BIKEN JOURNAL Vol. 14, 361-364, 1971

SHORT COMMUNICATION

ELECTRON MICROSCOPIC OBSERVATIONS ON DENGUE TYPE 2 VIRUS

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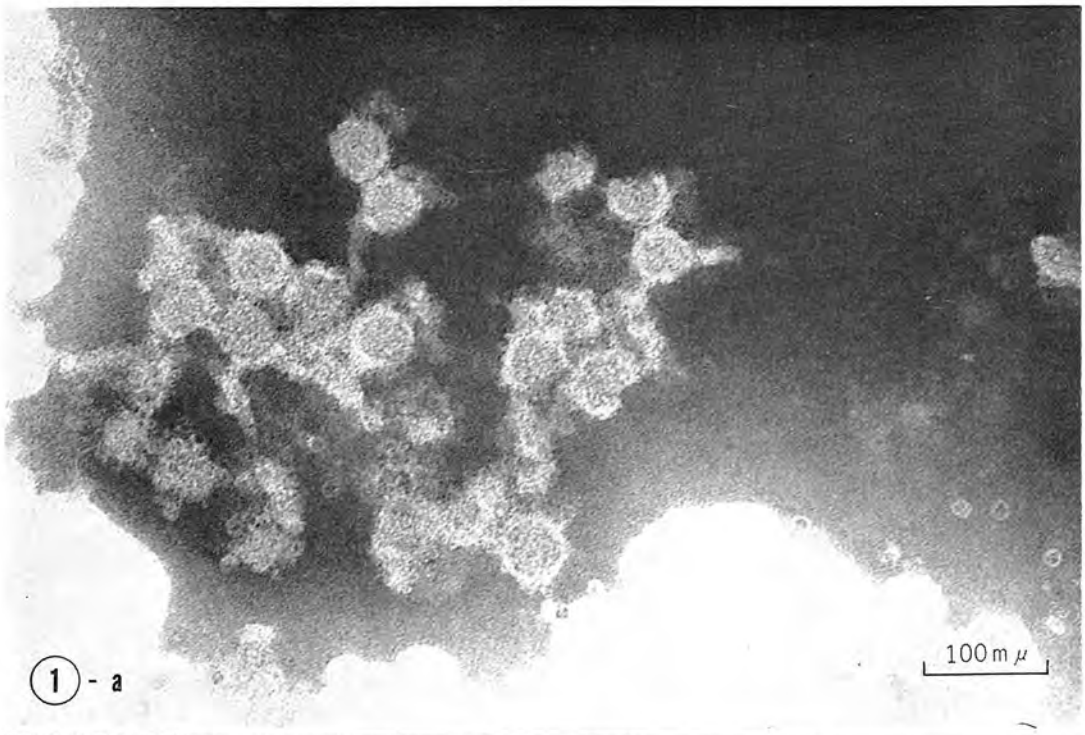
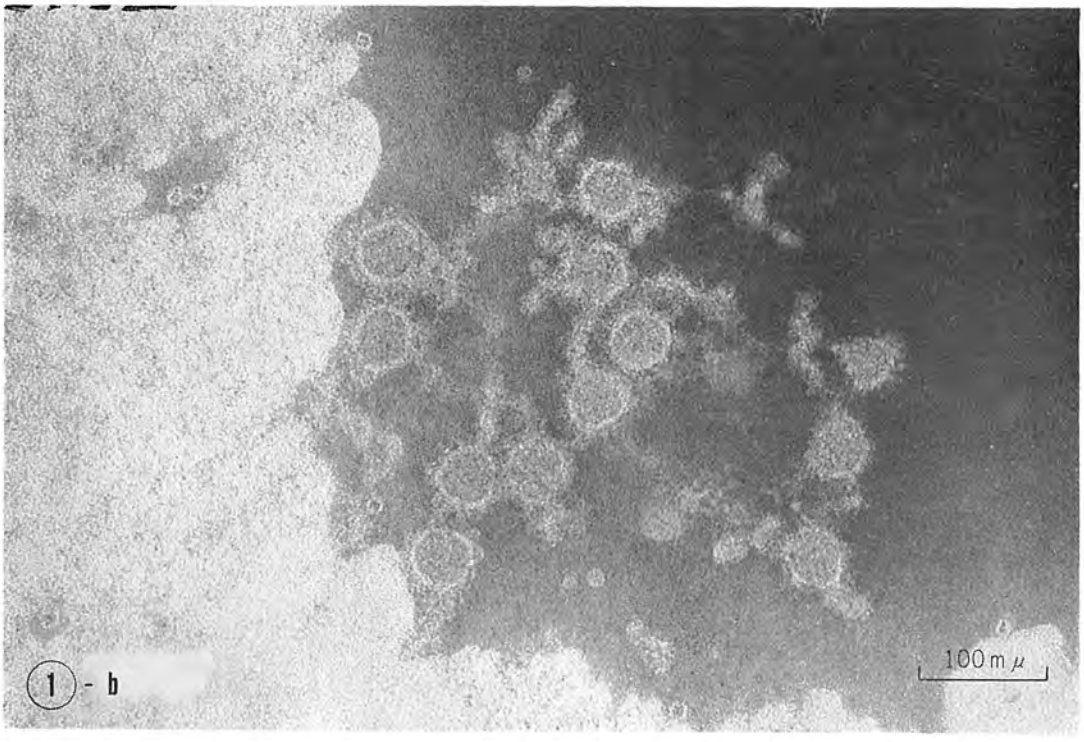
(Received May 1, 1971)

The electron microscopic observations on ultrathin sections of cultured cells infected with dengue type 1 and 2 viruses have been reported (Oyama et al., 1967). Recently, Horzinek et al (1969) reported studies on the structure and symmetry of the nucleocapsid after in vitro decoating of Sindbis virus particles, von Bonsdorff et al. (1969) reported the inner structure of group A and B arboviruses by electron microscopy, and Slávik et al. (1970) reported on the morphology and structure of tick-borne encephalitis virus. Moreover, Smith et al. (1970) described the physical and biological characters of dengue type 2 virus. This paper reports electron microscopic studies on the morphology of the virion and the hemagglutinating component of dengue type 2. The starting material was obtained from suckling mouse brains infected with dengue type 2 virus, Tr. 1751 strain, given by courtesy of Dr. Theiler of the Rockefeller Foundation. Then a 20% suspension in borate buffer-saline, pH 9.3, was prepared. The suspension was centrifuged at 8,000 rev/min for 20 min and the supernatant was treated with protamine sulfate (5 mg/ml) in a cold bath for 15 min. Then the mixture was centrifuged at 8,000 rev/min for 20 min. The clear supernatant was centrifuged at 40,000 rev/min for 120 min in a Spinco model L centrifuge, using rotor 40, to precipitate virus particles. The pellet was suspended in 1.5 ml of 0.05 M tris-buffer pH 8.7, and sonicated twice at 10 kc for 5 min. The sonicated preparation was centrifuged at 3.000 rev/min for 10 min, and 0.5 ml of the supernatant virus suspension was layered on a 5–45% sucrose gradient in 0.05 M tris buffered saline as described previously (Nishimura et al., 1964; Kitaoka et al., 1965, 1967). The preparation was centrifuged in the SW-39 swinging rotor of a Spinco preparative ultracentrifuge at 30,000 rev/min for 75 min. The main band formed in the lower part of the tube. This was thought to have the highest infectivity and HA activity from comparison with results on dengue viruses (Stevens et al., 1965) and JE virus (Kitaoka and Nishimura, 1965; Nishimura and Kitaoka, 1964). The main band was removed through a long needle and dialysed against redistilled water in the cold room overnight. It was examined using an electron microscope.

The sample was put on grids and fixed. After negative staining with 2% phosphotungstic acid, the structure of the dengue type 2 virus was observed under an electron microscope model JEM-TA, as described by Nishimura et al. (1967). In Fig. 1 and 2 the virion of dengue type 2 appears spherical and is about 48–55 mμ in diameter. It is covered by an



① - a



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FIGURES 1a and 1b. *Dengue type 2 virus (Virions, envelopes and HA particles).*

amorphous, apparently removable sticky envelope. Several virions seem to be aggregated together. There is a so-called white ring, which is an electron dense, apparently rigid ring of about 4–5 mμ width surrounding the inner core, as seen in Japanese encephalitis (JE) virus (Nishimura et al., 1967). It is difficult to estimate the width of the envelope because it separates from the surface during preparation of the test sample. It is noteworthy, however, that there are 2 kinds of subunit particles. One is seen on the surface of some virions and has a central hollow about 6–7 mμ wide. The other is 13–14 mμ in diameter. From the structure of JE virus, both particles probably have HA activity since this is usually on the surface of virions with envelopes. Fig 2 shows small sized HA components on the surface of a virion and large sized components free of the envelope. Further studies are required on whether the large particles originate from small ones. There is no evidence that these particles are incomplete viruses.

This work was done in 1968. We would like to thank Dr. Oikawa of the Laboratory of Japan Electron Optics Co. Ltd., for his kind support in this work.

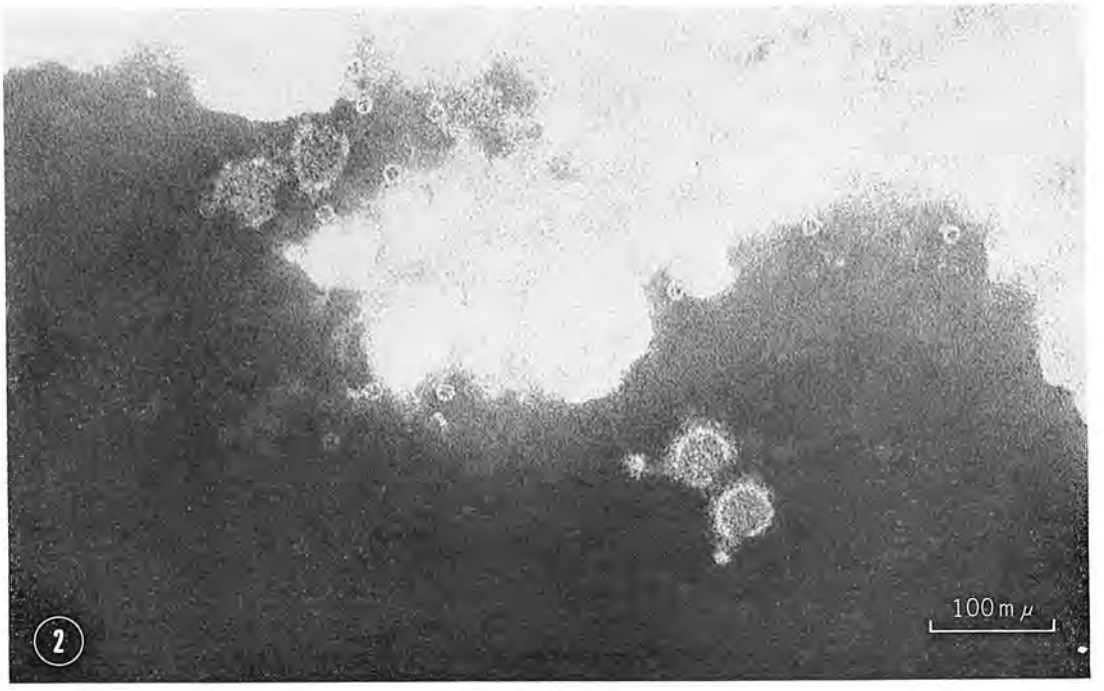

FIGURE 2. *Free HA particles associated with virions and envelopes.*

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