Replication of PARA (Defective SV40)-Adenoviruses in Simian Cells

By F. RAPP* AND MARYANN JERKOFFSKY

Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas 77025, U.S.A.

(Accepted 10 March 1967)

SUMMARY

Human adenovirus types 1, 2, 3, 5, 6, 7, 12, 14, 16, and 21 failed to replicate beyond input titres in green monkey kidney (GMK) cells. Co-infection of the monkey cells with papovavirus SV40 stimulated replication of all the adenoviruses tested. Addition of the defective SV40 genome in the PARA particle carried by an adenovirus type 7 population enabled all the adenoviruses to replicate in the GMK cells. Growth kinetics of the adenoviruses in GMK cells co-infected with SV40 were similar to the kinetics of the virus following addition of PARA. A latent period of 16 to 24 hr was followed by an exponential increase in infectious virus between 24 and 48 hr after inoculation. Synthesis of the PARA component closely paralleled that of the adenoviruses. Both the adenoviruses and PARA remained largely cell-associated throughout the growth cycle. During the replication of the transcapsidants, the titre of the adenovirus was always greater than the titre of the PARA component. Ratios were generally in the order of 500 infectious units of adenovirus per infectious unit of PARA. These results differed from those obtained with the parent PARA-adenovirus type 7 population in which the adenovirus to PARA ratio was approximately 3:1.

INTRODUCTION

A number of human adenoviruses do not appear to replicate in simian cells, although synthesis of tumour (T) antigen (Feldman, Butel & Rapp, 1966; Malmgren et al. 1966) and viral DNA (Rapp, Feldman & Mandel, 1966b) occurs following infection of the cells. In the presence of SV40, these viruses are able to complete the replicative cycle and the cells produce virus capsid antigen (Feldman et al. 1966; Malmgren et al. 1966) and infectious progeny (Beardmore et al. 1965; Butel & Rapp, 1966a; Easton & Hiatt, 1965; Feldman et al. 1966; Lewis et al. 1966a; Lewis, Prigge & Rowe, 1966b; Rabson et al. 1964; Rowe et al. 1965).

Several studies have characterized a human adenovirus type 7 carrying defective SV40 genomes (PARA) in adenovirus capsids (Huebner et al. 1964; Rapp et al. 1964b; Rowe & Baum, 1964). These genomes were originally detected by the ability of this PARA-adenovirus population to induce the synthesis of SV40 T antigen. Although the adenovirus was able to replicate in human cells, neither PARA nor the adenovirus replicated in green monkey kidney cells in the absence of the other (Boeyé, Melnick & Rapp, 1966; Butel & Rapp, 1966a; Rowe & Baum, 1965). Using the techniques necessary to achieve transcapsidation (Rapp, Butel & Melnick, 1965b),

* American Cancer Society Professor of Virology.
it was shown that the genome in PARA could be transferred to adenovirus type 2 (Rapp et al. 1965b; Rowe, 1965) and that during this procedure, the coat assumed by PARA was that of the helper adenovirus.

Although the growth kinetics of PARA-adenovirus 7 have been described (Butel & Rapp, 1966a), quantitative studies on the transcapsidants have not been made. The present investigation describes the transfer of PARA to a number of adenovirus types. The effect of the presence of the defective PARA virus on the replication of the adenoviruses in simian cells was compared to that of the complete genome of SV40.

METHODS

Cells. Primary African green monkey kidney (GMK) cells were grown in 1 and 16 oz prescription bottles and in 60 x 15 mm. plastic Petri dishes. The cells were grown in Melnick’s lactalbumin hydrolysate medium (M-H) with 2% (v/v) calf serum and 0-08% (w/v) NaHCO3. All media used contained 100 units of penicillin and 100 µg. of streptomycin per ml. The cells were generally used after incubation for approximately 1 week at 37°. Human embryonic kidney (HEK) cells were grown in 35 x 10 mm. plastic Petri dishes. These cells were grown in Melnick’s lactalbumin hydrolysate medium (M-E) with 10% (v/v) foetal bovine serum and 0-23% (w/v) NaHCO3. KB cells were grown in Eagle’s minimal medium plus 10% (v/v) calf serum and 0-08% (w/v) NaHCO3.

Viruses. Adenovirus types 1, 5, 6, 14, and 16 were the prototype virus strains obtained from the Communicable Disease Center, Atlanta, Georgia. They were propagated twice in KB cells and once in HEK cells in our laboratory. Adenovirus type 21 was obtained from the American Tissue Culture Collection and was also propagated twice in KB cells and once in HEK cells. Adenovirus type 3 had been obtained from Wyeth Laboratories and had been subcultured 4 times in KB cells, plaque-purified twice in HEK cells, and then propagated once in the same cells. The adenovirus type 7 (H) and the adenovirus type 2 were obtained from Dr Benyesh-Melnick, who had isolated them from clinical specimens. The history of the PARA-adenovirus type 7 (stock SP2) has been previously described in detail (Huebner et al. 1964; Rapp et al. 1964b; Rowe & Baum, 1964). The virus had been propagated 22 times in primary rhesus kidney cells and 7 times in primary GMK cells. The virus used in this study was subcultured twice more in GMK cells. The PARA-adenovirus type 2 and PARA-adenovirus type 12 are transcapsidants prepared from the PARA-adenovirus type 7 and previously described (Rapp et al. 1965a). SV40 was the Baylor reference strain used in previous studies reported from this laboratory (Rapp et al. 1965a, 1964a).

Virus assays. Adenoviruses were titrated on HEK cells in plastic Petri dishes as previously described (Boeyé, Melnick & Rapp, 1965; Feldman, Melnick & Rapp, 1965) All dilutions were inoculated in duplicate. The virus was added in 0-1 ml. amounts and allowed to adsorb at 37° for 1 hr with frequent manual rotation. At the end of the adsorption period, 1-5 ml. of overlay containing 1% (w/v) agar and 10% (w/v) foetal bovine serum in Eagle’s medium was added; the final concentration of NaHCO3 was equivalent to 0-23% (w/v). A second overlay containing neutral red was added one week later and plaques were counted 3 days after that (on the 10th day after inoculation).

The titre of PARA was obtained by plaque assay in GMK cells in plastic Petri dishes. A saturating amount of helper adenovirus was added as previously described
PARA (defective SV40)-adenoviruses (Butel, Melnick & Rapp, 1966a; Butel & Rapp, 1966a; Rapp et al. 1965b) because PARA requires a co-infecting adenovirus for plaque formation. The helper adenovirus did not induce plaques in the absence of PARA. After adsorption for 1 hr at 37°, 5 ml. of overlay (ingredients as described above) were added. One week later, a second overlay containing neutral red was added. Plaques were counted on the 10th to 12th day after virus inoculation.

Serology. Antisera against the various adenoviruses were prepared in rabbits. Adenovirus stocks were grown in KB cells; the original harvests contained between $10^9$ and $10^{10}$ plaque forming units (p.f.u.) per culture. The viruses were purified by density gradient centrifugation on a preformed gradient of caesium chloride. The material was centrifuged for 3 hr in a Spinco Model L centrifuge at 100,000 g. The bands at 1.34 g./ml. were removed by side puncture. The material from the band was diluted to 2 ml. with 2-amino-2-hydroxymethylpropane-1,3-diol (tris) buffer (0.025 M, pH 7.4) and dialysed against 0.85 ~ NaCl for at least 1 hr. Half of the material was injected intramuscularly into each of two rabbits. Four weekly injections were given and the rabbits were exsanguinated 1 week after the final injection. The sera were inactivated at 56° for 30 min., and generally used at a dilution of 1/20.

Neutralization tests were made by mixing equal volumes of virus and antiserum and incubating them at 37° for 1 hr. Virus was then assayed by plaque count as described above; for some of the studies, virus was placed on cells growing on coverslips in Petri dishes. After suitable times of incubation, the cells on the coverslips were fixed in acetone at room temperature for 3 min. and then treated with hamster serum containing antibody against SV40 T antigen. After washing the cells, anti-hamster gamma globulin labelled with fluorescein isothiocyanate was added and allowed to react with the antigen + antibody complex. The detailed procedure for these immunofluorescence tests for the detection of SV40 T antigen was described by Rapp et al. (1965a, 1964a).

RESULTS

Replication of adenoviruses in the absence and presence of SV40

Initial studies were made to determine whether any of the adenoviruses could replicate in GMK cells in the absence of SV40. At the same time, the replication of the viruses in the presence of SV40 was investigated. The viruses were added in 0.1 ml. amounts to GMK cells growing in 1 oz bottles. The input multiplicity of the adenoviruses ranged from 0.7 to 1 p.f.u./cell based on titres obtained in HEK cells. Higher input multiplicities were often cytotoxic and were therefore avoided. After adsorption for 1 hr at 37°, the cells were washed twice with tris buffer and 5 ml. of M-H without calf serum were added. Two cell cultures were inoculated for each sample to be tested. Cells were harvested at various times after inoculation, and disrupted by 2 cycles of quick-freezing in a solid CO₂ + alcohol bath followed by thawing at 37°. The cell debris was removed by centrifugation and the supernatant fluid tested for virus. It had previously been determined that adenovirus types 2, 7 and 12 were unable to replicate in GMK cells in the absence of SV40. One-step growth curves were constructed for the non-oncogenic adenovirus types 1, 5 and 6 (Fig. 1); these viruses are in the high (56 to 60 moles %) guanine + cytosine (G + C) group (Piña & Green, 1965). All three viruses went into eclipse within 6 hr after inoculation of the cultures. Adenovirus types 5 and 6 did not replicate in the absence of SV40. Although
adenovirus type 1 appeared to replicate slightly, the titre at 72 hr after inoculation was not higher than the input titre; this phenomenon has been observed with other viruses and it was observed that the titres never exceeded input titres and no increase was observed between the 3rd and 7th day after inoculation. However, in the presence of SV4o added at an input multiplicity of about 1 p.f.u. per cell simultaneously with the adenoviruses, all three adenoviruses replicated. Titres increased sharply between 24 and 48 hr after inoculation and continued to increase up to 72 hr after inoculation, but not after this time.

![Graph](image1)

Fig. 1. Replication of human adenovirus types 1, 5, and 6 in green-monkey kidney cells in the absence and presence of SV4o.

![Graph](image2)

Fig. 2. Replication of human adenovirus types 3, 14, 16, and 21 in green-monkey kidney cells in the absence and presence of SV4o.

Replicate cultures of green-monkey kidney cells growing in 1 oz bottles were inoculated with 0.7 to 1 p.f.u. of adenovirus per cell. Half the cultures were simultaneously inoculated with 1 p.f.u. of SV4o per monkey cell. Four cultures were harvested at each designated time by quick-freezing the contents of the bottles in a bath of alcohol + solid CO₂. The contents were then thawed and the process repeated. The infectious adenovirus content of the fluids was determined by plaque formation in human embryonic kidney cells.

Similar experiments were then made with adenovirus types 3, 14, 16 and 21 (Fig. 2); the DNA of these viruses has an intermediate (50 to 53 moles %) G+C content (Piña & Green, 1965). In the absence of SV4o, none of these viruses increased beyond the input titre. Again, all viruses replicated extensively between 24 and 48 hr after inoculation when SV4o was used to co-infect the cells.

These studies revealed, therefore, the inability of many human adenoviruses to replicate to any degree in GMK cells in the absence of SV4o, although they did so very effectively when the monkey cell was co-infected with SV4o. It was therefore decided...
PARA (defective SV40)-adenoviruses

to add PARA to these viruses to determine whether the information supplied by the defective SV40 genome carried by PARA would enable them to replicate in GMK cells.

Transcapsidation of PARA by various adenoviruses

PARA-adenovirus type 7 was used as a source of PARA. The virus (consisting of PARA and adenovirus type 7) was inoculated to GMK cells co-infected with various adenoviruses. (Rapp et al. 1965b). This technique enhances the titre of the PARA-adenovirus 7 because at end-point dilutions, plaques are obtained only when PARA

Table 1. Effect of co-infection of simian cells by various adenoviruses on the plaque titre of PARA-adenovirus type 7

<table>
<thead>
<tr>
<th>Enhancing adenovirus</th>
<th>Titre of PARA-adenovirus 7, ( \log_{10} ) p.f.u.</th>
<th>Enhancement of titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.7</td>
<td>—</td>
</tr>
<tr>
<td>Type 1</td>
<td>6.5</td>
<td>63 times</td>
</tr>
<tr>
<td>Type 2</td>
<td>6.1</td>
<td>25 times</td>
</tr>
<tr>
<td>Type 3</td>
<td>6.2</td>
<td>32 times</td>
</tr>
<tr>
<td>Type 5</td>
<td>5.4</td>
<td>5 times</td>
</tr>
<tr>
<td>Type 6</td>
<td>6.6</td>
<td>80 times</td>
</tr>
<tr>
<td>Type 7 (H)</td>
<td>6.4</td>
<td>50 times</td>
</tr>
<tr>
<td>Type 12</td>
<td>5.9</td>
<td>16 times</td>
</tr>
<tr>
<td>Type 14</td>
<td>6.2</td>
<td>32 times</td>
</tr>
<tr>
<td>Type 16</td>
<td>6.0</td>
<td>20 times</td>
</tr>
<tr>
<td>Type 21</td>
<td>5.9</td>
<td>16 times</td>
</tr>
</tbody>
</table>

Dilutions of the PARA-adenovirus 7 were inoculated to green-monkey kidney cells in the presence and absence of helper human adenoviruses. The viruses were added simultaneously. The helper human adenoviruses were used at inputs of 2 to 20 particles and 0.08 to 1.4 p.f.u. (based on titres in human embryonic kidney cells) per monkey cell. The human adenoviruses alone did not cause the formation of any plaques in the simian cells. Following the addition of an agar overlay, the cultures were incubated at 37°C in an atmosphere containing 5% CO₂ and plaques were counted 10 days after inoculation of the cultures.

and an adenovirus co-infect the same cell. The effect of various human adenoviruses used in this study on plaque formation by PARA-adenovirus 7 was therefore investigated under conditions providing maximum enhancement (Butel et al. 1966a) (Table 1). Without a helper adenovirus, the concentration of the PARA-adenovirus 7 was \( 10^{4.7} \) p.f.u./ml. In all instances where a helper adenovirus was supplied, the concentration of the PARA-adenovirus 7 was increased. Enhancement ranged from a fivefold increase (with adenovirus type 5) to an increase of 80-fold (with adenovirus type 6). Plaques at the endpoint were then picked and placed into 1 ml. of tris buffer. Stocks were grown in GMK cells and tested to determine whether transcapsidation had occurred. If transcapsidation had occurred, serum against adenovirus type 7 should no longer have neutralized any of the properties of the population but antisera against the helper virus should effectively have neutralized the ability of the virus to replicate. A simple test for this is the neutralization of cytopathic activity or the ability to induce the synthesis of SV40 T antigen.

Viruses derived experimentally were not susceptible to antisera against adenovirus type 7 or antiserum against SV40. However, antisera prepared against a specific helper adenovirus supplying the coat for PARA now neutralized completely the ability of the
virus to induce the synthesis of SV40 T antigen (Table 2). Additional tests were made with these antisera on a number of the PARA populations to determine the effect on the ability to induce plaques in GMK cells. Neither antiserum against adenovirus type 7 nor SV40-immune serum had any effect on the ability to produce plaques. However, the antiserum specifically directed against the capsid of the new PARA entirely neutralized more than 100 p.f.u. of the virus. Approximately fifty plaque-purified transcapsidants were tested. With one exception, the progeny and the plaques all appeared to carry the coat protein of the helper adenovirus. This is similar to previous results with PARA-7 and PARA-2 (Rapp et al. 1965b; Rowe, 1965).

Table 2. Effect of antiserum on the ability of PARA-adenovirus to induce synthesis of SV40 tumour in green-monkey kidney cells

<table>
<thead>
<tr>
<th>Virus</th>
<th>Normal rabbit serum</th>
<th>Anti-adeno 7 serum</th>
<th>Anti-homologous adeno serum*</th>
<th>Anti-SV40 monkey serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARA-adeno 1</td>
<td>+</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>PARA-adeno 3</td>
<td>+</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>PARA-adeno 5</td>
<td>+</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>PARA-adeno 6</td>
<td>+</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>PARA-adeno 7</td>
<td>+</td>
<td>0</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>PARA-adeno 12</td>
<td>+</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>PARA-adeno 14</td>
<td>+</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>PARA-adeno 16</td>
<td>+</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>PARA-adeno 21</td>
<td>+</td>
<td>+</td>
<td>o</td>
<td>+</td>
</tr>
</tbody>
</table>

The various PARA-adenoviruses were incubated with the antiserum for 1 hr at 37°. The virus+ serum mixtures were then placed on green monkey kidney cells growing as monolayers on coverglasses. Following incubation at 37° for 24 hr, the cultures were harvested and fixed for 3 min. in acetone. They were then reacted with the immunoreagents used to detect SV40 tumour antigen and examined microscopically for fluorescence.

+ = positive by immunofluorescence; o = negative by immunofluorescence.

*rabbit serum against the original enhancing virus.

Growth kinetics of PARA-adenovirus populations

As seen above, the adenoviruses used in this study were unable to replicate in GMK cells in the absence of SV40. After the addition of PARA, they were retested for this ability. The first studies were made on PARA-adenovirus type 2, PARA-adenovirus type 7, and PARA-adenovirus type 12, representing the non-oncogenic, weakly oncogenic, and oncogenic adenoviruses; the results have been published (Rapp, 1966). After the addition of PARA, all viruses tested were able to replicate in GMK cells. The titre of the adenovirus was always higher than that of PARA. Results obtained with PARA-adenovirus types 1, 5, and 6 are summarized in Fig. 3. A sharp increase in the titre of adenoviruses was obtained. The ratio of adenovirus types 1 and 6 to PARA was approximately between 500 and 1000 to 1 resembling that previously seen with adenovirus type 2 (Rapp, 1966). The difference between adenovirus type 5 and its PARA was somewhat smaller. The other adenoviruses tested (types 3, 14, 16 and 21 of intermediate G+C) gave similar results (Fig. 4). The titre of adenovirus increased sharply after an initial eclipse. The relative number of adenoviruses to PARA differed significantly. Two passages in GMK cells of the populations did not markedly
change these ratios. Adenovirus progenies obtained by cloning in human embryonic
kidney cells required the presence of SV40 to replicate in GMK cells.

These studies were repeated with PARA-adenovirus types 7 and 12 to determine
whether the adenovirus and PARA remain cell-associated. Before freezing and thawing
of the cells at various times following inoculation, the supernatant fluid was harvested
and titrated. The cells were washed repeatedly with tris buffer and then frozen and
thawed to liberate cell-associated virus. These studies yielded results very similar to

the ones previously obtained with these viruses. There was a rise in cell-associated virus
24 hr after inoculation with a maximum at about 48 hr. There was always less extracelluar virus. Ninety per cent of both adenovirus and PARA remained cell-associated
throughout the course of the experiments which were terminated 96 hr after inoculation of the cultures (Fig. 5, 6). Similar results were obtained with PARA-adenovirus type 2.

Transcapsidation of a human isolate of adenovirus type 7

The low ratio (3:1) of adenovirus type 7 to PARA in the parent population (Fig. 5) and the higher ratios obtained with other viruses suggested a possible close relationship between the original adenovirus type 7 and its PARA. It was therefore decided to transfer PARA to a fresh human isolate of adenovirus type 7 (H) and to determine the adenovirus to PARA ratio during the growth cycle of the transcapsidated population. Throughout the growth cycle, the ratio of adenovirus to PARA never exceeded 10:1 and was usually about 3:1, the same as the values obtained for the parent population. Thus, the parent adenovirus type 7 is not unique, although the low ratio of adenovirus to PARA in type 7 strains is noteworthy. It was possible, however, that the low ratio of adenovirus type 7 and PARA resulted from the constitution of the original inoculum. To overcome this, PARA-adenovirus 7 was diluted with additional
PARA (defective SV40)-adenoviruses

adenovirus to obtain a ratio of 100 adeno 7 infectious units per PARA component. This virus was then added to GMK cells for a modified growth experiment. Again, ratios during the growth cycle were very low and the additional adenovirus input apparently neither depressed the replication of PARA nor gave an advantage to the adenovirus resulting in the large discrepancy seen with other PARA-adenovirus populations. However, the necessity to assay PARA and the adenoviruses on different cell types made it difficult to evaluate possible differences in plating efficiencies that may have contributed to the observations.

DISCUSSION

It has been clearly demonstrated in this paper and elsewhere that most, if not all, human adenoviruses cannot replicate significantly in GMK cells (Beardmore et al. 1965; Boeyé et al. 1966; Butel et al. 1966a, b; Butel & Rapp, 1966a; Feldman et al. 1965, 1966; Rabson et al. 1964; Rapp, 1966; Rapp et al. 1966b). The adenoviruses tested thus far, however, including types 2, 7 and 12, can induce the synthesis of adenovirus tumour antigen (Feldman et al. 1966) and virus specific DNA (Rapp et al. 1966b) in the abortive cycle in these cells. Contamination or deliberate co-infection of the cultures with SV40 (Beardmore et al. 1965; Butel & Rapp, 1966a; Easton & Hiatt, 1965; Feldman et al. 1966; Lewis et al. 1966a, b; Morris et al. 1966; Rabson et al. 1964; Rowe et al. 1965) or the defective SV40 genome carried in the PARA particle (Boeyé et al. 1966; Butel et al. 1966a; Butel & Rapp, 1966a; Rapp, Tevetia & Melnick, 1966c; Rowe & Baum, 1965) enables adenoviruses to complete the replicative cycle in the simian cells. The formation of virus-specific DNA during the abortive adenovirus cycle suggests that the SV40 genome helps a late step in the replication of the adenoviruses. This hypothesis is further supported by the recent discovery (Butel et al. 1966b) that an adenovirus type 7 adapted to growth in GMK cells carries with it a monkey-adapting component that is also encased in an adenocapsid. Like PARA, the monkey-adapting component can be transferred by transcapsidation to other adenovirus populations; and is necessary for replication of these adenoviruses. When this determinant is removed from the adenovirus population, the viruses no longer replicate in the GMK cells. Since the monkey-adapting component does not specify the induction of the synthesis of SV40 tumour antigen, it is obvious that this papovavirus determinant is not required for adenovirus replication in the simian cells. However, complementation of these genomes with adenovirus appears to be unusual since Feldman et al. (1966) have shown that other DNA viruses able to replicate in GMK cells do not enhance the replication of adenovirus type 7 and type 2.

The growth curves for the adenoviruses in GMK cells in the presence of SV40 or PARA are very similar. Although SV40 can replicate autonomously, PARA replicates in parallel with the adenovirus but not in its absence (Butel & Rapp, 1966a). The striking difference in the ratio of infectious units of adenovirus and infectious units of PARA in populations other than the parent adenovirus type 7 deserves further exploration. This may be a consequence of the incorporation of adenovirus type 7 DNA in the PARA particle which is transferred to the other populations with the SV40 genome during transcapsidation. Such a possibility has previously been suggested from staining reactions induced by transcapsidants (Rowe & Pugh, 1966), kinetics of ultraviolet inactivation of PARA (Butel & Rapp, 1966b), and DNA
homology studies (Reich et al. 1966). However, resolution of this problem will await the separation in purified form of either PARA or PARA-DNA; attempts by density gradient centrifugation in rubidium chloride (Rowe et al. 1965) and caesium chloride (Butel & Rapp, 1966b) have failed but others are in progress.

The consequences of the observation that animal viruses can serve as carriers of foreign determinants may be very large. Complementation of a foreign virus genome with the adenoviruses enables the adenoviruses to replicate in otherwise non-susceptible host cells. The addition of genetic information with oncogenic properties to such virus populations confers oncogenicity; thus, transfer of PARA to adenovirus type 2, a non-oncogenic adenovirus, confers oncogenicity on this virus when it is inoculated into newborn hamsters (Rapp et al. 1966a; Rapp, Melnick & Levy, 1967). Preliminary results with other PARA populations suggest that other non-oncogenic adenoviruses when made to carry PARA also become oncogenic. Vaccine strains of adenoviruses adapted to growth in simian cells will therefore require careful examination for foreign genetic information. The type of analysis presented in this paper as well as some of those cited above (Boeyé et al. 1966; Butel et al. 1966a, b; Butel & Rapp, 1966a; Huebner et al. 1964; Morris et al. 1966; Rapp et al. 1964b; Rowe et al. 1965; Rowe & Baum, 1964) are useful and necessary steps in evaluating viruses for genetic homogeneity.

This investigation was supported in part by Public Health Service research grants CA-04600 and CA-10036 from the National Cancer Institute, research grant AI-05382, and training grant 5 T I A174 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

REFERENCES


Boeyé, A., Melnick, J. L. & Rapp, F. (1965). Adenovirus-SV40 'hybrids': Plaque purification into lines in which the determinant for the SV40 tumor antigen is lost or retained. Virology 26, 511.


PARA (defective SV40)-adenoviruses


(Received 15 December 1966)