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Chemical Structure of African Swine Fever Virus Investigated by Electron Microscopy

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The morphological development of African swine fever virus in a line of pig kidney tissue culture cells (PK 13) was described previously (1). In order to confirm earlier information on the type of nucleic acid present in this virus (2), two isolates, LISBON-57 (3) and HINDE (4), grown in tissue cultures were embedded in glycol methacrylate before treatment with enzymes (5). The methods used were described by Zambernard & Vatter (6) in their study of virus particles described by Lucké (7) in renal tumours of leopard frogs. Infected PK 13 cells in prescription bottles were harvested 48 hr after inoculation at an input multiplicity of approximately 10 plaque forming units (p.f.u.)/cell. Both 10 % formalin and 1 % glutaraldehyde in Sørenson's buffer pH 7·2, were used for 15 min. fixation at 4° before embedding in glycol methacrylate (6). The enzyme solutions were as follows: 0·5 % (w/v) pepsin (2 × crystallized, Mann Research Laboratory) in distilled water adjusted to pH 8·0 with 0·1 N-NaOH; 0·5 % trypsin (w/v) (2 × crystallized, Worthington Biochemical Corp.) in distilled water adjusted to pH 8·0 with 0·1 N-NaOH; 0·3 % (w/v) ribonuclease (RNase) (3 × crystallized, salt-free, Worthington Biochemical Corp.) in 0·1 M-sodium acetate pH 6·4, and 0·3 % (w/v) deoxyribonuclease, (DNase) (1 × crystallized, salt-free, Worthington Biochemical Corp.) in 0·1 M-sodium acetate at pH 7·0. Sections were floated on the enzyme solutions, either directly or after mounting on carbonized formvar coated grids, and incubated from 30 min. to 4 hr at 37°. After treatment, the sections were rinsed thoroughly with distilled water and stained 15 to 30 min. with saturated uranyl acetate. Micrographs were made with an RCA-EMU-3G microscope at 50 kv.

Sections were selected which contained appropriate numbers of virus particles, and both formalin- and glutaraldehyde-fixed specimens were treated with the enzymes for equal periods of time. Both virus isolates reacted in the same manner. Preservation of cell cytoplasmic detail was more successful with glutaraldehyde than with formalin. For comparison, Pl. 1, fig. 1 illustrates both mature and immature African swine fever virus embedded in Epon and stained with uranyl acetate, while Pl. 1, fig. 2, at lower magnification, shows the poorer preservation of cytoplasmic detail of infected tissue embedded in glycol methacrylate.

Pl. 1, figs 3 and 4 illustrate the results of 2 hr RNase and 2 hr trypsin digestion, respectively. In neither was any change seen in the virus structure. On the other hand Pl. 2, figs 5 A and 5 B show the effect of ½ hr and 1 hr pepsin digestion, respectively. The action of pepsin was to remove the outer hexagonal membrane of the virus, leaving only the densely staining central core.

Pl. 2, figs 6 A and 6 B illustrate DNase digestion for 1 and 4 hr, respectively. The DNase was not effective on glutaraldehyde-fixed cells and these are formalin-fixed. Removal of the central core of several virus particles was evident at 1 hr and had become more general after 4 hr. However, in no section were all the particles affected.

This study agrees with the finding by Aldinger *et al.* (2) that African swine fever

virus contains infectious DNA. The sharply delineated hexagonal outer membrane of the virus, which is a distinctive morphological feature (1), contains protein and the only site of DNA is the central core of the virus; ribonucleic acid is apparently not present. These observations parallel those of Zambernard & Vatter (6) for virus in renal tumours of leopard frogs.

The PK13 cells were obtained from Dr S. H. Madin, Naval Biological Laboratory, Berkeley, California.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. African swine fever virus particles, complete (arrows) and incomplete, as they appear in Epon embedded thin sections. Magnification mark = 1 μ.

Fig. 2. African swine fever virus particles embedded in glycol methacrylate after floating on water for 2 hr. Virus (arrows) has normal appearance. Magnification mark = 1 μ.

Fig. 3. African swine fever virus particles (arrows) after 2 hr digestion with RNase. No apparent structural changes. Magnification mark = 1 μ.

Fig. 4. African swine fever virus particles (arrows) after 2 hr digestion with trypsin. No apparent structural changes. Magnification mark = 1 μ.

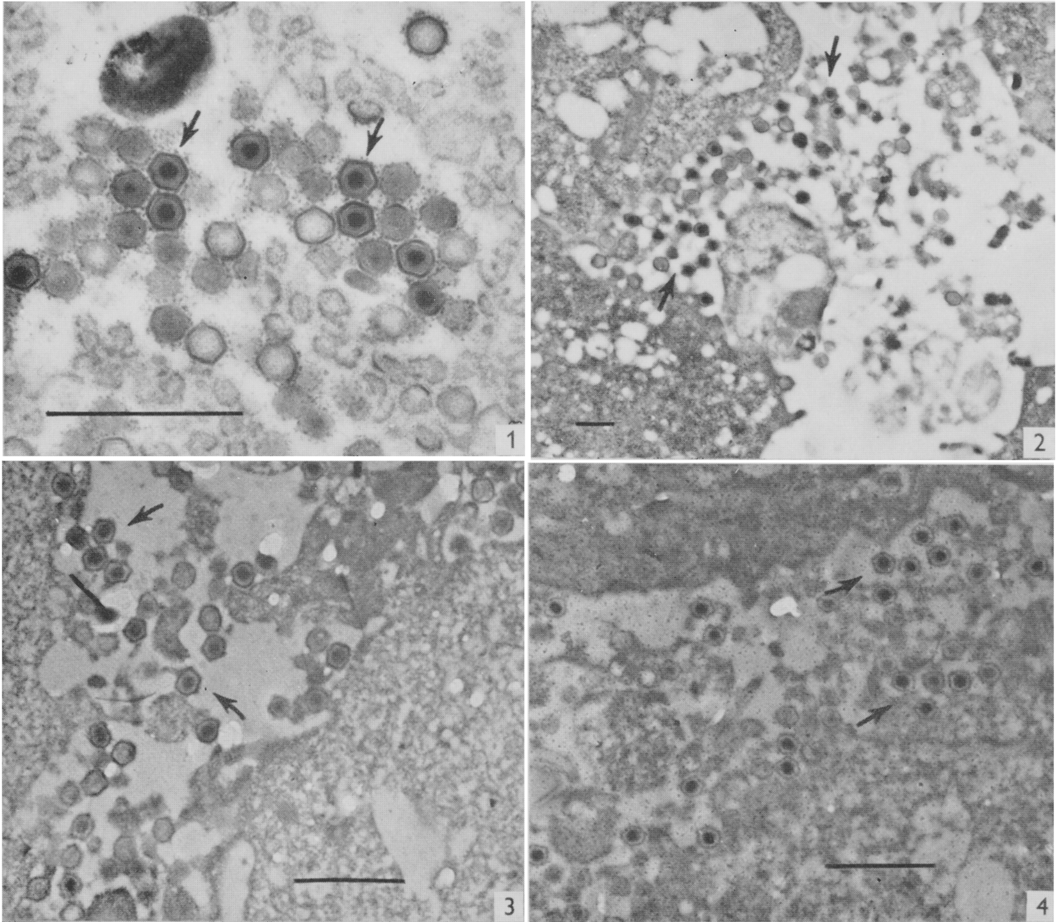
PLATE 2

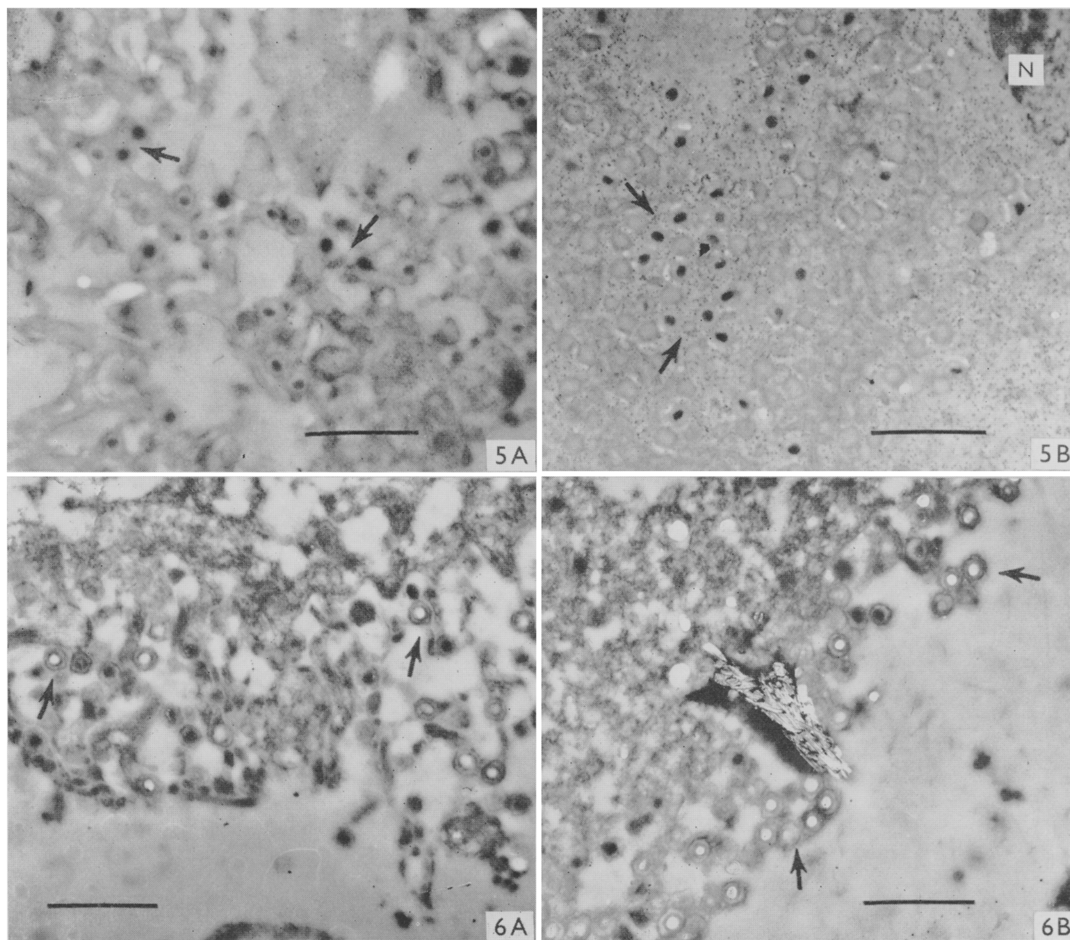
Fig. 5A. African swine fever virus particles after ½ hr digestion with pepsin. Some particles (arrows) have had outer hexagonal shell removed. Magnification mark = 1 μ.

Fig. 5B. African swine fever virus particles after 1 hr digestion with pepsin. All virus particles (arrows) have outer shells removed. N = cell nucleus. Magnification mark = 1 μ.

Fig. 6A. African swine fever virus particles after 1 hr digestion with DNase. Some particles (arrows) have inner core completely removed. Magnification mark = 1 μ.

Fig. 6B. African swine fever virus particles after 4 hr digestion with DNase. Most particles have inner cores removed (arrows). Magnification mark = 1 μ.





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