STUDIES OF ADENOVIRUS SV40 HYBRID VIRUSES

II. Defectiveness of the Hybrid Particles*

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The E46+ strain of adenovirus type 7 (Ad. 7) has been demonstrated to be a form of intergeneric hybrid between Ad. 7 and SV40 viruses, in that SV40 genetic material is enclosed in an Ad. 7 capsid (1–4). However, it was shown that a standard pool (E46) of this virus consisted of a mixture of adenovirus particles with and without SV40 DNA; the evidence for this was the finding that the limiting dilution of a titration of pool E46 consisted of Ad. 7 which did not induce SV40 antigen formation (2). This report describes negative attempts to recover, in pure form, the hybrid component of the mixture, and further experiments which indicate that the hybridized virus particles are defective.

Materials and Methods

Virus. The origin and passage history of the E46 preparation of the L.L. strain of Ad. 7 have been described previously (1); virus originating from this pool is referred to as E46+ strain. A serial passage line originating with E46 was carried in cell cultures of African green monkey kidney (AGMK) in an attempt to enrich the hybrid portion of the mixed virus population (2); virus stocks were prepared at various passage levels and were found to induce SV40 neoantigen in a high proportion of cells (2, 4). The passage level beyond the E46 pool is identified by "AG" followed by the number of passages. Thus, E46AG8 is the eighth AGMK passage beyond pool E46, and E46AG9L2 is the ninth AGMK passage, of which the last two were made at limiting titration dilutions.

The non-hybrid line of virus obtained from pool E46 by limiting dilution passage in human embryonic kidney (HEK) cells is referred to as E46−. This virus was propagated in HEK cultures, the standard pool used here being made from HEK cultures inoculated with virus from a second serial limiting dilution passage.

In referring to individual virus particles, adenovirus particles which induce SV40 neoantigen synthesis will be referred to as ⊕ particles, and those which do not, as ⊖ particles.

Plaque Procedures.—The plaque procedure was that of McAllister and Goodheart (5). Primary cultures of HEK (obtained from Microbiological Associates, Bethesda, Maryland, and Flow Laboratories, Rockville, Maryland) were dispersed with trypsin and the cells washed, counted and dispensed into 50 mm plastic Petri dishes (Falcon Plastic Company,

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Los Angeles) at 600,000 cells per dish in 5 ml of medium. Growth medium was 20 per cent inactivated agammaglobulinic calf serum in Eagle's basal medium No. 2 (BME) with 2x vitamins and amino acids, with glutamine, penicillin, and streptomycin. When cell sheets were confluent, they were washed once with BME, and inoculated with 0.5 ml of virus dilution. After 1 hour absorption at 37°C, with gentle agitation every 15 minutes, the cultures were overlaid with 5 ml. of growth medium in 0.9 per cent Noble agar. The cultures received additional overlays of 2.5 ml of the nutrient agar at 3 and 7 days; the latter contained neutral red at a concentration of 1:8,000. Plaques were counted on the 7th through 9th days.

AGMK plaque titrations were done by essentially the same technique, differing only in that the cells were grown in medium containing 10 per cent fetal bovine serum in BME, and in more recent tests the overlay agar contained 10 per cent rather than 20 per cent agammaglobulinic calf serum.

Well isolated plaques were picked with a Pasteur pipette and the agar plug extracted in 1 ml of BME. Negative areas were also sampled, and were almost always negative for virus.

Virus Quantitation by Neoantigen Induction.—The procedure for quantitating Ad. 7 and SV40 neoantigen-inducing particles by immunofluorescent staining at the end of the first cycle of infection has been described previously (4). Briefly, Petri dish cultures of HEK cells growing on coverslips were inoculated with 0.1 ml of an appropriate dilution of virus; 21 to 24 hours later the coverslips were fixed in cold acetone, dried, and broken in half. One portion was stained, by the indirect fluorescent antibody procedure, with serum from hamsters carrying Ad. 12 tumors, and the other with SV40-tumored hamster serum. The proportion of cells showing specific staining was determined (2), and was converted to an estimate of virus titer ($\log_{10}$) by obtaining the log of the product of the number of cells per dish (10^6) times the fraction of cells stained times the dilution factor. The ratio of percentages obtained with the adenovirus and SV40 reagents (Ad./SV40-staining ratio) was used as a measure of the proportion of particles carrying SV40 genetic material.

For assay of materials such as plaque isolates where the virus titer was occasionally low and the presence or absence of SV40 antigen, rather than the staining ratio, was the desired information, the coverslips were harvested when the characteristic adenovirus cytopathogenicity first appeared.

RESULTS

Attempts to Obtain a Pure Line of ⊕ Particles.—As described previously (2) E46$^+$ appeared to consist of a mixture of ⊕ and ⊖ particles, since the limiting dilution fluids of a titration of preparation E46 in HEK cultures contained no virus capable of inducing SV40 neoantigen. The E46$^-$ virus thus obtained was also found to lack the ability to be propagated in serial passage in AGMK cultures. It thus appeared reasonable to attempt to obtain a pure ⊕ population by rapid passage of E46$^+$ in AGMK, where the ⊕ particles would have high selective advantage, followed by purification by limiting dilution passage in this tissue. E46$^+$ was carried for 7 undiluted virus passages in AGMK, followed by two successive limiting dilution passages (E46AG9L2); throughout the passage series the Ad./SV40-staining ratio remained at 1:2 to 2:1. When E46AG9L2 was titrated in HEK tube cultures, the virus obtained at limiting dilution was again negative for SV40 neoantigen induction.

Attempts were then made to purify ⊕ particles by plaque isolation. Forty-

four plaques of E46 obtained in HeLa and HEK cultures were supplied to us by Dr. R. M. McAllister and Dr. C. R. Goodheart and passed once in HEK cells; these were all negative for SV40 neoantigen induction. Of 24 HEK plaques (all containing infectious adenovirus) from E46AG8, tested directly for SV40 neoantigen induction, 21 were negative and 3 induced SV40 neoantigen in 0.01 to 0.03 per cent of cells; 2 of these 3 plaque isolates were passed to AGMK cultures, then HEK, and twice more in AGMK (AG1-HEK1-AG2) to attempt to enrich the hybrid portion of the population, but both passage lines were negative for SV40 neoantigen induction. Next, E46AG9L2 was used to obtain plaque isolates in HEK cells; of 18 plaques tested, 17 were negative and one induced SV40 neoantigen in a few cells. On passage of this latter isolate through the AG1-HEK1-AG2 passage series, fully active hybrid virus was obtained, but when this was again plaqued in HEK cells, all of 4 plaques tested were negative for SV40 neoantigen induction.

In contrast, when plaques of E46AG9L2 were obtained in AGMK cultures, all of 13 tested were positive for SV40 neoantigen induction, as expected from the inability of the non-hybrid virus to propagate efficiently. However, 12 HEK plaque isolates were obtained from 3 of these plaque-purified strains, and again all were negative for SV40 neoantigen induction.

Thus, of 102 plaque isolates obtained in human cells, as well as 8 limiting dilution harvests of various $E46^+$ preparations, including AGMK-limiting dilution and plaque purified material, none has yielded a genetically stable population of $\oplus$ particles. It should be pointed out that the titers of $E46^+$ preparations in AGMK are 1.5 to 2 $\log_{10}$ lower than the titer in HEK cells; thus, AGMK-limiting dilution and plaque isolates are not obtained at the true infectivity end-point and could be viewed as being $\oplus$ particles contaminated by an excess of $\ominus$. This was unlikely, however, since areas between plaques rarely yielded virus when inoculated into HEK cultures.

Multiplication and Antigen Induction by $E46^+$ and $E46^-$ Viruses in Monkey and Human Kidney Cell Cultures.—To attempt to explain the negative plaque purification results, it was felt necessary to investigate more fully the nature of HEK and AGMK infection by $E46^+$ and $E46^-$ viruses, with particular reference to the selective pressures in the two systems. For this purpose, growth curve and antigen induction studies were done.

For studies of the growth characteristics, replicate cultures were inoculated with comparable multiplicities of $E46^+$ and $E46^-$ virus. At 4 hours the tubes were rinsed five times with 2 ml of BME, and the maintenance fluid replaced; it was established that this washing procedure reduced the amount of virus below the level of detection by the antigen-induction method. At intervals tubes were harvested by freezing and thawing twice and scraping the cell debris into the fluid; titers were determined by one cycle antigen induction in HEK cells (Table I). Several points are noteworthy. In HEK cells, $E46^+$ and

E46⁻ viruses yielded comparable adenovirus titers and the yield of ⊕ particles from the former followed the same curve as adenovirus particles. In AGMK cells E46⁺ virus grew essentially as well as in HEK cells, but with a somewhat longer latent period as evidenced by the lower virus titers at 24 hours. In contrast, E46⁻ virus in AGMK produced a much lower virus titer, and with a

TABLE I

Growth Curves of E46⁺ and E46⁻ in Human and Monkey Cells

Cells	Day of harvest	Infectivity titer* of harvests						
		Virus and multiplicity of infection (p.f.u./cell)						
		E46⁻ (M, 0.55)	E46 (M = 0.15)			E46AG8 (M = 0.21)		
		Ad. 12 HS‡	Ad. 12 HS	SV40 HS	Ad./SV40-staining ratio	Ad. 12 HS	SV40 HS	Ad./SV40 staining ratio
HEK	1	$10^{4.4}$	$10^{4.7}$	$10^{4.2}$	2.7	$10^{4.6}$	$10^{4.0}$	3.8
	2	$10^{5.1}$	$10^{5.6}$	$10^{4.9}$	4.5	$10^{5.5}$	$10^{5.0}$	3.0
	3	$10^{5.4}$	$10^{5.8}$	$10^{5.2}$	3.4	$10^{5.8}$	$10^{5.5}$	2.2
	4	$10^{5.7}$	$10^{5.7}$	$10^{5.1}$	4.0	$10^{5.6}$	$10^{5.0}$	3.6
	5					$10^{5.8}$	$10^{5.4}$	2.6
AGMK	1	$<10^2$	$10^{2.8}$	$10^{3.1}$	1/2.0	$10^{2.6}$	$10^{3.0}$	1/2.5
	2	$<10^2$	$10^{5.1}$	$10^{5.0}$	1.1	$10^{4.8}$	$10^{4.9}$	1/1.2
	3	$<10^2$	$10^{5.2}$	$10^{5.4}$	1/1.5	$10^{5.0}$	$10^{5.3}$	1/1.6
	4	$10^{2.9}$	$10^{5.3}$	$10^{5.3}$	1/1.2	$10^{4.9}$	$10^{5.2}$	1/1.9
						$10^{5.0}$	$10^{5.3}$	1/2.1
AGMK preinfected with SV40§	1	$<10^2$				$10^{2.7}$	$<10^2$	
	2	$10^{4.9}$				$10^{4.6}$	$10^{4.6}$	1.0
	3	$10^{4.9}$				$10^{5.1}$	$10^{4.7}$	2.7
	4	$10^{5.0}$				$10^{4.8}$	$10^{4.5}$	2.2

* Titer per 0.1 ml as determined by first cycle antigen induction in HEK cells.

‡ HS, tumored hamster serum.

§ Cultures were infected with SV40 virus at a multiplicity of 30 $TCID_{50}$/cell 24 hours before infection with adenovirus. The harvests were assayed in the presence of SV40 rabbit antiserum.

prolonged latent period. In AGMK cultures preinfected with SV40 virus, the E46⁻ virus grew as efficiently as the E46⁺. SV40 preinfection did not potentiate the growth of E46⁺ virus, but did affect the Ad. 12/SV40-staining ratio produced by the progeny; the ratio was consistently less than one for the virus grown in non-SV40-infected cultures, and greater than one for the mixed infection progeny. The probable explanation for this difference in ratios is that

the ⊖ particles in the E46+ preparation were better able to initiate infection in the SV40-infected cultures than in cells not preinfected. In neither HEK nor AGMK was there an indication of selective growth advantage of ⊕ or ⊖ particles, as might be evidenced by a progressive change in progeny ratio in successive harvests.

The failure of non-hybrid virus to propagate well in AGMK was not limited to the E46− isolate. Many of the plaques of E46+ obtained in HEK cells were inoculated into AGMK cultures, and although all produced cytopathic changes, in no case did the cytopathogenicity progress to involve more than 20 to 30

TABLE II

Induction of Immunofluorescent-stainable Antigens by E46+ and E46− Viruses in HEK and AGMK Cell Cultures

Culture	Time after inoculation	Percentage of cells stained					
		E46AG9L2 HEK-1*			E46−‡		
		Ad. 12 HS§	SV40 HS	Ad. 7 RS	Ad. 12 HS	SV40 HS	Ad. 7 RS
	hours						
HEK	24	15	1.6	0.5	6.5	0‖	0.15
	30	18	4.7	2.0	34	0	2.0
	48	60	3.2	19	85	0	21
AGMK	24	3.0	5.0	0.25	4.2	0	0
	48	4.4	7.0	0.20	9.0	0	0
	72	6.8	11.3	1.5	12.6		0
	96	10.8	15.3	3.6	10.0		0

* Multiplicity of 0.4 p.f.u./cell.

‡ Multiplicity of 1.0 p.f.u./cell.

§ HS, tumored hamster serum; RS, rabbit hyperimmune antiserum.

‖ 0, <0.01 per cent.

per cent of the cells, and the CPE eventually regressed. In contrast, the plaque isolates obtained in AGMK cells produced rapidly progressive cytopathic effects.

The ability of the E46+ and E46− preparations to induce antigens in HEK and AGMK was studied by infecting coverslip cultures and staining at various intervals (Table II). Induction of adenovirus antigens in HEK was comparable for the two viruses. In AGMK cells the E46− virus induced adenovirus neoantigen as efficiently as the E46+ virus, but did not induce Ad. 7 viral antigen. Thus, the failure of E46− virus to grow efficiently in AGMK cells was not related to early events in infection such as penetration or uncoating, but to a step necessary for viral antigen synthesis.

Evidence for Defectiveness of Particles Carrying SV40 Genetic Material.—In attempting to interpret the above findings, three main hypotheses had to be considered. First, E46$^+$ preparations may consist of a mixture of genetically stable complete (*i.e.*, non-defective) ⊕ and ⊖ particles, with the latter in excess. This hypothesis is difficult to reconcile with the fact that the Ad./SV40-staining ratios of AGMK -grown virus are generally one or less; with the adenovirus titer as measured by antigen induction generally being only one $\log_{10}$ lower than the infectivity titer, then 10 per cent or more of the HEK plaque isolates should have been positive. This model is also unlikely because of the limited growth potential of ⊖ particles in AGMK cells, though potentiation by the SV40 material from ⊕ particles would favor their growth.

Second, it is conceivable that ⊕ particles are fully infectious, but a high proportion of the progeny are revertants to ⊖. This model would predict rapid loss of ⊕ particles on serial high multiplicity passage in HEK cells, which did not occur. When E46AG7 was carried for 4 serial passages in HEK cells by undiluted virus passage, the Ad./SV40-staining ratio remained in the range of 2 to 3, and when a preparation with a ratio of 10 was similarly passed twice in HEK cells, the ratios produced by the progeny of the first and second passages were 12 and 18, respectively.

The third hypothesis is that ⊕ particles are defective, meaning that individual particles are unable to give rise to infectious progeny; new ⊕ particles would be formed only by cells sustaining dual infection with a ⊕ and a ⊖ particle. In AGMK cells, in which the ⊖ particles are also defective, only the multiply-infected cells would release virus, which would consist of both ⊕ and ⊖ particles in presumably comparable amounts. When plaqued in HEK cells, only the ⊖ particles would initiate plaques. Thus, this model provides a satisfactory explanation of the plaque data presented above. In addition, this model makes several crucial predictions which are subject to experimental testing; *i.e.*, that the proportion of ⊕ particles produced by HEK cells should decrease with a decrease in multiplicity of infection, and that plaque production by E46$^+$ in AGMK should follow a 2-hit dose-response curve.

The relationship of multiplicity of infection and proportion of ⊕ particles produced by HEK cells would be distinctly different depending on the defectiveness or completeness of the ⊕ particles. If they are fully infectious, and if there is little selection against them in HEK, the proportion of ⊕ particles produced, as measured by the Ad./SV40 ratio, should be constant at all dilutions of a titration, except at the limiting dilutions where only ⊖ particles might be present. If the ⊕ particles are defective, the number in the progeny would depend on the frequency of mixed infections, and there would be a continual increase in Ad./SV40-staining ratio with serial dilutions of the inoculum. Two types of experiment were done to study this question. First, E46$^+$ preparations were titrated in HEK tube cultures, the tubes harvested at complete

CPE, and the fluids tested for first cycle Ad./SV40-staining ratio in HEK coverslip cultures (Table III). There was clearly a progressive, rather than precipitous, rise in ratio with dilution. To minimize the possibility that selection pressure against ⊕ particles was responsible for the results, a similar type of experiment was done, but the cultures were harvested shortly after inoculation to obtain the progeny of the first cycles of infection (Table IV). Again, the ratios rose progressively after multiplicity fell well below one. If a dual infection by a ⊕ and a ⊖ particle is required for production of ⊕ par-

TABLE III

Ad./SV40-Staining Ratios Produced by Harvests of Serial Dilutions of E46⁺ Virus Titrated in HEK Cultures

Virus	Dilution	Multiplicity (p.f.u./cell)	Day of harvest	Assay of harvest*		
				Per cent of cells stained		Ad./SV40 ratio
				Ad. 12 HS‡	SV40 HS	
E46	10^{0}	$10^{-0.8}$	5	6.0	2.9	2.1
	10^{-1}	$10^{-1.8}$	6	8.8	1.4	6.3
	10^{-2}	$10^{-2.8}$	7	80	2.3	35
	10^{-3}	$10^{-3.8}$	10	2.3	0	>230
	10^{-4}	$10^{-4.8}$	12	25	0	>2500
E46AG8	10^{-2}	$10^{-0.2}$	6	11.9	4.0	3.0
	10^{-3}	$10^{-1.2}$	7	17.2	1.9	9.1
	10^{-4}	$10^{-2.2}$	7	23.8	0.83	29
	10^{-5}	$10^{-3.2}$	11	90	0.067	1340
	10^{-6}	$10^{-4.2}$	14	60	0	>6000
	10^{-7}	$10^{-5.2}$	14	60	0	>6000

* The titration tubes were harvested at ++++ CPE, and the harvests assayed by inoculation of a 10^{-1} dilution into HEK coverslip dishes.

‡ HS, tumored hamster serum.

ticles, the Ad./SV40 ratio should approximate the reciprocal of the multiplicity[1]; although the sources of error in the present techniques are too great to permit proper testing of this quantitative prediction, the data in Tables III and IV do show a general trend for the ratios to rise about 10-fold with each 10-fold decrease in multiplicity.

By the second prediction, that the dose-response relationship of a plaque titration of E46⁺ in AGMK should follow 2-hit kinetics, the number of plaques should decrease with the square of the dilution factor rather than with the

[1] When multiplicity, m, $\ll 1$, the number of singly hit cells, n_1, equals mc, where c is the total number of cells. The number of dually infected cells, n_2, equals m^2c. Thus, $n_1/n_2 = 1/m$.

TABLE IV

Ad./SV40-Staining Ratios Produced by Early Harvests of Titrations of E46⁺ Virus in HEK Cultures

Material inoculated			Material assayed										
			24 hr. harvest				48 hr. harvest				72 hr. harvest		
Virus	Dilution	Multiplicity (p.f.u./cell)	Dilution assayed*	Per cent Staining		Ad./SV40 ratio	Dilution assayed	Per cent staining		Ad./SV40 ratio	Dilution assayed	Per cent staining		Ad./SV40 ratio
				Ad. 12 HS‡	SV40 HS			Ad. 12 HS	SV40 HS			Ad. 12 HS	SV40 HS	
E46AG7, Exp. 1	10^{-1}	$10^{-0.1}$	$10^{-0.7}$	8.2	6.9	1.2	$10^{-0.2}$	13.0	6.9	1.9				
	10^{-2}	$10^{-1.1}$	$10^{1.3}$	2.7	1.7	1.6	10^{1}	80	35	2.3				
	10^{-3}	$10^{-2.1}$	$10^{1.6}$	0.01	0		$10^{1.3}$	6.9	0.73	9.5				
E46AG7, Exp. 2	10^{-1}	$10^{-0.1}$	10^{0}	0.62	0.33	1.9								
	10^{-2}	$10^{-1.1}$	$10^{1.3}$	0.03	0.05	§								
	10^{-3}	$10^{-2.1}$					$10^{1.5}$	4.2	0.06	70	10^{1}	5.5	0.03	180
	10^{-4}	$10^{-3.1}$					$10^{1.5}$	0.25	0	>25	$10^{1.5}$	0.75	0	>75
	10^{-5}	$10^{-4.1}$					$10^{1.5}$	0	0		$10^{1.5}$	0	0	

* The inoculum is referred to the standard volume of 0.1 ml; thus, a dilution of $10^{1.3}$ means that the assay plate received 2.0 ml.

‡ HS, tumored hamster serum.

§ Percentages in this low a range were not considered suitable for calculation of ratios.

first power as in the usual 1-hit system. Plaque titrations of several E46+ preparations were done in AGMK using closely spaced dilutions; the results are shown in Table V. It is clear that the number of plaques fell more rapidly than expected by the dilution factor. Two methods were used for statistical

TABLE V

Dose-response Relationship of E46+ Preparations in AGMK Plaque Titrations

Relative dilution of inoculum	Test No. and starting dilution				
	1 ($10^{-2.7}$)	2 ($10^{-2.9}$)	3 ($10^{-2.9}$)	4 ($10^{-2.5}$)	5 (10^{-4})
1	104*	211	347	306	
1:2	35	59	113	109	
1:4	9	15	41	23	
1:8	2	7	15		
1:16			3		
1:1					29
1:3.16					3
1:10					0

* Total number of plaques on two plates, each inoculated with 0.5 ml of virus dilution.

TABLE VI

Summary of Hypothetical Response of Individual Cells to Infection with ⊕ and ⊖ Particles in E46+ Preparations

Type of cell	Infecting particle	Response			
		Induction of neoantigen		Production of infectious virus	
		Adenovirus	SV40	⊖	⊕
HEK	⊖	+	−	+	−
	⊕	?	+	−	−
	⊖ and ⊕	+	+	+	+
AGMK	⊖	+	−	Trace	−
	⊕	?	+	−	−
	⊖ and ⊕	+	+	+	+

analysis of these data. In one, the expected number of plaques at the lowest dilution was determined by a minimum chi² method for 1-hit and 2-hit models (6), and the deviations from the expected number of plaques at subsequent dilutions evaluated by chi². In all five titrations the data were significantly different from the 1-hit model ($p < 0.001$ in four instances), while in three they did not differ significantly from the 2-hit model. In the two instances

(titrations No. 3 and 4 in Table V) where the data did not fit either the 1-hit or 2-hit model, the deviation was less for the 2-hit. In contrast, six plaque titrations of E46+ in HEK cells, in which plaque production depends only on adenovirus, all showed significant ($p < 0.001$) deviation from the 2-hit model, and no significant deviation (p 0.2 to 0.9) from the 1-hit model. The second method of analysis consisted of fitting a straight line to a log-log graph of number of plaques *versus* dilution; the slope of the line gives the average number of hits per plaque. For the five AGMK titrations, the slopes ranged from 1.57 to 1.97, with a mean of 1.82. In contrast, the 6 HEK plaque titrations of E46+ preparations gave slopes of 0.82 to 1.05, with a mean of 0.96. Thus, both methods of analysis indicate that two particles (presumably one ⊕ and one ⊖) are required for initiation of a plaque in AGMK cells, while only one particle (⊖ as indicated by the plaque isolation studies) is required in HEK cells. The deviation of some of the AGMK titrations towards values somewhat lower than 2 hits is possibly explained by aggregation of virus particles.

DISCUSSION

The findings presented here leave no alternative but that the ⊕ particles in E46+ preparations are defective. This model is compatible with all the experimental findings with this virus, whereas it is not possible to reconcile other models with the failure to obtain pure strains of ⊕, the tendency for the proportion of ⊕ in an HEK harvest to be inversely proportional to the multiplicity of infection, and the 2-hit kinetics in AGMK cells.

This concept, together with the demonstration by Rabson *et al.* (7) that adenovirus replication in monkey cells is facilitated by SV40 infection, which was confirmed for Ad. 7 in the present studies, permits formation of a coherent picture of the dynamics of E46+ infection of AGMK and HEK cells (Table VI). Individual virus particles of E46+ are capable of inducing neoantigen synthesis in both AGMK and HEK cells, as indicated by the 1-hit kinetics of neoantigen formation (4). ⊖ particles induce an adenovirus neoantigen, and ⊕ particles induce SV40 neoantigen and possibly the adenovirus antigen as well. In AGMK cultures, cells infected with a single virus particle, whether ⊕ or ⊖, cannot replicate complete virus; infection with a ⊖ particle is blocked at some step between uncoating and formation of viral structural proteins. The ⊕ particles presumably cannot replicate because of defectiveness for adenovirus genetic material. In cells with multiple infection, the ⊖ supplies the necessary complement of adenovirus genes, and the ⊕ supplies an SV40-coded material which facilitates the adenovirus growth. The virus progeny of such a cell will consist of both ⊖ and ⊕ particles. This interpretation may be an oversimplification, since it is not proved that the 2-hit kinetics of plaque formation in AGMK necessarily mean that plaques form only from those cells receiving a ⊕ and ⊖. If the ⊕ particles are randomly defective, two ⊕ hits

could conceivably supply the entire adenovirus genome. This model of AGMK infection is thus compatible with the basic observations that antigen induction in AGMK is 1-hit, plaque production is 2-hit, AGMK plaques all contain ⊕ and ⊖ particles, and the infectivity titer of E46+ is about 2 logs lower in AGMK than in HEK.

The ⊕ particles are defective in HEK cells as well, but the ⊖ particles are fully infectious. As in AGMK cultures, a dually infected cell will liberate both ⊖ and ⊕ particles, but HEK cells infected with a single ⊖ particle will liberate ⊖ progeny. Thus, the character of the progeny of a mass culture will depend on the proportion of multiply and singly infected cells.

In a number of respects the defectiveness of the Ad. 7-SV40 hybrid virus is analogous to that of the Bryan Rous sarcoma virus, in which system the propagation of the Rous gene is dependent on dual infection with a non-defective avian leucosis virus (8).

SUMMARY

E46+ virus purified by limiting dilution and plaque isolation in AGMK cells consists of a mixture of hybrid and non-hybrid virus particles. Of 102 plaque isolates obtained in HEK cells, 98 contained no detectable hybrid virus, while 4 contained a small proportion of hybrid virus. Plaque isolates from AGMK cells uniformly induced SV40 neoantigen, but when these plaque isolates were plaqued in HEK cells the progeny were again non-hybrid adenovirus. It thus appears impossible to obtain a pure line of hybrid virus.

The proportion of hybrid virus produced in HEK cells fell progressively with decreasing multiplicity of infection, and plaque induction by E46+ virus in AGMK followed 2-hit kinetics, indicating that the hybrid virus particles are defective and require dual infection with non-hybrid virus for their propagation.

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