that V antigen synthesis represents a terminal or near terminal synthetic step in virus replication and thus the close agreement of V antigen and infectious virus inactivation is to be expected.

We have restricted our discussion to biological methods of effecting differential synthesis of T and V antigens. These methods clearly provide a valuable initial procedure for T antigen purification studies since V antigens constitute a substantial portion of the total protein of adenovirus infected KB cells. Essentially similar results have been found by Rhim and associates.[1]

Summary. Adenovirus T and virion antigen syntheses are readily separable by use of DNA antagonists, e.g., FUDR, thermal separation, and UV irradiation of virus. Of these, the thermal separation procedure was most effective, and coupled with the use of FUDR, should provide a relatively simple procedure for large-scale preparations of T antigens for sero-epidemiological surveys.

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Temperature Dependence of the Synthesis of Adenovirus Tumor and Viral Antigens* (32763)

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Specific complement-fixing (CF)(1) and fluorescent antibody T (tumor) antigens (2) have been described in adenovirus-induced hamster tumors or in adenovirus-transformed hamster or rat cells (1–5). Synthesis of the T antigen (6) also occurs in cells infected with adenoviruses; however, the antigen appears early in the cytolytic cycle, before the appearance of the virus itself (2,7,8). The adenoviruses have recently been divided into three subgroups, A, B, and C, based upon their oncogenic properties in newborn hamsters, their T antigens, and their DNA base compositions (9–11).

Studies designed to produce virion-free T antigen by attempting to block adenovirus virion synthesis using 5-FUDR (8,12) and cytosine arabinoside (13) have been moderately successful; however, it has been suggested (10) that the production of working amounts of adenovirus T antigen free of structural virion antigens may prove to be more difficult than the production of virion-free SV-40 T antigens (14–16). Recent reports described the selective production of

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adenovirus T antigen by ultraviolet irradiation of virus (17) and by abortive infection of cells of simian and rabbit origin (18).

Kitahara and Melnick reported that thermal separation of the synthesis of SV-40 T and viral (V) antigens resulted in the production of virion-free SV-40 T antigen (16).

In this study, it is shown that at different temperatures the synthesis of adenovirus T and V antigens proceeds at different rates, and at low temperature (24°C) adenovirus T antigen, like SV-40 T antigen, can be produced in the relative absence of V antigen.

Materials and Methods. Tissue cultures. Cell cultures of KB and RK-1 (19) were obtained from Microbiological Associates, Inc., and were maintained at 37°C on Eagle's minimum essential medium in Earle's balanced salt solution containing 3% agamma calf serum (obtained from Hyland Laboratories, Los Angeles, Calif.), 4 m*M* of glutamine, and 100 U of penicillin and 100 μg of streptomycin/ml. A line of African green monkey kidney *(Cercopithecus aethiops)* cells (Vero) was obtained from Dr. Y. Yasumura, Chiba, Japan, in 1964, and maintained first at the Laboratory of Tropical Virology, National Institutes of Health, Bethesda, Md., and later in this laboratory. The Vero cells were subcultured and maintained in Medium 199 supplemented with 5% fetal bovine serum (20).

Virus. Adenovirus seed stocks were obtained from the American Type Culture Collection. They had been passed through human cell cultures, embryonic kidney (HEK), KB, and/or HeLa cells, before use. Virus pools and all T antigen preparations were tested and found negative for adeno-associated viral (AAV) antigens (21).

Virus titrations were done in HEK roller tube cultures employing 3 roller tubes per each 10-fold dilution. End points were calculated by the Reed-Muench formula (22).

Antigen. Cell cultures were inoculated with various adenoviruses at the multiplicity of infection stated in the text and incubated at different temperatures (24, 30, 37, and 40°C) in a stationary position. These cultures were harvested at various stages of cytopathic effect (CPE) by slow speed cell sedimentation as previously described (23). All antigen titers given are based on 5–10% cell suspensions. Tumor extracts (10%) were used as positive control materials for tumor serum identification. Control antigens consisted of uninoculated cell pack preparations, or extracts of hamster tumors induced by unrelated viruses —SV-40 and the Schmidt-Ruppin strain of Rous Sarcoma Virus.

Antisera. Hamsters bearing tumors induced by whole cell transplants from virus induced tumors developed serum antibodies which were either narrow-reacting (measuring virus-specific tumor and T antigen only) or broad-reacting (reacting also with specific virion or viral subunit antigens). As soon as tumors reached diameters of 50 mm or more, the hamsters were bled from the jugular vein and the serum was separated according to standard procedure (1). Group reference serum consisted of pools of human convalescent sera reactive with standard adenovirus viral antigen.

Complement fixation. Complement fixation tests were carried out in the microtiter technique described previously (1). Titers were recorded as reciprocals of the highest dilution giving 3+ to 4+ fixation of 1.8 units of complement.

Results and discussion. Development of adenovirus T and V antigens in vero cells incubated at different temperatures. Vero cultures were inoculated with 1 TCD_{50} per cell of adenovirus type 31 and incubated at 37, 30, or 24°C. They were harvested and tested for antigenic activity at various intervals after inoculation. When the infected Vero cells were maintained at 37°, T antigen appeared between 12 and 24 hours after infection, several hours before V antigen was first detected. During the first few days, both T and V antigens increased at similar rates. The T antigen reached its maximum level in 2–4 days after infection, and remained at that level for several days. During this time, V antigen titers increased, and reached a plateau as the viral cytopathic effect reached completion (Fig. 1).

When the infected Vero cells were incubated at 30°, both antigens were detected a few days later than in the 37°C cultures, and a longer time was required at 30°C for the T antigen to reach the maximum level found in the 37°C cultures.

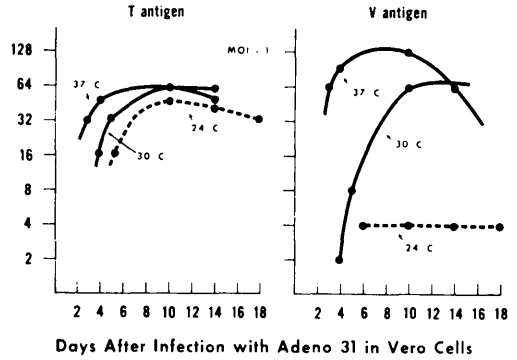

FIG. 1. Development of adenovirus type 31 T and V antigens in adenovirus type 31-infected Vero cells incubated at different temperatures. Input multiplicity was 1 TCD_{50} per cell.

At 24°C, the production of V antigen was very low, T antigen was first detected several days later than in 37° cultures, and 10 days were required at 24° for the T antigen to reach maximum levels (Fig. 1). Low levels of V antigen found at 24°C presumably resulted from residual antigen in the inoculum, since they were present in amounts directly proportional to the input multiplicity, and were removable by washing the inoculum.

When the infected cultures were first incubated at 37°C and then transferred to 24° at one day after infection, synthesis of T antigen continued, while that of the V antigen stopped (Fig. 2).

In other experiments, infected cultures were first incubated at 24°C and then transferred to 37° at 6 and 10 days post infection. There was an 8-fold increase of V antigen within 2–4 days after transfer to 37°C, while the

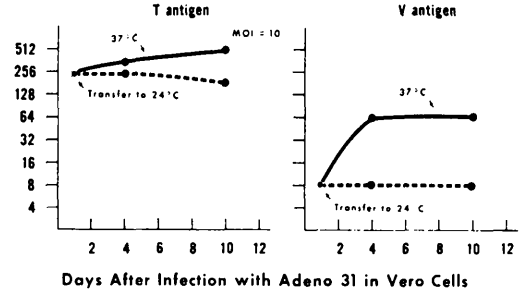

FIG. 2. Changes in synthesis and persistence of adeno type 31 T and V antigens after transfer of infected Vero cultures from 37 to 24°C. Multiplicity of infection was 10 TCD_{50} per cell.

synthesis of T antigen was apparently delayed (Fig. 3).

Similar results of thermal separation were obtained with adenovirus types 12 and 18 in Vero cells (Table I). However, such thermal separation was not observed with B group adenoviruses (types 3, 7, 14, 16, 21) in Vero cells. No T antigen was produced at 24°C, although a low level of V antigen was found. At 37°C, there was a low level of T antigen production, along with large amounts of V antigen.[1]

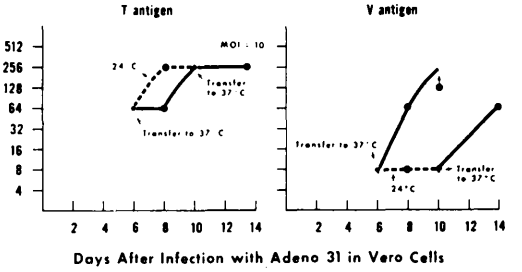

FIG. 3. Changes in synthesis and persistence of adeno type 31 T and V antigens when infected Vero cultures were first incubated at 24° and then transferred to 37°C. Multiplicity of infection 10 TCD_{50} per cell.

Development of adenovirus T and V antigens in RK-1 cells incubated at different temperatures. Titers of group A adenovirus T and V antigens in RK-1 cells incubated at different temperatures (37, 30, and 24°C) are shown in Table I. When RK-1 cells infected with adenovirus type 12 were maintained at 37°C, T antigen titers continued to rise for 7 days after maximum CPE, stayed at peak levels (1:256) for another week, and then decreased. The amount of V antigen found was usually low, and probably resulted from residual antigen in the inoculum (18).

When the infected RK-1 cells were incubated at 30°C, T antigen was detected 48 hours later than in 37° cultures, and reached a peak titer of 1:512, which was higher than that found in the 37° cultures at maximum CPE. (Table I). At 24°C, T antigen was detected 72 hours later than in the 37° cultures, and at least 3 weeks were required for

[1] Rhim, J. S., Schell, K., Snyder, T. J., Turner, H. C., and Huebner, R. J., in preparation.

TABLE I. Production of V and T Antigen in Cell Cultures Inoculated with Various Oncogenic Adenoviruses.

Type	Cells	MOI[a]	Temp. (°C)	Day of harv.	CF titer[b] Human ref. serum 26186 VAG	Hamster anti-ad. 12 tumor serum pool 16 TAG	Hamster anti-ad. 7 tumor serum pool 6 TAG
31	Vero	10	24	8	8	256	4
			37	4	64	256	4
	RK-1	32	30	15	8	>64	8
			37	11	(4)	64	4
18	Vero	3.2	24	4	0[c]	8	—
			30	3	0	>32	—
			37	3	>16	32	—
	RK-1	3.2	24	22	0	(32)	<4
			30	22	0	128	8
			37	15	0	4	—
12	Vero	32	24	5	8	16	—
			30	4	32	16	—
			37	2	64	16	—
	RK-1	32	24	9	2	32	(4)
				15	2	32	(8)
				21	4	128	8
			30	7	(8)	32	(8)
				12	4	512	(16)
				22	4	256	16
			37	7	8	128	16
				14	4	256	32

[a] MOI = multiplicity of infection.

[b] CF titer = reciprocal of dilution giving 3+ to 4+ fixation of 1.8 units of complement (in parentheses indicates 2+).

[c] 0 = <2; — = not done.

the T antigen to reach its maximum level (1:128).

Adenovirus types 18 and 31 incubated at different temperatures also yielded much more T antigen than V antigen, and titers of these preparations at 30°C were higher than those obtained at 24° (Table I). In contrast, adenovirus type 7 did not produce significant amounts of T antigen in RK-1 cells: The V antigen titers were often equal to or higher than those of the T antigen at the various temperatures.

Development of adenovirus T and V antigens in KB cells incubated at different temperatures. Thermal separation experiments were further extended to adenoviruses in KB cells. The result with adenovirus type 7 is shown in Fig. 4. T antigen without V antigen was obtained in the 24°C cultures within 72 hours after inoculation. The T antigen titers continued to rise with incubation, accompanied, however, by the development of high titers of V antigen. This contrasts with the results obtained with A group adenoviruses in Vero and RK-1 cells. Thus, in our limited tests in KB cells the differences between viral and T antigen titers were insufficient to be useful in the CF test which requires that at least four units of antigen be used.

Gilden and associates[2] on the other hand found KB cells quite effective for preparation of T antigens using the thermal separation method.

[2] Gilden, R. V., Kern, J., Lee, Y. K., and Huebner, R. J., in preparation.

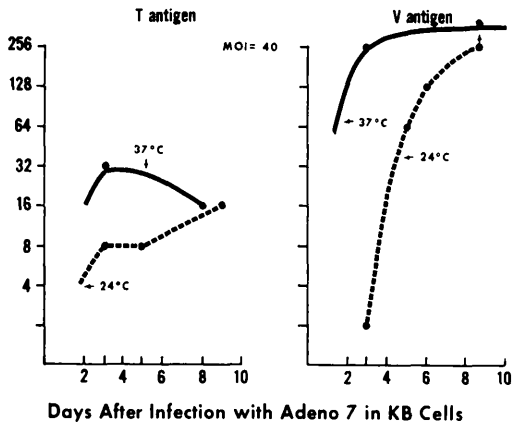

FIG. 4. Development of adenovirus type 7 T and V antigens in adenovirus type 7-infected KB cells incubated at 24 and 37°C. Input multiplicity was 40 TCD_{50} per cell.

Cross reactivity of adenovirus T antigens between A and B oncogenic groups. Group A adenovirus (types 12, 18, and 31) T antigen preparations in RK-1 and Vero cells showed considerable reactions with type 7 tumor sera (Table I). The KB grown T antigens, in contrast, were free from any detectable cross reaction. This unexpected finding deserves further study. Cross reactions between antigens in the two groups have hitherto been observed only by employing immunofluorescent staining techniques (2) or with very few selected hamster sera in the CF test (24).

Summary. The synthesis of adenovirus T and V antigens in infected cell cultures incubated at different temperatures was studied. At low temperature (24°C) development of V antigens was limited, while significantly larger amounts of T antigen were produced. Raising the incubation temperature from 24 to 37°C led to a rapid increase in V antigen. Highest titers of T antigen for adenovirus types 12, 18, and 31 were obtained in Vero and RK-1 cells incubated at 30°C. The antigen produced in the cultures was found to be unusually cross-reactive with type 7 tumor antibody. Thermal separation of the synthesis of adenovirus T and V antigens in cell cultures should provide a method for the production of adenovirus T antigens.

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