times) in the mouse was demonstrated by comparing cummulative urinary excretion ratios obtained after oral and intravenous drug administration.

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DNA Biosynthesis in Monkey Kidney Cells Infected With PARA (SV40)-Adenoviruses.* (32486)

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Recent studies have shown that a human adenovirus type 7 which grows in African green monkey kidney (GMK) cell cultures carries a portion of the SV40 genome(1-3). The virus population was found to consist of at least two types of particles(4,5), one being a normal adenovirus and the other containing the defective SV40 genome in an adenovirus capsid; the replication of these particles is interdependent in simian cells(6). This SV40-adenovirus 7 "hybrid" was called PARA-adenovirus 7(7).

Physical separation of the PARA particle (containing the SV40 genome) and the respective adenovirus in a mixed population has been unsuccessful(6,8-10). The SV40 determinants carried by the PARA-adenovirus include those coding for the induction of SV40 tumor (T) antigen(2,3) and transplantation rejection antigens(11). These properties can be transferred to other adenovirus types(7,12) and such transcapsidants breed true. Although PARA and adenovirus virions replicate in parallel, information on biosynthesis of deoxyribonucleic acid (DNA) by the infected cells has not yet been obtained. The present investigation was therefore undertaken to follow the synthesis of DNA in cultures of GMK cells inoculated with PARA-adenovirus 7, or with one of the transcapsidants PARA-adenovirus 2 or PARA-adenovirus 12, and to characterize the DNA from the infected cultures.

Materials and methods. Cells. GMK cells were grown in 16-oz bottles using Melnick-Hanks' lactalbumin hydrolysate medium with 2% calf serum as previously described(13). In some instances, GMK cells were also grown in 60 mm plastic petri dishes or on 22 mm square coverslips placed in the petri dishes. After 10-12 days of growth, the primary cultures were nourished with Eagle's minimal medium for 24 hours before the experiments were started.

Viruses. Stocks of PARA-adenovirus 7 and its transcapsidants were prepared in GMK cells as described(7). In each experiment, the virus was allowed to adsorb for 1.5 hours at 37C. The input multiplicity for PARA was adjusted to 1 to 3 plaque-forming units

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(PFU) per cell; the adenovirus component was always higher and varied with the population used.

Uptake of radioisotope into deoxyribonucleic acid (DNA). Primary GMK cells were exposed to 0.2 mg/ml of cold uridine for 24 hours. Following virus inoculation, the cultures were exposed to 4-6 μc/ml of H^3-thymidine (TdR) (14.5 c/millimole, New England Nuclear Corp.) in 2 ml of Eagle's minimal medium for one hour at various intervals. The radioactive medium was then removed and the cell sheet scraped into cold tris buffer (0.025 M, pH 7.6). DNA was hydrolyzed from the cells according to the procedure of Schmidt and Thannhauser(14) with certain modifications as described below. The cell suspension was centrifuged (2000 $\times$ *g*) at 4C and the cell pellet precipitated by cold 5% trichloroacetic acid (TCA). After the removal of the acid soluble pool, the pellet was washed 3 times with cold 5% TCA and the DNA hydrolyzed with 5% TCA at 90C for 45 minutes. The supernatant was removed after centrifugation (2000 $\times$ *g*) and the amount of DNA and radioactivity determined. The DNA content was measured using the diphenylamine reaction(15). The uninoculated control cultures were treated similarly. Two cultures were used for each sample analyzed.

Isopycnic analysis of DNA. DNA was extracted from virus infected and uninfected cultures according to the procedure of Rapp, Feldman and Mandel(16). DNA content in the partially purified nucleic acid samples in SSC (0.15 M NaCl and 0.015 sodium citrate) was determined by ultraviolet absorbances at 260 and 280 mμ. Two to 4 μg of DNA were analyzed by CsCl density gradient centrifugation (44,770 rpm for 24 hours) in a Spinco model E analytical ultracentrifuge equipped with monochromatic and direct ultraviolet photoelectric scanner at 265.4 mμ. In some instances, densitometer tracings were made from the photographic films. The DNA of *Bacillus subtilis* bacteriophage SP8 (furnished by Dr. Manley Mandel) having a density of 1.742 g/ml was used as reference DNA. The guanine plus cytosine (GC) content of each peak was calculated according to the procedure of Schildkraut, Marmur and Doty(17).

Measurement of radioactivity. The amount of H^3 or C^{14} counts present in a known volume of the sample was assayed with a liquid scintillation spectrometer (CPM200, Beckman Instruments). Dioxane phosphor mixture was used to count the radioactivity present in aqueous samples. Toluene scintillation fluid (4.0 g PPO and 0.1 g POPOP to every liter of toluene) was used to count the radioactivity in 0.01 ml fractions obtained from the DNA samples centrifuged to equilibrium in CsCl. Wherever mentioned, counts per minute were converted to disintegrations per minute as already described(16).

Results. Time sequence of DNA synthesis. The effect of PARA-adenovirus 2, PARA-adenovirus 7 and PARA-adenovirus 12 on the uptake of H^3-TdR by GMK cells was measured. In a series of experiments, uninoculated and inoculated cultures were given a one-hour pulse with H^3-TdR at different times following inoculation of the virus. The results from one such experiment are presented in Fig. 1. The infected cultures generally incorporated the label into the DNA fractions at a somewhat lower rate than the uninfected cultures until 15 hours post-infection. This was followed by a rapid increase in radioisotope incorporation 18 hours after inoculation of

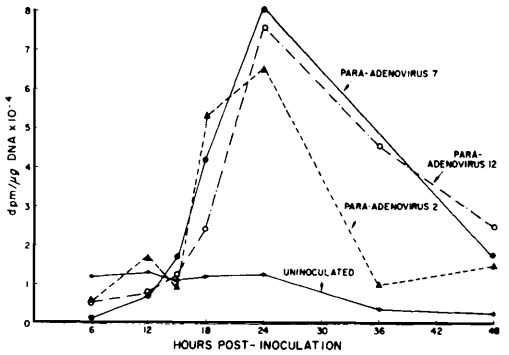

FIG. 1. Synthesis of DNA by cultures of GMK cells inoculated with different PARA-adenoviruses. The multiplicity of infection was 100, 10, and 40 plaque-forming units (PFU) per cell for adenovirus 2, 7, and 12, respectively and that of PARA virus was about 1 PFU percell. Replicate cultures were pulsed for one hour with 4-6 μc of H^3-thymidine (TdR) at various intervals. At the end of the pulse periods, cells were harvested, DNA hydrolyzed, and H^3-TdR incorporation determined.

the viruses. At 24 hours, there was approximately 6-8 fold increased uptake of TdR into the DNA in the cultures inoculated with either PARA-adenovirus 7 or its types 2 and 12 transcapsidants when compared to the uninoculated controls. This increase was then followed by a decreased incorporation of the radioisotope at 36 and 48 hours post-inoculation.

The increase in DNA biosynthesis in the infected cells was further demonstrated by radioautographic studies carried out with PARA-adenovirus 7. The cultures were exposed for 1 hour to 2 μc of H^3-TdR at various intervals after inoculation of the virus and processed as previously described(18). Nuclei showing 10 or more grains were counted. Again, radioisotope incorporation in the inoculated cells was lower than in the uninoculated cultures harvested at 6 and 12 hours post-infection (Table I). However, a

TABLE I. Radioautographic Studies on H^3-TdR Incorporation in GMK Cells Infected with PARA-Adenovirus 7.*

Hr post-inoculation	% Nuclei labeled following	
	No virus	PARA-adenvirus 7
6	17	6
12	15	9
18	18	28
24	17	70
36	5	65
48	—	21

* Secondary cultures of GMK cells grown on 22 × 22 mm coverslips were inoculated with PARA-adenovirus 7 at a multiplicity of 40 plaque-forming units of adenovirus 7 and 1 plaque-forming unit of PARA per cell. The cultures were pulsed with H^3-TdR (2 μc/dish) for 1 hr at various intervals after inoculation. At the end of the pulse time, the medium was removed, cultures washed, air-dried, fixed and radioautographs prepared. Radioautographs were developed after one week. Eight random fields were examined and the % of nuclei showing 10 or more grains was determined. Figures are based on averages derived from 100 to 250 cells per value given.

higher percentage of cells in the inoculated cultures synthesized DNA at 18 hours after infection and onwards. Radioisotope incorporation in the nuclei of infected cultures was maximal at 24 hours following virus inoculation. The pattern of radioactive labeling in the nuclei was generalized and the grains were present in the entire nuclear structure of the infected cells (Fig. 2). In a companion

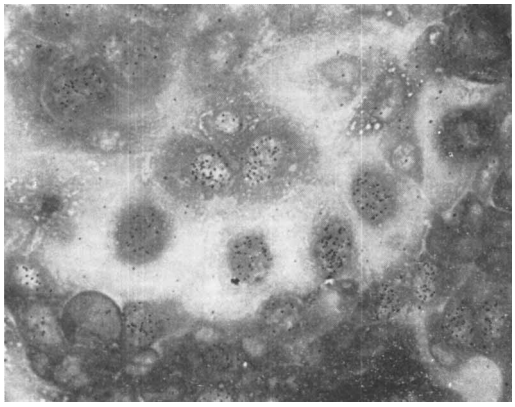

FIG. 2. Radioautograph of H^3-TdR pulse-labeled GMK cells, 36 hours post-infection. PARA-adenovirus 7 inoculated culture showing many black grains over the entire nuclear area in a large number of cells. Table 8 gives details of the experiment. × 160.

experiment, cultures harvested at 30 hours post-inoculation for the demonstration of SV40 T antigen by immunofluorescence techniques revealed that about 70% of the nuclei contained SV40 T antigen.

Characterization of the DNA. The results of preceding experiments made it obvious that GMK cell cultures inoculated with PARA-adenoviruses show increased DNA synthesis when compared with uninoculated control cultures. Experiments were therefore performed to determine whether this increase in DNA synthesis represented virus DNA, and whether the adenovirus and PARA DNA components could be separated.

The DNA was extracted from GMK cell cultures at various intervals after inoculation and subjected to analytical equilibrium centrifugation in gradients of CsCl. The results of this study are graphed in Fig. 3 and 4. The DNA samples obtained 1 hour after virus inoculation yielded only one band plus the reference DNA band. This experimental band had a buoyant density of 1.699 g/ml and a corresponding GC content of 40 moles %; this was identical with the band obtained from DNA extracted from uninoculated cultures and was therefore identified as GMK DNA. However, the DNA extracted from cultures 41 hours after inoculation of PARA-adenovirus 2 (Fig. 3) showed a distinct band in addition to the reference and host-cell DNA

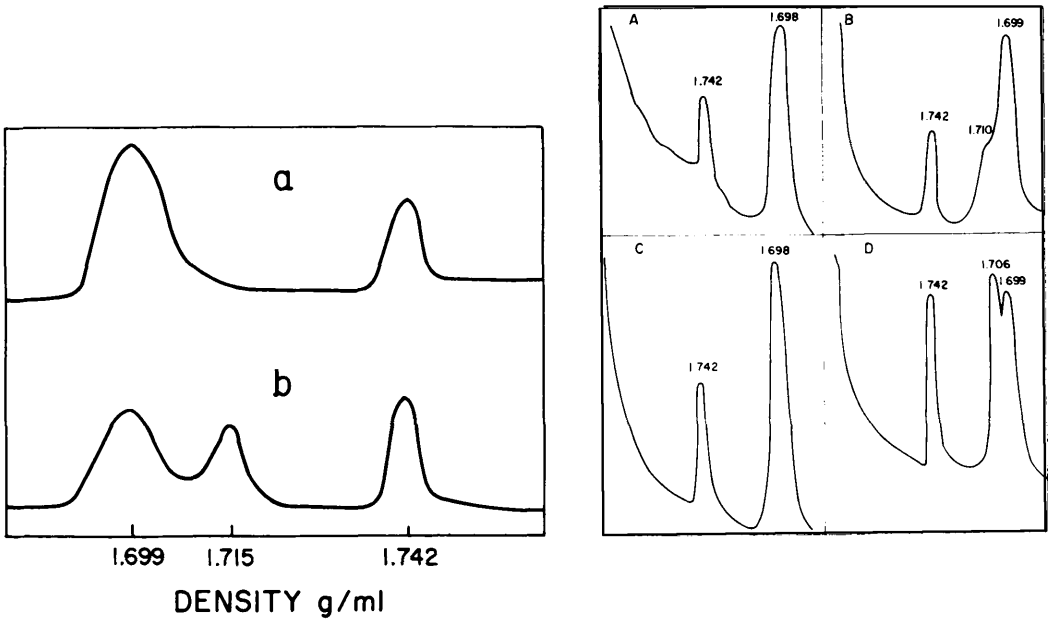

FIG. 3. Tracings of direct photoelectric scannings of extracted DNA from GMK cells inoculated with PARA-adenovirus 2 at equilibrium in CsCl gradients. The multiplicity of infection was about 46 PFU (adenovirus 2) and 1 PFU (PARA) per cell. a) DNA extracted 1 hr after inoculation. b) DNA extracted 41 hrs after inoculation. Reference DNA of *Bacillus subtilis* bacteriophage SP8 (density, 1.742 g/ml) is at right.

FIG. 4. Microdensitometer tracings (Joyce Loebl) from photographic films of extracted DNA centrifuged to equilibrium in CsCl gradients. The multiplicity of infection was about 10 PFU per cell for adenovirus 7 or 12 and 1 PFU per cell for PARA. a) DNA from GMK cultures inoculated 1 hr previously with PARA-adenovirus 7. b) DNA from GMK cultures inoculated 41 hr previously with PARA-adenovirus 7. c) DNA from GMK cultures 1 hr after inoculation of PARA-adenovirus 12. d) DNA from GMK cultures 41 hr after inoculation of PARA-adenovirus 12.

bands. The buoyant density of this band was found to be 1.715 g/ml, consistent with a GC content of 56 moles %. This was identical to the results obtained by Rapp *et al*(16) for non-productive infection of GMK cells with adenovirus 2 and agrees with the results of Green and Piña(19) for adenovirus 2 DNA.

Similarly, DNA extracted from PARA-adenovirus 7 and PARA-adenovirus 12 inoculated GMK cell cultures at 41 hours after infection (Fig. 4) showed peaks of virus DNA at a buoyant density of 1.710 g/ml and 1.706 g/ml corresponding to GC contents of 51 moles % and 47 moles %, respectively. These densities are in good agreement with those obtained previously for adenovirus 7 and adenovirus 12 DNA(16,20).

PARA-adenovirus 2 DNA was labeled with H^3-TdR by growing the virus in prelabeled GMK cells in the presence of the radioisotope. The virus was purified by centrifuging it twice to equilibrium in gradients of CsCl. DNA was extracted using the procedure of Green and Piña(19). About 10 μg of the DNA was then centrifuged to equilibrium in CsCl and the tube was emptied from the bottom in 0.1 ml fractions. As graphed in Fig. 5, the radioactivity appears as a sharp peak at a density of 1.714 g/ml, consistent with that of adenovirus type 2 DNA. Again, only one peak was obtained.

Discussion. Although PARA-adenovirus populations consist of phenotypically identical particles, they have been found to carry two sets of distinct genetic information(1-3). The present investigation, undertaken to follow DNA biosynthesis in cultures of GMK cells inoculated with such particles, showed an initial decrease followed by a rise in DNA biosynthesis between 15-18 hours post-infection; maximum synthesis of DNA as measured by incorporation of TdR into DNA and

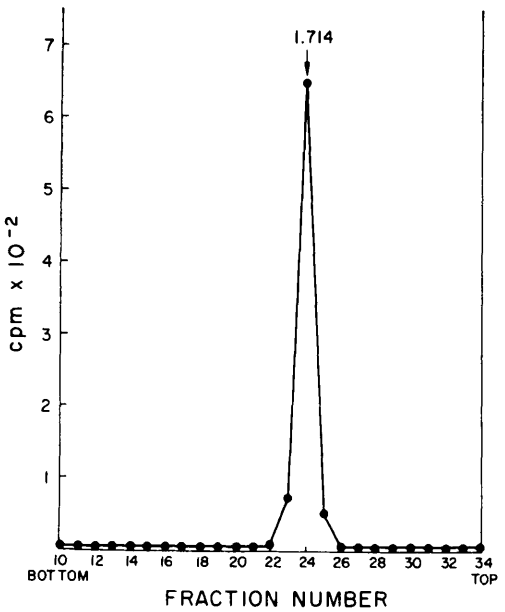

FIG. 5. Density of DNA extracted from PARA-adenovirus 2. Approximately 10 μg of purified DNA extracted from purified PARA-adenovirus 2 labeled with H^3-thymidine was centrifuged to equilibrium in CsCl. Fractions were collected from the bottom of the tube in 0.1 ml amounts and the radioactivity and density of each fraction was determined.

by radioautography occurred about 24 hours following inoculation of the cultures. A similar depression followed by increased DNA synthesis was also observed by Rapp *et al*(16) in GMK cells abortively infected with adenovirus types 2 and 12. The increase in DNA synthesis observed also coincides with an increase in thymidine kinase activity observed in our laboratory (Bresnick and Rapp, unpublished experiments) and by Kit *et al*(21) in GMK cells between 16-40 hours after infection with PARA-adenovirus 7. The observations presented here therefore reveal that DNA biosynthesis in GMK cells following infection with PARA-adenoviruses is similar to that observed during abortive infection of the same cells with the same adeno serotypes(16).

The presence of PARA virus DNA which contains a portion of the SV40 genome was not detected with the methods employed. It was therefore not possible to determine whether PARA virus DNA bands close to the adenovirus DNA or to the host-cell DNA, since the DNA of both GMK cells and SV40 have similar buoyant densities(22). However, DNA obtained from purified PARA-adenovirus 2 previously labeled with H^3-TdR yielded only one peak of radioactivity at a density of 1.714 g/ml in CsCl, suggesting that the PARA DNA probably bands very close to the adenovirus DNA and the difference, if any, cannot be resolved by isopycnic analysis. Other investigators(23,24) using other methods have come to a similar conclusion for PARA-adenovirus type 7. These studies have included attempts(23) to separate adenovirus and SV40 DNA in the "hybrid" population by density gradient centrifugation followed by hybridization of the DNA with virus-specific complementary RNA. Though artificial mixtures of SV40 and adenovirus DNA were readily separated by these procedures, "hybrid" DNA hybridized with both adenovirus and SV40-specific RNA.

Summary. Studies conducted to follow biosynthesis of deoxyribonucleic acid (DNA) in cultures of African green monkey kidney (GMK) cells inoculated with PARA (SV40)-adenoviruses 2, 7, and 12 showed an initial depression in incorporation of H^3-thymidine into the DNA. Levels of DNA started to increase after 15 hours, reaching a maximum in the virus inoculated cultures 24 hours post-infection. Extraction of the DNA from infected cells, followed by their identification by isopycnic analysis in CsCl gradients, revealed the presence of virus DNA with a density corresponding to the adenovirus serotype used. DNA samples from GMK cultures inoculated with PARA-adenovirus 7 or its transcapsidants, PARA-adenovirus types 2 and 12, yielded bands with peak densities of 1.710 g/ml [adenovirus type 7 DNA with 51 moles % guanine + cytosine (GC)], 1.715 g/ml (adenovirus type 2 DNA with 56 moles % GC), or 1.706 g/ml (adenovirus type 12 DNA with 47 moles % GC), respectively as well as a band at a density of 1.699 g/ml (GMK DNA with 40 moles % GC). A separate band that might have been distinctive for the DNA of the PARA particle was not detected.

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Inhibition of Adjuvant Arthritis by Statolon.* (32487)

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The parenteral administration of mycobacterial adjuvant into rats produces a disease characterized by polyarthritis, tendinitis and periostitis(1,2). Less constant features of adjuvant-disease or adjuvant arthritis (AA) include inflammation of portions of the eye, genital tract and skin. While the etiology of this disease is unknown, it is postulated that cellular or delayed hypersensitivity may play an important role in pathogenesis since: (a) the induction of tolerance to mycobacterial antigen in the neonatal period can inhibit subsequent production of "adjuvant-disease"(3), (b) there is a characteristic ten day latent period prior to the onset of arthritis(1), (c) the disease can be passively transferred between highly inbred rats by intact lymphoid cells(4), and (d) anti-rat lymphocyte serum inhibits the appearance of arthritis(5).

While it is possible that adjuvant activates a latent infection, against this is the fact that AA may be induced in gnotobiotic animals(6), and its onset is not inhibited by a variety of antibiotics(1). This evidence does not exclude the possibility of a virus or virus-like organism playing a role in the pathogenesis of AA. The effect of statolon (a broad spectrum anti-viral agent) was studied in this experimental model of arthritis in order to provide indirect evidence for virus or virus-like participation in the pathogenesis of AA.

Methods and material. Male, inbred, albino

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