces	+	+	+	++	-	#	-	+
	Fibrin	+	+	++	-	-	-	++	-
Inclusions	Hepatic cells	++	#	++	#	#	#	#	++
	Endothelium	++	#	#	#	#	#	#	++
	Bile duct epithelium	-	-	-	-	-	-	-	+

DISCUSSION

The following three forms of the disease were exhibited after the inoculation of H.c.c. virus. 1) Fatal severe form: the animals manifested severe symptoms and either died or were killed in a highly moribund condition from the 2nd to 6th day after inoculation [28.6% (8/28)]. 2) Mild and inapparent form: the animals showed mild symptoms or no clinical signs of illness, but C.F.T. for H.c.c. was positive; MATSUDA strain — 46.4% (13/28), YAMAGUCHI strain — 60% (6/10). 3) Negative form: the animals showed no signs of illness, no characteristic pathological findings and negative results in C.F.T. during observation period (OSAMURA et al.).

The fatal severe form is a typical hepatitis. It fully corresponds with the fatal fulminated or the severe non-fatal form which was described by DECAMP and other authors^{25,26}. It seems that this form is a transitional form of RUBARTH'S

TABLE 3. *Frequency of Nuclear Inclusions in the Various Cells*

CELLS	CASE NO.								%
	1	2	3	4	5	6	7	8	
Liver parenchymal cell	++	##	++	##	##	##	##	++	100
Liver endothelium	++	##	##	##	##	##	##	++	"
Bile duct endothelium	-	-	-	-	-	-	-	+	12.5
Spleen endothelium	-	+	-	+	.	+	+	+	71.4
Spleen reticulum cell	+	+	-	++	.	+	-	+	"
Spleen adventitia cell	-	+	-	-	.	-	-	-	14.3
Glomerulus endothelium	++	++	+	++	++	++	++	+	100
Myocardium endothelium	+	+	+	+	+	+	+	+	"
Valvular endothelium	-	-	-	-	-	+	-	-	12.5
Bronchial epithelium	-	-	-	-	-	-	-	-	0
Lung endothelium	+	+	-	+	+	+	+	-	75
Tonsil epithelium	+	+	+	-	+	+	-	-	62.5
Tonsil, reticulum cell and endothelium	-	-	-	-	+	+	+	-	37.5
Lymph node endothelium	+	+	+	+	+	+	++	+	100
Lymph node reticulum cell	+	+	+	+	+	+	++	+	"
Thymus endothelium	+	.	-	+	+	.	+	.	80
Adrenal cortex epithelium	-	-	+	-	.	.	-	-	16.7
Adrenal endothelium	-	+	+	-	.	.	++	-	50
Brain endothelium	+	++	+	+	++	+	++	+	100
Pharynx and larynx endothelium	-	-	-	-	-	+	-	-	12.5
Salivary gland endothelium	-	+	-	-	-	-	-	-	"
Tongue endothelium	-	-	+	+	-	+	+	-	50
Oesophagus endothelium	-	-	-	-	-	-	+	-	12.5
Stomach endothelium	-	-	+	+	+	-	-	+	50
Intestine endothelium	-	-	+	-	+	-	-	-	25
" serosa epithelium	-	-	+	-	+	-	-	-	"
Skeletal muscles, endothelium and histiocyte	+	-	-	+	-	-	-	-	"
Ovarium endothelium	-	.	-	.	.	-	++	.	"
Bladder, endothelium and serosa epithelium	-	-	-	-	+	+	-	-	"
Bone marrow, endothelium and reticulum cell	+	-	-	+	-	-	-	-	"

cases which rapidly terminated in death, or of cases with a more protracted lethal course.

The pathological findings in this form nearly correspond with those found in the spontaneous cases, but there are considerable differences in individual lesions. All cases investigated showed the earlier stage of hepatitis with a 2~6 days clinical course.

In RUBARTH's cases which rapidly terminated in death, relatively moderate, but rather diffusely spread, regressive changes with a great abundance of nuclear inclusions were observed. In his cases a more protracted lethal course usually

showed far advanced regressive changes and sheer necrosis. These findings are similar to those in the present author's spontaneous cases.⁵⁾ In the cases discussed here, centrolobular hepatic necrosis was not found as in spontaneous cases. Focal necrosis finally appeared in the 4th day after inoculation but its location was not constant. The size of the lesions was small; they were sporadically distributed in the liver lobules. But hepato-cellular necrosis was found in an earlier stage. Isolated liver cells became round, showing pyknosis and karyorrhexis, and then a marked eosinophilic cytoplasm appeared in the cells. They showed "eosinophilic coagulative necrosis" or "MALLORY bodies"²⁰⁾. These bodies were observed in almost all cases; they appeared increasing during the progression of the disease. After MALLORY bodies had appeared, focal necrosis occurred. Therefore, the appearance of MALLORY bodies is to be regarded as one of the most important findings of the initial changes in the earlier stage of hepatitis.

In the previous paper⁵⁾, the present author discussed the important role of hypoxemia in the formation of central hepatic necrosis, but in these cases, as the signs of hypoxemia, certain changes were observed. Specifically in case 7, on the 4th day after inoculation, numerous large rounded hyalinous droplets were found to be markedly acidophilic and positive to PAS and the ALLOXAN-SCHIFF method tests. These changes were regarded as being indicated by the hepatic hyaline droplets. It is an interesting fact that these changes correspond with ALTMANN's experimental cases of hypoxemia in low-pressure and E. R. FISHER's and B. FISHER's cytoplasmic inclusions after arterialization in dogs.

As a viral inflammation, KUPFFER cells and sinusoidal endothelia were activated, but the activity was less pronounced than in spontaneous cases. As a inflammatory cell reaction, polymorphonuclear cells were observed in the hepatic sinusoids in almost all cases. Then to a slight degree, mononuclear cells and lymphocytes appeared in the hepatic sinusoids.

In one case (No. 48) killed on the 6th day after inoculation, a granulomatous cell proliferation of endothelial cells was observed in the liver with the findings by MIYAKE et al. of epidemic hepatitis in man and KÜHN's "Spätknötchen."

The reticular fibers of the liver were almost intact. Proliferation and loss of the reticular fibers were not recognized.

Basophilia (Pentose nucleic acid: PNA) of hepatic cytoplasm, especially in MALLORY bodies and necrotic foci disappeared as it did in spontaneous cases. Basophilia (PNA) partially decreased in the centrolobular parts of the liver. Conversely, R.E.S. cells in the sinusoids were rich in PNA.

Post mortem findings, as a rule, were similar to those of spontaneous cases: Edema as a circulatory disturbance seemed to play the leading part in the disease. Subserous edema in the gall-bladder was regularly observed and increased fluids

of the body cavities, *perihepatitis fibrinosa acuta*, edema in the thymus and pancreas, dilatation and stagnation of portal lymph vessels and edema in the lymph nodes, were also conspicuously present.

Histopathologically, parenchymatous degeneration was of less extent than in spontaneous cases, but hepatic focal necrosis, necrobiotic changes in the spleen, the lymph nodes, the tonsils and the follicles of the digestive canals were found. The reticulo-endothelial system was activated in almost all parts of the body, such as with *lymphadenitis catarrhalis*, and with alveolitis in the lungs.

The distribution of characteristic nuclear inclusions of this disease, as listed in table 3, showed not only in the liver cells and endothelia, but also in all germinal layers. Especially, nuclear inclusions were found in the bile duct epithelia. This finding is a new one except for KRIESEL's description of it in enzootic fox encephalitis. Nuclear inclusions were also found in the serosa epithelia of the digestive canals and the urinary bladder, and in the histiocytes of the interstitium of skeletal musculature. Inclusions in the vascular endothelia, appeared in the liver, the spleen, the kidneys, the myocardium, the lungs, the lymph nodes, the adrenal glands, the tonsils, the pharynx, the larynx, the digestive glands, the tongue, the oesophagus, the stomach, the small intestine, the thymus, the pancreas, the urinary bladder, the ovaries, the skeletal musculature, the bone marrow, the brain and the spinal cord. Consequently it may be suggested that the virus of H.c.c. has extensive affinity to vascular endothelia. In the experimental cases, nuclear inclusions were more abundant than in spontaneous cases, but no inclusion bodies could be seen in the bronchial epithelium or the epithelium of *membrana nictitans*. The occurrence of nuclear inclusions in the ependymal cells was not observed in these cases, as it was in the experimental cases of GREEN et al.¹¹⁾ with enzootic fox encephalitis.

COFFIN et al., by means of specific fluorescent antibody, indicated that the intranuclear inclusions of H.c.c. contain high concentrations of viral antigen and clarified the mode of development in the nuclear inclusions.

The present author believes that H.c.c. virus is increased chiefly in the endothelium, and then in the hepatic nucleus, in view of the distribution of nuclear inclusions in various organs.

As the nuclear inclusions often appear in the degenerating or degenerated cells, that finding may be considered to support the view that multiplication of the virus is accompanied by destruction of these cells.

True encephalitic changes were not observed in this disease, as has already been reported by the present author,⁵⁾ and the changes in the central nervous system are regarded as secondary changes due to the vascular damage.

COFFIN et al. indicated that no brightly fluorescent nuclei comparable to

those found elsewhere could be found in glial, ependymal, or nerve cells; but the inclusions in the vascular endothelium contained high concentrations of antigen as a result of the use of specific fluorescent antibody. Therefore, they considered that the brain lesions were the result of vascular damage, and not indicative of viral invasion of the central nervous system itself.

Two types of inclusions, viz., granular and homogeneous types, were observed in this disease. In experimental cases, the former type was widely distributed. These inclusions appeared to have been developed from a smaller granule to larger granules and finally to have become homogeneous inclusion bodies. Therefore, granular inclusion bodies are regarded as the developing type and homogeneous inclusion bodies as the mature type.

In mild and inapparent form, no characteristic changes caused by this disease and no intranuclear inclusions were observed, but reactive changes were noted. This is the form of the disease in recovered cases and seems to correspond to the form in spontaneous case. DECAMP and other authors classified this disease in forms, fatal fulminating, severe non-fatal, mild, and inapparent forms. In the mild form; there is a slight clinical illness usually not recognized by the owner of the animal; temperature is moderately elevated, moderate lethargy and anorexia are present, and photophobia with serous eye discharges. In the inapparent form, no clinical symptoms of illness are noticeable, but puppies later develop specific antibodies in serum. Both of these forms are identical to those examined here.

Pathologico-anatomically, enlargement of the lymph nodes, general anemia, hyperplasia of splenic follicles, general edema and interstitial nephritis were generally observed, but characteristic changes were not, except for the changes in the one case which was observed for a long period of time, in which the changes were similar to those in spontaneous cases.

Histopathologically, there were observed: *lymphadenitis catarrhalis* with marked phagocytic changes, activity of vascular endothelia and adventitial cells in the brain, hyperplasia of splenic follicles and red pulp, and proliferation of R.E.S. cells in the wall of the alveoli in the lungs.

In the liver, in general, R.E.S. cells were quiescent, but granulomatous small foci of endothelial cells were distributed in the cases of 50~62 days course. These changes were similar to those of the fatal severe form on the 6th day after inoculation, and were considered a recovered form of the latter.

Double nuclei in the liver cells were often noted as regenerative changes; the arrangements of the liver cell cords were irregular. In the case of prolonged duration, hepatic sinusoids and DISSE's spaces were dilated and R.E.S. cells were activated; polymorphonuclear infiltration in the sinusoids and vacuolar degener-

ation in the liver cells were also conspicuous.

The wall of the gall-bladder showed marked edema, but nuclear inclusions were not found here nor in the liver.

DECAMP states that sequelae ordinarily occur with canine distemper, but usually they are not observed in H.c.c. This fact probably indicates that the present disease is not accompanied by severe nervous damages or liver cirrhosis. It appears that animals with this disease either died with fatal severe infection or recovered with *restitutio ad integrum*. It seems that most cases resist the disease and do not develop to liver cirrhosis.

It is questionable as to whether liver cirrhosis caused by H.c.c. has been observed to date. But there are only two reports of chronic H.c.c. without liver cirrhosis, one by HODGMAN and LARIN and one by LARIN. On the other hand, SEIBOLD and BAILEY investigated acute, subacute and chronic canine hepatitis of unknown cause and different from H.c.c. It is interesting to note that regeneration of liver cells and newly formed bile ducts were observed in his chronic cases.

The most noticeable changes in this form now under study were the lesions of the kidneys; all cases except two showed *pyelitis catarrhalis chronica*. POPPENSIEK, alone and working with BAKER, demonstrated that the virus of H.c.c. has been recovered from urine at intervals of time extending from three days after inoculation to a long period afterward. They also considered that the finding of proliferative changes in the kidneys during the febrile period and a focal interstitial nephritis after apparent recovery suggests that the source of virus eliminated in the urine is in the kidneys. STÜNZI also mentioned a focal interstitial nephritis in the late stage of his experimental cases. The present author experienced four cases of focal interstitial nephritis. As a focal interstitial nephritis is frequently found in apparently healthy dogs, it is dangerous to connect this change with H.c.c. virus. *Pyelitis catarrhalis chronica* was frequently observed in the mild and inapparent form, but it is problematical as to whether this was a simple result of H.c.c. virus. As the author observed one case of acute purulent pyelitis in the fatal severe form, he considers that there is a possibility of association between virus and bacteria as the cause of the changes.

SUMMARY

The author made histopathological investigation of experimental infectious canine hepatitis and gained the findings stated below.

Depending upon clinical symptoms, pathological findings and the results of C.F.T. after inoculation, the following three forms of the disease were demonstrated: 1) fatal severe form, 2) mild and inapparent form, 3) negative form.

The fatal severe form is a typical hepatitis and this form is considered as the initial stage of hepatitis. In this form, on the second day after inoculation, MALLORY bodies appeared, and on the fourth day after inoculation, focal necrosis occurred. The location of focal necrosis was not constant and the size of affected areas was small. Central hepatic necrosis, such as in spontaneous cases, was not found in experimental cases. On the fourth day after inoculation, hyaline droplet degeneration, a granulomatous cell proliferation of endothelial cells in the liver was found. The reticular fibers in the liver were intact in almost all cases. In the regressive parts of the liver, decrease or disappearance in PNA were observed, but conversely, R.E.S. cells in the sinusoids were increased in PNA.

The characteristic nuclear inclusions of this disease occurred in almost all germinal layers, such as vascular endothelia (mesoderm), adventitial cells, reticulum cells and histiocytes (mesenchyma), liver cells, bronchial epithelia, bile duct epithelia (entoderm), mucosa epithelia in the tonsils (ectoderm), and serosa epithelia in the digestive canals and urinary bladder, epithelia in the heart and epithelia in the adrenal cortex (mesoderm). No inclusion bodies could be seen in the bronchial epithelium and epithelium of *membrana nictitans* as in spontaneous cases. The occurrence of nuclear inclusions in the ependymal cells was not observed as in experimental cases by GREEN et al.

In mild and inapparent form nuclear inclusions were not found. But reactive changes were noticeable, although characteristic changes were not. It appears that recovered cases of H.c.c. take this form and that it corresponds to cases of inapparent infection in spontaneous cases. *Pyelitis catarrhalis chronica* was especially frequently observed in this experimental cases.

In the negative form, no characteristic changes nor reactive changes were observed.

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EXPLANATION OF PLATES

PLATE I.

- Fig. 1. Case No. 7. Focal hepatic necrosis. Hematoxylin-eosin stain (H.-E.) $\times 60$.
- Fig. 2. Case No. 6. Nuclear inclusions in the liver. H.-E. $\times 1000$.
- Fig. 3. Case No. 7. Eosinophilic necrosis (E) (MALLORY bodies) in the liver cells. H.-E. $\times 1000$.
- Fig. 4. Case No. 7. Hyaline droplet degeneration (H) in the liver. H.-E. $\times 1000$.
- Fig. 5. Case No. 8. Cellular foci (KÜHN's "Spätknötchen") in the liver. H.-E. $\times 1000$.
- Fig. 6. Case No. 8. Nuclear inclusions in the bile duct epithelia. g: Granular inclusion, h: Homogeneous inclusion. H.-E. $\times 1000$.

PLATE II.

- Fig. 7. Case No. 2. Nuclear inclusion in the glomerular endothelium in the kidney. H.-E. $\times 1000$.
- Fig. 8. Case No. 6. Alveolitis of the lung and nuclear inclusion in the alveolar endothelium. H.-E. $\times 1000$.
- Fig. 9. Case No. 6. Nuclear inclusion in the adventitial cell in the spleen. H.-E. $\times 1000$.
- Fig. 10. Case No. 4. Nuclear inclusion in the reticulum cell in the bone marrow. H.-E. $\times 1000$.
- Fig. 11. Case No. 7. Nuclear inclusions in the reticulum cells and sinus endothelia in the lymph node. H.-E. $\times 1000$.
- Fig. 12. Case No. 3. Nuclear inclusions in the cortex epithelium in the adrenal gland and polymorphonuclear emigration. H.-E. $\times 1000$.
- Fig. 13. Case No. 3. Nuclear inclusion in a histiocyte in the subendocardiac region. H.-E. $\times 1000$.
- Fig. 14. Case No. 1. Nuclear inclusion in the epithelium of the tonsil. H.-E. $\times 1000$.
- Fig. 15. Case No. 8. Nuclear inclusion in the vascular endothelium in the brain. H.-E. $\times 1000$.



