Further Observations on the Structure of Influenza Viruses A and C

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SUMMARY

The reticular structure previously described on the original TAYLOR 1233 strain of influenza C was found on two further strains of influenza C.

In negatively stained preparations of influenza C three kinds of particles with different surface morphology have been found: particles with 'spikes', typical of myxovirus, particles with a hexagonal reticular surface and 'naked' particles with no reticular structure.

The fine structures of influenza viruses A and C as seen in thin sections were compared. In walls of influenza A the structures encountered from the surface inwards were: a layer of spikes hollow and open at both ends; an electron-transparent layer of uniform width; and a finely granulated layer. The electron-transparent layer of influenza C virus was much less prominent; the spikes were irregularly placed and often absent. On sectioned influenza C filaments a hexagonal pattern was seen. The internal component of influenza A virus measured about 6 nm. in diameter and sometimes appeared to be hollow. The internal component of influenza C measured about 9 nm. across and appeared to be helical.

On the basis of these results the taxonomic position of influenza C has been questioned.

INTRODUCTION

The conventional picture of influenza A and B is that of irregularly shaped bags or hollow filaments invested with a series of spikes upon the surface. Several authors have described a reticular structure on the wall of influenza C (Waterson, Hurrell & Jensen, 1963; Archetti, Jemolo & Steve-Bocciarelli, 1967; Flewett & Apostolov, 1967). Choppin & Stoeckenius (1964) illustrated a somewhat similar structure on one particle of a simian para-influenza virus, SV5. Three newly isolated strains of influenza C afforded us the opportunity to compare their morphology with that of the original TAYLOR strain.

We used positive staining of thin sections to determine whether the structures observed by negative staining could be made visible with positive staining and to gain new information on the structure of the walls and of the internal components of influenza A and C virus.
METHODS

Viruses. Influenza A1/PR 8, A/PERSIAN GULF an unnamed 1968 A2 strain isolated and in Birmingham were propagated by inoculation of a 10⁻³ dilution into the allantoic cavities of 10-day-old chick embryos.

Influenza C/TAYLOR 1233, the recently isolated influenza C/JHB 4/67 (kindly supplied by Dr H. G. Pereira) and influenza C/PARIS/1/67 were propagated by inoculation of 10⁻³ dilutions into the amniotic cavities of 10-day-old chick embryos.

Preparation for electron microscopy. For negative staining two methods were used. In some preparations untreated amniotic or allantoic fluid was mixed with an equal volume of 2 % (w/v) potassium phosphotungstate, pH 7; a thin film of the mixture was allowed to dry on grids coated with either carbon or formvar membranes. In the others, the virus suspension was allowed to dry on a formvar membrane, washed three times in distilled water and then 'stained' for 10 sec. in phosphotungstate.

To obtain pellets for embedding and section cutting, influenza A virus from allantoic fluids was first aggregated by the method of Apostolov & Fishman (1967) and then centrifuged. Influenza C amniotic fluid was centrifuged at 120,000 g for 90 min. To separate the filaments, influenza C amniotic fluid was centrifuged at 3500 rev./min. for 20 min. The pellets were fixed in 6 % (w/v) glutaraldehyde in 0.05 M-phosphate buffer, pH 7.0, for 20 min., further fixed in Palade's fixative for 30 min. and washed in Palade's buffer (without OsO₄) for 30 min. ‘Maraglas’ was used for embedding. The sections were stained with 1 % uranyl acetate in alcohol for 30 min. and then in Reynold's lead citrate for 20 min. The time of staining was fairly critical; over-staining obscured fine detail.

RESULTS

Negatively stained preparations of influenza C/JHB 4/67 strain

This strain is characterized by numerous very long filaments, a feature even more marked than in the TAYLOR 1233 strain. In negatively stained preparations, on almost all the particles, both spherical and filamentous, the surface pattern of hexagons and pentagons could be seen (Pl. 1a, b). Numerous breaks in the reticulum were found

EXPLANATION OF PLATES

Plate 1a, b are of influenza C viruses, negatively stained with aqueous potassium phosphotungstate 2 % (w/v), pH 7.0.

Plate 1c-f and Pl. 2a-e are of sections through virus particles fixed in glutaraldehyde and then in osmic tetroxide, embedded in Maraglas and stained with lead citrate.

PLATE 1

(a) A filament consisting of an amorphous structure of fairly uniform width invested partially with a sleeve of hexagonal network, some of which has become detached. Spikes are attached to the sleeve.

(b) A broad filament invested with a hexagonal network to which spikes are attached.

(c, d, e) Influenza A/PR 8. A layer of spikes extending radially surrounds an electron-transparent zone 3 to 4 nm. thick. Beneath this is an electron-dense layer of small granules or vesicles about 4 nm. in diameter, forming the inner layer of the virus wall. Within the wall can be seen a narrow filamentous (c, d) or round (e) internal component, about 6 nm. in diameter.

(f) Influenza A/PR 8. Some particles, often larger than most, have similar walls but appear to be empty.
(a) Influenza C/JHB. The filaments are obliquely cross-banded at intervals of 11 nm., when the section has passed tangentially.

(b) On tangentially sectioned filaments can be seen a hexagonal pattern of similar dimensions to that revealed by negative staining.

(c) Influenza C TAYLOR strain. Distribution of spikes around some particles is irregular. The transparent layer is narrow or undetectable. The nanogranular layer is similar to that in influenza A. The internal component seen in transverse section appears round, about 9 nm. diameter.

(d) Influenza C/TAYLOR. The internal components appear filamentous, uniformly about 9 nm. wide.

(e) The internal component appears to have a periodic, probably helical, substructure.

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exposing an amorphous surface (Pl. 1 b). As in the TAYLOR strain, particles with the 'conventional' influenza surface structures, particles invested with a hexagonal reticular structure, and 'naked' particles were found in the JHB and PARIS strains.

**Electron microscopy of thin sections of influenza viruses A and C**

**The wall of influenza A virus particles.** In the walls of particles where the section had passed at or near the equator, three layers could be identified:

1. A layer of spikes on the surface, 8 nm. long. In places they were seen to be hollow and open at both ends (Pl. 1 c to e).
2. An electron-transparent layer of uniform thickness beneath the outer layer of spikes. It appeared to be completely empty and was a constant feature in sections of influenza A virus particles (Pl. 1 d).
3. An inner layer of approximately the same thickness as the transparent layer. This layer was electron-dense and appeared to be composed of small micelles or granules approximately spherical in shape, about 4 nm. in diameter—the nanogranular or micellar layer (Pl. 1 c, f).

**The internal structure of influenza virus A.** An internal structure within influenza A and B viruses has been detected in negatively stained preparations (Hoyle, Horne & Waterson, 1961; Apostolov & Flewett, 1965; Lovas & Takatsy, 1965) as a coiled structure in the form of a helix of varying numbers of turns approximately 60 nm. in diameter; the strand of the helix had a diameter of 5 to 6 nm. with perhaps a helical substructure. These authors noted the internal component in negatively stained preparations only in a very small proportion of particles. But in most sectioned particles the internal component was visible as a number of thread-like structures irregularly distributed within the space enclosed by the wall. The width of the component in cross-section was remarkably constant at approximately 6 nm. (Pl. 1 e). Some particles appeared to be completely empty, although possessing the normal structure of the influenza virus wall (Pl. 1 f). The internal component of influenza A as seen in sections did not appear to be arranged in an obvious helix.

**The wall of influenza C virus particles.** This differed from the wall of influenza A in the spiky layer: the spikes on many particles were often irregularly distributed and were sometimes absent. These appearances presumably correspond to the 'naked' particles or particles with bare areas seen in the negatively stained preparations. Beneath the spiky layer the electron-transparent layer, so prominent in influenza A, was in sections of influenza C much thinner and often not seen. In longitudinal sections of filaments a hexagonal pattern was sometimes seen, perhaps corresponding to the hexagonal network seen by negative staining (Pl. 1 g, h).

Transverse banding, often oblique, at an interval of 11 nm. was seen on filaments lying with their long axis in the plane of this section (Pl. 2 b). Spikes were attached at the ends of the bands and sometimes appeared as double narrow parallel lines, suggesting that they might be hollow.

**The internal structure of influenza virus C.** No internal structure could be seen within filaments except at the ends, which were often dilated to a somewhat larger diameter than the rest of the filament. In sections of predominantly spherical particles the great majority contained an internal component in the form of a filament of regular width, which on cross-section appeared to be tubular. The diameter of the component was remarkably constant at about 9 nm. (Pl. 2 c). In some preparations its appearance
suggested that it might have a helical substructure (Pl. 2). The dimensions of the internal component were the same whether the section was through a spherical particle or through one end of a filament.

**DISCUSSION**

The results obtained from negatively stained preparations and from sections of virus pellets complement each other in producing a fuller picture of the morphology of influenza A and C virus particles. Our concept of the morphology of these two viruses is summarized in the two drawings (Fig. 1, 2).

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**Fig. 1.** Schematic presentation of the influenza A and B virions.

**Fig. 2.** Schematic presentation of the structure of the influenza C filament.
The difference in morphology of the two viruses is clear. Three complete and well-defined layers could be seen in all sections of influenza A, while a large proportion of the influenza C particles were without a spiky layer. The electron-transparent layer in influenza A sections was of constant appearance and uniform thickness and in influenza C sections was very thin and often not detectable. The appearances described here were sufficiently constant for micrographs of sections of influenza A and C to be correctly distinguished by inspection.

The granular layer which is common to both viruses appears to be composed of small granules or spaces of about 4 nm. across with electron-dense walls. A structure composed of a layer of micelles 4 nm. in diameter has been postulated for some cellular membrane systems (Sjöstrand, 1963; Sjöstrand & Elfvin, 1964); the evidence for the micellar organization of membrane ultrastructure has been reviewed by Lucy (1968).

The internal component of influenza C virus as far as we know has not hitherto been visualized. It appears to be composed of a filament of helical substructure. Its diameter of 9 nm. is different from the diameter of the internal component of influenza A (6 nm.).

Division of myxoviruses into the influenza and parainfluenza groups appears to have been made, at least in part, upon the morphology of the internal component (Waterson, 1962). Apart from morphology, influenza C differs from influenza A and B in growing poorly in the allantoic cavity of eggs, in agglutinating fowl erythrocytes poorly or not at all at room temperature, and in not agglutinating human, guinea-pig or embryonic chick erythrocytes, and (as far as the strains which have been investigated are concerned) in serological stability also. Furthermore it does not appear to cause epidemic influenza.

There appears to be a case for taxonomic separation of influenza C virus from influenza A and B viruses. A new name may eventually be needed for myxoviruses with a reticular surface structure and a nucleocapsid of 9 nm. diameter.

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REFERENCES


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