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9 July 1965

Oncogenicity of the Simian Adenoviruses

Abstract. *Five of 17 adenoviruses of rhesus or cynomolgus monkey origin induced tumors in newborn hamsters. The tumors appeared between 42 and 280 days after subcutaneous inoculation and had the general characteristics of lymphomas. The tumors were specific by cross-complement fixation tests. An adenovirus recovered from* Cercopithecus *monkeys appeared to be highly oncogenic; all 23 inoculated hamsters developed tumors within 30 to 40 days.*

The oncogenicity of human adenoviruses for newborn hamsters has been reported (*1, 2*), and we have observed that 6 of 18 simian adenoviruses behave similarly. Five of these were part of the SV (simian virus) series and were of rhesus or cynomolgus monkey origin (*3*). The other, SA7, was isolated from an African green monkey (*4*). All tests were performed with prototype strains.

Small groups of newborn hamsters were inoculated with one of each of the 18 simian adenoviruses, or with one of two known oncogenic agents, adenovirus 12 and SV40. Each animal received 0.05 ml of virus subcutaneously, in the dorsal cervical area. Tumors were obtained in the general area of the inoculation with five of the viruses in the SV series, with SA7 (Table 1), and with the known tumor viruses. On the basis of incidence of tumors and duration of latent periods, SA7 appeared to be the most oncogenic, followed by SV20 and SV38. SA7, furthermore, produced multiple, rapidly growing subcutaneous tumors, while the other viruses produced single masses. What effect, if any, the virus dose had on the degree of oncogenicity was not determined. None of the other viruses produced tumors in these initial tests, although Huebner *et al.* (*5*) previously reported that SV1 (M-1) produced tumors in hamsters. Because of the limitations in our studies—in number of animals inoculated, virus concentrations and periods of observation—the possibility cannot be excluded that other simian adenoviruses are oncogenic.

The capacity of most of these viruses to produce tumors has been confirmed by further studies. Only SV37 and SV38 have failed to produce tumors in repeated tests, but the animals involved have only been under observation for 90 days, and much longer latent periods than this were noted in the original study. A summation of the results of all inoculations to date is presented in Table 2.

The oncogenicity of SA7 was not only readily confirmed for baby hamsters, but was also observed for suckling rats. Tumors were obtained in 3 of 21 inoculated animals. In addition to producing tumors in hamsters, SV20 produced a tumor in one of 15 newborn C3H mice after a 92-day latent period. Tumors produced by SA7, SV20, SV33, SV34, and SV38 were successfully transplanted to weanling hamsters.

Tumors produced by these simian adenoviruses were locally invasive, but not lethal within the experimental period, except for the rapidly growing SA7 tumors, which did kill some hamsters. Metastases generally were not observed, but one animal bearing an SV20-induced tumor and killed for pathologic examination did have a small metastasis to the brain. Histopathologically, the tumors produced by the various viruses except for the SV34 tumor, were similar and more like tumors produced by adenovirus (*2*) than like the fibrosarcomas produced by SV40 virus. The tumors were undifferentiated neoplasms with some characteristics of lymphomas of the reticulum-cell type although, few, if any, reticulum fibers were present. The actual cell of origin was not determined. The SV34 tumor was somewhat more differentiated, with some areas resembling a spindle-cell sarcoma. Electron micrographs of SV34 revealed a second particle, smaller than the adenovirus; in this respect, it was unique among the viruses in the oncogenic group (Fig. 1). Similar particles were seen in other simian adenoviruses which did not produce tumors. Representative fields of the histological sections of tumors produced by SV20, and SV34, and by SV40, for comparison, are shown in Fig. 2.

Attempts to recover or to demonstrate the presence of SV20 in tumors produced by the virus failed. Homogenates were inoculated into susceptible cell cultures, and explanted cultures were prepared from the tumor tissue. After sedimentation procedures, electron microscope examination, which readily showed particles in fluids from SV20-infected tissue culture, failed to

Table 1. Oncogenicity of simian adenoviruses in newborn hamsters. The viruses in the SV series were grown in strain LLC-MK2 cells; and SA7 in BS-C-1 cells. Inoculation of newborn hamsters with 10^6 or more viable cells of either strain failed to produce tumors.

Virus	Inoculations (No.)	Virus dose ($\log_{10}$)	Survivors beyond 30 days/ No. with tumors	Time of tumor detection (days)
SV20	12	6.75	6/3	53–62
SV33	8	6.00	6/1	229
SV34	8	5.50	6/1	110
SV37	7	*	6/1	231
SV38	9	4.83	8/3	154–280
SA7	23	7.50	23/23	31–41

* Not determined.

Table 2. Compilation of oncogenic findings in hamsters with simian adenoviruses, adenovirus 12, and SV40. All animals were inoculated within 24 hours of birth with 0.05 ml of undiluted virus and observed until death or until killed for histopathology.

Virus	Inoculations (No.)	40-day survivors/No. with tumors	Time of tumor detection (days)
SA7	83	47*/46	28–48
SV20	250	141/54	40–211
SV33	31	25/2	82–229
SV34	62	48/3	110–330
SV37	23	20/1	231
SV38	36	14/3	154–280
SV40	75	39/24	121–251
AV12	29	21/3	55–89

* Includes animals killed for histopathology between 30 and 40 days.

reveal virus in tumor homogenates. Tumor antigens and antiserums also failed to show cross-reactions in complement-fixation (CF) tests with the homologous virus and its specific antiserum. Similar studies with SA7-tumor tissue also gave negative results.

Although the viruses used in these studies were grown in cell strains free of SV40, it was necessary, because of their origins and passage histories, to eliminate any possible role of SV40 (*6*) in the oncogenic effect observed. Attempts to demonstrate the presence of SV40 in the seed pools by inoculation of *Cercopithecus* monkey kidney cells and by electron microscope examination were negative. Representative electron micrographs are illustrated in Fig. 1. The histology of these tumors, except of those induced by SV34, also lent support to these findings.

Preliminary CF testing (*7*) suggested that these tumors produced by virus were type specific and that there was no cross-reaction with SV40-tumor antigen or antiserum. Saline extracts of the tumors were prepared as antigens (*8*), and serums were obtained from tumor-bearing and normal animals. The results of these tests are shown in Table 3. Cross-reactions were not done with all reagents since the supply was limited. Where such tests were done, the tumors appeared to be type specific, and tumor antigens and antiserums did not react with the homologous viral reagents. None of the adenovirus-induced tumor antigens or antiserums cross-reacted with those of SV40 tumors (Table 3). Moreover, serum from the hamster bearing the SV33-induced tumor did not fix complement with adenovirus 12-, SV20-, or SV38-tumor antigens. The homologous antigen was not prepared. Antigen and serum from the first animal to develop an SV34 tumor were lost before CF tests were performed. Similar material obtained from the second animal failed to react in the CF test. The serum from this animal also failed to react with SV40-tumor antigen.

In addition to concern over the possibility of contamination of the seed viruses with intact SV40 virus, there has also been mention of a further problem. Huebner *et al.* (*9*) recently introduced the idea that "hybridization" between adenoviruses and SV40 virus was possible. These authors described such a phenomenon involving the human adenovirus type 7, strain L.L. According to their report, however, tumors produced by the "hybrid virus" strain contained SV40-tumor antigens, and serum from these animals contained SV40-tumor antibodies, determined by complement fixation. Our failure to detect SV40-tumor antigens in the tumors induced by these simian adenoviruses or SV40-tumor antibodies in serum from tumorous animals, seemed to preclude the possibility that we were working with SV40 hybrid strains similar to the strain described by Huebner *et al.*

The significance of these findings may extend beyond the demonstration of virus oncogenicity for laboratory animals. A high incidence (62 to 82 percent) of antibody to SV20 was recently reported (*10*) in a New Guinea human population where there were

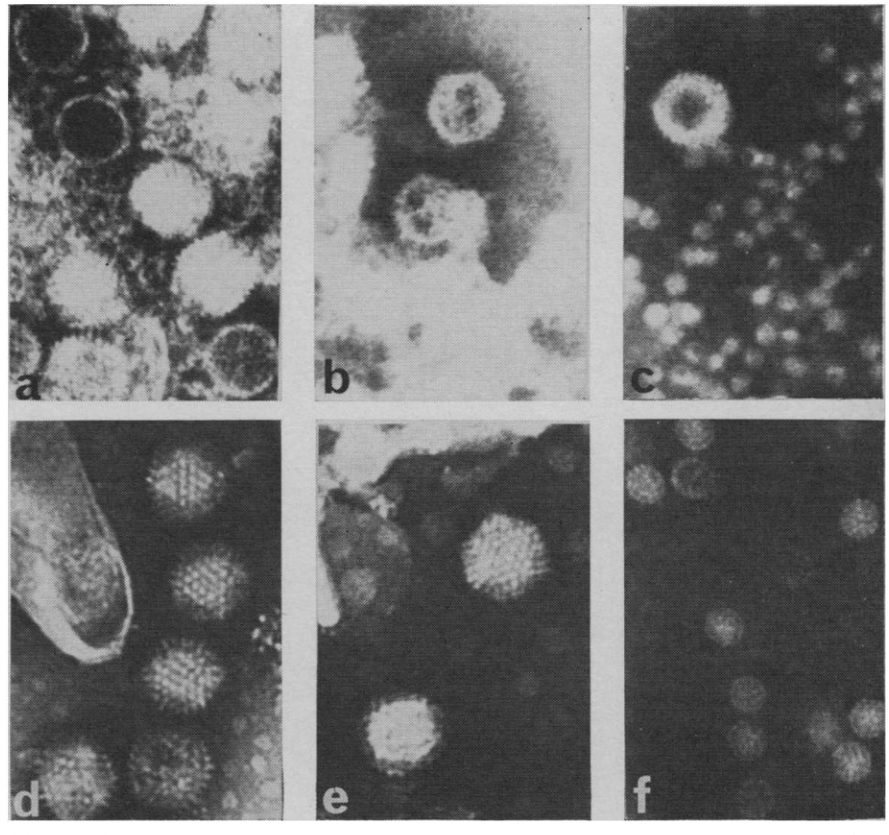

Fig. 1. Oncogenic simian viruses, negatively stained (phosphotungstate). *a*, SV20; *b*, SV33; *c*, SV34; *d*, SV37; *e*, SV38; *f*, SV40 (× 200,000).

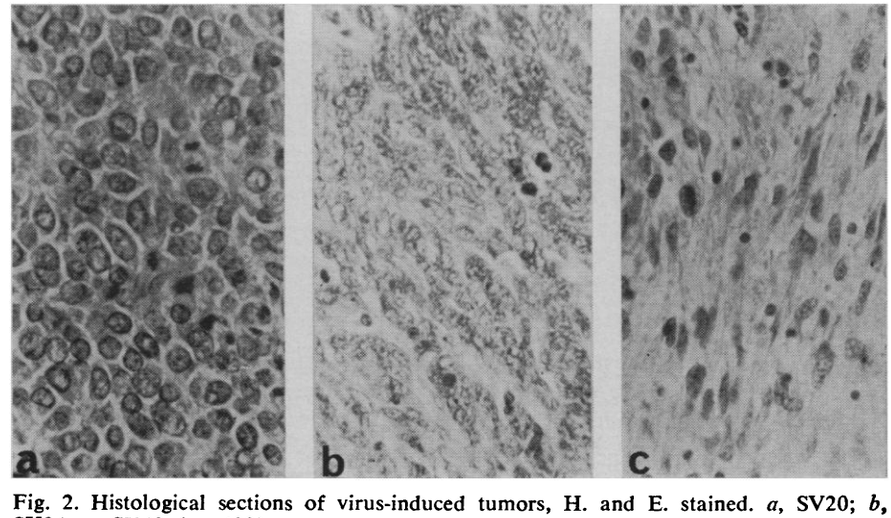

Fig. 2. Histological sections of virus-induced tumors, H. and E. stained. *a*, SV20; *b*, SV34; *c*, SV40 (× 400).

Table 3. Complement-fixation tests with virus and tumor antigens and antiserums. Virus antigens (V) were live viruses grown in tissue culture. Antiserums to virus were prepared by immunization of rabbits with live virus. Tumor antigens (T) were saline extracts of virus-induced tumors, and serums were obtained from tumor-bearing hamsters. Results are expressed as reciprocals of the highest dilution of antiserum reaction with antigen.

Antigens	Serums							
	SV20V	SV20T	SV38T	SA7V	SA7T	SV40T	SV40T†	Normal
SV20V	64	<2	*	*	*	*	<2	<2
SV20T	<2	32	4	*	<2	<2	<2	<2
SV38T	*	4	32	*	*	*	*	*
SA7V	*	*	*	32	<2	*	*	*
SA7T	*	<2	*	<2	32	4	*	<2
SV40T	*	<2	*	*	<2	256	*	<2
SV40T‡	<2	<2	*	*	<2	*	*	<2

* Not tested. † This serum was supplied by Dr. Bernice Eddy, NIH. ‡ This antigen was supplied by Dr. David Axelrod, NIH.

no naturally occurring primates other than man. A lower incidence (19 percent) was reported in a U.S.A. population. These findings, combined with the demonstration of a potent viral oncogen (SA7) occurring in primates in Africa, suggest that epidemiological studies of the relationship of SV20 and SA7 to lymphomas of the Burkitt type, and to other malignancies in these divergent geographical areas, would be of considerable interest.

Earlier reports on the oncogenicity of SV40 for baby hamsters had a profound effect on the production and testing of virus vaccines prepared in cell cultures from monkey kidney, as judged by the more stringent regulations that have been imposed upon manufacturers of such products. Although the simian adenoviruses do not appear as contaminants in primary cultures of monkey-kidney as frequently as does SV40, our findings point to possible contamination of products for human immunization with at least seven different viruses of demonstrated oncogenic potential. Thus, the program recommended by the Committee on Tissue Culture Viruses and Vaccines (appointed by the director of the National Institutes of Health) for the valuation of serially propagated cell cultures for vaccine preparation (*11*) should be vigorously pursued.

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13 September 1965; revised 25 October 1965

Bacterial Contamination of Some Carbonaceous Meteorites

Abstract. *Three types of bacteria were isolated from samples of two carbonaceous chondrites and identified as common contaminants that are widely distributed.*

As an extension of other work done in our laboratory on carbonaceous chondrites (*1*), it was considered of interest to determine to what extent, if any, these meteorites are contaminated by ordinary viable microorganisms. The following three meteorites were selected for investigation. The Orgueil (Wiik type I) received from A. Cavaillé, the Murray (Wiik type II) obtained from E. P. Henderson, and the Mokoia (Wiik type III) secured from C. B. Moore. Using standard microbiological techniques, we had no difficulty in obtaining bacterial cultures from samples of the Murray and Mokoia but were unable to detect the formation of any bacterial colonies from samples of the Orgueil.

Small fragments of each meteorite (*2*) were removed aseptically and placed in carefully weighed sterile containers. One milliliter of sterile water was added, and the tubes were placed on a rotary shaker for 30 minutes. The supernatant was carefully removed and plated on nutrient agar. The plates were incubated 24 to 48 hours at 37°C. The colonies were counted on a Quebec counter. The meteorite fragments were then dried, reweighed, and ground to a powder with a mortar and pestle that had been washed in acid or autoclaved. (In other tests the fragments were ground without any prior treatment). One milliliter of sterile water was again added, and the sample was treated as before.

The results are summarized in Table 1. Microbiological analyses of the Murray samples gave consistent results, whereas analyses of the different Mokoia samples were more variable; no viable growth could be found in any of the Orgueil samples tested under either aerobic or anaerobic conditions. Some Orgueil samples were incubated at room temperature for 2 weeks with no additional change. We cannot say, however, whether more prolonged incubation might have shown growth of fungi or other microorganisms that require unusually long incubation periods for germination.

As shown in Table 1, the approximate average contamination of the samples ranges from 1800 (Mokoia) to 6000 (Murray) bacteria per gram of meteorite. The organisms isolated from the Murray were identified as *Bacillus cereus* and *B. badius* (in a ratio of 5 to 1) and the organism from the Mokoia as *Staphylococcus epidermidis*. Identification was accomplished by extensive testing according to the descriptions given in Bergey's manual. No organisms were isolated from the solvent or the agar.

It appeared that a much higher bacterial count, in comparison with the count from the supernatant fractions, could be obtained by spreading the washed meteorite granules on nutrient agar; hence affinity between the meteorite particles and the bacteria is suggested. This observation is in line with reports by other investigators of bacteria and other microorganisms that grow out from hard rocks and minerals into nutrient media (*3*). The spreading of the meteorite particles on agar plates was not employed for determining the bacterial counts because of the additional work and the difficulty