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Studies of the Mechanism of Enhancement of Human Adenovirus Infection in Monkey Cells by Simian Virus 40

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The defect which prevents human adenovirus replication in monkey cells and the mechanism whereby this restriction is overcome by coinfection with simian virus 40 (SV40) have been studied. Adenovirus capsid proteins are not synthesized efficiently in monkey cells in the absence of SV40. Adenovirus "enhancement" by SV40 was found to be the product of increased efficiency of replication by a small percentage of cells. This enhancement effect apparently occurred only when SV40 and adenovirus infected the same cells. These findings suggest that the replicative block occurs prior to virion assembly, and an accompanying report seeks to locate the site of restriction more precisely at the post-transcriptional level.

Infection of monkey cells by human adenoviruses results in abortive replication in which the adenovirus is able to penetrate the cell, uncoat viral deoxyribonucleic acid (DNA), and synthesize some virus-specific ribonucleic acid (RNA) (1), early proteins (24), and new viral DNA (18, 20). However, the amount of infectious virus produced in this type of infection is very small (18). The yield of adenovirus can be increased 1,000-fold or more if the monkey cells are simultaneously infected with simian virus 40 (SV40). This increase of infectious adenovirus, in the presence of SV40, has been termed "enhancement" (18, 20). This paper describes the results of highly specific methods of peptide analysis used to examine the production of adenovirus proteins in unenhanced and enhanced infection. These analyses show capsid protein synthesis to be deficient in the unenhanced infection. The accompanying paper by Fox and Baum (4), through a comparison of RNA synthesis in single and double infection, indicates that the block to efficient adenovirus replication is post-transcriptional.

MATERIALS AND METHODS

Cells. Primary human embryonic kidney (HEK) cells, and primary African green monkey kidney (AGMK) cells, were purchased from Flow Laboratories, Rockville, Md. HEK cells, obtained in suspension, were grown in roller bottles, and AGMK cells were obtained as monolayers. CV-1 cells, an epithelial subline of AGMK cells cloned by J. Robb, were supplied by J. Maio.

All cells were grown to confluence by using Eagle minimal essential medium (MEM) supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 mg/ml), and 2 mM glutamine. At the time of infection, confluent monolayers were refed with MEM containing 2% agammaglobulinic calf serum. Only cells found free of mycoplasma contamination by culture were used.

Viruses. Previous publications have described the preparation and passage history of the E46$^-$ strain of adenovirus type 7 (Ad7) (23), as well as those of adenovris type 2 (Ad2) (15). Pools of both adenoviruses for use in these experiments were produced by passage in HEK cells at high multiplicity of infection (MOI). Unless otherwise specified, infected cells were harvested by scraping the monolayer when all cells showed cytopathogenic effect. Cell pellets were prepared and virus was extracted, as previously described, with the aid of the fluorocarbon solvent trichlorotrifluoroethane (T. F. Genetron, E. I. Dupont Co., Wilmington, Del; 20, 22). Purified virus was obtained from the aqueous phase of this extraction by two cycles of equilibrium centrifugation through CsCl (22).

Adenovirus to be used as a marker in the electrophoretic analyses of proteins was radioactively labeled with [^{3}H]leucine, [^{3}H]valine, and [^{3}H]threonine (Schwartz BioResearch, Orangeburg, N.Y.) and was purified as described above. This virus was stored in CsCl at 4 C and remained intact for 2 to 3 months.

SV40 strain 777 (3) was obtained from Paul Black and was passaged at low multiplicity to produce stocks in either AGMK or CV-1 cells.

Antisera. Hexon, penton, and fiber capsomeres were purified from cells infected with Ad2 by use of diethylaminoethyl-cellulose chromatography and electrophoresis on acrylamide gels, as previously described (8). Rabbit sera containing antibodies to either the purified hexon, penton, or fiber capsid proteins of Ad2 were prepared by published methods (6). Since the penton capsomere contains fiber antigenic determinants as well as penton base, purified fiber antigen was used to remove antibodies to fiber from the anti-penton serum. After absorption, anti-penton serum no longer yielded precipitin lines in an immunodiffusion assay when tested against purified fiber antigen. The specificity of the anti-hexon serum has been demonstrated (7). Serum containing antibodies to rabbit gammaglobulin was prepared by hyperimmunization of sheep as previously described (7).

Unenhanced and enhanced adenovrius infection of monkey cells. AGMK or CV-1 cell monolayers were singly infected with adenovirus or doubly infected with adenovirus and SV40 as described in a previous publication (20). MOI values of Ad2, Ad7, and SV40 were 3, 10, and 10 plaque-forming units (PFU)/cell, respectively.

Immunofluorescence and infectious center assays. Two methods were used to measure the percentage of a population of monkey cells which participated in an unenhanced or enhanced adenovirus infection. The indirect fluorescent-antibody method was used to assay infected monkey cells which had been grown on cover slips and which were harvested and acetone-fixed at various times after infection (17). The cells were first treated with the rabbit anti-hexon, -penton, or -fiber serum described above, and then were reacted with fluorescein-conjugated goat anti-rabbit gamma globulin. Rhodamine-conjugated bovine serum albumin was used as a counterstain. At 21, 28, and 48 hr after single or double infection, the percentage of cells on a cover slip showing specific immunofluorescence with each antiserum was determined.

A modification of the method of infectious center plaque assay, described by Bishop and Koch (2), was also used to determine the number of cells in a population producing infectious adenovirus after unenhanced or enhanced infection. Six hours after infection of monkey cell monolayers in petri dishes, unadsorbed input virus was removed by five changes of medium. Infected cells were rapidly trypsinized and were serially diluted in 0.9% noble agar in MEM containing 10% agammaglobulinic calf serum. Two-fold dilutions of monkey cells in 0.6 ml of agar were plated onto monolayers of HEK cells in petri dishes. When this agar had solidified, an additional 5 ml of the agar medium overlay was pipetted gently into the dish. Seven days later an additional 2.5 ml of agar medium containing neutral red dye was overlaid, and, the following day, plaques were counted. As a control, infected cells, which had been killed by disruption after viral absorption but before new virus could be produced, failed to register as infectious centers.

Radioactive labeling, fluorocarbon extraction, and immunoprecipitation of infected monkey cells. At 48 hr after infection of CV-1 or AGMK cells, the medium was replaced with MEM containing [^{14}C]-leucine, [^{14}C]valine, and [^{14}C]threonine (Schwartz BioResearch), and one-twentieth the usual concentration of nonradioactive valine, leucine, and threonine. Twenty-four hr later, cells were harvested and cell packs were prepared. These cell packs were lysed and subjected to fluorocarbon extraction as described for virus purification. An amount of serum required to precipitate all of a specific antigen from a sample of the aqueous phase of the fluorocarbon extract was determined by performance of quantitative precipitin curves (7). Anti-hexon, -penton, and -fiber sera were reacted for 2 hr at 4 C with samples of each aqueous phase containing viral proteins, and the antigen-antibody complexes were then completely precipitated by adding sheep anti-rabbit gamma globulin and by continuing the incubation for 16 hr at 4 C. The resulting immunoprecipitates were washed twice in phosphate-buffered saline, solubilized with 2% sodium dodecyl sulfate (SDS) and 1% mercaptoethanol, and were layered on 6.5 by 200 mm cylindrical gels containing 5% acrylamide and 0.1% SDS (13). Before solubilizing the precipitates, purified [^{3}H]-labeled Ad2 virus was added as a marker. Electrophoresis was carried out at 70 v for 16 hr, the gels were fractionated with a Savant gel divider, and the [^{3}H] and [^{14}C]radioactivity of each gel fraction was measured using a Beckman liquid scintillation counter.

Samples of monkey cells infected with Ad2 or Ad7, unenhanced or enhanced by coinfection with SV40, and labeled with [^{14}C] amino acids were also treated with 10% trichloroacetic acid. The radioactive precipitates were pelleted by centrifugation at 10,000 $\times$ *g* for 10 min. The pellets, consisting of about 2.5 $\times$ 10^5 cells, were washed twice with 5% trichloroacetic acid, and once with acetone, and then were drained dry. Pellets were redissolved in 50 μliters of a solution containing 1% SDS, 0.1% 2-mercaptoethanol, 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 10% glycerol, and 0.001% phenol red. The *p*H of the resulting solution was adjusted to 7 to 8 with ammonium hydroxide, and the samples were heated at 100 C for 1 min.

Electrophoresis of these whole-cell precipitates was performed in a slab gel apparatus patterned after that of Reid and Bieleski (21). The gels contained the SDS-disc buffer system previously described (10, 14), and were 1.5 mm thick, 130 mm wide, and 92 mm high. They were formed by pumping a 20-ml gradient of 7.5 to 30% acrylamide by using a gradient maker similar to that of Leif and Vinograd (11). This gradient slab gel was overlaid with 4% acrylamide-SDS-disc gel into which 26 sample wells, 3 mm wide and 1.5 mm apart, were cast with a comblike Plexiglas spacer. The distance from the bottom of the sample well to the top of the acrylamide gradient was 9.5 mm.

A 10-μliter amount of each sample (the extract of 5 $\times$ 10^4 cells) was introduced into each sample well and was subjected to electrophoresis at 50 v for 16 hr, during which time the total current dropped from 12 to 3 ma. Gel slabs were stained for 1 hr in 0.2% Coomassie Brilliant Blue in 50% methanol and 7% acetic

acid, and then were decolorized in a transverse electrophoretic de-stainer (Canalco, Inc., Rockville, Md.) for 30 min. Gels were dried and radioautography was performed as described previously (14).

Assay of infectious adenovirus produced in unenhanced and enhanced infections. The yields of infectious adenovirus from all enhanced or unenhanced infections of monkey cells were measured by plaque titration in HEK cells (12). The titrations were done both in the presence and in the absence of neutralizing antiserum to SV40, and yielded identical results.

RESULTS

Production of infectious adenovirus in monkey cells. Monkey kidney cells infected with adenovirus alone, or coinfected with adenovirus and SV40, were assayed for their yield of infectious adenovirus. The relative yields of adenovirus in AGMK and CV-1 cells are shown in Table 1. In all cases there was at least 1,000-fold more Ad2 or Ad7 produced in the enhanced infection as compared to the unenhanced. Similar results have been reported in the past (9, 18, 20). The virus assays were repeated here to provide a necessary positive control for the results of infectious center and immunofluorescence assays.

Immunofluorescence assays of infected monkey cells. Attempts were made to employ an indirect fluorescence assay to measure the capsid protein production in unenhanced and enhanced infection of monkey cells. Cells were stained with hexon, penton, or fiber serum at 21, 28, and 48 hr after infection. In all cases, cells coinfected with Ad2 and SV40 showed much brighter fluorescence than did those infected with Ad2 alone. In the case of hexon reaction in both enhanced and unenhanced infection, the staining consisted of cytoplasmic dots early in infection (21 hr), but, by 48 hr, there was an additional clumped nuclear staining which was actually brighter in intensity than was the cytoplasmic stain at that point. The same pattern was noted with anti-penton and anti-fiber serum, except that no staining appeared until about 24 hr after infection. Although doubly infected cells always fluoresced much brighter than singly infected ones, singly infected cells did show some cytoplasmic and nuclear fluorescence with specific anti-hexon, -penton, and -fiber sera. Figure 1 shows typical patterns of nuclear and cytoplasmic staining with anti-hexon serum at 48 hr after Ad2 infection.

Staining was totally absent when the infection was performed in the presence of the inhibitors of DNA synthesis (5-fluorodeoxyuridine, or cytosine arabinoside), thereby differentiating the capsid immunofluorescence from adenovirus T antigens which are formed in the absence of new DNA synthesis (25).

TABLE 1. *Comparison of adenovirus yield and percentage of cells infected in unenhanced and enhanced infection of monkey cells*

Type of cells[a]	Type of infection[b]	Adenovirus yield (PFU/ml)[c]	Infectious centers/10^3 cells	Cells positive in fluorescence assay (%)
AGMK	Ad2	$10^{5.7}$	4	3.9
AGMK	Ad2 + SV40	$10^{9.5}$	4.2	6.3
AGMK	Ad7	$10^{5.0}$	5	0.6
AGMK	Ad7 + SV40	$10^{8.6}$	5.5	1.8
CV-1	Ad2	$10^{4.2}$		0.4
CV-1	Ad2 + SV40	$10^{8.8}$		1.0
CV-1	Ad7	$10^{2.7}$		
CV-1	Ad7 + SV40	$10^{7.9}$		

[a] AGMK = African green monkey kidney; CV-1 = an epithelial subline of AGMK.

[b] Ad2 = adenovirus type 2; Ad7 = adenovirus type 7; SV40 = simian virus 40.

[c] PFU, plaque-forming units.

These immunofluorescence studies showed that the ratio of cells containing antigens in the enhanced infection to those showing adenoviral antigens in the unenhanced infection did not exceed 3:1 (Table 1). Therefore, the 1,000-fold enhanced viral yield appeared to be the result of increased production of infectious virions without comparable increase in the total number of cells producing virus.

Attempts were made to verify these findings by using an infectious center assay to determine how many cells in each type of infection were capable of producing infectious adenovirus. Although this assay system was less sensitive than immunofluorescence, similar results to those seen in fluorescent-antibody experiments were found. There was approximately the same low percentage of infectious centers in the doubly infected cells as in the singly infected cells (Table 1).

Electrophoretic analysis of adenoviral protein synthesis during enhanced and unenhanced infection. Immunofluorescence examination of infected cells had indicated that some capsid proteins were synthesized in the unenhanced infection although quantitation relative to enhanced infection was impossible. The gradient slab gel technique described above provided an opportunity for semiquantitative analysis of viral protein synthesis in monkey cells. The method offered sufficient sensitivity and resolution to permit direct examination of whole-cell extracts.

Figure 2 shows the slab gel electrophoresis of an enhancement experiment in which Ad2-infected AGMK cells were studied. Although exact

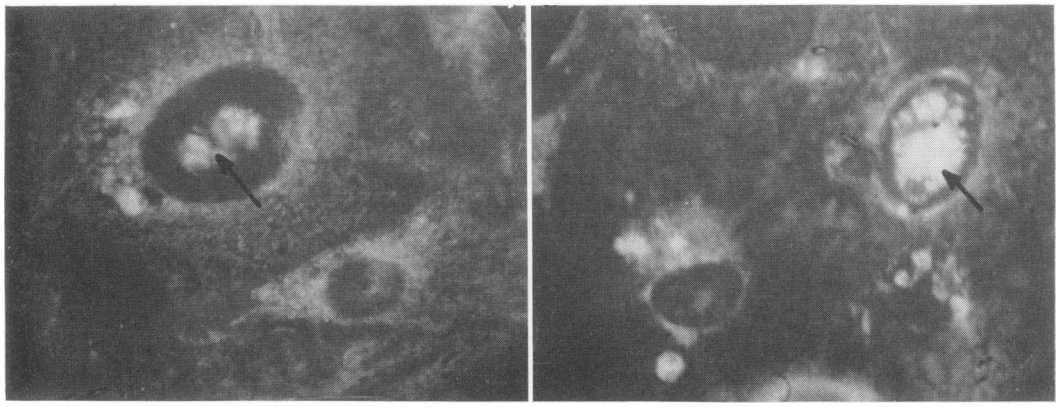

FIG. 1. *Immunofluorescence of AGMK cells infected with Ad2 (unenhanced, left), or with Ad2 and SV40 (enhanced, right). Forty-eight hours after infection, cells were stained by the indirect fluorescent-antibody technique with rabbit anti-hexon serum and fluorescein-conjugated goat anti-rabbit gammaglobulin. Singly infected cells (left) contain fine granular cytoplasmic stain. Some of them (arrow) also contain clumped nuclear stain. Doubly infected cells (right) contain granular and clumped cytoplasmic stain as well as nuclear clumps (arrow) which are brighter than that in the unenhanced preparation, and which occupy almost the entire nucleus. Magnification:* × *485.*

quantitation is not possible from these radioautographs, it is seen that the hexon, penton, and fiber protein bands are synthesized to a greater extent in the enhanced as compared to the unenhanced infection. Results of studies involving Ad7 in AGMK and Ad2 in CV-1 were similar to those shown.

A small amount of material migrating with hexon is evident in the unenhanced infection. This finding is consistent with the low level of viral replication previously described (20), as well as with the fluorescence and infectious center assays cited above.

The band in Fig. 2 denoted "SV" represents a major structural component of SV40. This band appears more prominent in cells infected with SV40 alone than in those infected with adenovirus and SV40. This finding is in agreement with results of unpublished experiments in our laboratory, and those published by Friedman et al. (5) showing that the yield of infectious SV40 virus is reduced in the presence of adenovirus coinfection.

There are also protein bands, marked with arrows in Fig. 2, which appear after adenoviral infection of human and monkey cells but which may not be incorporated into the virion. The band marked "1→" is also detected in the SDS-acrylamide gel system to be discussed, but it is not precipitable with antisera against the capsid antigens. The band marked "2→" is more clearly demonstrable in the unenhanced infection and has a mobility similar to that of the "P" antigen described by Russell and Skehel (26). Band "3→"

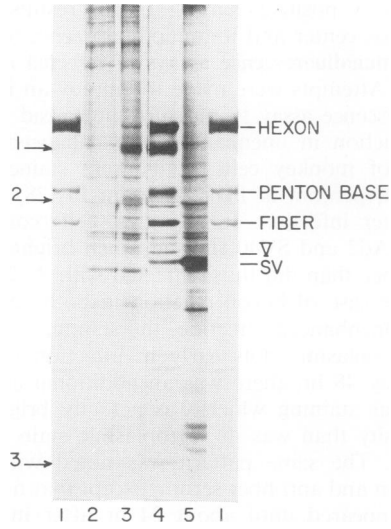

FIG. 2. *Electrophoretic pattern of whole-cell lysates from enhanced and unenhanced Ad2 infection of AGMK monkey cells. Following infection with Ad2, or with Ad2 + SV40, cells were radioactively labeled with [*^{14}*C]amino acids. Cells were precipitated with 10% trichloroacetic acid and the solubilized precipitates were electrophoresed at 50v for 16 hr on 7.5 to 30% gradient acrylamide slab gels. Radioautography of dried gels was carried out for 48 hr. 1, Ad2-infected KB cell marker; 2, uninfected CV-1 cells; 3, Ad2-infected CV-1 cells; 4, Ad2- and SV40-infected CV-1 cells; 5, SV40-infected CV-1 cells. The band labeled "SV" corresponds to the major capsid protein of SV40. The bands marked with arrows are non-capsid proteins of adenovirus produced in enhanced and unenhanced infection.*

(Fig. 2) is similar in mobility to an early protein seen in Ad2 lytic infection of human cells (*unpublished data*).

Quantitation and immunologic identification of viral polypeptides. Although the gradient slab gel technique confirmed the impression that appreciably more capsid protein was synthesized in the enhanced infection, it was desirable to establish further the identity of the protein bands, and to measure more accurately the ratio of capsid protein production in the two infections. We employed the technique of immunoprecipitation of radioactively labeled viral proteins followed by electrophoresis of the dissociated precipitate on SDS-acrylamide cylindrical gels. These gels could be fractionated, and the radioactivity in each fraction could be accurately counted. Electrophoresis of known adenovirus protein on the same gels allowed identification of the material in each fraction.

It was found that both enhanced and unenhanced adenovirus infection of monkey cells were even more inefficient at terminating cell protein synthesis than was the lytic infection of human cells (27). We attempted to improve the discrimination of viral capsid proteins with the use of specific antisera, but again found that immunoprecipitated radioactivity, that might be assumed to be viral in a lytic system, did not co-migrate electrophoretically with a viral marker. The specificity of immunoprecipitation was greatly improved by first extracting infected cell lysates with Genetron. This extraction procedure yielded an aqueous phase much enriched for adenoviral proteins.

Figure 3 shows the peptide pattern from CV-1 cells which were infected with either Ad2, Ad2 plus SV40, or SV40 alone. Following fluorocarbon extraction, the aqueous phase was treated

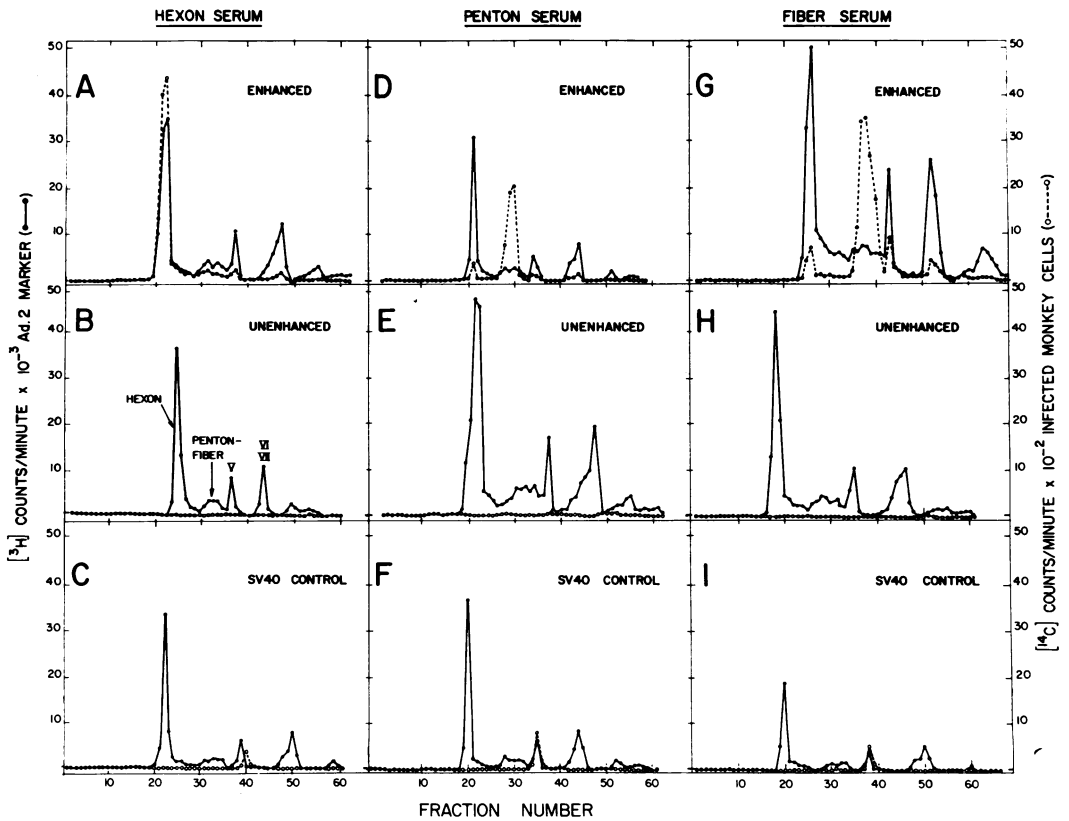

FIG. 3. *Electrophoretic pattern of* [^{14}C] *adenoviral proteins from unenhanced and enhanced Ad2 infection of CV-1 monkey cells* (○). *Radioactively labeled infected cells were extracted with fluorocarbon solvent, and viral proteins were immunoprecipitated from the aqueous phase with antiserum to the hexon, penton, or fiber-antigen of Ad2. Electrophoresis of solubilized immunoprecipitates was carried out in the SDS-acrylamide system at 70v for 16 hr on 5% acrylamide gels.* [^{3}H] *proteins of purified Ad2 virus grown in human cells were added as a marker* (●), *to each gel before electrophoresis.*

with specific antisera and the dissociated precipitate was subjected to electrophoresis on 5% acrylamide gels. Enhanced infection (panel A) is seen to yield an easily demonstrable polypeptide, precipitated with hexon serum, and migrating with [^{3}H]-labeled hexon. Unenhanced infection (panel B) and SV40 infection (panel C) do not yield such a polypeptide. SV40 infection produces a lower molecular-weight polypeptide which is partially precipitated by all of the adenovirus antisera, and which is present in large quantities in nonimmunoprecipitated SV40-infected material (Fig. 2).

Figure 3 also shows the results of electrophoresis of similarly processed singly and doubly infected CV-1 cells treated with anti-penton or anti-fiber serum. Once again, specifically immunoprecipitated polypeptides migrating in the region of penton and fiber proteins (which are not clearly distinguished on this type of gel) are synthesized in the enhanced (panels D and G) but not in the unenhanced (panels E and H) or SV40-infected cells (panels F and I). As has been previously noted in human cells lytically infected with Ad2 (*unpublished results*), anti-fiber serum precipitated a small amount of hexon antigen from infected monkey cells.

It appeared from these studies that abundant amounts of the major capsid proteins of adenovirus were synthesized in the enhanced infection but were not produced in the unenhanced infection of monkey cells. It can be calculated that, in the case of hexon, 0.1 to 1.0% of the level detectable in the enhanced infection would have been readily detected by these techniques in the unenhanced infection, if present. That is, the enhanced infection produced at least 100- to 1,000-fold more hexon than did the unenhanced infection.

In evaluating the above results, it was considered that fluorocarbon extraction and immunoprecipitation might have selected for a small nonrepresentative proportion of the total viral protein present in the cell lysate. Results of gradient slab gel electrophoresis of whole-cell lysates (Fig. 2) gave some assurance that immunoprecipitation was not artifactually selective. This slab gel method also was used to show that the aqueous phase of the fluorocarbon extraction contained most of the adenovirus proteins and, therefore, was appropriate material in which to measure these proteins.

Fluorocarbon extraction yielded three phases: an aqueous phase containing about 9% of the initial radioactivity, a fluorocarbon layer containing less than 1% of the initial radioactivity, and a turbid gelatinous interphase which comprised about 90% of the starting radioactivity. To determine the distribution of virus-specific and cell-specific proteins in these phases, samples of aqueous phase and aqueous-Genetron interphase were prepared for electrophoresis on gradient slab gels as described above. The results of radioautographic analysis of these gels (Fig. 4) indicate that, although the aqueous phase of the fluorocarbon extraction contained only 9% of the total counts, it comprised almost all of the material identified as viral capsid protein.

Role of SV40 in enhancement. Attempts were made to establish the identity of the enhancing principle of SV40 responsible for the increase in adenovirus yield. Experiments of ours (*unpublished data*) and the work of Friedman et al. (5) appeared to indicate that early SV40 proteins were responsible for enhancement. We attempted to enhance adenovirus infection of monkey cells

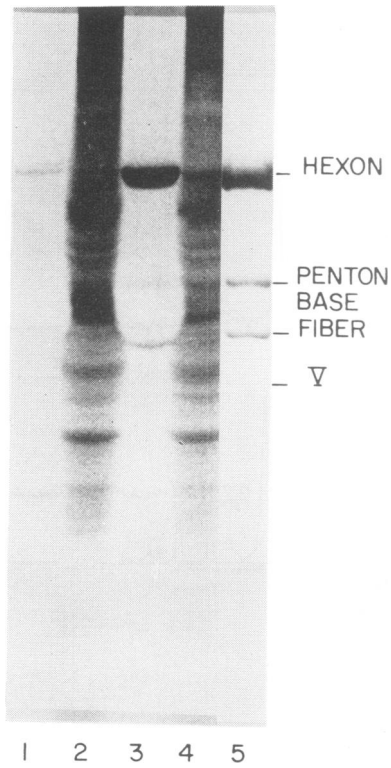

FIG. 4. *Electrophoretic pattern of aqueous phases, and Genetron-aqueous interphases from enhanced and unenhanced Ad2 infection of CV-1 monkey cells. The procedure was as described for Fig. 2 except that fluorocarbon extraction preceded trichloroacetic acid precipitation. 1, Aqueous phase of unenhanced infection; 2, aqueous-Genetron interphase of unenhanced infection; 3, aqueous phase of enhanced infection; 4, aqueous-Genetron interphase of enhanced infection; 5, Ad2-infected KB cell marker (whole-cell lysate).*

with products of SV40 infection in two ways. AGMK cells infected with SV40 virus at a MOI of 20 PFU/cell were allowed to progress to complete cytopathogenic effect. Infected cells were lysed by rapid freeze-thawing and sonic treatment, and particulate material including virions was sedimented by centrifugation at 60,000 × *g* for 16 hr. The supernatant fluid of this centrifugation, shown to be free of SV40 virus by plaque titration on AGMK cells, was added to monolayer cultures of AGMK cells, which were then infected with Ad7 at a MOI of 10 PFU/cell. Replicate AGMK monolayers were infected with Ad7 alone, or with Ad7 and the pellet formed by the above centrifugation. Virus yields at 72 hr after infection were measured by plaque titration in HEK cells. There was no significant difference in the amount of virus produced in cells infected with Ad7 alone from those treated with SV40 virus-free lysate. Cells coinfected with the pellet of the 60,000 × *g* centrifugation and Ad7 showed the usual 1,000-fold enhancement.

To minimize the possibility that failure to detect the SV40-enhancing principle was due to its degradation during preparation, the following additional experiments were carried out. AGMK cells were grown parabiotically in an apparatus obtained from Bellco Glass Co. (Vineland, N.J.). Monolayers of AGMK growing on cover slips in modified Leighton tubes were separated from one another by a gradacol membrane (pore size—25 nm). Monolayers on one side of the membrane were infected with Ad7, and cells on the other side were infected with SV40. Control chambers were infected with adenovirus alone. The tubes were constantly agitated at 37 C for 7 days, at which time the supernatant fluid was harvested from the sides of the chamber infected with adenovirus and was titrated for infectious virus. No enhancement was noted.

DISCUSSION

After the discovery by Rabson et al. in 1964 (18) that SV40 infection of monkey cells enhanced the replication of adenoviruses in these cells, a study was undertaken to determine the step in adenovirus replication which might be blocked in monkey cells. It was found that adenovirus specific T antigen (24), DNA (19, 20), and RNA (1) were made in approximately equal amounts in unenhanced and enhanced infections, although 1,000-fold more adenovirus was produced in the enhanced infection. From these data, it was inferred that steps preceding DNA replication, i.e., adsorption, penetration, and uncoating of viral genome, took place normally in monkey cells.

It appeared then that the block to efficient replication occurred at some step during or following RNA transcription. Previous experiments, showing approximately the same total amount of adenoviral RNA in enhanced and unenhanced infection (1), seemed to militate against the presence of major transcriptional defects in the unenhanced RNA. It appeared then that the defect was either in processing the RNA, in protein synthesis, or in assembly of synthesized proteins. Therefore, we undertook an analysis of the production of adenoviral capsid proteins in this system.

Although less efficient than poliovirus, human adenoviruses are relatively competent at terminating cellular protein synthesis in the permissive lytic infection of human cells (27). The shut-off of cell protein synthesis is, however, quite inefficient in the infection of monkey cells. In addition, we have found that the enhanced infection produces SV40 proteins which cross-react with many adenovirus sera. These two factors render inaccurate the quantitation of adenovirus proteins by immunoprecipitation without some prior purification of the antigens and subsequent characterization of the precipitate.

To eliminate much of the contaminating cellular and SV40 proteins, we employed a fluorocarbon extraction procedure which is a part of a standard purification scheme for adenovirus (22). We found this extraction method to be very effective in separating many cellular and SV40 proteins from those of adenovirus, which partitioned in the aqueous phase. By using specific antisera, we were further able to quantitatively precipitate the radioactively labeled capsid proteins of adenovirus from the aqueous phase and then to measure their relative amounts after electrophoresis on SDS-acrylamide gels. Results of these experiments showed that adenoviral capsid proteins were made in very small amounts, in the absence of SV40 coinfection, but were easily detectable and measurable as a result of SV40 enhancement. It is our estimate that at least 100- to 1,000-fold more capsid protein is made in the enhanced infection.

The possibility that the different amounts of capsid proteins detected in unenhanced and enhanced infections were artifacts of the extraction or immunoprecipitation steps was excluded by direct electrophoresis experiments in which whole-cell lysate was subjected to electrophoresis on gradient slab gels. While these conditions were "non-selective," the resolution was high enough to distinguish viral capsid proteins from contaminating cell and SV40 proteins. This method demonstrated efficient synthesis of adenoviral proteins only in the enhanced infection.

Very small amounts of material migrating at the location of viral capsid proteins were detected in the singly infected cells subjected to electrophoresis on slab gels. This finding is consistent with the detection of some specific fluorescence staining of singly infected cells and with previous experiments which have shown that virus replication does proceed at a very low level in singly infected monkey cells (20). The discrepancy in capsid protein synthesis is of the same order of magnitude as that of synthesis of infectious adenovirions, and explains the low viral yield in the unenhanced infection.

Combined with the findings of approximately equivalent DNA (19, 20) and RNA (1) synthesis in the unenhanced and enhanced AGMK infections, the protein data indicate a block at a preassembly level.

Therefore, it would appear that human adenoviruses are capable of adsorbing to and penetrating monkey cells. Following this, the DNA is sufficiently uncoated to allow transcription of some messenger RNA (mRNA), translation of early proteins, and synthesis of new viral DNA.

At this point in the replicative pathway, either some species of mRNA is inefficiently transcribed or, if all mRNA is made, it is defectively translated, modified, or remains unbound to ribosomes, with the end result being inefficient viral protein synthesis and minimal yield of infectious virus. These studies, therefore, point to a block either at transcription or at a post-transcriptional level.

An accompanying study by Fox and Baum (4) examines some of the species of adenovirus-specific RNA synthesized in enhanced and unenhanced infection of monkey cells to establish the exact nature of the block. By using hybridization competition and gel electrophoresis of RNA, this study indicates no detectable difference in several species of early and late adenovirus RNA synthesized in unenhanced and enhanced monkey cell infection, and suggests a post-transcriptional block to replication.

The role of SV40 in overcoming this block has yet to be elucidated. The Ad7-SV40 hybrid virus $E46^+$ (23) can be said to enhance the growth of adenovirus in monkey cells, since its presence in mixed populations of nonhybrid and hybrid virus allows the continued propagation of nonhybrid adenovirus in these cells. Presumably, it is the presence of the partial SV40 genome in the hybrid virion which permits adenovirus replication to take place. Since the hybrid virion is not able to transcribe "late" SV40 mRNA (16), to code for late SV40 capsid proteins, or to yield infectious SV40 (23), it is reasonable to assume that an early function of SV40 is capable of enhancing adenovirus growth. Mutants of SV40 virus which are deficient in late function but able to enhance no doubt would prove useful in establishing this as fact.

We have attempted to see if simultaneous infection of a cell is necessary for enhancement, or if products of an SV40-infected cell can enhance adenovirus growth in a different monkey cell. Our results indicate that SV40 must infect the same cell as adenovirus for enhancement to occur. Furthermore, SV40 seems to cause an increased adenovirus yield from a relatively fixed number of cells.

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