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Multiplication of Polyoma Virus
II. Source of Constituents for Viral Deoxyribonucleic Acid and Protein Synthesis

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Previous studies (L. M. Kozloff, Cold Spring Harbor Symp. Quant. Biol. **18**:209, 1953; S. S. Cohen, Federation Proc. **20**:641, 1961) with the *Escherichia coli* T-even bacteriophage system have demonstrated the cessation of host deoxyribonucleic acid (DNA) synthesis, with subsequent degradation and reutilization of these products for viral DNA synthesis. Consequently, an interest has arisen concerning the source of small molecules for the synthesis of animal viruses. Various investigators have demonstrated that these molecules are predominantly obtained from medium constituents (J. E. Darnell and L. Levintow, J. Biol. Chem. **235**:74, 1960; J. E. Darnell et al., Virology **13**:271, 1961; N. P. Salzman and E. D. Sebring, Virology **13**:258, 1961; H. Eagle and K. A. Piez, J. Biol. Chem. **235**:1095, 1960; Federation Proc. **20**:151, 1961; E. R. Pfefferkorn and R. Clifford, Virology **23**:217, 1964), although some molecules are drawn from pre-existing host constituents (E. R. Pfefferkorn and H. S. Hunter, Virology **20**:433, 1963; Virology **20**:446, 1963). The present study was undertaken to determine whether constituents for the synthesis of a tumor virus were drawn from the medium or from pre-existing host material. The system investigated was pre- and postlabeled primary mouse embryo cultures that were infected with polyoma virus. H^3-thymidine was used to determine the nucleic acid source, and H^3-valine, the protein source. Specific isotope incorporation into polyoma virus was determined after the cell-free lysates were concentrated and purified in cesium chloride density gradients.

The experiment was performed by prelabeling 24-hr cultures of primary mouse embryo cells with either 1.5 μc of H^3-thymidine or 2.0 μc of H^3-valine per ml of medium (Eagle's medium with 5% fetal calf serum), and allowing the cultures to proceed for an additional 40 hr. Isotopically labeled medium was removed and replaced with unlabeled medium for 12 hr to deplete the cell pool of the respective labels. The cultures were infected with the wild-type polyoma [600 plaque-forming units (PFU) per cell], and the virus was adsorbed for 3 hr in a CO_2 humidified incubator (37 C). Unadsorbed virus was removed by washing three times with Hanks balanced salt solution, followed by the addition of unlabeled Eagle's medium. In the postlabeled experiment, the isotope was added to the cultures after polyoma infection. The pre- and postlabeled cultures received identical label exposure and input of viral multiplicity, and were harvested 40 hr after infection. Carrier polyoma virus (10^8 PFU) was added to each of the respective preparations to aid in the viral purification.

Virus was purified by E. Winocour's (Virology **19**:158, 1963) method, modified as follows. Virus was liberated from the infected cells by the method of L. V. Crawford (Virology **18**:177, 1962), followed by centrifugation at 8,000 $\times$ *g* in a Sorvall (SS-3 superspeed) centrifuge for 30 min to remove the cellular debris. The infected tissue culture fluid was concentrated by centrifugation in a Spinco ultracentrifuge (model L, SW 25 rotor) at 80,000 $\times$ *g* for 3 hr. The viral pellet was resuspended to 5% of the original volume in phosphate-buffered saline (PBS, 0.01 M PO_4, *p*H 7.2, 0.15 M NaCl) and treated with deoxyribonuclease (30 μg/ml), ribonuclease (30 μg/ml), $MgCl_2$ (10^{-3} M), and trypsin (0.1 mg/ml), for 30 min at 37 C. The enzymes were removed by centrifugation in a Spinco ultracentrifuge (SW 39 rotor) at 156,000 $\times$ *g* for 3 hr. Viral pellets were resuspended to 1% the original volume in tris(hydroxymethyl)aminomethane (Tris) - buffered saline (TBS, 0.01 M Tris-HCl, *p*H 7.2; 0.15 M NaCl), and dialyzed against TBS for 3 hr to remove unincorporated label. The virus was further purified by using a pre set CsCl density gradient (1.20 to 1.40 g/ml) and centrifuging (Spinco, SW 39 rotor) at 156,000 $\times$ *g* for 18 hr. Fractions of 12 drops each were collected with a model D density flow fractionometer (Instrumentation Specialities Co., Lincoln, Nebr.) and assayed for radioactivity (counts per minute) and

hemagglutination (HA). The complete virus band which demonstrated maximal HA and counts per minute was dialyzed against TBS to remove CsCl, and was further purified in a second CsCl density gradient as previously described. Fractions of 12 drops each were collected and assayed for counts per minute, PFU, and HA.

HA titrations were standard twofold dilutions of polyoma made in PBS, with guinea pig red blood cells (0.35% final concentration) as the indicator system. Plaque assays were performed on primary mouse embryo cultures. Infected monolayers were covered with 8.0 ml of an equal mixture of 2× Adeno Medium [a modification of adenovirus medium used by W. Lawrence and H. S. Ginsberg, University of Pennsylvania (*personal communication*) found to maintain monolayers for extended periods; 2× Adeno Medium: Earles salts (10× stock), 200 ml; amino acids (50× stock), 40 ml; vitamins (100× stock), 20 ml; lactalbumin hydrolysate, 5.0 g; peptone (Difco), 10 g; calf serum, 100 ml; deionized water to 1,000 ml; antibiotics: penicillin, 200 units; streptomycin, 0.2 mg; kanomycin, 0.010 mg per ml of medium] + 2% purified agar. After 5 days of incubation in a CO_2 atmosphere at 37 C, 2.5 ml of the above medium-agar mixture was fed to the cultures. On day 11, 2.5 ml of the above mixture containing 0.01% neutral red was added. Plaques were distinctly visible 24 hr later. Radioactivity was measured in a Packard Tricarb liquid scintillation counter. Purified polyoma virus densities were calculated from refractomer readings by the method of J. Weigle et al. (Brookhaven Symp. Biol. **12**:125, 1959).

Purified progeny virus was investigated as to its specific isotopic incorporation. The respective gradients (Fig. 1 and 2) were each found to possess one distinct band with a buoyant density of 1.316 g/ml. In both the pre- and the postlabeled H^3-thymidine experiment (Fig. 1), similar results were obtained on the basis of HA and PFU. However, a distinct difference was observed in specific isotopic incorporation. The viral progeny obtained from the prelabeled cells had only 7% of the counts per minute found in the postlabeled experiment. This indicated that the label was predominantly taken from the medium and incorporated into de novo polyoma DNA, resulting in the labeled infectious particle.

When H^3-valine was used to determine the source of protein constituents for polyoma capsid synthesis, it was found that the greatest specific incorporation occurred also in the postlabeled experiment (Fig. 2). The progeny obtained from the prelabeled cells contained only 12% of the radioactivity (H^3-valine) found in the postlabeled progeny.

From these experiments, it was evident that polyoma infection did not significantly degrade host constituents and specifically reutilized these products for virion synthesis. The minute amount

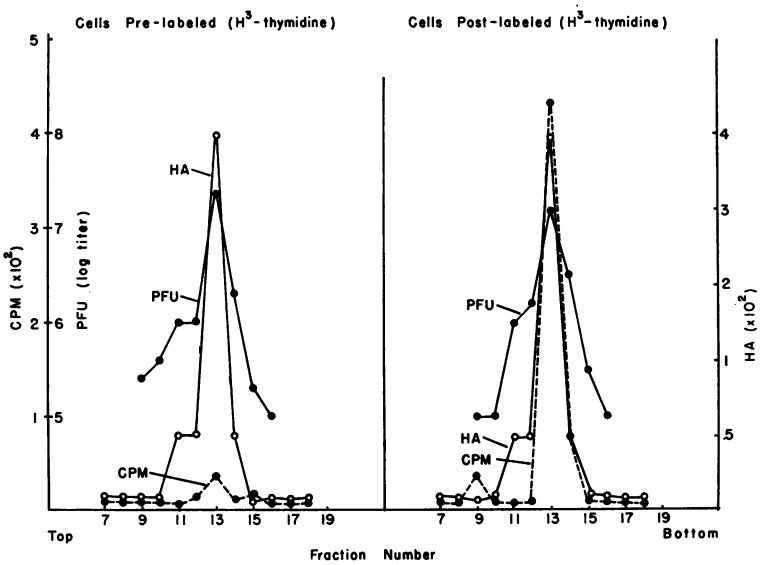

FIG. 1. *CsCl gradients from polyoma-infected pre- and postlabeled (H^3-thymidine) cultures. The experiment was performed as described in the text. In both the pre- and postlabeled gradients, the peak fraction had a bouyant density of 1.316 g/ml.*

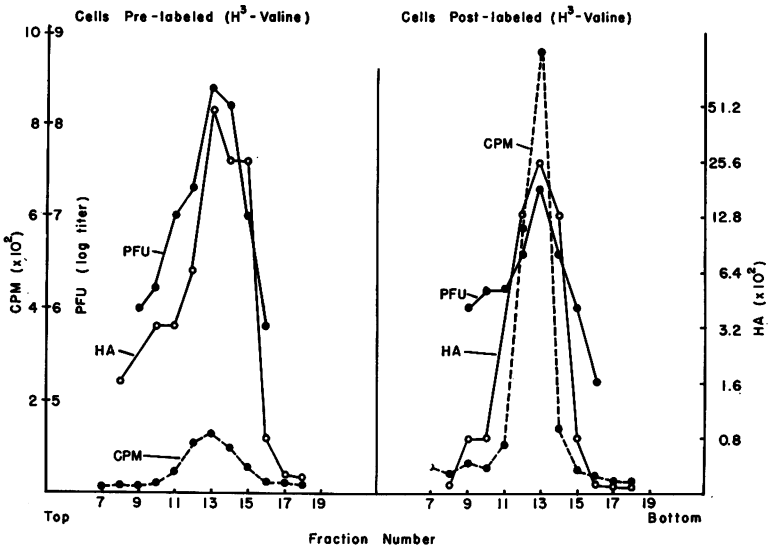

FIG. 2. *CsCl gradients from polyoma-infected pre- and postlabeled (H^3-valine) cultures. The experiment was performed as described in the text. In both the pre- and postlabeled gradients, the peak fraction had a bouyant density of 1.316 g/ml.*

of incorporation found in the progeny obtained from the prelabeled cultures was possibly due to cell death and autolysis of the labeled host constituents. In addition, the normal rate of protein turnover may also be a contributory factor in the H^3-valine experiment. However, it was obvious that the constituents for polyoma protein and nucleic acid synthesis came predominantly from cellular pools which were supplied by the medium.

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