evidence of etiological relationship than is the isolation of the original inoculated virus from the tumor, since it is well known that tumors offer good sites of multiplication of unrelated viruses. Whether superinfection of one tumor by another tumor virus would induce the cellular antigen characteristic of the superinfecting virus and therefore give false evidence of an etiological relationship in the resistance type of test has not been investigated.

Evidence similar to that reported here for the presence of a new cellular antigen in SV 40 induced tumors and the specificity of SV 40 and polyoma hamster tumors has been found by Defendi(12) and Koch and Sabin (13) and is reported in this issue of the Proceedings.

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Received February 7, 1963. P.S.E.B.M., 1963, v113.

Specificity of Virus-Induced Resistance to Transplantation of Polyoma and SV 40 Tumors in Adult Hamsters.* (28260)

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Habel(1,2) and Sjögren *et al.*(3,4) independently demonstrated that a previous inoculation of adult mice with polyoma virus grown in mouse embryo tissue cultures induced a resistance to transplantation of isologous mouse tumors induced by polyoma virus but not of spontaneous or carcinogen-induced isologous tumors. Various experiments by both Habel and Sjögren led to the conclusion that this resistance was due to the presence in the mouse polyoma tumor cells of specific cellular antigen(s) that resulted from infection of the cells with polyoma virus but was unrelated to the viral antigens. Moreover, since the polyoma tumor antigen(s) was present in tumors in which neither polyoma virus antigens nor infectivity were demonstrable by the techniques employed, it appeared that the tumor specific antigen was a consequence of a genetic modification of cells in which the genome of the tumor-inducing virus was no longer demonstrable. For this reason it appeared highly desirable to carry out additional studies on the specificity of this tumor antigen to determine especially whether other DNA or RNA viruses multiplying in mouse embryo tissue cultures or in the living host would or would not give rise to a cellular antigenic modification that might also produce resistance to transplantation of polyoma virus induced tumor cells.

Most of the resistance tests in mice have been carried out with 10^4 or more tumor cells, because smaller numbers either produced no tumors or did so irregularly. The number of cells used for challenge was critical in demonstrating the resistance phenomenon since with

* This investigation was supported by research grant No. C-4557 from the National Cancer Institute, Public Health Service.

10^6 cells most or all of the virus pre-treated mice also developed tumors. When Habel (1,2) performed similar tests in a few hamsters with comparable numbers of hamster tumor cells of polyoma virus origin, the resistance was demonstrable only by a difference in the size of tumors at a given time after transplantation. During the course of our work on the possible presence of a "malignant transforming principle" in hamster polyoma tumors, presumably free of residual viral genome, we found that 1 to 10 tissue cultured tumor cells were tumorigenic in adult hamsters. Accordingly it was of interest to determine whether the phenomenon of transplantation resistance could be demonstrated more definitively in hamsters by the use of small numbers of cells for challenge, and also whether such tests on adult hamsters after infection with other viruses would throw further light on the specificity of this important phenomenon.

Materials and methods. Female, Syrian hamsters weighing about 70 g were obtained from a local breeder who stated that his stock was derived from a single pair by random breeding. They were kept in individual cages. *Tumors.* The *polyoma* hamster fibrosarcoma was obtained from Dr. Karl Habel in its 23rd transplant passage and was reported to be free of demonstrable virus. The tumors used in the present experiments were from the 49th to the 74th transplant passages. Sera from untreated hamsters that developed tumors after inoculation of 2 or 10 cells had no polyoma hemagglutination inhibition antibodies (HAI) in a 1:8 dilution, while the sera of hamsters receiving the single inoculation of polyoma virus had HAI titers of 256 to >4,096. The *SV 40* hamster fibrosarcoma was obtained from Dr. Bernice Eddy in its 16th transplant passage and in the present experiments was used in the 20th to the 28th passages. Undiluted sera from hamsters bearing these tumors had no demonstrable neutralizing antibodies for SV 40 virus by the plaque reduction test. As a control for these virus-induced hamster sarcomas we obtained from Dr. J. G. Fortner a hamster fibrosarcoma (F. Sa. No. 3 B) that appeared at the site of multiple injections of sodium cholate and had undergone 102 transplant passages. The tumors used in the present experiments were from the 111th to the 130th transplant passages. All tumors were maintained in this laboratory by transplantation at approximately 2-week intervals by subcutaneous inoculation of 0.5 ml of a 20% suspension of minced, grossly non-necrotic tumor tissue. *Preparation of tumor cells for challenge.* About 2 g of minced, grossly non-necrotic tumor tissues were treated with 0.2% trypsin, and 40 ml of a 1:200 suspension of the washed cells in growth medium were planted in 32-ounce prescription bottles. Eagle's minimal essential medium (MEM) + 10% calf serum was used for the polyoma and F. Sa. No. 3 B tumor cells, and the same medium + 30% calf serum for the SV 40 tumor cells. The polyoma and F. Sa. No. 3 B tumor cells were harvested by trypsinization 2 to 4 days after planting when a confluent cell sheet was present, while the SV 40 tumor cells were harvested 1 day after planting when a loose network of spindle-shaped cells was present. The suspended cells were counted in a hemocytometer. Approximately 95% of the cells were viable by the trypan blue test. The cells were diluted in MEM + 10% calf serum to contain the desired number of cells in 0.1 ml. To prevent adhesion to the glass the cells were inoculated immediately after preparation, the suspension being shaken every 5 minutes during the period required for the inoculation of large numbers of animals. *Viruses used.* The *polyoma virus* (strain 7-2) was obtained from Dr. Karl Habel and was propagated in this laboratory in mouse embryo tissue culture (METC) in MEM + 5% horse serum. The *SV 40 virus* (strain 777) was obtained from Dr. Paul Gerber (NIH). It was propagated in this laboratory in the stable line of cercopithecus kidney cells (BS-C-1) in medium 199 + 2% calf serum and titrations were carried out in the same cells, the final titers being based on readings obtained 40 days after inoculation. The *reovirus, type 3* (Dearing strain) was isolated in this laboratory, and after 7 passages in primary cynomolgus kidney tissue cultures was passaged once in METC in MEM + 5% horse serum. The titer of this virus in METC

was $10^{6.2}$ TCD_{50}/ml. The *vesicular stomatitis virus* (Indiana strain) was obtained from Dr. Werner Henle and passaged in METC in MEM + 5% horse serum; it had a titer of $10^{7.5}$ TCD_{50}/ml in METC. *Adenovirus, type 12* (Huie strain), originally isolated by Dr. S. Kibrick in human kidney and passaged 2 or more times in monkey kidney, had undergone 20 passages in HeLa or KB cells at the National Institutes of Health. *Adenovirus, type 7A* (strain S-1058), originally isolated by Dr. Alexis Shelokov from a throat swab, had undergone 9 passages in HeLa or KB cells at the National Institutes of Health. Both adenoviruses were supplied by Dr. R. M. Chanock. They were propagated and titrated in this laboratory in H. Ep. 2 cells in Eagle's basal medium (BME) + 5% chicken serum. Adenovirus, type 12 had a titer of $10^{5.7}$ TCD_{50}/ml and adenovirus type 7A had a titer of $10^{7.3}$ TCD_{50}/ml. The *herpesvirus* (Schooler strain) was isolated and passaged in this laboratory in primary, young rabbit kidney cultures. For this work it was passaged once in H. Ep. 2 cells in BME + 5% chicken serum, and yielded a titer of $10^{7.9}$ TCD_{50}/ml in H. Ep. 2 cells. The *poliovirus, type 1* (LSc, 2ab strain) in commercially produced, SV 40-free, oral vaccine was passaged once in H. Ep. 2 cells in BME + 5% chicken serum, and yielded a titer of $10^{7.3}$ TCD_{50}/ml in these cells. Each time a lot of virus was prepared an aliquot of the same uninoculated tissue cultures was incubated until the virus was harvested; the uninoculated tissue cultures were then frozen and thawed 3× and the centrifuged fluids were used as a control in the same experiments in which the centrifuged virus suspension was used. *Transplantation resistance tests.* All viruses and the corresponding control tissue cultures were inoculated undiluted in a dose of 0.5 ml intra-abdominally, except adenovirus, type 12 of which 1.0 ml was inoculated because the titer was low. After intervals indicated in the Tables, the hamsters were inoculated with the tumor cells. The hamsters were palpated weekly for the appearance of tumors which were measured when present.

TABLE I. Tumorigenic Capacity of Different Numbers of Cells from Polyoma, SV 40 and Sodium Cholate (Fortner F. Sa. No. 3 B) Hamster Tumors On Subcutaneous Inoculation in Random Inbred Adult Hamsters.

Tumor cells used	Treatment of hamsters	Incidence of tumors and day (in parentheses) on which maximum number had appeared in group receiving indicated number of cells							
		10^6	10^5	10^4	10^3	10^2	10^1	$10^{0.3}$	
Polyoma tissue culture	Cortisone*	—	—	5/5 (14)	5/5 (21)	9/10 (28)	7/9 (42)	10/17 (35)	
	None	—	—	—	—	—	15/20 (42)	4/8 (42)	
F. Sa. No. 3 B tissue culture	Cortisone	—	—	—	—	5/5 (24)	5/5 (38)	1/5 (31)	
	None	—	—	4/4 (14)	4/4 (21)	4/4 (21)	4/4 (28)	—	
SV 40—original tumor	None	2/2 (7)	4/4 (22)	4/4 (22)	10/10 (43)	6/10 (50)	2/10 (57)	3/10 (64)	
SV 40—tissue culture	None	—	—	—	10/10 (26)	9/10 (33)	7/9 (26)	4/9 (85)	

* Beginning on the day of inoculation of cells, 2 mg of cortisone were given subcutaneously twice a week for a period of 6 wk.

Results. Tumorigenic capacity of small numbers of cells. The data in Table I show that even 2 polyoma tumor cells produced progressively growing tumors in 50 to 60% of adult hamsters and the incidence and time of appearance of the tumors was the same in the untreated and cortisone-treated hamsters. With the F. Sa. No. 3 B tumor cells there was no significant difference in the tumorigenic capacity in untreated and cortisone-treated hamsters, both as regards numbers of cells required and time of appearance. With small numbers of SV 40 tumor cells more tumors were produced with cells after a single passage in tissue culture than with trypsinized cells from the original tumor, and the tumors also appeared earlier. Based on these data it was decided to use 10 cells from the first passage in tissue culture in adequate numbers of hamsters as the standard challenge test for resistance.

Transplantation resistance induced by polyoma and SV 40 viruses. The results of individual tests are given in Table II chiefly because the incidence of polyoma tumors in untreated controls varied over too wide a range in the later tests and also to show the reproducibility of the data. The 197 hamsters that were pre-treated with polyoma virus showed distinct transplantation resistance in all 7 tests with 10 polyoma tumor cells, and also in single tests with 2 cells and 100 cells. The resistance manifested itself either by complete prevention of tumor formation or by a decrease in incidence to a maximum of 10%. The incidence of polyoma tumors among the 119 hamsters that were pretreated with an aliquot of the same mouse embryo tissue culture cells (METC) in which the polyoma virus was grown, was always slightly lower than in the 136 untreated hamsters but the difference was not statistically significant. Tests for HAI polyoma antibody in the sera of 48 hamsters pre-treated with METC were all negative (<8). Moreover, while there was no difference between the average largest diameter of the tumors at 6 weeks in the untreated (46 mm) and the METC pretreated (44 mm) hamsters, it was distinctly less (26 mm) in the 7 measurable tumors which appeared in the polyoma virus pre-treated hamsters. Pre-treatment with polyoma virus had no effect on the incidence or size of tumors produced by 10 F. Sa. No. 3 B hamster tumor cells; 100% of the 38 polyoma virus pre-treated hamsters developed these tumors as compared with 90% of 40 untreated hamsters and 94% of 47 pre-treated with METC.

Pre-treatment of adult hamsters with SV 40 virus also produced distinct resistance to transplantation of 10 SV 40 tumor cells, which in 5 different tests was evident by a variable reduction in the incidence of tumors that was usually less marked than that observed in the polyoma virus *vs* the polyoma tumor system. In 76 untreated hamsters the incidence of tumors after inoculation of 10 SV 40 tumor cells was 76% ($\pm$ 14%); in 32 hamsters pre-treated with the cercopithecus BS-C-1 tissue culture cells in which the SV 40 virus was grown, it was 81% ($\pm$ 12%), while in the 120 hamsters pre-treated with SV 40 virus the incidence of tumors in different tests varied from 7% to 55% (average 33%) without reference to the interval between pre-treatment and challenge. Moreover, there was little, if any difference in the average of the largest diameters of the tumors at 6 weeks after transplantation among the untreated hamsters (29 mm), the BS-C-1 pre-treated (35 mm) and SV 40 virus pre-treated hamsters (27 mm). In one of 2 tests in which 15 hamsters were pre-treated with METC the incidence of SV 40 tumors was the same as in the untreated controls (87%), while in the second test with 16 hamsters it was 37% as compared with 62% in the untreated controls; the average largest diameter of the tumors at 6 weeks was 34 mm. Pre-treatment of hamsters with SV 40 virus had no effect on the incidence or size of tumors produced by 10 F. Sa. No. 3 B hamster tumor cells; 100% of the 15 SV 40 virus pre-treated hamsters developed these tumors as compared with 85% of 13 untreated, 87% of 15 pre-treated with BS-C-1 tissue culture, and 93% of 15 pre-treated with METC.

The tests for a possible relationship between the antigens in the polyoma and SV 40 tumor cells yielded different results in different experiments, without reference to the interval between pre-treatment and challenge

TABLE II. Effect of Pre-Treatment of Adult Hamsters with Polyoma Virus, SV 40 Virus, And Corresponding Uninoculated Tissue Cultures on Transplantation of Small Numbers of Cells from Tissue Cultures of Polyoma, SV 40, or Sodium Cholate (F. Sa. No. 3 B) Hamster Tumors.

Tumor cells used for challenge	Date of test	Interval,* days	No. of tumor cells	% of hamsters with tumors 8 wk after challenge in group pre-treated with: None	Polyoma virus	SV 40 virus	METC†	BS-C-1‡
Polyoma	9-21-61§	38	2	50 (8) ‖	0 (10)	—	33 (9)	—
	"	"	10	75 (20)	10 (20)	—	47 (19)	—
	11-29-61§	54	10	89 (19)	7 (15)	—	77 (13)	—
	1-16-62	43	10	50 (10)	7 (30)	—	49 (41)	—
	5-14-62	19	10	53 (15)	7 (29)	20 (15)	33 (15)	67 (15)
	7-16-62	42	10	44 (16)	0 (16)	56 (16)	35 (17)	71 (17)
	"	"	100	80 (5)	0 (5)	75 (4)	60 (5)	100 (5)
	9-21-62	38	10	33 (15)	0 (14)	0 (15)	—	—
	10- 6-62	22	10	18 (28)	3 (29)	27 (30)	—	—
	"	44	10	" "	0 (29)	24 (29)		
SV 40	5-15-62	20	10	87 (15)	60 (15)	55 (29)	87 (15)	93 (15)
	7-17-62	43	10	62 (16)	56 (16)	35 (17)	37 (16)	70 (17)
	"	"	100	100 (5)	100 (4)	60 (5)	100 (5)	100 (5)
	9-21-62	38	10	80 (15)	50 (14)	7 (15)	—	—
	10- 5-62	21	10	77 (30)	79 (29)	13 (30)	—	—
	"	43	10	" "	87 (30)	45 (29)	—	—
Total for SV 40		20-43	10	76 (76)	74(104)	33(120)	61 (31)	81 (32)
Fibrosarcoma	11-29-61§	54	10	89 (19)	100 (14)	—	92 (12)	—
No. 3 B	1-16-62	43	10	100 (8)	100 (10)	—	95 (20)	—
(Fortner)	5-14-62	19	10	87 (8)	100 (9)	100 (10)	90 (10)	80 (10)
	7-16-62	42	10	80 (5)	100 (5)	100 (5)	100 (5)	100 (5)

* Interval between pre-treatment and challenge.

† METC = aliquot of mouse embryo tissue culture used for preparation of polyoma virus employed in same test.

‡ BS-C-1 = stable line of cercopithecus monkey kidney tissue culture—aliquot of 1st used for preparation of SV 40 virus used in test.

§ The hamsters in this test were observed for only 7 wk after challenge.

‖ Numbers in parentheses refer to number of hamsters in test.

Note: Polyoma virus—preparation A, containing $10^{7.6}$ TCD_{50}/0.5 ml was used in the tests of 9-21-61, 11-29-61, 5-14-62 and 5-15-62; preparation B containing $10^{7.0}$ TCD_{50}/0.5 ml was used in the test of 1-16-62; preparation C, containing $10^{6.7}$ TCD_{50}/0.5 ml was used in the tests of 7-16-62, 9-21-62, 10-5-62 and 10-6-62. SV 40 virus—preparation A, containing $10^{8.5}$ TCD_{50}/0.5 ml was used in the tests of 5-14-62, 5-15-62 and 7-16-62; preparation B, containing $10^{7.8}$ TCD_{50}/0.5 ml was used in the tests of 9-21-62, 10-5-62 and 10-6-62.

(Table II). Thus in the experiments of 5-14-62 (19-day interval) and 9-21-62 (38-day interval) there was evidence of significant transplantation resistance to polyoma tumor cells in hamsters pre-treated with SV 40 virus, while in the experiments of 7-16-62 and 10-6-62 the incidence of tumors in the SV 40 virus pre-treated hamsters was even slightly higher than in the untreated controls. Moreover, the average largest diameter at 6 weeks was not significantly different for the polyoma tumors which appeared in the SV 40 virus pre-treated hamsters (41 mm) and in the untreated controls (46 mm). In the combined experiments of 5-14-62 and 9-21-62, 23/45 (51%) of the hamsters, that were untreated or were pre-treated with the BS-C-1 tissue culture in which the SV 40 virus was grown, developed polyoma tumors compared with 3/30 (10%) of those pre-treated with SV 40 virus ($\chi^2 = 13.4$, $P = <0.001$). In the reverse system when pre-treatment with polyoma virus was tested for its effect on transplantation of SV 40 tumor cells there was again some indication of resistance in the tests of 5-15-62 and 9-21-62, when 16/29 (55%) of the hamsters pre-treated with polyoma virus developed SV 40 tumors as compared with 38/45 (84%) of the hamsters that were untreated or were pre-treated with

TABLE III. Effect of Pre-Treatment of Adult Hamsters with Different Viruses and Corresponding Uninoculated Tissue Cultures on Transplantation of 10 Cells from Tissue Cultures of Polyoma, SV 40, or Sodium Cholate (F. Sa. No. 3 B) Hamster Tumors.

Date of test	Interval*	Pre-treatment	% of hamsters with tumors 8 wk after challenge with indicated tumor cells		
			Polyoma	SV 40	F. Sa. No. 3 B
1-16-62	43	None	50 (10)‖	—	100 (8)
		METC†	49 (41)	—	95 (20)
		Reovirus 3 in METC	48 (23)	—	100 (10)
		Polyoma in METC	7 (30)	—	100 (10)
4- 5-62	54	None	50 (8)	—	—
		METC	75 (8)	—	—
		Vesicular stomatitis (Ind.) in METC	75 (8)	—	—
9-21-62	38-44	None	33 (15)	80 (15)	90 (10)
		Human epithelioma No. 2 (H.Ep. 2)	27 (15)	87 (15)	90 (10)
		Poliovirus I in H. Ep. 2	27 (15)	67 (15)	100 (8)
		Herpesvirus " "	20 (10)	70 (10)	100 (5)
		Adenovirus 12 " "	20 (30)	67 (30)	90 (10)
		" 7A " "	23 (30)	63 (30)	100 (10)
		Polyoma in METC	0 (14)	50 (14)	—
		SV 40 in BS-C-1‡	0 (15)	7 (15)	—

Legends same as in Table II.

the METC in which the polyoma virus was grown ($\chi^2 = 7.7$, $P = <0.01 > 0.001$). However, in 2 other experiments (7-17-62 and 10-5-62—Table II) pretreatment with polyoma virus had no effect on the transplantation of 10 SV 40 tumor cells.

Effect of pre-treatment with other RNA and DNA viruses on resistance to transplantation of different tumors. Reovirus, type 3 (an RNA virus) and vesicular stomatitis virus were tested chiefly because they grew and produced a cytopathogenic effect in METC, the tissue culture used for growing the polyoma virus, and thus could help to check on the possibility that the antigen(s) involved in the transplantation resistance phenomenon might arise in METC as a by-product of the growth of any virus. Since the vesicular stomatitis virus also multiplied in the intra-abdominally inoculated hamsters, as was evident from the fact that 12 of the 20 hamsters died with signs of encephalomyelitis, it served to indicate whether or not multiplication of other viruses in the tissues of adult hamsters may also produce some antigenic change that would result in increased resistance to transplantation of polyoma tumor cells. All hamsters inoculated with reovirus, type 3 remained well and the sera of 6 tested for HAI antibody all yielded significantly high titers of 256 to 512; while this antibody response might result from the original mass of antigen ($10^{5.9}$ TCD_{50}), the properties of this virus are such as to suggest the possibility of inapparent infection in adult hamsters. The data in Table III show that neither of these viruses produced any resistance to transplantation of polyoma tumor cells.

When Trentin *et al.*(5) reported that adenovirus, type 12 produced tumors in newborn hamsters and that adenovirus, type 7A did not, it became of interest to determine the effect that these DNA viruses would have on the transplantation of polyoma, SV 40 and F. Sa. No. 3 B tumor cells. Since the adenoviruses were grown in H. Ep. 2 cultures, the tests were controlled not only with the uninoculated H. Ep. 2 cultures but also with another DNA virus (herpesvirus) and an RNA virus (poliovirus) grown in these cells. It is noteworthy that the herpesvirus infected the hamsters producing a fatal encephalomyelitis in 15 of the 40 intra-abdominally inoculated animals. The results shown in Table III indicate that none of these viruses produced any significant resistance to transplantation of either the virus-induced polyoma and SV 40 tumors or the chemically-induced (spontaneous?) F. Sa. No. 3 B tumor. In the same test both the polyoma and SV 40 viruses produced good resistance against their corresponding tumors, as well as some suggestive,

TABLE IV. Evidence that Progeny of Hamster Polyoma Tumor Cells Which Grew Progressively in Polyoma Virus Treated and Immune Adult Hamsters Possess the Distinctive Polyoma-Induced Cellular Antigen.

History of tumor used for challenge	% of hamsters with tumors 8 wk after challenge with 10 cells of indicated origin in	
	Controls*	Polyoma-treated†
Derived from 10 cells in untreated hamster in test of 1-16-62	39 (13)	0 (13)
Derived from 10 cells in polyoma virus pre-treated and immune hamster in test of 1-16-62	46 (13)	0 (12)

* These hamsters had previously received either mouse embryonic tissue culture or Reovirus 3 in mouse embryonic tissue culture and failed to develop tumors after implantation of 10 polyoma hamster tumor cells in the test of 1-16-62.

† These hamsters had previously received polyoma virus and failed to develop tumors after implantation of 10 polyoma hamster tumor cells in the test of 1-16-62.

though not statistically significant, cross resistance against each other.

Antigen in polyoma tumor cells which produce tumors in polyoma immune hamsters. It was first of all established that the small number of polyoma virus pre-treated hamsters, in which 10 polyoma cells were able to produce progressively growing tumors, had developed HAI polyoma antibodies in titers comparable to those in the hamsters which exhibited resistance. To determine whether the polyoma tumors which developed in the polyoma virus pre-treated hamsters may be the result of a selection of cells that are devoid of the distinctive tumor antigen(s), a large tumor (45 × 35 × 30 mm) was removed 43 days after transplantation of 10 cells in a polyoma immune hamster, and a 20% suspension of its cells was transplanted into untreated hamsters. Twelve days later the resulting tumors were used for initiating the tissue culture which supplied the cells for the experiment shown in Table IV. A large tumor from an untreated hamster in the same test was similarly passaged to supply the control cells for the experiment shown in Table IV. The results indicate that no selection of antigen-free tumor cells had occurred.

Absence of cytotoxic activity in sera of polyoma-immune resistant hamsters. Habel (1) tested for cytotoxic activity of sera from polyoma immune mice and hamsters that exhibited resistance to polyoma tumors by adding such sera, with or without guinea pig complement, to fully grown tube cultures of the tumor cells and observing for clumping or lysis of the cells on incubation. He found no cytotoxic activity. It seemed desirable to use a more sensitive procedure, to obtain an answer to this very important question. The serum pool tested for cytotoxic activity was derived from 19 hamsters, pre-treated with polyoma virus and resistant to challenge with 10 polyoma tumor cells. Trypsinized tumor cells were washed and diluted in phosphate buffered saline to contain 20,000-2,000-200 or 20 cells per 0.1 ml. The various cell suspensions were mixed with an equal volume of normal or tumor resistant hamster serum diluted 1:5 in phosphate buffered saline containing guinea pig complement in 1:8 dilution. After incubation at 37°C for 30 min, 0.1 ml of each mixture was inoculated into each cheek pouch of two hamsters. The hamsters were given 2 mg of cortisone subcutaneously twice a week for 3 weeks, beginning with the day of inoculation of the tumor cells. The results (Table V), failed to reveal any evidence of cytotoxic activity. It is noteworthy that the same mixtures that were inoculated in the hamsters were also examined by the trypan blue technique without finding any difference in the number of stained cells in the various mixtures.

Discussion. The results of the present study provided additional evidence for the specificity of a distinctive antigen or antigens in the progeny of tumor cells originally induced by viruses. The use of only 10 tumor cells in the test for transplantation resistance provided a sensitive measure of this phenomenon, and yet other viruses with infective capacities in hamsters failed to produce resis-

TABLE V. Absence of Cytotoxic Activity in Sera of Polyoma Virus Pre-Treated Adult Hamsters Which Resisted Implantation of Small Numbers of Hamster Polyoma Tumor Cells.

Tumor cells tested	Cells incubated with	No. of cheek pouches with tumors at 21 days after inoculation with indicated No. of cells			
		10,000	1,000	100	10
Hamster polyoma	Phosphate buffered saline	—	4/4 (20)*	4/4 (14)	2/4 (4)
	Normal hamster serum + complement	4/4 (25)	4/4 (18)	4/4 (17)	4/4 (13)
	Polyoma tumor resistant hamster serum + complement	4/4 (22)	4/4 (26)	2/2 (13)	4/4 (19)
Hamster F.Sa. No. 3 B	Phosphate buffered saline	—	—	3/4 (4)	0/4
	Normal hamster serum + complement	—	—	2/2 (8)	2/4 (6)
	Polyoma tumor resistant hamster serum + complement	—	—	4/4 (12)	1/2 (4)

* Figures in parentheses refer to largest avg diameter of tumors in mm.

tance against the polyoma or SV 40 tumors, while the polyoma and SV 40 viruses regularly produced resistance against their corresponding tumor cells but not against a hamster fibrosarcoma of either spontaneous or sodium cholate origin(6). On the other hand, we cannot now say with certainty that there is no relationship between the antigens in the polyoma and SV 40 tumor cells, because in 4 tests with polyoma virus *vs* SV 40 tumor cells and in 4 tests with SV 40 virus *vs* polyoma tumor cells, the combined results of 2 tests in each case gave statistically significant evidence for a cross relationship, while in the other 2 tests there was no relationship. When the results of all tests are combined there is no evidence for a common antigen in the polyoma-SV 40 systems. Klein *et al.*(7) also reported that while Gross lymphoma tumors contained a distinctive antigen, it apparently was unrelated to that contained in polyoma tumor cells. Thus it now seems quite clear that in the transplantation resistance phenomenon we now have a tool whereby a "fingerprint" of the virus that originally produced the tumor can be identified, even though *infective* virus may no longer be demonstrable. Although Habel(2) concluded that the distinctive, cellular antigen produced by polyoma virus in mice is different from that produced by the same virus in hamsters, more definitive and quantitative experiments are needed to establish this important point.†

Our failure to demonstrate cytotoxic activity in the sera of polyoma virus pre-treated, tumor-resistant hamsters is to be contrasted with the recent report of Slettenmark and Klein(8) in which a comparable procedure clearly showed the presence of specific cytotoxic antibody for Gross lymphoma cells in the serum of mice pre-treated with these but not other tumor cells.

Our demonstration that the progeny of 10 polyoma hamster tumor cells which formed large tumors in occasional, polyoma-immune adult hamsters nevertheless possessed the distinctive polyoma-virus-induced, cellular antigen is consistent with the unpublished results of Sjögren (quoted by G. Klein in his paper at the International Microbiological Congress, Montreal, August, 1962) who failed to obtain mouse polyoma tumor cells free of the distinctive antigen by protracted passage in pre-treated polyoma resistant mice.

The recent report of Atanasiu *et al.*(9) that immunization of newborn hamsters with single doses of polyoma virus *shortly after birth* rendered 23/31 animals completely resistant at 120 to 535 days of age to the transplantation of 3×10^5-2×10^6 polyoma tumor cells that had been passaged in tissue cul-

† Further studies, reported by Habel (*Federation Proc.*, 1963, v22, 438) after this paper was submitted for publication, indicated the presence of a common antigen in the tumors produced by polyoma virus in mice and hamsters.

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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Received February 7, 1963. P.S.E.B.M., 1963, v113.

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

* This work was supported in part by grants from Am. Cancer Soc., and Nat. Cancer Inst., USPHS. hamster^{PV} or hamster^{SV40}.

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