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ULTRASTRUCTURAL STUDY OF CELL CULTURES INFECTED WITH SWINEPOX AND ORF VIRUSES

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SUMMARY Monolayer cells of a porcine kidney cell line were infected with the PP-1 strain of swinepox virus, while secondary or third subcultured monolayer cells of African green monkey kidney were inoculated with the Iwate BT-9 strain of orf virus. Those infected cells were fixed when CPE became remarkable in the monolayers and examined by electron microscope.

In the cytoplasm of cells infected with both viruses, various immature forms of viral particles in different developmental stages were observed. Micells were detected very close to the opening of immature particles. Mature particles associated with a double membrane were frequently observed. From those observations it was suggested that the developmental sequence of both viruses is essentially the same as that of vaccinia (Dales, 1973).

Besides various virus forms as well as factories, the following ultrastructural changes were noted in swinepox infected cells, i.e. 1) intranuclear inclusions which consist of very fine filaments, 2) fibrillar structures with cross striations located in the nuclear inclusions, and 3) similar striated fibrillar structures in or just adjacent to virus factories (B type inclusions) in the cytoplasm. Those observations made with in vitro cells are in good accordance with the descriptions by previous investigators on in vivo materials. Accordingly those ultrastructural changes characterize the swinepox infection.

INTRODUCTION

Extensive information has accumulated on structure and developmental sequence of vaccinia and some other poxviruses and also on morphological alterations of cells infected with those viruses (Dales, 1973). On the other hand, swinepox and orf viruses have so far received less attention, although some aspects of ultrastructural study of these viruses have

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been reported. Namely, electron microscopic studies of epidermal cells of swinepox infected pigs have been presented by several investigators (Reczko, 1959; Cheville, 1966; Conroy and Meyer, 1971; Šmíd et al., 1973; Teppema and de Boer, 1975), while surface as well as inner structures of orf virus were examined by negative staining technique (Nagington and Horne, 1962; Nagington et al., 1964; Peters et al., 1964). Nevertheless, ultrastructural studies of in vitro cultured cells infected with both viruses are very few (Conroy and Meyer, 1971). Therefore, we initiated investigations first to study the ultrastructural alterations of cultured cells after infection with these viruses and secondly, to compare the developmental features of the viruses with those of other poxviruses, especially of vaccinia. This report describes fibrillar structures observed in swinepox infected cells and also some developmental features of swinepox and orf viruses.

MATERIALS AND METHODS

1. *Viruses and cells*

The PP-1 strain of swinepox virus and the Iwate BT-9 strain of orf virus were used. An established cell line of porcine kidney cells, which was obtained from Osaka Prefectural Institute of Public Health at the 318 subculture level and further cultivated in our laboratory, was used for infection with the former virus, while secondary or 3rd subcultures of African green monkey kidney cells served as host for infection with the latter virus. Both kinds of cells were nourished with Eagle's MEM supple-

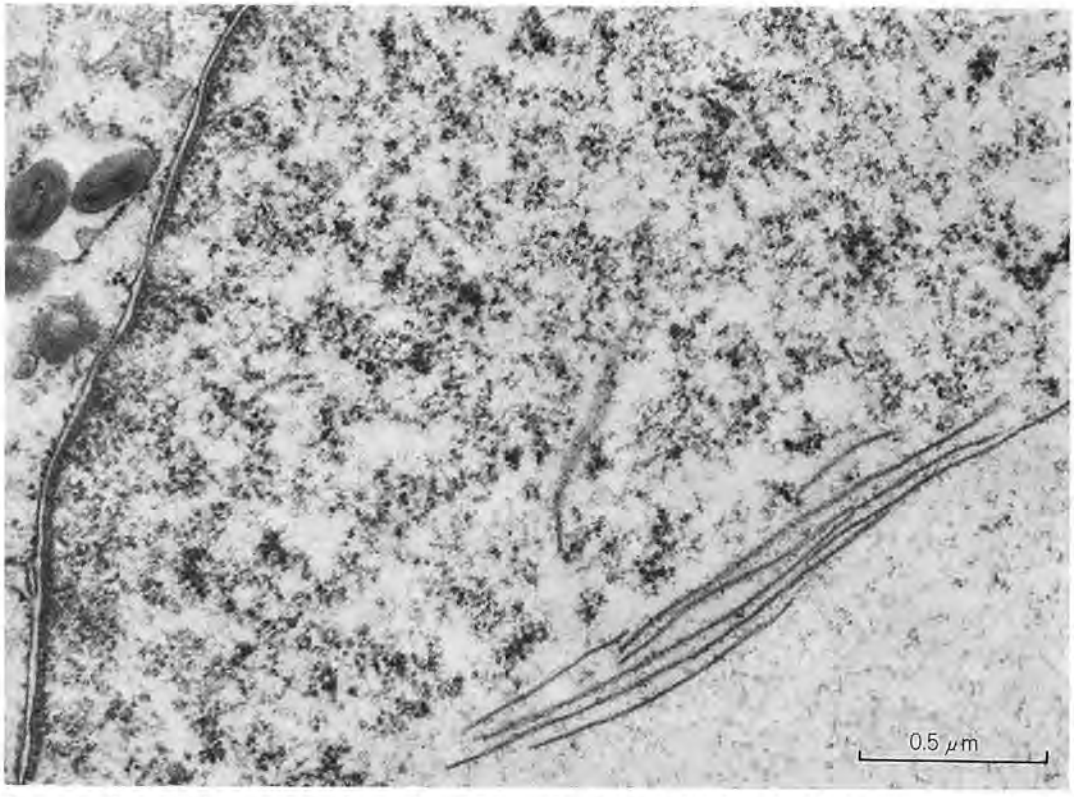


FIGURE 1. Parts of the nucleus and cytoplasm of a porcine kidney cell infected with the PP-1 strain of swinepox virus. A portion of an intranuclear inclusion which consists of very fine filaments is seen at the lower and right part. Seven to 8 fibrillar structures which run in parallel are also seen.

mented with approximate 10% calf serum.

2. *Preparation of infected cells*

Essentially the same procedures as described by Nii and Yasuda (1976) were also applied for present investigations. Briefly, when an advanced degree of CPE appeared in monolayers after infection with either of the above viruses, the cells were scraped off the dishes into the medium and centrifuged at low speed. The resulting pellets were fixed for 1 hr in 1% glutaraldehyde, washed well, fixed for 45 min in 1% osmium tetroxide, dehydrated, then embedded in epoxy resin.

3. *Sectioning and electron microscopic observation*

Sections were made with a glass knife in an LKB microtome and stained with uranyl acetate and lead citrate. A Hitachi 11 Ds electron microscope was used with an accelerating voltage of 75 kv.

RESULTS

1. *Fibrillar structures in the nucleus of porcine kidney cells infected with swinepox virus*

Most of Fig. 1 is occupied by the nuclear area of a porcine kidney cell infected with swinepox virus. In the lower and right part of the picture a portion of a nuclear inclusion is seen, which is characterized by the presence of very fine filaments. Just along the borderline between this inclusion area of rather low electron density and the neighbouring area abundant in electron dense material of host cell chromatin, 7 to 8 fibrillar structures which run roughly in parallel are seen. Figure 2 illustrates at a higher magnification a part of the same fibrillar structures shown in Fig. 1

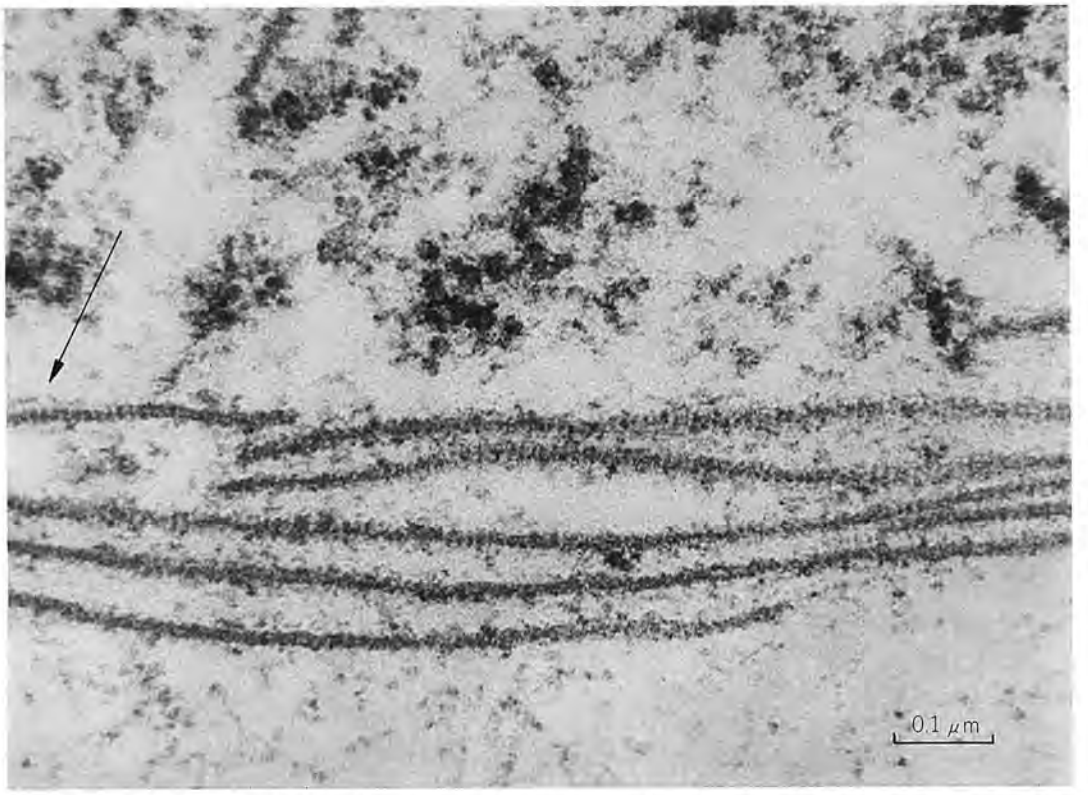


FIGURE 2. A higher magnification of a part of Fig. 1. Several fibrillar structures are shown. Cross striations look to be most clear at a point indicated by an arrow.

and it is demonstrated that these structures have cross striations, clearly visible especially at the point indicated by the arrow.

2. *Fibrillar structures in the cytoplasm of porcine kidney cells infected with swinepox virus*

In and just adjacent to the virus factories, i.e. the so-called B type inclusions originally named by Kato et al. (1959), bundles of fibrillar structures were frequently observed. At both the upper and lower parts in the middle of Fig. 3, a bundle of fibrillar structures is observed just adjacent to the one virus factory which occupies the left two thirds of this figure. Each bundle of the structures is indicated by a long arrow. Figure 4 shows cross striations of the fibrillar structures observed in a higher magnification.

3. *Ultrastructure of swinepox and orf viruses in connection with their development*

1) Swinepox virus

In Fig. 3 several foci each consisting of irregular reticulate material of moderate electron density are seen. Surrounding each foci there are several arc or semicircular membranous structures, some of which partially enclose a portion of the reticulate material as indicated by a short arrow. Also a lot of viral particles in rather earlier developmental stages

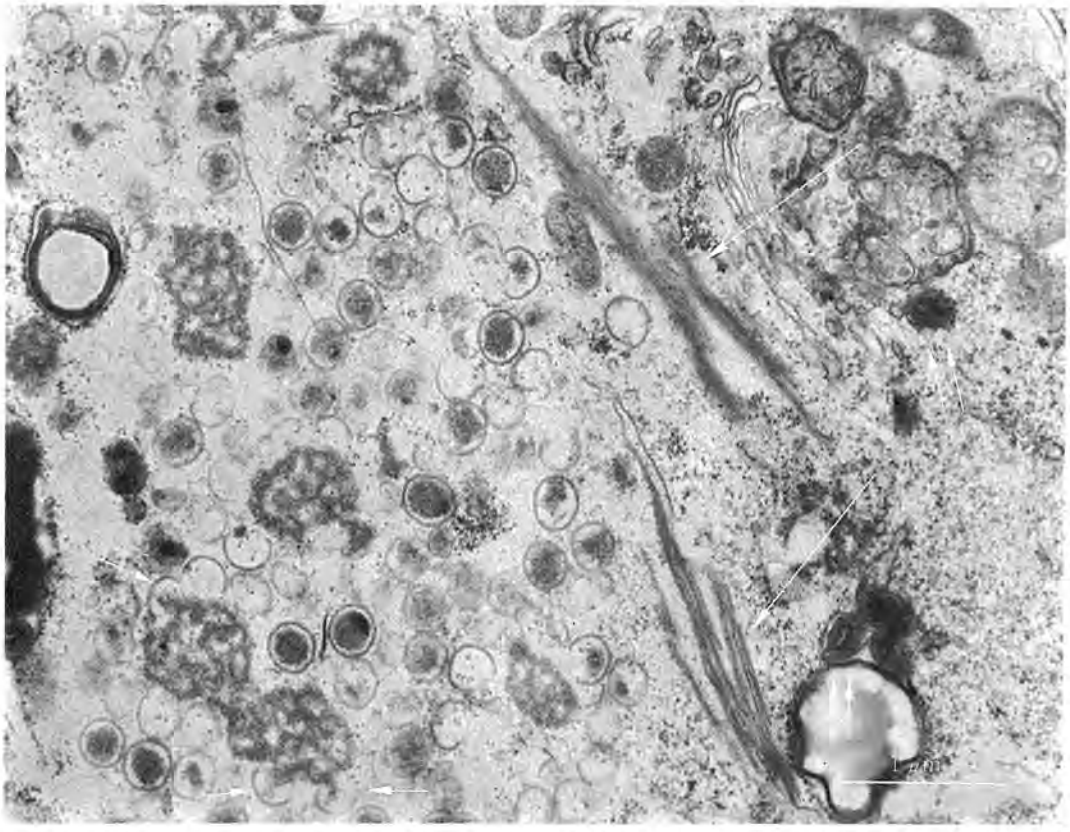


FIGURE 3. Part of the cytoplasm of a porcine kidney cell infected with the PP-1 strain of swinepox virus. A virus factory (B type inclusion) occupies the left two thirds of this figure. Long arrows indicate a bundle of fibrillar structures, and semicircular membraneous structures each indicated by a short arrow partially enclose a portion of the reticulate material. Mature particles are indicated by double short arrows.

are seen. Some appear to be just after completion of their limiting membranes, having completely enclosed circular contours and containing the viroplasm of similar electron density as the reticulate material, and some contain a nucleoid of high electron density as well as the viroplasm. Thus most of the particles seen in this picture reveal immature forms but a few in the right part (double arrows) look to be mature.

On the other hand most of the particles seen in Fig. 5 have mature forms and are located in close association with vacuolar membranes in the cytoplasm, some being in the vacuoles. A few particles found in the cytoplasmic matrix (short arrow) are immature and enclose a nucleoid.

2) Orf virus

In Fig. 6 several immature particles of orf virus are shown. Vesicular structures with irregular shapes or micells, previously described by some investigators (Dales and Mosbach, 1968; Grimley et al., 1970; Fil et al., 1974) are seen (arrow) just close to the opening of one particle which consists of incomplete circular double membrane structures but is seemingly devoid of viroplasm. One particle in the center of the figure contains a nucleoid as well as viroplasm.

Figure 7 shows numerous mature particles

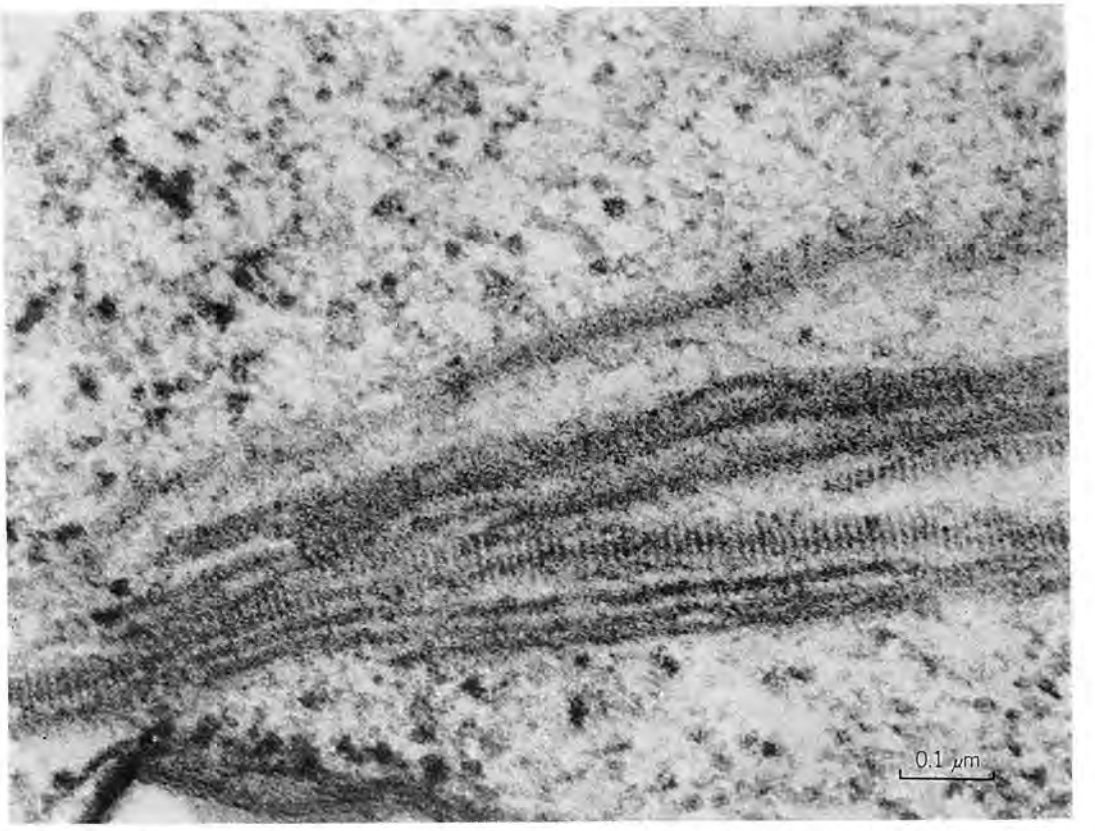


FIGURE 4. A higher magnification of a part of a lower bundle of fibrillar structures shown in Fig. 3. Two or three fibrillar structures show clear cross striations.

of orf virus located in the cytoplasm. Many of them are associated with a double membrane in variable length. For instance, one particle (short arrow) is partly and two particles each indicated by a long arrow are almost enclosed by the membrane. Occasionally two particles are wrapped together by a common double membrane. Each two mature particles indicated by a thick arrow are completely enclosed seemingly by an electron dense thick membranous structure. These, exactly comprise the outer and inner membranes. However, it is uncertain whether each particle would finally be enclosed by the inner membrane or whether the twin particles enclosed by the common double membranes would be transported to the plasma membrane as they are and released from the cell.

DISCUSSION

Blackmore and Abdussalam (1956) first described the appearance of cytoplasmic and intranuclear inclusions in epidermal cells of a swinepox infected piglet. Since then, these morphological alterations of cells associated with virus infection have been confirmed by many investigators; namely, light microscopically by Cheville (1966), Goto et al. (1968) and Garg and Meyer (1972, 1973), and electron microscopically by Reczko (1959), Cheville (1966), Nakamatsu et al. (1968), Conroy and

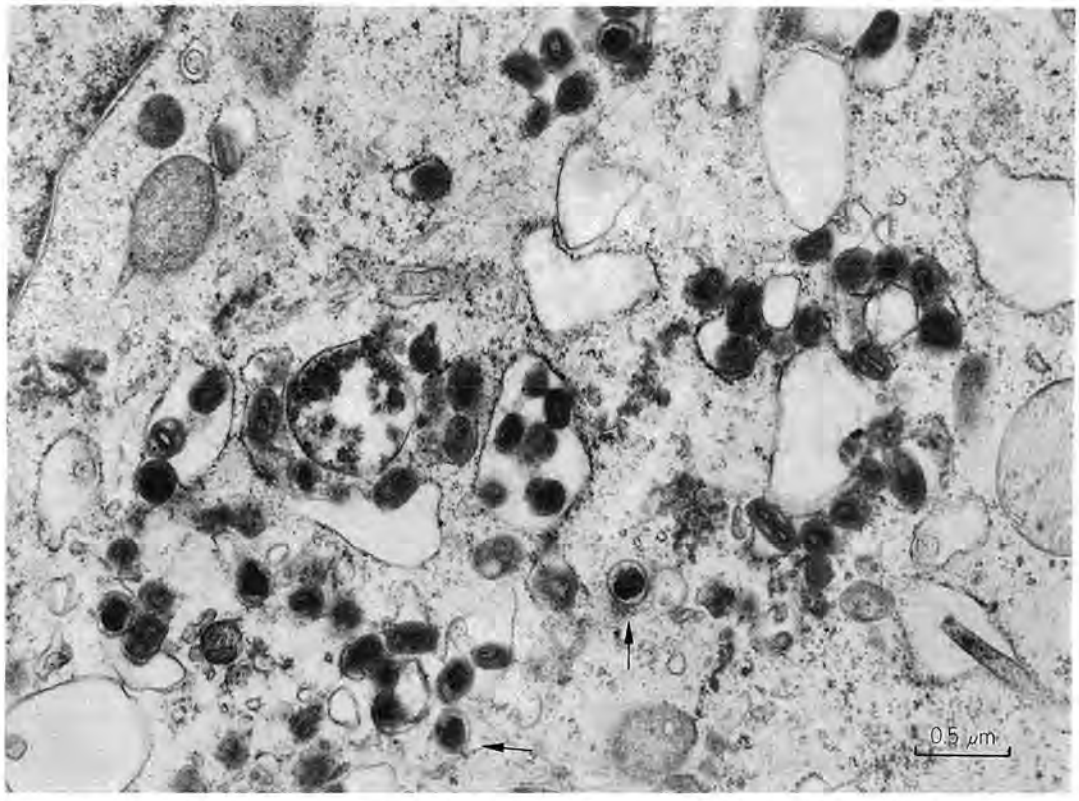


FIGURE 5. Parts of the nucleus and cytoplasm of a porcine kidney cell infected with the PP-1 strain of swinepox virus. Most of the figure is occupied by the cytoplasmic portion.

Many mature particles are closely associated with membrane systems in the cytoplasm and some are seen in the vacuoles. A short arrow each indicates an immature particle with a nucleoid.

Meyer (1971), Šmíd et al. (1973) and Teppema and de Boer (1975). Their electron microscopic studies have revealed that nuclear inclusions consist of very fine filaments and frequently, fibrillar or lamellar structures with cross striations made appearances in the inclusions. However, it must be noted that those ultrastructural findings were obtained with materials of infected pigs although Conroy and Meyer (1971) examined cultured cells infected with swinepox. Accordingly, we wanted to study whether such ultrastructural changes as observed in infected epidermal cells would also appear in tissue culture cells when infected. As a result, we recognized the main morphological alterations described by previous investigators, i.e. intranuclear inclusions, striated fibrillar structures in association with these areas and the similar structures in the cytoplasm. Thus it was confirmed that those ultrastructural changes characterize the swinepox infection.

Besides swinepox the appearance of intranuclear inclusions or vacuoles was noted in junco pox (Beaver and Cheatham, 1968) and sheep pox (Plowright and Ferris, 1958; Murray et al., 1973), although striated fibrillar structures were not found in intranuclear inclusions by the latter viruses. However, it is unknown why those three viruses induce such intranuclear alterations and conversely why many other poxviruses do not.

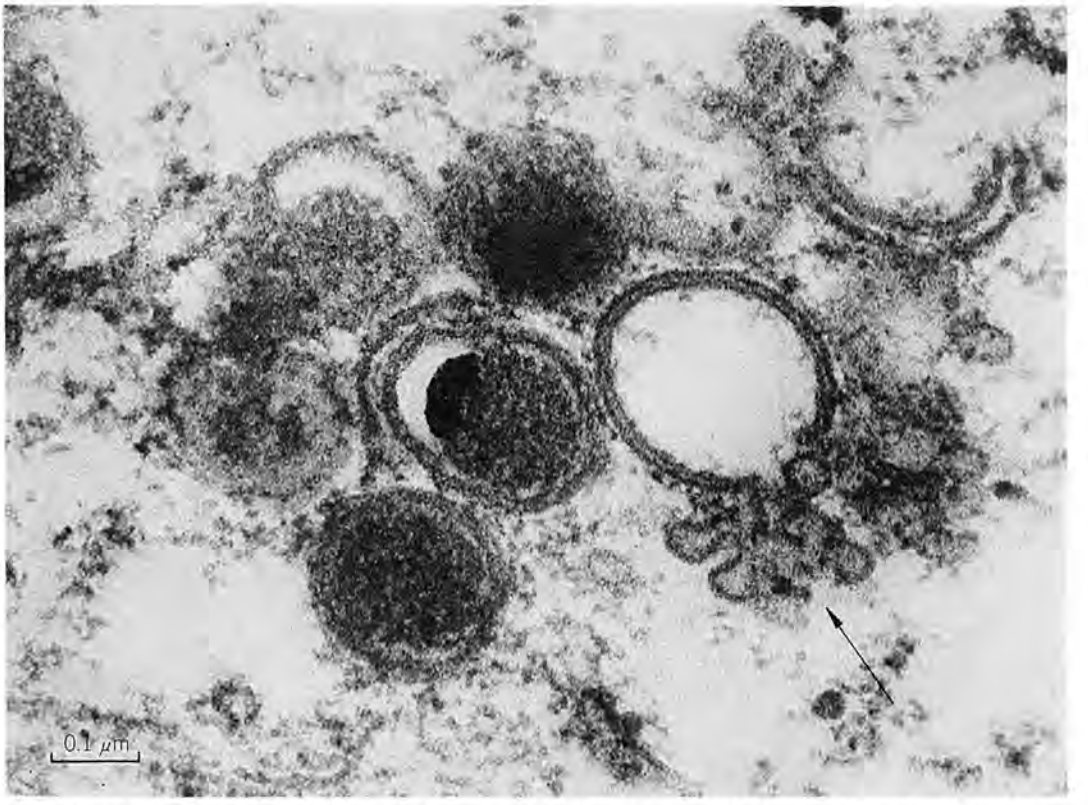
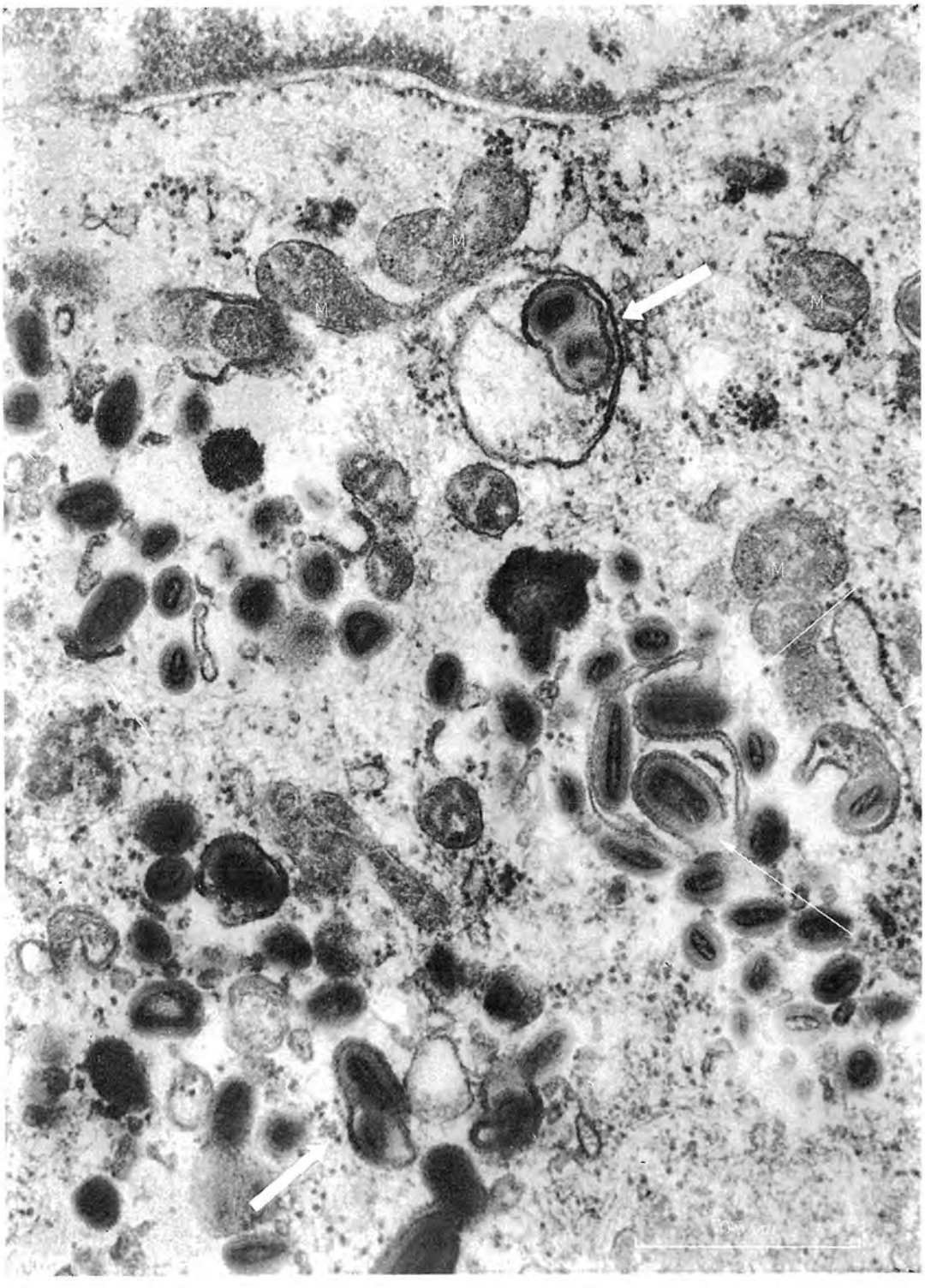


FIGURE 6. Several immature particles of orf virus, observed in a portion of the cytoplasm of an African green monkey kidney cell infected with the Iwate BT-9 strain of the virus. An arrow indicates micells and one particle in the center encloses a nucleoid as well as viroplasm.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Garg and Meyer (1973) studied nuclear and cytoplasmic inclusions of swinepox with fluorescent antibody technique and observed viral antigen in the cytoplasm but never found them in the nucleus or in the nuclear inclusion. Thus their experimental results seem to rule out the direct relation of intranuclear inclusion to the synthesis of viral components, but rather suggest that it is associated with a degenerative process. Nevertheless, the appearance of fibrillar structures with cross striations in the nuclear inclusion suggests a relation with virus replication. Recently it was demonstrated that purified nuclei, isolated from HeLa cells appropriately infected with vaccinia virus synthesized large amounts of virus DNA in vitro (LaColla and Weissbach, 1975; Bolden et al., 1975). Therefore it would be interesting to examine what biochemical events occur in cells infected with such poxviruses that produce nuclear inclusions.

Whether fibrillar structures with cross striations found in the cytoplasm are antigenically and ultrastructurally the same as those in the nuclear inclusion is not yet known. Such fibrillar structures as seen in the cytoplasm of swinepox infected cells were reported with some other poxviruses, namely, Shope fibroma (Bernhard et al., 1954; Banfield and Kasnic, 1971), Yaba (de Harven and Yohn, 1966), ectromelia (Leduc and Bernhard, 1962) and fowlpox (Simpson, 1969), and it was pointed out that there was a close resemblance between the surface subunits of fibrillar or lamellar structures and those covering the immature viruses. Thus it might be that the fibrillar structures represent some aberrant forms of the virus concerned.

Our present experiments on swinepox and orf viruses were not performed under the condition of one step virus growth and so it is rather difficult to compose a developmental sequence of both viruses. However, ultrastructures of various viral forms observed in cells infected with both viruses were in accordance with those of the correspondents reported with many other poxviruses, especially vaccinia (Dales, 1973), and therefore it is strongly suggested that the developmental sequence of both viruses is essentially the same as that of vaccinia.

As shown in Figs. 5 and 7, mature particles of swinepox and orf viruses are closely associated with membrane structures in the cytoplasm and it is presumed that egress of both viruses takes place in a similar manner to that of vaccinia (Ichihashi et al., 1971; Morgan, 1976).

There are no extensive studies on developmental features of orf virus. Therefore, further studies to clarify this problem and also the ultrastructures of mature particles of this virus in comparison with other poxviruses are currently in progress.

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FIGURE 7. Parts of the cytoplasm and nucleus of an African green monkey kidney cell infected with the Iwate BT-9 strain of orf virus. Numerous mature particles are seen and many are associated with a double membrane. A short arrow indicates a particle partially enclosed, while each long arrow shows the one almost enclosed with the double membrane. Two particles (thick arrow) are wrapped together by common double membranes. M: mitochondria.

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