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Expression of Aleutian Mink Disease Antigen in Cell Culture[1]

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Infection of CRFK feline kidney cells with Aleutian disease virus leads to production of virus-induced antigen(s) in the nucleus which could be demonstrated by the fluorescent-antibody technique. The number of fluorescent nuclei was linearly dependent on the dilution of the inoculum, but rarely exceeded 20% of the cells. Aleutian disease nuclear antigen was only transiently detectable. The virus-induced antigen was detected after infection of cells of several divergent species; however, the CRFK line of feline kidney cells was the most susceptible. Inhibitor studies indicated that deoxyribonucleic acid synthesis, ribonucleic acid synthesis, and protein synthesis were required for viral antigen production. Cell growth was also a requirement for synthesis of viral antigen. An in situ radioimmune assay was used to measure binding of ^{125}I-labeled mink anti-Aleutian disease virus to infected cells and competition with unlabeled sera. The system is suitable for quantitation of infectivity.

Aleutian disease (AD), a persistent viral disease of mink, has been very difficult to characterize because titration of the virus in vivo is expensive and cumbersome; often mink are naturally infected. AD antigen can be quantitated by immunodiffusion or counterimmunoelectrophoresis (CEP) (7, 8); however, the viral antigens must be laboriously purified and concentrated considerably before assaying.

The virus of Aleutian disease (ADV) is a naked, icosohedral particle; estimates of the diameter of the particle have ranged from about 20 to 30 nm (6, 9, 16, 17, 21). Virus purified in CsCl gradients has been recently determined to be 27 ± 3 nm when stained with uranyl acetate and measured with internal standardization on the electron microscopic grids (17). The buoyant density of infectious virus assayed in mink and AD antigen assayed by CEP has been determined by Chesebro et al. to be 1.38 g/cm^3 (6). Cho and Ingram reported a density of 1.36 g/cm^3 for CEP antigen (11). Yoon et al. isolated virus at a density of 1.33 g/cm^3 (21). Infectivity in mink has been shown to be resistant to lipid solvents, anionic detergents, and heat inactivation at 56°C (5, 13).

Infection of mink results in a large production of antibody with circulating immune complexes (18) containing low-avidity antibody (A. J. Kenyon and J. E. Gander, Fed. Proc. 34:870, 1975), which is not capable of neutralizing infectivity in the host (20). Several lines of evidence suggest that the gammapathy which is associated with the disease is not the overproduction of antibody directed exclusively against ADV. Barnett et al. found a lack of correlation of "nuclear" antigens and antinuclear antibodies with the degree of gammapathy (2). Secondly, Bloom et al. showed that the titers of anti-ADV antibody bore no correspondence to the levels of serum globulin (4). Finally, levamisole treatment of infected mink depressed antibody titers to ADV independently from the gammapathy (A. J. Kenyon, R. Kassel, G. Notani, and E. C. Hahn, Fed. Proc. 35:569, 1975).

A major goal has been to cultivate ADV in a cell culture system. Basrur et al. (3) reported production of CPE in mink testicular cells in culture. Yoon et al. described replication of ADV in L cells (22) and mink kidney cell cultures (21). Porter et al. (D. D. Porter, A. E. Larsen, N. A. Cox, H. G. Porter, and S. C. Suffin, Fed. Proc. 34:947, 1975) reported passage of ADV in the CRFK feline kidney cell line. Unfortunately, none of these reports has as yet been confirmed. Our recent search for an optimal cell culture system for detection and titration of ADV infectivity has used fluorescent-labeled antibody to detect the production of virus-induced antigens in CRFK cells. The experiments reported here describe properties of the in vitro assay system for the detection of ADV.

MATERIALS AND METHODS

Source of materials. Feline kidney cells, CRFK (12), were provided by Walter A. Nelson-Rees. Mink lung cells were purchased from the American Type Culture Collection. NGB cells and H-1 virus were kindly supplied by H. Toolan, K. Ellem, and S.

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Rhode, Putnam Memorial Hospital Institute for Medical Research. ADV, Pullman strain, was supplied by T. McGuire, University of Washington, Pullman; Canadian AD antigen was supplied by F. J. Depauli, Ontario Veterinary College, Guelph.

Carrier-free ^{125}I and ^{131}I were purchased from New England Nuclear. Fluorescein isothiocyanate (FITC) was obtained from Calbiochem. Tryptophanase, a tryptophan-degrading enzyme, was a gift of Joseph Roberts, Sloan-Kettering Institute. Other chemicals were purchased as follows: acrylamide and chloramine-T from Eastman Organic Chemicals; actinomycin D from Merck, Sharp and Dohme; arabinosyl cytosine (cytosyl) from Upjohn; methotrexate from Squibb; cordycepin (3'-deoxyadenosine) from Sigma; and chamber slides with eight wells from Labtek.

Virus inoculum. The Connecticut strain of ADV was passed by intraperitoneal inoculation of 1 ml of a stock of 10% liver homogenate stored at −70°C. Spleens were removed after 8 to 10 days and frozen. Thawed spleens were homogenized with a Tenbroeck tissue grinder in 8 volumes of phosphate-buffered saline (PBS), pH 7.5. The homogenate was frozen and thawed four to five times with a dry ice-acetone bath. The suspension was cleared for 20 min at 9,000 × *g*. The supernatant was blended with freon (2 parts suspension: 1 part freon) for 5 min in an ice-jacketed Waring blender. The aqueous phase was removed after centrifugation for 20 min at 9,000 × *g* and layered onto 30% (wt/wt) sucrose in PBS. Virus was pelleted by centrifugation at 5°C for 3 h at 80,000 × *g* or 2 h at 175,000 × *g*. The pellet was resuspended in medium without serum to represent 1 ml per g of tissue and tested for AD antigen-forming activity.

Cell culture and virus inoculation. Most cells were cultured in Eagle minimal essential medium with added nonessential amino acids and 10% calf serum. All primary cultures except ferret fetal liver were maintained in the same medium supplemented with 5% tryptose phosphate broth. Ferret fetal liver cells were cultured in RPMI 1640 with 5% fetal bovine serum, 1 mM sodium pyruvate, 2 mM extra glutamine, and 0.01 mM 2-mercaptoethanol.

For ADV assay, CRFK cells were trypsinized with 0.25% trypsin-0.2% sodium ethylenediaminetetraacetate and seeded on chamber slides at 6,000 to 8,000 cells/cm well at 42 to 44 h before infection. This method yielded a partially synchronized population of cells in S-phase at the time of infection. Sera for culture supplement were selected which showed superior growth-promoting properties for CRFK cells. Routinely, wells were inoculated with 0.2 ml of ADV inoculum or dilution in medium. Additional complete medium was added after 1 h of adsorption. After 3 to 5 days of incubation at 37°C, the chamber slides were washed in PBS and fixed in cold, dry acetone for staining with fluorescein-conjugated mink anti-ADV.

Preparation of anti-ADV reagent. Mink immunoglobulin (Ig) was purified from pooled serum collected from infected ranch mink showing marked gammapathy and CEP titers against AD antigen. The serum was cleared of virus and viral immune complexes by centrifugation at 150,000 × *g* for 2 h. Saturated ammonium sulfate (ultrapure, Schwarz/Mann), pH 7, was added to make a 40% saturated solution. The precipitate formed at 5°C was collected by centrifugation and washed in 50% saturated ammonium sulfate. The dissolved precipitate was dialyzed against 0.01 M sodium phosphate, pH 7.5, cleared by centrifugation, and chromatographed on diethylaminoethyl-cellulose. IgG-containing fractions were concentrated at 15 mg/ml and frozen.

Since the validity of the assay depends on the mink immunoglobulin containing the anti-ADV activity, the purity of this reagent was examined. When ^{125}I-labeled anti-ADV was run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, only a single peak of 50,000 cpm was seen corresponding to IgG. Immunoelectrophoresis of both purified mink IgG and mink serum which were reacted with rabbit IgG raised against the mink IgG showed only a single precipitin line to each, indicating that the purified IgG was not contaminated with other serum proteins. As little as 50 μg of the purified mink anti-ADV per ml formed a single precipitin band in an immunodiffusion plate run against purified AD antigen (titer: 1:32 by CEP).

IgG was coupled to FITC by the method of Goldstein et al. (14). The acetone-fixed cells were hydrated with PBS and reacted with FITC-coupled mink IgG for 30 min in a humidified CO_2 incubator. Excess reagent was removed by washing in PBS. Fluorescent cells were counted in replicate fields of area 0.427 or 0.650 mm^2. All values represent the mean number of fluorescent nuclei per field ± the standard deviation of the mean for the 20 to 50 fields. Titers of infectivity in the system are expressed in terms of fluorescent nucleus-forming units per ml. Where the number of cells per field varied by more than 10%, replicate cell counts were made, and the results were expressed as percentage of positive nuclei.

Binding of iodinated antibody. CRFK cells in tubes were infected to yield 20% of the cells positive for nuclear antigen. After 5 days of incubation, infected and control cells were washed in PBS, fixed in methanol, air-dried, and stored at −15°C. Before assay, the tubes were rinsed in 2 ml of PBS. Mink anti-ADV IgG was iodinated by the chloramine-T method (15). Two micrograms of ^{131}I-labeled mink IgG (specific activity, 2.5×10^5 cpm/μg) were added to each tube in 0.1 ml of PBS and incubated for 45 min at 37°C. The tubes were washed five times in PBS to remove unbound IgG and counted (Nuclear-Chicago, model 1185 gamma counter).

RESULTS

Demonstration of ADV nuclear antigen. Infection of mink, ferret, or feline cells with ADV resulted in the appearance of nuclear antigen demonstrable with FITC-coupled anti-ADV. Figure 1 shows fluorescent nuclei in CRFK cells 3 days after infection. The appearance of the fluorescent nuclei was variable: specked, globular, and uniformly stained nuclei have been commonly seen. There was a general progression from specked to uniformly stained nu-

FIG. 1. *ADV nuclear antigen demonstrated by fluorescent antibody. Feline kidney cells (CRFK) on cover slips were infected with ADV for 3 days. Cells were fixed in cold acetone, dried, and stained with FITC-conjugated mink anti-ADV.*

clei with time after infection. Occasionally, only the nucleolar region stained. No reaction of the FITC-coupled anti-ADV with surfaces of infected cells was observed when living preparations were examined at 3 days after infection.

A number of cell types from different species were tested for their relative susceptibility to ADV. The results (Table 1) indicate that mink and ferret and primary kitten kidney cells were similarly susceptible to ADV. Mouse cells were refractory, as were the human cells with the exception of NBG cells. Of all cells tested, CRFK cells were the best indicator of ADV antigen synthesis. The increased susceptibility of the CRFK cells was seen as a more rapid appearance of the nuclear antigen after infection and a higher percentage of cells stained. Macrophages from both mink and guinea pigs displayed cytoplasmic staining after exposure to ADV; however, no nuclear fluorescence was observed. Peritoneal macrophages and bone marrow cells from infected mink showed cytoplasmic and very rarely nuclear fluorescence. In all cell systems tested, fluorescence was transitory, fading within 2 to 4 days after the maximum was reached.

Using CRFK cells as the indicator system, the amount of virus capable of producing antigen was determined for various virus preparations (Table 2). All samples except those from gut usually yielded virus capable of initiating nuclear antigen. Virus was detected in liver samples from chronically infected ranch mink and samples of other strains of ADV obtained from different laboratories. No staining of uninfected cells from any source or cells infected with herpes simplex virus, adenovirus type 2, simian virus 40, EMC, or H-1 parvovirus has been observed.

Binding of iodinated antibody. In studies where FITC-coupled anti-ADV was competed during incubation with unlabeled serum and immunoglobulin from infected mink, the competing antibody reduced the nuclear fluorescence; however, the reduction was in intensity rather than percentage of positive cells. Since intensity of fluorescence was difficult to quantitate, an in situ radioimmunoassay was used to measure the amount of iodinated antibody bound to fixed, infected, or uninfected cells under various conditions (Table 3). In both experiments, the amount of bound ^{131}I-labeled anti-

body was reduced to near the background level bound to uninfected control cells when unlabeled anti-ADV was added during incubation. Binding was unaffected by the presence of calf serum.

TABLE 1. *Host range susceptibility to ADV*

Cell type	No. of samples tested	Relative susceptibility[a]
Normal adult mink kidney	3	+
ADV-infected mink kidney	7	+
Mink lung	5	+
Adult mink bone marrow	1	+
Adult ferret kidney	2	+
Ferret embryo	2	+
Ferret fetal liver	2	+
Ferret fetal kidney	1	+
HeLa	1	−
Human epithelium	1	−
NBG, simian virus 40-transformed human	2	+
CV-1, African green monkey kidney	3	−
3T3 (mouse)	2	−
L (mouse)	2	−
Kitten kidney (primary)	1	+
CRKF, feline kidney	>100	++
Mink peritoneal macrophages	2	+[b]
Guinea pig peritoneal macrophages	2	+[b]

[a] Growing cells were infected with ADV and fixed for fluorescent antibody after 3 to 5 days. Relative susceptibility: −, no fluorescent nuclei found per >5,000 cells scanned; +, 0.001 to 1% positive; ++, 1 to 25% positive.

[b] Cytoplasmic fluorescence.

TABLE 2. *Source of virus*

Source	No. samples tested (no. of tests)[b]	Relative activity[c]
Infected mink spleen (8–10 days pi[a])	53 (18)	+++
Infected mink liver (8–10 days pi)	11 (6)	++
Infected mink gut (8–10 days pi)	8 (8)	−
Chronic mink spleen	25 (1)	+
Chronic mink liver	28 (2)	+
Pullman ADV	1 (1)	++
Canadian ADV antigen	2 (2)	++

[a] p.i., Postinfection.

[b] Numbers in parentheses indicate the number of independent assays.

[c] Samples were assayed on CRKF cells. Relative activity: −, no fluorescent nuclei per >5,000 cells; +, 0 to 0.1% positive; ++, 0 to 1% positive; +++, 0 to 25% positive. All values were compared on the basis of activity per gram of tissue in the starting material.

TABLE 3. *Binding of ^{131}I-labeled AD antibody to ADV-infected cells*[a]

Expt	Sample	Incubation	cpm bound
1	ADV inf.[b]	^{131}I-AB	8,850
	ADV inf.	^{131}I-AB + 1/20 mink anti-ADV IgG	2,200
	Control cells	^{131}I-AB	1,700
2	ADV inf.	^{131}I-AB	1,329
	ADV inf.	^{131}I-AB + 1/20 mink anti-ADV IgG	573
	ADV inf.	^{131}I-AB + 1/200 mink anti-ADV IgG	810
	ADV inf.	^{131}I-AB + 1/20 chronic AD serum	411
	ADV inf.	^{131}I-AB + 1/200 chronic AD serum	598
	ADV inf.	^{131}I-AB + 1/20 bovine IgG (57%)	1,470
	Control cells	^{131}I-AB	663

[a] Iodinated antibody (5×10^5 cpm/tube) was incubated with or without unlabeled serum. Unbound antibody (AB) was removed by repeated washing before counting.

[b] inf., Infected.

Dose response to ADV. Some of the spleen inocula were toxic to CRFK cells at high concentrations, thereby reducing the expected number of susceptible cells. Consideration was given to the possibility that this toxicity was related to certain mink being infected with ADV at the time of experimental infection. This is illustrated in Fig. 2, where the dose response for virus isolated from various mink is shown.

Inoculum was prepared from two chronically infected mink and two asymptomatic mink. Both chronic mink had high titers of antibody to ADV in an immunodiffusion test; the control mink had no demonstrable titer. All four mink had been experimentally infected with ADV 10 days earlier. Dilutions of the pooled spleen inocula obtained from these animals were tested on CRFK cells for 4 and 8 days. In the case of inoculum from asymptomatic mink, the response was linear and reproducible for both assay periods. Virus from the chronic mink produced the same fraction of positive nuclei at the highest dilution tested, but produced a reduced response at the more concentrated levels. With longer incubation, however, there was a tendency for the curve for virus from chronic mink to approach linearity. The toxicity of some inocula was so severe that undiluted material was cytocidal for the cells. Attempts at reducing the toxicity, when present, have been unsuccessful in spite of purification of virus.

Abortive nature of the infection of CRFK cells. The time course of formation of ADV nuclear antigen is shown in Fig. 3. Immediately after infection, some particulate antigen

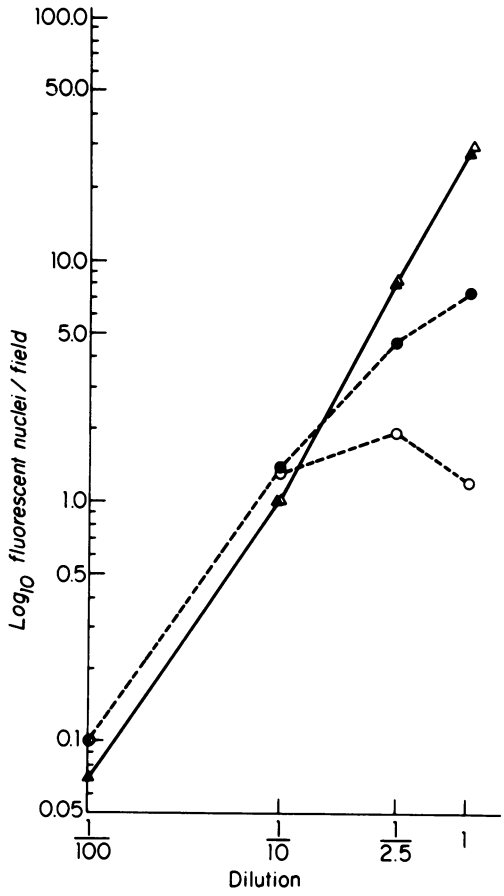

FIG. 2. *Dose dependence of antigen production. Spleens from experimentally infected mink were taken 10 days after inoculation. Isolated virus was diluted in medium and tested on CRFK cells. Symbols: Triangles, virus from mink not persistently infected at the time of inoculation; circles, virus from mink which were chronically infected at the time of inoculation; open symbols, CRFK cells fixed 4 days after infection; closed symbols, cells fixed 8 days after inoculation.*

could be detected in the cytoplasm and adsorbed on the culture surface between cells, presumably the result of persisting antigenic material from the inoculum. The first appearance of nuclear antigen was detected 3 days after infection. Maximal numbers of infected cells were observed 3 to 5 days after infection. The second small peak at 5 days was partially the result of proliferation of infected cells. Often daughter cells were detected which displayed identical nuclear fluorescent patterns. The cultures were given fresh medium on day 6. This was followed by renewed cell growth and an increase in the number of fluorescent nuclei. In experiments where fresh medium was not added, fluorescence faded until it was no longer detectable.

Incubation of infected cultures at 33°C sometimes resulted in a larger percentage of infected cells, but the cells did not survive well at that temperature, and results were often not reproducible.

Several attempts were made to passage the virus at the time of maximal response. At most, only three passages were achieved. The fraction of labeled nuclei decreased at each passage so that no proof of actual replication was obtained.

Effect of inhibitors on antigen production.

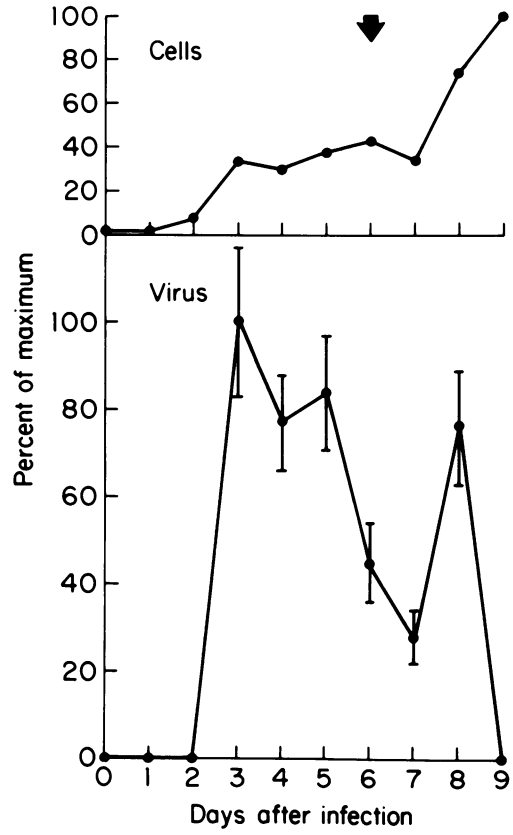

FIG. 3. *Time course of formation of nuclear antigen. Cells were seeded in a 60-mm petri dish containing glass cover slips. After 42 h, 1.3 × 10⁴ fluorescent nucleus-forming units (FNU) of ADV was adsorbed for 1 h. Medium was added, and the culture was incubated for 9 days with the addition of 4 ml of fresh medium on day 6 (arrow). Cover slips were removed each day, fixed in cold acetone, and stored at −14°C. The average yield of fluorescent nuclei per unit area was determined based on 40 areas. The cover slips were washed and stained with Giemsa. Cells were enumerated in replicate areas of 0.028 mm². The maximum virus titer on day 3 was 9.0 × 10³ FNU/culture; the maximum number of cells was 60,000/cm².*

Various inhibitors were used to assess the dependency of ADV antigen production on nucleic acid and protein synthesis. The inhibitors were selected to achieve inhibition of: deoxyribonucleic acid synthesis (Ara-C, hydroxyurea, and methotrexate), ribonucleic acid synthesis dependent on a double-stranded deoxyribonucleic acid template (actinomycin D), and protein synthesis [cycloheximide, tryptophanase, which stops protein synthesis by reducing an essential amino acid, and cordycepin, which interferes with poly(A)-containing messenger ribonucleic acid]. As shown in Table 4, all inhibitors that were tested prevented or reduced the appearance of the nuclear antigen.

Requirement for cell growth. The inhibitor studies suggested that cellular macromolecular synthesis was required for expression of ADV nuclear antigen. Other experiments showed that serum deprivation after infection, a treatment that slowed the growth rate of the cells, also prevented ADV antigen formation. To further study the dependency of antigen synthesis on cell growth, different number of CRFK cells were infected with ADV. The number of cells and number of fluorescent nuclei were determined. Mean values of these counts and the percentage of positive cells are shown in Table 5. The percentage of positive cells increased with the number of allowed cell doublings. The highest reproducible relative yield of positive cells was observed where 10,000 cells were seeded and underwent 2.3 doublings. Seeding much fewer than 10,000 cells resulted in erratic cell growth, and the small numbers of cells made quantitation of these samples difficult.

TABLE 4. *Inhibitors of ADV antigen synthesis*[a]

Compound	Conc	Mean fluorescent nuclei/field
Ara-C	10 μg/ml	0
		0
Hydroxyurea	2mM	0
		0
MTX	10^{-6} M	0.039
		0.018
MTX	10^{-7} M	9.97 ± 0.90
		9.72 ± 0.72
Actinomycin D	0.03 μg/ml	0
		0
Actinomycin D	0.001 μg/ml	0.098
		0.214
Cycloheximide	50 μg/ml	0
		0
TDase[b]	0.1 IU/ml	0.154
		0.81
Cordecypin	15 μg/ml	8.90 ± 61
		2.92 ± 69
Control		10.88 ± 1.04
		14.49 ± 0.78

[a] Cells were treated with the concentrations shown for 24 h after ADV adsorption. Medium was then replaced, and the infected cells were incubated further for 2 days before fixing for FA staining.

[b] TDase is an enzyme which degrades extracellular tryptophan effectively inhibiting cellular protein synthesis.

TABLE 5. *Dependence of antigen production on cell growth*[a]

No. of cells seeded per well	No. of cells/well at term[b]	No. of FN/well at term[b]	% Positive cells
40,000	63,000 ± 2,400	2,400 ± 200	3.8
40,000	61,000 ± 2,200	1,900 ± 140	3.0
20,000	65,000 ± 4,500	3,400 ± 260	5.2
20,000	65,000 ± 4,900	4,300 ± 230	6.5
10,000	53,000 ± 3,100	4,300 ± 290	7.9
10,000	45,000 ± 4,700	2,800 ± 540	6.2
5,000	8,700 ± 1,000	1,200 ± 120	13.7
5,000	3,300 ± 600	100 ± 30	3.2
2,500	1,500 ± 300	80 ± 30	5.18

[a] Cells were seeded at different densities and infected after 44 h. All cells were fixed at 3 days after infection.

[b] Both mean number of cells and mean number of fluorescent nuclei per field were used to calculate the number per cm^2 well. The standard deviation of the mean is given for each value. FN, Fluorescent nuclei.

When the percentage of positive cells is compared to the initial number of cells present (Fig. 4), a linear relationship is suggested. Moreover, the values extrapolate to the value 67,000 cells per cm^2 well, at which point no fluorescent nuclei would be expected. Although CRFK cells do not display much contact inhibition of cell division, this value (67,000 cells per well) is close to the saturation density achieved in this and other experiments.

DISCUSSION

The demonstration of ADV nuclear antigen(s) in infected CRFK cells essentially confirms and extends the results of Porter et al. (Fed. Proc. 34:947, 1975). Although complete infectivity has not as yet been demonstrated at 37°C or lower temperatures of incubation, antigen could be adequately quantitated in infected cell cultures. Using this as an assay for ADV, similar antigen production was detected with virus preparations from tissues of experimentally infected mink, chronically infected animals from mink ranches, and preparations of ADV from other laboratories. Highest titers were found in spleens of mink at 8 to 10 days after experimental infection. This specificity of the antibody reaction and the assay was indicated by the ability of sera from infected animals to compete with iodinated antibody. Similar fluorescent reagents prepared in other laboratories have been used to demonstrate ADV antigens in other cell systems (19) and in vivo (19, 20). Except for rare nuclear staining, cells and tissues from infected animals usually

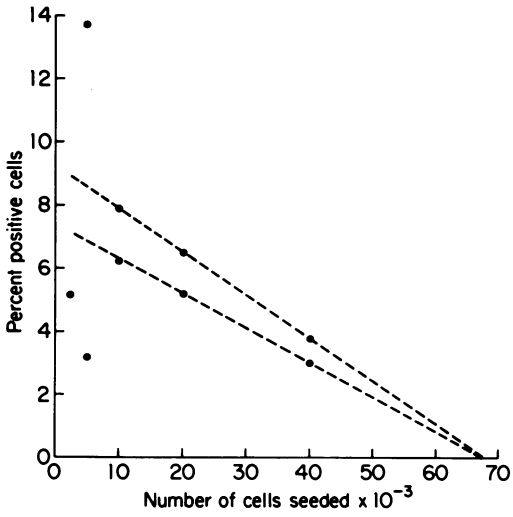

FIG. 4. *Dependence of nuclear ADV antigen production on cell density at the time of seeding. The data from Table 5 were plotted to show the relation of antigen to allowable cell growth.*

showed cytoplasmic staining antigens. This could represent either ingested antigen or cytoplasmic sites of antigen synthesis. No fluorescence was ever observed when cells infected with other viruses or uninfected cells were stained with fluorescein-labeled mink anti-ADV.

For all cell types tested for sensitivity to ADV, there was never evidence of virus replication or observable cytopathic effect in living cultures or after staining fixed preparations. This contradicts earlier reports concerning the cytopathic effect produced during replication of ADV in L cells and mink kidney cells (21, 22) and the reported replication of ADV in CRFK cells (Porter et al., Fed. Proc. 34:947, 1975).

The effects of inhibitors (Table 3) and dependence of antigen expression on cell growth (Fig. 3 and 4) indicate that there are cellular requirements upon which viral antigen synthesis depends. It is not possible on the weight of these results, however, to say what physiological state or phase of the cell cycle is required. The small size of ADV, production of antigen exclusively in the nucleus, its various physical and chemical properties (5, 11, 13), and the dependence of antigen synthesis on cell growth would be consistent with this virus being a parvovirus, as has been suggested by others (6, 11). If this is so, then ADV probably requires actively dividing cells, in particular cells in S-phase, a characteristic of other parvoviruses (1). Proof of this requires further characterization of the virus.

The low percentage of positive cells in infected cultures of CRFK cells deserves comment. High-titered preparations of virus titered in CRFK cells between 10^4 and 10^5 infectious units per gram of splenic tissue. The strain used here has routinely titered in mink to 10^6 mean infectious doses (16), so there is an apparent disparity between the sensitivity of CRFK cells and mink. At present it is not known whether such a disparity is due to nonpermissiveness of the cultured cells, defectiveness of the virus, some kind of interference, or a combination of these. Despite the apparent lower sensitivity of the CRFK cells, an advantage of the cell culture assay is that it only takes 3 to 4 days to obtain a result compared to 5 to 10 weeks for titration in mink. Although only about 10^4 infectious units were normally detected per gram of tissue, this assay is over 100 times more sensitive than CEP, which can detect titers of only about 64 CEP units per gram of tissue. In addition, samples can be tested in cell culture without the exhaustive purification of antigen required before the CEP assay is meaningful.

As to why the CRFK cells are more permissive for ADV than other cultured cells, the best possibility is that the CRFK cell line is rapidly growing and does not exhibit much contact inhibition of division at confluency. Other cell types tested did stop growing at confluency. In looking for the in vivo site of viral replication, a dividing population of cells would be a likely possibility. The availability of the CRFK cell assay for ADV should facilitate study of the cell-virus relationships and reasons for the persistence of the infectious process in the host.

ACKNOWLEDGMENTS

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