Virus Infection of Cells in Mitosis II. Measles Virus Infection of Mitotic HEp-2 Cells.* (26324)

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During studies of Human Epidermoid Carcinoma No. 2 Cells (HEp-2) infected with measles virus and stained with fluorescent antibody or acridine orange, we found a number of mitotic cells containing measles antigen. In addition, mitotic figures were present within virus induced syncytia. The experimental data reported here demonstrate that the virus antigen in the mitotic cells resulted from virus multiplication and that mitotic cells are readily recruited into syncytia induced by measles virus.

Materials and methods. The Edmondston strain of measles virus was passaged 23 times in HEp-2 cells prior to these experiments. HEp-2 coverslip cultures were prepared in Leighton tubes seeded with 60,000-80,000 cells suspended in tissue culture fluid consisting of 10% calf serum in mixture 199. Infected coverslip cultures were maintained in tissue culture fluid consisting of 1 to 10% calf serum in mixture 199, as indicated in text. Generally, syncytia formed more readily if infected HEp-2 cells were maintained with media containing high serum concentrations.

Acridine orange staining of coverslips was performed by the method of Armstrong and Niven(1). Fluorescent antibody was prepared from a pool of human immune gamma globulin selected for its high content of measles neutralizing antibody and conjugated to fluorescein isothiocyanate by the method described by Marshall(2). For better resolution of cellular detail, the coverslip preparations stained with fluorescein labeled gamma globulin were counterstained with Lissamine rhodamine (RB-200) conjugated with bovine albumin(3).

Localization of measles antigen in mitotic cells. Infectious tissue culture fluid (ITCF) was cleared of cell debris and clumps of virus by ultracentrifugation in a density gradient designed to minimize resuspension of pelleted material by diffusion or handling. Four-ml volumes of undiluted ITCF (2% calf serum in mixture 199) titering $10^{5.2}$ $TCID_{50}$ per ml were carefully layered into lusteroid tubes over 2 layers of 0.5 ml of sucrose, 21% w/w and 37% w/w, respectively. The tubes were then centrifuged for 2 hours at 35,000 rpm in a SW-39 Rotor driven by a Spinco Model L Ultracentrifuge. At the end of the ultracentrifugation the top 3 ml from 2 tubes were pooled, diluted 5-fold in mixture 199 with 1% calf serum and added in 1-ml volumes to 27 coverslip cultures of HEp-2 cells. The top 3 ml from a third tube, which contained "used" tissue culture fluid from an uninfected culture, was similarly diluted and added to 12 coverslip cultures, which served as controls. At intervals of 8, 22, 52 and 73 hours after incubation at 37°C, 7 infected and 3 control coverslip cultures were fixed in cold (−60°C) ethyl alcohol and stained with fluorescein labeled antibody.

Thorough examination of all control cultures exposed for 8 hours to the supernatant from ITCF did not show any yellow-green fluorescence. Specific fluorescence was first detected in an average of 30 well dispersed cells per coverslip in cultures fixed 22 hours after infection. The number of infected mitotic cells in each coverslip varied from one to 4. These were in metaphase, anaphase or telophase; infected prophase cells were not seen.

The appearance and site of localization of measles antigen in HEp-2 cells at rest have been described(4). Generally, we found that use of rhodamine counterstain permitted better visualization of cellular detail after staining with fluorescein labeled antibody. In our studies virus antigen was frequently present in both nucleus and cytoplasm of cells in in-

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terphase. In the nucleus, virus antigen appeared as dispersed small spherical granules distinct from nucleoli (Fig. 1). Virus antigen in cytoplasm of interphase cells was usually seen as a diffuse powdery fluorescence; less frequently, areas of specific fluorescence took the form of elongated or oval bodies arranged in a circle or semi-circle around the nuclei. In mitotic cells, virus antigen occasionally was present in the form of several small fluorescent granules adjacent to chromosomes but more frequently just under the cell membrane (Fig. 2-4). In general, mitotic cells were readily differentiated from interphase cells by their size and shape. In addition, chromosomes, which did not retain the rhodamine counterstain, were sharply silhouetted against the intense red-orange fluorescence of cytoplasm.

Coverslip cultures fixed and stained at 52 and 73 hours after infection showed an increased number of infected cells. In both groups, but particularly in coverslip cultures fixed at 73 hours, there were small syncytia and numerous foci of infection each consisting of 2-10 cells showing specific fluorescence. It is of interest to note that in some syncytia containing 5-10 nuclei, only 1 or 2 of these nuclei showed specific fluorescence. Similarly, virus antigen present in the cytoplasm appeared to be near some, but not all, nuclei contained in the syncytium. Infected cells in metaphase, and less frequently in anaphase and telophase, were seen in both sets of cultures. In 3 coverslip cultures fixed at 52 hours after inoculation, infected mitotic cells constituted 2.4, 5 and 8%, respectively, of total infected cells.

Recruitment of mitotic cells into syncytia induced by measles virus. Twenty-four hours after seeding with HEp-2 cells, 18 coverslip cultures were each exposed to 100 $TCID_{50}$ of measles virus suspended in mixture 199 with 1% calf serum and incubated for 72 hours. The medium in 6 coverslip cultures was then replenished with 10% calf serum in mixture 199; the remainder received 10^{-7} M colchicine (Nutritional Biochemical Co., Cleveland, Ohio) in the same medium.

Examination of coverslip cultures stained with acridine orange 96 hours after infection revealed the presence in both colchicine treated and untreated cultures of abundant syncytia containing as many as 20 nuclei each. In cultures without colchicine 3 of 68 syncytia contained a dense mass of material with the characteristic bright yellow fluorescence of DNA. This mass appeared to be similar in size and color to the chromosomes of uninfected cells in metaphase, but more irregular in shape. Nearly 70% of syncytia counted in infected cultures treated with colchicine contained at least one, but usually several such masses (Fig. 5). In these syncytia some masses appeared to be identical in size and shape to aggregated chromosomes of cells in metaphase seen in untreated cultures. Others were smaller, more irregular in shape, and appeared to be amorphous clumps of chromosomes.

These studies on recruitment of mitotic cells into infected syncytia were repeated several times with variations in the time at which the cultures were fixed and stained. In one experiment, colchicine was withdrawn after 24 hours of exposure. In another experiment, uninfected cells arrested in metaphase by colchicine were seeded among cells in previously infected cultures. In these latter experiments, it was again evident that treatment with colchicine markedly increased the number of mitotic cells recruited into infected syncytia.

Discussion. The conclusion that measles antigen present in mitotic cells resulted from virus multiplication is based on the following evidence: (1) Ultracentrifugation served to clear the inoculum of cell debris, clumps of virus antigen, and most of the virus. The absence of specific fluorescence in cells exposed to the inoculum for 8 hours supports the contention that virus antigen was formed *de novo* following infection of cells in the coverslip cultures. (2) It seems unlikely that measles virus antigen was merely released from degenerated cells and subsequently ingested by mitotic cells. At 22 hours after infection very few cells (average of 30 in each coverslip culture) exhibited specific fluorescence, but those that did contained as many as 6 discrete fluorescent granules distributed

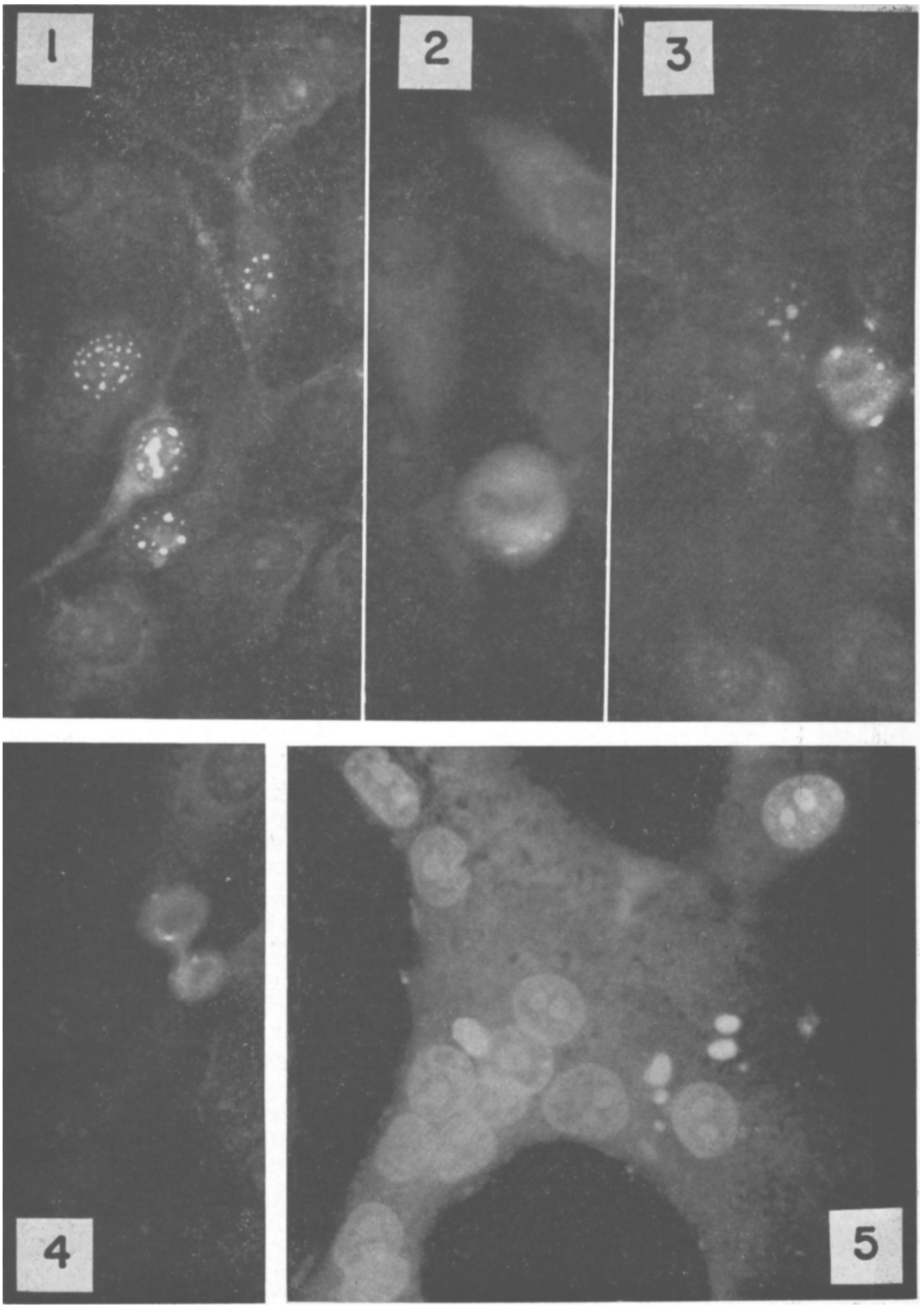

FIG. 1. Measles virus antigen in nuclei and cytoplasm of interphase cells. 480×. Fluores-

cein labeled antibody stain, counterstained with Lissamine rhodamine conjugated to bovine albumin.

FIG. 2. Granules of measles virus antigen in metaphase cell. 720×. Fluorescein labeled antibody stain, counterstained with Lissamine rhodamine conjugated to bovine albumin. The unstained chromosomes are silhouetted against the intense red-orange rhodamine fluorescence of the cytoplasm.

FIG. 3. Granules of measles virus antigen in late anaphase or telophase cells. A portion of a syncytium of HEp-2 cells is also shown. 480×. Fluorescein labeled antibody stain, counterstained with Lissamine rhodamine conjugated to bovine albumin.

FIG. 4. Measles virus antigen in daughter cells (late telophase or early interphase). Note that virus antigen is present in both daughter cells. 480×. Fluorescein labeled antibody stain, counterstained with Lissamine rhodamine conjugated to bovine albumin.

FIG. 5. Mitotic figures and clumps of chromosomes (in the photomicrograph they appear as white oval bodies without internal structure) in syncytium of HEp-2 cells induced by measles virus. 480×. Stained with acridine orange.

in their cytoplasm. It is our contention that it is unlikely that so many granules could be ingested by a single cell in a culture in which very few cells showed evidence of infection unless the mitotic cells ingested whole infected cells. Careful examination failed to reveal additional nuclei or other evidence that a whole cell was ingested. Nevertheless, demonstration of measles antigen in mitotic cells is not by itself evidence of mitotic division of infected cells. The data are also consistent with the hypothesis that a cell may become infected at any stage in the mitotic cycle (including interphase), and that infection causes mitotic arrest.

It is of interest that very different results were obtained in experiments with the MP strain of Herpes simplex virus, as described elsewhere(5). In these studies, HEp-2 cells in prophase appeared to be more susceptible to recruitment into syncytia induced by Herpes simplex virus than were cells in metaphase; infected cells in anaphase and telophase were not seen. Mitotic cells arrested in metaphase by colchicine were also resistant to infection. However, these cells readily fused to form giant cells after withdrawal of the drug. From comparisons of HEp-2 cell cultures infected with measles and Herpes simplex virus, it seems reasonable to postulate that (1) susceptibility of HEp-2 cells to virus infection may vary depending on their stage in the mitotic cycle and, (2) susceptibility to infection of cells at each mitotic stage may vary from one virus to another.

Summary. Fluorescein labeled antibody staining of HEp-2 cell cultures infected with an inoculum of measles virus free of cell debris and other particulate matter revealed the presence of measles virus antigen in cells in metaphase, anaphase and telophase. Mitotic cells were shown to be readily recruited into syncytia induced by measles virus, both in absence and presence of colchicine. These observations contrast with the results of similar studies made in HEp-2 cell cultures infected with Herpes simplex virus and indicate that cells in different stages of the mitotic cycle are not uniformly susceptible to infection with different viruses, or even the same virus.

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