IMMUNOFLUORESCENT STUDIES OF ADENOVIRUS 12 TUMORS AND OF CELLS TRANSFORMED OR INFECTED BY ADENOVIRUSES

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Plates 63 to 66

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Tumors induced in hamsters by adenovirus type 12 (Ad. 12) (1) continually elaborate virus-specific antigens, although never forming infectious virus (2, 3). The presence of viral antigens was first inferred from the development of antiviral antibodies in the serum of tumorous hamsters (2), and subsequently antigens were demonstrated directly in tumor extracts by complement fixation (CF) (3) and immunodiffusion tests (4) against serum of tumorous hamsters and rats. A spectrum of antibodies was observed in the hamster sera, including CF antibodies to the Ad. 12 C antigen (5), to an antigen present in viral harvests which is distinct from C antigen, and to an antigen present in tumors and in cellular fractions of Ad. 12-infected tissue cultures (6), as well as neutralizing antibody. However, no antibody is present for the group-reactive A antigen (5); its lack of formation is probably responsible for the absence of infectious virus, since the A antigen constitutes part of the protein coat of the virion (7).

In order to obtain additional information on the proportion of cells elaborating antigen and on the intracellular localization of the various antigens, we applied fluorescent antibody techniques, using sera of tumor-bearing hamsters and of rabbits immunized with viral antigens. Cell systems which were studied included Ad. 12-induced hamster and mouse tumors, and human and hamster cell cultures infected with various adenoviruses.

Materials and Methods

Tissue Cultures.—Primary cultures were prepared from transplanted hamster tumors by trypsinization, and grown on coverslips in Petri dishes as described previously (8).

Primary hamster embryo (HaE) and human embryonic kidney (HEK) cultures were obtained from Microbiological Associates, Bethesda, either seeded out on coverslips in Petri dishes or in bottles from which secondary cultures were prepared in our laboratory. All cul-

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tures were maintained on 5 or 10 per cent heated agamma calf serum (Hyland Laboratories, Los Angeles) in Eagle's basal medium (BME) with penicillin and streptomycin.

Viruses.—The prototype adenovirus strains (9) grown in KB cell cultures were used for infection of HaE and HEK; undiluted virus suspension was clarified by centrifugation at 2000 RPM for 20 minutes and 0.2 ml was added to each dish containing 4.0 ml of medium. This dosa contained $10^{7.3}$ to $10^{8.0}$ $TCID_{50}$ for types 1, 2, 5, 7, and 12, and $10^{5.8}$ for type 18.

With one exception mentioned in the text, adenovirus tumors were induced with the prototype (Huie) strain of Ad. 12.

Antisera.—A transplant line of Ad. 12–induced hamster tumor was used for preparation of hamster antiserum. Sera were collected as late as possible after subcutaneous transplantation of tumor. Several hyperimmune sera were prepared by inoculating hamsters intraperitoneally with 5 weekly doses of viable tumor fragments and collecting sera when the animals were moribund. The majority of tests described here were carried out with individual sera of the hyperimmunized hamsters; these sera had complement-fixing antibody titers of 1:160 or greater against Ad. 12 hamster tumor antigen, 1:20 to 1:80 against Ad. 12 viral antigen, and were negative for neutralizing antibody and for CF antibody to purified Ad. 12 A and C antigens.

Control sera consisted of pooled sera from normal hamsters, hamsters bearing transplanted SV40-induced tumors, or hamsters which received Ad. 12 virus at birth but did not develop tumors.

Rabbit antisera to purified Ad. 12 A and C antigens were kindly supplied by Dr. H. G. Pereira.

Sera were stored at −20°C and were used as a routine in indirect immunofluorescent tests at a 1:5 dilution, without heat inactivation.

Conjugates.—Fluorescein-labeled goat antiserum to hamster globulin fraction, supplied by Baltimore Biological Laboratories, Baltimore, was used in the indirect test. It was diluted 1 in 10 in a hamster brain extract (20 per cent in phosphate-buffered saline pH 7.2, clarified 2000 RPM/15 minutes), held in the cold for 1 hour, and then centrifuged at 15,000 RPM/15 minutes in a Spinco rotor 40. Anti-rabbit conjugate of ovine origin (Progressive Laboratories, Baltimore) was used at 1:20 or 1:40 dilution.

Dr. M. David Hoggan kindly supplied a conjugated rabbit antiserum to adenovirus type 12 and a conjugated Ad. 12 hamster tumor serum (No. 6252) which contained CF antibodies to Ad. 12 tumor (titer >1:160) and viral (titer 1:20) antigens. The rabbit antiserum was prepared by hyperimmunization with crude culture fluids from KB cell cultures infected with the Huie strain; the conjugate was absorbed with KB cells prior to use.

Immunofluorescent Tests.—Except for tests with hamster serum 6252 and the conjugated rabbit Ad. 12 antiserum, all tests were done by the indirect procedure. The procedures employed for the direct and indirect tests have been described elsewhere (8).

RESULTS

Fluorescent Antibody Staining of Adenovirus 12 Tumor Cells in Vivo and in Vitro.—Cryostat sections of a transplanted hamster tumor were stained with the conjugated CF-positive hamster tumor serum (No. 6252). A high proportion of cells in the viable portions of tumor demonstrated specific fluorescent staining in the form of yellow-green rod-like cytoplasmic flecks. The fibrous capsule and necrotic areas within the tumor were negative. No fluorescence occurred in sections of SV40-induced hamster tumors stained with the same con-

jugated antiserum. Attempts to use the indirect method on tissue sections gave unsatisfactory results.

Tissue cultures of Ad. 12 hamster tumors readily showed fluorescence when stained by either the direct or indirect procedure with sera from tumorous hamsters; the indirect procedure was used for the great majority of subsequent studies. The most striking and consistent pattern, seen with both the direct and indirect conjugates, was the presence in the cytoplasm of numerous fluorescent particles which were fine, elongate, irregular in size, and sometimes slightly curved (Fig. 1). Although most of these particles were scattered at random in the cytoplasm, there was often one lying adjacent to the nuclear membrane. Fluorescent particles were present in almost all cells with the morphology of tumor cells, while connective tissue cells did not stain (Fig. 2). The tumor cells without fluorescent flecks demonstrated the generalized homogeneous fluorescence described below. The number of flecks per cell was generally much greater than in tumor sections; this may be due to differences in the sera or procedures used.

A second type of fluorescent staining, seen when the hyperimmune hamster sera to Ad. 12 tumor were used, was numerous fine fluorescent flecks in the nucleus in addition to the cytoplasmic flecks (Fig. 3). Compared with the latter, the flecks in the nucleus were more slender, generally curved, and less intensively fluorescent. This form of nuclear fluorescence was therefore often partly obscured by the more prominent and often overlying cytoplasmic flecks and by occasional diffuse fluorescence of the entire nucleus (Figs. 2 and 3). A third type of fluorescence was homogeneous staining of both the nucleus and cytoplasm, with a variable number of small dark areas, of the general size and shape of nucleoli, in the nucleus. While this type of staining was generally of light to medium intensity, occasional groups of cells were brightly fluorescent.

Absorption of hamster antiserum with Ad. 12 tumor extract markedly reduced both nuclear and cytoplasmic staining, while absorption with SV40 tumor or normal hamster tissue had no effect. With higher dilutions of hamster antiserum, only cytoplasmic flecks stained.

Observations of the immunofluorescent reactions of a number of combinations of tumor cell preparations and antisera indicated that variation occurred in the relative amount of each type of fluorescence. However, the cytoplasmic flecks were the most consistent pattern.

Large numbers of cytoplasmic flecks identical in appearance with those in hamster tumor cells induced by the prototype Ad. 12 virus were seen in hamster tumor cells induced with the 10534 strain of Ad. 12 (2) and in BALB/c mouse tumor cells (received from Dr. Alan Rabson) (10) induced by the Ad. 12 prototype.

Fluorescence of Ad. 12 hamster tumor cells grown *in vitro* occurred with

hamster sera which possessed CF antibodies to Ad. 12 tumor antigen as a result of either transplantation of tumor or induction of tumor by virus. Negative results were obtained with sera of hamsters which failed to develop tumors after inoculation of Ad. 12 virus, with sera from hamsters with SV40-induced tumors, and with normal hamster serum. Sera from Ad. 12 tumor-bearing hamsters which produced fluorescence in Ad. 12 tumor cells were negative with SV-40-transformed cells of C3H mouse, rabbit, hamster, and porcine origin, polyoma hamster tumor, normal hamster cell line BHK-21, and normal hamster embryo cultures.

In Vitro "Transformation" of Hamster Embryo Cells by Ad. 12 Virus.—Tube cultures of hamster embryo cells (Lot 70331, Microbiological Associates) were infected with 10^7 $TCID_{50}$ of Ad. 12 virus. Maintenance was in 10 per cent heated agamma calf serum in BME with antibiotics. The infected and control cells were serially transferred 3 times by trypsinization at 18 to 24 day intervals. After the first trypsinization the tubes were held in an atmosphere of 5 per cent CO_2. Although the cells in each line gradually deteriorated, those of the virus-infected line consistently showed slightly better appearance, both in number and condition of cells, even when the same number of cells was planted per tube. Eleven days after the 3rd trypsinization (74 days after the original inoculation of virus) a small focus of about a dozen cells was observed in 1 of 3 surviving tubes. The focus morphologically resembled those of hamster Ad. 12 tumor cells grown in culture (11) as well as the cells transformed by Ad. 12 *in vitro* described by McBride (12). Within 7 days, proliferation of the focus was quite evident, while other cells in the culture remained unaltered. Two similar foci were subsequently found on a coverslip preparation of the same passage level; both foci gave a positive result when tested with Ad. 12 hamster tumor serum in the indirect fluorescent antibody test. The fluorescence of the cells of one focus resembled that of Ad. 12 tumor cells already described, in that there were typical cytoplasmic fluorescent flecks. However, there were also many other cytoplasmic granules which were smaller, less elongate, more regular in size, and more numerous. The second focus contained only these smaller cytoplasmic inclusions. Other cells of the culture were fibroblasts either isolated or in small groups and generally in poor condition; the majority were clearly negative in the fluorescent test, but a few possessed some faintly fluorescent cytoplasmic granules of unknown significance. A coverslip preparation of the same batch of cells, not containing a proliferative focus, was tested with conjugated rabbit antiserum to Ad. 12 virus with negative results.

Cells of the original proliferative focus have been maintained through 9 additional tissue culture passages, and have continually elaborated Ad. 12 tumor CF and fluorescent antigens. Cells of the 6th passage produced progressively growing tumors within 11 days after subcutaneous inoculation of 10^6 cells into 1- to 2- day-old hamsters.

Studies of Early Stages of Infection of Hamster Embryo and Human Embryonic Kidney Cells with Ad. 12 Virus.—Hamster embryo cells grown *in vitro* undergo abortive infection when inoculated with Ad. 12 virus; an antigen reacting in CF with sera of Ad. 12 tumor-bearing hamsters is formed, but no detectable A or C antigen or infectious virus is produced and no clear-cut cytopathic effect (CPE) occurs (6). In contrast, HEK cell cultures produce high titers of infectious virus, as well as the three above mentioned CF antigens.

Hamster embryo cell cultures were harvested at various intervals from 6 hours to 10 days after inoculation with $10^{7.8}$ $TCID_{50}$ of Ad. 12 virus per Petri dish (multiplicity of infection approximately 10); staining was done by the indirect procedure, using a hyperimmune hamster Ad. 12 tumor antiserum. At 6 hours no fluorescence was present. However, at 24 hours approximately 50 per cent of cells showed fine nuclear fluorescent flecks and many cytoplasmic flecks. At 2 days a high proportion of cells was involved, fluorescent material being present in nucleus or cytoplasm or both (Fig. 4). After this time progressively fewer cells demonstrated antigen; on the 6th day few nuclei still contained flecks, and the cytoplasmic fluorescent material appeared more amorphous and irregular. This degenerative process continued up to 10 days, the last observation of the experiment. No definite cytopathic effect was observed at any time.

Duplicate coverslips at each time period were stained with conjugated rabbit antiserum to Ad. 12; all were negative. Positive material harvested at 2 days failed to stain with SV40 hamster tumor serum, while the whole series was negative with control hamster serum. Also, the same Ad. 12 hamster tumor serum failed to produce fluorescence in uninoculated HaE cells.

Similar tests were made on HEK cells which were infected with Ad. 12 virus and harvested 24 hours later when CPE was beginning. Almost 100 per cent of cells showed fluorescence which was basically similar to that described in HaE cells; *i.e.*, fluorescent flecks in the nucleus and/or cytoplasm (Fig. 5). There was variation in the intensity of fluorescence of the nuclear flecks, some being fine and light and others thicker and brighter. Also, in some cells there was a homogeneous, more diffuse, fluorescence of the nucleus in addition to the flecks, and this fluorescence sometimes involved the cytoplasm. Occasional cells contained large tangled masses of fluorescent fibrils in the cytoplasm. When stained with the conjugated rabbit Ad. 12 antiserum, the infected HEK cultures showed the characteristic type of nuclear fluorescence described with other adenoviruses (13) in a small proportion of cells. This fluorescence involved the entire nucleus apart from the nucleolus, was finely granular, and gave no indication of flecks; no cytoplasmic flecks were observed.

Uninfected HEK cells in the same test did not fluoresce when stained with the Ad. 12 hamster or rabbit sera, and the infected HEK cells were negative when SV40 hamster tumor serum or normal hamster serum was used.

Studies of Hamster and Human Cell Cultures Infected with Other Adenoviruses.—Hamsters bearing Ad. 12–induced tumors do not develop antibodies to the adenovirus group antigen, in that there is no CF reaction with viral antigens of adenovirus types other than 12 and 18 (3). To determine if the antibody detected in hamster serum by the immunofluorescent procedure was similarly non–group-reactive, studies were made of cells infected with several other adenovirus types. In a preliminary test, adenovirus types 1, 2, 5, 7, and 18, and as controls, reovirus type 3 and polyoma virus, were inoculated into both hamster embryo and HEK cell cultures. The hamster embryo material was harvested at 2 days and the HEK at 24 hours. All cells were tested with Ad. 12 tumor hamster serum containing CF antibodies to both tumor and viral antigens.

The hamster embryo cells inoculated with types 7 and 18 showed fluorescence essentially similar to that seen with Ad. 12, except that the flecks were predominantly nuclear (Figs. 6 to 8) rather than cytoplasmic. In addition, occasional cells contained tangled masses of fluorescent fibrils in the cytoplasm (Fig. 6). Type 5 produced faint fine fluorescent flecks in many nuclei and some large amorphous highly fluorescent cytoplasmic inclusions, while with type 1 only a small percentage of cells showed faint nuclear flecks, with no cytoplasmic staining. With type 2, scattered nuclei contained fluorescent material of a granular rather than fleck-like appearance, and the cytoplasm was negative. Cells inoculated with reovirus 3 and polyoma, and uninoculated cells, were negative. No flecks occurred when a serum pool from hamsters bearing SV40 tumors was used on each of the infected materials; likewise, conjugated rabbit antiserum to Ad. 12 virus failed to stain any of the hamster material.

Similar results were obtained with the HEK cells infected with types 1, 7, and 18, in that nuclear flecks developed which stained with Ad. 12 hamster tumor serum; in addition, cytoplasmic flecks were common with type 18. Cells inoculated with types 2 and 5, reovirus 3 or polyoma, as well as uninoculated cells, were negative. When stained with the conjugated Ad. 12 rabbit antiserum, HEK cultures infected with Ad. 18 showed faint nuclear fluorescence like that with Ad. 12, in a few cells, while cultures infected with types 1, 2, 5, and 7 were negative. This finding was not unexpected in view of the relatively type-specific antibody responses of immunized rabbits, both in neutralization and CF tests (14, 15); the conjugated Ad. 12 rabbit antiserum did not react in CF with purified A antigen of type 1 adenovirus.[1]

A sequential study was made of the development of antigen in HaE cells infected with adenovirus types 7 and 2. With type 7, nuclear fluorescent flecks were rare at 6 hours, were seen in scattered cells at 24 hours, were present in a large number of cells at 2 days, and were still present on the 6th day, the last

[1] This antigen was provided by Dr. Julius A. Kasel.

time point tested. Type 2 also produced fluorescence, but the development was slower than with types 12 and 7. Cells were completely negative at 4 hours, and at 1 day only a few scattered nuclei showed fluorescent flecks. The number of cells involved increased slowly between 1 and 5 days during which time no CPE was seen. The appearance was quite different at 9 days, when CPE was evident. Fine fluorescent flecks were present in a high proportion of nuclei, and many cells also contained cytoplasmic flecks or fluorescent cytoplasmic inclusions, variable in size and irregular in shape, such as were seen with type 5 infection in the earlier experiment.

Preliminary Attempts to Identify Fluorescent-Stainable Antigens.—At least 4 distinct soluble CF antigens have been associated with Ad. 12 virus: the group reactive A antigen, the type-specific C antigen, the Ad. 12 tumor antigen(s), and the non-C antigen in viral harvests reactive with serum of tumorous hamsters (3, 5).

Three phases of antibody production are generally observed in hamsters bearing Ad. 12 tumors. The initial, and eventually highest titered response, is to tumor antigen, followed by response to the non-C antigen in viral harvests. Very late sera contain antibody to C antigen, but at no time are antibodies to A antigen detected. Attempts were made to identify the fluorescent-stainable antigens by comparing their reactions with rabbit antisera to purified A and C antigens and with hamster antisera showing the first and second stage of response.

As mentioned in previous sections, rabbit antiserum to standard Ad. 12 viral preparation gave staining of the entire nucleus of HEK cells infected with Ad. 12, but did not stain Ad. 12 tumors or hamster embryo cultures undergoing abortive infection with Ad. 12. Likewise, rabbit antisera to purified Ad. 12 A and C antigens did not stain the hamster tumors or Ad. 12–inoculated hamster embryo cultures, but stained the Ad. 12–infected HEK cells in a manner indistinguishable from the antiviral serum.

A comparison was made of the reactivity of the three types of cell preparation with 2 hamster sera, one ("broad reacting") having high titer CF antibody to both tumor and viral antigens, and the other ("narrow reacting") having a similar high titer to tumor antigen but no reaction with viral antigen. In Table I an attempt is made to grade the intensity of fluorescence observed. The broad serum stained large numbers of cytoplasmic flecks in all 3 systems. The narrow reacting serum demonstrated comparable numbers of cytoplasmic flecks in the Ad. 12 tumor and Ad. 12–infected HEK cells; however, on Ad. 12–inoculated hamster embryo cells, the number of cytoplasmic flecks was markedly reduced in comparison with the broad serum. In a second experiment, using the same 2 sera on a different batch of infected hamster embryo cultures, the contrast between the broad and narrow sera was even more marked.

Staining of nuclear flecks revealed a different pattern (Table I), in that the

broad and narrow reacting sera stained comparable numbers in both the infected hamster and human cell cultures, while in tumor cells the nuclear flecks were more evident with the broad than with the narrow serum. However, in view of the low level of intensity of the flecks in the tumor cells, we do not attach much significance to this observation.

Although a definite difference was seen between the reactivity of the broad and narrow sera in relation to cytoplasmic flecks in hamster embryo cultures, we are reluctant to equate the difference in staining with the difference in CF

TABLE I

Patterns of Fluorescent Staining with Antisera to Different Ad. 12 Antigens

Material	Rabbit antisera			Sera of Ad. 12 tumor-bearing hamsters			
				Cytoplasmic flecks		Nuclear flecks	
	A	C	Viral	Broad reacting serum*	Narrow reacting serum*	Broad reacting serum	Narrow reacting serum*
Ad. 12 tumor	−	−	−	++++	++++	+	Trace
Ad. 12-infected HaE TC	−	−	−	++++	Trace to +	++++	++++
Ad. 12-infected HEK TC	++++‡	++++‡	++++‡	++++	++++	++++	++++
SV40 hamster tumor	−	−	−	−	NT§	−	NT
Control HaE TC	−	−	−	−	−	−	−
Control HEK TC	−	−	−	−	−	−	−

* The broad reacting serum had CF antibody titers of 1:640 or greater against Ad. 12 tumor antigen and 1:40 against Ad. 12 viral antigen. The narrow reacting serum titered 1:640 *vs.* tumor antigen and less than 1:10 *vs.* viral antigen. Both sera were negative for neutralizing antibody (1:5 dilution) and for CF antibody to Ad. 12 A and C antigens (1:10 dilution).

‡ Fluorescence of entire nucleus; no flecks.

§ NT, not tested.

reactivity. However, it can be concluded that the cytoplasmic flecks formed in hamster embryo cells represent a different antigen than those in tumor and HEK cells. Furthermore, none of the stainable flecks in any of the 3 systems was associated with A or C antigen.

DISCUSSION

Table II summarizes the patterns of staining in the various systems studied.

Throughout this work the specificity of the immunofluorescent reaction is a matter of basic importance. It was established that fluorescent antibody staining was specifically and consistently obtained with Ad. 12 tumor cells and Ad. 12 tumor hamster antiserum. However, it was more difficult to establish that the reaction was specifically related to Ad. 12 virus rather than to some hypothetical contaminating agent in the system. Evidence excluding a role of a contaminant was obtained by the study of primary induction of tumors. When newborn hamsters were inoculated with Ad. 12 virus, the sera of those develop-

ing tumors and CF antibodies to tumor antigen were positive in the fluorescent antibody test with tumor cells, while sera from those not developing tumors or CF antibodies were negative. The chances are highly unlikely of a contaminant exhibiting the same latency as Ad. 12 virus, selective localization in Ad. 12 tumor cells, and an inability to produce antibodies in hamsters in the absence of Ad. 12 tumors. Also, tumors induced by two strains of Ad. 12 demonstrated the

TABLE II

Summary of Patterns of Fluorescent Staining of Adenovirus Tumors and Infected Cells

	Serum of hamster-bearing Ad. 12 tumor*			Rabbit anti-Ad. 12 virus		
	Cytoplasmic flecks	Nuclear flecks	Entire nucleus	Cytoplasmic flecks	Nuclear flecks	Entire nucleus
Ad. 12 tumor						
Hamster	+	+	±	−	−	−
Mouse	+	−	−			
Ad. 12 "transformed" HaE TC	+	−	−			
Infected HaE TC						
Ad. 12	+	+	−	−	−	−
Ad. 1 and 2	− or ±	+	−	−	−	−
Ad. 5, 7, and 18	+‡	+	−	−	−	−
Infected HEK TC (24 hrs.)						
Ad. 12	+	+	±	−	−	+
Ad. 1 and 7	−	+	−	−	−	−
Ad. 2, 5	−	−	−	−	−	−
Ad. 18	+	+	−	−	−	+

* Hamster hyperimmunized by repeated intraperitoneal inoculation of viable tumor.

‡ Cytoplasmic fluorescence with type 5 was in amorphous masses.

antigen. The presence of antigen of similar appearance in tumor cells of both hamster and mouse origin indicated an association with virus rather than a host reaction.

In both the hamster and mouse tumors, virtually every tumor cell showed fluorescence. The morphology of the antigen was unique, being distinctly different from that observed in acutely infected cells stained with antiviral antisera, and to our knowledge not resembling any viral fluorescence previously described. Since the fleck type morphology was seen in both direct and indirect tests, and both in frozen sections of tumor and in tissue culture preparations fixed by several methods, it does not seem likely that it represents a technical artifact.

The pattern of staining of Ad. 12–inoculated hamster and human cell cultures by hamster sera closely resembled that of the tumor cells. Thus, like the CF test (6), the fluorescent antibody test has not given evidence of antigens in tumor cells which are not found in acutely infected cells. A striking finding in these studies was the contrast between hamster and human cells in elaboration of A and C antigens. It seems possible that the hamster cells, both tumor cells and abortively infected tissue culture cells, synthesize the same virus-specific precursor proteins as do human cells, but are incapable of carrying out subsequent syntheses necessary for formation of at least one virus-structural protein.

It was not possible to determine the relationship, if any, of the fluorescent-stainable antigens to those reacting in the CF test, other than to rule out the A and C antigens. The failure to detect C antigen in tumor cells was unexpected, since some tumorous hamsters do eventually develop specific anti-C antibodies (5).

The finding of fluorescent antigen reacting with Ad. 12 hamster tumor serum, but not with antiserum to crude Ad. 12 viral preparation in HaE cells inoculated with a variety of other adenovirus types suggests that these viruses induce an additional common antigen which is apparently distinct from the A antigen.

SUMMARY

Complement-fixing (CF) antibody–positive sera from hamsters bearing adenovirus type 12 (Ad. 12)–induced tumors revealed specific immunofluorescent stainable antigens in essentially all Ad. 12 hamster tumor cells. The antigens were primarily in the form of cytoplasmic flecks; less frequent staining was seen as nuclear flecks or homogeneous staining of nucleus and cytoplasm of a small proportion of cells. Tumor cells did not stain with rabbit antisera to crude Ad. 12 virus or A and C antigens. The hamster serum also stained cytoplasmic flecks in an Ad. 12–induced BALB/c mouse tumor and Ad. 12–"transformed" hamster embryo tissue culture cells.

The hamster serum also stained fleck-shaped antigens in hamster and human cell cultures inoculated with homologous and heterologous adenovirus types, although the hamster cells did not react with the rabbit Ad. 12 antiserum.

Attempts to identify the fluorescent-stainable fleck-shaped antigens indicated that they are not previously recognized viral antigens and that the cytoplasmic antigens formed in hamster cell cultures inoculated with Ad. 12 are different from those in tumors and in acutely infected human cell cultures.

We are indebted to Dr. Robert J. Huebner and Mr. William T. Lane for supplying some of the materials used in these studies, and to Dr. Roger Wilsnack for the anti-hamster conjugate. Mr. Charles W. Shifler rendered valuable technical assistance.

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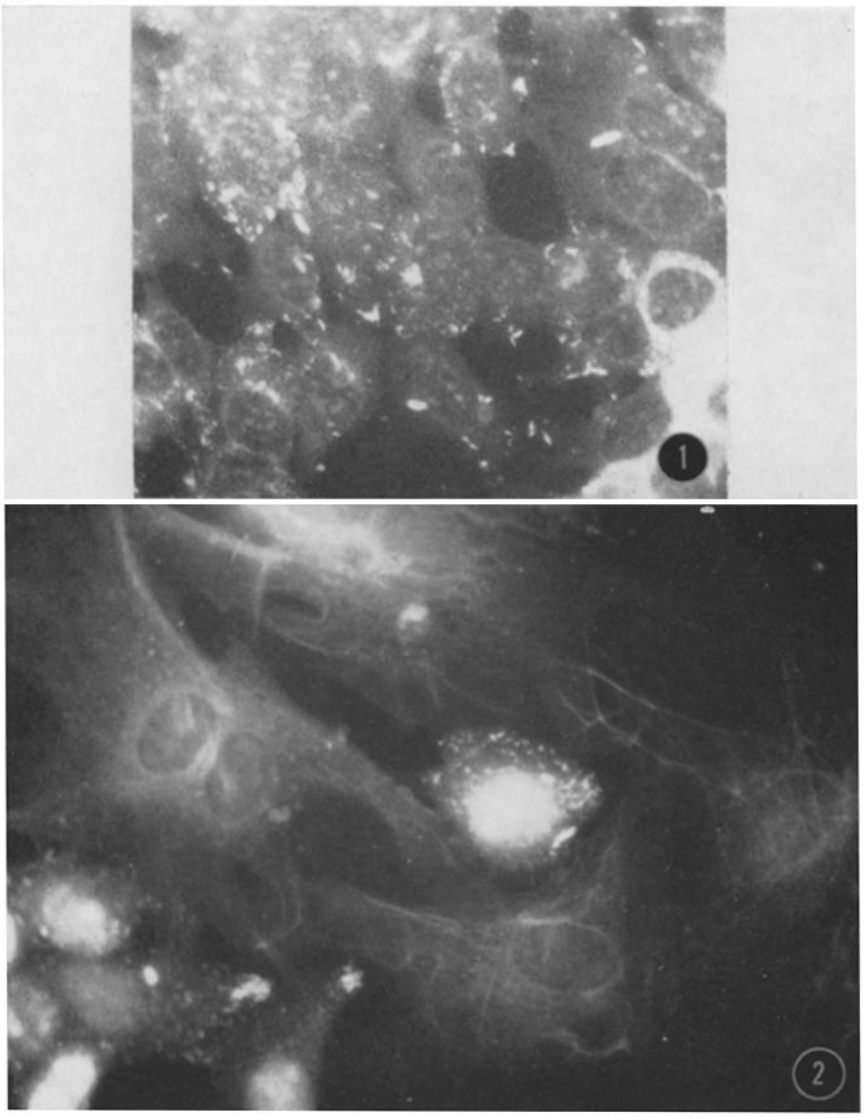
EXPLANATION OF PLATES

PLATE 63

FIGS. 1 and 2. Ad. 12 hamster tumors grown in tissue culture; stained in indirect test with hyperimmune Ad. 12 hamster tumor serum. × 540.

FIG. 1. Note large numbers of cytoplasmic fluorescent flecks, no staining of nuclei.

FIG. 2. Area showing tumor cells and large connective tissue cells. Note nuclear and cytoplasmic fluorescence in tumor cells, no staining of connective tissue cells.

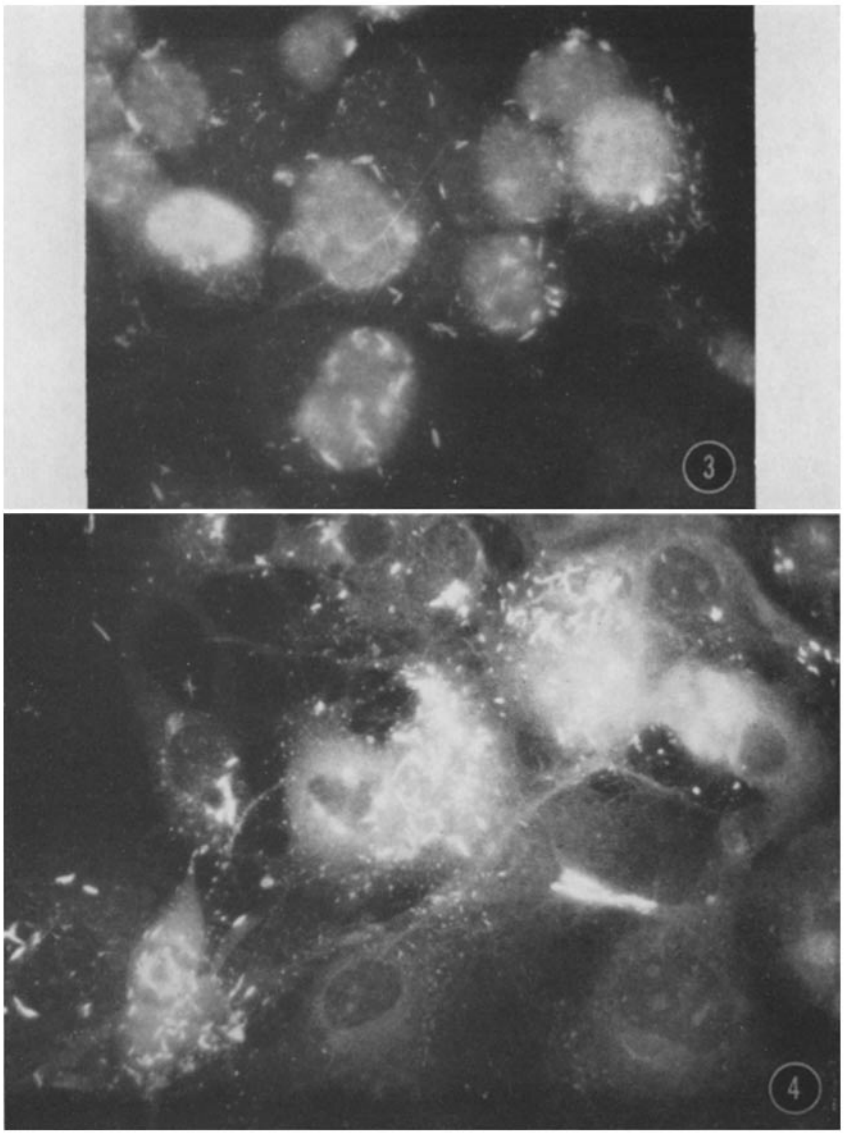


(Pope and Rowe: Adenovirus 12 tumors)

PLATE 64

FIG. 3. Ad. 12 hamster tumors grown in tissue culture; stained in indirect test with hyperimmune Ad. 12 hamster tumor serum. Tumor cells showing nuclear and cytoplasmic flecks with homogeneous staining of nuclei. × 540.

FIG. 4. Hamster embryo tissue culture cells 2 days after inoculation with Ad. 12 virus; stained in indirect test with broad reacting Ad. 12 hamster tumor serum 8314. Note intensely stained nuclear and cytoplasmic flecks. × 540.

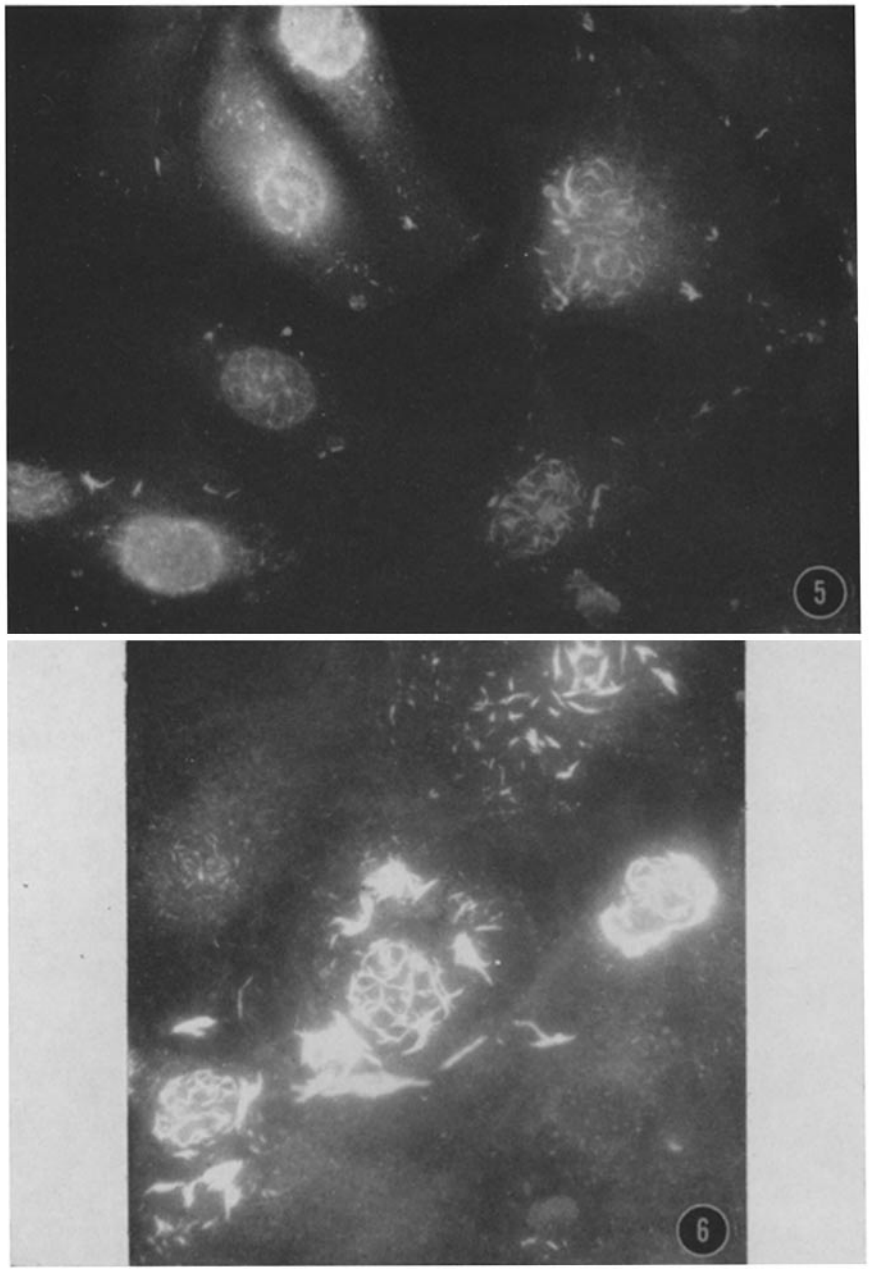


(Pope and Rowe: Adenovirus 12 tumors)

PLATE 65

FIG. 5. Human embryonic kidney tissue culture cells 1 day after inoculation of Ad. 12 virus; same staining as in Fig. 4. Note preponderance of nuclear flecks. × 540.

FIG. 6. Hamster embryo tissue culture cells 2 days after inoculation of Ad. 18 virus; same staining as in Fig. 4. Note large numbers of nuclear flecks and tangled masses of cytoplasmic fibrils. × 540.

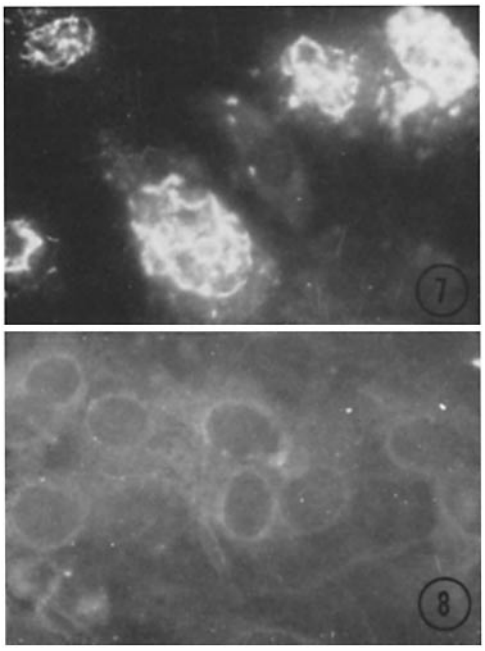


(Pope and Rowe: Adenovirus 12 tumors)

PLATE 66

FIG. 7. Hamster embryo tissue culture cells 2 days after inoculation of Ad. 7 virus; same staining as in Fig. 4. Note intense nuclear flecks, few cytoplasmic flecks. × 540.

FIG. 8. Uninfected hamster embryo tissue culture cells, stained in indirect test with hyperimmune Ad. 12 hamster tumor serum. The print was overexposed to show cellular outlines. × 540.



(Pope and Rowe: Adenovirus 12 tumors)