Effect of Ultraviolet Irradiation and Actinomycin D on Polyoma Virus Replication in Mouse Embryo Cell Cultures

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Ultraviolet irradiation and actinomycin D impair the capacity of mouse embryo (ME) cells to support the replication of polyoma virus, but not of encephalomyocarditis (EMC) virus. The loss in capacity for polyoma virus synthesis was an "all-or-none" effect and followed closely upon the loss in cellular capacity for clone formation. Cells treated with either agent produced polyoma "T" antigen, but did not synthesize polyoma structural protein. Infection of untreated ME cells with polyoma virus produced marked stimulation of both deoxyribonucleic acid (DNA) synthesis and ribonucleic acid (RNA) synthesis. ME cell cultures irradiated with ultraviolet for 30 sec at 60 $\mu\text{W}/\text{cm}^2$ or treated with actinomycin D at 0.1 $\mu\text{g}/\text{ml}$ for 6 hr prior to infection were incapable of synthesizing DNA or RNA, even after infection with polyoma virus. Irradiation of cells during infection produced cessation of synthesis of both RNA and DNA. Addition of actinomycin D during infection did not inhibit DNA synthesis but abolished RNA synthesis and reduced the yield of polyoma virus to 10% of that in untreated infected cultures. Both agents lost the ability to prevent replication of a full yield of polyoma virus when administered 30 hr after infection or later. The period after which neither agent inhibited polyoma replication corresponded with the period at which maximal RNA synthesis in untreated infected cultures had subsided. It can be concluded on the basis of the data presented that the functional integrity of the mouse embryo cell genome is required for the replication of polyoma virus, but not for EMC virus. Whereas the requirement for cellular DNA-dependent RNA synthesis for polyoma virus replication has been demonstrated, the exact nature of the host-cell function remains to be elucidated.

Mouse embryo (ME) cell cultures infected with polyoma virus exhibit a marked increase in deoxyribonucleic acid (DNA) synthesis compared with uninfected control cultures. This increase has been shown to be due to the induction of both cellular and viral DNA synthesis (1, 4, 7-9, 12, 16, 17, 19, 20). It is not clear whether the induction of cellular DNA synthesis is essential to the replication of progeny virus in polyoma-infected cells. Minowada (13) and Gershon et al. (9) have reported that X-irradiated mouse embryo cells are incapable of synthesizing DNA, but support polyoma virus replication to the same extent as unirradiated control cultures. These investigators also observed that X-irradiated cells show increased levels of DNA synthesis after infection with polyoma virus (9, 12). Logan and Whitmore (11) found that cobalt-60 irradiated ME cell cultures lost the capacity to support

replication of polyoma virus. The capacity for the support of polyoma virus synthesis was more radioresistant than was the cloning capacity of the cells. The results of experiments with mitomycin C (6) and with actinomycin D (5) suggested that damage to ME cell DNA impairs the capacity of the cells to replicate polyoma virus. Our experiments, in which the effects of ultraviolet irradiation and actinomycin D treatment before and after infection of ME cells with polyoma virus were studied, support and extend the evidence that polyoma replication in ME cells requires a functional host-cell genome. The results of the experiments further indicate that loss of cellular capacity to support virus replication probably reflects impairment in cellular DNA-dependent RNA synthesis.

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MATERIALS AND METHODS

Polyoma virus. Virus used in these studies was originally obtained from Sarah E. Stewart, National Cancer Institute, Bethesda, Md. The virus has been maintained by serial passage in Swiss (Texas Inbred Mice Co., Houston, Tex.; TIMCO) mouse embryo cell cultures. High-titer virus stocks for experiments were prepared by a method similar to that described by Winocour (18). Titers of these stock virus preparations varied from 1×10^8 to 4×10^8 plaque-forming units (PFU)/ml.

Encephalomyocarditis (EMC) virus. EMC virus was a gift from D. R. Dubbs of Baylor University College of Medicine, Texas Medical Center, Houston, Texas 77025. Virus stocks were grown and titrated in primary ME monolayer cultures. Titers of EMC virus stocks varied from 1×10^7 to 5×10^7 PFU/ml.

Tissue culture media and solutions. Growth medium for all experiments was Eagle medium (modified) with Hanks' saline solution (NaHCO_3 concentration = 1.3 g/liter) supplemented with 0.5% lactalbumin hydrolysate and 20% bovine serum (v/v). Maintenance medium was Eagle medium (modified) with Earle's saline solution supplemented with 0.5% lactalbumin hydrolysate and 2% bovine serum. Overlay medium for plaque titrations was Eagle medium (modified) with Hanks' saline solution (NaHCO_3 concentration = 2.6 g/liter), supplemented with 0.5% lactalbumin hydrolysate and 2% bovine serum, and containing 1.0% agar. Neutral red agar overlay was 0.85% NaCl containing 1% agar and 0.01% neutral red. Actinomycin D was generously supplied by Hoffman-La Roche, Inc., Nutley, N.J. The compound was dissolved at 12.5 mg/ml in acetone and subsequently diluted in maintenance medium to desired concentrations.

ME cell cultures. Embryos were aseptically removed from Swiss (TIMCO) mice in the 16th to 19th day of pregnancy. Cells were suspended in growth medium and seeded at 10^7 cells per dish in Falcon tissue culture (60 by 15 mm) dishes. In some instances, 100-mm Pyrex petri dishes were used. These were seeded with 3×10^7 cells per dish. The cultures were used when monolayers were contiguous, usually after 4 days of incubation at 37 C in a humidified 5% CO_2 atmosphere.

Plaque titrations. Polyoma plaque titrations were performed with ME monolayer cultures in 60- by 15-mm Falcon dishes. Cultures were washed once with PBS and each plate inoculated with 0.5 ml of an appropriate virus dilution in PBS. After a virus adsorption period of 1 hr, each culture received 5 ml of overlay medium. Cultures received 3 ml "feedings" of the same medium at 3 and 6 days of incubation (37 C in a humidified 5% CO_2 atmosphere). At 8 days of incubation, 3 ml of neutral red-agar was added to each culture. Plaques were counted at 9 days.

Ultraviolet irradiation of cultures. Monolayer cultures of ME cells were washed once with PBS and all fluid aspirated from the cultures prior to irradiation. Irradiation was administered at room temperature

with a Westinghouse germicidal (type A) lamp. Desired dosages of UV light were given by exposing the cells for various periods of time to a radiation intensity of 60 μw per cm^2 as measured by a UV actinometer (International Light, Inc.).

Isotope experiments. Thymidine- H^3 (H^3 -TdR; specific activity, 16 c/mmmole) and uridine- H^3 (H^3 -UR) (specific activity, 10 c/mmmole) were obtained from New England Nuclear Corp., Boston, Mass., or Schwarz BioResearch Inc., Orangeburg, N.Y. Stock solutions of 10 μC /ml were prepared and appropriately diluted for experiments. For pulse-labeling experiments, cultures in 100-mm petri dishes received 2.5 μC /culture. After 1 hr of incubation in the presence of one of the tritiated metabolites, the cultures were washed twice with cold 0.4 N perchloric acid. The cells were suspended in 0.5 ml of 0.4 N perchloric acid and hydrolyzed for 15 min at 100 C. The hydrolysates were cooled and 20 μl of each sample was applied to a 0.5-inch square of Whatman no. DE-81 paper. The dried squares were placed in Wheaton scintillation vials and the samples were eluted from the papers by addition of 0.2 ml of absolute methanol. Toluene (10 ml) containing 4 g of Omnifluor (New England Nuclear Corp.) per liter was added to each counting vial. The samples were counted in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

Serological methods. Antiserum to polyoma V (structural) antigen was prepared by injecting concentrated, partially purified polyoma virus into rabbits. For immunofluorescence tests, the antiserum was fractionated and the gamma globulin portion conjugated with fluorescein isothiocyanate.

Complement-fixation (CF) tests for polyoma T antigen and hemagglutinin titrations were carried out by the microtiter method described by Sever (15). Polyoma T standard antigen and hamster antipolyoma T serum were obtained from Flow Laboratories. Polyoma-infected and control ME cells for CF tests were washed twice with PBS and suspended in Veronal buffered saline at approximately 5×10^7 cells/ml. The cell suspensions were sonically treated for 10 min at 10 kc in a Raytheon sonic oscillator, and the crude lysates were used as antigens in the CF test. Hemagglutinin titrations were done against 0.25% guinea pig erythrocyte suspension.

RESULTS

Effect of ultraviolet irradiation on ME cell cloning capacity and ability to support replication of polyoma or EMC virus. ME cell monolayer cultures were irradiated at a UV intensity of 60 $\mu\text{w}/\text{cm}^2$ for 15, 30, or 45 sec. Immediately after irradiation, replicate cultures were tested for capacity to clone or to support the replication of polyoma or EMC virus. Cloning was carried out by dispersing irradiated or control cells with 0.02% ethylenediaminetetraacetic acid (EDTA) in Ca- and Mg-free phosphate-buffered saline (PBS; pH 7). Cell suspensions were counted, diluted in growth medium, and seeded in 60- by 15-

mm Falcon dishes. Clones were counted after 10 to 14 days of incubation. Cloning efficiency of ME cells under these conditions varied from 3.7 to 9.6%. ME cellular capacity for support of EMC virus growth was tested by infecting irradiated and control cultures with EMC virus at approximately 20 PFU/cell. EMC virus-infected cultures were harvested 24 hr after infection. Cultures were stored in stoppered tubes at -70°C until they were titrated for EMC plaques. The effect of UV irradiation on ME cellular capacity for the support of polyoma virus was determined by infecting irradiated and control monolayer cultures with polyoma virus (20 PFU/cell). After a 1-hr adsorption period, the cultures were washed once with PBS and 3.0 ml of maintenance medium was added to each culture. After 18 hr of incubation, the cultures were treated for 30 min with antipolyoma rabbit serum in a 1:50 dilution to reduce residual inoculum virus. The cultures were then washed twice with PBS, fresh maintenance medium was added to each culture, and the cultures reincubated for a total period of 51 hr, shown previously to be the time required for a single cycle of polyoma virus growth under these conditions (5). Infective-center analyses were performed on cultures infected as above, except that antibody treatment was applied immediately after the adsorption period. Cells were then dispersed with EDTA, counted, serially diluted in PBS, and plated on ME monolayers.

FIG. 1. Effect of UV irradiation on ME-cell cloning capacity and ability to support replication of polyoma or EMC virus. ME monolayer cultures were irradiated at $60 \mu\text{w}/\text{cm}^2$ for the indicated times, then infected with polyoma or EMC virus. Plating efficiency of untreated control = 5.6%. Polyoma yield in untreated, infected control = 9.3×10^7 PFU/culture. Polyoma infective centers in untreated, infected controls = 15 infective centers/100 cells. EMC held in untreated, infected controls = 2.3×10^8 PFU/culture. Symbols: ●, cloning capacity; ▲, polyoma replication; ○, polyoma infective centers formation; and, ■, EMC replication.

Plaques were developed at 8 days after plating and counted on the 9th day (Fig. 1). The cells were unimpaired in the capacity for EMC virus replication, even at the maximal UV dose. Cellular capacity for polyoma replication, however, declined with increasing UV dose, both in total virus production and in formation of infective centers, at the same rate as the loss of cloning capacity. This observation is in contrast to the findings of Logan and Whitmore (11) with cobalt-60-irradiated ME cells, in which the capacity for virus replication was more radioresistant than cloning capacity. This difference may be due to differences in the biological effects induced by nonionizing and ionizing radiation.

Effect of actinomycin D on the replication of polyoma virus in infected ME cells. We wondered whether the UV irradiation of ME cells was unique in impairment of the replication of polyoma virus or whether actinomycin D which interferes with cellular DNA (14) has a similar effect. ME cell monolayers in 60- by 15-mm Falcon dishes were treated with either 0.1 or 0.3 μg of actinomycin D per ml for 1 or 6 hr prior to infection with either polyoma virus or EMC virus. After exposure to the drug, the cultures were washed with PBS and infected with one of the viruses. The cultures were incubated for a period corresponding to a single cycle of virus replication. Polyoma virus-infected cultures were incubated for 51 hr and EMC virus-infected cultures were incubated for 24 hr. The cultures were then harvested, frozen, thawed, and titrated on ME cell monolayers (Fig. 2). It can be seen that the

FIG. 2. Effect of actinomycin D on replication of polyoma virus in infected ME cells. Cultures were treated with either 0.3 (broken line) or 0.1 μg (solid line) of actinomycin D per ml, then washed and infected with polyoma (●) or EMC (■) virus. Polyoma-infected cultures were titrated after 51 hr of incubation, and EMC-infected cultures were titrated after 24 hr of incubation.

capacity to support the replication of polyoma virus was impaired in ME cells treated with actinomycin D. Loss in the capacity of the cells to replicate polyoma virus was a function of both the concentration of actinomycin D and the duration of exposure. ME cultures similarly treated showed negligible impairment in the cellular capacity to support the replication of EMC virus. These results indicate that actinomycin D treatment, as well as UV irradiation, produces a loss in ME cellular capacity to support the replication of polyoma virus.

Polyoma V and T antigen synthesis in ME cells irradiated or treated with actinomycin D. In an attempt to determine whether polyoma T antigen and structural proteins were synthesized in irradiated ME cells, the production of these antigens was studied in cells receiving various doses of UV irradiation.

Synthesis of polyoma virus structural antigen was determined by irradiating ME cell monolayers on cover slips, infecting with polyoma virus at an estimated input multiplicity of 20 PFU/cell, then staining with fluorescein-conjugated, anti-polyoma rabbit serum after 51 hr of incubation. The number of stained cells was determined with the aid of a Zeiss fluorescence microscope. A total of 500 cells were counted for each point. The number of positive cells decreased as a function of increasing ultraviolet dose (Fig. 3). These re-

FIG. 3. Effect of UV irradiation on ME cellular capacity to support synthesis of polyoma V (structural) antigen. ME cells grown on cover slips were irradiated at $60 \mu\text{w}/\text{cm}^2$ for the indicated times. At 51 hr post-infection, the cover slips were fixed in acetone and stained with fluorescein-conjugated antipolyoma rabbit serum. Fluorescent cells were counted. Each point represents the mean of three separate experiments. A total of 500 cells from each group were counted for each experiment.

sults indicate that synthesis of polyoma structural protein was abolished or reduced to levels below those detectable by immunofluorescence. It appears from these data that the UV-sensitive, host-dependent function in polyoma virus replication occurs prior to structural protein synthesis.

Since the T antigen is one of the first demonstrable virus-specific components to appear in polyoma-infected cells (10), it was important to determine whether this antigen was synthesized in ME cells irradiated or treated with actinomycin D and subsequently infected with polyoma virus.

ME cultures in 100-mm petri dishes were irradiated at $60 \mu\text{w}/\text{cm}^2$ for 15, 30, 45, or 60 sec. In addition, replicate cultures were treated with 1.0, 0.3, or $0.1 \mu\text{g}$ of actinomycin D per ml for 1 or 6 hr. Treated cultures and untreated controls were infected with polyoma virus at an input multiplicity of 20 PFU/cell. After 51 hr of incubation, the cultures were harvested and titrated for polyoma T antigen as described. The 50% complement fixation end points of these samples were between 1:16 and 1:32 in the untreated infected controls and all treated samples. Our results indicate that T antigen synthesis is not reduced by UV irradiation or actinomycin D treatment of cells before infection.

The results of hemagglutinin titrations carried out concomitantly with the "T" antigen titrations indicated that there was a UV and actinomycin D dose-dependent loss in the production of polyoma hemagglutinating protein. This observation is in agreement with the results of immunofluorescence experiments (Fig. 3).

Effect of ultraviolet irradiation and actinomycin on DNA synthesis in uninfected and polyoma infected ME cells. The observation that UV irradiation or actinomycin D treatment impaired the capacity of mouse embryo cells to replicate polyoma virus posed the question of whether cells thus treated would show the characteristic stimulation of DNA synthesis after infection with polyoma virus. ME cells irradiated for 30 sec at $60 \mu\text{w}/\text{cm}^2$ and subsequently infected with polyoma virus lost their ability to synthesize DNA, as measured by incorporation of $\text{H}^3\text{-TdR}$ (Fig. 4). Similarly, ME cell cultures pretreated for 6 hr with $0.1 \mu\text{g}$ of actinomycin D per ml failed to show significant levels of $\text{H}^3\text{-TdR}$ incorporation whether or not infected with polyoma virus. These observations led to experiments designed to determine the effect of irradiation or actinomycin D treatment at various times during the period of viral DNA synthesis. Polyoma-infected and uninfected ME cultures were either irradiated for 30 sec at $60 \mu\text{w}/\text{cm}^2$ or treated with actinomycin D at $0.1 \mu\text{g}/\text{ml}$ at the times indicated

FIG. 4. Effect of UV irradiation on DNA synthesis of ME cells irradiated prior to polyoma infection. ME monolayer cultures were irradiated for 30 sec at $60 \mu\text{w}/\text{cm}^2$. Irradiated and unirradiated cultures were either mock-infected or infected with polyoma virus at 20 PFU/cell. At 3, 5, 15, 19, 23, 27, 31, 35, 39, 47, 51, and 55 hr after infection, duplicate cultures from each group were exposed to tritiated thymidine for 1 hr. Cultures were then washed with cold 0.4 N perchloric acid and acid-precipitable radioactivity was determined. Symbols: $\circ$, unirradiated, uninfected cultures; $\square$, irradiated, uninfected cultures; $\bullet$, unirradiated, infected cultures; and, $\blacksquare$, irradiated, infected cultures.

in Fig. 5. At 1 hr later, $2.5 \mu\text{C}$ of $\text{H}^3\text{-TdR}$ was added to each culture. After a 1-hr $\text{H}^3\text{-TdR}$ pulse, cultures were prepared and acid-precipitable radioactivity was determined. Ultraviolet light administered between 6 and 36 hr after infection markedly reduced DNA synthesis in both infected and uninfected cultures, as was the case with cultures irradiated before infection (cf. Fig. 4). Actinomycin D treatment, however, did not alter DNA synthesis in uninfected cells and even appeared to stimulate incorporation in the infected cells. Note that, although DNA synthesis was not inhibited in the presence of actinomycin D, production of infectious virus in the treated cultures was inhibited by 90% (Fig. 5).

Effect of UV and actinomycin D treatment on RNA synthesis in uninfected and polyoma-infected ME cells. The UV irradiation or actinomycin D treatment of ME cells results in an impairment of cellular ability to synthesize RNA, as measured by uridine incorporation. In experiments analogous

to the $\text{H}^3\text{-TdR}$ -uptake studies already described, the effect of irradiation and actinomycin treatment on $\text{H}^3\text{-UR}$ incorporation by uninfected and polyoma-infected ME cultures was determined. Note that a marked alteration in the pattern of RNA synthesis was observed in polyoma-infected cells as compared with uninfected controls (Fig. 6). In uninfected cultures, a small peak of uridine incorporation was observed at 30 hr after infection. The infected cultures exhibited a marked increase in the level of $\text{H}^3\text{-UR}$ incorporation over uninfected controls, with a maximal rate reached at 24 hr after infection. This altered pattern was observed in eight separate experiments. The level of incorporation fell sharply after 30 hr and approached background at 48 hr after infection, a point nearing the end of the polyoma virus replication cycle. In the irradiated cultures and those treated with actinomycin D, there was negligible incorporation of uridine. In the treated infected cultures, small peaks were

FIG. 5. Effect of UV irradiation or actinomycin D on DNA synthesis of infected ME cells when applied at various times during the polyoma virus replication cycle. Polyoma-infected and uninfected ME cultures were either irradiated for 30 sec at $60 \mu\text{w}/\text{cm}^2$ or treated with actinomycin D at $0.1 \mu\text{g}/\text{ml}$ at the times indicated. At 1 hr later, $2.5 \mu\text{C}$ of $\text{H}^3\text{-TdR}$ was added to each culture. After a 1-hr pulse, cultures were washed with cold 0.4 N perchloric acid and acid-precipitable radioactivity was determined. Symbols: $\circ$, untreated, uninfected cultures; $\bullet$, untreated, infected cultures; $\square$, irradiated, uninfected cultures; $\blacksquare$, irradiated, infected cultures; ∇ , actinomycin D-treated, uninfected cultures; and $\blacktriangledown$, actinomycin D-treated, infected cultures.

FIG. 6. Effect of UV irradiation or actinomycin D on RNA synthesis of infected ME cells when applied at various times during the polyoma virus replication cycle. Polyoma-infected and uninfected ME cultures were either irradiated for 30 sec at $60 \mu\text{w}/\text{cm}^2$ or treated with actinomycin D at $0.1 \mu\text{g}/\text{ml}$ at the times indicated. At 1 hr later, $2.5 \mu\text{C}$ of $\text{H}^3\text{-UR}$ was added to each culture. After a 1-hr pulse, cultures were washed with cold 0.4 N perchloric acid and acid-precipitable radioactivity was determined. Symbols: ○, untreated, uninfected cultures; ●, untreated, infected cultures; □, irradiated, uninfected cultures; ■, irradiated, infected cultures; ▽, actinomycin D-treated, uninfected cultures; and, ▼, actinomycin D-treated, infected cultures.

observed at 24 hr after infection, at which time maximal incorporation was also observed in the untreated cultures. These peaks were probably due to RNA synthesis by a small number of undamaged cells in the treated cultures. Treated uninfected cultures showed levels of $\text{H}^3\text{-UR}$ incorporation only slightly above the background. Thus, both UV irradiation and actinomycin treatment produced rapid cessation of RNA synthesis in ME cells whether infected or not.

Effect of UV irradiation and actinomycin D treatment at various times during infection on the production of infectious polyoma virus. The point at which the last essential UV- and actinomycin D-sensitive syntheses occurred during the polyoma replication cycle was determined in the following manner. ME cells were infected with polyoma virus, and, at various times, they were frozen to determine the amount of infectious virus produced at that point, irradiated (30 sec at 60

$\mu\text{w}/\text{cm}^2$) and frozen to measure the amount of virus directly inactivated by irradiation, irradiated, or treated with actinomycin D (final concentration = $0.3 \mu\text{g}/\text{ml}$) and reincubated for the remainder of a single virus replication cycle and then frozen. All cultures were subsequently thawed and titrated for polyoma plaques. From the maturation curve (Fig. 7) for infected untreated cells, it may be seen that new virus was detectable by 18 hr after infection. The cultures which were irradiated and immediately frozen showed no significant differences from nonir-

FIG. 7. Effect of UV irradiation and actinomycin D treatment at various times during infection on the production of infectious polyoma virus in ME cells. ME-cell monolayer cultures were infected with polyoma virus ($20 \text{ PFU}/\text{cell}$) and at the indicated times were treated as follows: (●) frozen to determine the amount of infectious virus produced at that point; (□) irradiated for 30 sec at $60 \mu\text{w}/\text{cm}^2$ and reincubated; and (▼) treated with actinomycin D at a final concentration of $0.3 \mu\text{g}/\text{ml}$ and reincubated. After addition, actinomycin D remained in the cultures until the end of the experiments. Treated cultures were frozen at 48 hr. All cultures were then thawed and titrated for polyoma plaques.

radiated cultures frozen at the same time. Therefore only the nonirradiated controls are shown in Fig. 7. The curves for the UV-irradiated and actinomycin D-treated cultures are similar. They show that both UV and actinomycin D failed to reduce the yield of infectious polyoma virus when administered at 30 hr or later after infection. This observation correlates well with the results of the previous experiments which demonstrate that RNA synthesis was virtually complete in infected cells by 30 hr after infection.

DISCUSSION

The salient features of the results presented in this paper are as follows.

Both UV irradiation and actinomycin D produce impairment in the capacity of mouse embryo cells to support the replication of polyoma virus, at levels of treatment at which neither agent significantly reduces cellular capacity for EMC virus synthesis. The loss in capacity for polyoma virus synthesis is apparently an all-or-none effect, since treated cells do not give rise to infective centers and produce no polyoma structural antigen.

The synthesis of polyoma T antigen is not impaired by either UV irradiation or treatment with actinomycin D.

Cells which have lost the capacity to synthesize polyoma virus following treatment prior to infection are impaired in their ability to synthesize both DNA and RNA, as measured by uptake of tritiated thymidine or uridine. Irradiation of cells during infection virtually abolishes the synthesis of both DNA and RNA. Actinomycin D stops RNA synthesis within 1 hr after addition of the drug to ME cell cultures, but does not inhibit DNA synthesis during this period.

Both agents lose the ability to prevent the replication of a full yield of infectious polyoma virus when they are administered at 30 hr or more after infection. This point corresponds with that at which polyoma virus-induced stimulation of RNA synthesis in infected cultures subsides.

It can be concluded on the basis of these experiments that the functional integrity of the mouse embryo cell genome is required for the replication of polyoma virus. That the effect does not reflect general impairment of ME cellular functions is indicated by the continued ability of the cells to support EMC replication after treatment with either UV or actinomycin D. The effect on polyoma virus replication in treated cells appears to occur very early in infection, prior to the onset of viral DNA and viral structural protein synthesis. The decrease in the titer of adsorbed inoculum-polyoma virus during the first

18 hr after polyoma infection and the synthesis of T antigen in the treated cells indicate that penetration and uncoating of the polyoma virus proceed normally after irradiation or exposure of the ME cell to actinomycin D. The production of T antigen in the treated cells also suggests that the synthesis of new DNA is not required for "T" antigen synthesis. This finding further supports the observation (10) that the viral genome carries the information for the synthesis of T antigen.

The nature of the required host-cell function(s) is not as yet clear. The close parallel between the loss in cloning ability of the cells and loss in the capacity for polyoma synthesis suggests that cellular DNA synthesis, or at least the cellular ability to synthesize DNA, is necessary for polyoma virus replication. This suggestion is further supported by the observation that treated cells incapable of synthesizing virus were also unable to synthesize measurable quantities of DNA, even after infection with polyoma virus. The effect of UV differs in this respect from that of X-irradiation, as reported by Minowada (12) and Gershon et al. (9). Our findings support those of Ben-Porat, Kaplan, and Tennant (3) who observed that ME cells treated with 5-fluorouracil prior to infection with polyoma virus lost the ability to synthesize DNA and to support the replication of polyoma. They suggested the possibility that cellular DNA synthesis was necessary for polyoma replication. Note that UV irradiation inhibited DNA synthesis when applied to any point during the period of maximal incorporation of thymidine, whereas actinomycin D did not inhibit DNA synthesis once it had been initiated, at least during the 1-hour period covered in our experiments. It would appear, therefore, that an effect on DNA synthesis alone could not account entirely for the inhibitory effect of both agents.

The results of the experiments show that mouse embryo cells exhibit a markedly altered pattern of RNA synthesis after infection with polyoma virus. The principal feature of the changed pattern was a marked stimulation in the overall rate of RNA synthesis, with maximal uptake of uridine occurring at 30 hr after infection.

This finding is in agreement with that of Fried and Pitts (8) who observed stimulation of RNA synthesis in polyoma-infected ME cells which were rendered metabolically quiescent by incubation in low serum-containing medium.

Benjamin (2) detected the synthesis of polyoma-specific messenger RNA in cells transformed or productively infected with polyoma virus with the aid of hybridization techniques. It is probable that the increased level of RNA synthesis ob-

served in our experiments represents production of both cellular and virus-specific RNA.

Treatment with UV or actinomycin D inhibited RNA synthesis in both polyoma-infected and uninfected cultures, as determined by measurement of H^3 -UR incorporation. The capacity for synthesis of at least one type of messenger RNA remained in the treated cells, however, since they were capable of supporting polyoma T antigen synthesis after infection. It is of interest to note that both agents failed to prevent synthesis of a full yield of polyoma virus when they were administered to the infected cultures after the period of peak RNA synthesis. Since treatment during the period of virus synthesis would result in inhibition of both cellular and viral functions, it cannot be determined at present whether the last essential synthesis is controlled by the host cell or viral genome. Actinomycin D is an inhibitor of messenger RNA synthesis (14) and shares with UV the ability to prevent RNA synthesis and polyoma virus replication in ME cells. It appears therefore that cellular DNA-dependent RNA synthesis may be required for the formation of polyoma structural components and infectious virus. Further study is required to elucidate the role of cellular genetic apparatus in polyoma virus synthesis. Studies on the nature of the UV-sensitive host functions are currently in progress.

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