ture for over 2 years, and that 11/16 hamsters pre-treated as adults with extracts of these tumor cells were also completely resistant to challenge with similar large numbers of cells, is surprising in the light of Habel's (1,2,10) inability to prevent tumor formation in pre-treated hamsters receiving 10^4 to 10^6 cells. The reported data(9), however, do not indicate the minimal number of the tissue culture passaged polyoma tumor cells required to produce tumors in untreated hamsters.

Summary. Specificity of the transplantation resistance phenomenon was tested by using only 10 tissue cultured polyoma, SV 40, and F. Sa. No. 3 B hamster tumor cells in sufficiently large numbers of adult hamsters to yield definitive results. Pre-treatment with polyoma virus regularly produced resistance to transplantation of polyoma tumor cells but not of F. Sa. No. 3 B tumor cells; there was evidence of some resistance to SV 40 tumor cells in only 2 of 4 tests. Pre-treatment with SV 40 virus regularly produced resistance to SV 40 tumor cells, although in some tests of a lower order than in the polyoma virus *vs* polyoma tumor system, but not to F. Sa. No. 3 B cells; there was evidence of some resistance to polyoma tumor cells in only 2 of 4 tests. Pretreatment with adenoviruses, types 12 and 7A, herpesvirus and poliovirus type 1 produced no resistance against any of the 3 tumors tested. Reovirus, type 3, and vesicular stomatitis virus (Indiana strain) propagated in mouse embryo tissue culture, the same cells in which the polyoma virus was grown, produced no resistance against the polyoma tumor cells. The progeny of 10 polyoma hamster tumor cells which formed large tumors in occasional, polyoma-immune adult hamsters, nevertheless, were shown to possess the distinctive, cellular antigen. A sensitive test failed to reveal any cytotoxic antibody in the serum of polyoma-immune hamsters that resisted transplantation of polyoma tumor cells.

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Effect of SV_{40} Virus Immunization on Growth of Transplantable SV_{40} and Polyoma Virus Tumors in Hamsters.* (28261)

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Specific antigens have been demonstrated in several tumors of viral origin(1-4). The recognition of such antigens is based on the relative resistance of animals immunized with a tumor virus or with homologous tumor cells to the transplantation of cells from tumors induced by the same virus. Present evidence indicates that these antigens are cellular, not viral(5,6).

It would be of obvious importance to determine whether a similar phenomenon could be demonstrated with other tumor viruses such as SV_{40}. In addition, since SV_{40} and polyoma virus share many properties in common(7) including their deoxyribonucleic acid

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core, their similar size and structure, and their ability to induce sarcoma in Syrian hamsters, it would also be of interest to determine whether cross immunity can be demonstrated against the tumors induced by these 2 viruses. The experiments reported here were designed to answer these questions.

Method and materials. Viruses. The polyoma virus used for immunization was prepared in mouse embryo cultures and had a titer of 10^7 PFU/ml; it was the third passage in mouse embryo fibroblast of a seed (M-11-9a) obtained from Dr. E. A. Mirand (Roswell Park Memorial Inst., Buffalo, N. Y.). The SV_{40} virus was from a pool prepared in African green monkey cells with a titer of 5×10^5 $TCID_{50}$/ml.

Tumors. The polyoma tumor (*"A"*) was a transplantable sarcoma derived from a subcutaneous tumor harvested from a 40-day-old hamster inoculated at birth with polyoma virus. It was at the 54th and 57th transplantation passage, respectively, in the 2 experiments reported here. No polyoma virus could be isolated from this anaplastic tumor after its second passage, nor did polyoma hemagglutination inhibiting antibodies (H.I.) appear in any of the hamsters in which it had been serially transplanted since its derivation almost 3 years ago. The SV_{40} tumor (*#12*) was received from Dr. B. E. Eddy (Nat. Inst. Health, Bethesda, Md.) and was derived from a tumor that originated in 1960 in a hamster after inoculation of *Lot 12* SV_{40} virus(8). The 43rd and 46th passages were used in these experiments. This tumor is also a virus-free sarcoma as indicated by testing tumor cell extracts on African green monkey cell cultures in this laboratory.

Adult hamsters,† 4-5 months old, were immunized with a single subcutaneous injection of 1 ml of the virus pool in the left thigh and challenged with the tumor inoculum 35-45 days later. The subcutaneous, rather than intraperitoneal, inoculation of the virus was selected since, after intraperitoneal inoculation of polyoma virus, some hamsters die because of rupture of liver hemangiomata(9). Animals inoculated with polyoma virus were tested at the time of tumor challenge for the presence of polyoma H.I. antibodies; titers of the sera ranged between 1:250 to 1:1024 with a median of 1:512. The tumor to be used for the challenge was removed from the host animal, trypsinized and maintained *in vitro* in Eagle's medium supplemented with 10% calf serum for 2 or 3 days prior to animal inoculation. This procedure helped to eliminate dead cells and necrotic material. The cells were harvested, viable cells were counted (with 0.25% trypan blue) and appropriate dilutions were made in phosphate buffered saline; 0.5 ml of the cell suspension was inoculated into each animal in the right laterodorsal nuchal region. The animals were palpated weekly and the size of the tumors were recorded. The hamsters were divided into the following groups:

Group I: Non-immunized animals inoculated with polyoma tumor cells. Group II: Non-immunized animals inoculated with SV_{40} tumor cells. Group III: Animals immunized with polyoma virus and challenged with polyoma tumor cells. Group IV: Animals immunized with polyoma virus and challenged with SV_{40} tumor cells. Group V: Animals immunized with SV_{40} virus and challenged with SV_{40} tumor cells. Group VI: Animals immunized with SV_{40} virus and challenged with polyoma tumor cells.

Results. The results of 2 experiments are reported in Table I, where the first line in each group indicates the results of the first experiment and the second line, the results of the second experiment. Results were similar in both experiments and will be considered together.

Groups I and II—Control animals. All animals inoculated with either the SV_{40} or the polyoma induced tumor cells developed tumors, with the exception of 3 animals that received 10^3 cells of the polyoma tumor. The SV_{40} tumor appeared to be more malignant than the polyoma tumor since tumors developed in 7/7 animals inoculated with 10^2 cells. The median time for the tumors to become palpable (0.2-0.3 cm) was fairly similar in the 2 groups: 10 days with 10^5, 14 days with 10^4, 18 days with 10^3 and 23 days with 10^2 cells.

† Lakeview Hamster Farm, Neufield, N. J.

TABLE I. Effect of SV_{40} and Polyoma Virus Immunization on Subcutaneous Transplantation of SV_{40} and Polyoma Virus Induced Tumors.*

Challenge	Immunization	Cell dosage 10^2	10^3	10^4	10^5
Polyoma tumor	None		8/10	10/10	10/10
			6/7	7/7	6/6
	Polyoma virus		0/9	8/10	10/10
			2/7	5/5	6/6
	SV_{40} virus		9/10	10/10	10/10
			7/7	7/7	6/6
SV_{40} tumor	None	—	7/7	7/7	6/6
		7/7	7/7	6/6	—
	Polyoma virus	—	7/7	7/7	6/6
		6/7	7/7	6/6	—
	SV_{40}	—	3/5	6/7	5/6
		1/7	6/7	6/6	—

* Three- to 4-mo-old animals, challenged 40-45 days after virus inoculation. The polyoma tumor was at the 54th passage in first experiment and at the 57th in second experiment. The SV_{40} tumor was at the 43rd and 46th respectively.

Groups III and V—Immunized animals challenged with homologous‡ tumors. Using 10^5 cells, there were no differences in incidence among the various groups; all animals developed tumors, and though a slight delay was observed in the time of appearance compared to the control groups, it was not considered significant. At lower cell dosage it became quite apparent that hamsters immunized with polyoma or SV_{40} virus showed some degree of protection against challenge with the corresponding tumor cells. The protection appeared complete at dosages of 10^2 and 10^3 cells in which many animals (23 out of 35) did not show any tumor even after a period of observation of 3 months, while in the control or hetero-immunized groups, all but 5 of the 76 animals developed tumors. The frequency of complete protection was similar in the polyoma and SV_{40} immunized groups and of the same order as that observed with other polyoma tumors or with cells transformed by polyoma virus *in vitro* (10). A degree of protection was also observed in homo-immunized animals challenged with 10^4 cells in that the appearance of tumors was delayed and the size smaller in comparison to the control and hetero-immunized groups. The median time for the appearance of tumors with 10^4 cells was 19 days compared to the control median of 14 days. Once tumors appeared, their rate of growth was similar to the control or hetero-immunized groups. It was noticed that large tumors in these groups occasionally became necrotic with sloughing-off of large tumor masses and ulceration, but the same phenomenon was also observed in control animals, and it does not appear to have an immunological basis.

Groups IV and VI—Immunized animals challenged with heterologous tumors. The results in this group were identical to the control groups. Animals immunized with polyoma virus were just as susceptible to the challenge with SV_{40} tumor cells as the control and vice versa. Even delay of appearance of tumor could not be demonstrated in these groups. This is particularly evident from Fig. 1—an example from Exp. 1 reported in Table I—in which the rate of appearance of tumors in 6 groups of animals is recorded: no difference was found between polyoma immunized and control hamsters upon challenge with SV_{40} tumor cells.

Discussion. In these experiments it has been demonstrated that immunization of adult hamsters with SV_{40} virus induces resistance against the transplantation of a limited number of SV_{40} tumor cells, a phenomenon also

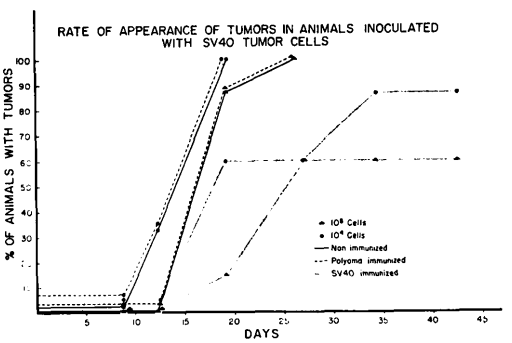

FIG. 1

‡ Homologous and heterologous are used in their etymological meaning, with no reference to intra or interspecies transplantation antigens. *Homologous tumor:* tumor induced by the same virus against which the animals were immunized. *Heterologous tumor:* tumor induced by a different virus against which the animals were immunized.

If tumor antigens are proved to be similar to histocompatibility antigens, then hamsters immunized with polyoma virus or SV_{40} virus could be indicated as $hamster^{PV}$ or $hamster^{SV40}$.

observed with polyoma virus immunized animals challenged with polyoma tumor cells. Furthermore, it has been shown that there is no cross-protection between polyoma virus immunization and SV_{40} tumor cells, and between SV_{40} virus immunization and polyoma tumor cells. Similar findings are reported by Habel and Eddy(11) and by Koch and Sabin(12).

The interpretation of these results is compatible with the original hypothesis(5) that immunization with a tumor virus induces the appearance of new specific antigen(s), characteristic for the tumor induced by that virus, and that small numbers of tumor cells inoculated into such immunized animals are rejected by a mechanism similar to a second set reaction. Data supporting such a hypothesis have also been obtained in experiments with thymectomized mice and hamsters(13). In this sense, these antigens could be considered as equivalent to weak histocompatibility antigens. The lack of cross immunization between SV_{40} and polyoma tumors also excludes the possibility that such antigens could represent a non-specific Forssman antigen that has been found to increase in polyoma-induced hamster kidney tumors(14). The degree of resistance against tumor cells conferred by the virus immunization appears to be limited in hamsters to a small number of challenging cells and effective only during the initial phase of cell implantation and early growth, as demonstrated by the fact that the rate of growth, once the tumors become palpable, is similar in control and immunized groups. In mice, the degree of protection conferred by virus immunization is much higher(1,2,10). Since the same phenomenon has been found to be present with hamster cells transformed *in vitro* by polyoma(10,15), it seems evident that when cells morphologically transformed *in vitro* by a tumor virus are tested for transplantability *in vivo*, recipient test animals should not have been infected with that virus and/or the challenging dose should be high enough to overcome a possible resistance induced by a previous inadvertent virus infection.

The number of virus-induced tumors in which specific antigen(s) appear is now quite large; their specificity characterizes these tumors as a distinct group from chemically induced tumors(16). Indeed, in some cases such as polyoma and SV_{40} tumors from which infectious virus cannot be recovered, the presence of such antigen(s) may be the only vestige of their viral origin. The antigen(s) appears to be an essential constituent of the tumor cells since it can still be demonstrated even after numerous passages of the tumor *in vivo* (up to 3 years) under conditions in which selection pressure by the host should favor loss of the antigen(s). On the other hand, there is some evidence that virus immunization is more effective against recently transformed cells *in vitro*(15) or against primary tumors(10).

Final conclusions as to the nature of such antigen(s) and as to their relevance in terms of the tumor virus-cell relationship must be suspended until these antigens can be isolated and identified in an *in vitro* system.

Summary. Adult hamsters immunized with SV_{40} or polyoma virus were challenged with graded doses of virus-free polyoma or SV_{40} tumor cells. Animals immunized with SV_{40} virus were resistant to a small inoculum of SV_{40} tumor cells, and animals immunized with polyoma virus were resistant to a small inoculum of polyoma tumor cells. There was no cross protection between polyoma virus immunization and SV_{40} tumor cells or between SV_{40} virus immunization and polyoma tumor cells, even at the minimum cell dose required to give tumors in all the animals.

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Absorption of $Sr^{90}SO_4$ from the Lung.* (28262)

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In experiments with mice it was found that Sr^{90} appeared in the skeleton soon after inhalation of $Sr^{90}SO_4$ aerosols(1). Since $SrSO_4$ is not very soluble in water, rapid transport of Sr across the alveolar membrane was not expected. Therefore, it was speculated that $Sr^{90}SO_4$ was largely solubilized in the acid environment of the gastrointestinal tract(2) which receives a large fraction of inhaled material cleared from the respiratory tract, and that this accounted for the rapid accumulation in the skeleton. To test this, it was necessary to evaluate the role of the lung in affecting the solubilization and translocation of $Sr^{90}SO_4$.

Methods. In a female dog, weighing about 25 kg, the trachea and lower respiratory tract were isolated from the gastrointestinal tract by surgically preparing a ventrocervical tracheal fistula. The laryngeal stump of the trachea was closed with sutures and the distal stump leading to the lung was sutured to the underlying muscle and tissues to assure a patent lumen regardless of the posture of the animal. After allowing one week for post operative healing the dog was allowed to inhale a $Sr^{90}SO_4$ aerosol through the fistula for one hour. A total of 8.3 μc Sr-Y^{90} was deposited. Blood samples were withdrawn *via* an intravenous catheter introduced through the jugular vein to the base of the heart. All feces and urine were collected daily, and all tissue taken upon sacrifice of the animal 7 days post exposure. Gauze pads were held in place over the fistula to filter inhaled air and to collect mucus cleared from the respiratory tract. The gauze pads were changed frequently and were analyzed for Sr-Y^{90}. The dog received 24 hour professional care, water *ad libitum,* and a normal diet of commercial dog food. The dog was sacrificed by exsanguination at the common carotid artery while under pentothal sodium anesthesia.

Results. The levels of Sr-Y^{90} in one ml of blood are plotted in Fig. 1 as per cent of the total Sr-Y^{90} deposited. Immediately after exposure about 0.006% of the Sr-Y^{90} deposited was present in 1 ml of blood, or 12% in all of the blood, assuming a total blood volume of 2 liters. From the 3rd to the 24th hour after exposure the radioactivity content of the blood decreased exponentially and was maintained from one day to one week after exposure at about 0.0004% per ml of blood. This probably suggests that an equilibrium between blood and tissue content of Sr-Y^{90} was reached and maintained after the first day.

The daily clearance of Sr-Y^{90} from the trachea and the excretion in urine and feces are also shown in Fig. 1. The Sr-Y^{90} collected on the gauze pads was probably cleared from the lung by mucus-ciliary processes. Four per cent of the total Sr-Y^{90} deposited was eliminated in this manner on each of the

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