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SHORT COMMUNICATION

RE-ESTIMATION OF THE VIRION DENSITY OF JAPANESE ENCEPHALITIS VIRUS

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Nishimura et al. (1968) reported that the virion density of some strains of Japanese encephalitis (JE) virus from mouse brain was 1.30–1.31 or 1.34–1.35 g/cc. The virion density of dengue 2 virus, which is also a group B arbovirus, obtained from infected sucking mouse brain was reported to be 1.22 g/cc (Stevens and Schlesinger, 1965; Smith et al., 1970). We invested this discrepancy in the report values for the virion densities of similar viruses and this paper reports an amended value for the virion density of JE virus from mouse brain in CsCl solution of 1.24–1.25 g/cc, based on the results of repeated experiments described here.

Two strains of JE virus, Nakayama-NIH and Mizushima, were used. Both strains were grown in suckling mouse brains.

The virus was partially purified by a slight modification of the method of Kitaoka and Nishimura (1963); that is, a 20% suspension of infected suckling mouse brain in 0.5 M borate buffered saline, pH 9.1–9.3, was centrifuged at 6,000×g for 30 min and protamine sulfate (2–5 mg/ml) was added to the supernatant. After 15 min in a cold bath, the mixture was centrifuged at 6,000×g for 30 min and the supernatant obtained was recentrifuged at 105,000×g for 120 min to precipitate viral components. The pellet thus obtained was used for further analyses.

The pellet was resuspended in 0.02 M Tris buffer, pH 7.7 (TB), and clarified by centrifugation at 800×g for 10 min. Concentrated CsCl solution in TB was added to the supernatant to make a final CsCl concentration of 35–40%. The mixture was centrifuged at 105,000×g for 19 hr in the SW-39 rotor of a Beckman model L ultracentrifuge at 5 C. Fractions were collected dropwise from the bottom of the tube. To determine the buoyant density of the virions, the density (Bruner and Vinograd, 1964), hemagglutinating (HA) activity (Clarke and Casals, 1958), and infectivity

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titer (PFU) in primary chick embryo cell cultures (Oya and Okuno, 1967), of each fraction were measured.

The result of isopycnic density gradient centrifugation in CsCl is shown in Fig. 1. The infectivity was distributed in a range of density of ρ (g/cc)=1.20–1.27 with three peaks at ρ=1.22, ρ=1.25. and ρ=1.27. Of these the second peak was the highest and the third was a minor peak of variable size. HA activity was located between ρ=1.20 and ρ=1.26, being highest at ρ=1.20 and decreasing with increase in density. Two shoulders are observed at ρ=1.22 and ρ=1.25, suggesting the existence of two peaks of activity at these points.

These observations suggest that the partially purified sample contains the following viral components:

(1) Fraction 1.20 (ρ=1.19–1.20); only HA activity positive
(2) Fraction 1.22 (ρ=1.21–1.22); both HA activity and infectivity positive
(3) Fraction 1.25 (ρ=1.24–1.25); both HA activity and infectivity positive
(4) Fraction 1.27 (ρ=1.27–1.28); infectivity positive; HA activity not detectable; variable fraction

Of these four components, components (2) and (3) with infectivity and HA activity seem to have the characteristics of JE virions. To prove the existence of these two components, the two fractions of ρ=1.23 and ρ=1.24–1.25 obtained by CsCl density gradient centrifugation were each subjected to isopycnic density gradient centrifugation after readjusting their CsCl concentration.

The results are shown in Figs. 2A and 2B. The heavier (ρ=1.24–1.25) fraction showed only one peak of infectivity at ρ=1.24, but two peaks of HA activity at ρ=1.19 and ρ=1.24 (Fig. 2A), suggesting that a non-infective fraction (ρ=1.19) is derived from the original ρ=1.24 fraction.

The lighter (ρ=1.23) fraction showed a major peak of infectivity at ρ=1.22 and a minute peak at 1.24 which might be due to contamination with the heavier component. In this case most of the HA activity was located at ρ=1.19. There were also two small peaks at ρ=1.22 and ρ=1.24, the former like a shoulder and the latter rather sharper (Fig. 2B). Thus in both fractions high HA activity was observed in the ρ=1.19 fraction without infectivity.

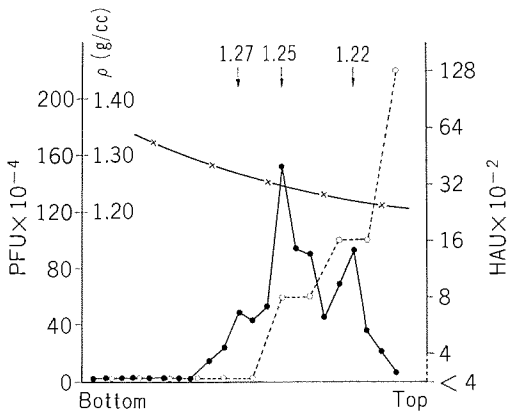


FIGURE 1. *Distribution of infectivity and HA activity after CsCl isopycnic centrifugation of partially purified Japanese encephalitis virus.* ●, *infectivity (PFU/0.4 ml);* ○, *HA titer at pH 6.4;* ×, *medium density (g/cc).*

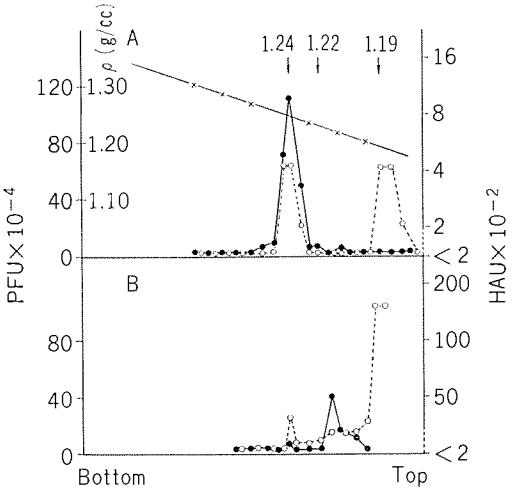


FIGURE 2. *Recentrifugation of the fractions obtained from the experiment shown in Fig. 1, in CsCl. A: fraction ρ=1.24–1.25 g/cc; B: fractions $\rho \leqslant$1.23 g/cc.* ●, *infectivity (PFU/0.4 ml);* ○, *HA titer at pH 6.4;* ×, *medium density (g/cc).*

Figs. 3 and 4 show the electron microscopic appearances of the fractions. The samples were dialysed overnight against water in cold after fractionation on the density gradient column and then negatively stained with 2% potassium phosphotungstate.

For reference Figs. 3a and 3b show the morphology of JE virions (Mizushima strain), further purified from the $105{,}000 \times g$ pellet by sucrose density gradient (5–40%; linear) centrifugation. Similar 40–50 nm particles were only seen in the $\rho = 1.25$ fractions obtained by CsCl density gradient centrifugation (Fig. 4a, 4b), while fractions $\rho = 1.30$–1.31 contained only amorphous components without complete architecture (Fig. 4c, 4d).

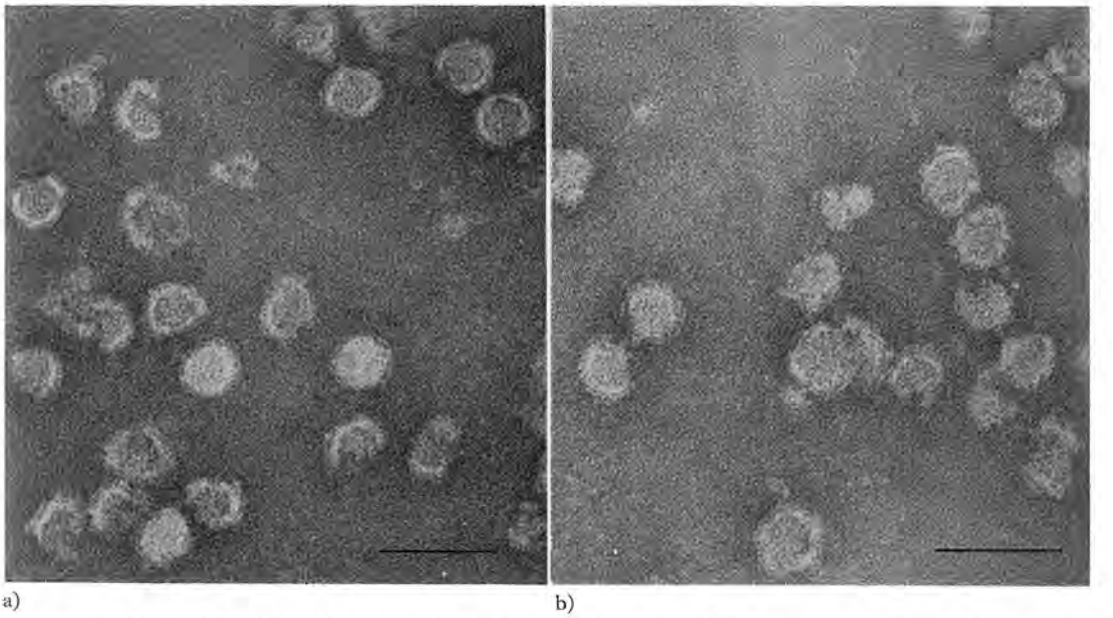
The above data indicate that the buoyant density of JE virus is $\rho = 1.21$–1.22 and $\rho = 1.24$–1.25. Similar values were obtained in further experiments using two other strains of JE virus (Q–180 and JaGAr–01) from infected suckling mouse brain.

Three possible explanations for this dual density of JE virion might be considered:

(1) There are essentially two kinds of virions with different densities in infected mouse brain.
(2) The actual density of virions is 1.24–1.25, while those with a density of $\rho = 1.21$–1.22 are artifacts. (possibly due to attachment of some lighter substances such as lipoproteins derived from host cells.)
(3) The actual density of virions is $\rho = 1.21$–1.22 while the other groups are artifacts due to structural change occurring during their preparation.

As a source of virions we used infected suckling mouse brain, in which multiplication of the virus is not synchronous, so the preparation may contain virions at various maturation stages and these could be expressed as a population of different densities. This could explain the heterogeneity of the virions on assumption (1).

On the other hand, in a control experiment, we have confirmed that the virion density of JE virus grown in BHK21 cells is 1.24 g/cc. This finding and the result shown in Fig. 2A strongly support assumption (2).



a) b)

FIGURE 3. *Purified JE virion (Mizushima strain) prepared by differential and sucrose density gradient centrifugation. Virus band was stained with potassium phosphotungstate after dialysis against water; scale, 1,000 nm.*

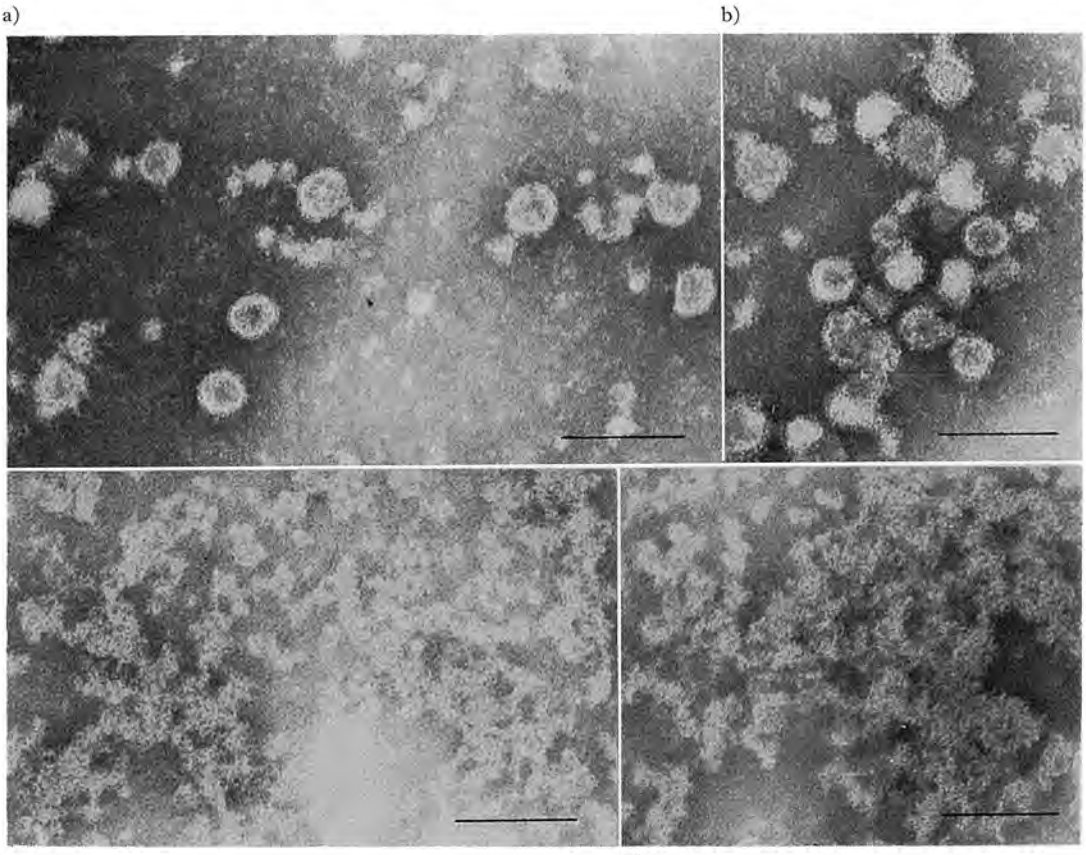


FIGURE 4. *Electron microscopic appearances of the fractions obtained by CsCl isopycnic centrifugation. a and b, fractions ρ=1.26 g/cc; c and d, fraction ρ=1.30–1.31 g/cc; potassium phosphotungstate stain; scale, 1,000 nm.*

Aaslestad et al. (1968) reported that eastern equine encephalitis virus caused new products of ρ=1.23 from the complete type virion, which has a density of 1.20 g/cc in CsCl isopycnic centrifugation. This suggests the validity of assumption (3).

Thus, for the dualism of JE virus virion density can not yet be fully explained. However, we would like to postulate that the virion density of JE virus grown in suckling mouse brain is 1.24–1.25 g/cc, instead of the formerly reported value of 1.30–1.31 g/cc or 1.34–1.35 g/cc and that there is secondary population of JE virions, which has density of 1.21–1.22 g/cc. The components in the fractions of ρ=1.27–1.28 and ρ=1.30–1.31 may be degraded forms of virions and the heaviest components in the fraction of ρ=1.34–1.35 may be the naked ribonucleoprotein core of the virion (Igarashi, 1969).

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