Interactions of Polyoma and Mouse DNAs

I. Lytic Infection of Bromodeoxyuridine-Prelabelled Mouse Embryo Cells

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On CsCl isopycnic centrifugation of the DNA extracted from secondary mouse embryo (ME) cultures grown in the presence of 5-bromodeoxyuridine (B UdR) and 5-fluorodeoxyuridine (FUdR) for 40 h, 10 to 25% of the DNA was found to be unsubstituted, 70 to 80% was bromouracil-hybrid DNA, and 5 to 10% was heavy DNA. These results together with cell number determinations, autoradiography, and Feulgen microspectrophotometry revealed three types of cells in these cultures: (i) 60 to 80% of the cells replicated their DNA once, divided, and then stopped mitotic activity, (ii) 5 to 10% were going through a second round of DNA replication; whereas (iii) 10 to 30% did not replicate DNA during the BUdR-FUdR exposure. After the transfer of these cultures to normal medium (without BUdR-FUdR), up to 20% of the cells resumed DNA synthesis asynchronously within 60 h, but no increase in cell number was observed. BUdR-FUdR-treated cultures, which were infected with polyoma virus in the absence of the thymidine analogues, supported a lytic infection to the same extent as did untreated ME cultures. This was concluded from the similar number of cells, which were induced to synthesize DNA, from the similar replication rate of the viral DNA, from the similar number of cells containing polyoma capsid proteins, and from the similar yields of progeny virus determined by hemagglutination and plaque formation. Thus, BUdR-prelabelled ME cultures are suitable for the investigation of interactions of the polyoma and mouse genomes during the lytic infection.

Halogenated thymidine analogues such as 5-bromodeoxyuridine (B UdR) may be incorporated into viral (14), bacterial (3), and mammalian (10) DNA and thereby increase their buoyant density in CsCl isopycnic centrifugation. Thus, BUdR has been used to investigate the mode of DNA replication in various systems (e.g., 4, 11, 14). In mammalian cells, BUdR incorporation is enhanced by the simultaneous presence of 5-fluorodeoxyuridine (FUdR), which after intracellular phosphorylation inhibits the endogenous thymidylate synthesis (22). After one round of replication in the presence of BUdR-FUdR, bromouracil (BU) substitutes for most or all of the thymine in the newly synthesized strand of the “BU-hybrid” DNA. In some cases a second round of replication may take place leading to “heavy” DNA, in which essentially all thymine is replaced by BU (1).

Density labeling of mouse cellular DNA with BUdR prior to polyoma virus infection should facilitate investigations of host-virus interactions, because the mouse and polyoma DNA can then be easily separated by CsCl isopycnic centrifugation. It was essential, however, to see first whether the incorporated analogue influences the course of infection.

Primary mouse kidney cells, which are used in this laboratory for most of the studies on the lytic polyoma infection, do not incorporate BUdR into their DNA in sufficient amounts to change the buoyant density (24), because these cells possess very low levels of thymidine kinase (15). Mouse embryo (ME) cells, however, which are also permissive for polyoma virus, incorporate it readily. In this paper the properties of BUdR-labeled ME cultures are described. We have also attempted to clarify some divergence in earlier reports (1, 2, 16, 22) about the effects of BUdR (or 5-iododeoxyuridine) on cell multiplication and DNA replication during and after exposure to the analogues. Finally, it is demonstrated that BUdR-prelabelled ME cultures support a lytic infection by polyoma virus to the same extent as do untreated ME cultures.

MATERIALS AND METHODS

Cell culture media and buffers. Eagle medium with a fourfold concentration of amino acids and vitamins was used (26). Unless otherwise stated, the
medium was supplemented with 10% calf serum (CS) for growth of uninfected cultures and 10% horse serum (HS) for infected cultures (both sera were obtained from Microbiological Associates, Bethesda, Md.).

The buffer for washing cell cultures was TD (25) supplemented with 100 μg of streptomycin sulfate per ml and 500 U of penicillin G per ml. Phosphate-buffered saline (PBS) is 0.8% NaCl, 0.02% KCl, 0.115% Na₂HPO₄, 0.02% KH₂PO₄, 0.001% MgCl₂·6H₂O, 0.001% CaCl₂ (pH 7.4).

Cultures of primary and secondary ME fibroblasts. ME fibroblasts were prepared from 12- to 14-day-old embryos of Swiss albino CR 1 mice. Large plastic dishes (9.4-cm diameter) were seeded with 4 x 10⁷ to 5 x 10⁷ cells and incubated at 37°C in a water-saturated atmosphere containing 5% CO₂. Seventy-two hours after plating the primary cultures were trypsinized (0.2% trypsin [Difco] 1:250, 0.8% NaCl, 0.03% KCl, 0.0073% Na₂HPO₄, 0.002% KH₂PO₄, 0.0015% phenol red, and supplemented with antibiotics as for TD, pH 6.8). For the secondary ME cultures, which were used in all experiments, 5 x 10⁶ to 7 x 10⁶ cells were seeded per large dish. Cover slip cultures were obtained by seeding 10⁶ to 1.5 x 10⁷ cells into 3.5-cm plastic dishes containing a glass cover slip (15 by 15 mm).

For low serum cultures (7), cells were plated in Eagle medium with 1% CS and cultured in the presence or absence of BUdR-FUDR. After virus infection they were maintained in medium with 1% HS. The low serum concentration did not significantly change the BUdR incorporation into the mouse DNA or the cell proliferation during the BUdR-FUDR exposure, but markedly decreased the growth rate of untreated ME cells (see Fig. 1B).

Standard method for density labeling of ME cells with BUdR. The cells were seeded as described above in medium containing 5 μg of BUdR per ml (Sigma Chemical Corp.) and 15 μg of FUDR per ml (a gift from Hoffmann-La Roche, Basel, Switzerland) and incubated for 40 h. The medium was then removed, and the cell monolayers were washed once with TD and fed with medium free of BUdR-FUDR for 8 to 10 h to exhaust the intracellular pool of the base analogues. Cells treated this way will be referred to as BUdR-prelabeled ME cells (ME-BU cells).

Infection with polyoma virus. After removal of the medium, the cultures were infected with 30 to 50 PFU/cell using a stock polyoma lysate (wild-type strain) of about 10⁸ PFU/ml. After 2 h of adsorption, the cultures were washed once with TD and maintained in Eagle medium. To obtain uninfected control cultures ("mock-infected") the same procedure was applied, but an equal volume of sterile medium was used instead of the polyoma lysate during the period of adsorption.

Radioisotopic incorporation of precursors. For radioisotopic labeling of DNA, [³H]thymidine or [³H]thymidine was added to the culture medium, which only contained serum when cells were cumulatively labeled for periods exceeding 10 h. The amounts of radioisotopes and the labeling times are mentioned in the text. At the end of the labeling period, the radioactive medium was removed, the cultures were washed three times with prewarmed TD, and incubated in medium without serum for 1 h ("post-labeling") (20, 24) before the cells were either fixed or lysed.

Cover slip cultures to be used for autoradiography were washed and fixed as described earlier (20). To determine the radioisotopic incorporation, the fixed cultures were placed into toluene scintillator and counted. They were then washed with toluene, air-dried, and mounted. The procedure used for autoradiography was described earlier (20).

Feulgen microspectrophotometry. Cover slip cultures were washed once with PBS and fixed with 70% ethanol for 30 min at room temperature. Feulgen microspectrophotometric analysis was performed by G. Haemmerli at the Division of Cancer Research, Pathology Institute, University of Zürich, Switzerland (9).

Determination of polyoma capsid proteins. Uninfected and polyoma-infected cover slip cultures were washed twice with PBS, fixed, and assayed for synthesis of polyoma capsid proteins by indirect immunofluorescence as previously described (20).

DNA extractions. The culture (or post-labeling) medium was removed, and the cultures were washed once with TD. The cells were lysed by adding 1.5 ml of 0.6% sodium dodecyl sulfate (SDS)-0.01 M EDTA, pH 7.5, to a large dish. After 15 min at room temperature, the viscous lysate was collected.

For total DNA extracts, i.e., cellular DNA from uninfected cultures and cellular plus viral DNA from infected cultures, the DNA was sheared by pipetting the lysate 10 times through a 2-ml tissue culture pipette (opening 1.5 mm). The sheared cellular DNA sediments between 30S and 60S (see Fig. 5). SDS and proteins were precipitated by adding 1/4 volume of 5 M NaCl and storing at 4°C for 12 to 15 h. The mixture was centrifuged at 12,000 x g for 30 min in the cold. The supernatant fluid containing the DNA was used for further physicochemical analysis.

Viral and low-molecular-weight cellular DNA was selectively extracted from similar SDS lysates by the procedure described by Hirt (12).

CsCl density gradient equilibrium centrifugation. The total DNA extract from two large dishes of ME-BU cells was adjusted to a density of 1.75 g/cm³ with CsCl. Samples of 3 ml were then centrifuged in a Spinco SW26 rotor at 40,000 rpm for 30 h at 20°C. Fractions were collected and assayed for absorbance and radioactivity.

Sedimentation velocity analysis of DNA extracts. Samples of the total DNA extracts from polyoma-infected ME and ME-BU cultures were sedimented through a solution of CsCl with a density of 1.50 g/cm³ as described earlier (12, 18).

Polyoma virus quantitation. The medium and the remaining cells from two large dishes were collected 81 h after infection. After freezing and thawing three times, the cell debris was collected by centrifugation at 3,000 x g for 15 min and resuspended in 2 ml of phosphate buffer (0.001 M, pH 7.9). The resuspended pellets were homogenized in a Sorvall microhomogenizer at 25,000 rpm for 10 s and treated with 0.2 ml of receptor-destroying enzyme (Microbiological Associates, Bethesda, Md.) overnight at 37°C. After a second centrifugation to remove the cell debris, the
supernatants were pooled. The virus titer was determined by the hemagglutination (HA) test (6). The infectivity was determined by a plaque assay using confluent monolayers of primary baby mouse kidney cells and the techniques described earlier (5).

Radioisotopes. [Methyl-5,3H]thymidine (specific activity 20 Ci/mmol) and [6-3H]-B UdR (specific activity 10 Ci/mmol) were purchased from NEN Chemicals, Frankfurt, Germany.

RESULTS

Multiplication of ME cells during and after exposure to BUdR-FUdR. Secondary ME cultures were grown in the presence or absence of BUdR-FUdR. After 40 h of incubation the medium containing BUdR-FUdR was removed and replaced by normal medium (without BUdR-FUdR). At the times indicated in Fig. 1 the number of cells per culture was determined. In medium supplemented with 10% CS (Fig. 1A), untreated cultures showed the expected increase in cell number, whereas the cell number in the cultures grown in the presence of BUdR-FUdR increased only by 50%. No further increase was detected after the transfer of the cultures to normal medium for the next 48 h. There was no cytopathic effect observed during this time.

Figure 1B shows the same experiment using low serum (1%) medium. By this method similar cell numbers were obtained both in the BUdR-FUdR-exposed cultures and in the untreated cultures. This was an advantage for the comparison of the synthesis of polyoma DNA and the production of progeny virus in ME-BU and ME cultures. No difference in morphology was observed between the BUdR-FUdR-exposed and the untreated cells.

DNA synthesis in BUdR-FUdR-exposed ME cultures. Secondary ME cultures were grown on cover slips in the presence of BUdR-FUdR under standard conditions. The number of DNA synthesizing cells during the BUdR-FUdR exposure was determined by cumulative labeling with [3H]BUdR (1 μCi/ml). Twenty-four and forty hours after plating, the cells were fixed and processed for autoradiography. It was found that 71% of the nuclei were labeled at 24 h and 72% at 40 h. Untreated cultures grown in medium supplemented with 10% CS and cumulatively labeled by the addition of 1 μCi of [3H]thymidine per ml had 84% of the nuclei labeled at 24 h and 91% at 40 h after plating, whereas untreated cultures in low serum (1%) medium gave results similar to the BUdR-FUdR-exposed cultures (70% both at 24 and 40 h after plating). It is likely that the remainder of the cells in the BUdR-exposed cultures (and in the untreated cultures grown with 1% CS), i.e., 25 to 30%, did not synthesize DNA during the prelabeling period (see below).

The rate of [3H]BUdR incorporation and the changes in the number of DNA synthesizing cells with increasing time of BUdR-FUdR exposure were determined by pulse labeling experiments. Cultures grown on cover slips in the presence of BUdR-FUdR were labeled with [3H]BUdR (5 μCi/ml) for 2.5 h at various times between 24 and 120 h after plating. Both the rate of radioisotopic incorporation and the number of DNA synthesizing cells determined by autoradiography dropped rapidly between 24 and 48 h. After 48 h, they leveled off, and the labeled nuclei remained between 6 and 3% up to 120 h. It will be shown subsequently that mostly heavy DNA was formed during this period.

To determine where in the cell cycle the majority of the BUdR-FUdR-exposed cells were arrested, the DNA content of individual cells was estimated by Feulgen microspectrophotometry. Figure 2A shows the typical pattern of ME cells actively growing in medium with 10% CS fixed 20 h after plating. About 15 to 20% of these cells had DNA values between 2n and 4n, which means that they were actually replicating their DNA (S-phase). In contrast, cultures grown in the presence of BUdR-FUdR
and fixed 40 h after plating had no cells in the S-phase, but showed a bimodal distribution with 85 to 90% of the cells exhibiting absorbance values corresponding to a 2n DNA complement, whereas the remaining cells contained about twice that amount of DNA (Fig. 2B). The reason for the small shift to higher absorbance observed both in the 2n and 4n peaks is unknown. It might reflect the observation that a few cells apparently initiate a second cycle of DNA replication, which leads to the formation of heavy DNA.

The results reported thus far suggest that, in BUdR-FUdR-exposed cultures, 60 to 80% of the plated cells replicated their chromosomal DNA once and subsequently divided; thereafter essentially all cells lost the ability for further mitosis even after the transfer to normal medium (Fig. 1A and B).

**Analysis by CsCl density gradient equilbrium centrifugation of the DNA from BUdR-labeled cultures.** (i) In the analytical ultracentrifuge. The DNA extracted from ME-BU cells showed three main bands and two minor bands (Fig. 3). The largest fraction of the DNA banded at a buoyant density of 1.753 g/cm³ and corresponded to “BU-hybrid” DNA (HL). The other two major peaks corresponded to unsubstituted “light” DNA (LL), buoyant density 1.702, and to fully substituted “heavy” DNA (HH), buoyant density 1.800. The two minor peaks with buoyant densities of 1.724 and 1.764 represent the two forms of the BU-hybrid satellite DNA (HL and LH) (2). In this gradient the unsubstituted (LL) and the fully substituted (HH) satellite DNA were not resolved.

(ii) In the preparative ultracentrifuge. In preparative CsCl density gradient equilibrium centrifugations only the three main peaks (HH, HL, and LL) were resolved both by optical density readings at 260 nm (Fig. 4) and radioactivity measurements of the individual fractions.
Note that also in untreated cultures not every cell became labeled with \(^{[3]H}\)-thymidine during 40 h (see results above).

The results in Table 1 show, however, an increase in the amount of HH DNA which was apparently formed by replication of HL DNA. This is also indicated by the similar amount of \(^{[3]H}\)BUdR incorporated into the HL and HH peak. Because the radioactivity in peak HH did not exceed that in peak HL, it is furthermore concluded that the HH DNA itself does not replicate any more. HH DNA is mouse chromosomal DNA, because (i) autoradiography showed that \(^{[3]H}\)BUdR was incorporated only in the nucleus; (ii) HH DNA could not be extracted selectively by the method of Hirt (15); (iii) it did not form dense (superhelical) bands in an ethidium bromide-containing CsCl gradient (21); and (iv) it could not be distinguished from HL DNA in the electron microscope (17), both types showing long linear fragments of DNA. It is concluded therefore, that most or possibly all of the cells that continued to incorporate \(^{[3]H}\)BUdR under prelabeling conditions maintained for more than 40 h were going through a second round of DNA replication (see also Fig. 2B). Presumably, these cells became tetraploid and contained HH and HL DNA.

**Evidence for DNA synthesis in a fraction of BUdR-prelabeled cells after the transfer to normal medium (without BUdR-FUDR).** After the transfer of ME-BU cells to normal medium, no increase in total DNA per culture was detected by the diphenylamine reaction (8) for periods up to 70 h. Evidence for DNA replication in a fraction of cells was obtained, however, by other methods as follows.

(i) Cumulative labeling of ME-BU cells with \(^{[3]H}\)thymidine (1 \(\mu\)Ci/ml) for various periods from the time of the transfer up to 60 h and analysis by autoradiography showed a gradual increase of labeled nuclei from 2% at 12 h to 12% at 36 h and 20% at 60 h after the transfer (experiment with 10% CS). In another experiment (1% HS) 16 to 20% of the cells became labeled during 60-min pulses with \(^{[3]H}\)thymidine (2 \(\mu\)Ci/ml) made between 30 and 50 h after the transfer.

(ii) The analysis by Feulgen microspectrophotometry of ME-BU cultures fixed at 36 h after the transfer to normal medium indicated that a fraction of cells (10 to 15%) had DNA measures between 2n and 4n and was therefore synthesizing DNA (Fig. 2C).

(iii) At various times after the transfer to normal medium, BUdR-prelabeled cultures were labeled with \(^{[3]H}\)thymidine (2 \(\mu\)Ci/ml) and the DNA was extracted as indicated in

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**FIG. 4. Example of a preparative CsCl density gradient equilibrium centrifugation of DNA extracted from ME-BU cells.** The DNA was extracted from two large dishes of BUdR-prelabeled ME cells (standard conditions) 2 h after the transfer to normal medium (first sample in Table 2). The extract was adjusted with solid CsCl to a density of 1.748 g/cm\(^3\), and 3 ml of it was centrifuged in a Spinco SW 65 rotor at 40,000 rpm for 30 h at 20 C. The density gradient was determined in a separate tube with the same amount of CsCl solution of the same density. The amount of DNA in each peak (see Table 2) was determined by weighing the area under the peak after subtraction of the background (dashed line).

For more than 10 independent cultures labeled with BUdR under standard conditions (40 h) the relative amounts of the DNA in the three main bands were estimated graphically from the UV light absorption curve. The values found were, for HL DNA 70 to 80%, for LL DNA 10 to 25%, and for HH DNA 5 to 10%.

In another experiment we wanted to detect possible quantitative changes of the DNA in the three main peaks in cultures kept under prelabeling conditions for more than 40 h. For this purpose cells were grown in the presence of BUdR-FUDR for 46, 77, and 125 h, the medium being changed every 48 h; \(^{[3]H}\)BUdR (5 \(\mu\)Ci/ml) was present in the prelabeling medium for the last 6 h before extraction. The results shown in Table 1 indicate that there was no change in the amount of LL DNA during the period of observation. This rules out the possibility that the majority of the cells containing unsubstituted DNA would replicate DNA and divide normally, but would not incorporate BUdR due to absence of thymidine kinase. Therefore, most or possibly all of these cells did not replicate their DNA during the time of observation (125 h).
Table 2. After a preparative CsCl density gradient equilibrium centrifugation, the amount of DNA in each peak was estimated graphically from the optical density profile (see example in Fig. 4). The results in Table 2 indicate a considerable, but rather slow shift of DNA from peak HL to peak LL, with increasing time after the transfer. In this experiment there was again no increase in total DNA per culture after the transfer to normal medium. In the [3H]-thymidine-labeled samples, essentially all of the radioactivity was found in two peaks coinciding with peak HL and peak LL of the optical density profile. The presence of radioactivity in both peaks indicated replication of HL DNA, but there was always more radioactivity in peak LL than in peak HL. This might be due to replication of LL DNA in a few cells that did not incorporate BUdR during the prelabeling period and/or to repair processes which act selectively on the H strand replacing BUdR by thymidine.

All the results reported here are compatible with the idea that, after the transfer of ME-BU cultures to normal medium, individual cells start to synthesize DNA very asynchronously. Apparently, cells having incorporated BUdR and cells with unsubstituted DNA are both able to initiate DNA replication. Thus there is no irreversible inhibition of DNA replication in the larger part of the BUdR-prelabeled cells. For as yet unknown reasons replication of HL DNA seems to proceed very slowly over a period of several days.

**Productive infection of BUdR-prelabeled ME cells with polyoma virus.** To compare the time course of infection and the production of progeny virus in ME-BU and untreated ME cultures, the cells were grown and maintained after infection in low serum (1%) medium, in order to have similar cell numbers in the two cultures (see Fig. 1B).

At different times after infection, the number of DNA synthesizing cells was determined by autoradiography, and the number of viral capsid antigen containing cells was determined by immunofluorescent staining. The ME-BU and ME cultures had the same percentage of DNA synthesizing cells and of viral capsid protein containing cells at 20, 30, and 44 h after infection (Table 3). Twenty hours after infection, 30% of the cells were induced to synthesize DNA by the polyoma infection. This number did not increase at later times. At 44 h after infection, 25% of the cells contained viral capsid proteins.

The rate of synthesis of viral DNA was

**Table 1. Synthesis of HL and HH DNA in ME cells exposed to BUdR-FUdR**

<table>
<thead>
<tr>
<th>Total time of BUdR-FUdR exposure (h)</th>
<th>Time of [3H]BUdR labeling (5 μCi/ml) (h)</th>
<th>DNA under the peaks (%)</th>
<th>Radioactivity under the peaks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>40–46</td>
<td>5.3 78.3 16.4</td>
<td>43 57 —</td>
</tr>
<tr>
<td>77</td>
<td>71–77</td>
<td>14.5 70.6 14.9</td>
<td>43 57 —</td>
</tr>
<tr>
<td>125</td>
<td>119–125</td>
<td>21.0 63.4 15.6</td>
<td>49 51 —</td>
</tr>
</tbody>
</table>

*After a preparative CsCl density gradient equilibrium centrifugation the relative amounts of DNA (UV light absorbance) and of radioactivity in each peak were determined.

**Table 2. Replication of HL DNA in ME-BU cultures after transfer to normal medium**

<table>
<thead>
<tr>
<th>Time after transfer (h)</th>
<th>DNA*</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>HH (%)</td>
</tr>
<tr>
<td>Extraction</td>
<td>Label*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>144.3</td>
<td>7.6</td>
</tr>
<tr>
<td>14</td>
<td>12-13</td>
<td>124.6</td>
</tr>
<tr>
<td>26</td>
<td>24-25</td>
<td>124.4</td>
</tr>
<tr>
<td>50</td>
<td>48-49</td>
<td>117.1</td>
</tr>
<tr>
<td>7 days</td>
<td>104.4</td>
<td></td>
</tr>
</tbody>
</table>

*The amount of DNA in each peak was determined graphically from the optical density profile (see Fig. 4); the total amount of DNA is expressed in arbitrary units of area weight.
* A 2-μCi amount of [3H]thymidine per ml.
* More than 95% of the total radioactivity in the gradient was associated with the HL and LL peaks of the optical density profile.
TABLE 3. Virus-induced synthesis of DNA and synthesis of viral capsid proteins in ME-BU and in untreated ME cells

<table>
<thead>
<tr>
<th>Time after infection (h)</th>
<th>Infection</th>
<th>Autoradiography (% labeled nuclei)</th>
<th>Immunofluorescence (% labeled nuclei)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ME</td>
<td>ME-BU</td>
</tr>
<tr>
<td>20</td>
<td>I</td>
<td>53.9 (6.6)</td>
<td>55.1 (10.4)</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>21.5 (4.7)</td>
<td>20.4 (5.0)</td>
</tr>
<tr>
<td>30</td>
<td>I</td>
<td>52.3 (4.2)</td>
<td>51.6 (6.8)</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>18.6 (3.4)</td>
<td>16.6 (4.7)</td>
</tr>
<tr>
<td>44</td>
<td>I</td>
<td>47.6 (3.5)</td>
<td>44.3 (4.6)</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>15.3 (1.4)</td>
<td>16.1 (2.4)</td>
</tr>
</tbody>
</table>

* I, Infected; MI, mock-infected.

a Nuclei (600) were counted on each of two cover slips.

b In parenthesis is the total radioactivity per cover slip in (counts per min) x 10^-4 (mean of two cover slips).

e Estimated from the relative amounts of radioactivity incorporated into polyoma DNA I (20S) during a 1-h pulse with 5 µCi of [3H]thymidine per ml. After sedimentation velocity analysis of the total DNA extracts (Fig. 5) the counts in the 20S peak were calculated as the percentage of the counts in all fractions. At 22 h after infection, 10.3% (mean of two extracts) were in 20S polyoma DNA in ME-BU and 8.6% were in ME cultures. At 36 h after infection, the values were 9.4% and 12.8%, respectively.

Finally, the amount of progeny virus produced on ME-BU and ME cultures was determined by the hemagglutination and the infectivity assay. After infection for 81 h, 1,400 HA units/ml were found both in the ME-BU and ME cultures. The infectivity titer was, however, 30% lower in the ME-BU than in the ME culture, which gave a titer of 2.5 x 10^8 PFU/ml. It is reported in the accompanying paper (23) that all polyoma preparations obtained from ME-BU cultures always contained more than 50% pseudovirions. This finding could explain the lower infectivity titer resulting from the ME-BU cultures.

These results indicate that the lytic infection proceeds essentially the same in ME-BU and ME cultures with respect to time course and production of progeny virus.

**DISCUSSION**

In secondary ME cultures which were grown in the presence of BUdR-FUdR for 40 h, three types of cells were observed. (i) Most cells went through one cycle of DNA replication, divided, and then stopped further mitotic activity; i.e., they did not start a second round of DNA replication, if maintained in the presence of BUdR-FUdR for up to 120 h. These cells contained HL DNA, which accounted for 70 to 80% of the DNA after 40 h of BUdR labeling.

(ii) Ten to 30% of the cells did not replicate their DNA during the BUdR-FUdR exposure. Neither an increase nor a decrease in the amount of LL DNA was observed during maintenance of the cultures in BUdR-FUdR for up to 120 h. Cultures seeded at higher cell densities (10 x 10^6 to 15 x 10^6 cells per large dish) usually had a higher proportion of LL DNA, up to 40% after 40 h of BUdR labeling.

(iii) About 5 to 10% of the cells had initiated a second round of DNA replication. Most likely...
these cells became tetraploid and contained both HH and HL DNA. The amount of HH DNA in the culture increased with prolonged exposure to BUDR-FUdR. This and the results obtained by autoradiography indicated that, between 40 and 120 h of BUDR labeling, again a few percent of cells were able to initiate a second round of DNA replication.

In cultures labeled with BUDR for 40 h and then transferred to normal medium, a small but gradually increasing number of cells reached 20% started to synthesize DNA within 60 h, but no increase in cell number was observed. From the quantitative changes of HL and LL DNA and the rate of [3H]thymidine incorporation, it was concluded that both cells with HL and with LL DNA could resume DNA replication. One week after the transfer, almost 70% of the HL DNA was shifted to peak LL, which indicates that there was no irreversible inhibition of DNA replication in the larger part of the BUDR-containing cells. The situation is thus similar to that observed in L-cells (2) and murine mast tumor cells (19) which had been prelabeled with BUDR or 5-iododeoxyuridine, respectively. Several questions remain open about the inhibitory effect of halogenated thymidine analogues on DNA replication in mammalian cells. In ME cells the inhibition caused by BUDR incorporation is apparently not an absolute one, but a long period is required for the cells to eliminate the analogue. It is not known to what extent replication and repair processes are involved; neither do we know whether the cells will eventually reacquire the ability to divide. Studies in this direction are rendered difficult because of the heterogeneous cell population in the BUDR-FUdR-treated cultures and the slow and asynchronous response of the cells after the transfer. On the other hand, the data presented show that polyoma infection of BUDR-prelabeled cultures induces stimulation of DNA synthesis that is similar to that observed in untreated cultures with respect to the incorporation of radioactive precursors and the number of cells induced to synthesize DNA (see also reference 16).

BUDR-prelabeled ME cells infected with polyoma virus in the absence of the analogue produce progeny virus. Direct evidence for this is given by the fact that progeny virus obtained from BUDR-prelabeled cultures contains pseudovirions with fragments of HL mouse DNA. From such virus preparations pure polyoma DNA III could be isolated and new information on the formation of polyoma pseudovirions was obtained (see accompanying paper [23]).

Finally, the results reported here show that there is practically no quantitative difference between the polyoma infection of untreated and BUDR-prelabeled ME cultures. Therefore, these cells can be used to study interactions between viral (polyoma) and host (mouse) genomes in the lytic infection, e.g., the "integration" of polyoma DNA into the mouse chromosomal DNA (H. Türlir, Experientia 28:752, 1972, and manuscript in preparation).

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LITERATURE CITED


