THE CELLULAR CHANGES PRODUCED IN TISSUE CULTURES BY HERPES B VIRUS CORRELATED WITH THE CONCURRENT MULTIPLICATION OF THE VIRUS*

By MAGDALENA REISSIG, M.D., and JOSEPH L. MELNICK, PhD.

(From the Section of Preventive Medicine, Yale University School of Medicine, New Haven)

Plates 16 to 25

(Received for publication, November 8, 1954)

Virus-infected cells often manifest characteristic morphological changes, but there is still considerable question as to how far these alterations correspond to the actual presence of virus particles. The situation in regard to herpes simplex virus has received considerable attention. Although intranuclear inclusions are found in infected cells (1–3), several workers have reported that the virus itself is not associated with the nucleus (4–7). Recently Scott and his coworkers (8, 24) have found that herpes virus is found within the cell nucleus in significant amounts only in the early stages of infection, before the appearance of inclusion bodies.

It seemed to us that a contribution to this problem might be made by studying cells in tissue culture infected with a herpes virus, in which a relationship could be established among the cellular alterations seen in the optical microscope, the presence of virus particles within the cells visualized in the electron microscope, and the extent of virus multiplication determined by infectivity titrations. A study of this kind requires that a virus-host cell system be utilized in which all the cells are equally susceptible to the virus, and in which all can be infected within a brief period by exposure to a sufficiently large inoculum. Such a homogeneous system is also desirable for electron microscopic studies (in which the total areas of tissue examined are very small), in order to insure that the samples are truly representative of infected cells taken at different stages after infection.

For the present investigation, a member of the herpes group, B (or herpes B) virus was studied in cells derived from the natural host of the virus, the monkey. Monolayer cultures of monkey kidney epithelium provided a system which permitted uniform infection of the culture; and the enumeration of infective herpes B virus particles in such cultures could be accomplished by plaque counting (9). Renal epithelial cells in culture offer a further advantage in that they lack the complex morphological elements present in more differentiated

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

cells, which after infection might themselves become altered and difficult to distinguish from the products of virus multiplication. The present work, then, concerns electron microscopic (E.M.) and histological observations made together with tests of the amount of infective virus produced.

Materials and Methods

Virus.—A strain of herpes B virus isolated from a monkey in the local colony (10) and adapted to monkey renal tissue culture was selected for study. The source of virus for the present work was the infected fluid phase harvested from the fourth tissue culture passage. Its titer of 10^{-6} was determined by plaque counting on monolayer cultures as already described (9). The pattern of cytopathogenic degeneration observed for this virus is very similar to that seen by Scott *et al.* (8) in tissue cultures of corneal epithelium inoculated with the virus of herpes simplex.

Tissue Culture Methods.—Monolayer cultures of kidney epithelial cells from adult *rhesus* monkey were prepared essentially by the trypsinization method described by Youngner (11). The cells were suspended, and grown, in a medium consisting of 0.5 per cent lactalbumin enzymatic hydrolysate, 2 per cent calf serum, and 97.5 per cent Hanks's balanced salt solution. The cultures were grown on glass in 16 × 150 mm. test tubes that had one side flattened near the base over a rectangular area of 15 × 40 mm.[1] In order to be able to withdraw the cells from the tubes without altering their shape or spatial relations, a coverslip, 11 × 22 mm. in size, was placed on the flattened area before adding the cell suspension. Each culture was started by 1 ml. of suspension containing about 600,000 cells.

Preparation of Coverslips.—For the cultures to be used for histological observation the coverslips were cleaned in "7x" detergent, rinsed, and dried, and sterilized overnight by ultraviolet irradiation. For E.M. studies, coverslips coated with a film of a polyvinyl resin (formvar) were prepared by a procedure essentially similar to that described by Porter (12). The clean coverslips were dipped in a 0.3 per cent solution of formvar, allowed to dry, and sterilized by ultraviolet irradiation.

Infection of Cultures.—After 4 or 5 days at 35°C. in the stationary position, a monolayer of cells had grown on the flat area, most of which was occupied by the coverslip. The cultures were then washed with Earle's solution and each inoculated "*in situ*" with 0.9 ml. of virus suspension and 0.2 ml. of nutrient medium. The dose used was calculated so that at least 2 infectious units were available per cell. With this virus, when the number of available cells exceeds the number of particles, plaque formation is readily observed, as reported elsewhere (9). In contrast to this, when the number of infective virus units inoculated exceeds the number of cells in the culture, no plaque formation should be observed, and actually none was found in these experiments, cell degeneration due to the virus being general.

Method of Following the Developmental Cycle.—Tissue cultures were harvested at different time intervals after inoculation, 6 tubes being removed after each selected interval, 3 of them for histological examination and 3 for E.M. examination. This was done at 2, 4, 6, 10, 14, 18, 24, 36, and 48 hours after inoculation. The fluids were then harvested for the titration of infective virus, after which the coverslips were removed from the tubes. This was accomplished by sliding under the coverslip a metal spatula, one of the ends of which had been tapered and bent at a 45° angle. The wet coverslip remained attached to the spatula and could be easily removed from the tube in this manner. Uninoculated cultures were harvested at the same time as the last infected culture, as controls for possible non-specific cellular degeneration. Titrations of virus in the culture fluid were made by a method described elsewhere (9).

---

[1] Obtained from Microbiological Associates, Bethesda.

Preparation of Specimens for Electron Microscopy.—After removal from the culture tubes, the coverslips were transferred for fixation to 2 per cent buffered osmium tetroxide in Earle's balanced salt solution at pH 7.4, essentially as described by Palade (13). The cells, still attached to the glass, were immersed in the osmium fixative for 20 minutes in a covered Petri dish.

Since the infected cells become swollen and consequently too thick for direct E.M. observation, a technique was devised which enabled us to cut sections of them about 0.05 μ thick. For this purpose the coverslips were transferred to another dish containing Earle's balanced salt solution and, under a dissecting microscope, the whole film was freed from the glass. This was done by breaking the continuity of the film close to the edges of the coverslip by means of a sharpened jeweler's forceps and peeling it away. As surface tension forces make the film difficult to handle, it is important that these manipulations be performed under a sufficient depth of fluid to prevent the film from floating to the surface. The films were not used for direct observation in the electron microscope; they served only as temporary supports for the cells prior to their embedding. Consequently their thickness was not critical and, contrary to usual procedure, slightly thicker plastic films were prepared, because they could be peeled off and handled more readily.

The cells, held together by the underlying film, were dehydrated by rapid passage through a series of graded alcohols (15 minutes in each change) and then transferred to a mixture of 1:9 *N*-methyl:*N*-butyl-methacrylate. The film, with cells still adhering, was then gently folded with the aid of a glass rod and placed in the bottom of a gelatin capsule. The capsule was filled with the same methacrylate mixture, to which 1 per cent of 2, 4 benzoyl peroxide had been added as a catalyst and polymerized overnight at 45°C. (14).

Sections about 0.05 μ thick were cut from the tissues with glass knives, using a thermal expansion microtome designed by Porter and Blum (15). The sections were collected on 10 per cent acetone in distilled water and immediately mounted on collodion-covered copper grids. They were examined in an R.C.A. type EMU 2A electron microscope without removal of the embedding medium. Electron micrographs were taken at original magnifications of 1900 to 6000.

Histological Examination.—After removal from the culture tubes, the coverslips were washed in Earle's balanced salt solution for a few minutes, and fixed for 1 hour either in buffered osmium tetroxide or Zenker's acetic acid solution. Although the buffered osmium solution is known to be a poor fixative for the demonstration of inclusion bodies (3), it was included in order to compare the histological picture with the electron microscopic observations. Different samples from each harvest were fixed in the two solutions and then stained with hematoxylin and eosin. Thicker sections from the same tissue blocks used for electron microscopy were also examined in the light microscope. These were mounted on glass slides, and the embedding medium was then removed by immersion in acetone. They were observed unstained by phase microscopy or after staining with Giemsa solution.

RESULTS

Morphological Changes

Uninfected Cultures.—Normal cells from monolayer cultures of monkey kidney epithelium had a homogeneous cytoplasm in which mitochondria and a few fat droplets were clearly visible either in the natural state, with the phase microscope, or after fixation in buffered osmium tetroxide and staining with hematoxylin and eosin. Under these conditions the nuclei appeared round and homogeneous with one or two dark, well defined nucleoli (Fig. 1). After Zenker fixation and staining with hematoxylin and eosin, the cytoplasm appeared

slightly granular, mitochondria were no longer visible, and the nuclei were somewhat shrunken, with the chromatin arranged in fine strands. Vertical sections of these cells when observed in the electron microscope had the appearance shown in Figs. 5 and 6. Numerous round and elongated elements with a clear center bound by a single membrane were found in the ground substance of the cytoplasm. They ranged in diameter from 50 to 100 mμ and they corresponded to sections of the endoplasmic reticulum. Smaller granular elements were scattered throughout the cytoplasm. In many of the sections mitochondria were readily identified and occasionally fat droplets were also seen. The nuclei appeared oval or rounded and had a fine internal structure surrounded by a membrane of uniform thickness. In particularly thin sections the two layers forming this membrane were clearly distinguished. Nucleoli were present in many of the sections. As shown by the vertical sections, the thickness of normal cells sectioned vertically was no greater than 2 or 3 μ (Figs. 5 and 6).

Infected Cultures.—The first noticeable changes consisted in swelling and vacuolization of the infected cells, which occurred about 2 hours after virus inoculation. Similar cells were occasionally seen in control cultures, although never in such large numbers. It is possible that they represent only a reaction of the cells to the introduction of a foreign substance in the culture tubes, regardless of its infectivity. Under the electron microscope the cytoplasm of these swollen cells appeared less dense than that of the normal ones, and consequently the mitochondria and elements of the endoplasmic reticulum stood out much more clearly (Fig. 7). In some cells vacuoles were seen, either empty or filled with a material made up of granules of varying size and electron density. These vacuoles were lined by what looked like a condensation of the cytoplasm; on very rare occasions they were seen in control cells. No special significance could be attached to them. Although a large number of cells harvested 2 and 4 hours after inoculation was examined under the electron microscope, nothing could be found in them that appeared morphologically related to virus particle formation.

From 4 to 6 hours after inoculation most of the cells began to show typical nuclear changes (Fig. 2). The nucleoli underwent a marked increase in volume, they became irregular in shape, and they appeared much lighter in color, both in the living state under the phase microscope, and after staining with hematoxylin and eosin. In some cells, in which presumably the infection had advanced further, the nucleoli had a finely granular structure or were actually replaced by granules. This phenomenon is very difficult to observe with the electron microscope in sectioned cells. Because of the extreme thinness of the section, it does not follow that the absence of the nucleolus at the level examined means that it has disappeared from the cell. Furthermore, its irregular and poorly defined appearance could correspond simply to a tangential section through its outer surface.

From 6 to 10 hours after inoculation the regular chromatin pattern of the

normal nucleus became greatly altered: clear areas made their appearance in the central zone of the nucleoplasm, as is shown in Fig. 8. In some instances, the initial stage of this process could be observed before the complete disappearance of the nucleoli. As these patches increased in size the chromatin became condensed in coarse strands (seen best in thick sections) located chiefly at the periphery of the nucleus. From the increase in electron and optical density, a real condensation of chromatin seemed to occur, rather than its mere disappearance from the central area of the nucleus. As the infection progressed, the chromatin became restricted to a narrow zone just inside the nuclear membrane (see Figs. 9, 11, 12). This phenomenon started in cells from 10 to 14 hours after inoculation. At about this time clusters of closely packed round particles, having dense membranes, began to appear in this marginated chromatin. Their sizes ranged from 60 to 130 mμ and some of the larger ones contained a dense central body measuring about 30 mμ in diameter. Since many of the smaller particles were closely packed together and virtually in contact with each other, as shown in Figs. 13 and 14, the differences in size were considered real and not an artifact caused by subtangential sectioning. In the marginal zone of the nucleus, irregular dense bodies were found (Figs. 8, 11, and 12). These were rarely in contact with the nuclear membrane. Most of them were embedded in the marginated chromatin where they stood out because of their higher electron density and much coarser granular structure. Isolated particles appeared in the central, clear zone of the nucleoplasm (as can be seen in Figs. 8, 9, 11, and 12). They varied in size from 60 to 100 mμ. Most of them consisted of a dense "nucleus" surrounded by a membrane but some of the particles seemed to consist of membranes alone, free of the central body. In addition to particles with single membranes, "nucleated" particles with double membranes were present in the nucleus, particularly at late stages of infection. Their size was slightly larger than the single coated particles and they ranged from 100 to 180 mμ (see Fig. 17). Single and double coated particles also appeared between the two layers of the nuclear membrane (see Figs. 15 and 16).

At this stage particles like those just described, either isolated or in clusters, began to appear in the cytoplasm of the infected cells (Figs. 9 and 10). They also had a dense center surrounded by either one or two membranes. Those having a single coat measured from 60 to 100 mμ, and those with two membranes were slightly larger, measuring from 100 to 180 mμ.

Particles were also seen on the external surface of the infected cells. They first appeared 10 hours after virus inoculation and at this period they were found in only a few cells in the culture. The number of particles per cell as well as the number of cells involved increased greatly in the following hours. Particles were associated only with those cells which exhibited margination of the nuclear chromatin and intracellular particles. They were in close contact with the cell membrane and seemed to be bound to it, since they were not washed away during the fixing and embedding process. In some cases they were found in

slight depressions of the cell membrane. Except for a small number of particles, 60 to 80 mμ in diameter, the extracellular particles ranged from 130 to 180 mμ, and most of them appeared to have a central "nucleus" surrounded by a double membrane. A few of these extracellular particles were formed by two dense central bodies, each having its own inner membrane and surrounded by a single external membrane (Figs. 21 and 22). Similar "binucleated" particles were found on several occasions in the cytoplasm of infected cells, scattered among "mononucleated" particles of the usual kind (Fig. 20).

In the late stages of infection, 24 to 36 hours after inoculation, further cellular changes occurred. As seen in Figs. 11 and 12, the amount of chromatin decreased until most of the nuclei appeared as clear vesicles surrounded by a double membrane. The nuclear membrane became wavy and irregular and exhibited numerous finger-like prolongations. If such nuclear projections without chromatin happened to be sectioned so that no connection with the nucleus was seen, they could not be identified as such. Numerous polynucleated cells first appeared about 18 hours after virus inoculation and they continued to increase in number in later stages of infection (Fig. 18). The mechanism of their formation remains unknown, but at least in some cases they seemed to originate from single mononucleated cells, since their nuclei sometimes were joined by prolongations from the nuclear membrane (Fig. 19) and bilobulated nuclei were frequently seen.

It is noteworthy that no structure identifiable as the classical type A intranuclear inclusion body could be observed in the electron micrographs. However, typical inclusions were readily seen in the nuclei of infected cells fixed in Zenker's fluid and stained with hematoxylin and eosin, and an attempt was made to correlate this histological picture with the electron microscopic image. When the cells were fixed in buffered osmium tetroxide and stained with hematoxylin and eosin, the altered nuclei were intensely stained by the eosin but no clearly outlined intranuclear inclusions were found. The difference in cells fixed in Zenker's and in osmium tetroxide may be seen by comparison of Figs. 3 and 4. The same failure to observe intranuclear inclusions was noted when sections of the same blocks of tissue used for electron microscopy were stained with Giemsa solution after removal of the embedding. Earlier workers have reported that detection of inclusion bodies depends to a large extent on the shrinking properties of the fixatives and preservatives used (3). This was also true for our material when, after Zenker's fixation followed by immersion of the cultures for 24 hours in 80 per cent alcohol, the clear central areas of the nucleus underwent a slight retraction, separated from the marginated chromatin, and appeared as inclusion bodies. In the early stages of infection, when the chromatin is condensed into several strands leaving clear areas between them, each of these clear areas contained an inclusion which stains bluish pink with hematoxylin and eosin. As the infection progressed and the chromatin became restricted to a narrow margin about the nuclear membrane, the inclusion

stained darker with eosin and differentiated into a fully formed type A inclusion body, filling most of the nuclear space.

Appearance of Infective Virus in the Culture Fluids.—A number of experiments were performed as outlined under Methods, in which the number of plaque-producing units was determined at varying times after the inoculation of the cultures. One of these is summarized:—

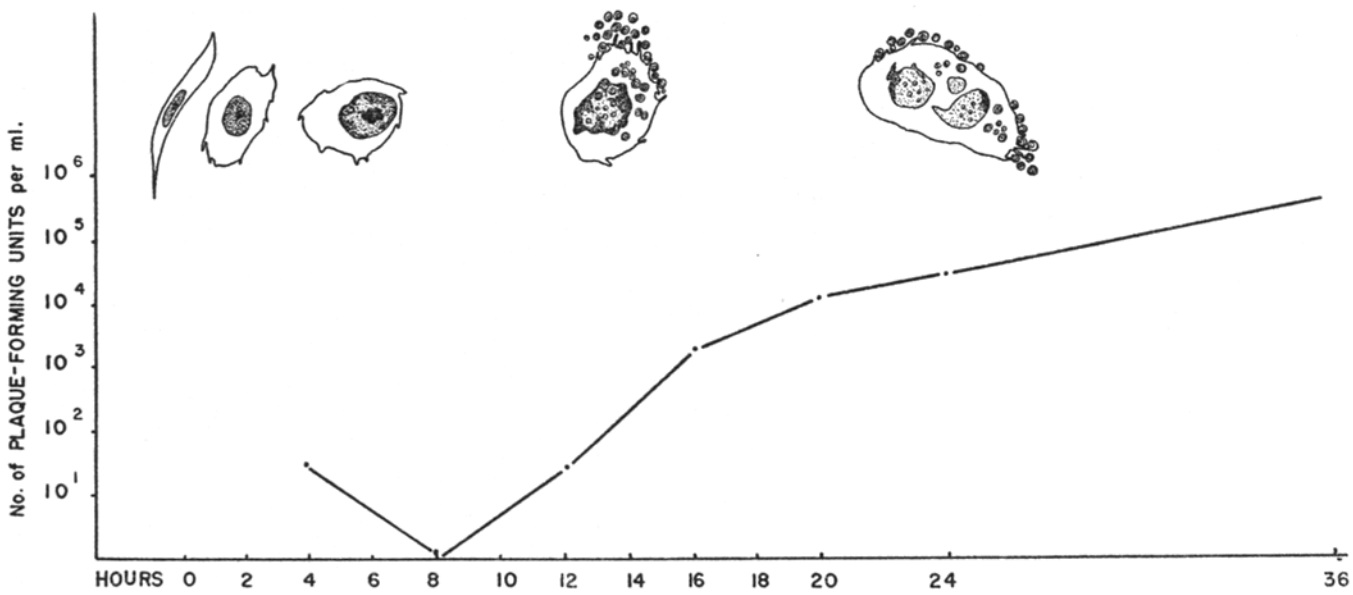
A number of cultures were each inoculated with 25,000 plaque-producing units of virus. After an adsorption period of 2 hours, the inoculum was removed, the cultures washed with balanced salt solution, and fresh nutrient medium was added to the cultures. Periodically, as shown in Text-fig. 1, aliquots were removed and titrated. The amount of virus in the fluid phase was found to increase significantly sometime between the 12th and 16th hour after inoculation. After the 16th hour, the increase continued but it was more gradual.

DISCUSSION

In this study a definite relationship was found to exist between the appearance of characteristic particles within the infected cells and the release of infective virus in the culture medium. In Text-fig. 1 we have attempted to correlate these two sets of data. When herpes B virus was added to the cultures, it disappeared from the supernatant fluid in the course of 4 to 8 hours. In these first hours, which correspond to the so called latent period of virus multiplication, no changes were detected which could be morphologically related to virus particle formation. Particles began to appear in the cells and on their external surfaces about 10 hours after virus inoculation, and this phenomenon took place concurrently with a rise in virus titer in the culture fluid. Nuclear margination of the chromatin was constantly associated with the appearance of the particles. At late stages of infection, cells lost most of their nuclear chromatin and the rate of increase of infective virus fell off.

It appears from these observations that in the case of herpes B virus both the cell nucleus and the cytoplasm play an important role in the process of virus multiplication. The gradual disappearance of the nuclear chromatin suggests that this material may perhaps be utilized for virus particle formation. From their study of chorioallantois infected with the virus of herpes simplex, Morgan and coworkers (16) have advanced the hypothesis that a dense central body surrounded by a single membrane is formed in the nucleus, later finding its way to the cytoplasm through disruptions of the nuclear membrane; and the second external coat of the virus is formed in the cytoplasm. But the process appears to be more complex, at least in the case of herpes B virus growing in primate epithelial cells. The same wide variation in particle size exists both in the nucleus and in the cytoplasm, and particles with one and two surrounding membranes are found in both places. This suggests that particle formation may take place in both locations, perhaps simultaneously.

This study has thrown some light on the matter of inclusion bodies. By comparing the electron micrographs with ordinary histological observations, the inclusion bodies were found to correspond to the central clear zone of the



Text-Fig. 1. Herpes B virus infection of cultures of monkey kidney epithelium. Schematic diagrams to illustrate the correlation of morphological changes with the appearance of infective virus in the culture fluid. The appearance of characteristic particles in the cells is coincident with the rise in virus titer.

nucleus in which particles—for the most part of the smaller type with only one surrounding membrane,—are found. It is conceivable, as has been suggested by Morgan and his coworkers for herpes simplex, that the smaller particles correspond to incomplete, or non-infective, forms of the virus particle. If this is the case, negative results of infectivity tests alone would be insufficient to rule out the presence of particles related to the virus, and other methods would have to be employed. Further work remains to be done on the subject.

In the present study the characteristic particles which appear in the infected cells have been considered to be virus particles. Several facts support this statement: (*a*) the particles have not been found in normal cells; (*b*) they are first seen in the infected ones 6 to 10 hours after virus inoculation; (*c*) they appear on the external surface of the cells at about the time new infective virus first becomes detectable in the culture fluid; (*d*) their size range is compatible with the observations made on particles isolated from herpes-infected tissue and considered to be the virus (17–19); (*e*) similar particles have recently been found in the chorioallantois of chick embryos infected with herpes simplex virus (16).

The question arises as to the mechanism by which virus particles are released from infected cells. A significant proportion of the extracellular particles must correspond to mature virus, since they are first seen at about the same time new infective virus becomes detectable in the culture fluid. They make their appearance a few hours before the beginning of cell destruction is visible in the histological preparations. The last observation is in line with the findings of Dulbecco and Vogt (20) on western equine encephalomyelitis and of Cairns (21) and others (22, 23) on influenza. By indirect methods these workers have arrived at the conclusion that release of virus from cells occurs in a gradual and continuous manner by a process which does not, by itself, cause immediate destruction of the cells. Viruses have been found within the double membrane surrounding the infected nucleus, and it is not unlikely that they are in transit from the nucleus into the cytoplasm. We have no direct observations as to whether virus leaves the cell without disruption of the cell membrane. Particles are found in large numbers in close relation with the cell membrane, either in the cytoplasm or on the external surface of the cells, sometimes in depressions closely adapted to their shape, but no large disruptions of the membrane were found through which the virus might have escaped. Although finger-like projections of the cytoplasm were sometimes observed, they were of a much smaller diameter than the virus, and particles have never been seen intimately associated with them.

It might be argued that in our experiments the virus seen on the external surface of the cells came from the original inoculum or was produced in neighboring cells which had already been destroyed. However, no particles were seen on the external surface of cells from specimens harvested in the first 6 hours after inoculation, as would be expected if they belonged to the original inoculum.

They first appeared in the 10 hour specimen, when cell destruction had not yet occurred, and only on the surface of those cells showing typical nuclear alterations and intracellular particles. Although external to the cells, the particles observed were definitely associated with them, for the fixation and dehydration procedure failed to wash them away.

SUMMARY

A sequential study is reported of the morphological changes occurring after herpes B virus infection of cells as revealed in ultrathin sections under the electron microscope. Monolayer cultures of renal epithelial cells prepared from the natural host of the virus, the monkey, were infected, and the cellular alterations were correlated with the appearance of infective virus in the culture fluids.

The morphological changes consisted in swelling of the cells and disappearance of the nucleolus, followed by margination and gradual decrease of the nuclear chromatin. The inclusion material corresponded to the clear central areas of the nucleus, where the chromatin had disappeared. In the late stages of infection this inclusion material filled the nucleus and formed a classical type A inclusion body.

Characteristic particles appeared in the nucleus and cytoplasm of the infected cells a few hours after inoculation. They had a dense center surrounded by one or two membranes. Those with one membrane ranged in size from 60 to 100 mμ and those with two from 120 to 180 mμ. Particles showing the same wide variation in size and structure were seen both in the nucleus and in the cytoplasm. They were first visible on the external surface of the swollen but intact cells at about the same time new infective virus became detectable in the culture fluid.

A small number of the extracellular, and cytoplasmic, virus particles appeared "binucleated," containing two central bodies, each having its own membrane, both being surrounded by a single external coat. About 180 mμ in diameter, they were randomly distributed among the "mononucleated" particles.

BIBLIOGRAPHY

1. Lipschutz, B., *Wien. med. Woch.*, 1921, **71**, 231.
2. Goodpasture, E. W., *Am. J. Path.*, 1925, **1**, 1.
3. Cowdry, E. V., *Arch. Path.*, 1934, **18**, 527.
4. Bang, F. B., *Bull. Johns Hopkins Hosp.*, 1950, **87**, 511.
5. Francis, T., and Kurtz, H. B., *Yale J. Biol. and Med.*, 1950, **22**, 579.
6. Ackermann, W. W., and Kurtz, H., *J. Exp. Med.*, 1952, **96**, 151.
7. Johnson, R. B., and Ackermann, W. W., *Proc. Soc. Exp. Biol. and Med.*, 1954, **86**, 318.
8. Scott, T. F. McNair, Burgoon, C. F., Coriell, L. L., and Blank, H., *J. Immunol.*, 1953, **71**, 385.
9. Black, F. L., and Melnick, J. L., *J. Immunol.*, 1955, in press.
10. Melnick, J. L., and Banker, D. D., *J. Exp. Med.*, 1954, **100**, 181.

11. Youngner, J. S., *Proc. Soc. Exp. Biol. and Med.*, 1954, **85**, 202.
12. Porter, K. R., *J. Exp. Med.*, 1953, **97**, 727.
13. Palade, G. E., *J. Exp. Med.*, 1952, **95**, 285.
14. Newman, S. B., Borysko, E., and Swerdlow, M., *J. Research Nat. Bureau Standards*, 1949, **43**, 183.
15. Porter, K. R., and Blum, J., *Anat. Rec.*, 1953, **117**, 685.
16. Morgan, C., Ellison, S. A., Rose, H. M., and Moore, D. H., *J. Exp. Med.*, 1954, **100**, 195.
17. Evans, A. S., and Melnick, J. L., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71**, 283.
18. Coriell, L. L., Rake, G., Blank, H., and Scott, T. F. McNair, *J. Bact.*, 1950, **59**, 61.
19. Munk, K., and Ackermann, W. W., *J. Immunol.*, 1953, **71**, 426.
20. Dulbecco, R., and Vogt, M., *J. Exp. Med.*, 1954, **99**, 183.
21. Cairns, H. J. F., *J. Immunol.*, 1952, **69**, 162.
22. Henle, W., Liu, O., and Finter, N. B., *J. Exp. Med.*, 1954, **100**, 53.
23. Ackermann, W. W., and Maassab, H. F., *J. Exp. Med.*, 1954, **99**, 105.
24. Gray, Alan, and Scott, T. F. McNair, *J. Exp. Med.*, 1954, **100**, 473.

EXPLANATION OF PLATES

Key to figures:—

C —cytoplasm
N —nucleus
Cm—cell membrane
Nm—nuclear membrane
mi —mitochondrion
ch —marginated chromatin
e —endoplasmic reticulum
np and *p*—nuclear particles
cp —cytoplasmic particles
ep —extracellular particles
v —vesicles full of particles, attached to the nuclear membrane
dp —particle with a "double nucleus"

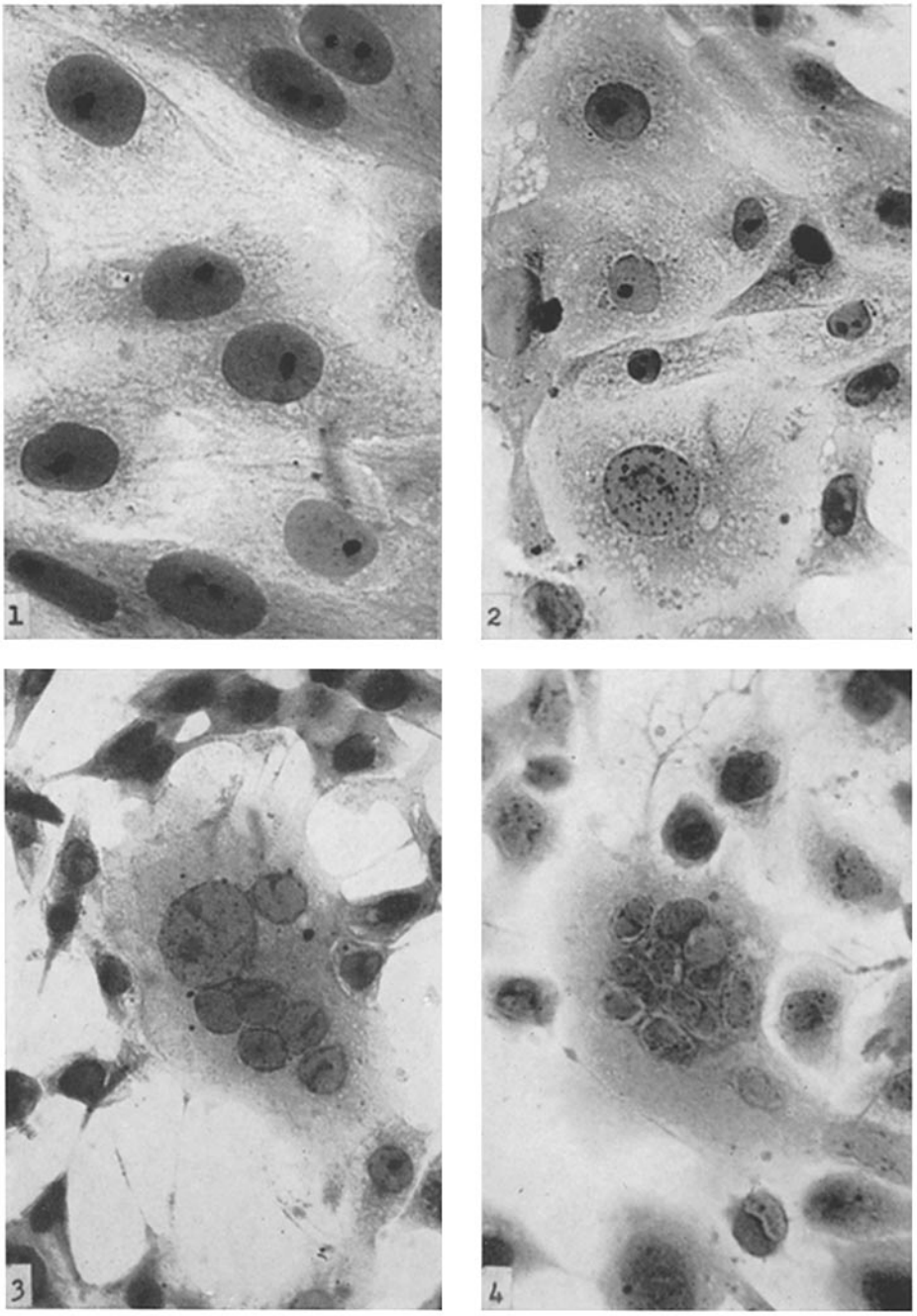
PLATE 16

FIG. 1. Normal tissue culture cells from monkey kidney epithelium fixed in buffered osmium tetroxide and stained with hematoxylin and eosin. ×970.

FIG. 2. Similar cells 6 hours after inoculation with herpes B virus. At upper left a cell with a swollen nucleolus. In the cell at lower left the nucleolus has disappeared and dense granules are to be seen in the nucleus. Increased vacuolization of the cytoplasm is evident. Fixed in buffered osmium tetroxide and stained with hematoxylin and eosin. ×670.

FIG. 3. Cells from a similar culture, 36 hours after inoculation with herpes B virus, fixed in buffered osmium tetroxide and stained in hematoxylin and eosin. The mild action of the buffered osmium has failed to cause retraction of the central zone of the nucleus and intranuclear inclusions are not apparent. ×490.

FIG. 4. A multinucleated cell similar to that shown in Fig. 3 but fixed in Zenker's fluid and stained with hematoxylin and eosin. The central zone of the nucleus has retracted and typical intranuclear inclusions are present. ×490.



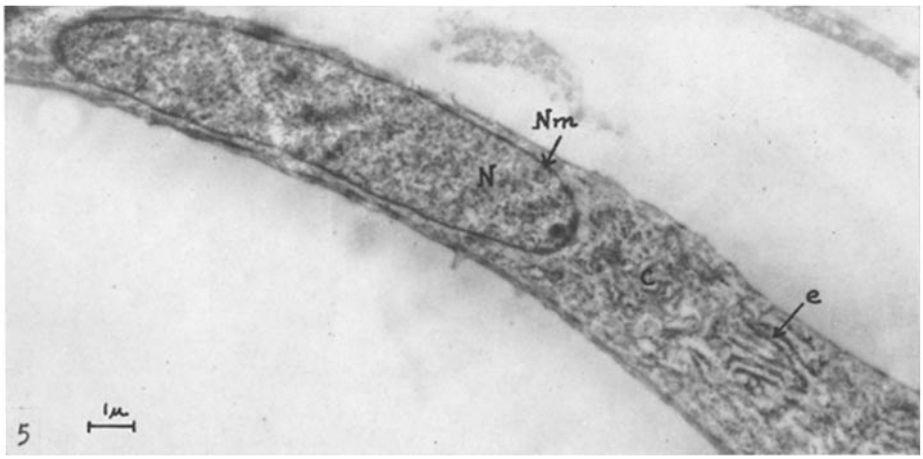
(Reissig and Melnick: Herpes B virus and cellular changes)

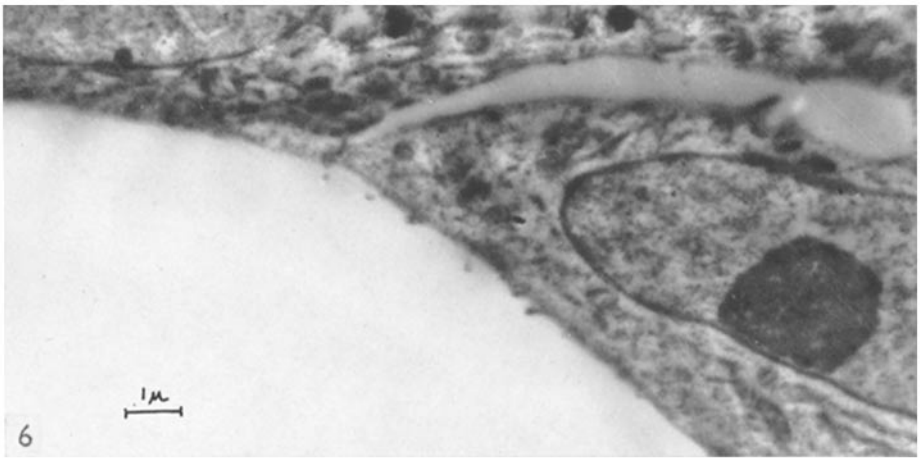
PLATE 17

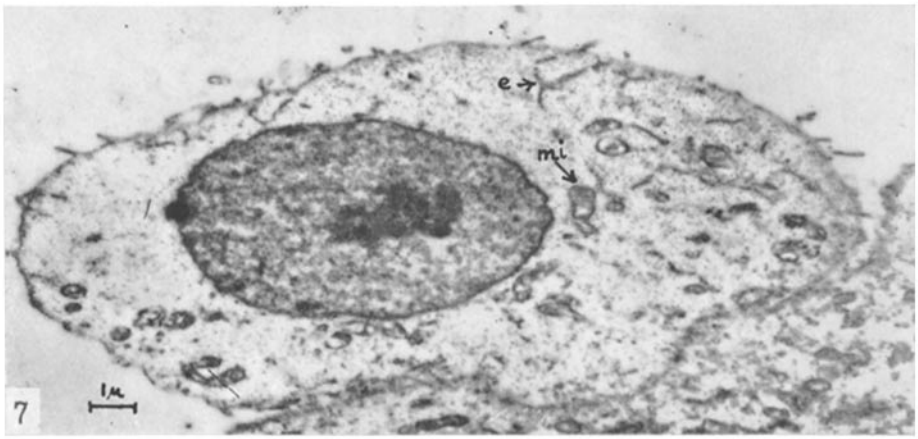
FIG. 5. Electron micrograph of an ultrathin section of a tissue culture cell from normal monkey kidney epithelium, fixed in buffered osmium tetroxide at pH 7.4. The cell surface is smooth and regular. The nucleus has a fine regular structure. At lower right elongated double lamellar elements of the endoplasmic reticulum can be seen in the cytoplasm. ×6100.

FIG. 6. Portion of two sectioned cells from a normal culture of monkey kidney epithelium. The nucleolus can be seen in the cell to the right. ×7500.

FIG. 7. Electron micrograph from a similar cell 2 hours after inoculation with herpes B virus. The cell is swollen and a few finger-like prolongations appear on its external surface. ×6100.



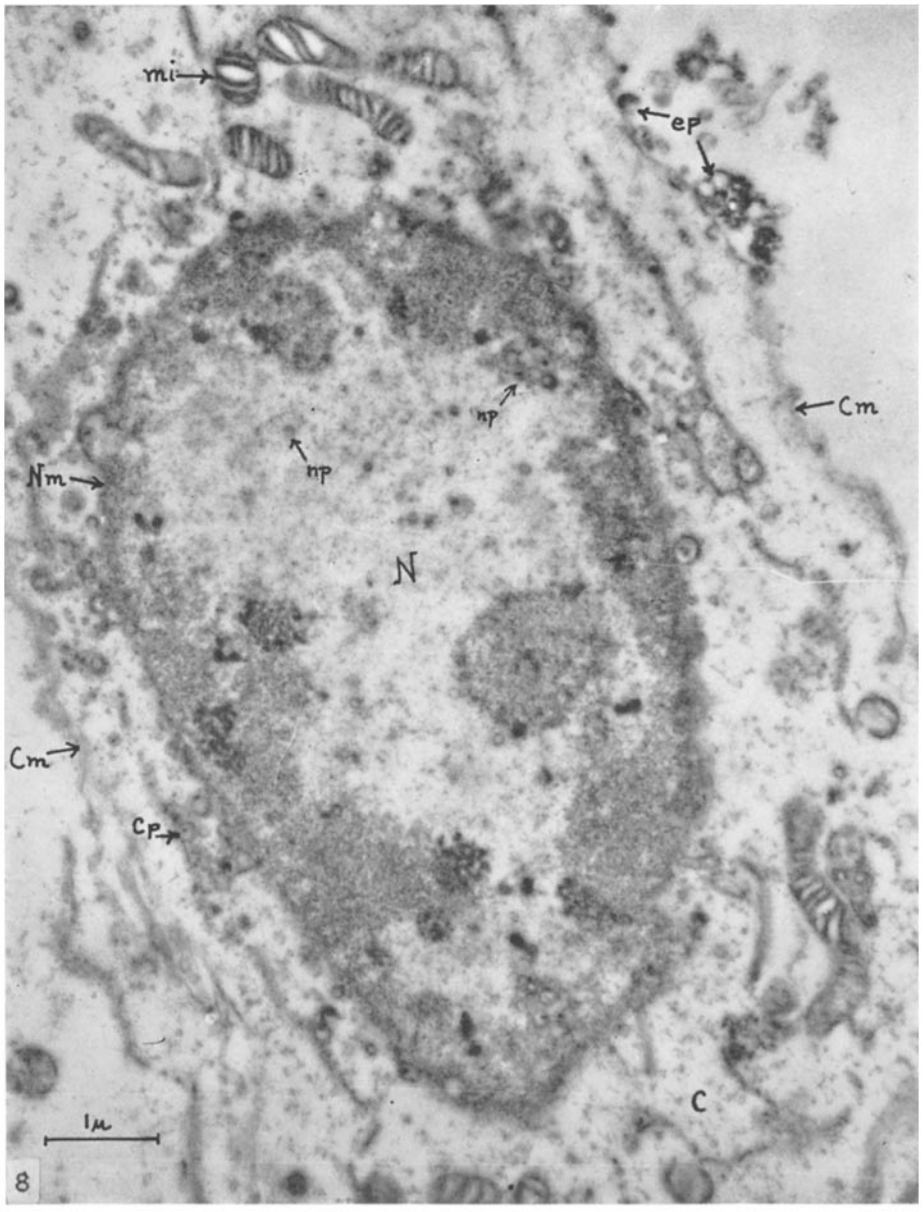




(Reissig and Melnick: Herpes B virus and cellular changes)

PLATE 18

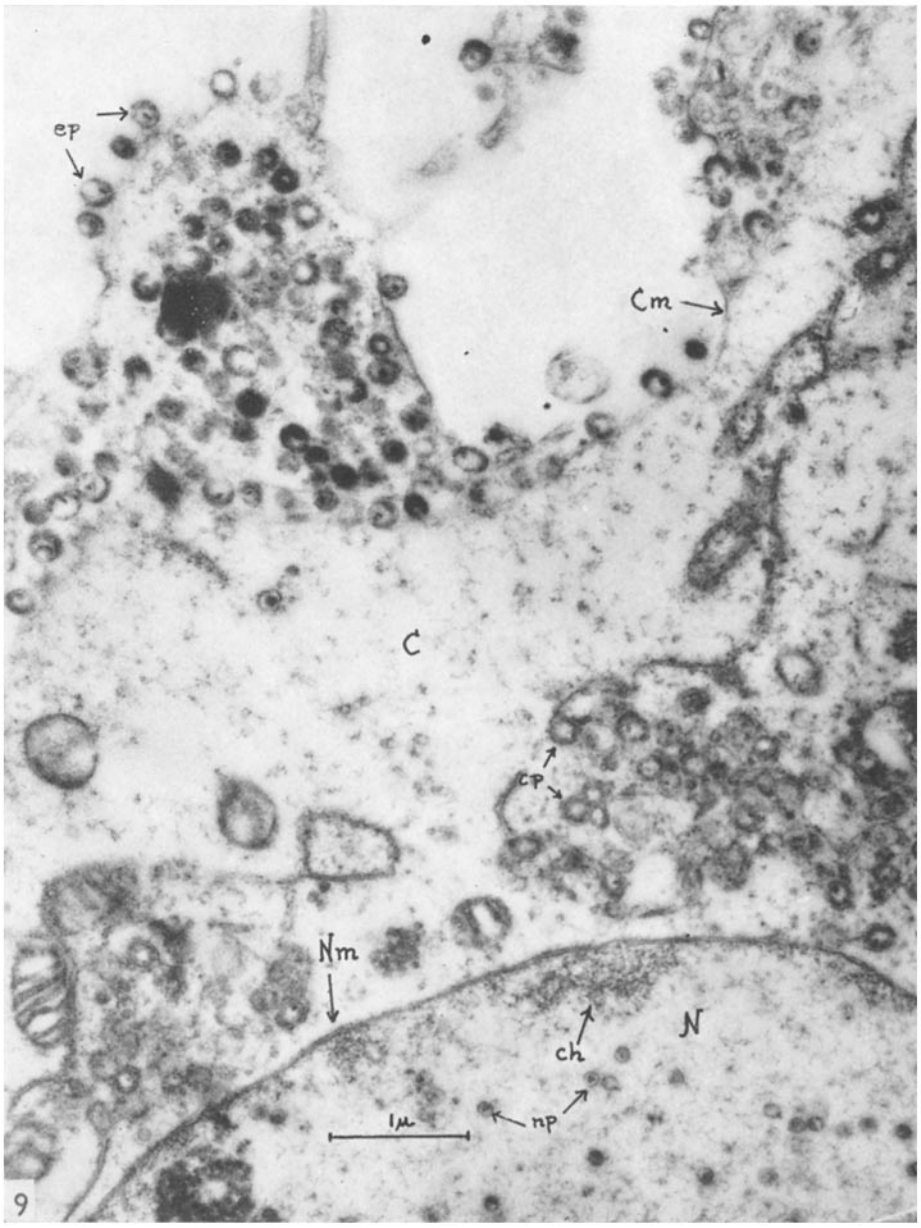
FIG. 8. Part of a cell 14 hours after inoculation. Note the margination of the chromatin and the presence of particles characteristic of the infected cell. ×13,800.



(Reissig and Melnick: Herpes B virus and cellular changes)

PLATE 19

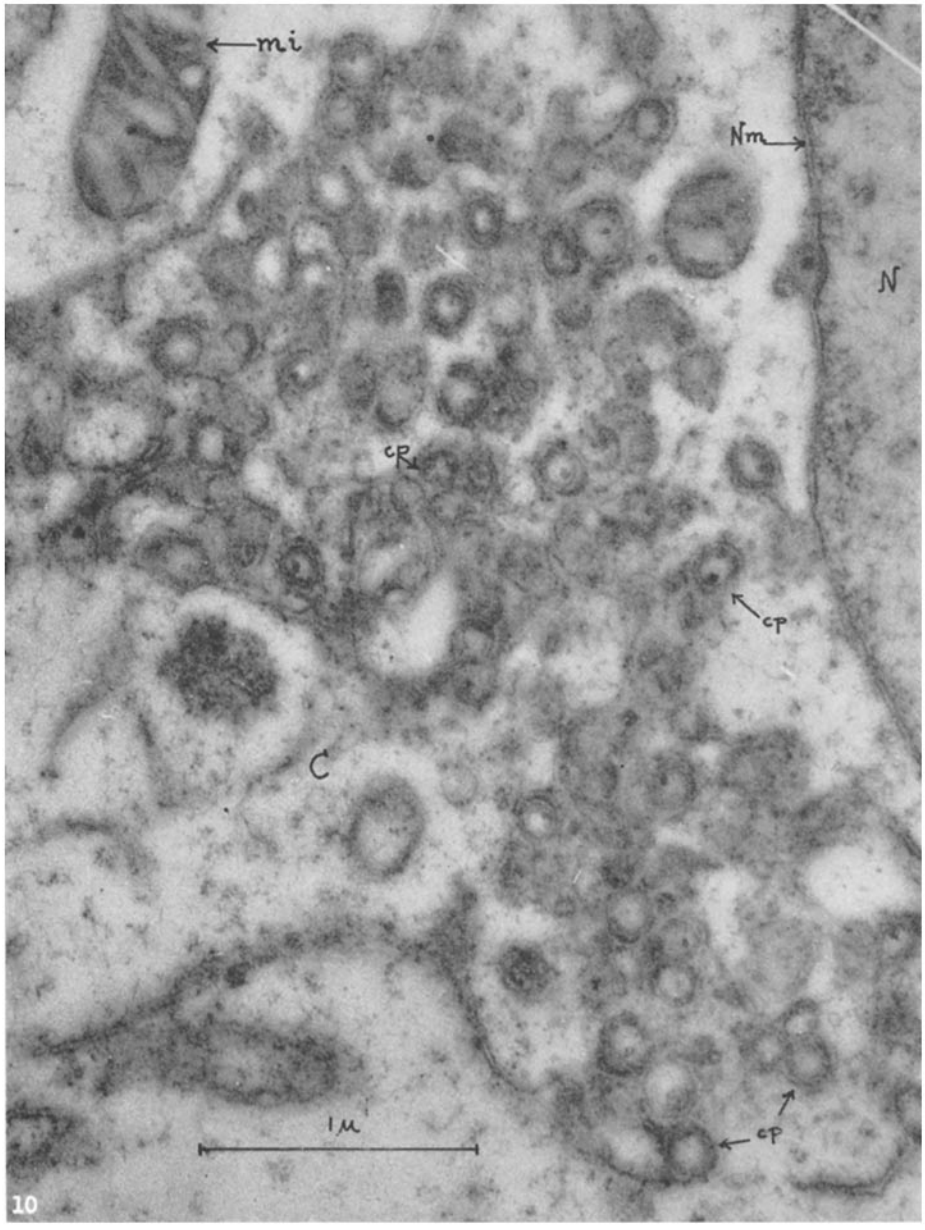
FIG. 9. Another cell harvested 14 hours after virus inoculation. Particles can be seen in the nucleus, the cytoplasm, and on the external surface of the cell. ×18,100.



(Reissig and Melnick: Herpes B virus and cellular changes)

PLATE 20

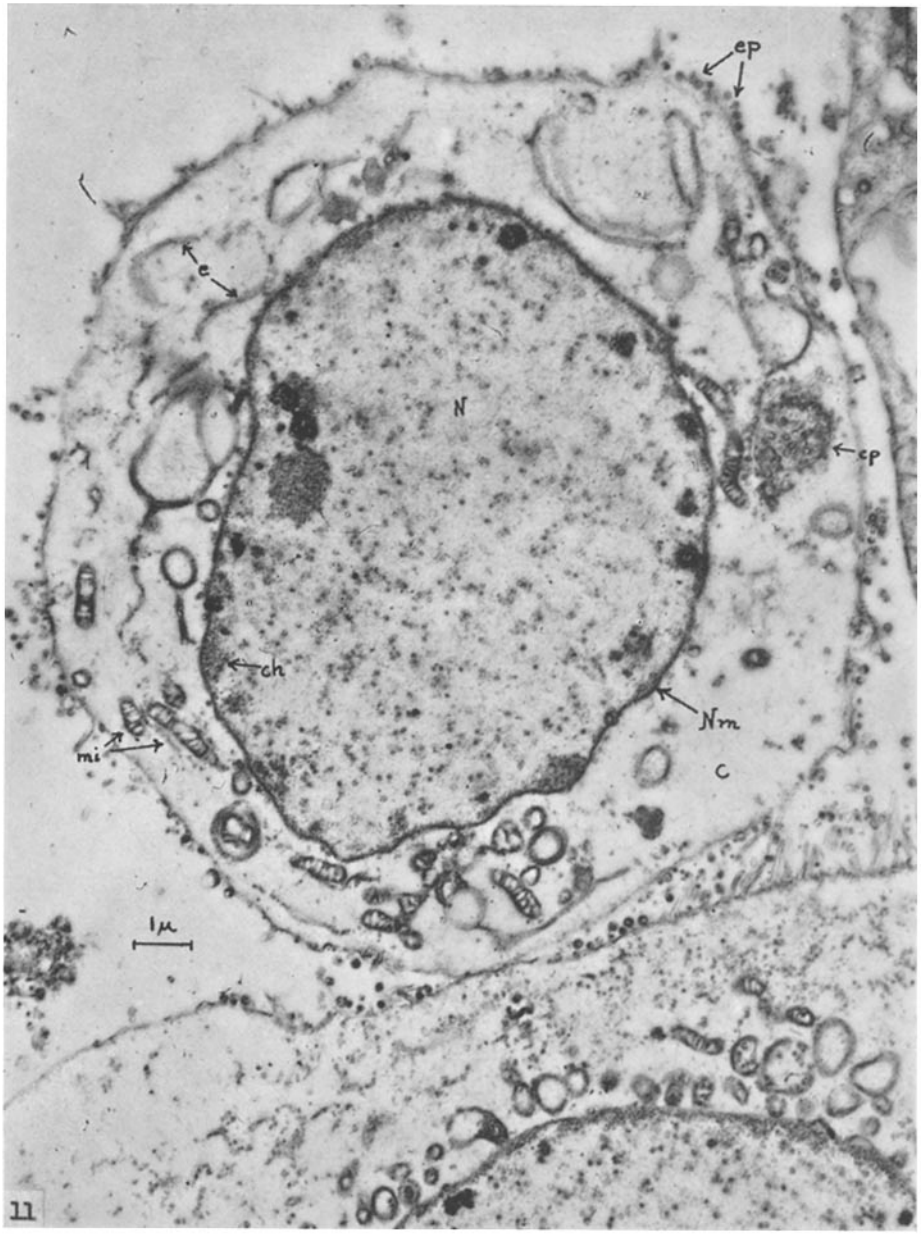
FIG. 10. Enlargement of an area of the cell shown in Fig. 9. There is great variation in the size of the cytoplasmic particles, some of which appear to be enclosed within double membranes. ×36,000.



(Reissig and Melnick: Herpes B virus and cellular changes)

PLATE 21

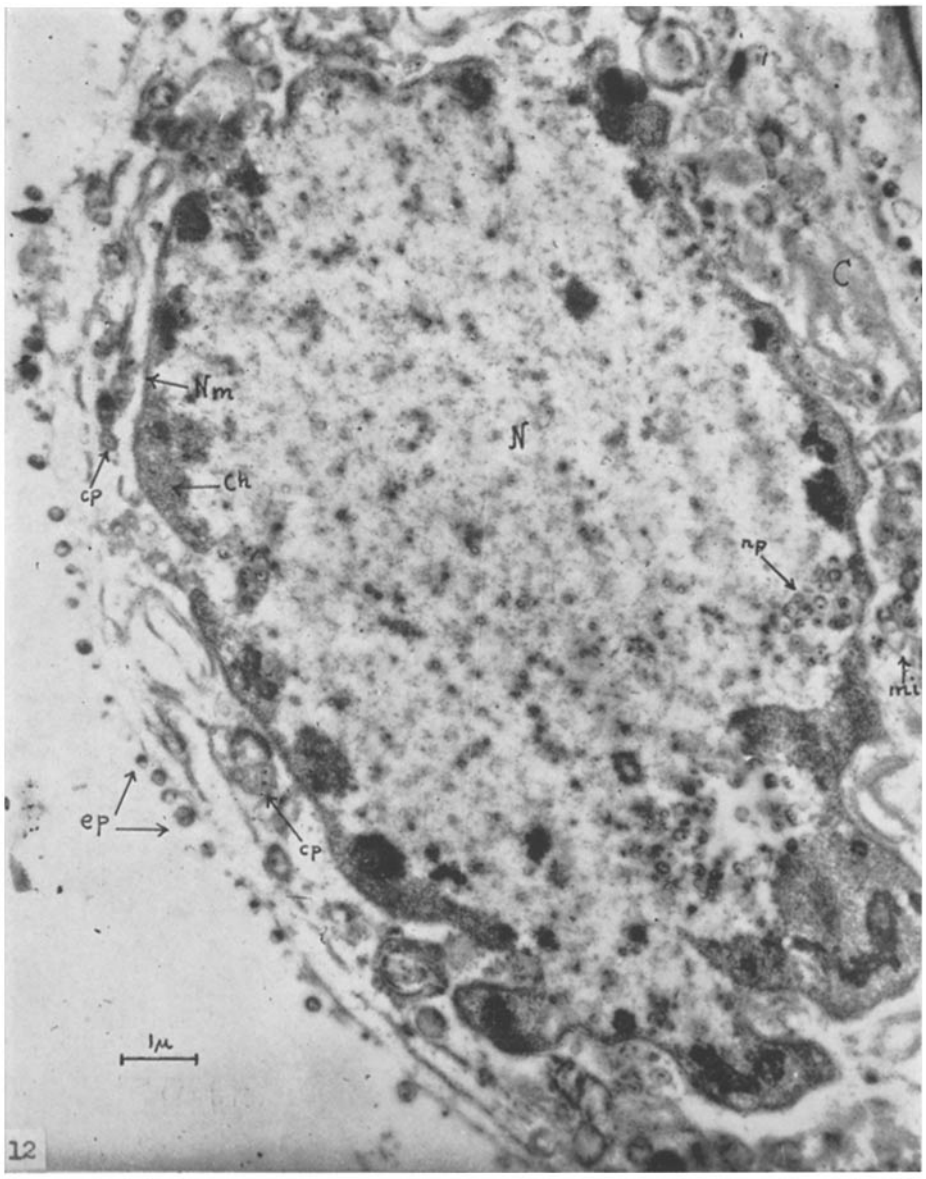
FIG. 11. Cell 24 hours after virus inoculation. Most of the nuclear chromatin has disappeared and scattered particles of the small type (60 to 130 mμ) are numerous in the central zone of the nucleus. A cluster of particles and double lamellar elements are seen in the cytoplasm. Extracellular particles are numerous. At lower right numerous microvilli from a contiguous cell are seen. ×7200.



(Reissig and Melnick: Herpes B virus and cellular changes)

PLATE 22

FIG. 12. Cell 36 hours after virus inoculation. The swollen nucleus exhibits numerous irregular projections of its surface. Clusters of intranuclear particles of the large type (130 to 180 mμ) are present in the periphery of the nucleus. Particles of this order of magnitude are also seen in the cytoplasm and on the external surface of the cell. ×9600.



(Reissig and Melnick: Herpes B virus and cellular changes)

PLATE 23

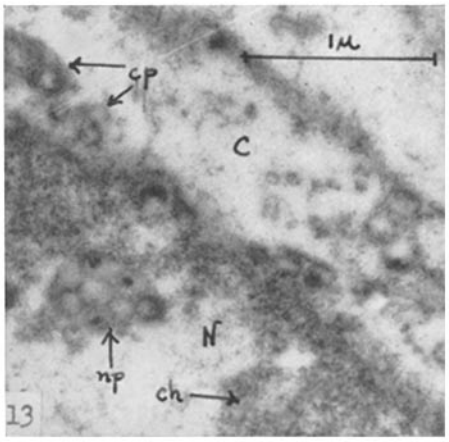
FIG. 13. Enlargement from the upper peripheral zone of the nucleus of the cell shown in Fig. 8. A cluster of intranuclear particles is seen in the region of the chromatin masses. ×25,500.

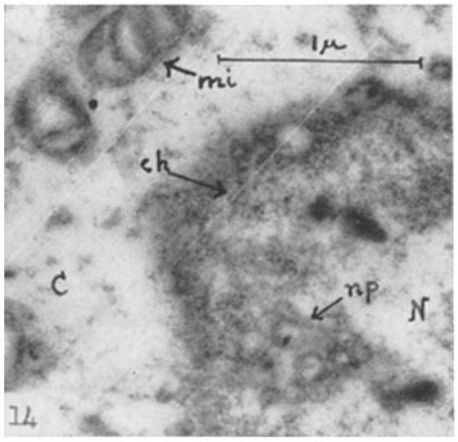
FIG. 14. Enlargement of a zone from the lower right portion of the nucleus of the same cell, showing another cluster of intranuclear particles. Since the particles are closely packed, their differences in size can be considered real and not artifacts due to subtangential sectioning. ×26,000.

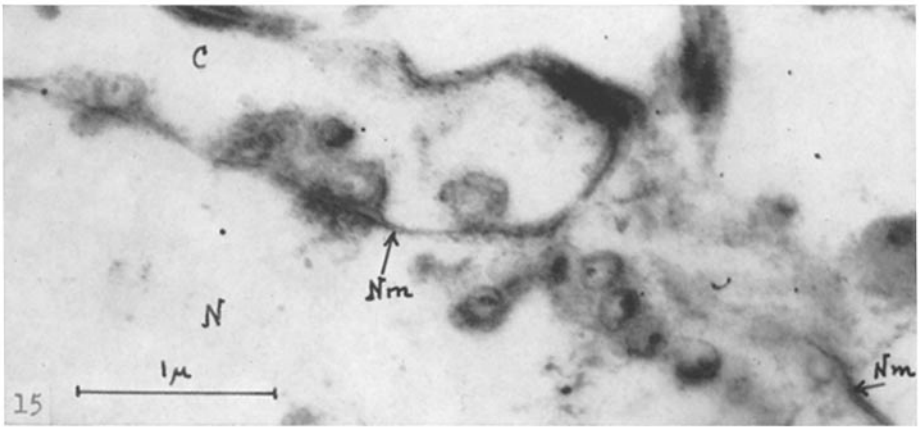
FIG. 15. Enlargement of the peripheral zone of the nucleus of a cell harvested 36 hours after virus inoculation. Particles can be seen in the nucleus and also between the two layers of the nuclear membrane. The particles between the nuclear membranes appear to have two surrounding coats. ×26,700.

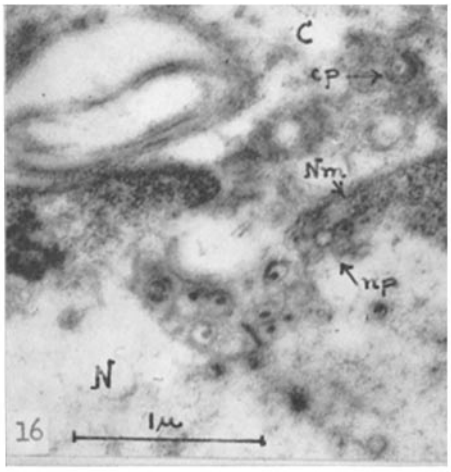
FIG. 16. Cluster of particles in the left portion of the nucleus of the cell shown in Fig. 12. Particles of the larger variety (150 to 180 mμ), some with two coats, are seen between the two layers of the nuclear membrane. A group of much smaller particles is seen at the right of them, in the nuclear chromatin. ×23,600.

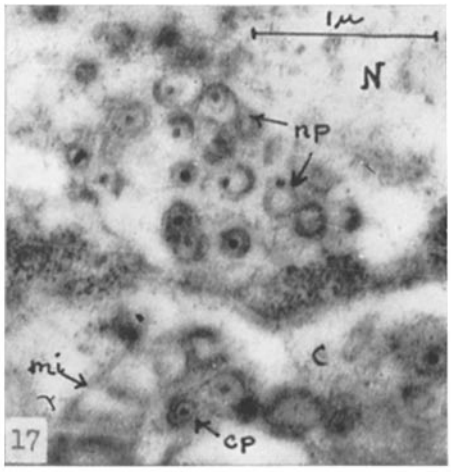
FIG. 17. Another cluster of intranuclear particles from the lower portion of the nucleus of the same cell. Particles with two surrounding membranes are present. ×23,900.







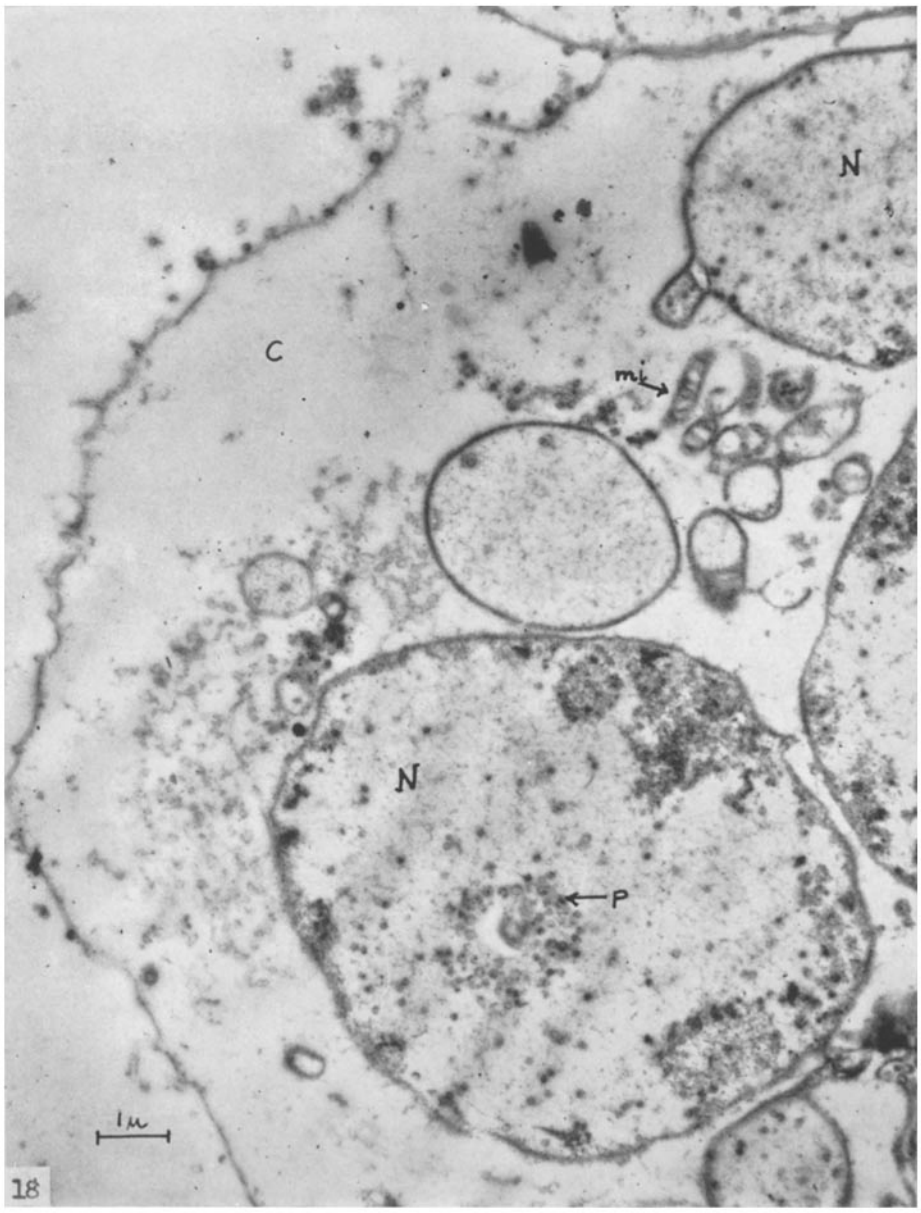




(Reissig and Melnick: Herpes B virus and cellular changes)

PLATE 24

FIG. 18. Polynucleated cell from a culture 36 hours after inoculation with herpes B virus. Particles are present in the nucleus, which has lost most of its chromatin. A few mitochondria are seen in the clear, empty cytoplasm. ×8900.



(Reissig and Melnick: Herpes B virus and cellular changes)

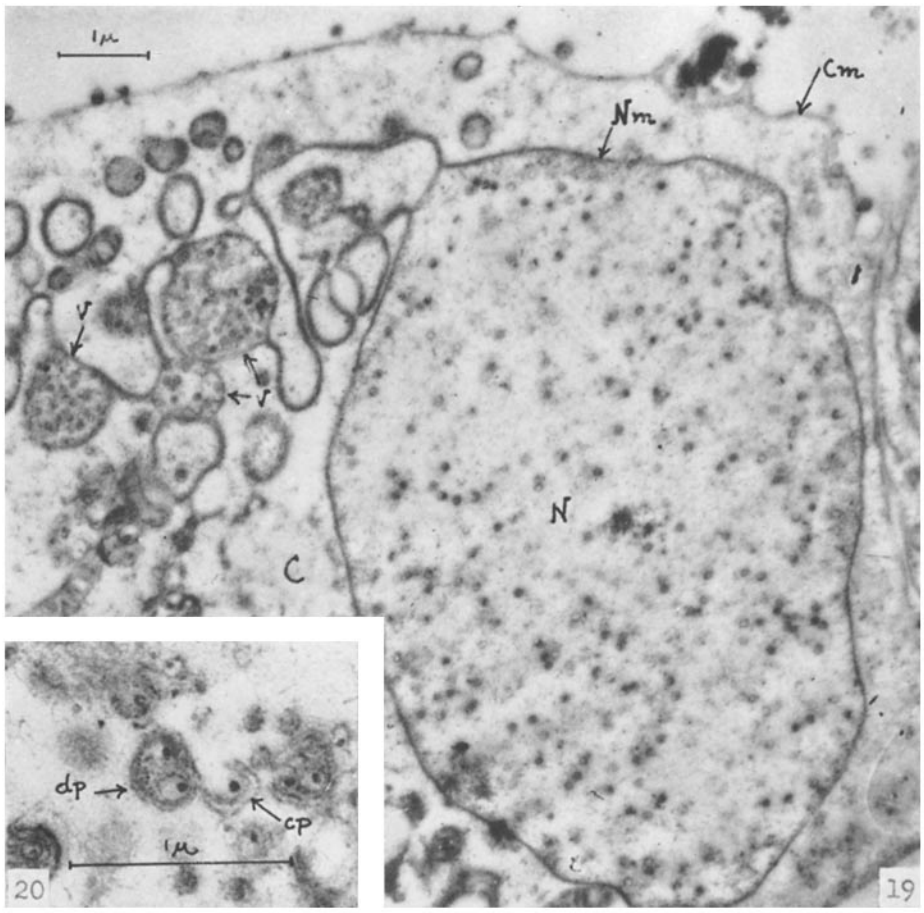
PLATE 25

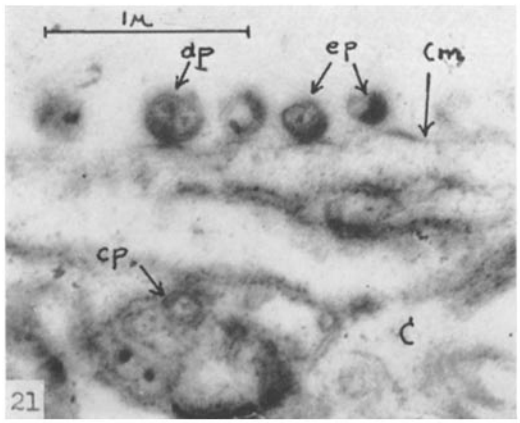
FIG. 19. Portion of a cell from a culture 36 hours after inoculation. The nucleus has lost all of its chromatin and contains numerous isolated particles of the smaller kind (about 100 mμ). Several vesicles full of small particles (such as those in the nucleus) are present. They are connected to each other as well as to the nuclear membrane. ×11,900.

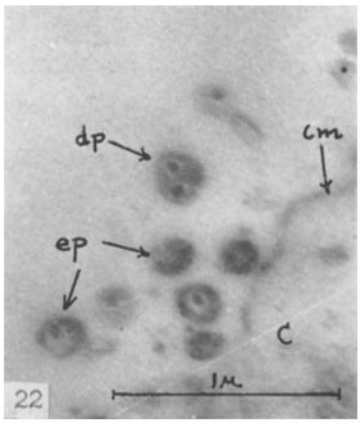
FIG. 20. Portion of the cytoplasm of a cell 36 hours after inoculation. A "binucleated" particle is seen amidst other particles of the usual kind. ×29,000.

FIG. 21. Zone of the external surface of a cell from a culture 36 hours after inoculation, showing particles attached to it. One of these extracellular particles has two dense centers and two inner membranes surrounded by a single external membrane. ×26,500.

FIG. 22. Portion of the external surface of another cell 36 hours after inoculation. An extracellular particle with two dense central bodies is seen in the vicinity of particles with single nucleus-like central bodies. ×29,300.







(Reissig and Melnick: Herpes B virus and cellular changes)