IN VITRO STUDIES ON POLYOMA VIRUS INFECTION, WITH
SPECIAL REFERENCE TO DEOXYRIBONUCLEIC ACID
SYNTHESIS AS DETERMINED BY AUTORADIOGRAPHY*1
(Plates VI~VIII)

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Synopsis

DNA synthesis in polyoma virus-infected mouse embryo cultures was studied by
means of autoradiography and correlated cytological and immunofluorescent analyses.
In confirmation of previous reports concerning the "transformation" of infected
cultures, continuously-growing cell populations were obtained. Nevertheless, these
cell populations were found to be merely "polyoma virus carriers," capable of pro-
ducing parotid tumors after inoculation into newborn mice, but not transplantable as
neoplastic cells.

The autoradiographic results obtained indicated that de novo synthesis of viral DNA
takes place. Increased DNA synthesis in polyoma virus infection was concluded to
be a primary response caused by active synthesis of viral DNA. In addition, it was
evident that synthesis of host-cell DNA also continued to a certain extent.

Polyoma virus, which is capable of inducing multiple tumors in a variety of animals
in vivo, can multiply in mouse embryo tissue culture with a cytopathogenic effect.29) The virus consists of deoxyribonucleic acid (DNA) as a nucleic acid constituent6,28) and multiplies first in the nucleus of the host cell.2,7,14,33)

Despite the great interest in DNA metabolism in polyoma virus-infected cultures
in connection with the fact that in vitro malignant conversion of the infected cultures
has been demonstrated,8,24,26,30,31) little information1,4,7,17,18) is available thus far on
DNA synthesis in the light of polyoma virus-host cell interaction. Recent advances
in autoradiographic technique make it possible to study changes in the synthetic
activity of DNA at the cellular level.

The experiments described here were designed to elucidate in vitro interaction of
polyoma virus with its host cell in regard to DNA synthesis by means of autoradio-

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graphy using incorporation of tritiated thymidine. In addition, cytological, cytochemical, and immunofluorescent studies were carried out.

**MATERIALS AND METHODS**

**Tissue Culture** Swiss mouse embryo cultures were used as primary cultures. They were grown at 37°C in a Roux bottle, with Medium 199 supplemented with 10% calf serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. After establishment of a satisfactory cell layer (about 3~7 days), the cells were dispersed by trypsinization, and 5~10 x 10^5 cells were seeded into each of a number of Leighton tubes containing the same nutrient medium (1 ml in volume). One day after seeding of the secondary mouse embryo cells, the virus was inoculated. The cultures were fed daily. Duplicate or triplicate sets of cultures were used in all the experiments described here.

**Virus** The polyoma virus stock used was 2610-9 (1:12,800 hemagglutinating units (HAU)/ml). The polyoma virus strain was originally obtained from Dr. S. E. Stewart of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland, U.S.A. In all experiments, inoculation of the virus was carried out at a multiplicity ranging from 10 to 15 x 10^-4 HAU/cell at 37°C for 3 hours.

**Isotope** Tritiated thymidine, 0.36 c/mM specific activity (Schwarz Lab., Mt. Vernon, N.Y., U.S.A.), was used. The nutrient medium, containing tritiated thymidine at a level of 0.02 μc/ml, was applied to the culture. Tritiated thymidine was present in the culture for 1 hour at various times after virus inoculation. After incubation with the isotope, the culture was thoroughly washed three times with phosphate-buffered saline and was fixed immediately. When a subsequent incubation was required, the third washing was done with a non-radioactive medium in place of the phosphate-buffered saline and incubation was continued thereafter with the non-radioactive medium supplemented with thymidine at a level of 10 μg/ml.

**Hemagglutination Test** In order to determine the amount of virus release, the culture fluid was subjected to the hemagglutination (HA) reaction. The test was carried out with 2-fold serial dilutions of the sample in saline, 0.5 ml in volume, starting at a 1:10 dilution. The test tubes thus prepared were then heated at 56°C for 30 minutes to unmask the virus^1^ and then 0.5 ml of 0.4% guinea pig red cell suspension was added to each tube. Hemagglutinin titer is expressed as the reciprocal of the last dilution of the sample showing complete hemagglutination after standing overnight at 4°C.

**Cytological and Cytochemical Procedures** At various periods during the course of experimentation, the cells (which were grown on a coverslip inserted in Leighton tubes) were washed three times with phosphate-buffered saline and were fixed directly with modified Carnoy's fixative (1 part acetic acid; 3 parts absolute ethanol; v/v) for
15 minutes at room temperature. Until the end of that particular experiment, the fixed specimens were allowed to stand in tubes to which 1 ml of 70% ethanol was added. They were then removed and allowed to dry at room temperature. For convenience in handling, each coverslip (cell-side up) was attached to a 1×3 inch glass microscope slide with 1 drop of Permount.

Such prepared specimens were then stained with Hematoxylin-Eosin or with Feulgen nuclear stain, or were used in the autoradiographic studies. In some instances, before Feulgen stain or autoradiographic preparation, treatment of the cells with deoxyribonuclease (DNase) (crystallized twice, Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.) was carried out for 1 hour at room temperature in 0.1M phosphate buffer (pH 7.0), containing 100 μg DNase/ml in the presence of Mg ion (0.003M MgSO₄). For the Feulgen nuclear stain, the cells were hydrolyzed in 1N HCl at 60°C for 8 minutes, stained with the Schiff reagent at room temperature for 1.5 hours, and were finally counterstained with Fast Green.

Fluorescent-antibody Staining In order to compare the percentage of the infected cells with the percentage of DNA-synthesizing cells at various periods of infection, fluorescent-antibody staining was used to detect the presence of viral antigens which may have been produced in the infected cells. The fluorescent-antibody staining employed was carried out by the indirect method. The fluorescein-labeled goat sera against rabbit γ-globulin was purchased from Microbiological Associate, Baltimore, Md., U.S.A., and the rabbit antipolyoma-sera were obtained from 5 rabbits immunized with a partially purified polyoma virus preparation. Both antisera were preadsorbed with washed secondary mouse embryo culture cells free from virus infection and with nutrient medium. The infected or non-infected specimens, which were fixed with cold acetone for 10 minutes, were incubated with each of these sera for 1 hour, first with goat sera, then with rabbit sera, in a moist chamber at room temperature. After each incubation with the sera, the specimens were washed with phosphate-buffered saline for 10 minutes at room temperature. The stained specimen, which was mounted with buffered glycerol (pH approximately 7.0), was then observed under a Leitz ultraviolet microscope at 500× magnification with an UV light source of HBO 200 (Osram). Percentage of fluorescent-stained cells was obtained from counts in a total cell population of at least 500 cells. Two determinations at different fields, chosen randomly, in each preparation were carried out.

Autoradiographic Preparation The autoradiographic technique employed was the dipping-coating method of Messier and Leblond. Eastman Kodak NTB 3-type nuclear track bulk emulsion was supplied by the Research Laboratory of the Eastman Kodak Company, Rochester, N.Y., U.S.A. Autoradiographs were exposed for 3 days and were developed with D-19 developer (Eastman Kodak). A modified Giemsa stain was used on the autoradiographs after completion of the photographic process.
Observation of the autoradiographs was carried out under a bright-field microscope at 1,000× magnification with oil immersion or at 500× magnification. The percentage of nuclear labeling was obtained from the number of labeled cells counted in a total cell population of at least 500 cells. Two determinations, with the field of view chosen randomly, were also done for each autoradiograph. Cytological localization of autoradiographic grains was observed simultaneously.

**Experimental Results**

Preliminary experiments were first conducted to clarify the various parameters of the experimental system in this study. These experiments indicated several interesting variables involved in the successful multiplication of polyoma virus in tissue culture and supported the results obtained by other investigators. First, the greater the content of calf serum (between 2% and 40%) in the nutrient medium, the greater the yield of virus from the infected cultures. Second, frequent feeding of the cultures appeared to be favorable for enhanced virus production. Third, the greater the inoculum size of the virus introduced to the culture, the greater the yield of virus and the earlier the appearance of cytopathogenic effect. Fourth, cultures at a younger age in incubation also appeared to be more favorable for virus multiplication.

Polyoma virus infection in either mouse or hamster embryo culture results in "transformation" of the culture possessing a tumor-producing activity. In order to study this phenomenon further, a number of experiments were carried out. A continuously-growing cell population (Photos 3 and 4) after polyoma virus infection was obtained only in the culture grown in Medium 199 supplemented with 20% calf serum, whereas it hardly developed from the cultures grown in Medium 199 supplemented with 2, 10, or 40% calf serum. There was also a reproducible sequence of events under these conditions in that an extensive cytopathogenic effect was noticed throughout the infected culture during the first to second week of infection. This was followed by development of a growing-cell population in the next few weeks of incubation. Such a growing-cell population replaced eventually the degenerating-cell population exhibiting cytopathogenic effect in the early period of infection. It thus became possible to subculture indefinitely the polyoma virus-infected cell population, as is done with other established strain of tissue culture cells. During the maintenance of the culture, cytopathogenic effect was negligible as compared with that observed in the early period of incubation. Virus production, as determined by the hemagglutination reaction, was maintained at a high rate even during the early period of development of the growing-cell population, but decreased gradually with time and finally became negative. A small amount of virus production persisted,
however, as was shown by fluorescent-antibody staining and infectivity titration with mouse embryo cultures. The cell populations obtained revealed epithelial-type morphology (Photo 3), but in some instances fibroblast-type morphology was noted (Photo 4). These cell populations have been maintained for more than 1 year.

These cells at varying periods of incubation were injected into a number of mice, about 1 month of age, which had been irradiated by X-rays (450 r) a few hours before cell injection. The results as illustrated in Table I show that none of the mice injected developed a tumor at the injection site during a 4-month period of observation. Nevertheless, newborn mice (less than 24 hours of age) which were injected with these cells developed parotid tumors with a high rate of incidence during the observation period (experiment No. 503 in Table I). It would seem that the injected

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Days after infection</th>
<th>Virus titer (HAU/0.1 ml)</th>
<th>Average inoculum $\times 10^6$ cells/mouse</th>
<th>Age of mouse (days)</th>
<th>X-Ray conditioning</th>
<th>No. of mice with tumor</th>
<th>No. of mice inoculated</th>
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<td>174</td>
<td>0</td>
<td>1.5</td>
<td>13</td>
<td>—</td>
<td>0/4</td>
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<tr>
<td></td>
<td>174</td>
<td>40</td>
<td>1.6</td>
<td>13</td>
<td>—</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td>501</td>
<td>191</td>
<td>160</td>
<td>0.3</td>
<td>30</td>
<td>+</td>
<td>0/4</td>
<td></td>
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<tr>
<td></td>
<td>191</td>
<td>40</td>
<td>1.3</td>
<td>30</td>
<td>+</td>
<td>0/10</td>
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</tr>
<tr>
<td>503</td>
<td>224</td>
<td>0</td>
<td>1.2$^b$</td>
<td>less than 24 hrs.</td>
<td>—</td>
<td>0/3</td>
<td></td>
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<tr>
<td></td>
<td>224</td>
<td>0</td>
<td>2.1$^b$</td>
<td>less than 24 hrs.</td>
<td>—</td>
<td>2/6$^c$</td>
<td></td>
</tr>
<tr>
<td>504</td>
<td>263</td>
<td>160</td>
<td>4.0</td>
<td>44</td>
<td>+</td>
<td>0/6</td>
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<td>35</td>
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<td>290</td>
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<td>1.8</td>
<td>36</td>
<td>+</td>
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<td>308</td>
<td>0</td>
<td>5.8</td>
<td>32</td>
<td>+</td>
<td>0/6</td>
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</tr>
</tbody>
</table>

**Table I. Comparison of Mouse Embryo Culture Cells infected or non-infected with Polyoma Virus in regard to their Capacity to induce Tumors in Swiss Mice**

In the table above:
- **a)** Free virus in 0.1 ml of tissue culture fluid at the inoculation time.
- **b)** Volume of inoculum = 0.2 ml.
- **c)** Parotid tumors, not a tumor at the inoculation site.

**Control**

<table>
<thead>
<tr>
<th>Incubation (days)</th>
<th>Expt. No.</th>
<th>Days after infection</th>
<th>Virus titer (HAU/0.1 ml)</th>
<th>Average inoculum $\times 10^6$ cells/mouse</th>
<th>Age of mouse (days)</th>
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<th>No. of mice inoculated</th>
</tr>
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<tr>
<td>500</td>
<td>174</td>
<td>0</td>
<td>0.1</td>
<td>13</td>
<td>—</td>
<td>0/1</td>
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<td></td>
<td>15</td>
<td>0</td>
<td>0.4</td>
<td>44</td>
<td>+</td>
<td>0/2</td>
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</tr>
<tr>
<td>501</td>
<td>191</td>
<td>0</td>
<td>0.5</td>
<td>30</td>
<td>+</td>
<td>0/1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>32</td>
<td>0</td>
<td>1.3</td>
<td>30</td>
<td>+</td>
<td>0/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>503</td>
<td>224</td>
<td>0</td>
<td>1.0$^b$</td>
<td>less than 24 hrs.</td>
<td>—</td>
<td>0/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>0</td>
<td>2.1$^b$</td>
<td>less than 24 hrs.</td>
<td>—</td>
<td>0/3</td>
<td></td>
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</table>

Total 0/14
cells were of the "virus carrier" type, rather than "transformed," i.e. neoplastic cells. A recent report by Barski et al.3) noted such a "virus carrier" state. Further investigation on this matter is in progress.

**Cytological and Cytochemical Observations** Microscopical observations were also made on living and stained specimens throughout the course of experimentation. There were no apparent differences between the infected and control cultures during the first 3–5 days. Thereafter, many rounded cells in the infected cultures appeared to be scattered among normal-appearing cells. These rounded cells tended to stick together, forming clumps, and many showed refractive cytoplasmic vacuoles in various sizes. These alterations progressed with time, and soon these cells were observed to be detaching from the glass surface. At this time, the process was recognized as an obvious cytopathogenic effect (Photo 2). The appearance of this effect was found to be closely related to the appearance of virus in the culture fluid, as determined by the hemagglutination test. Actually, both the positive hemagglutination reaction and cytopathogenic effect were found during the third to fifth day of infection, depending upon the size of the inoculating dose of the virus. Nevertheless, there were always normal-appearing cells present in varying numbers, along with degenerating cells. The normal-appearing cells seemed to be capable of multiplying while extensive cytopathogenic effect was taking place in other cells. This mitotic activity appeared to be not directly related to virus growth, as the hemagglutination titer of the culture fluids rose rapidly at first and then decreased gradually as cytopathogenic effect became widespread. It was further observed that many infected cells were increased in size, with enlarged, swollen, and irregular nuclei, prominent nucleoli (Photo 6), margination of chromatin, increase or decrease of the nuclear material, or vacuole formation in nuclei, nucleoli, or cytoplasm, and that there were many cells showing pycnosis, shrunken nuclei, or phagocytizing activity. In addition, a large quantity of cellular debris and many fibrous structures were noticed. Some of these alterations, however, were found to a lesser extent in the control culture at a later period in a long-term cultivation. Basophilic foci, which are usually Feulgen-positive, were occasionally observed in the nuclei of the infected cells, but not in the nuclei of the control cells. These foci were of varying sizes and shapes, and were more dense than normal nuclear material (Photo 6). In some instances, acidophilic material was found in the nucleus or cytoplasm in both the infected and the control cells, and thus did not appear to be associated exclusively with polyoma infection. This material appears to constitute disintegrated cellular fragments phagocytized by intact cells. On the whole, cytopathogenic effect was recognized as a specific morphological manifestation of polyoma infection, as compared with the insignificant cell damage present in the corresponding control cultures. It was further noted that in the infected culture many altered nuclei, which were obviously degenerative, revealed a stronger
Feulgen stain than normal-appearing cell nuclei did. DNase digestion removed the Feulgen staining material from both the infected cells and controls.

**Observations by Autoradiographic and Fluorescent-antibody Techniques** In order to ascertain the synthetic activity of DNA in the infected culture at various stages of virus infection, nutrient medium containing tritiated thymidine was added to the culture for 1 hour at varying times after virus inoculation. Some of the cultures were also observed by fluorescent-antibody staining in addition to the autoradiographic analysis to locate cells in which viral antigens were present. The results are shown in Fig. 1.

The tissue cultures were of secondary mouse embryo cells and, therefore, would be considered to be mainly of fibroblastic-type cells. During a certain period of incubation, immediately after trypsin dispersion, connective tissue cells from the rabbit in a stationary culture condition have been shown to undergo a cell division which is partially synchronous. Generally, a stationary phase is reached soon after an initial growth phase and thereafter the number of cells in the culture gradually decreases. This effect in regard to synthetic activity of the cellular DNA was observed as illustrated in Fig. 1. The percentage of labeled cells in the non-infected culture showed a considerable fluctuation with the age of the culture after seeding of the cells. Although the magnitude of this effect varied somewhat from experiment to experiment, the percentage of labeled cells was at a low level one day after seeding of the trypsin-dispersed cells of secondary mouse embryo culture and it rose to a peak on the next day. Thereafter, it was followed by a fluctuation of the percentage of labeled cells and the percentage decreased gradually when the incubation time was extended. The sequence of events which may be responsible for such a pattern of DNA synthesis was found to be as follows: The first division cycle of the cells under this particular culture condition seemed to proceed in a partially synchronized fashion and was usually followed by several such cycles. Synchronization was lost, however, during further growth of the cells.

The percentage of labeled cells in the infected cultures showed a pattern similar to that of the non-infected culture up to the third day of infection (Fig. 1). Thereafter, the percentage of labeled cells in the infected cultures rose to a peak on the fifth day of infection and was maintained at about the same level in the subsequent period of incubation. During the first two days after virus infection, the culture was negative in both cytopathogenic effect and hemagglutination reaction. Shortly thereafter, substantial virus release and an increasing degree of cytopathogenic effect were observed in the infected culture, but not in the control, for the duration of the experiment (Fig. 1).

Strong evidence of virus multiplication in the infected cells was the appearance of viral antigens. Fluorescent-antibody staining was carried out at various times after
infection and the results obtained (Photo 7) were essentially the same as those previously reported.\textsuperscript{14,33} Polyoma virus antigens were first observed in the infected nuclei (at 24 hours after the infection), as fluorescent particles of various sizes. Shortly thereafter (3~5 days after the infection), the infected cells revealed diffuse distribution of fluorescence throughout their nuclei. Furthermore, in the period of advanced infection (5~7 days after the infection), some of the cells with cytoplasmic fluorescence, either in a particulate or diffuse distribution, showed a rather less intensive or no fluorescence in their nuclei. In some of these cells cytoplasmic degeneration was found to be evident. At this time, around 4 or 5 days after infection, a considerable amount of virus release was noted and soon thereafter cytopathogenic effect was apparent. In this period and subsequent periods of infection, where an extensive cytopathogenic effect was present, a large quantity of particulate fluorescent materials, presumably cellular fragment, in addition to many cells with fluorescent

\textbf{Fig. 1.} Autoradiographic analysis of labeled cells (DNA-synthesizing cells) in polyoma virus-infected and non-infected cultures and immunofluorescent analysis of the infected cultures

- infected ○ non-infected × HA titer: -→+++ increasing degree of cytopathogenic effect (CPE)
- * percentage of fluorescent-stained cells presented is the lowest estimation, since much cellular debris with fluorescence made accurate counting difficult. Control cultures showed some non-specific fluorescence (autofluorescence), but none in the nucleus.
nuclei, was observed to be scattered in the culture with no correlation in their morphology.

The percentage of fluorescent nuclei as shown in Fig. 1 was determined on 1, 3, and 5 days after the infection. A marked increase in the percentage of fluorescent nuclei was observed on the fifth day and this seemed to coincide with the increased percentage of DNA-synthesizing cells, the increased virus release, and the progressively spreading cytopathogenic effect.

It is well known that tritiated thymidine is incorporated efficiently and solely into the DNA molecule. Therefore, under normal condition, only the nucleus of the cells should show an autoradiographic reaction. In the early period when no cytopathogenic effect was evident, autoradiographic grains were localized exclusively over the nucleus of both the infected and control cells (Photo 8). In some instances, the cultures that showed cytopathogenic effect were incubated with tritiated thymidine for 1 hour and were fixed 24 hours later. Some grains were observed in the vicinity of the nuclear membrane, in addition to the nuclear labeling (Photo 9). In contrast, such a sequence of changes in grain distribution was never found in the cells of the control cultures.

It seemed likely that the autoradiographic grains seen in the infected cells might represent both host-cell and viral DNA. In order to differentiate between the two, advantage was taken of the fact that DNA in a complete virus particle is resistant to DNase digestion, whereas host-cell DNA is not.\textsuperscript{4,6} DNase-treated preparations should have revealed only grains caused by such a viral DNA. It was found that DNase digestion before autoradiographic preparation did not remove all of the label in the infected cells and that there were still areas containing autoradiographic grains in the nuclei (Photo 10). In contrast, DNase digestion completely removed all of the label in the control cells. This finding also indicates that the grains seen in the present autoradiographic studies represent both host-cell and viral DNA.

Furthermore, the observation that the increase in the number of DNA-synthesizing cells was paralleled by an increase in the fluorescent-staining nuclei in accordance with the progressive spread of polyoma virus infection would strongly support the concept that \textit{de novo} synthesis of viral DNA takes place in the infected nuclei.

**DISCUSSION**

The autoradiographic studies reported here indicate that the increased percentage of DNA-synthesizing cells in mouse embryo cultures infected with polyoma virus is due to newly synthesized host-cell and viral DNA. The results obtained from the experiment with DNase treatment supports the interpretation that \textit{de novo} synthesis of viral DNA takes place by utilization of tritiated thymidine in the nutrient medium. Consequently, the autoradiographic reaction may well represent, in part, viral DNA.
itself. Nevertheless, some uncertainties still remain. The extent of autoradiographic reactions in the infected culture after DNase treatment seemed to be far less than the amount expected from consideration based on the growth pattern of the virus. Since digestion with DNase was done after fixation, it is possible that the histological fixative used may denature cellular or viral protein. Thus the DNase-resistant nature of polyoma virus particles, presumably due to their protein coat, might then be partially lost and some of the viral DNA might be removed by the action of DNase. On the other hand, DNA synthesis of vaccinia virus studied by biochemical\(^{25}\) or autoradiographic technique,\(^{6}\) or both,\(^{19,27}\) have shown that synthesis of viral DNA precedes the completion of mature virus particles. An analogous process of virus formation in polyoma virus would then account for the action of DNase on the virus, its effectiveness would depend on the degree of completion of virus particles, and would presumably be much more effective for the incomplete than for the complete virus. This explanation may well account for the variations observed in the present studies. On the other hand, evidence indicating \textit{de novo} synthesis of viral DNA has been obtained in our earlier autoradiographic study.\(^{21}\) DNA synthesis in the polyoma-infected cultures, in which the synthetic activity of host-cell DNA had been suppressed by X-ray irradiation before virus inoculation, was observed to a marked extent, indicating viral DNA synthesis. In contrast, DNA synthesis in similarly irradiated control cultures was at a very low level. Finally, cytological observation of the autoradiographic grain distribution also supports this concept of labeling of viral DNA. Most of the cells that incorporated tritiated thymidine during the one-hour incubation period with the isotope (24 hours prior to the histological fixation) were degenerating and presumably no longer capable of multiplying. Under these conditions, it is unlikely that the labeling seen in the nucleus represented newly synthesized host-cell DNA. Therefore, it is most likely that it represents newly synthesized viral DNA.

Although these data indicate the occurrence of viral DNA synthesis in polyoma-infected cultures, the continuation of synthetic activity in host-cell DNA was also evident in the infected cultures. This is not surprising, since the presence of cells capable of multiplying was observed.

In some virus infections, the virus-host cell relationship causes an increase in DNA synthesis in the host cells.\(^8,15,22,32\) Spectrophotometric observations on DNA variation in virus-infected cells are available.\(^{16}\) It is also a common feature of virus-infected tissue that both degenerative and proliferative changes occur simultaneously or sequentially during the course of infection.\(^{23}\) The degenerative changes in the infected cells may actually stimulate DNA synthesis in the non-infected cells. The increase in the cells showing DNA synthesis in our infected cultures may be attributed, in part, to the nutrient release due to progressive cytopathogenic effect.
Although it is not yet possible to state the quantitative relationship between host-
cell and viral DNA synthesis in polyoma virus-infected cultures, it is suggested by
the present data that the increase in DNA synthesis observed in the polyoma virus-
infected cultures is primarily related to virus multiplication.

We wish to thank Drs. T. S. Hauschka and P. Stelos of this Institute for their helpful advice
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REFERENCES

Inst., 26, 331 (1961).
EXPLANATION OF PLATES VI~VIII

Photo 1. Mouse embryo culture, 7th day after seeding. Hematoxylin-Eosin (H-E) stain ×200.

Photo 2. Mouse embryo culture, 7th day after polyoma virus infection, showing cytopathogenic effect. Many enlarged cells with prominent nucleoli, a considerable number of rounded or degenerating cells, and a large quantity of cellular debris are scattered throughout the picture. H-E stain. ×200.

Photo 3. Polyoma virus-infected mouse embryo culture incubated in Medium 199 supplemented with 20% calf serum (18th day after infection), showing a continuously-growing cell population with epithelial-type morphology. Note the cytopathogenic effect at the upper portion of the picture. Hemagglutination titer was 640 HAU/0.1 ml of the culture fluid. Living state. ×250.

Photo 4. Another example of the infected culture (24th day after infection) under the same condition as for Photo 3, showing a continuously-growing cell population with fibroblastic-type morphology. Note the cytopathogenic effect at the upper-right portion of the picture. Hemagglutination titer was 320 HAU/0.1 ml of the culture fluid. Living state. ×250.


Photo 6. Nucleus showing characteristic changes due to polyoma virus infection. Enlarged nucleus contains several prominent nucleoli (usually Feulgen-negative) and an amorphous denser material (usually Feulgen-positive) in the upper-right portion of the nucleus. H-E stain. ×1,260.

Photo 7. Fluorescent-stained nucleus, 1 day after polyoma virus infection, showing particulate fluorescence throughout the nucleus except nucleolar areas. Fluorescent-antibody stain. ×1,200.

Photo 8. Autoradiograph of a polyoma virus-infected cell on the 5th day after infection, showing nuclear labeling with tritiated thymidine. Modified Giemsa stain. ×2,000.

Photo 9. Autoradiograph of a polyoma virus-infected cell on the 7th day after infection, showing nuclear labeling and labeling in the vicinity of the nuclear membrane with tritiated thymidine. Modified Giemsa stain. ×2,500.

Photo 10. Autoradiograph of a polyoma virus-infected cell on the 7th day after infection, showing nuclear labeling with tritiated thymidine after DNase treatment. Modified Giemsa stain. ×2,000.