

VARIATION IN MORPHOLOGY OF EPHEMERAL FEVER VIRUS

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ABSTRACT

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Electron microscope studies on the three ephemeral fever virus (EFV) strains EFV1, EFV13 (South Africa) and Japanese bovine epizootic fever (BEF) virus (Strain YHK) showed that the normally cone-shaped South African EFV, may, under certain circumstances, appear bullet-shaped while the Japanese EFV which is usually bullet-shaped may appear as a cone. These findings suggest that ephemeral fever virus is pleomorphic.

INTRODUCTION

Electron microscopic studies on the prototype ephemeral fever virus (EFV1) isolated by Van der Westhuizen (1967) revealed that the virion is cone-shaped in thin sections of infected BHK21 cells (Lecatsas, Theodoridis & Erasmus, 1969). Similar studies on EFV isolates in Japan and Australia have shown that the infective particles are bullet-shaped (Ito, Tanaka, Inaba & Omori, 1969; Holmes & Doherty, 1970). Aberrant forms of the Australian EFV strain were observed by Holmes & Doherty (1970).

Though there are morphological variations between these three isolates, serologically they appear to be almost identical (Lecatsas, Theodoridis & Els, 1969). At present the cause of morphological variation is not known, but some recent findings indicate that each isolate may appear in either one or the other, or both, of these forms. Similar observations have been reported by Australian workers (Murphy, Taylor, Mims & Whitfield, 1972).

The present communication emphasizes the pleomorphism of the particle in both negative contrast and thin sections and, in addition describes the form and variation in length of spikes covering the envelope of mature particles of two isolates of the virus, EFV1 and the Japanese BEF strain YHK.

MATERIALS AND METHODS

(1) Cells

Monolayers of BHK21-C13 cells or Vero cells (African green monkey kidney cells) grown in roller tubes with Eagle's medium supplemented with 5% bovine serum (negative for EFV) were used in these studies. The maintenance medium was supplemented with 1% foetal calf serum.

(2) Virus

The three EFV isolates employed in these experiments were the South African isolate EFV1, EFV13 and the BEF virus strain YHK originating from Japan. Cross neutralization between EFV1 and EFV13 strains has been shown by serum neutralization tests in mice (Theodoridis unpublished observations, 1972).

- (a) Virus strain EFV13 was derived from an ox experimentally inoculated with blood from a natural case of the disease. Blood was collected at the peak of the febrile reaction in 1% sodium citrate and centrifuged for 30 minutes at 2 000 g

in a refrigerated centrifuge at 4°C. The leucocyte-platelet fraction was aspirated, suspended in phosphate buffer containing peptone and lactose* (BLP) and stored at -70°C. Roller tube cultures of BHK21 cells were seeded with 0.2 ml of the leucocyte suspension and a blind passage of frozen and thawed tissue culture cells resuspended in the medium was carried out every third or fourth day until cytopathic effects (CPE) became evident at the third passage.

For electron microscopy, monolayers of BHK21 cells infected with EFV13 of the fifth and seventh passage were harvested 44 hours after inoculation when CPE was evident in all the cells.

After five passages in BHK21 cells EFV13 was adapted to grow in Vero cells. At the seventh passage in Vero cells, cultures were also examined in the electron microscope.

- (b) EFV1 serially propagated in BHK21 cultures for 17 passages was used to infect BHK21 monolayers and the cells were used for electron microscopy 44 hours after inoculation.
- (c) The Japanese BEF virus isolate was received at the nineteenth passage in BHK21 cells. In this laboratory it was carried through three intracerebral passages in infant mice and stored as a 10% infective mouse brain suspension in the lyophilized form at -20°C. This material was inoculated onto monolayers of BHK21 cells and after four to five serial passages, cultures showing 100% CPE within 44 hours of inoculation were harvested and examined with the electron microscope.

(3) Electron microscopy

- (a) EFV-infected cells were scraped off the glass into the medium with a long needle. This suspension was centrifuged at low speed; the resultant pellet was prefixed in 3% glutaraldehyde for 1 hour and thereafter postfixed in 1% osmium tetroxide in Millonig's buffer for a further hour (Millonig, 1961). Subsequently, the cells were dehydrated in graded ethanol dilutions, cleared in propylene oxide and embedded in Epon 812 as described by Lecatsas & Weiss (1969). Thin sections were cut on a Reichert Om U 2 ultramicrotome and double stained in uranyl acetate and lead citrate.

* Seitz filtered solution of 0.2M phosphate buffer with 2% peptone and 10% lactose

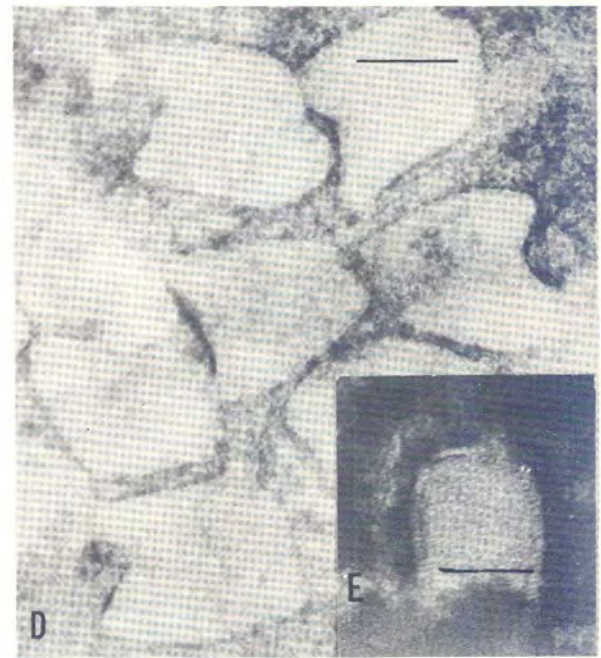
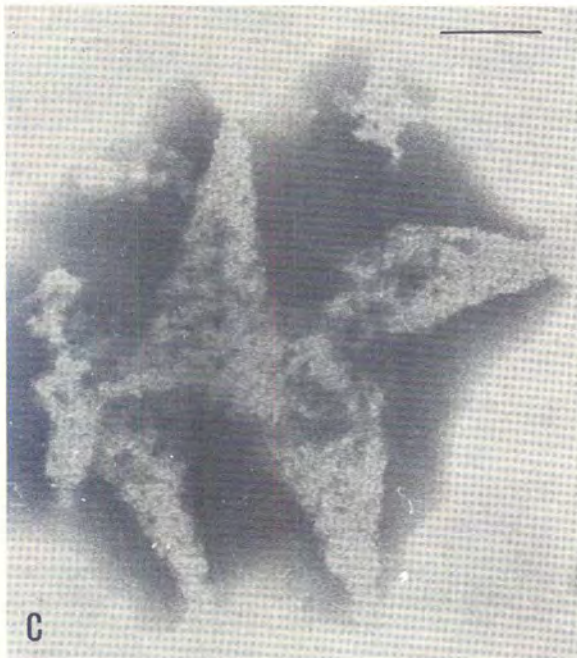


FIG. 1A, 1B and 1C. EFV13 in Vero cells negatively stained with 3% PTA at pH 6. Note both cone-shaped and bullet-shaped forms of the virus. FIG. 1D, 1E. EFV13 in BHK21 cells negatively stained with 3% PTA at pH 6. Note both cone-shaped and bullet-shaped forms of the virus. Bar 80 nm.

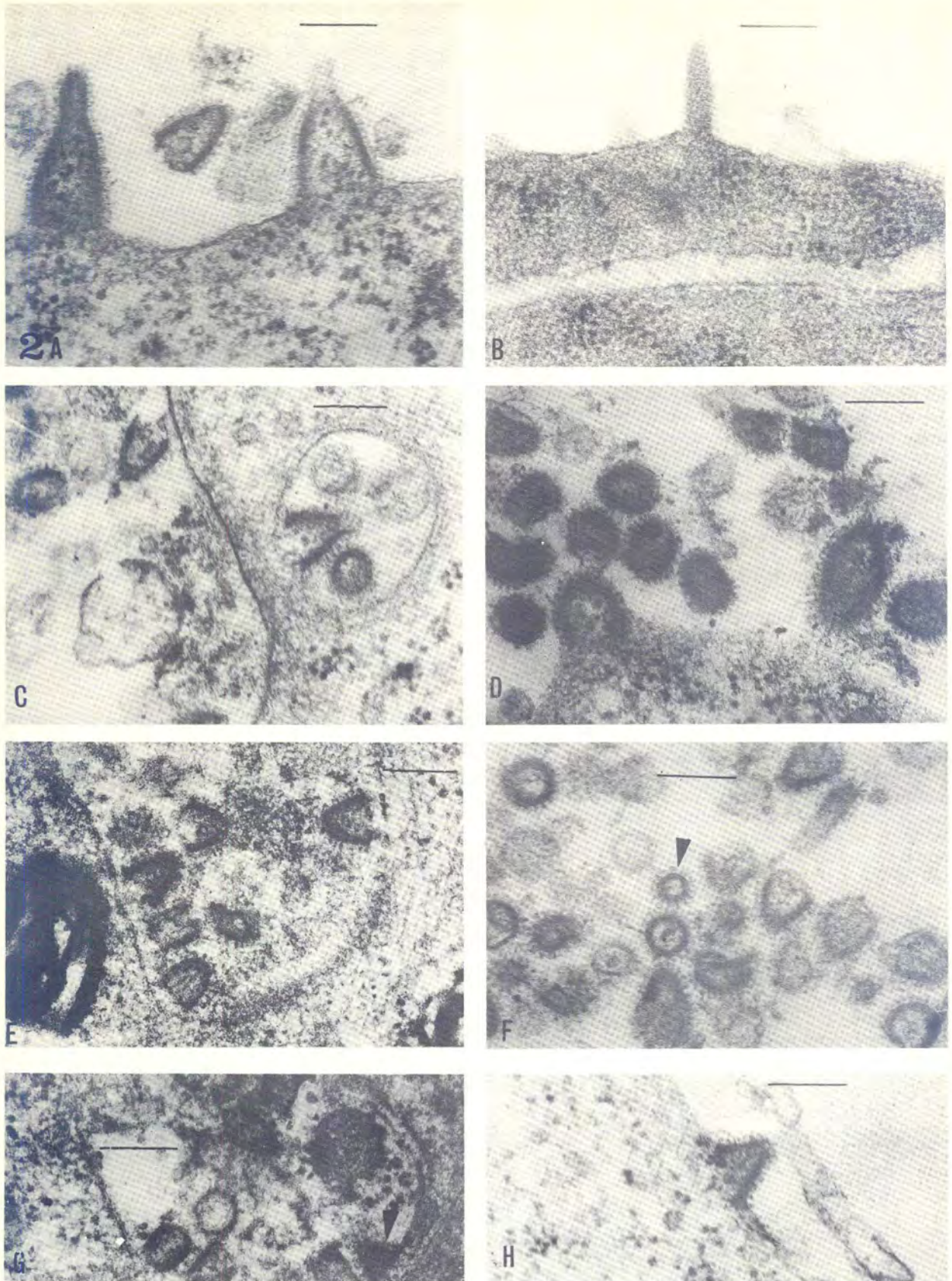


FIG. 2A, 2B, 2C, 2D, 2E, 2F, 2G and 2H. BEF virus in sections of BHK21 cells. FIG. 2A. Aberrant form of cone-shaped virus particle. FIG. 2B. Bullet-shaped form of virus lacking well-defined spikes on surface. FIG. 2C, 2E and 2G. Intracytoplasmic vesicles containing virus particles. Note attachment of virus to limiting membrane of vesicle in FIG. 2G (arrow). FIG. 2D, 2F, 2H. Virus particles with prominent surface projections. Note double membrane (arrow) character of electron dense portion of virus particle in FIG. 2F.

(b) For negative contrast studies EFV-infected cells suspended in Eagle's medium were pelleted at low speed and, after decanting, the medium was drained off by mounting the tube on filter paper. A portion of the pellet was mixed with a small quantity of distilled water and a drop of this suspension mixed with a drop of 3% phosphotungstic acid (PTA) at pH 6. A drop of this mixture was transferred to a formvar carbon coated grid, the excess fluid blotted and the grid allowed to dry. Grids were examined in a Siemens Elmiskop 1A electron microscope at an accelerating voltage of 80 kV and an instrumental magnification of 40 000. Particle diameters were determined on negatives using a Nikon model 6C comparator.

RESULTS

The negatively stained preparations (Fig. 1A to 1E) indicate that a mixture of both bullet-shaped and cone-shaped forms of EFV13 can occur in both BHK21 and Vero cells. The presence of spikes on the surface of virus particles is clearly demonstrated in Fig. 1E. The preparations of BEF virus (Strain YHK) show that the virus possesses surface spikes with a height of $17,7 \pm 2$ nm (Fig. 2F). The preparations of EFV1, however, (Fig. 3A and 3B) show projections of a considerably shorter length, viz. $12,4 \pm 0,7$ nm.

Thin sections of BEF indicated the pleomorphic character of the virus harvested at 44 hours (Fig. 2A and 2B). Bullet-shaped particles occurred predominantly in cells with a dense cytoplasmic matrix (Fig. 2B). Such particles often lack the well-defined spikes in other sections of this isolate (BEF). Aberrant virus particles are illustrated in Fig. 2A. The development of bullet-shaped BEF virus particles from intracytoplasmic vesicles was reported by Ito *et al.* (1969) and although such development was confirmed the particles in this investigation were predominantly cone-shaped (Fig. 2C, 2E, 2G). The development of conical forms from the limiting membrane of such vesicles is clearly illustrated in Fig. 2G. This developmental stage was not seen in sections of EFV13.

DISCUSSION

Electron micrographs of the ephemeral fever virus strains EFV1, EFV13 (South Africa) and YHK (Japan) show distinct variation in the morphology of this virus. While EFV1 appeared mainly as cone-shaped particles, EFV13 of the fifth tissue culture passage presented a mixed population of bullet- and cone-shaped particles in approximately equal propor-

tions. Ito *et al.* (1969), however, who reported only bullet-shaped virions in their studies using BHK21 cells, cultured the organisms at 33 to 34°C. This Japanese strain, which consisted mainly of bullet-shaped particles (Ito *et al.*, 1969) appeared as cone-shaped units when cultured in BHK21 cells at 37°C. Consequently we suggest that the temperature at which the organism is cultured may be one of the factors which control the shape of the virus. Since the results in BHK21 and Vero cells were identical, the type of cell does not have any effect on the shape of the virus.

Moreover, the present investigation also showed that the length of the spikes varies in the different strains.

Recently Murphy *et al.* (1972) reported that Australian and Japanese EFV isolates appear as bullet forms early during multiplication and cone-shaped particles late in infection cycle and are of the opinion that the shape of the virus is dependent on the time of harvesting. Our experience with this virus has shown that, when EFV-infected BHK21 cells are harvested between 40 and 48 hours after infection, various proportions of both morphological forms of the virus are found and thus indicates that it is pleomorphic.

Our present observations suggest, therefore, that EFV shows a tendency towards pleomorphism and that the temperature at which it is cultured may be one of the factors which influence it.

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REFERENCES

HOLMES, I. H. & DOHERTY, R. L., 1970. Morphology and development of bovine ephemeral fever virus. *J. Virol.*, 5, 91-96.
 ITO, Y., TANAKA, Y., INABA, Y. & OMORI, T., 1969. Electron microscopic observations of bovine epizootic fever virus. *Natn. Inst. Anim. Hlth. Q., Tokyo*, 9, 35-44.
 LECATSAS, G., THEODORIDIS, A. & ELS, H. J., 1969. Morphological variation in ephemeral fever virus strains. *Onderstepoort J. vet. Res.*, 36, 325-326.
 LECATSAS, G., THEODORIDIS, A. & ERASMUS, B. J., 1969. Electron microscopic studies on bovine ephemeral fever virus. *Arch. ges. Virusforsch.*, 28, 390-398.
 LECATSAS, G. & WEISS, K. E., 1969. Formation of Wesselsbron virus in BHK21 cells. *Arch. ges. Virusforsch.*, 27, 332-338.
 MILLONIG, G., 1961. Advantages of phosphate buffer for OsO₄ solution in fixation. *J. appl. Phys.*, 32, 16J.
 MURPHY, F. A., TAYLOR, W. P., MIMS, C. A. & WHITFILDE, S. G., 1972. Bovine ephemeral fever virus in cell culture and mice. *Arch. ges. Virusforsch.*, 38, 234-249.
 VAN DER WESTHUIZEN, B., 1967. Studies on bovine ephemeral fever. *Onderstepoort J. vet. Res.*, 34, 29-40.

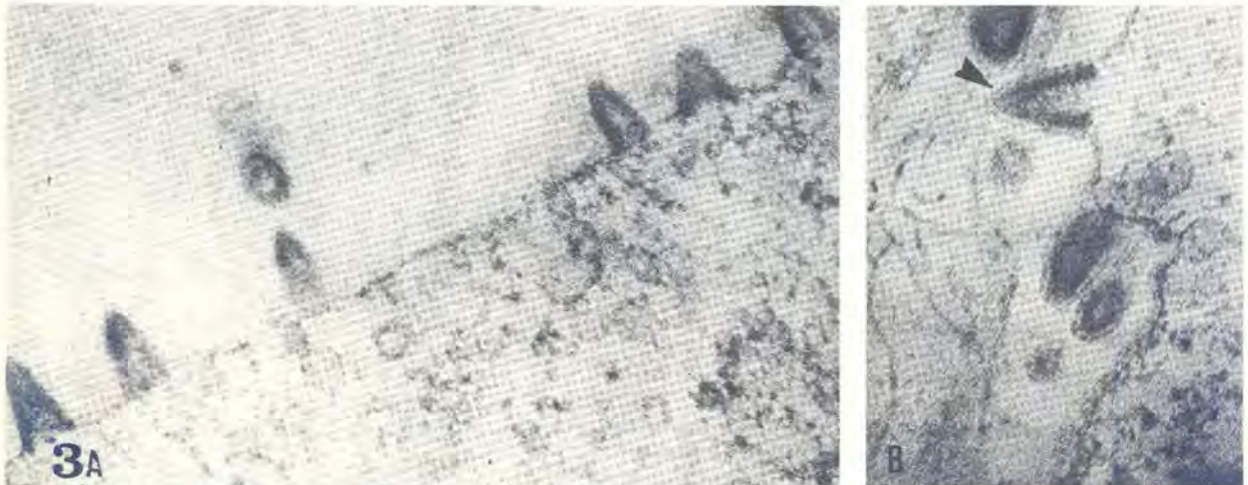


FIG. 3A and 3B. EFV1 in sections of BHK21 cells. Note membrane (arrow) with short spikes covering virus particles in FIG. 3B, which differs from membrane in figures depicting BEF virus, which have longer spikes.