Infection of Adult Mouse Macrophages *in Vitro* with Cytomegalovirus* (33399)

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Chronic cytomegalovirus infections occur in man and a number of lower species (1). The mechanism by which the virus persists for prolonged periods in the animal host is unknown. In addition, the kinetics of cytomegalovirus growth in tissues and the factors which affect dissemination are poorly defined. Thus far *in vitro* studies which might assist in the elucidation of cytomegalovirus pathogenicity have been carried out almost exclusively in fibroblast cultures prepared from fetal tissue. The present study was undertaken in an attempt to define the growth properties of mouse cytomegalovirus (MCMV) in peritoneal macrophages derived from adult animals. Similar studies on the interaction of viruses with leukocytes and macrophages have provided information which has contributed to our understanding of the pathogenesis of other viral diseases (2).

Methods and Materials. Cell cultures. Trypsin dispersed cells from 14- and 18-day-old fetuses were grown in Eagle's basal medium containing 10% heated (56°, 30 min) calf serum and antibiotics. The monolayers were trypsinized 48 hr later and secondary cultures were prepared in glass tubes or 35-mm plastic dishes.

Peritoneal macrophages. Five ml of Eagle's medium with 20% heated calf serum were injected into the peritoneal cavities of pathogen-free Swiss mice (Charles River Breeding Laboratories, Wilmington, Massachusetts) which had been killed by cervical disarticulation. The fluid was removed after approximately 1 min with a capillary pipette. Foreign substances were not introduced into the peritoneum to provoke the release of cells. Harvests from several animals were pooled and introduced into glass tubes or plastic dishes. After stationary incubation for 1 hr at 37°, the preparations were washed 3 times and fresh medium added. This procedure removed most lymphocytes and polymorphonuclear leukocytes; large numbers of macrophages remained adherent to the glass or plastic surface. The medium in all cultures was changed at intervals of 3 days. Phagocytic activity of the cells was determined at intervals throughout the experiment by adding a dilute suspension of washed carbon black particles 2 hr prior to fixation of the cell sheet. Macrophage cultures in tubes were fixed in Bouin's solution, removed from glass in a collodion film (3) and stained with hematoxylin and eosin.

Virus. The Smith strain of MCMV (4), obtained from Dr. Wallace P. Rowe of NIH, was grown in newborn mice and passaged 4 times in fibroblast cultures. Plaques were selected for the first 2 passages. Pools of virus were prepared by harvesting cells and medium after the appearance of extensive cytopathic effect. The virus suspension was clarified by light centrifugation and the supernate was stored in ampuls at —65°. The plaque assay of Henson *et al.* with minor modifications was used routinely to determine virus titers (5).

Infection of Cultures. Experiments to quantitate virus adsorption were carried out in plastic dishes containing 1-day cultures of either macrophages or fibroblasts. The cells were exposed at 25° to 120 plaque-forming units (pfu) of MCMV in 0.4 ml of medium. At intervals thereafter the fluid in individual plates was aspirated and titered for unadsorbed virus. The number of cells which had taken up virus could not be determined by quantitation of infectious centers because it was difficult to remove intact macrophages from the plastic surface.

Virus production and cytopathic effect were studied by inoculating 1-day tube cul-

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tures of both macrophages and fibroblasts with varying dosages of virus. At intervals thereafter, the medium was titered and the cells were removed for staining in a collodion film (3). Empty tubes or tubes containing cells which had been heated at 56° for 30 min were used to determine the rate of inactivation of the virus under the same conditions. Some macrophage cultures were exposed to carbon particles before the inoculation of virus.

Results. Peritoneal macrophages in uninfected cultures exhibited a characteristic appearance over a 15-day period of observation. The cells were crowded but never confluent. Mitoses were not found and there was no apparent increase in number. Spreading of the cytoplasm of the cells was observed in young cultures. Although macrophages often became elongated, they maintained a random arrangement which was distinct from the parallel pattern of fibroblasts in culture (Fig. 1A). As shown in Fig. 1B, most macrophages retained the ability to phagocytize carbon particles for 15 days. Fibroblasts phagocytized carbon very poorly.

The rate of adsorption of cytomegalovirus to cultures of macrophages and fibroblasts was approximately the same. Adsorption rates could not be determined with accuracy because of the spontaneous uptake of virus by the plastic of the culture dishes (Fig. 2). Characteristic intranuclear inclusions and nonspecific cytopathic effect first became apparent in macrophage cultures 3 days after the inoculation of virus. At this time the number of cells exhibiting changes was roughly proportional to the size of the inoculum. Subsequently, affected cells increased in numbers and intranuclear inclusions appeared in all cells by from 6 to 15 days after inoculation (Fig. 1C). Cells with MCMV inclusions lacked phagocytic properties when exposed to carbon black. On the other hand, macrophages which had taken up carbon particles before inoculation developed viral inclusions as readily as did cells maintained in carbon-free medium (Fig. 1D). Specific cytopathic effect appeared in fibroblast cultures 1 day after inoculation and involved the complete cell sheet within a period of from 2 to 5 days.

The MCMV replicated in macrophages after inoculation with a wide range of virus dosage (Fig. 3). During the first 72 hr there was a slight decrease in virus titer which approximated the virus loss attributable to thermal inactivation. Subsequently cultures consistently showed increases in virus titer regardless of the inoculum size. All cultures produced extracellular virus titers of 2–4 $\times$ 10^7 pfu/ml over the course of 15 days. Fibroblasts yielded similar quantities of virus but at a faster rate (Fig. 3).

Discussion. Our results show that cultured macrophages from the mouse peritoneal cavity are uniformly susceptible to infection by MCMV and produce large quantities of extracellular virus. Although the total amount fabricated by the macrophages approximated that made by fibroblasts, the appearance of cytopathic effect and the release of virus by the cells was relatively slow. It remains to be determined whether or not these differences reflect intrinsic properties of the two different types of cell or the interrelation of these cells in the cultures. Direct spread of virus from cell to cell probably is more efficient in a confluent monolayer of fibroblasts than in a nonconfluent macrophage culture. On the other hand the age of the animals from which cells were derived may influence the initial interaction of virus with cells or the virus synthetic process.

McGavran and Smith presented ultrastructural evidence showing that human cytomegalovirus particles are engulfed after release from the nucleus by lysosome-like structures in the cytoplasm (6). Although these workers failed to determine whether or not virus is degraded by lysosomal enzymes, they observed that only small amounts of infectious virus can be recovered from cells possessing many lysosomes. Using MCMV-infected mice Ruebner *et al.* found that virus persists for prolonged periods in salivary gland cells which contain few lysosomes but is present for only short periods of time in the lysosome-rich parenchymal cells of the liver (7). Monocyte-like cells from the mouse peritoneal cavity were found by Cohn and

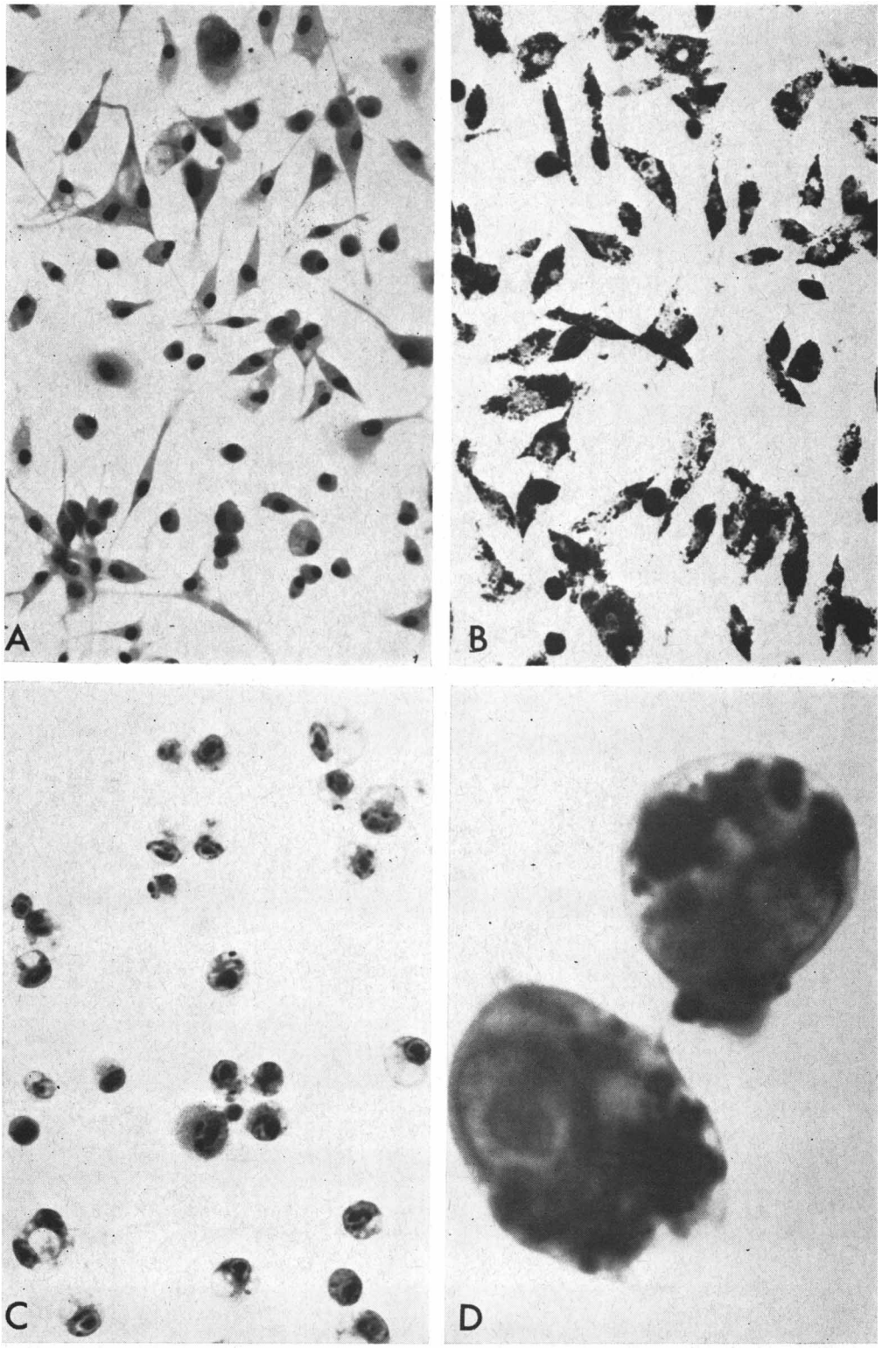
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FIG. 1A. Uninfected adult mouse peritoneal macrophages on day 16 of culture. (B) Uninfected macrophage on day 16 of culture. The cells were fixed 2 hr after exposure to a dilute suspension of carbon black. (C) Macrophages on day 16 of culture after inoculation with 10^2 pfu of MCMV on the first day. (D) Macrophages 3 days after inoculation with 10^6 pfu of MCVC. The cells were exposed to carbon black and were washed before virus inoculation. Note the intranuclear inclusion bodies and intracellular carbon particles.

Benson to differentiate into macrophages when cultured *in vitro* (8). During this process increasing numbers of lysosome-like organelles and hydrolases of many types accumulate in the cytoplasm. We feel that the demonstrated production of large amounts of MCMV by cultured macrophages is evidence against the concept of an inverse relationship between the presence of lysosomes in the cell and the release of complete infectious virus particles.

Virus-infected mouse peritoneal macrophages possess the ability to produce substantial amounts of interferon *in vitro* (9). It seems likely that interferon did not play an important role in multistep MCMV infection in our macrophage cultures since the amount of virus in the culture fluids increased despite infrequent changes of medium and the use of small virus inocula. Under these circumstances interferon might be expected to accumulate in the medium (10). Osborn and Medearis claim that MCMV lacks the capacity to initiate interferon production in mouse tissues (11). This question was not explored in our studies.

Bang and Warwick believe that macrophages play an important role in the pathogenesis of mouse viral hepatitis (12). Replication of West Nile virus in macrophages from different strains of mice has been correlated with *in vivo* susceptibiltiy (13). Although histologic studies indicate that macrophages of the lung and spleen are infected by MCMV the significance of these cells as sites of virus multiplication in the intact animal is not known. Indeed, the observations of Henson *et al.* suggest that macrophages limit the spread of MCMV in the parenchyma of the liver (14). Our findings *in vitro* indicate that macrophages are not only susceptible but possess the capacity to produce large amounts of virus. Thus, they may contribute to persistence of the infection or dissemination of the virus *in vivo*.

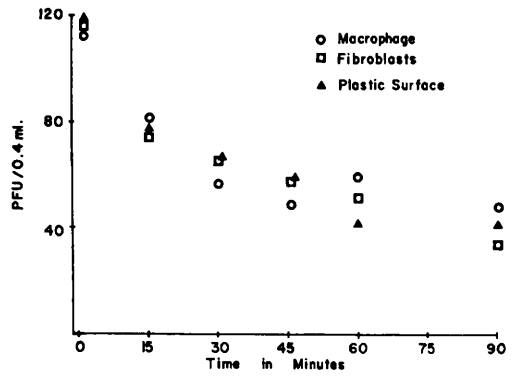

FIG. 2. Adsorption of MCMV by 1-day-old plate cultures of adult mouse peritoneal macrophages and fetal fibroblasts. Results with plastic dishes not containing cells are also shown. Virus disappeared from each inoculum at approximately the same rate.

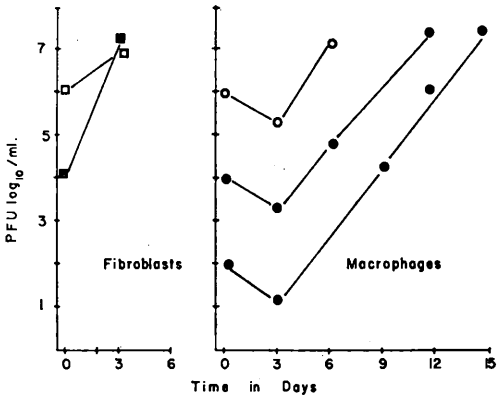

FIG. 3. Extracellular virus production by adult mouse peritoneal macrophages and fetal fibroblast cultures inoculated with 10^6, 10^4 and 10^2 pfu of MCMV.

Summary. Adult mouse peritoneal macrophages were infected with the Smith strain of mouse cytomegalovirus. Sequential observations were made on the development of cytopathic changes and the release of virus from cells. Virus production by macrophages and the appearance of typical intranuclear inclusions was slower than in mouse fibroblast cultures although the total amount of virus

produced by the two types of cells was roughly equivalent. Macrophages in culture retained their phagocytic properties and were susceptible to the virus after uptake of carbon black. On the other hand, infected cells failed to phagocytize foreign carbon particles.

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Passive Cutaneous Anaphylactic-Like Reactions in Young Chicks* (33400)

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It has been reported that chickens, unlike guinea pigs and other mammals, fail to undergo anaphylactic reactions (1–4). Although several investigators have reported on immediate hypersensitivity reactions resembling systemic anaphylaxis (5–11), corroborative evidence for the anaphylactic reaction has been lacking, e.g., sensitization by passive antibody.

In the present study using chicken antiserum, we showed that a passive cutaneous anaphylactic-like (PCA-like) reaction could be elicited only in the skin of newly hatched and young chicks and not in the skin of the adult chicken.

Materials and Methods. Chicken antibovine gamma globulin sera (anti-BGG) were obtained from adult white leghorn chickens after two injections of the antigen, 50 days apart; serum samples were collected 8 days after the last injection. Chicken antirabbit gamma globulin sera (anti-RGG) were obtained from hyperimmunized chickens which were bled 11 days after the final injection. Of 7 precipitating antisera tested (5 anti-BGG and 2 anti-RGG), 6 gave PCA-like reactions in young chicks; one anti-BGG serum was negative. For comparing the reactivities of the young and the adult chickens, one anti-BGG and one anti-RGG serum were selected for testing. These sera contained 43 and 12 μg of precipitin/10 μl, respectively.

Results. Consistent positive reactions were obtained in the young chicks when 10 μl of anti-BGG or anti-RGG serum were injected intracutaneously and 0.6 mg of the specific antigen per ml of blood volume was injected 2 hr later intravenously. Each 0.6 mg of antigen was dissolved with 0.02 ml of saline and 0.04 ml of 0.4% solution of Evans blue.

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