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ELECTRON MICROSCOPIC OBSERVATIONS ON FL CELLS INFECTED WITH HERPES SIMPLEX VIRUS II.ENVELOPMENT[1]

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SUMMARY FL cells infected with one laboratory strain (-GCr Miyama) and three freshly isolated strains (Iwa, Ko and Watanabe) of herpes simplex virus were fixed at late stages of infection. Thin sections of the cells were prepared and examined with an electron microscope. The following observations were made. Envelopment of herpes smiplex virus occurred by budding into the perinuclear cisterna from the inner nuclear membrane, by association with double membrane structures in the cytoplasm and by budding into cytoplasmic vacuoles from the membranes of these vacuoles. Budding of virus particles into nuclear vacuoles which seemed to communicate with the perinuclear cisterna was also observed. However, no budding of the virus at the cell surface was seen. There are two pathways for egress of enveloped particles of herpes simplex virus from the sites of envelopment to the extracellular fluid. One is by a process of " reverse phagocytosis " and the other is through the cisternae of the rough-surfaced endoplasmic reticulum. Most envelopes of virus particles in the proximity of the inner nuclear membrane appeared as electron dense circular membranes, while envelopes of particles found in cytoplasmic vacuoles and in the extracellular space had projections or globular structures on them and occasionally a clear unit membrane structure was seen. The different morphological appearances of the envelopes are discussed.

INTRODUCTION

Envelopment of herpes simplex virus was first noted by Morgan et al. (1954) who examined thin sections of infected chorioallantoic membranes by electron microscope and observed that the nuclei of infected cells contained particles with capsids while in the cytoplasm most particles had an envelope. From this finding it was suggested that the initial site of viral development was the nucleus and particles with nucleocapsids appeared to acquire an envelope upon release into the cytoplasm. The enveloped form thus produced was supposed to represent the mature virus. This initial study was confirmed by two independent studies

1 A part of this work was presented at the Annual Virus Symposium held in the Virus Research Institute, Kyoto University, on July 5, 1968 and at the 16th Annual Meeting of the Society of Japanese Virologists in Fukuoka, on October 9, 1968.

made by Morgan et al. (1959) and by Falke et al. (1959) and both groups presented evidence that the particles with capsids were enclosed by the nuclear membrane. On the other hand Epstein (1962) reported that envelopment occurred at the cellular surface as well as in cytoplasmic vacuoles. Similarly, Siminoff and Menefee (1966) reported that envelopment occurred in Golgi bodies, at the cell surface or occasionally within the nucleus. More recently Darlington and Moss (1968) studied the envelopment process of three representative herpesviruses; equine abortion, pseudorabies, and herpes simplex, and emphasized the role of the inner nuclear membrane.

Our previous investigation on the sequence of development of herpes simplex virus supported the concept that envelopment occurred in the nucleus and cytoplasm. However, envelopment of the virus by budding from the plasmic membrane could not be confirmed (Nii et al., 1968a). This report describes further studies on envelopment of herpes simplex virus using FL cells infected with this agent.

MATERIALS AND METHODS

1. *Virus and cells*

Four strains of herpes simplex virus, i.e. -GCr Miyama (Nii and Kamahora, 1961 b), Iwa, Ko and Watanabe (Nii and Ono, 1971) were examined in this study. The first virus is a laboratory strain originally isolated from the vesicular fluid of *herpes labialis* (Nii and Kamahora, 1961 a), while the latter three are fresh isolates each also from a patient with *herpes labialis*. FL cells were used as host cells for virus infection.

2. *Samples for electron microscopy*

Two samples of FL cells infected with the Miyama strain were used, which were made on different occasions. The one prepared using FL cells 20 hr after infection at an adsorbed multiplicity of approximate 10. The other was the same specimen used as in the previous investigation (Nii and Ono, 1971) and this had been prepared with FL cells 40 hr after infection with about the same dose of the virus. The same samples of FL cells each infected with Iwa, Ko and Watanabe strains that were used in the previous investigation also served for this study.

The other procedures for infection of cells were described previously (Nii and Ono, 1971).

3. *Electron microscopic technique*

The same technique was used as described previously (Nii and Ono, 1971; Nii, 1971).

RESULTS

1. *Envelopment of herpes simplex virus by budding from the inner nuclear membrane*

Fig. 1 and 2 illustrate stepwise envelopment of single viral particles at the surface of the nucleus. Half of particle "A" is enclosed in the inner nuclear membrane. Particle " B " is almost completely enveloped and the envelope of particle " C " is connected to the nuclear membrane by a narrow pedicle. The envelope of particle " D " appears to be just separating from the nucleus. Thus it seems that the budding process of single viral particles of herpes simplex occurs in the order A→B→C→D.

Occasionally viral particles were observed in close association with reduplicated nuclear membranes, as shown in Fig. 3.

Fig. 4–8 show that some viral particles acquire envelopes by budding from the inner nuclear membrane into nuclear vacuoles. The nuclear vacuoles seen in Fig. 4 and 5 are adjacent to the surface of the nucleus and presumably these communicate with the perinuclear cisterna. In Fig. 4 three relatively well defined particles and one indistinct particle are seen enclosed in a common membrane which seems to be continuous to the vacuolar membrane. One of the particles is almost completely enclosed in the common membrane and protrudes into the vacuole while more than half of the other two particles are covered. Thus the four particles have a grape-like appearance. In the nuclear vacuole of Fig. 5 one enveloped particle and an aggregate of particles enclosed in a common membrane are seen. The latter viral particles consist of capsids and

electron dense cores. Fine filaments as well as tail-like structures (Nii et al., 1968a; Nii, 1971) are interwoven with these particles. The perinuclear cisterna in Fig. 4 and 5 is dilated and is very obvious in Fig. 5.

In Fig. 6 many enveloped particles are seen in the space between the vacuolar and reduplicated membranes, which seem to be located in the nuclear matrix. Most of the particles have capsids and electron dense cores while a few have empty capsids. Particle A (single arrow) is in the process of budding and the membrane surrounding it has increased in density. Two particles (double arrow) form a dumb-bell shape their envelopes being connected together by electron dense material.

Fig. 7 illustrates the budding process into a nuclear vacuole which is continuous with the perinuclear cisterna. The vacuole occupies most of the right part of the picture, where numerous viral particles are seen. Many of them are single enveloped particles while a few particles are connected together in tubular membranes, looking like peas in a pod (Shipkey et al., 1967; Nii et al., 1968a). A single particle (single arrow) and a tubular membrane (double arrow) containing at least two empty capsids have almost separated from the vacuolar membrane. Fig. 8 shows the budding of particles in small vacuoles or tubules in the nucleus. Those vacuoles or tubules seem to communicate with the perinuclear cisterna. One particle (arrow) is just in the process of budding while two particles which form a dumb-bell shape are seen surrounded by a vacuolar membrane.

2. *Envelopment of herpes simplex virus in the cytoplasm*

Two morphological types of envelopment of herpes simplex virus in the cytoplasm were differentiated. One was envelopment of viral particles by association with a double membrane consisting of two parallel membranes with not enough space between them, while the other was envelopment by budding of viral particles from the cytoplasmic membrane into the vacuoles.

Fig. 9–12 illustrate the first type of envelopment. In the cytoplasm shown in Fig. 9 at least two of three neighbouring particles are associated with a double membrane. In Fig. 10 five particles are seen located in close proximity to a perinuclear cisterna and two of them (arrows) are enclosed in a double membrane. In Fig. 11 an empty capsid has a double membrane on either side and in Fig. 12 one hexagonal capsid, located rather near the cellular surface, is almost enclosed in a membrane. The origin of these double membranes is unknown but one possibility is that they are derived from Golgi vesicles. One particle indicated by an arrow in Fig. 13 is seen to be almost completely enclosed in a double membrane. Thus the inner lamella of the double membrane seems to participate in formation of envelopes of virus particles while the outer counterpart becomes a vacuolar membrane which contains the particles. Fig. 13–15 show the envelopment of viral particles by budding from the vacuolar membrane in the cytoplasm. A capsid (double arrow) enclosing an electron dense core in Fig. 13 and an empty capsid (large arrow) in Fig. 14 are just budding into cytoplasmic vacuoles. These vacuoles seem to communicate with the perinuclear cisterna. Another particle in Fig. 14 (small arrow) is acquiring an envelope from the inner nuclear membrane. In Fig. 15 one particle (large arrow) protrudes far into the vacuole and another particle (small arrow) seen at the right of the picture has a bag-like envelope.

3. *Egress of viral particles from infected cells*

In the cytoplasmic portion in Fig. 16 two particles (small arrows) in the process of envelopment and many single particles each closely surrounded by a vacuolar membrane are seen. Irregular cytoplasmic projections are seen at the cell surface and many vacuoles just inside the plasma membrane contain one or several virus particles. This picture suggests the following process for egress of viral particles. First, naked particles acquire envelopes in the cytoplasm and then cytoplasmic vacuoles

enclosing these particles move towards the cell surface. On the way some vacuoles may fuse to form larger vacuoles and this fusion is remarkable near the plasma membrane. Finally, after reaching the cell surface these vacuoles open to the exterior of the cell and release the particles outside the cell. Thus particles (large arrow) in an indentation in the plasmic membrane are possibly in the process of being released from the cell.

Fig. 17 shows an aggregate of closely packed, enveloped particles in the cytoplasm. One smooth vacuolar membrane encloses these particles. However, no nucleocapsids are observed in the rest of the cytoplasm. Therefore, the particles may have acquired their envelopes from the inner nuclear membrane and may be moving towards the cell surface by reverse phagocytosis.

In the cytoplasm shown in Fig. 18 several enveloped particles are located in branched tubules. Fig. 19 shows a long tubule stretching from near the perinuclear cisterna to the cell surface. One particle (arrow) is seen to be budding at the nuclear surface. These two figures show another possible route for egress of viral particles to the exterior of the cell and the tubules enclosing enveloped particles are thought to be derived from the preexisting rough-surfaced endoplasmic reticulum.

Fig. 20 shows particle "A" in a small vacuole, particle " B " in an indentation of the plasma membrane and two particles " C " and " D " at the cell surface. These particles probably represent different stages in the release of virus from the cell.

No budding of virus from the plasma membrane was seen, although a single capsid was extremely rarely observed at the top of a cytoplasmic projection, as shown in Fig. 21.

4. *Morphology of enveloped particles*

Fig. 22 illustrates numerous enveloped particles in a nuclear vacuole most of which have electron dense cores. One particle (arrow) has a core of low electron density. However, very few enveloped particles with this kind of viral core, were encountered in cytoplasmic vacuoles or outside the cell.

The envelopes of all particles observed in this picture appear as a circular, electron dense membrane. More detailed examination of enveloped particles located in the close proximity of the inner nuclear membrane revealed that envelopes of some particles appeared to consist of three layers, i.e., an outermost layer of low electron density, a middle transparent layer and an inner, electron dense layer. This is shown in the inset figure in Fig. 22. The outermost layer appears to consist of fine globules or projections and occasionally is seen as a broken line while the innermost layer is seen as a thick electron dense membrane. Therefore, it seems that in most particles found near or at the inner nuclear membrane the outermost layers are not clearly seen and only the innermost layers are conspicuous, as seen in the numerous particles in Fig. 22. Particles enclosed in such electron dense membranes were found in tubules in the cytoplasm, originating from rough-surfaced endoplasmic reticulum as well as in nuclear vacuoles and in the perinuclear cisterna.

The envelopes of the particles seen in Fig. 23 and 24 are a little different from those in Fig. 22. Namely, the envelopes of the particles observed in the cytoplasmic vacuole shown in Fig. 23 show clearer projections (Wildy et al., 1960) or fine globular structures on them and their unit membrane structure is also apparent. The extracellular particles in Fig. 24 clearly show the unit membrane structure of viral envelopes. On the contrary this structure was difficult to recognize in the envelopes of viral particles found in the nucleus and at the perinuclear cisterna.

Enveloped empty capsids were frequently observed in the cytoplasm and outside the cell. Fig. 25 shows two such particles, one in the cytoplasm and the other in the extracellular space. This undoubtedly indicates that empty capsids are also enveloped.

DISCUSSION

Morgan et al. (1954) first reported that the nucleocapsid of herpes simplex virus was formed in the nucleus and appeared to acquire an envelope upon release into the cytoplasm. They suggested that the enveloped nucleocapsid represented the mature virus. Extensive studies by electron microscopy have been made by various investigators on the development of various herpes viruses and now there seems to be general agreement that the nucleocapsid of herpes viruses is formed in the nucleus and it can be enveloped either at the nuclear membrane or in the cytoplasm.

Available data on the envelopment of herpesviruses are listed in Table 1. Regarding herpes simplex virus Morgan et al. (1959) and Falke et al. (1959) emphasized the role of the nuclear membrane, whereas Epstein (1962) found no evidence of budding from the nuclear membrane but instead observed budding from the plasma membrane or from vacuolar membranes in the cytoplasm. Siminoff and Menefee (1966) supported all these possible budding processes but rarely observed envelopment at the inner nuclear membrane. More recently Darlington and Moss (1969) reviewed the envelopment of herpesviruses on the basis of their own work and the work of other investigators and some of their conclusions were as follows. "Envelopment appears to be a nonspecific process in that the nucleocapsid may be enveloped whenever it comes into contact with a cell membrane. The primary site of envelopment is at the nuclear membrane. However, if the nucleocapsid gets out of the nucleus, it may become enveloped at cytoplasmic or plasma membrane sites." Previously we reported that envelopment of herpes simplex virus occurred most frequently in close proximity to the nuclear surface, although the process was also encountered within the nuclear matrix and in the cytoplasm, but envelopment of the virus by budding from the cell surface was not observed (Nii et al., 1968a). The present studies on four strains of herpes simplex virus, i.e. one laboratory and three freshly isolated strains of the virus, each isolated from a patient with *herpes labialis* confirmed the above result. Judging from my experimental results and from the data on envelopment of various herpesviruses listed in the Table, envelopment at the cell surface seems to take place very rarely if at all.

With regard to whether inner nuclear membrane, cytoplasmic vacuoles or the plasma membrane, is most important in envelopment of herpes simplex virus the following points should be remembered. 1) Different virus strains and host cells may differ in appearance under the electron microscope. In connection with this it is interesting that Schwarz and Roizman (1969a) reported that HEp-2 cells infected with the G strain (type 2 herpes simplex virus) contained numerous nucleocapsids in the cytoplasm without envelopes or with partial envelopes from a relatively early stage of infection. This is not a common finding with type 1 strains of the virus and they thought that the type 2 virus might be enveloped in the cytoplasm. 2) The main sites of envelopment may also depend on the viral multiplicity at the time of infection and the stage of infection. Nucleocapsids have been observed in the cytoplasm even in cells infected with type 1 herpes simplex virus but the time of their appearance differs in different experimental systems. Watson et al. (1964) and we (Nii et al., unpublished data) observed both naked and enveloped particles in the cytoplasm at an early stage of infection (6 hr). On the other hand Darlington and Moss (1968) observed occasional particles without envelopes (naked) in the cytoplasm late in infection (24 hr or later), which were usually associated with breaks in the nuclear membrane. In the latter case the nuclear membrane must be the most important in envelopment of the virus and Darlington and Moss reported that unless the nucleus is ruptured the nuclear membrane is the primary site of envelopment of herpesvirus.

The envelopmental process is thought to be essentially the same for all the various herpes-

TABLE 1. *Sites of envelopment of herpesviruses*

Subgroup A herpesvirus

Virus	Authors	Year of publication	Site of envelopment		
			Nucleus[a]	Cytoplasm[b]	Cell surface[c]
Herpes simplex virus	Morgan et al.	1959	+[d]		
	Falke et al.	1959	+		
	Epstein	1962	−[d]	+	+
	Luse et al.	1965	+		
	Nii et al.	1966	+	+	
	Siegert and Falke	1966	+		
	Siminoff and Menefee	1966	±[d]	+	+
	Shipkey et al.	1967	+		
	Darlington and Moss	1968	+	+	
	Bedoya et al.	1968	+	+	
	Nii et al.	1968	+	+	
	Leestma et al.	1969	+	+	
Pseudorabies virus	Felluga	1963	+	+	
	Darlington and Moss	1968	+	+	
Herpes B virus	Reissig and Melnick	1955	+	+	
Equine abortion virus	Darlington and James	1966	+		
	Darlington and Moss	1968	+	+	
Infectious bovine rhinotracheitis virus	Armstrong et al.	1961	+		
	Gratzek et al.	1966	+		

Subgroup B herpesvirus

Varicella-zoster virus	Becker et al.	1965	+[d]	+	
	Nii	1970	+	+	
	Cook and Stevens	1970	+	−[d]	
Human cytomegalovirus	Stern and Friedman	1960	+		
	Becker et al.	1965	+	+	
	McGavran and Smith	1965	+		
	Ruebner et al.	1965	+		
	Donnellan et al.	1966	+		
	Kawanishi et al.	1967	+		
EB virus	Epstein et al.	1965	+	+	
	Dalton and Manaker	1967	+	+	
	Yamaguchi	1969	+	+	+
Guinea pig cytomegalovirus	Middelkamp et al.	1967	+		
Mouse cytomegalovirus	Ruebner et al.	1964	+		
	Ruebner et al.	1966	+	+	
Porcine cytomegalic inclusion disease virus	Duncan et al.	1965	+	+	
	Valícek	1970	+	+	
Canine herpesvirus	Strandberg and Carmichael	1965		+	
	Strandberg and Aurelian	1969	+		
Herpes-type virus of the frog renal adenocarcinoma	Lunger et al.	1965	+	+	
	Zambernard et al.	1966	+	+	
	Stackpole	1969	+	+	
Herpes-type virus of Marek's disease	Nazerian and Burmester	1968	+		
	Epstein et al.	1968	+	+	
Turkey herpes virus	Nazerian et al.	1971	+	+	

◀

a Envelopment of herpes virus occurred in close proximity to the nuclear surface and within the nuclear matrix.

b Particles of herpes virus acquired envelopes by budding from vacuolar membranes in the cytoplasm, or were enveloped by association with double membrane structures (flattened vesicles; Leestma et al., 1969) or with unusual elements of endoplasmic reticulum (Becker et al., 1965), or within delimited amorphous cytoplasmic inclusions (Becker et al., 1965).

c Budding of herpes virus was observed at the plasma membrane.

d The symbols indicate respectively that envelopment of herpes virus at the site indicated was observed frequently (+) rarely (±) and never (−).

viruses described above. However, another interesting mechanism of virion formation was reported by Stackpole (1969) for a herpes-type virus causing renal adenocarcinoma in frogs. He observed particles with dense cores which were enveloped by budding through the inner membrane of the nuclear envelope, and these lost their envelope by pressing through the outer membrane into the cytoplasm. Viral particles in the cytoplasm acquired fibrillar coats and budded into enlarged vesicles, presumably formed from the Golgi complex. Therefore, in this case envelopment of the herpes-type virus appears to occur in the nucleus, but is temporary. However, the inner nuclear membrane as well as vacuolar membranes in the cytoplasm are both considered to be sites of permanent envelopment of many other herpes viruses.

Extensive examinations have been made of thin sections of cells infected with herpes simplex, but no naked nucleocapsids have so far been seen in the perinuclear cisterna. Thus the mechanism by which nucleocapsids enter the cytoplasm is still unknown although in late stages of infection they may stream into the cytoplasm through breaks in the nuclear membrane.

On infection with herpes simplex virus the nuclear and cytoplasmic membranes change antigenically to contain both host cell and viral protein (Nii et al., 1968c). This coincides with the fact that these membrane systems in the cells participate in envelopment of the virus. However, the nuclear membrane is certainly important in envelopment of herpes simplex virus judging from the initial site of contact with virus particles. Namely the nuclear membrane enfolds nucleocapsids or empty capsids and these are finally pinched off leaving the membrane intact. Occasionally remarkable reduplication of the membrane occurs but enveloped particles are not always associated with sites of reduplicated membranes. The budding process also takes place in vacuoles or tubules in the nucleus which are supposed to be indentations of the inner nuclear membrane. In the cytoplasm of infected cells two kinds of structures are concerned with envelopment of virus particles, namely small double membranes, which correspond to the flattened vesicles reported by Leestma et al. (1969) and cytoplasmic vacuoles. The double membranes are most frequently seen near the perinuclear cisterna but occasionally the membranes enclosing virus particles are found near the cell surface. These double membranes (flattened vesicles) may originate from Golgi vesicles. Double membranes of various lengths were seen enclosing particles and this suggested that these membranes may proliferate. Cytoplasmic vacuoles also varied in sizes and usually only one budding particle was seen in small vacuoles while large vacuoles contained numerous virus particles. Occasionally vacuoles seemed to communicate with the perinuclear cisterna, suggesting that they were derived from a dilated perinuclear cisterna. Alternatively, they may have arisen from the Golgi apparatus. Neither the double membranes nor the cytoplasmic vacuoles were associated with ribosomal particles.

There are at least three routes of egress of enveloped particles from infected cells to the extracellular fluid. First, some particles which acquire envelopes at the inner nuclear membrane become enveloped by the outer nuclear membrane and are released by a process of " reverse phagocytosis ", as originally proposed by Morgan et al. (1959). Fig. 17 seems to illustrate this process. Second, particles acquire their envelopes in the same way, but pass through a network of tubules derived from the rough-surfaced-endoplasmic reticulum. This pathway was described by Schwarz and Roizman (1969b). Fig. 18 and 19 show this process. Third, virus particles acquire envelopes in the cytoplasm by budding from vacuoles and are released by a process of reverse phagocytosis. Fig. 16 illustrates many single particles each enclosed in a vacuolar membrane and numerous particles being released at the cell surface. Thus viral particles are released in two main ways, namely by a process of reverse phagocytosis and through cisternae of the endoplasmic reticulum.

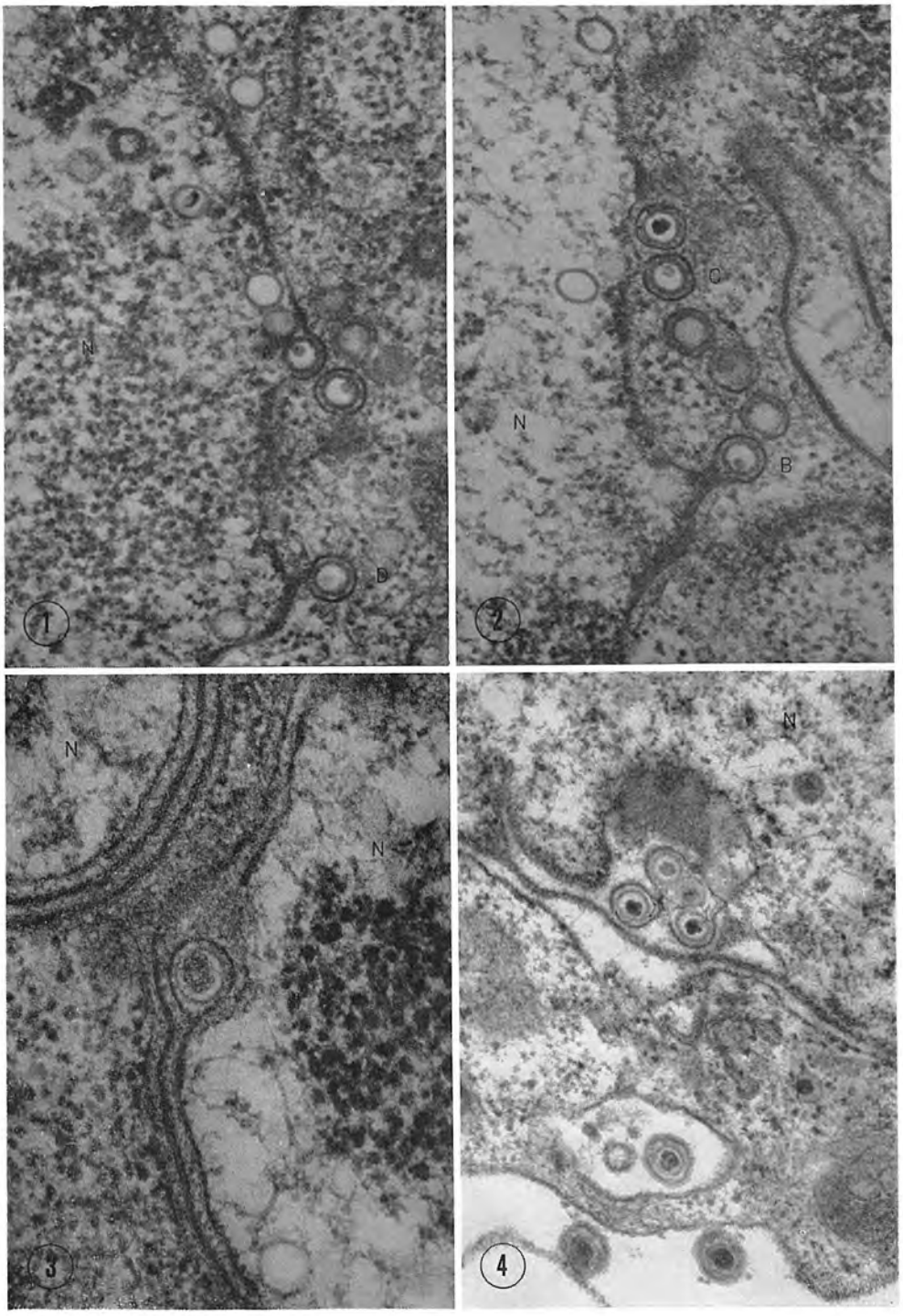
The envelopes of particles found in the nucleus, at the perinuclear cisterna and in the cisternae of the rough-surfaced endoplasmic reticulum differed in appearance from those of particles in the cytoplasmic vacuoles and in the extracellular spaces. The envelopes of the former particles appeared as electron dense circular membranes, especially at low magnification (Endo, 1967). On the other hand the envelopes of the latter particles had projections or globular structures and a unit membrane structure was occasionally seen very clearly. More detailed examination of enveloped particles found in the perinuclear cisterna revealed that some of the envelopes consisted of three layers; an outer layer of low electron density, a

▶

FIGURES 1 and 2. *FL cells 40 hr after infection with the -GCr Miyama strain of herpes simplex virus. The process of envelopment of single virus particles is illustrated step-wise. The capsid of particle A is half covered with the inner nuclear membrane. The capsid of particle B is almost enclosed in the membrane and the envelope of particle C is connected to the membrane by a narrow pedicle. The membrane of particle D is being pinched off. Fig. 1 × 53,000 Fig. 2 × 57,000*

FIGURE 3. *FL cell 62 hr after infection with the Ko strain of herpes simplex virus. One capsid with a core is enclosed by the inner nuclear membrane which shows remarkable reduplication × 73,000*

FIGURE 4. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. Four particles covered with a common membrane are seen budding into a vacuole just inside the nuclear surface. One of them is almost completely enveloped. × 46,000*


N
D
1
C
N
B
2
N
N
3
N
4

transparent thin middle layer and a thick, inner layer of high electron density. Only the innermost layers of the envelopes were clearly seen round most particles.

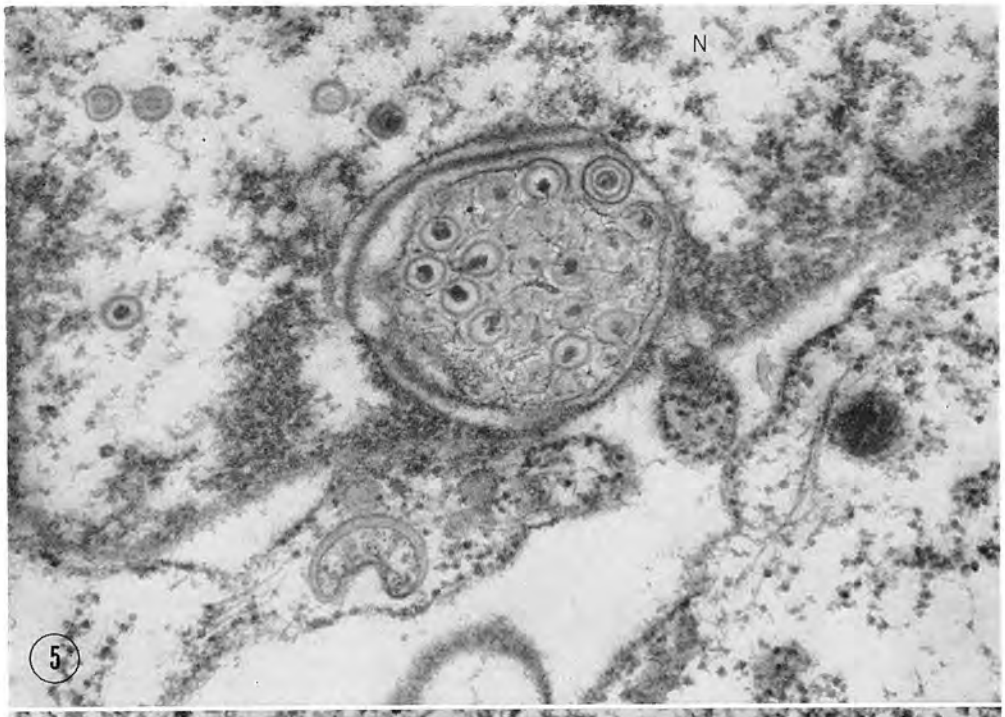
These morphological differences in the envelopes might be due to differences between the molecular structures of the inner nuclear and cytoplasmic vacuolar membranes, from which the envelopes were derived. Recent studies showed that, the saccules and vesicles of the Golgi apparatus are each surrounded by a smooth membrane without ribosomes, which is about 75 A thick, and has a characteristic unit membrane structure with three layers (Favard, 1969). On the other hand the unit membrane structure of the nuclear envelopes is rarely observed, suggesting that there is some fundamental difference at a molecular level between the membranes of the nuclear envelope and the plasma membrane or the membranes of the Golgi apparatus (Stevens and André, 1969). This problem will be discussed again in a subsequent paper.

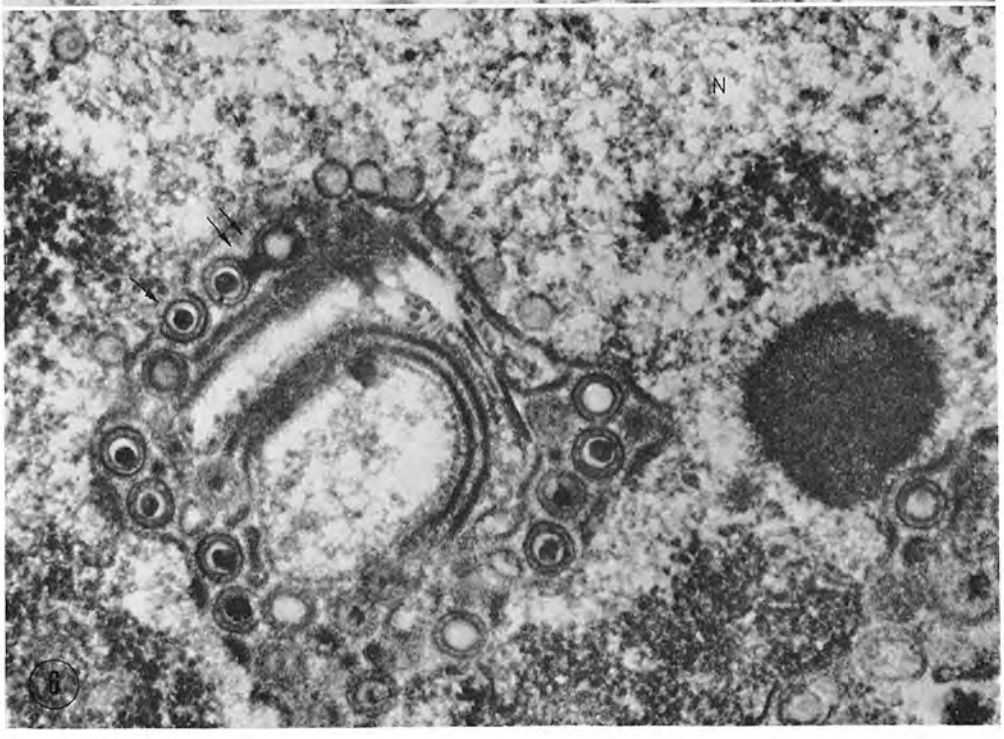
Many pictures (Fig. 6, 7, 10, 14, 16 and 17) show envelopment of empty capsids, suggesting that the envelopment process is not selective. However, very few enveloped particles had a core of low electron density (Fig. 22) although there were numerous capsids with this type of core in the nuclear matrix. Further studies are necessary on whether there is any relation between envelopment of herpes simplex virus and acquirement of electron density of its core (Nii et al., 1968b) and between virus envelopment and modification of the configuration of the virus capsid.

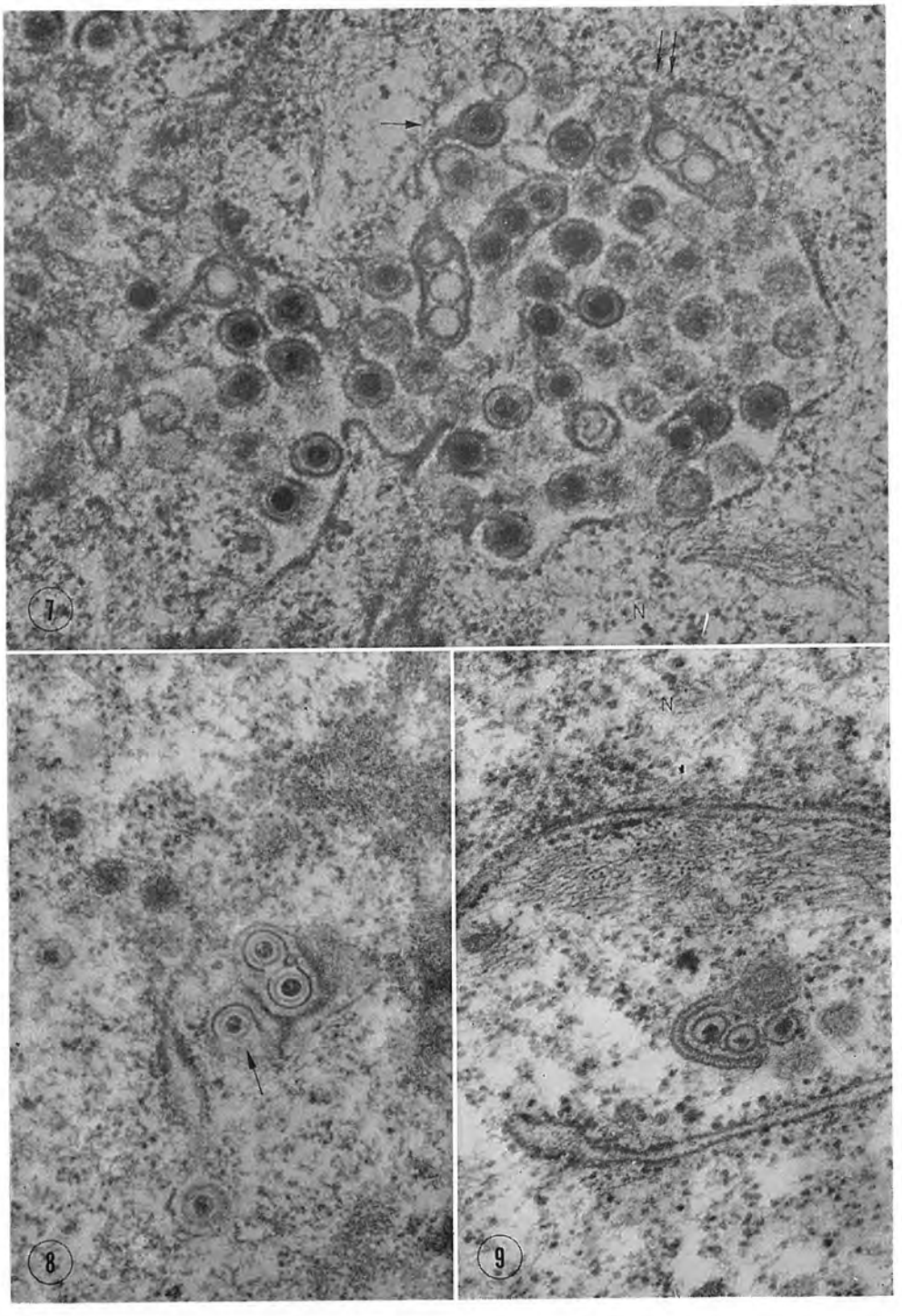
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FIGURE 5. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. An aggregate of virus particles covered with a common membrane and one enveloped particle are seen in a nuclear vacuole. All the particles have electron dense cores. Tail-like and filamentous structures are seen interwoven among an aggregate of the virus particles. Remarkable dilatation of the perinuclear cisterna is seen.* × *55,000*

FIGURE 6. *A part of the nucleus of an FL cell 40 hr after infection with the -GCr Miyama strain of herpes simplex virus. The nuclear surface is seen in the low right corner of the picture. Numerous enveloped particles are seen in a space between the vacuolar and reduplicated membranes. One particle (single arrow) is in the process of envelopment and envelopes of two particles are connected by electron dense material giving a dumb-bell shape (double arrow). Some particles have no cores.* × *55,000*


N
5


N
6


7
N
8
N
9

◀

FIGURE 7. *FL cell infected with the Watanabe strain of herpes simplex virus. A particle (single arrow) and a tubular membrane (double arrow) containing at least two empty capsids are budding from the vacuolar membrane in the nucleus. The latter look like peas in a pod. The vacuole contains tubules enclosing virus particles as well as numerous single enveloped particles and opens to the dilated perinuclear cisterna.* × *48,000*

◀

FIGURE 8. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. One particle (arrow) is budding into a small vacuole in the nucleus. Two particles in a nuclear vacuole form a dumb-bell shape.* × *45,000*

FIGURE 9. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. A smooth surfaced double membrane in the cytoplasm is in close association with two capsids with electron dense cores.* × *48,000*

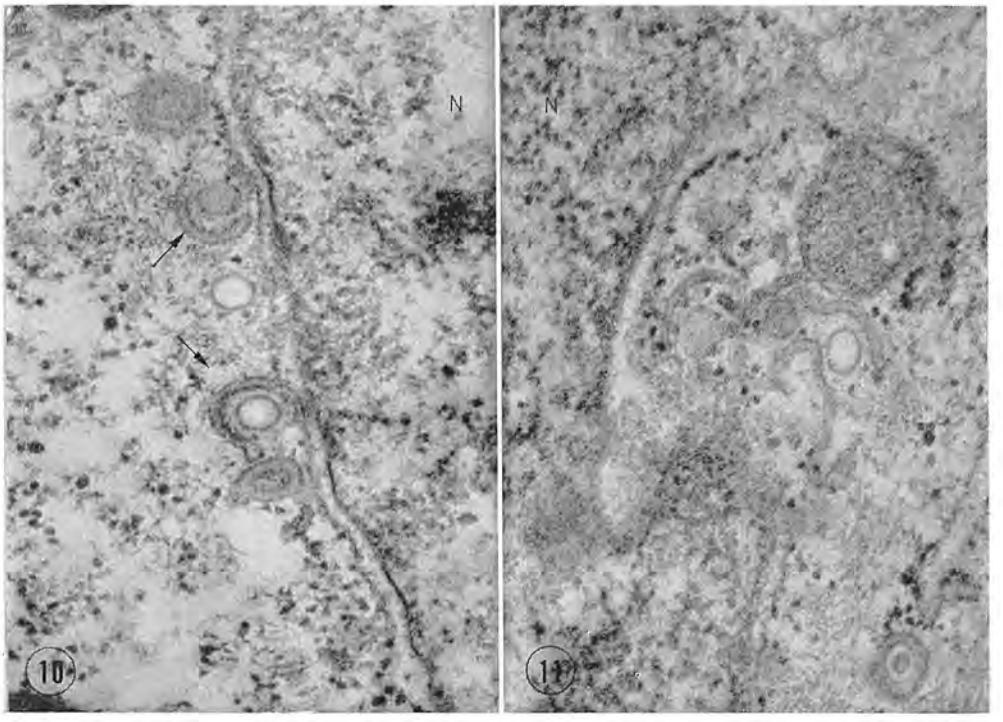


FIGURE 10. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. Two particles in the cytoplasm in close proximity to the perinclear cisterna are enfolded by double membranes.* × *58,000*

FIGURE 11. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. An empty capsid has a double membrane on either side of it.* × *55,000*

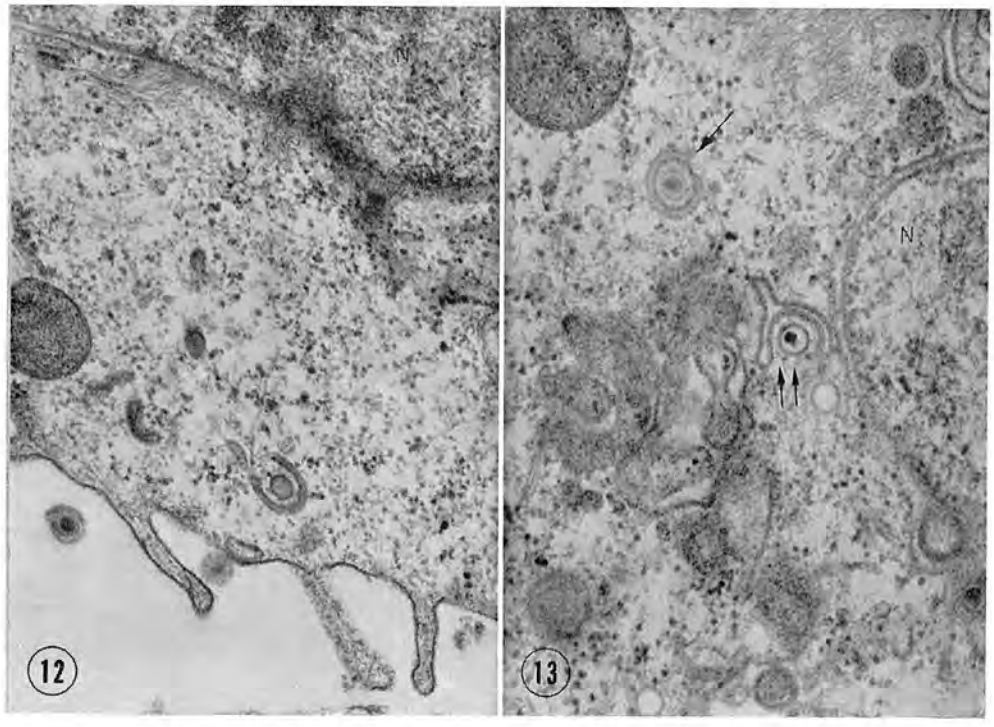


FIGURE 12. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. A hexagonal capsid near the cell surface is almost completely enclosed in a double membrane.* × *32,000*

FIGURE 13. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. One particle (single arrow) is almost completely enclosed in a double membrane and another particle (double arrow) is budding into a small cytoplasmic vacuole.* × *45,000*

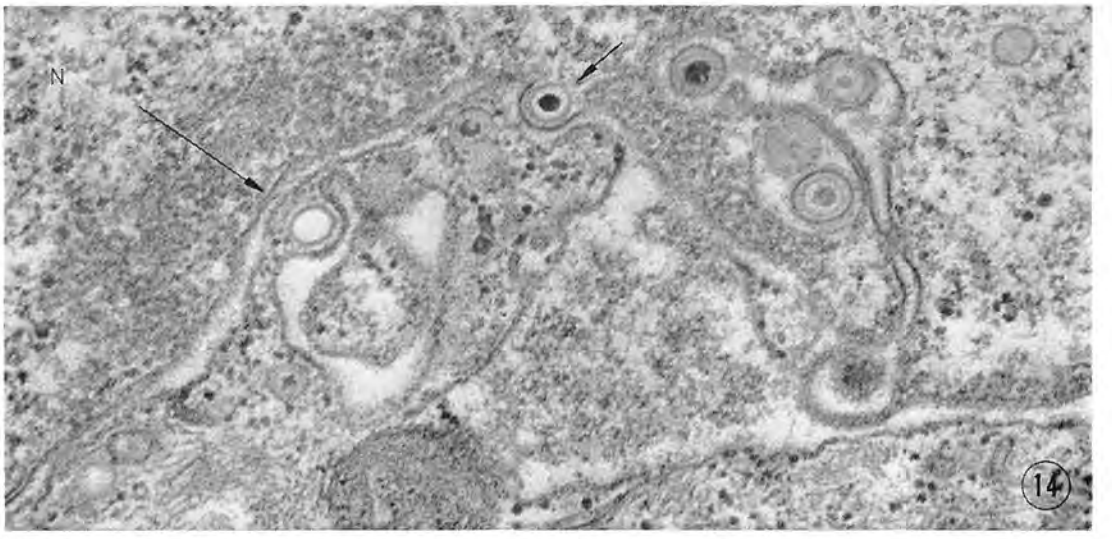


FIGURE 14. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. Budding is seen both at the vacuolar membrane in the cytoplasm (large arrow) and at the inner nuclear membrane (small arrow).* × *60,000*

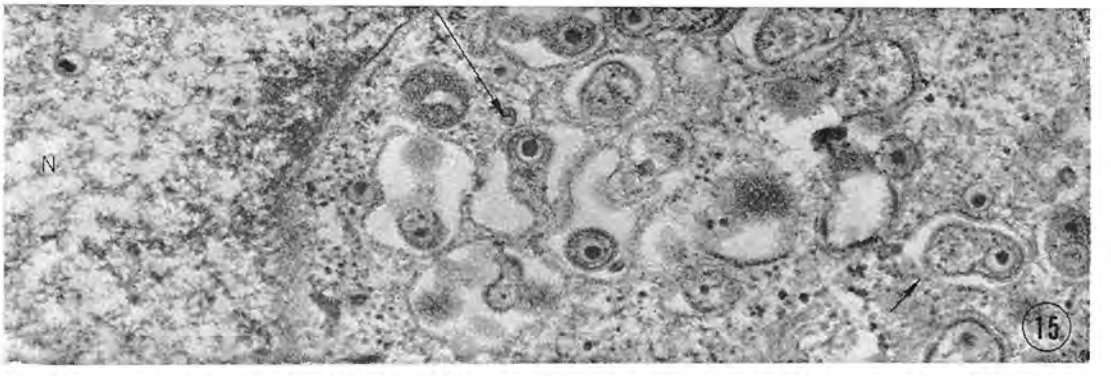


FIGURE 15. *FL cell 63 hr after infection with the Watanabe strain of herpes simplex virus. One particle (large arrow) has a bag-like envelope in a cytoplasmic vacuole and another particle (small arrow) with a similar envelope is seen in another vacuole.* × *41,000*

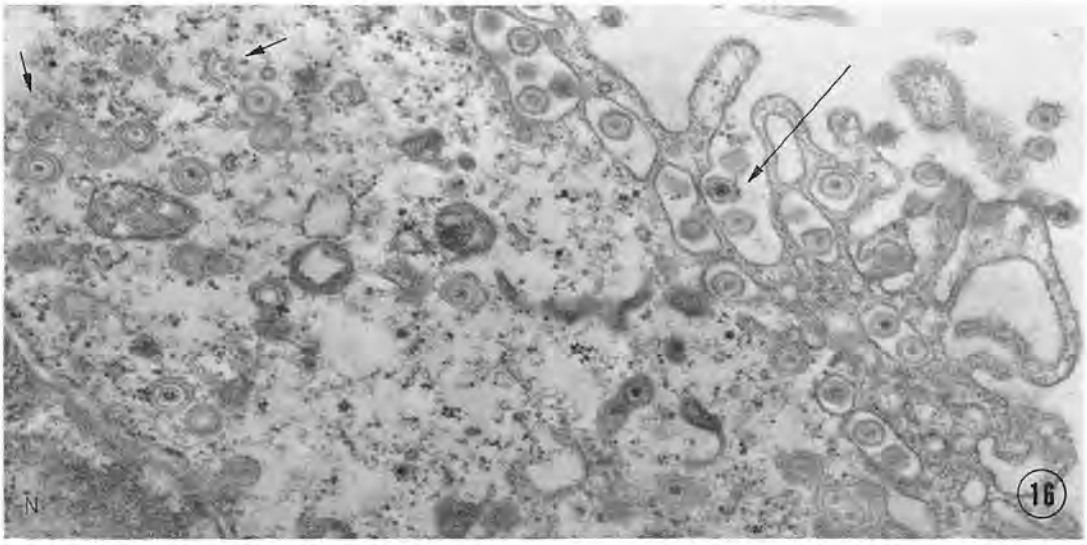


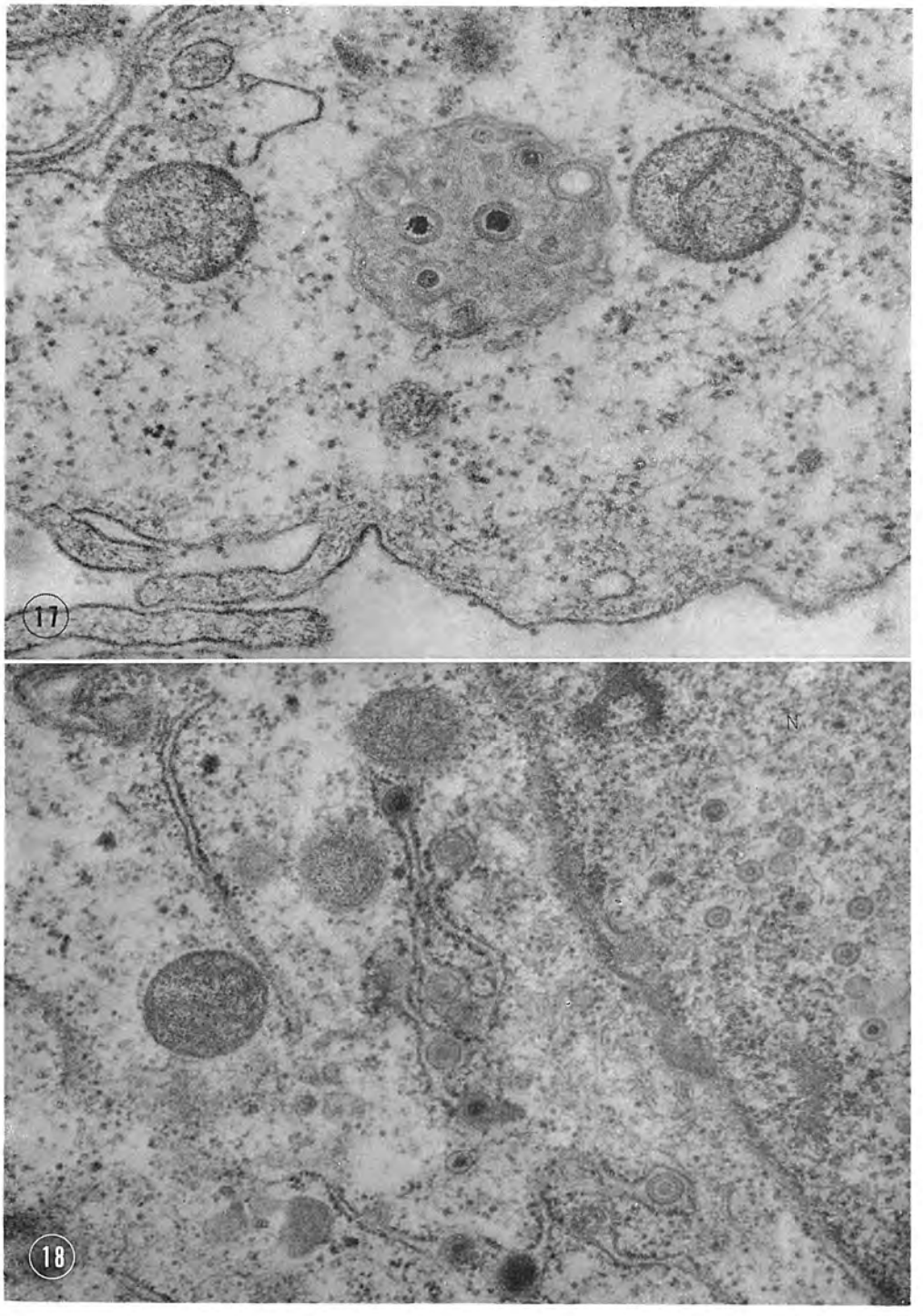
FIGURE 16. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. In the cytoplasm one particle is partially enclosed in a double membrane and another particle is almost entirely enclosed (both shown by small arrows). Many single particles are closely surrounded by a smooth-surfaced vacuolar membrane and appear as three concentric, circular membranes enclosing an electron dense core. At the cell surface irregular cytoplasmic projections protrude to the exterior of the cell and just inside the plasma membrane many small vacuoles, some of which open to the outside of the cell, contain one to more particles. Those findings strongly suggest that enveloped particles (large arrow) released by a process of reverse phagocytosis.* × *30,000*

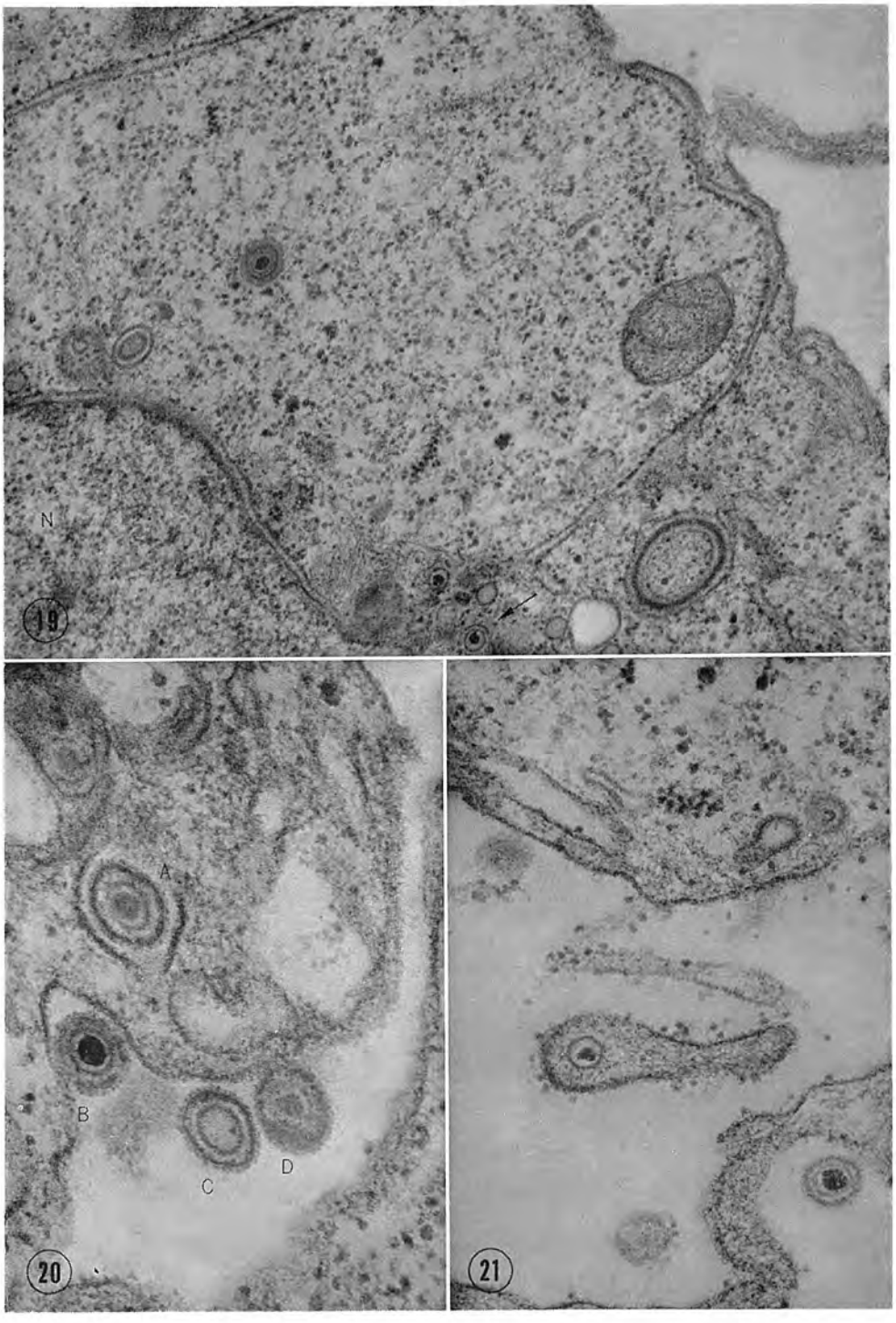
▶

FIGURE 17. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. An aggregate of enveloped particles are enclosed in a smooth-surfaced vacuolar membrane and are presumably moving towards the cell surface by a process of reverse phagocytosis. One capsid is empty.* × *54,000*

▶

FIGURE 18. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. Several particles are seen in branched tubules. These structures are thought to be derived from the rough-surfaced endoplasmic reticulum since they are associated with ribosomal particles. This picture suggests one pathway by which enveloped particles move from the perinuclear cisterna to the exterior of the cell.* × *40,000*


17
18
N


N
19
A
B
C
D
20
21

◀

FIGURE 19. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. One tubule stretches from near the perinuclear cisterna to the cell surface, suggesting one route for egress of virus particles from the cell. The membrane of the tubule appears to fuse with the plasma membrane at two points. One particle (arrow) is at the beginning of the budding process at the inner nuclear membrane.* × *40,000*

◀

FIGURE 20. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. Particle A is seen in a small cytoplasmic vacuole, particle B in an indentation of the plasma membrane and particles C and D are at the cell surface. These four particles may be in the process of being released from the cell.* × *73,000*

◀

FIGURE 21. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. One virus particle is seen inside a cytoplasmic projection close to its tip.* × *55,000*

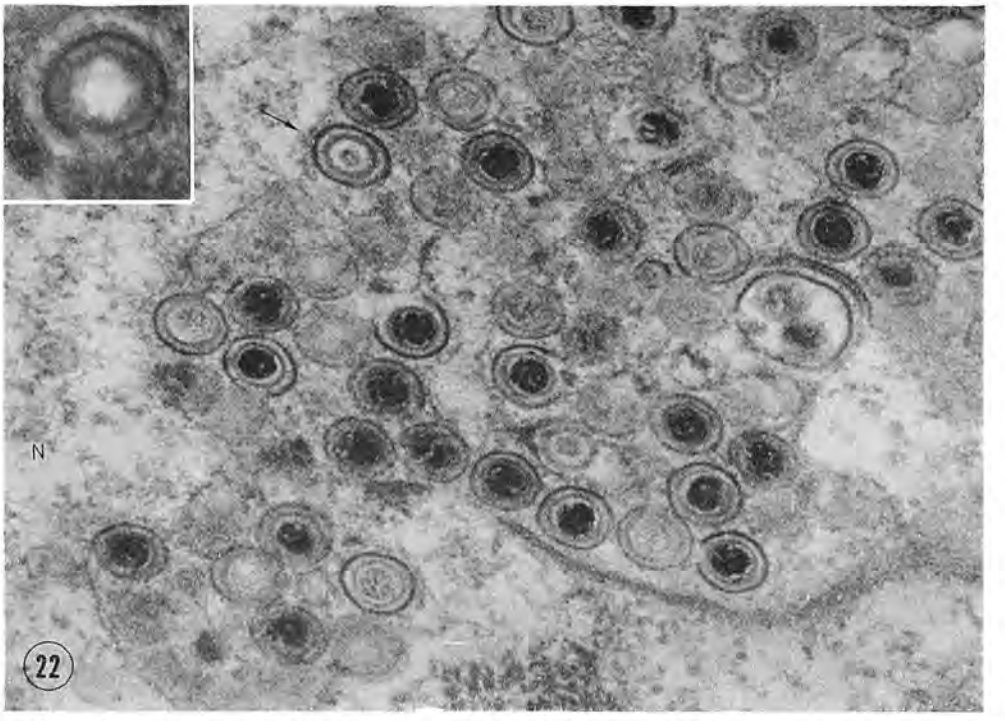


FIGURE 22. *FL cell 62.5 hr after infection with the Iwa strain of herpes simplex virus. Numerous virus particles are seen in a nuclear vacuole, the envelopes of which are each seen as an electron dense circular membrane. One particle (arrow) has a core of low electron density while the others have electron dense cores.* × *65,000*

The inset figure shows one enveloped particle in the perinuclear cisterna. The envelope is seen to consist of three concentric layers; an outermost layer of low electron density, a transparent, thin, middle layer and an electron dense, inner layer. The outermost layer appears to consist of fine globules or projections. × *110,000*

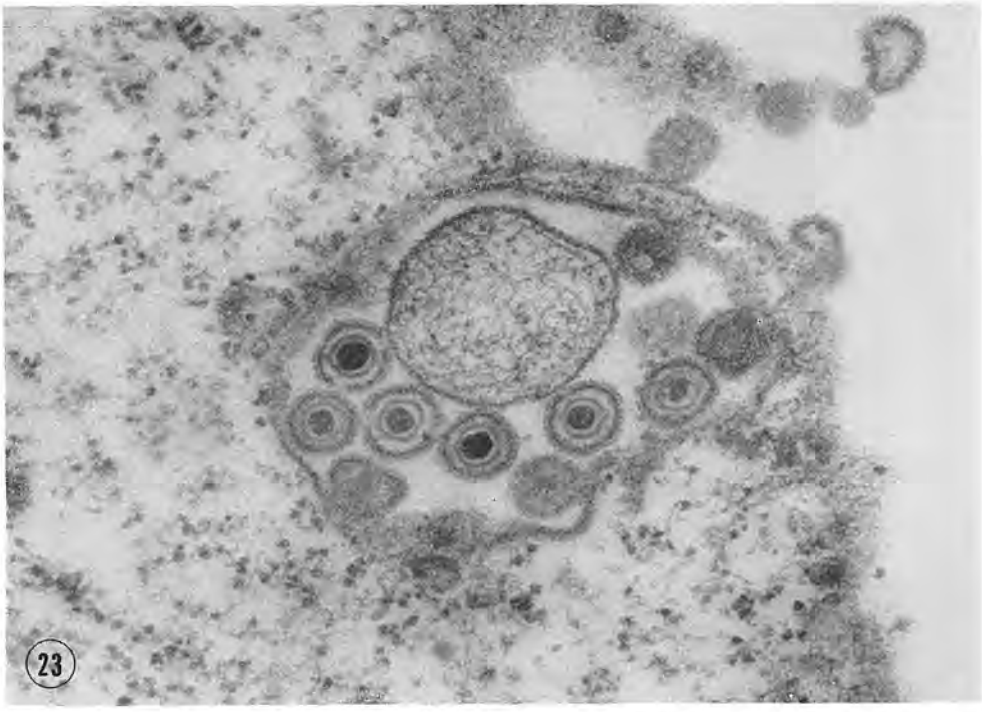


FIGURE 23. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. A cytoplasmic vacuole close to the plasma membrane contains several enveloped particles as well as a cytoplasmic mass in a membrane. Projections or fine globular structures are seen on the envelopes of these particles.* × *62,000*

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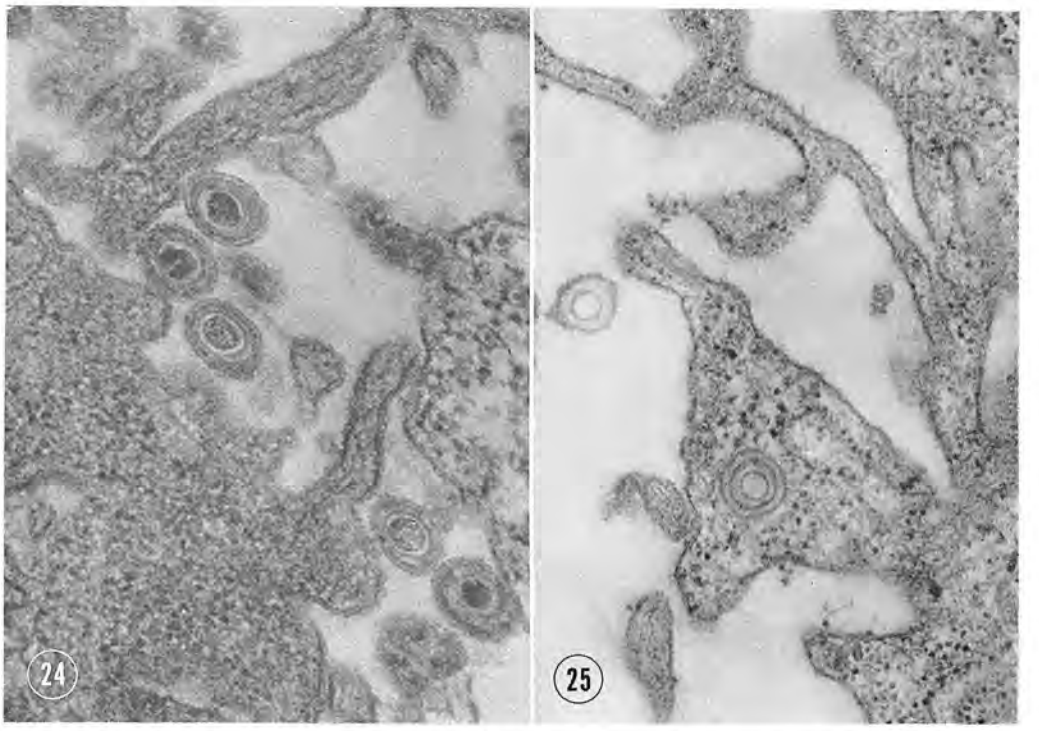


FIGURE 24. *FL cell 62 hr after infection with the Ko strain of herpes simplex virus. Envelopes of extracellular particles in this picture demonstrate a clear unit membrane structure.* × *53,000*

FIGURE 25. *FL cell 20 hr after infection with the -GCr Miyama strain of herpes simplex virus. Two particles, one in the cytoplasm and the other in the extracellular space have empty capsids.* × *41,000*

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