

MORPHOLOGICAL PROPERTIES OF DIFFERENT MEASLES VIRUS VACCINE STRAINS (*)

M. S. Shahrabadi*, H. Mirchamsy, A. Shafyi, S. Bahrami and M. Kamali
Razistate Serum Institute, Tehran, Iran, and* Department of Medical Bacteriology, University of Alberta, Canada

ABSTRACT

Five strains of measles virus and a strain isolated from a SSPE case were examined by electron microscopy. It was found that these strains differed morphologically with respect to their surface haemagglutinating spikes. Some strains, such as Edmonston and Sugiyama strains, contained long spikes with distinct stalks whereas the AIK strain lacked distinct spikes. The relationship of the surface haemagglutinating spikes of these strains and their ability to induce immunity is discussed.

Measles virus, a member of the family Paramyxoviridae (Fenner, 1976), is structurally similar to other paramyxoviruses (Choppin and Compans, 1975; Kingsbury, 1972; Morgan and Rapp, 1977; Waterson, 1965). These viruses are composed of an outer lipoprotein envelope containing short projections or spikes which cover the entire outer surface of the virion. Inside the envelope exists a linear, helical nucleocapsid that contains viral RNA and is the major component of the virion. In paramyxoviruses the surface envelope containing glycoproteins has haemagglutinating (HA), haemolytic (HL) and cell fusion activities (Waterson, 1965). The haemagglutinating activity is associated with the surface spikes (Norrby and Gollmar, 1975). A large surface glycoprotein (MW 79,000) is involved in haemagglutinating properties of measles virus (Tyrrell and Norrby, 1978).

The successful vaccination by live measles virus vaccine has significantly improved the immunity and protection against measles and reduced its complications (Krugman, 1977). Several attenuated strains of measles virus have been used for vaccination with variable degrees of protection (Mirchamsy *et al.*, 1977). We studied the morphological properties of these several strains of measles viruses used for vaccination and compared their surface structure with regard to the presence of haemagglutinating spikes by means of negative staining electron microscopy.

The strains collected by Dr Mirchamsy were obtained from the following sources. Edmonston strain isolated by Enders and Peeble (1954) was obtained

(*) Reprinted from: *Microbios letters* 14 39-46 1980

from Dr Makino, Kitasato Institute, Japan. The virus had been passaged 24 times in primary human kidney cells, 41 times in human amnion cells, six times in Vero cells and five times in MRC-5 cells (Matumoto *et al.*, 1962).

Schwarz strain which has been developed by passing the Edmonston virus 77 times in chick embryo cells was obtained from the Merieux Institute, France. Leningrad vaccine strain lot 69-6 was supplied by the Moscow Research Institute of Virus Preparation. A variant of Sugiyama strain called 5F100 was obtained from Dr S. Hashizume Chiba Serum Institute, Japan. The original strain of Sugiyama had been developed by Matumoto *et al.* (1962) by passing it 82 times in calf kidney cells. AIK-Cvaccine lot TV 12 was obtained from Dr Makino, Kitasato Institute. This strain originated from the Enders-Edmonston virus and was developed by passing it twelve times in primary sheep kidney cells at 33°C and cloning it with seven subsequent passages in chick embryo cells (Makino *et al.*, 1974). Subacute sclerosing panencephalitis (SSPE) virus was isolated from brain biopsies by co-cultivation with Vero cells (Mirchamsy *et al.*, 1978). The above strains were subculture three times in MRC-5 cells using Parker 199 medium containing antibiotics and 0.2% gelatin, except for the SSPE strain which was grown in Vero cells. Monolayers of cells were divided into five groups and infected with the above mentioned virus strains at a multiplicity of infection of 0.1 pfu/cell. Five to seven days after infection the liquid media of the infected cultures were harvested and centrifuged at $10,000 \times g$ for 10 min to sediment cell debris. The supernatant from the cells infected with different strains was centrifuged under the same conditions at $65,000 \times g$ for 1 h in a Beckman Ultracentrifuge. In some cases the viruses were pelleted over a 75% sucrose cushion and centrifuged as above. The band over the sucrose cushion was collected by puncturing the side of the tube with a needle connected to a syringe.

The virus strains were collected from the centrifuge tube and were resuspended in 0.5 ml of the growth medium at 4°C. Immediately after samples from each of the virus strains were negatively stained with 3% sodium phosphotungstate using formvar coated grids and examined in a Phillips EM 400 electron microscope. The conditions for culture growth, virus preparation and centrifugation were kept constant for all samples of different strains of viruses. In addition, an attempt was made to prepare all the electron microscope samples under similar conditions to avoid any artifact which might occur under different staining procedures. In each preparation a large number of virus particles were examined and photographs were taken of many particles at random and their size was measured from the photographs. Negatives were projected on a screen and the length of surface spikes was measured. There was no distinct difference between the virus pelleted in an ultracentrifuge tube and the virus collected on sucrose cushion.

From 230 particles of Edmonston strains of measles virus examined, they all appeared very pleomorphic with size ranging from 100 to 310 nm in

diameter. Some of the particles spherical but the majority had irregular shape (Figures 1 and 2). All particles were covered with an envelope containing projections or spikes about 25 ± 2 nm long. The envelope appeared thick and intact so that the internal virus nucleocapsid was not visible. Particles from the SSPE isolate were less heterogenous in shape. The 180 particles examined had a diameter of 90 to 170 nm and contained distinct spikes about 30 ± 3 nm long (Figure 3). Two hundred particles of Sugiyama strain virus were examined. They were pleomorphic, ranging in size from 130 to 280 nm with surface spikes similar to those from the Edmonston strain (Figure 4). Virus particles of Leningrad strain were 150 to 300 nm in diameter. Their surface projections were less pronounced and shorter (18 ± 2 nm long in 170 virus particles examined) than the above mentioned strains (Figure 5). A total of 230 Schwarz strain virus particles were examined. They were roughly spherical with a size of 140–240 nm and did not have projections with distinct stalks. Their surface was covered with protrusions about 14 ± 3 nm long. The internal nucleocapsid was visible in most particles (Figure 6).

Two hundred fifty virus particles from the ALK strain were examined. The particles were not pleomorphic, and they were spherical or oval in shape with a diameter of 160–190 nm. They lacked distinct surface spikes. It seemed that the loss of surface spikes made the envelope more permeable to the negative stain and as a result the internal nucleocapsid was visible (Figure 7). All the experiments including virus preparation, staining, and electron microscopy were repeated three times. The numbers mentioned above are the results of total particle counts and size measurements from three separate preparations. The morphological appearance of each virus strain was similar in all the preparations examined. In one case a sample of the AIK strain and the Edmonston strain were fixed in 0.1% glutaraldehyde for 2 min and then dialysed and used for electron microscopy, when similar differences in spike morphology as in Figures 1 and 7 were observed. Therefore, the morphological differences in various strains were not due to the alterations occurring during the handling and virus preparation. In some cases virus preparations were dialysed against 0.5 M ammonium acetate for 8h and then used for electron microscopy. No morphological differences were observed in virion structures before and after dialysis.

From the above observations it seems that changes in the structure of the envelope of the different strains probably occurred during the frequent passages of the virus in different host-cell systems. Whether the envelope changes were due to the host cells or resulted from alterations in the viral genome is not clear. To determine the haemagglutinating capacity of each strain, a microhaemagglutination test was applied using vervet monkey red blood cells. It is interesting to note that the AIK strain had very low haemagglutinate monkey red blood cells, whereas the Edmonston strain showed high haemagglutinating activity, (Table 1). The Schwarz strain had a haemagglutination titre less than the Edmonston strain. In measles virus,

Table 1 Haemagglutination capacity of different strains of measles virus

Strain 10 ⁷ pfu/ml	HA/0.025 ml
Edmonston	512
Leningrad	256
AIK	4
Sugiyama	256
Schwartz	128
SSPE	ND

Preparations of measles virus strains were partially purified and their concentration was adjusted to contain 1×10^7 Pfu/ml. The haemagglutination (HA) titre was calculated per 0.025 ml.

ND = Not done.

surface projections are involved in haemagglutinating (HA) activity and the absence of distinct spikes in the AIK strain may be the reason for its inability to haemagglutinate erythrocytes. Since all the vaccine strains used in this study have been shown to induce a relatively long-lasting immunity in vaccinated individuals (Mirchamsy *et al.*, 1966) it seems that HA antigen and surface spikes may not be critical for induction of immunity against measles. It will be interesting to analyse the glycoprotein content of the above strains and compare their haemolytic activity with their immunogenicity. This might clarify the role of some of the surface glycoproteins in immunization.

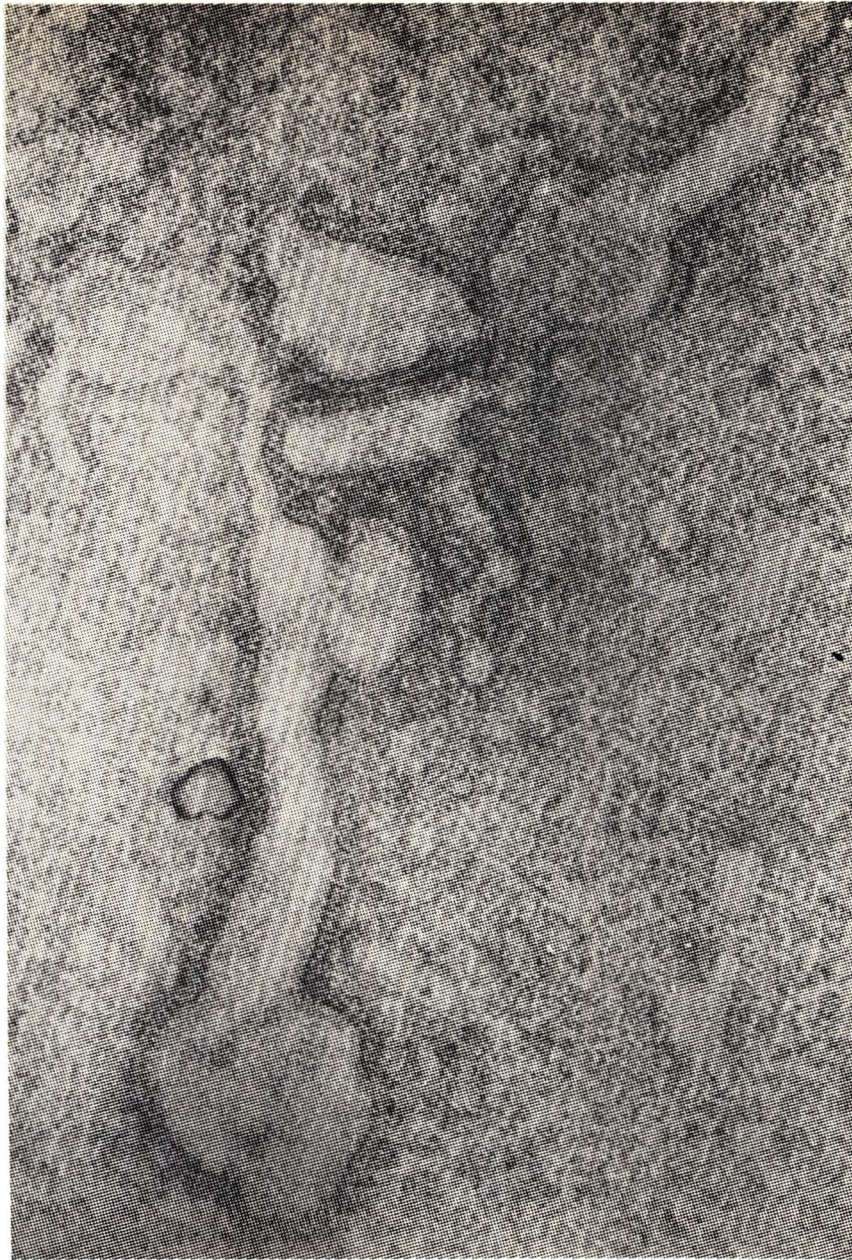


figure 1 Particles of Edmonston strain of measles virus negatively stained with sodium phosphotungstate showing pleomorphism of the virion. $\times 100,000$.

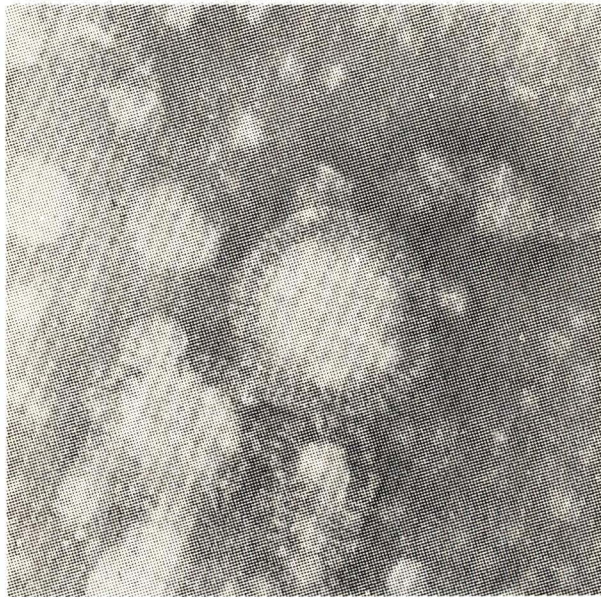
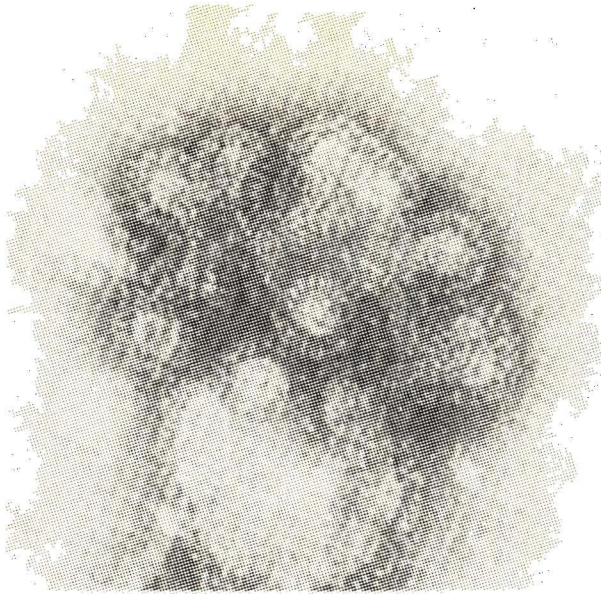


Figure 2 (top) Similar virus preparation as in Figure 1 showing a particle with distinct spikes. $\times 130,000$.

Figure 3 (bottom) Virus particles from SSPE isolate. The surface of virions are covered with long spikes with distinct stalks. $\times 120,000$.

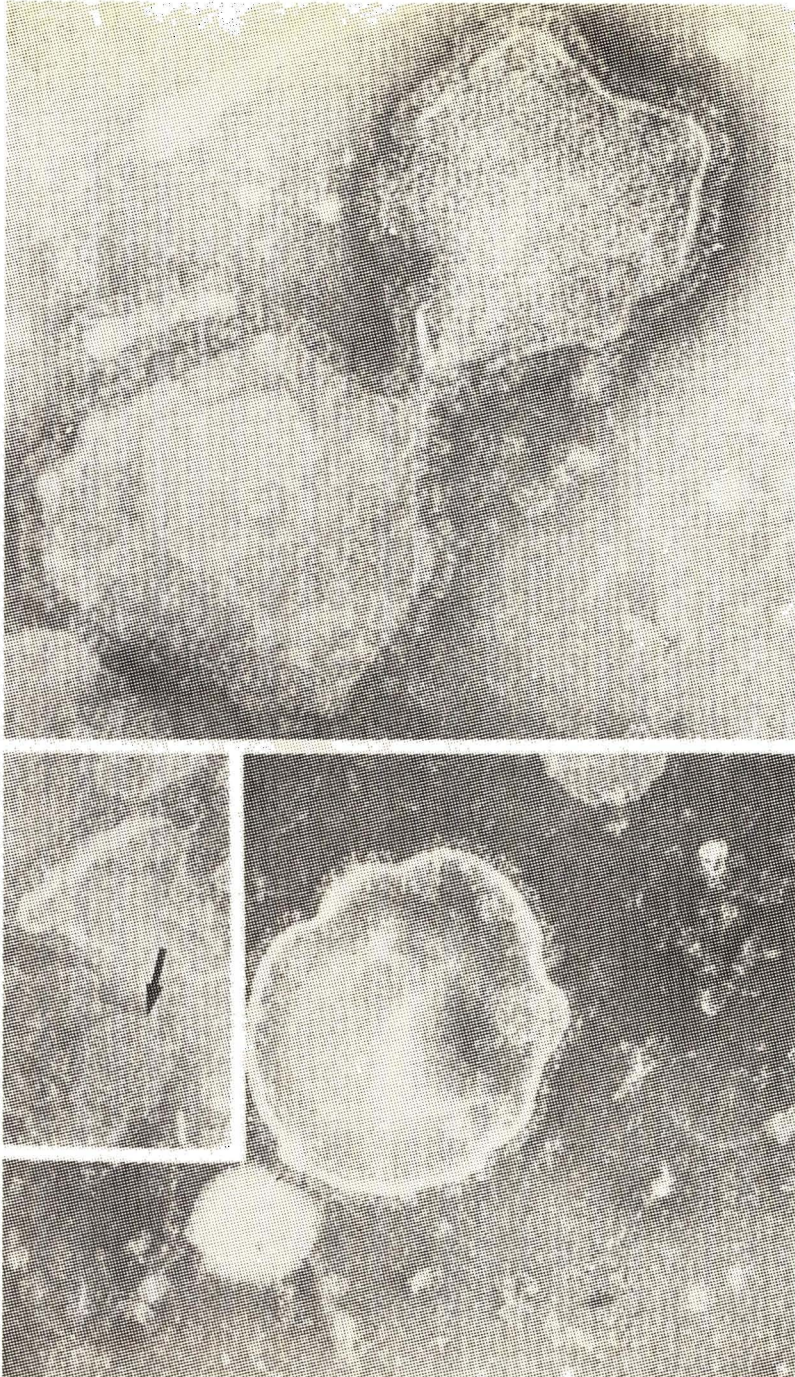


figure 4 (top) Avirus particle of Sugiyama strain with long surface spikes. $\times 120,00$.

Figure 5 (bottom) Leningrad strain of measles virus. The virion is covered with short projections. the *inset* shows a particle from which the surface projections have been removed and a hexagonal pattern is displayed (arrow). $\times 130,000$.

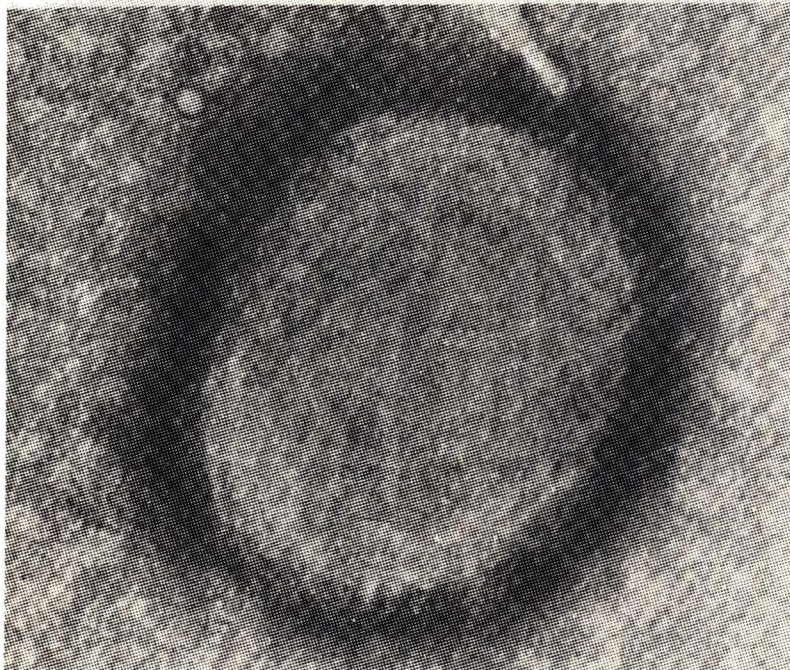
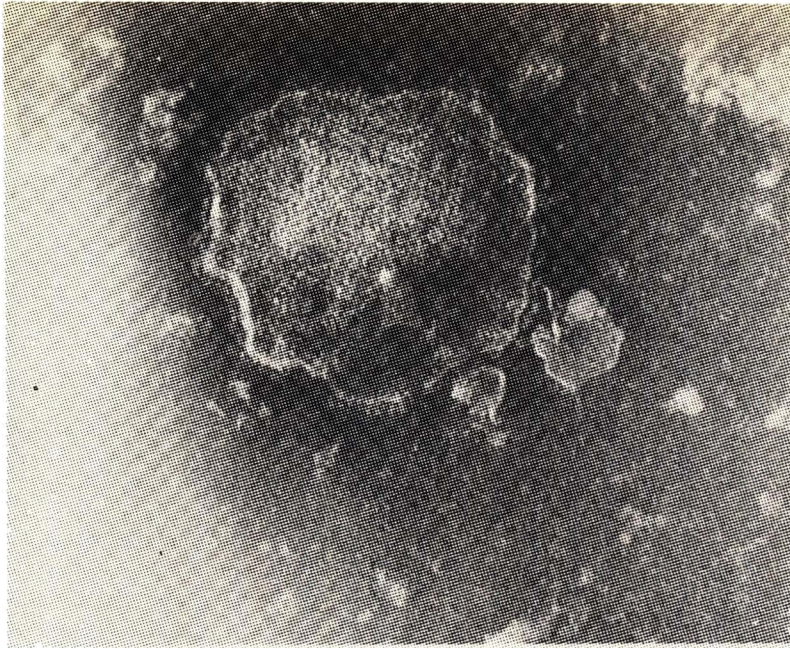


Figure 6 (top) Schwarz strain of measles virus with short surface protrusions. The internal nucleocapsid is visible. $\times 150,000$.

Figure 7 (bottom) Virus particle of AIK strain. The virion lacks distinct surface projections, and the internal nucleocapsid is visible. $\times 200,000$.

References

- CHOPPIN P. W. and Compans R. W. 1975. Reproduction of paramyxoviruses. P.95–178. In Comprehensive Virology. Edited H. Fraenkel-Conrat and R.R.Wagner. Vol. 4.Plenum Press, New York and London.
- ENDERS J. F. and peeble T. C. 1954. Propagation in tissue cultures of cytopathogenic agents from patients with measles. *Proc. Soc. exp. Biol. Med* 86 277–86.
- FENNER F. 1976. Classification and nomenclature of viruses. Second report of The international Committee on Taxonomy of Viruses. *Intervirology* 7 59–60.
- KINGSBURY D.W. 1972. Paramyxovirus replication. *Curr. Top. Microbiol. Immunol.* 59 1–33.**
- KRUGMAN S. 1977.Present status of measles and rubella immunization in the United States: a medical progress report. *J. Ped.* 90 1–12.
- MAKINO S. SASKI K. and Nakamura N. 1974. Studies on the modification of the live AIK measles vaccine. II. Development and evaluation of the AIK-C measles vaccine. *Kitasato Archives Exp. Med.* 47 13-21.
- MATUMOTO M. Mutai Y. Saburi R. Fjuii M. Minamitani M. and Nakamura K. 1962. Live measles virus vaccine: clinical trail of vaccine prepared from a variant of the Sugiyama strain adapted to bovine kidney cells. *Jap. J. Exp. Med.* 32 433-48.
- MIRCHAMSY H. Shafiy A. Bahrami S. Kamali M., Nazari P., Razavi J., Ahourai P. Fatemi S.and Amin-Salehi M. 1977. A comparative field trail of five measles vaccines produced in human diploid cells, MRC-5 *J. BIOL. Stand.* 5 1–18.**
- MIRCHAMSY H., Ahafiy A.,Bahrami S., Shahrabadi M. S., Kamali M., Ahurai P., Nazari P. and Drarakshshah I. 1978. Isolation and characterization of a defective measles virus from brain biopsies of three patients in iran with subacute sclerosing panencephalitis. *Intervirology* 9 106–18.**
- MORGAN E. M. and RAPP F. 1977. Measles virus and its associated diseases. *Bact Rev.* 41 636–66.
- NORRBY E. and Gollmar Y. 1975 Identification of measles virus-specific hemolysis inhibiting antibodies separate from from hemagglutination inhibiting antibodies. *Infec. Immun.* 11 231–9.
- TYRRELL D. L. G. and Norrby E. 1978 Structural polypeptides of measles virus. *J. gen. Virol.* 39 219–29.
- WATERSON A. P. 1965. Measles virus. *Arch. Gesamte Virus Forsch.* 16 57–80.