## Title

**STUDIES ON THE KINETICS OF THE NEUTRALIZATION REACTION BETWEEN JAPANESE ENCEPHALITIS VIRUS AND ANTISERUM**

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INFORMATION

The Hokkaido University granted the degree of Doctor of Veterinary Medicine to the following researchers September and December 1963 under a new regulation (1962) authorizing the granting of the Doctor's degree to qualified researchers who are not graduates of the Post-Graduate School.

September 30—Mr. N. HASHIMOTO, Mr. M. NAKAMATSU and Mr. M. SUGIMURA.
December 25—Mr. H. IZAWA, Mr. M. OHYA, Mr. T. TOKUI and Mr. M. TSUBOKURA.

The authors' summaries of the theses excepting Mr. TSUBOKURA's are as follows.

STUDIES ON THE KINETICS OF THE NEUTRALIZATION REACTION BETWEEN JAPANESE ENCEPHALITIS VIRUS AND ANTISERUM*

Nobuo HASHIMOTO

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Methods were described for obtaining plaque formation with Japanese encephalitis virus on chick embryo fibroblast monolayers. Overlay medium containing Gey's salts solution, 0.04 M tris buffer at pH 7.6, 0.5 percent lactalbumin hydrolysate, 0.3 percent tryptose phosphate broth, 1 percent Noble agar plus 5 percent calf serum, was optimal for the plaque formation of Japanese encephalitis virus.

Preliminary experiments revealed that the plaque assay method was at least as sensitive as the intracerebral inoculation of suckling mouse for detection of Japanese encephalitis virus, and that pure clones of this virus may readily be isolated from single plaques. Viral activity was only slightly affected between pH 6.5 and 9.0, but declined rapidly on either side of these limits.

These experiments have shown that the plaque assay method is suitable for kinetic studies of the interaction between Japanese encephalitis virus and its antibody.

The kinetics of the neutralization of Japanese encephalitis virus followed only a short exponential inactivation course. Under certain conditions, spontaneous recovery of infectious virus occurred with continued virus-antiserum incubation. These findings were more pronounced when rabbit antiserum was employed than

* The summary covers studies which were reported in part in the Annual Report Professional, 406 Medical General Laboratory, 135–143 (1960) and in “Virology”, 19, 261 (1963).
with guinea-pig antiserum. However, these characteristics were not altered by the use of hyperimmune sera, immune sera plus fresh normal guinea-pig serum, or selection of virus from persistent fraction. Using standard virus concentrations in the virus-antiserum mixture, the virus recovery increased as the pH of the mixture decreased. With higher concentrations of virus, however, recovery occurred even at high pH.

Virus surviving neutralization by rabbit antiserum resisted further neutralization by rabbit antiserum, but was sensitive to neutralization by guinea-pig antiserum, however, virus surviving neutralization by guinea-pig antiserum resisted further neutralization by both antisera.

These results suggest the possibility that two phenomena are in operation: dissociation of virus-antibody complexes and replacement with either blocking antibody which protects reneutralization by the homologous antisera, or with antibody which has the ability to alter the critical site on the virus in a manner which prevents further combination with neutralizing antibody specific for the same critical site regions.

HISTOPATHOLOGICAL STUDIES ON PARATUBERCULOSIS IN GOATS CENTERING AROUND THE FORMATION OF REMOTE LESIONS*

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Histopathological studies were carried out on 45 cases of naturally infected paratuberculosis of goats.

As the mode of development of the lesions, it was found that paratubercle bacilli invaded primarily in the intestinal mucosa and produced granulomatous proliferative inflammation and, subsequently, they reached the intestinal and mesenteric lymph nodes by way of the lymphatics. In this process, intimagranuloma, endolymphangitis and perilymphangitis in the lymph vessels were frequently observed. On the other hand, metastasis of the bacilli by way of the blood vessels was presumed. However, the author knew that it is insufficient to understand the pathology of the disease by only a interpretation of bacteremic events.

In regard to the remote lesions, very characteristic lesions were pointed out

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