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Cancer Induction in Hamsters by Human Type 12 Adenovirus. Effect of Age and of Virus Dose.* (27786)

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We have recently reported(1) that hamsters injected intrapulmonarily at birth with human type 12 adenovirus develop a high incidence of malignant sarcomas in the thorax and liver in from 1 to 3 months. The tumor-inducing activity is not lost by Selas 02 filtration or by tissue culture passage in HeLa cells. The induced tumors grew in and killed a high percentage of unconditioned young adult hamsters into which they were transplanted. Polyoma virus antibodies (HI) were not present in tumor bearing or control hamsters. Evidence was presented that the tumor inducing effect was not attributable to contamination with SV40 or other animal viruses, but was indeed associated with the human adenovirus. No such tumors occurred in hamsters injected with control tissue culture fluids or in control breeder hamsters. The present report deals with the effect of age at time of injection, and with the dose-response relationship.

Materials and methods are as previously reported(1) unless otherwise stated. Human type 12 adenovirus was propagated in human embryonic lung cells kindly supplied by Drs. Hayflick and Koprowski of the Wistar Institute, or in HeLa cells from Microbiological Associates, Inc. Virus titer was determined by cytopathic effect in tissue cultures of HeLa cells as previously described. The final reading was made on day 5. The titers given are therefore probably underestimates of the actual virus titer. Hamsters 8 days of age or younger were intrapulmonarily injected as previously reported. In hamsters of 2 weeks of age or older, the lung could not be seen through the chest wall and these were injected by inserting the needle 7 to 10 mm through the right ventral chest wall.

Results. In hamsters less than 24 hours old at time of injection 500 tissue culture infective doses (TCID) of virus produced tumors in 26 of 27 animals, and as little as 0.5 TCID produced a tumor in 1 of 5 animals. The 50% tumor dose lies somewhere between 0.5 and 50 $MTCID_{100}$ (Table I). Susceptibility to tumor induction decreased rapidly with increasing age at time of injection. After injection at 8 days of age 500 TCID induced tumors in only 2 of 9 animals. A dose of 1000 TCID produced one tumor in 8 hamsters injected at 2 weeks of age, but none in hamsters injected at 3 weeks of age or older. The tumors induced were, as previously reported(1), undifferentiated sarcomas at the site of injection, arising after a short latent period.

Discussion. The observed decrease in susceptibility with increasing age is in keeping with what is known of other tumor virus systems. It is possible that doses larger than 1000 TCID may result in tumor induction in hamsters older than 2 weeks at time of injection. It is even more probable that factors which depress immunological reactivity, such as whole body X-irradiation, may permit tumor induction following injection of virus into still older (irradiated) hamsters(2). If malignant transformation induced by adeno-

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TABLE I. Tumor Induction in Hamsters of Various Ages by Human Type 12 Adenovirus.

Virus titer ($MTCID_{100}$*/.1 ml)	Dose ml	Dose TCID	Age at inj. (days)	No. of tumor-developing hamsters / No. of inj. hamsters†	Days from inj. to death by tumor
1‡	.05	.5	<1	1/5	73
"	"	"	2–3	0/6	
"	"	"	7	0/2	
"	"	"	14	0/10	
"	"	"	19–21	0/9	
"	"	"	30–60	0/20	
100	.05	50	<1	16/19	33– 91
1000§	.05	500	<1	26/27	35–157 (59)‖
"	"	"	5	5/7	41– 78 (57)‖
"	"	"	8	2/9	70, 102 (86)‖
"	.1	1000	14	1/8	56
"	"	"	21	0/7	
"	"	"	39–43	0/6	
"	"	"	76–77	0/7	

* Minimum 100% tissue culture infective dose.

† No. of injected hamsters which survived longer than 1 mo.

‡ Supernatant of the tissue culture fluid of human embryonic lung cells inoculated with the virus; centrifuged at 1500 rpm for 10 min. The animals injected with this material have been observed for 316 days.

§ The cell-free filtrate of the tissue culture fluid of HeLa cells inoculated with the virus. The animals injected with this material have been observed for 195 days.

‖ Average.

virus type 12 is associated with the appearance of cancer specific antigen(s), as appears to be the case for polyoma virus induced tumors(3,4), it is possible that malignant transformation of cells at the site of injection of human adenovirus type 12 occurs in hamsters of all ages, but that clinically evident tumors appear only in the immunologically incompetent young hamster.

Summary. The susceptibility of newborn hamsters to induction of sarcomas at the site of injection of human type 12 adenovirus decreases rapidly with increasing age at time of injection. For a given age at the time of injection, the incidence of tumors induced is directly proportional to the dose of virus injected. The 50% tumor-inducing dose in hamsters injected when less than one day of age lies between 0.5 and 50 $MTCID_{100}$ of adenovirus under the conditions of these experiments. Increasing the dose of virus to 1000 $MTCID_{100}$ produced one tumor among 8 hamsters injected as late as 14 days of age, but none in 3-week or older hamsters.

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