

## Kinetic Neutralization Study of JE Viruses

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**OBJECTIVE:** The purpose of this study is to compare the antigens of selected JE virus strains isolated in Thailand, Vietnam, and Japan.

**DESCRIPTION:** Virus strains: Table I lists the strains employed in this study, together with brief information on their isolation and identification. Virus stocks were prepared as 20% suckling mouse brain suspensions in 0.75% bovine albumin in phosphate-saline (BAPS).

Antisera: Antiserum to the Thai strain (Cglt-1545) and to the Nakayama strain were prepared in rabbits by four intravenous inoculations of stock virus containing  $10^{5.0}$  plaque forming units. The viruses were given at 1-week intervals, and the animals were bled 15 days following the last inoculation. The serum was stored at  $-20^{\circ}\text{C}$ . Antisera to the remaining JEV strains listed in Table I were not employed in this study.

Virus assay: Virus was assayed by the standard plaque method in MK2 cells. A monolayer culture of MK2 cells in a 1 oz prescription bottle was washed with Hank's BSS (pH 8.3) and then inoculated with 0.3 ml of virus sample. After adsorption at  $37^{\circ}\text{C}$  for 90 minutes, the culture was overlaid with medium consisting of half strength minimal Eagles media (MEM), 2.5% inactivated calf serum and 1.5% noble agar (pH 8.3). The bottles were stained with neutral red on the 4th day after inoculation, and the plaques were counted on the 5th day.

### Neutralization kinetic method

The reaction mixtures contained approximately  $10^5$  PFU of virus and heat inactivated ( $56^{\circ}\text{C}$ , 30 min) high-titered antiserum at a dilution of 1:10. Fresh guinea pig serum was used to provide complement; it was added to reaction mixtures as a 10% dilution. In pilot experiments with or without guinea pig serum, the JEV antiserum effectively neutralized 90% of the homologous virus after 10 minutes incubation at  $37^{\circ}\text{C}$ . The reaction mixtures were placed in a  $37^{\circ}\text{C}$  water bath, and at 0, 1.25, 2.5, 5.0 and 10 minutes, 0.1 ml samples were removed and immediately diluted 100-fold in medium (M-199 with 5% inactivated calf serum, pH 8.3) which had been precooled to  $4^{\circ}\text{C}$ . Residual virus activity was determined as PFU in MK2 cells. Three plaque bottles were used for each time point. The % survival for each virus was plotted on log probit paper against time, and kinetic neutralization curves were drawn and compared.

**PROGRESS:** The neutralizing titer using JE (Nakayama) antiserum against the JEV strains listed in Table I indicated that homologous and Vietnam strains gave greater than 4-fold higher titers, and thus were neutralized more effectively than the 3 Thai strains. This suggested to us that the Thai strains may be antigenically different from the Vietnam and Nakayama strains. However, the passage levels of the JE strains were dissimilar, and there is some evidence that repeated passages of viruses in mice or tissue culture progressively alter the antigenic character of the virus. In the next experiment we have accordingly employed viruses which have identical mouse passage histories.

Kinetic neutralization assays were used to search for possible antigenic differences existing between different JEV strains of the same passage level. The results of a kinetic neutralization assay are shown in Figure 1.

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\*\* Deceased.

Three Thai strains, Cglt 1545, 40783 and 35929 and one Vietnam strain 34391, were studied. These strains were at 5th suckling mouse passage level. The JE (Nak) strain was not included in this experiment because the passage level was higher (SM 21). Antiserum prepared against Thai strain Cglt 1545 was used without guinea pig serum in the reaction mixture. The result shows no more than a 2-fold difference in percent virus survival between the 4 JEV strains at any time point along the kinetic neutralization curve. A 2-fold difference is not considered significant; thus there were no demonstrable antigenic differences between the homologous Thai strain, two other Thai strains, and the Vietnam strain.

In order to further exclude the possibility that the virus strains were different, we next employed complement (fresh guinea pig serum) in our system. Complement (or accessory factor found in fresh serum) has been shown to enhance the neutralizing capacity of antiserum in numerous virus-antibody systems. Use of fresh serum in this system may amplify and thus make apparent antigenic differences not demonstrable in the reaction mixture lacking fresh serum. The results of a kinetic neutralization assay employing fresh guinea pig serum is illustrated in Table 2. At 1.25 and 2.5 minutes no difference exists in the percent virus survival of the 4 JEV strains. At 5.0 and 10.0 minutes a difference in % survival does become more obvious. One Thai strain (40783) and the Vietnamese strain (34391) are neutralized to a greater degree (about 6 times more) than the Thai strains, 35929 and Cglt 1545. Although more than a 3-fold difference is considered significant, the 5 and 10 minute time points are not the most quantitatively sensitive areas on the neutralization curve, because only small numbers of virus remain infectious after 5 & 10 minutes incubation with antiserum. Moreover the homologous strain Cglt 1545 was seemingly less susceptible to its antiserum than two heterologous strains. These results should be confirmed by a checker-board (two-way cross) titration using homologous and heterologous antisera against each of the 4 virus strains. If true differences actually exist between neutralizability of the 2 groups of JEV strains, they appear to be minimal differences and may not be important biologically. Note that the addition of fresh guinea pig serum to the reaction mixture is associated with a greater reduction in percent virus survival at 10 minutes for all virus strains as compared to virus survival without fresh g.p. serum. This potentiation of heat inactivated antiserum by fresh serum is discussed more completely in another section of this Annual Report entitled, "The Effect of Serum Accessory Factor on JEV & Dengue Virus Neutralizing Antibody."

These preliminary results suggest there are no major antigenic differences among 3 Thai strains and one Vietnamese strain of JEV tested by a one-way neutralization kinetic assay.

Table 1. JE virus strains

Strain	Date of Isolation	Place of Isolation	Source	Passage Level	Nf. titer vs. JE (Nak) AS
Cglt 1545	Nov 68	Bang Phra, Thailand	Mosquito	SM <sub>6</sub>	220
40783	31 July 69	Chiengmai, Thailand	Human brain	SM <sub>2</sub>	640
35929	31 Oct 68	Udorn, Thailand	Human brain	SM <sub>3</sub>	400
34391	2 Aug 68	Vietnam	Human brain	SM <sub>3</sub>	<2560
JE (Nakayama) (Homologous)	?	Japan	?	SM <sub>21</sub>	3600

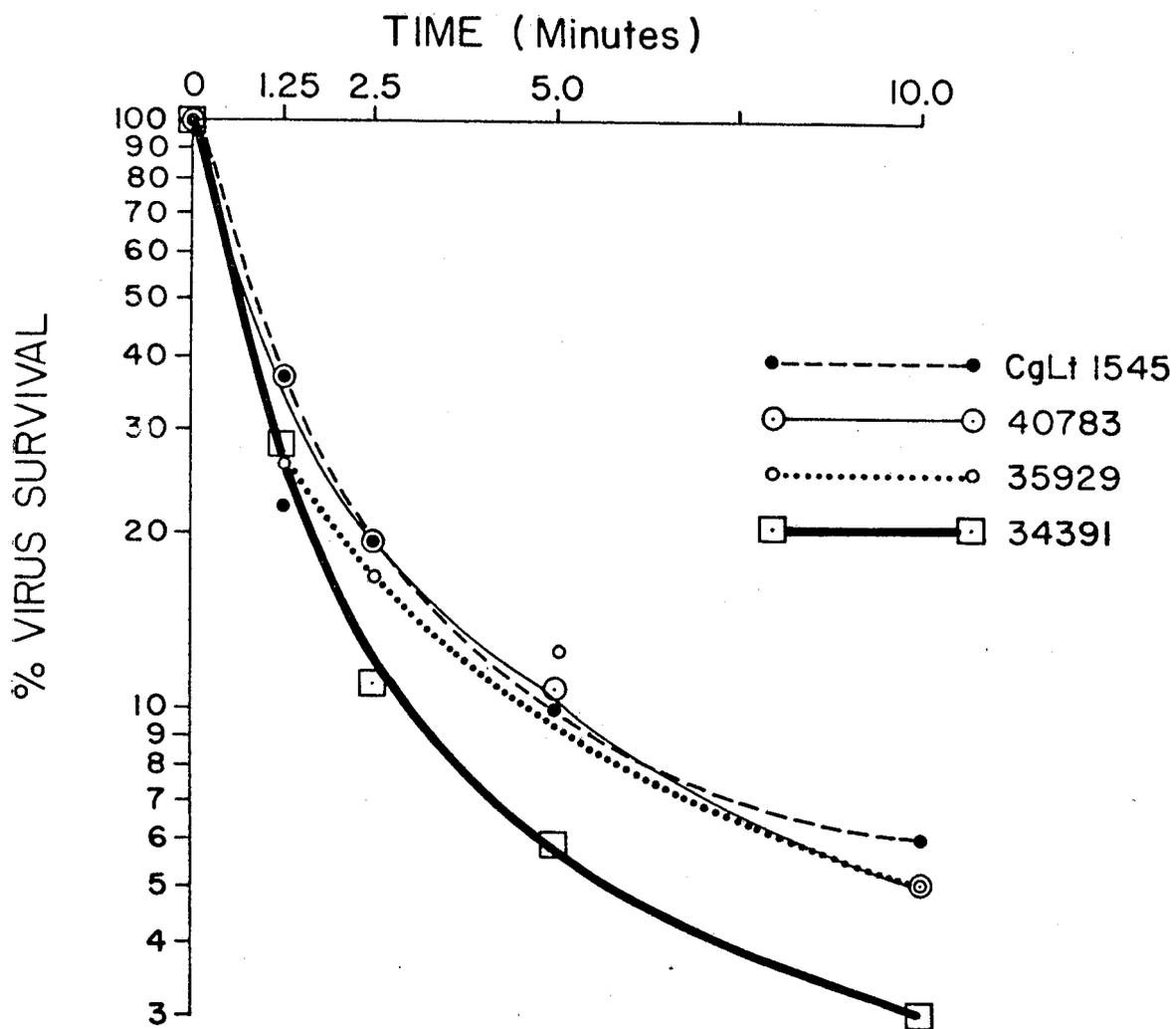


FIGURE I. Neutralization kinetic reaction of CgLt 1545, 40783, 35929 and 3439I vs CgLt 1545 antiserum (without guinea pig serum).

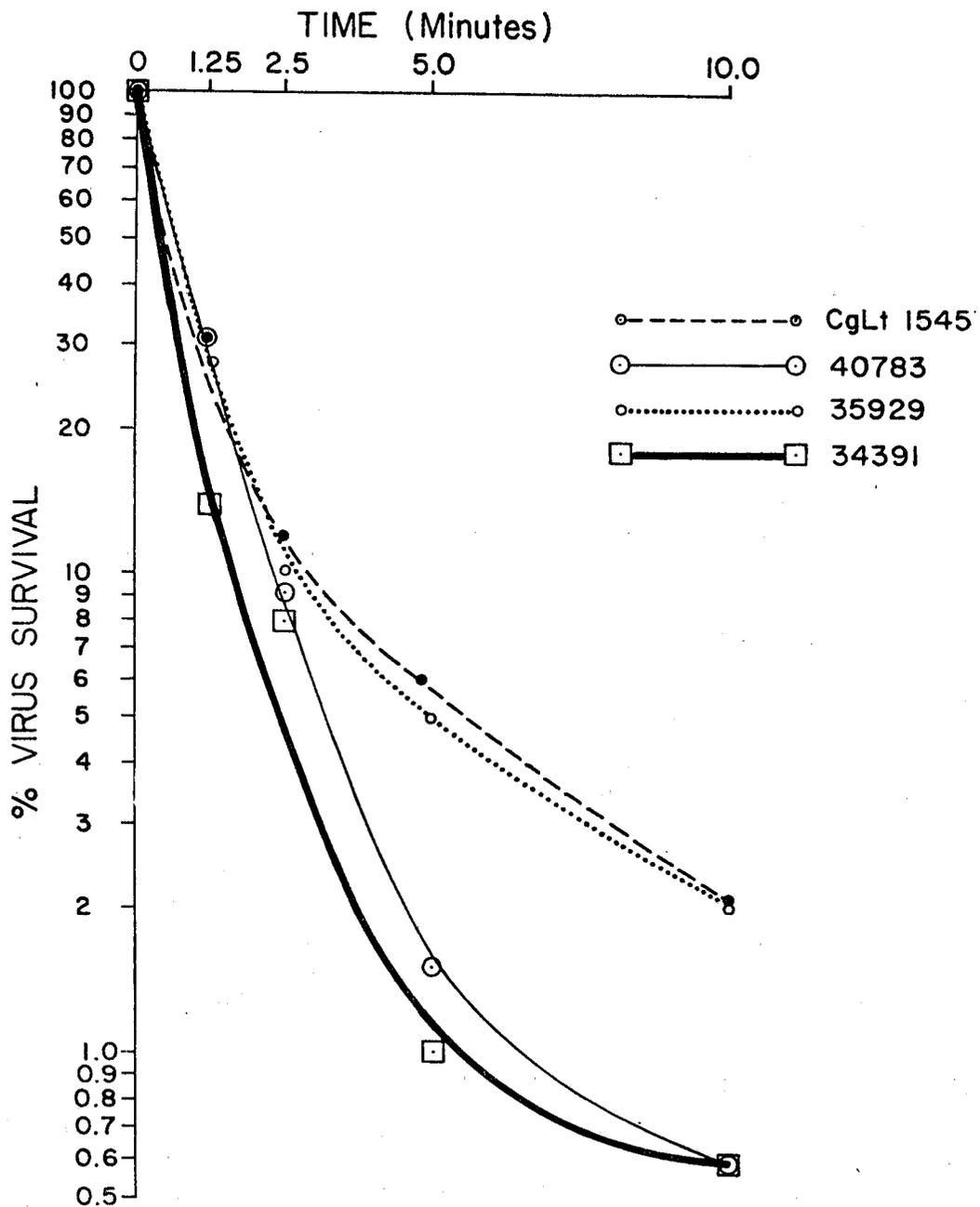


FIGURE 2. Neutralization kinetic reaction of CgLt 1545, 40783, 35929 and 34391 vs CgLt 1545 antiserum with 10% fresh guinea pig serum.