### Title

**STUDIES ON THE MULTIPLICATION OF INFECTIOUS CANINE HEPATITIS VIRUS IN TISSUE CULTURE**

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INFORMATION

Hokkaido University granted the degree of Doctor of Veterinary Medicine to the following researchers in March, June and December 1964 under a new regulation (1962) authorizing the granting of the Doctors degree to qualified researchers who are not graduates of the Post-Graduate School.

March 25 — Mr. Y. SHIMIZU
June 30 — Mr. M. MAKITA
December — Mr. M. HAYASHI and Mr. M. NAKAMATA

The authors' summaries of the theses are as follows:

STUDIES ON THE MULTIPLICATION OF INFECTIOUS CANINE HEPATITIS VIRUS IN TISSUE CULTURE

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Well defined plaques on dog-kidney monolayers with an agar overlay medium containing bovine albumin (Armour) were observed with three strains of infectious canine hepatitis virus. The effects of variations in the composition of medium are described. A considerable level of antibody titer to infectious canine hepatitis virus was found in bovine sera used for overlay medium in complement fixation tests. When such bovine serum was used for overlay medium, the plaque formation was inhibited.

In the course of infection in dog-kidney cells, infectious canine hepatitis virus produced the soluble antigen and hemagglutinin. The soluble antigen possessed high antigenicity in complement fixation test. Centrifugation experiments demonstrated there existed at least two sizes in hemagglutinin. One of them could not be sedimented even at 40,000 rpm for 2 hours and another which seemed to be identical with infective particles was recovered in the pellet fraction. From heat stability experiments, the latter may be considered a comparatively heat stable component of virus particles.

Growth curve experiments indicated that the viruses which were responsible for multiplication were adsorbed and penetrated into the cells 4 hours after inoculation. The new infectious viruses appeared within the cells between 12 to

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JAP. J. VET. RES., VOL. 13, NO. 2, 1965
18 hours, and the length of the latent period was 18 hours. The new virus particles were released outside of the cells following this latent stage. The estimated yield of virus particles was about 250 PFU per infected cell at 42 hours.

The remarkable difference in the infectivity: hemagglutinin ratio between extracellular phase and intracellular phase suggested that there exists at least two stages in the course of the virus multiplication.

In making the diagnosis of infectious canine hepatitis complement fixation test, hemagglutination inhibition test and agar gel precipitation test were all found useful using the antigen prepared from infected tissue culture fluid.

HISTOPATHOLOGICAL STUDIES OF GIZZARD EROSION IN DAY OLD CHICKENS*1

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Out of a large number of day old chickens which had been diagnosed as having weakness of the body, one hundred and two cases were sacrificed at random within 12 to 24 hours of hatch. The autopsies of all the cases revealed erosions of the gizzard. All of the cases were histopathologically investigated from a general point of view.

The most fundamental histo-pathogenetical processes in the gizzard erosions were the alteration of the vessel walls (edematous loosening to swelling and hyaline swelling to hyalinization) and the degeneration of the intramural nervous plexus (formation of globular substances, etc.); the degeneration of the nervous plexus was regarded as what took part in the alteration.

*1 The original report of this work will appear in this Journal in the near future.
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