

# Abortive and Productive Infections of Human Mononuclear Phagocytes by Type I Herpes Simplex Virus

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The ability of Type I herpes simplex (HSV) to replicate in normal human mononuclear phagocytes was investigated. Mononuclear leukocytes were obtained from the peripheral blood of patients by Ficoll-Hypaque gradient centrifugation, and the monocytes were isolated by allowing the cells to adhere to tissue culture dishes. The monocytes ( $10^{6.0}$  cells) were infected ( $10^{7.0}$  PFU HSV) either immediately after isolation or were cultured *in vitro* for varying numbers of days and were then infected. Inoculation of freshly isolated monocytes resulted primarily in an abortive infection. HSV antigens were produced by the cells, as determined by an indirect fluorescent antibody technique, and empty herpes capsid structures were detected by electron microscopy of the inoculated monocytes; however, no increase in virus titer was noted in the cultures. Inoculation of viable cells that had been maintained for 7 days in culture resulted in a productive infection. An increase in titer was noted 24 hours after inoculation, and normal virus maturation was documented by ultrastructural study of the infected cells. The experiments show that the interaction of HSV with human mononuclear phagocytes is complex, and the data suggest that whether or not the cell replicates infectious virus may depend on the functional activity of the cell. (*Am J Pathol* 91:119-136, 1978)

MONONUCLEAR PHAGOCYtic CELLS are generally regarded as being of fundamental importance in defending the host against viral invasion.<sup>1,2</sup> These cells play a central role in both the afferent and efferent limbs of the immune response, and any abnormality in their inability to function would profoundly affect resistance to infection. In experimental animals the susceptibility of the hosts to lethal herpes simplex,<sup>3-5</sup> hepatitis,<sup>6</sup> rabies,<sup>7</sup> and arbovirus<sup>8</sup> infections has been correlated with the ability of the virus to replicate in the mononuclear phagocytes.

Little is known concerning the effects viruses have on human mononuclear phagocytes. In a previous study we showed that herpes simplex virus (HSV) could depress the ability of freshly isolated human monocytes from responding to a chemotactic lymphokine.<sup>9</sup> In the experiments reported here, we sought to study in greater detail the interaction of Type I HSV with normal human monocytes by determining the morphologic

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effects of the infection and whether this cell has the capacity to replicate infectious virus.

## Materials and Methods

### Virus and Serums

HSV, strain CHR-HSV-3,<sup>10</sup> was isolated from a patient with recurrent herpes labialis and identified as Type I HSV by virus neutralization tests using immune rabbit serum<sup>11</sup> and by plaque morphology on chick embryo fibroblasts.<sup>12</sup> The virus was grown in primary rabbit kidney (PRK) cells and assayed as plaque-forming units (PFU) using an antibody overlay.<sup>11</sup>

Human serums were obtained from normal subjects with an AB blood type. Individuals were classified as immune or nonimmune on the basis of the presence of anti-HSV antibodies in their heat-inactivated serums.<sup>13</sup> Nonimmune donors had no detectable neutralizing activity in their serum when it was tested at 1:10 dilution; however, immune subjects had titers of greater than 1:100.

Rabbit serums were obtained from adult New Zealand white rabbits<sup>10</sup> either prior to immunization (normal rabbit serums) or 4 weeks after injection with PRK-grown HSV (immune serums). HSV neutralization titers of normal rabbit serum were less than 1:10, and those of immune serum were 1:10,000.

### Isolation of Human Monocytes

Fifty milliliters of venous blood was collected in heparinized (10 units/ml) (Upjohn Co., Kalamazoo, Mich.) syringes from HSV immune and nonimmune donors. The mononuclear leukocytes (MNL) were isolated from the whole blood by centrifugation on Ficoll-Hypaque gradients.<sup>14</sup> The cells were washed twice in medium (Eagle's minimal essential medium supplemented with 50 µg/ml streptomycin and 100 units/ml penicillin G) (MEM).<sup>\*</sup> The MNL were adjusted to a concentration of  $1 \times 10^7$  cells/ml in MEM containing 10% (V/V) nonimmune AB human serum (MEMHS); 1.0-ml portions of this suspension were placed into separate 60-mm plastic tissue culture dishes. After allowing the cells to incubate for 2 hours at 37 C, the nonadherent cells were removed, the dishes were washed twice, and the attached cells were used in the experiments. The adherent cell population consisted of greater than 95% monocytes, as judged by their ability to phagocytose latex particles,<sup>15</sup> the presence of nonspecific esterase in their cytoplasm,<sup>16</sup> and electron microscopic criteria.<sup>17</sup> After 7 days in culture, the adherent cells had retained their identity as mononuclear phagocytes, as evidenced by their phagocytic activity, resistance to trypsinization,<sup>18</sup> and the presence of nonspecific esterase in greater than 99% of the cells.

### Infection of Monocytes

On the day of isolation, as well as after various periods in culture, each dish of monocytes ( $1 \times 10^6$  to  $3 \times 10^6$  cells) was incubated for 2 hours at 37 C with 1 ml of medium containing  $10^{7.0}$  PFU of HSV. After washing twice, the monocytes were treated for 60 minutes with human anti-HSV serum (1:4 dilution) to neutralize the unpenetrated virus and to synchronize the infection. The cells were washed twice again and then cultured in MEMHS at 37 C in a CO<sub>2</sub> incubator. At the times indicated, samples were removed and portions were either a) frozen at -70 C for subsequent virus assay, b) fixed for electron

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\* Unless otherwise stated, all washings procedures and dilutions of cells, virus, and antisera were done in MEM.

microscopy, c) processed for fluorescent microscopic examination, or d) overlaid with  $10^{6.0}$  PRK cells in 2% (v/v) human anti-HSV for an infectious center assay.

On completion of each study, the frozen monocyte cultures were thawed, scraped from the bottom of the dish, and sonicated at 100 watts for 1 minute with a Sonifier Cell Disruptor (Model W135, Heat Systems Ultrasonics, Inc., Plainfield, N.Y.). After centrifugation at 600g for 10 minutes, the supernatants were appropriately diluted and assayed for virus content. Results are expressed as  $\log_{10}$  PFU/ml and any increase in titer greater than 0.3  $\log_{10}$  was considered significant. During the course of the experiments, monocytes and immune serums were obtained from several human volunteers. The variations in the initial amount of virus associated with the monocytes were due to fluctuations in the number of donor cells attached to the dish and in the human anti-HSV immune serums used to treat the infected monocytes.

#### Fluorescent Antibody and Electron Microscopic Studies

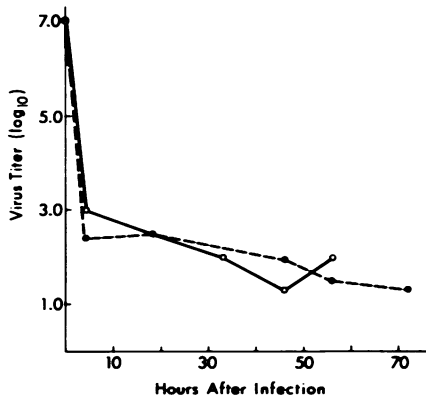
Infected and uninfected monocytes were examined for HSV antigens by an indirect fluorescent antibody (FA) method. Rabbit serums, obtained as previously described, were absorbed with uninfected human MNL and diluted 1:10 in phosphate-buffered (pH 7.3) sodium chloride solution (PBS) containing 4% (w/v) bovine serum albumin (BSA). Goat antirabbit IgG (heavy-chain specific) conjugated with fluorescein isothiocyanate (FITC) (Cappel Laboratories, Downingtown, Pa.) was absorbed with human  $\gamma$ -globulin conjugated to Sepharose 4B and diluted 1:2 in PBS-BSA. Twenty-four hours after being exposed to either HSV or medium (control), the monocytes were scraped from the dishes and fixed to glass slides by spinning at 260g for 10 minutes on a cytocentrifuge (Shandon Southern Instruments, Sewickley, Pa.). The air-dried slides were washed in PBS, fixed in acetone for 5 minutes at 25 C, and washed again in PBS. Each slide was then treated for 30 minutes with either normal or immune rabbit serum. Following washing in PBS, each slide was reacted with the FITC-conjugated anti-IgG for 30 minutes and washed again. The slides were examined with a Zeiss photomicroscope outfitted for transmission fluorescence microscopy using a primary KP490 (Zeiss) filter and No. 50 barrier filter. Cells were considered to contain HSV antigens if they manifested bright, apple-green nuclear and cytoplasmic fluorescence with the immune reagents.

Monocyte cultures were also examined for evidence of HSV replication by ultrastructural study. Infected and uninfected cells were fixed in 0.05 M sodium-cacodylate-buffered (pH 7.2) 4% (w/v) glutaraldehyde for 1 hour at 4 C and postfixed in 0.1 M *sym*-collidine-buffered (pH 7.3) 1% (w/v)  $\text{OsO}_4$ . Following *en bloc* staining with 0.12 M Veronal acetate-buffered (pH 4.7) 0.5% (w/v) uranyl acetate, the cells were dehydrated in increasing concentrations of ethanol. Cell suspensions were centrifuged, and the pellets were embedded in Epon 812. Thin and thick sections were cut on MT-1 Porter-Blum ultramicrotomes and stained with 7.5% uranyl magnesium acetate and 0.4% lead citrate. Representative fields were examined with a Hitachi HU-11E electron microscope at 75 kV.

## Results

#### Infection of Freshly Isolated Monocytes

To determine if HSV would replicate in monocytes, replicate cultures ( $10^{6.0}$  cells/dish) from an immune (C.D.) and a nonimmune (J.W.) subject were inoculated with  $10^{7.0}$  PFU of HSV immediately after isolation, and samples were assayed at sequential times for infectivity (Text-figure 1). After adding virus, cells were washed and treated with human anti-HSV



TEXT-FIGURE 1—Infection of freshly isolated human monocytes with HSV. Cells were infected for 2 hours with  $10^{7.0}$  PFU of virus, washed, treated with antibody, and washed again. At various hours after infection, samples were assayed for infectious virus. *Solid circles*, sample from patient CD; *open circles*, sample from patient JW.

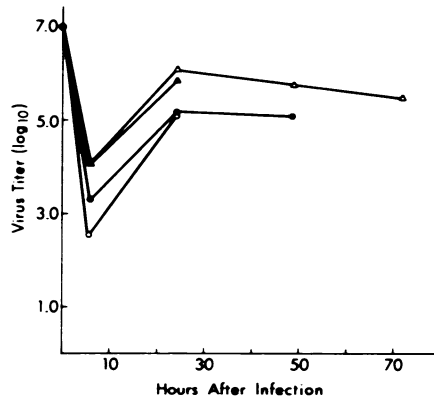
to remove the unpenetrated HSV. As shown, this procedure lowered the titer to approximately  $10^{2.5}$  PFU at 3 hours after infection; thereafter, a gradual decrease in the virus content of the cells took place. Although by the infectivity assay there was no evidence of virus growth in the freshly isolated monocytes, the cells incubated with HSV developed a cytopathologic effect. In contrast to the controls exposed to medium, the monocytes incubated with HSV underwent a vacuolar degeneration, and by 48 hours after inoculation they had detached from the bottom of the tissue culture dish. Thus, we elected to examine the virus-inoculated cells morphologically for evidence of infection.

#### Electron Microscopic and FA Studies of Monocytes Infected at the Time of Isolation

Uninfected monocytes after 2 days in culture had elongated nuclei with peripherally clumped chromatin and well-defined nuclear membranes (Figure 1). The cytoplasm contained many vacuoles and an irregular plasma membrane. In contrast to these uninoculated cells, monocytes exposed to virus on the day of isolation and then incubated for 48 hours had undergone extensive morphologic alterations. The cell nuclei assumed an oval profile, and their chromatin condensed with multiple irregular areas of clearing (Figures 2 and 3). Of special note was the absence of a well-defined nuclear membrane in these infected cells. Within the nuclear and/or cytoplasmic regions of cells were collections of 100-nm circular structures which resembled empty herpes virus capsids. Frequently, these viral structures were associated with stacks of membranous cytoplasmic profiles and were located at the periphery of the cell (Figure 3).

The freshly isolated monocytes inoculated with HSV were examined for the presence of HSV antigens by an indirect FA technique using rabbit

TEXT-FIGURE 2—Infection of human monocytes after 7 days of cultivation. Cells, kept in culture for 1 week, were incubated for 2 hours with  $10^{7.0}$  PFU of HSV, washed, treated with antibody, and then washed again. At various hours after infection samples were assayed for infectious virus. *Open triangles*, sample from patient CD; *solid triangles*, sample from patient JS; *solid circles*, sample from patient JP; *open circles*, sample from patient BB.

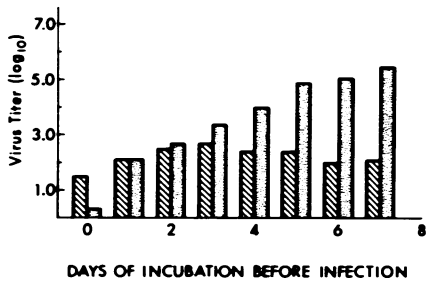


immune serums. Twenty-four hours after infection, the cells incubated with the anti-HSV serums exhibited nuclear and cytoplasmic fluorescence, whereas the uninoculated monocytes did not manifest fluorescence and neither did the infected cells when incubated with nonimmune rabbit serum. Thus, the experiments indicated that when freshly isolated human monocytes are exposed to HSV, the cells synthesize viral antigens but do not make infectious virus. Further experiments determined whether the monocytes would support a productive infection under other conditions.

#### Infection of *In Vitro* Cultivated Monocytes

As has been reported previously,<sup>19</sup> monocytes kept in culture for 7 days undergo marked morphologic and cytochemical alterations and acquire characteristics of macrophages. The cells flatten out on the bottom of the dish, manifest an increase in nuclear size with prominent nucleoli, develop more vacuoles in their cytoplasm, and show a marked increase in cytoplasmic acid phosphatase and nonspecific esterase content. Experiments determined whether these cultured cells would support HSV replication.

Replicate 7-day cultures ( $10^{5.0}$  cells/dish) from two immune (C.D. and B.B.) and two nonimmune (J.S. and J.P.) subjects were inoculated with virus and washed as described earlier. In contrast to the freshly isolated monocytes (Text-figure 1), the cells cultured for 7 days were shown to be capable of HSV replication (Text-figure 2). Six hours after infection, the titer dropped as before due to the washing procedures, but by 24 hours after infection, the cultured cells showed a 1.7 to 2.3  $\log_{10}$  increase in virus titer. HSV grew in immune as well as nonimmune subjects' cells, and the virus recovered in each case was identified as Type I HSV by neutralization tests<sup>11</sup> and plaque morphology on chick embryo fibroblast cultures.<sup>12</sup>



TEXT-FIGURE 3—Growth of the HSV in monocytes after various days of cultivation. Monocytes were isolated from peripheral blood of a non-immune subject (KT) and infected with virus after various days of culture. Samples are taken at 6 hours after infection to determine how much virus was bound to the cell before viral synthesis could take place. Additional cultures were assayed 48 hours after infection to determine how much virus had been synthesized. *Lined columns*, titer 6 hours after infection; *dotted columns*, titer 48 hours after infection.

#### Electron Microscopy of 7-Day Cultivated Cells Infected With HSV

Forty-eight hours after infection, many herpes virus particles could be identified within the nucleus (Figure 4). Few empty capsid structures were seen; the majority of the particles had dense central cores, and some had aligned themselves close to the nuclear membrane. Reduplication of the nuclear membrane was evident and enveloped capsid structures with dense cores could be seen in perinuclear cisternae (Figure 5).

#### Effects of *In Vitro* Cultivation of Monocytes on Their Ability to Replicate Infectious Virus

A final experiment determined how long the monocytes had to be kept in culture before they acquired the ability to synthesize infectious HSV. Duplicate monocyte cultures were infected after being kept in culture for various days. Samples were harvested 6 hours after infection to serve as a baseline on which to assess growth. Cultures were also taken at 48 hours after inoculation since this represented a time by which maximal infectivity was always obtained. An increase of 0.3 log<sub>10</sub> in virus titer of the 48-hour sample over that of the 6-hour sample was considered to represent growth of HSV. As shown, virus did not grow in the monocyte when the cells were infected on the day of isolation (Text-figure 3). The monocyte cultured for 1 or 2 days and then infected did not show any significant increase in virus titer. After 3 days of culture the monocytes gradually acquired the ability to synthesize infectious virus, which was maximal by 5 days after *in vitro* cultivation and changed little thereafter. Although the results shown were obtained by using just 1 nonimmune subject's cells, identical data were obtained from 2 patients who were immune.

An infectious center assay was also done to determine when in culture the monocytes were capable of replicating infectious virus. As before, monocytes were infected on the various days after isolation and treated with anti-HSV; however, instead of washing the cells and harvesting for virus assay, a suspension of 10<sup>6.0</sup> PRK cells in 2% anti-HSV was added to

the adherent, infected monocytes. After plating, the PRK cells attached to the bottom of the dish and formed a confluent monolayer surrounding the HSV-inoculated monocytes. As virus replicated in the human monocyte, these adjacent PRK cells became infected and after 48 hours had formed a plaque (ie, infectious center).

As shown in Table 1, monocytes ( $10^{6.3}$  cell/dish) inoculated with virus on the day of isolation formed very few infectious centers ( $3.7 \pm 1.5$ ). Cells from 4 immune and 2 nonimmune subjects were examined, and no relationship was found with regard to the state of immunity and the number of infectious centers produced. If the monocytes were incubated for 1 or 2 days and then exposed to HSV, infectious centers were always present. After 72 hours of culture, the number of monocytes that had acquired the ability to replicate HSV was so numerous that, due to confluency of the plaques, it was not possible to accurately determine the number of infectious centers produced.

### Discussion

Inoculation of cells with HSV does not always result in the formation of infectious progeny.<sup>20</sup> The virus may adsorb to the cell, penetrate it, and synthesize one or more viral components; however, if infectious virus is not produced, the interaction is considered abortive. We have shown that, regardless of the immune status of the donor, when  $1 \times 10^6$  to  $3 \times 10^6$  freshly isolated human monocytes are exposed to HSV, the vast majority of the cells were abortively infected. Viral antigens were synthesized in the cytoplasm and herpes virus capsid structures appeared in the monocytes (Figures 1 and 2), yet no increase in virus titer occurred and very few of the cells (less than 0.01%) formed infectious centers (Table 1).

An HSV infection may be abortive for a variety of reasons: a) The environment in which the infected cell is being cultured can be in-

Table 1—Quantification of the Ability of Cultured Human Monocytes to Replicate HSV, as Determined by an Infectious Center Assay

Days in culture before infection*	No. of determinations	No. of infectious centers per $3 \times 10^6$ monocytes†
0	12	$3.7 \pm 1.5$
1	9	$41 \pm 9$
2	6	$169 \pm 69$
3	6	TMTC‡

\* Monocytes ( $3 \times 10^6$  cells) were incubated with HSV ( $10^{7.3}$  PFU), washed, treated with anti-HSV; then  $10^6$  PRK cells in 2% anti-HSV were added. Infectious centers were assessed at 2 days.

† Average values obtained from 6 donors  $\pm$  SEM

‡ Too many to count, ie, greater than 300 plaques

sufficient for the normal virus maturation to take place. For example, the medium may be deficient in a certain amino acid,<sup>21</sup> or a drug<sup>22</sup> might have been added to the culture fluid, suppressing a metabolic event needed for virus synthesis. b) The virus may be a mutant<sup>23</sup> or a specific serotype<sup>24</sup> which is incapable of replicating in the cell because of the particle's genetic composition. c) The cell may be deficient in its ability to perform all the synthetic events required by the virus for the production of infectious particles.<sup>25</sup>

The reason Type I HSV produced an abortive infection in freshly isolated monocytes is unknown. It is unlikely that the manner in which the cells were cultured was the cause. Eagle's medium with 10% human serum is a rich nutrient solution in which, as we have shown, a variety of human cells are able to replicate HSV. Experiments using other media (RPMI 1640, medium 199, lactalbumin hydrolysate) and different serums (calf and fetal bovine) have also shown the HSV-human-monocyte interaction to be abortive. Equally improbable is that the virus strain used was the cause of the abortive infection. Our strain has been shown to grow readily in freshly isolated human leukemic leukocytes,<sup>26</sup> fetal kidney, lung, and skin cells as well as phytohemagglutinin-stimulated lymphocytes.<sup>26</sup> Experiments using two low-passaged Type I HSV serotypes have also shown to abortively infect freshly isolated human monocytes. Thus, the infection is abortive probably because of host-cell-dependent factors and because the freshly isolated monocyte is incapable of performing all the metabolic events required by the virus. In support of this premise is the fact that these same cells, when cultured *in vitro*, acquire the ability to replicate infectious virus (Figure 3, Table 1).

Morphologic study of the interaction of freshly isolated monocytes with HSV has provided insight as to what events are not taking place in the abortively infected cells. From electron microscopic studies by Schaffer with a variety of temperature-sensitive HSV mutants,<sup>23</sup> it was documented that three general "classes" of abortive infections can be recognized: Class A in which capsids are not formed, Class B in which capsid structures are seen but do not develop dense (DNA-containing) cores, and Class C interactions in which enveloped and dense nucleocapsid structures are present; yet the particles are still not infectious. Using this classification, we would tentatively classify the HSV-human-monocyte interaction as a Class B abortive infection. Capsid structures were formed, yet neither dense nucleocapsids nor enveloped particles were seen within the cells (Figures 2 and 3). This is similar to what has been described for the HSV-dog-kidney abortive infection.<sup>25</sup>

Ultrastructural changes were noted in the HSV-infected, freshly iso-



lated monocyte that had not been described previously with either productively or abortively infected cells. Most remarkable was the disappearance of the nuclear membrane. This structure is stimulated to undergo proliferation in productive HSV infections, and it is from this membrane that the virus receives its outer envelope which is believed by some to be needed for the virus to be infectious.<sup>27</sup> What happens to this structure in the abortively infected monocyte is unclear. It may have undergone dissolution in the cell, as suggested by the presence of residual cleftlike spaces around the nuclei,<sup>23</sup> or it might have migrated to the periphery of the cell. Membranous whorls were noted at the surface of the abortively infected monocyte, and empty capsids were associated with these structures. Perhaps these whorls represent remnants of nuclear membrane that was instructed by the virus to undergo reduplication. Further morphologic studies are needed to determine whether these changes are virus-directed or if they represent responses of the cell to injury.

In those cells in which abortive infection with HSV has been described,<sup>4,25</sup> the cells have not been shown to be able to change in culture such that they acquire the ability to synthesize infectious virus. In this respect normal human monocytes are unique. Cells inoculated after being kept in culture show virologic (Text-figures 2 and 3, Table 1) and morphologic (Figures 4 and 5) evidence of a productive infection. When peripheral blood monocytes are cultured, they undergo alterations such that they resemble mature macrophages. As has been described by us<sup>19</sup> and others,<sup>28</sup> this maturation is accompanied by an increased number of lysosomes in the cultured cells and a rise in the specific activity of acid phosphatase. Thus, by cytologic and biochemical studies, the cell that HSV productively infects is a human macrophage. In some fashion, probably by the induction of constitutive enzymes or structures, the cultured cell acquires the necessary equipment needed for the synthesis of infectious particles.

The significance of the observation that freshly isolated monocytes will not replicate HSV is unclear. Macrophages from the newborn mouse and susceptible strains of adult mice readily replicate the virus. Outbred adult mice and HSV-resistant strains have peritoneal macrophages which replicate the virus in a limited fashion. Thus, a correlation exists between the susceptibility animals have to HSV,<sup>3-5</sup> as well as other viral infections,<sup>6-8</sup> and the ability of the agents to productively infect the host's macrophage. Herpetic infections in humans vary from superficial oral and genital infections to lethal CNS, liver, and pulmonary infections. Some individuals have frequent recurrent local infections, whereas others have none.

Perhaps modulations in the susceptibility of mononuclear phagocytes to HSV may be responsible. From the data presented one might predict that in normal patients the peripheral blood monocytes would suppress dissemination by phagocytosing the particle and thereby becoming abortively infected. In the tissues, the same cells, if activated, might be able to synthesize infectious virus and thus propagate the infection. Further studies on the roles these cells may play in resistance of humans to herpetic infection are warranted.

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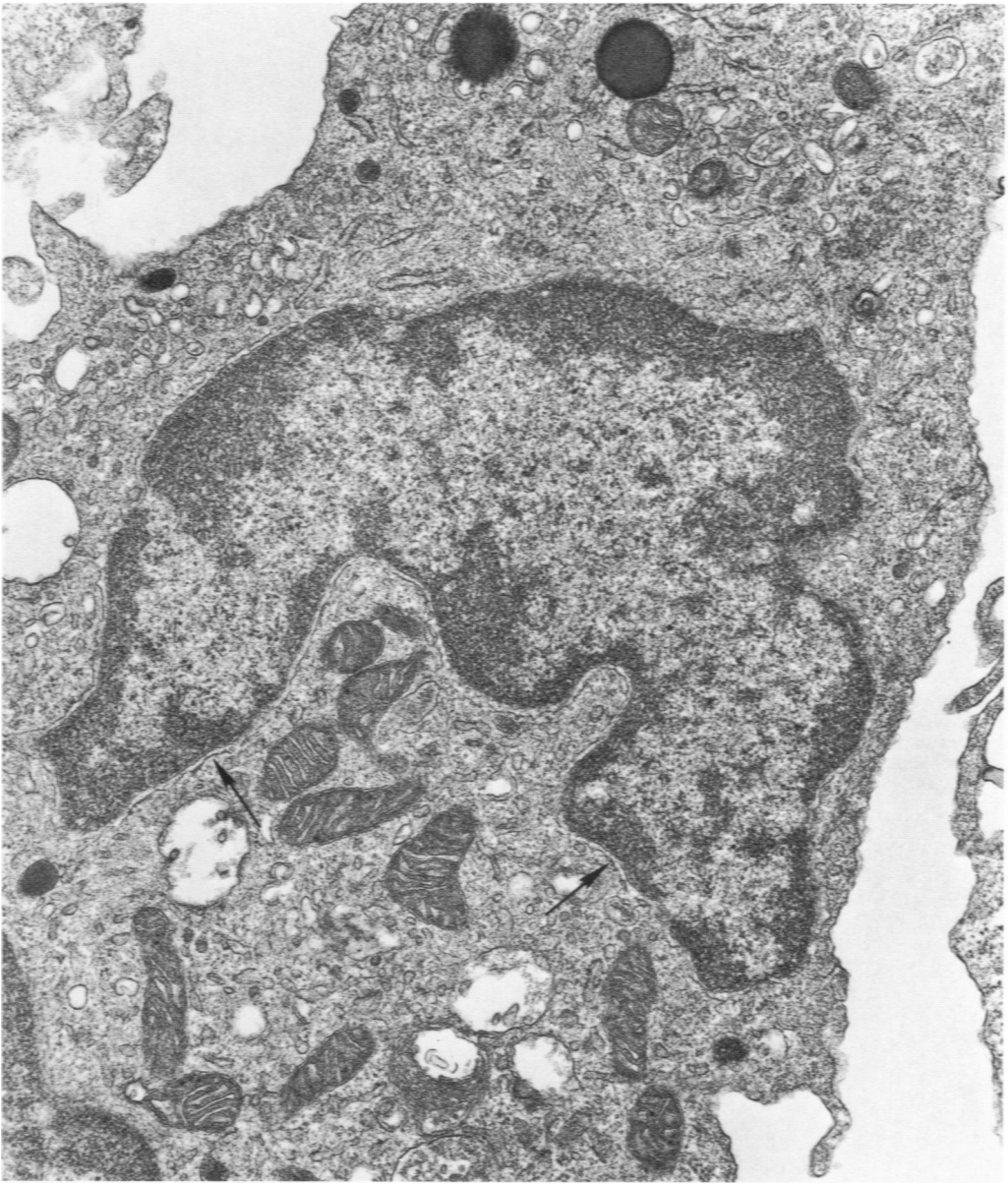
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### **Acknowledgments**

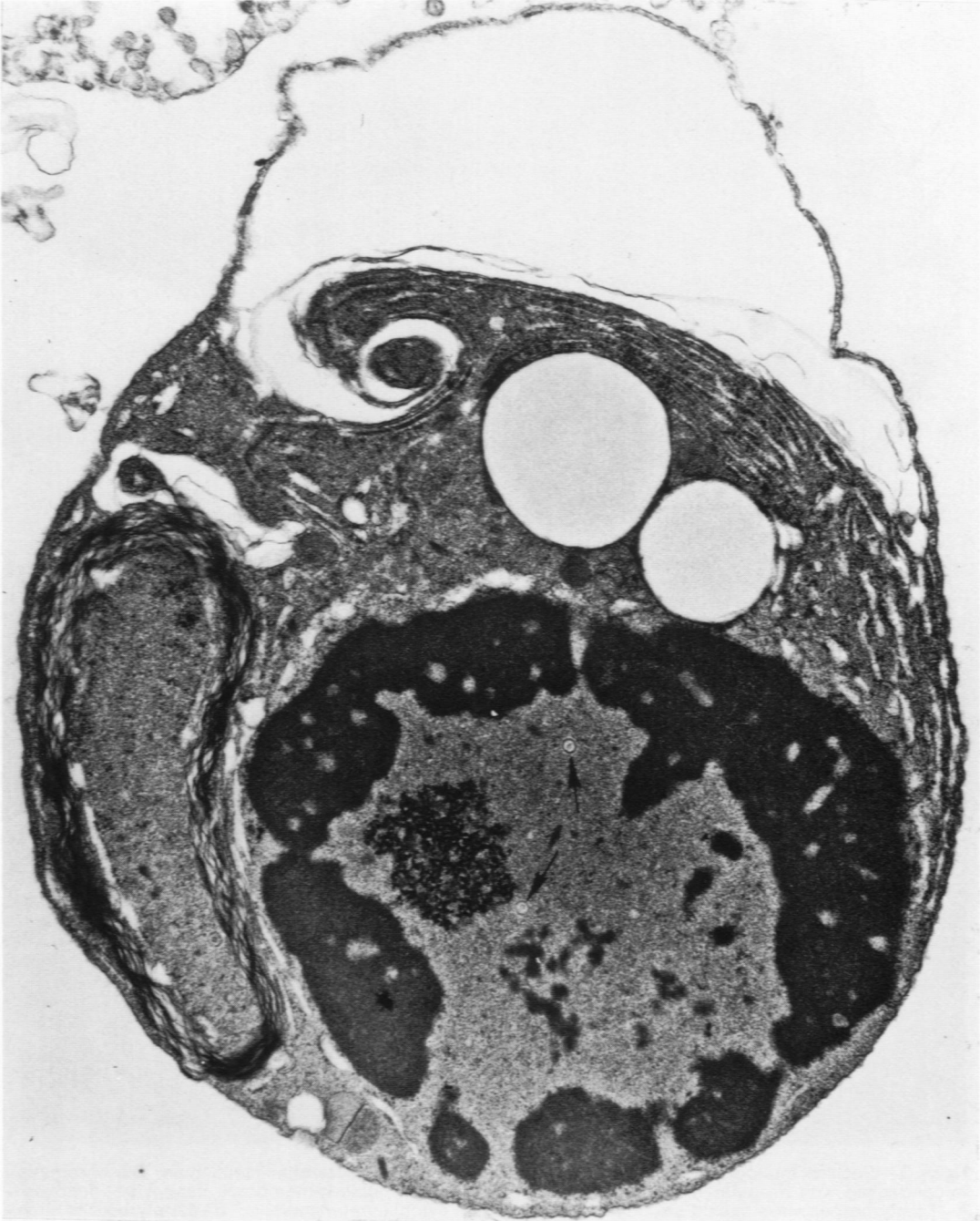
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*[Illustrations follow]*



**Figure 1**—Ultrastructural appearance of an uninfected, freshly isolated monocyte. The irregularly shaped nucleus has peripherally clumped chromatin and a defined nuclear membrane (*arrows*). Membrane-bound vacuoles are present in the cytoplasm as well as normal appearing mitochondrial profiles. (Uranyl magnesium acetate and lead citrate,  $\times 21,800$ )

**Figure 2**—Electron micrograph of a freshly isolated monocyte 2 days after infection with HSV. Marked nuclear alterations have occurred with loss of the nuclear membrane. Empty herpes virus capsid-like structures are present in the nucleus (*arrow*) and multilayered membranous profiles are found at the periphery of the cell. (Uranyl magnesium acetate and lead citrate, × 20,200)



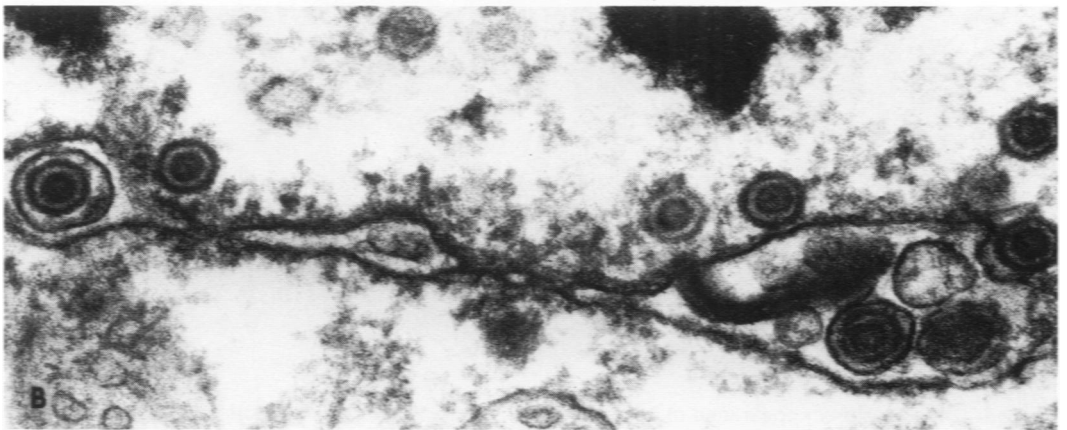
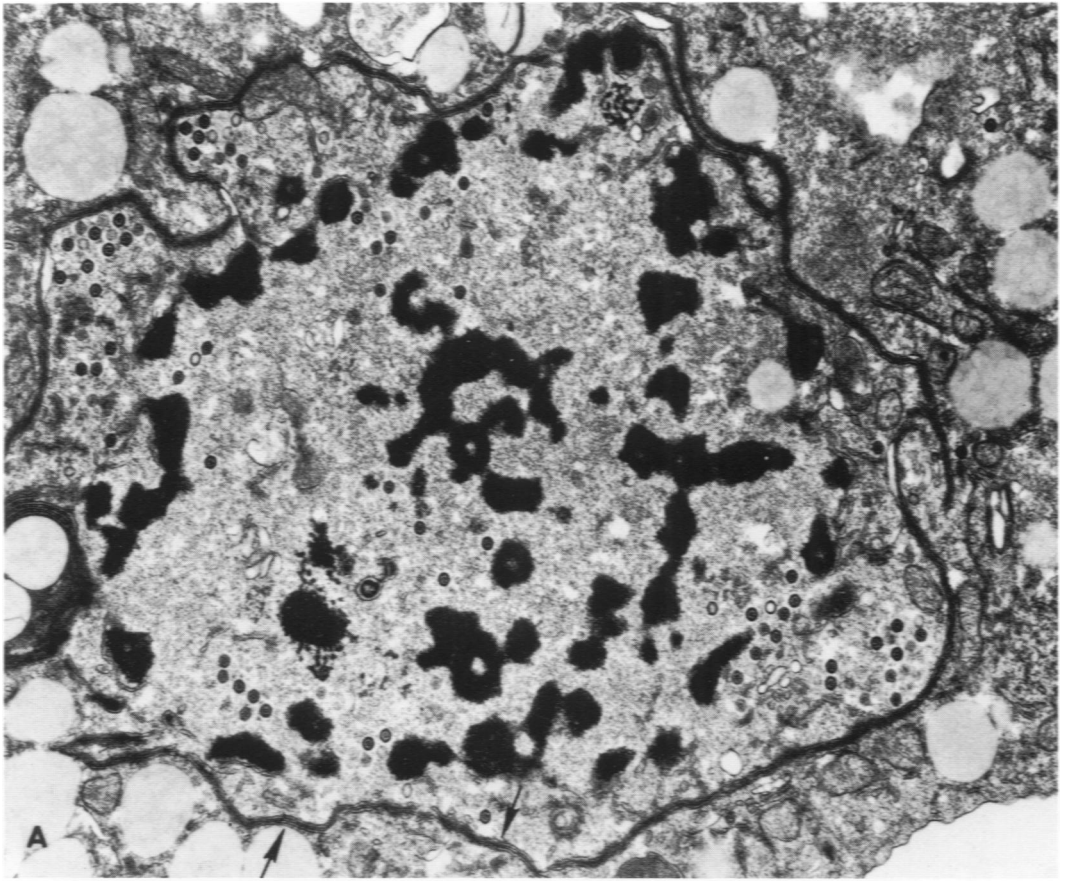


**Figure 3**—Electron micrograph of a freshly isolated monocyte 48 hours after inoculation. The chromatin is condensed with irregular areas of clearing. A well-defined nuclear membrane cannot be identified. **A**—Empty herpes virus capsid structures are free in the cytoplasm (*arrows*). **B**—Empty herpes virus capsid structures are associated with membranous profiles (*arrows*). (Uranyl acetate and lead citrate; **A**,  $\times 2300$ ; **B**,  $\times 50,600$ )





**Figure 4**—Ultrastructural appearance of 7-day-cultured monocyte after being infected with virus. Monocytes were kept in culture for 1 week and were then exposed to HSV and fixed for electron microscopic study 48 hours after infection. Multiple vacuolar structures are seen in the cytoplasm. The nucleus, at the lower edge of the cell, has a well-defined nuclear membrane, and many virus particles (*arrow*) can be seen. (Uranyl acetate and lead citrate,  $\times 19,400$ )



**Figure 5**—Electron micrograph of the nucleus of 7-day-cultured monocyte after infection with HSV. **A**—Many herpes virus capsid structures are seen, some of which have dense nucleoids. Duplication of nuclear membrane can be seen (*arrow*) and on the left it forms a tight whorl. **B**—A segment of the nucleus of another 7-day-cultured monocyte after infection with HSV. Herpes virus capsid structures are present in the nucleoplasm, and particles are seen adjacent to the inner leaflet of the nuclear membrane. Enveloped nucleocapsid structures with dense cores are present within the perinuclear cisternae. (Uranyl acetate and lead citrate; **A**,  $\times 10,200$ ; **B**,  $\times 96,800$ )