tionally infected despite the fact that the subjects were not isolated, and no serious sequellae of infection were observed. The infections all began at a known time and were rather uniform in their duration, so that a biological study on a new drug could be done with a minimum number of subjects and controls being exposed to new compounds with their uncertain toxicities. Despite previous tissue culture evidence of antiviral activity, this study did not confirm the value of trifluorothymidine or methisazone in preventing or treating adenovirus infection in man.

This study was made possible through the cooperation of the Florida Division of Correction, Raiford, Florida. The assistance of Mrs. Emily Ellison and Mr. Clarence E. Leach was greatly appreciated.

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Received Nov. 20, 1967. P.S.E.B.M., 1968, Vol. 127.

Human Cytomegalovirus. Observations of Intracellular Lesion Development as Revealed by Phase Contrast, Time-Lapse Cinematography* (32863)

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Human cytomegalovirus (CMV) induces characteristic cytoplasmic and intranuclear lesions in human fetal fibroblasts. In previous reports, cultured cells infected with human cytomegalovirus were studied by cytochemistry (1), autoradiography (2), and electron microscopy (3–5). These studies provided information regarding the development of cytopathic and cytochemical effects in tissue culture related to infection by this DNA virus.

To further characterize the sequential events leading to the development of the mature nuclear and cytoplasmic lesions, human fetal fibroblasts cultured in Rose chambers were infected with CMV and observed by phase contrast microscopy. Time-lapse cinematog-

* This investigation was supported by USPHS Research Grants 5 RO1 AI 03079 from the National Institute of Allergy and Infectious Diseases and 3 R01 CA 07991 from the National Cancer Institute.

raphy was employed for the first time to observe the cell changes associated with infection. The present report describes the changes observed in living cells.

Materials and Methods. Virus and cell cultures. The Rib strain of CMV (1), serologically related to the AD-169 strain, was used for this study and was in the one hundred-eighth passage in human fetal fibroblasts. This virus had originally been isolated from the urine of a male newborn who was suspected of having cytomegalic inclusion disease. The WI-38[1] cells used in this experiment were maintained in Eagle's basal medium containing 2% inactivated calf serum, penicillin (200 μg/ml), streptomycin (200 μg/ml), and kanamycin (100 μg/ml), and were incubated at 37°C. Confluent sheets of WI-38 cells growing in Rose chambers were infected with approximately 10^4 cell infectious units (6) of freshly harvested CMV.

Cinematography. Time-lapse cinematographic records were made on five separate, incubated cine units with Zeiss phase contrast optics (40× and 100× objectives) and a film-taking rate of 2–8 frames/min (7). The records were begun 14 hours prior to infection and continued for more than 90 hours following infection of WI-38 cells. Still-phase photomicrographs (4 × 5) were made of various preparations prior to and after infection with CMV.

Some individual cells which had been filmed before infection were relocated following inoculation of virus into the culture and subsequently photographed until cell death. A total of 2100 feet of 16-mm film was accumulated for analysis by direct observation and with the aid of an L-W photo-optical data analyzer. In the present report, certain film frames and still-phase photos were selected for the preparation of plates to illustrate the sequential morphological changes after a cell becomes infected. The dynamic alterations visualized in the time-lapse film, whose action is speeded up over 700 times when projected at 24 frames a second, cannot be appreciated fully by the still photographs.

Results. Uninfected control cells. By phase contrast microscopy, uninfected control WI-38 cells were seen to grow in monolayer sheets of fibroblast-like, fusiform cells arranged in parallel bundles. Nuclei were usually elongated with one or more apparent nucleoli and finely granular chromatin material; granules were diffusely distributed in the cytoplasm of some cells (Fig. 1). With cinematography there was apparent activity, or movement of granules and other components, within the cytoplasm of the uninfected cell.

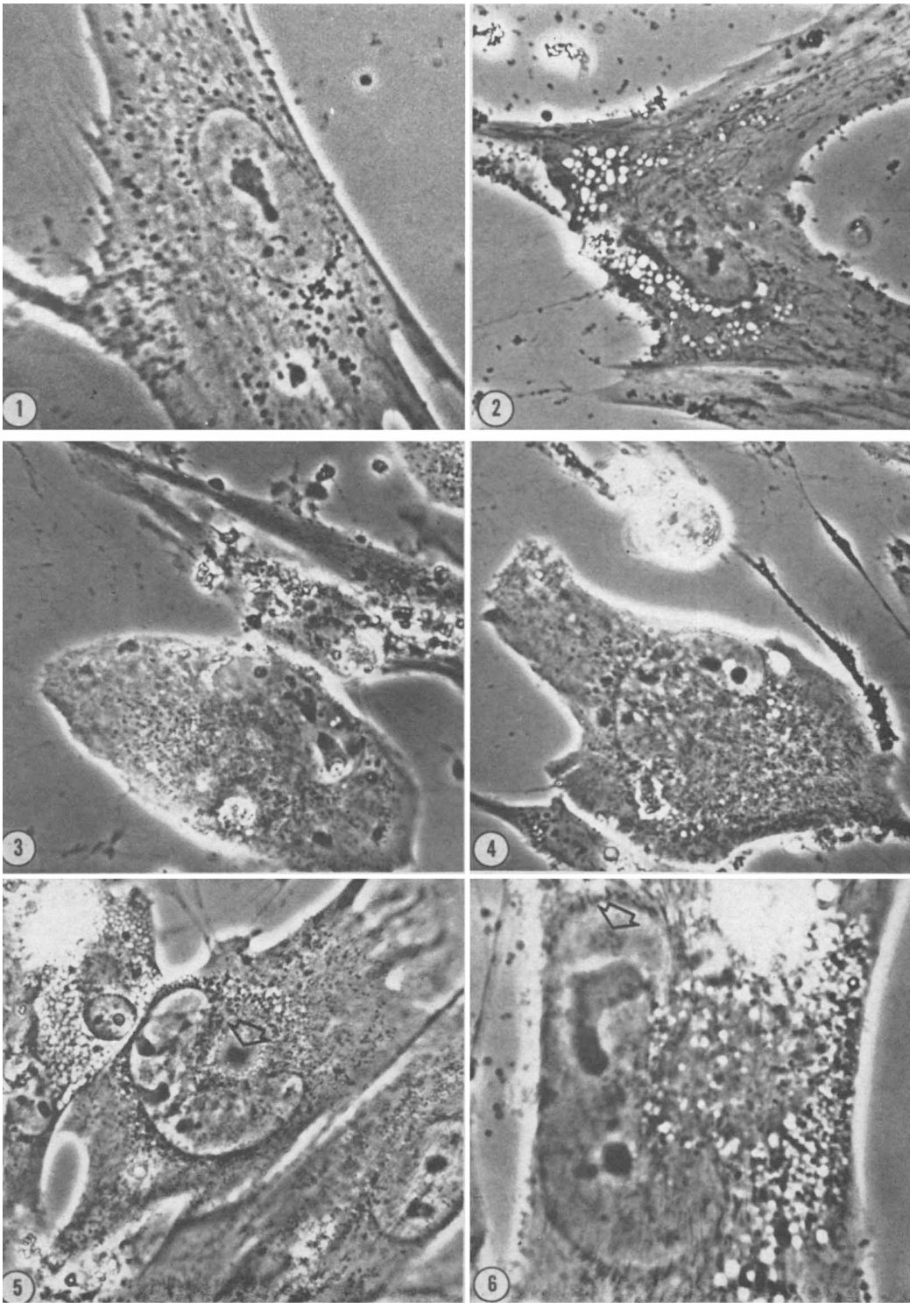
Changes in morphology of infected cells. Cellular changes observed after infection by phase contrast microscopy showed considerable synchrony. The sequence of changes has been arbitrarily divided into three stages.

Stage 1 (usually occurring 18–24 hours after infection). The earliest change seen after infection was a contraction and rounding of the cell followed by the appearance of an area of increased cytoplasmic activity (movement of granules and other components) adjacent to one pole of the nucleus, with apparent aggregation of cytoplasmic components (granules and vacuoles) in this area (Fig. 2). The cytoplasmic activity within this area then became more rapid and the area appeared to migrate *in toto* towards the center of the cell and to a paranuclear position. This area corresponded to the cytoplasmic inclusion described in earlier studies (1). Invagination of the nucleus followed, and the kidney-shaped nucleus was compressed against one edge of the cell (Fig. 3).

Stage 2 (usually 24–48 hours after infection). During this stage, there appeared to be rapid activity at the center of the cytoplasmic inclusion. The periphery of the lesion was made up of vacuoles which formed a halo about the highly active granular area. There was a slow rotation of the nucleus around the active cytoplasmic lesion. In one sequence, this movement seemed to encompass 180° (Figs. 3–5). This rotation took 4–8 hours and was considerably slower than nuclear rotation commonly observed in tissue culture with time-lapse filming. Dark oval or round areas which were smaller and more dense than the nucleoli gradually formed in the nucleus. These areas were believed to represent the intranuclear inclusions.

Stage 3 (usually 48–72 hours after infec-

[1] Microbiological Associates, Inc., Bethesda, Md.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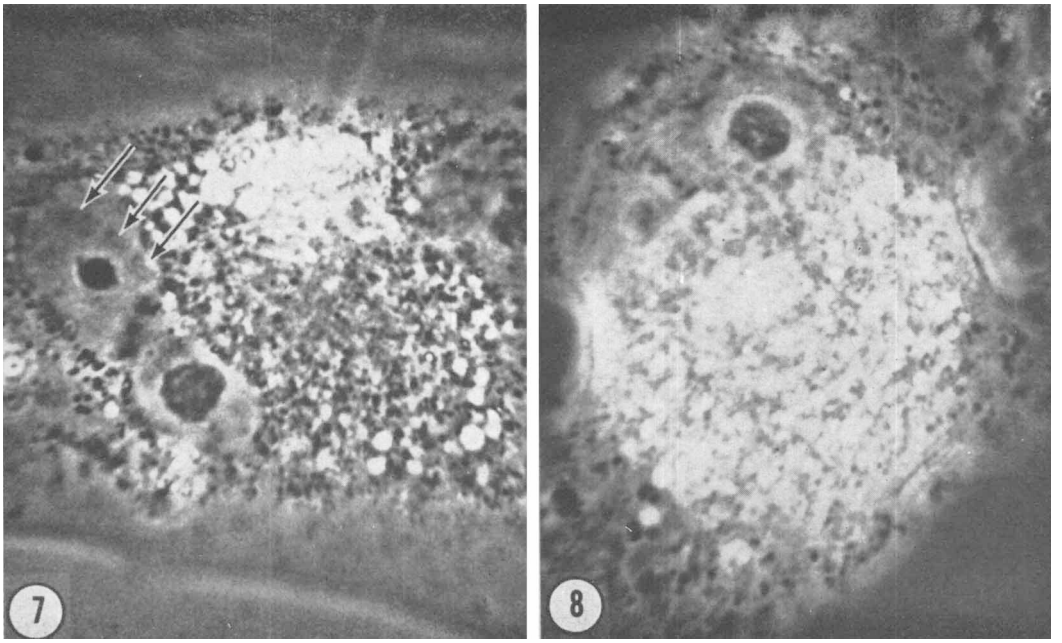

FIG. 7. WI-38 cell 88 hours after infection. The U-shaped nucleus seems to have turned upon itself. Nucleoli are obvious and arrows point to what could be intranuclear inclusions. The cytoplasmic lesion seems to dominate the cell. Photo abstracted from a time-lapse film. magnification: × 1500.

FIG. 8. Same cell as Fig. 7 at the moment of death, 112 hours after infection. The nucleus appears to have rotated clockwise in the previous 24 hours. Photo abstracted from a time-lapse film. magnification: × 1500.

tion). During this period, the intranuclear inclusions "matured" by developing into one or two separate bodies occupying the center of the nucleus, and separated from the distinct nuclear membrane by areas of less dense nucleoplasm. The single cytoplasmic inclusion became well defined and was surrounded by an increased number of vacuoles which

FIG. 1. Uninfected WI-38 cells in culture for 48 hours, showing an elongated nucleus with one large and two smaller nucleoli and finely granular cytoplasm. Photo abstracted from a time-lapse film; magnification: × 1500.

FIG. 2. WI-38 cell 14 hours after infection. Note the accumulation of vacuoles about the nucleus, especially at one pole. Photo by still-phase optics; magnification: × 950.

FIG. 3. WI-38 cell 30 hours after infection. Note the contracted cell at the bottom and the area of granules outlining the cytoplasmic lesion which impinges upon the nucleus in a paranuclear position. Several nucleoli are seen within the nucleus. Photo by still-phase optics; magnification: × 950.

FIG. 4. Same cell as Fig. 3, 54 hours after infection. Note the counterclockwise rotation of the nucleus which has become U-shaped. Photo by still-phase optics; magnification: × 950.

FIG. 5. Same cell as Fig. 4, 72 hours after infection. The nucleus seems to have rotated further, however the cell configuration has changed. The arrow points to a dense area in the cytoplasmic lesion, the significance of which is unknown. Photo by still-phase optics; magnification: × 950.

FIG. 6. WI-38 cell 77 hours after infection. Note the nucleus which seems to be compressed against the cell membrane and the paranuclear cytoplasmic lesion. Several nucleoli are seen as well as a mature nuclear inclusion (arrow). Photo by still-phase optics; magnification: × 2300.

appeared quite active, whereas the center of the lesion appeared "engorged" and inactive (Fig. 6).

Cell death. As the activity about the periphery of the cytoplasmic lesion apparently built up momentum, the center of the lesion became inactive, as did the nucleus (Fig. 7), and the cell suddenly exploded with a "mushrooming" effect. All activity ceased, cellular morphology became indistinct (Fig. 8), and the cell apparently died.

Discussion. Information obtained from this direct study on living cells corroborates the results of previous experiments with human CMV that have been reported from these, as well as other laboratories (1–3,6). The activity or movement of internal components within infected cells was much more apparent than within uninfected cells.

In the present studies, as well as in previous studies of fixed and stained cells, the earliest effects of infection on the cell were observed about 18 hours following infection. These effects consisted of contraction and rounding of the cell.

Seen in living cells, the lesion which appeared after infection consisted of an accumulation of granules and vacuoles in rapid motion. Cytochemical studies have shown that this lesion contains lipid, carbohydrate, and protein and is surrounded by a halo of RNA-containing material. Ruebner and others (4) have suggested that the cytoplasmic lesion might be Golgi vesicles dilated with dense material; however, it was not stained with the De Fano stain, a stain used to demonstrate the Golgi apparatus (1).

In living cells, the nuclear inclusions were observed to form at about the same time (48 hours) as has been reported previously using cytochemical techniques. That these inclusions are composed of DNA is well documented (1,2,4,5).

A previously unrecognized feature of CMV infected cells is the unusually slow rate of nuclear rotation, which is a consistent and dramatic feature of such cells. The slow rate of nuclear rotation began soon after infection and continued until the cell died. In the infected cells, the nucleus rotated 180° in 4–8 hours whereas in normal cells such a rotation, when it takes place, occurs in 5–10 min. The cause of this slow rate is unknown but could be due to the apparent attachment of the cytoplasmic lesion to the nucleus, since they rotate together. The kidney-shape of the infected nucleus may also be associated with the slow rate.

The death of cells observed in the film was most dramatic. Although it is possible that death does not occur *in vivo* as represented in the film, one might speculate that cell-to-cell transfer of virus could occur in this manner, i.e., extrusion of virus during the terminal "explosion" process.

Summary. The earliest change seen after infection of human embryo fibroblasts with human CMV was a contraction of the cell, followed by the appearance of an area of increased cytoplasmic activity adjacent to one pole of the nucleus. The area of cytoplasmic activity moved to a paranuclear position before it impinged upon and invaginated the nucleus. Slow rotation of the nucleus about the cytoplasmic lesion within the cell was apparent. Intranuclear inclusions, distinct from the nucleoli, appeared and matured before the cell exploded and all activity ceased.

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Received Nov. 22, 1967. P.S.E.B.M., 1968, Vol. 127.