with respect to the capacitance corresponding to the initial uniform concentration in the cell.

We have solved numerically the differential equation of diffusion for an initial exponential concentration gradient and have obtained solutions for the decay of the capacitance in terms of only one adjustable parameter, the average diffusion constant. The solutions agree with those obtained by MacCallum (*5*) using other methods for the same problem. The fit of the theoretical curve to the results at two temperatures is shown in Fig. 1. The corresponding diffusion constants (D), for five temperatures, are shown in Fig. 2; from replicate experiments we estimate their precision to be about ± 5 percent.

At 25°C we find D to be 2.0×10^{-7} cm²/sec. By classical methods, Tsvetkov *et al.* (*6*) found 2.2×10^{-7} cm²/sec for PBLG of molecular weight 114,000 in dimethylformamide at 21°C, and Spach *et al.* (*7*) found 2.7×10^{-7} cm²/sec for PBLG of molecular weight 135,000 in dimethylformamide (*8*) at 20°C. Since the viscosity, η_0, of dimethylformamide at 20°C, 0.84 centipoise, is almost identical to that of EDC (*9*), 0.83 centipoise, the agreement of our results with the data of the other two groups appears satisfactory.

Evidence that PBLG behaves as a hydrodynamic particle in solution is shown in Fig. 2. Although both D and η_0/T vary by almost a factor of 2 over the temperature range of measurements, their product is constant to within 10 percent, characteristic of a Stokes' law dependence.

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Immunological Studies on Urinary Bladder Tumors of Rats and Mice

Abstract. *Human neoplasms derived from the same tissue have been previously shown to have tumor associated antigens characterizing that tissue type. Evidence is now presented for the existence of analogous antigens common to both rat bladder papillomas and carcinomas, and for antigens common to mouse bladder carcinomas. Rats immunized with syngeneic urinary bladder papillomas, then challenged with a methylcholanthrene pellet inserted into the bladder, develop (4 to 6 months later) fewer primary bladder tumors than rats immunized with normal bladder tissue.*

The study, both by transplantation techniques in vivo and by tests in vitro, of antigens associated with chemically induced tumors suggests that such tumors have individually unique antigens that do not usually cross-react with other tumors induced by the same chemical (*1*). Tumor antigens associated with virus induced tumors, in contrast, are highly cross-reactive if two tumors are associated with the same virus. Tumor antigens associated with different viruses do not cross-react (*1*). Human tumors behave still otherwise; cross-reacting tumor associated antigens have been demonstrated for neoplasms from the same histological type of tissue; such antigens do not cross-react with antigen from neoplasms of different histological types (*2*). We refer to such antigens as tissue type specific (TTS) antigens.

We now report (i) that there are cross-reacting tumor associated antigens in methylcholanthrene induced bladder carcinomas of mice and in methyl-

Table 1. Effect of lymph node cells from mice sensitized against (or bearing) urinary bladder carcinomas, the same or different from the target tumors; C, carcinoma; S, sarcoma.

Target cells	Lymphocytes	No. of tumor cells per well (mean ± S.E.)	No. of wells	Reduction (%)	*P*
		Experiment 1			
5102 (C)	Normal	79.6 ± 4.6	32		
	5102 (C)	42.6 ± 3.7	16	46.5	<.001
	5591 (C)	46.7 ± 4.7	16	41.3	<.001
	5664 (S)	89.2 ± 7.2	24	−12.1	*
		Experiment 2			
5591 (C)	Normal	46.8 ± 3.0	32		
	5591 (C)	32.7 ± 3.3	16	42.4	<.001
	5102 (C)	44.8 ± 4.5	16	21.2	<.01
	5664 (S)	59.4 ± 5.2	24	−26.9	<.01
		Experiment 3			
5102 (C)	Normal	34.9 ± 1.6	24		
	5102 (C)	25.5 ± 1.8	24	26.9	<.001
	5331 (C)†	35.3 ± 3.6	16	− 1.1	*
	5664 (S)	47.1 ± 2.1	24	−35.0	<.01
		Experiment 4			
5331 (C)	Normal	19.7 ± 1.3	24		
	5331 (C)†	16.1 ± 1.1	24	18.4†	<.01
	5102 (C)	9.9 ± 0.9	16	49.9	<.001
	5664 (S)	17.2 ± 1.0	24	12.7	*
		Experiment 5			
7922 (S)	Normal	36.8 ± 1.8	24		
	7922 (S)	23.1 ± 1.7	24	37.2	<.001
	5331 (C)	35.4 ± 2.2	24	3.8	*
		Experiment 6			
7922 (S)	Normal	35.4 ± 1.8	16		
	7922 (S)	23.0 ± 1.3	24	35.2	<.001
	9206 (C)	33.2 ± 2.5	16	6.4	*
Fibroblasts	Normal	46.8 ± 2.3	8		
	7922 (S)	45.6 ± 2.0	8	2.6	*
	9206(C)	53.8 ± 2.7	8	−15.0	*
		Experiment 7			
9206 (C)	Normal	34.6 ± 1.3	16		
	9206 (C)	24.3 ± 1.3	24	29.8	<.001
	7922 (S)	39.5 ± 1.9	16	−14.2	*
Fibroblasts	Normal	36.9 ± 3.1	8		
	9206 (C)	43.1 ± 1.7	8	−16.8	*
	7922 (S)	40.3 ± 2.6	8	− 9.2	*

* Not significant ($P > .05$). † Lymph node cells obtained from animals irradiated with 300 r before the tumors were transplanted.

cholanthrene induced bladder papillomas and carcinomas of rats, and (ii) that the development of bladder papillomas can be significantly delayed by immunizing rats with the TTS antigen-carrying tissue before the carcinogenic stimulus is applied.

All studies were performed on brother-sister mated BALB/c mice obtained from our own colony, or on inbred Fisher rats (Simonsen Laboratories). Tumors were induced in rats with a pellet preparation consisting of 20 percent methylcholanthrene in paraffin (*3*). A similar technique as modified for mice was used (*4*) with the same type of pellet. Each rat received one pellet (weighing 30 ± 2 mg) inserted into its bladder; each mouse received a pellet (12 ± 2 mg) placed into a subcutaneously transplanted bladder from a syngeneic mouse.

Most of the rat tumors were papillomas and were obtained by surgical excision from living animals. One transplantable rat carcinoma (4909) was obtained by seeding the peritoneal cavity of the animal bearing the primary tumor with minced papilloma. Subsequent intraperitoneal and retroperitoneal deposits were transitional carcinomas (histologically) and were readily transplantable.

Tissue cultures were established from the primary tumors in the case of papillomas, and from first- or second-generation transplants in the case of carcinomas. Material from a sarcoma (PW-13) induced in rats by polyoma virus (*5*) was obtained from H. O. Sjögren. In the mice both carcinomas and sarcomas were produced. After histological confirmation of the tumor type, tissue cultures were established from first- or second-generation transplants.

All experiments testing for lymphocyte mediated tumor immunity or presence of blocking factors (*6*) in the serum (which would interfere with the lymphocyte mediated tumor immunity) were done by in vitro microcytotoxicity test with Falcon 3040 Microtest plates (*7*). Tumor target cells were established as described above; lymphocytes were added to the tissue culture in a ratio of lymphocytes to target cells of 2000 to 1. Lymphocyte mediated tumor immunity was demonstrated if the lymphocytes added to the target cells resulted in the death of some of the target cells. "Blocking" activity of serum was evidenced by a decrease of detectable cell mediated immunity—interference by the serum with the killing of the target cells. Samples were coded for scoring.

Results of experiments in vitro aimed at detecting cell mediated immunity to mouse bladder carcinomas are shown in Table 1. The addition of lymph node cells (LNC) from mice sensitized against the respective carcinomas, in all five cases resulted in the killing of a significant number of the carcinoma target cells. In three of four cases with LNC from mice sensitized against a bladder carcinoma other than the target tissue, there was also a significant killing of the target cells. This then demonstrates the presence of cell mediated immunity against these tumor cells. Lymph node cells from mice sensitized against sarcomas (two different sarcomas were included in the experiments) were not cytotoxic to the bladder carcinoma cells, but did destroy

Table 2. Effect of blood lymphocytes from rats sensitized against (or bearing) urinary bladder papillomas or carcinomas, the same or different from the target tumors. Experiments 7 and 8 were performed with the same lymphocyte suspensions and serums that were used in a "criss-cross" pattern as material for the experimental groups and controls, respectively. The ability of serum from tumor-bearing rats (as compared to normal healthy rats) to block (abrogate) cell mediated tumor immunity was calculated; C, carcinoma; P, papilloma; PW-13, polyoma.

Target cells	Lympho-cytes	Serum	No. of tumor cells per well (mean ± S.E.)	Wells counted	Reduction (%)	*P*
			Experiment 1			
5204 (P)	Normal		63.8 ± 1.5	40		
	4909 (C)		41.5 ± 2.0	16	34.9	< .001
	4940 (P)		35.0 ± 1.5	32	45.1	< .001
			Experiment 2			
4909 (C)	Normal		40.5 ± 1.7	40		
	4909 (C)		34.9 ± 1.4	24	13.8	< .05
	7803 (P)		30.0 ± 1.9	24	25.8	< .001
			Experiment 3			
4909 (C)	Normal		23.1 ± 1.0	32	0	
	4909 (C)		15.6 ± 1.0	24	32.6	< .001
	7803 (P)		7.0 ± 0.6	24	69.6	< .001
			Experiment 4			
5215 (P)	Normal		28.8 ± 1.5	48	0	
	4909 (C)*		28.3 ± 1.2	32	0	†
	7803 (P)		13.8 ± 0.9	16	52.2	< .001
			Experiment 5			
8722 (P)	Normal		14.6 ± 1.0	24		
	8706 (P)		9.5 ± 0.9	24	34.9	< .001
	8713 (P)		10.0 ± 0.7	16	31.5	< .001
Fibroblasts	Normal		104.1 ± 7.3	8		
	8706 (P)		109.4 ± 6.9	12	− 5.1	†
	8713 (P)		114.3 ± 5.4	12	− 9.8	†
			Experiment 6			
8725 (P)	Normal		14.1 ± 0.8	24		
	8706 (P)		7.6 ± 0.6	20	46.1	< .001
	8713 (P)		8.2 ± 0.6	16	41.8	< .001
Fibroblasts	Normal		77.0 ± 7.0	8		
	8706 (P)		85.8 ± 6.1	12	−11.4	†
	8713 (P)		108.0 ± 5.6	12	−40.3	< .001
			Experiment 7			
PW-13	Normal	Normal	103.4 ± 3.6	8		
	6207 (C)	Normal	96.9 ± 6.7	8	6.3	†
	PW-13	Normal	76.5 ± 4.9	8	26.0	< .001
	Normal	4909 (C)	106.4 ± 3.0	8		
	6207 (C)	4909 (C)	96.3 ± 4.5	8	9.5	†
	PW-13	4909 (C)	71.8 ± 2.8	8	32.5	< .001
	Normal	PW-13	94.4 ± 4.2	8		
	6207 (C)	PW-13	87.8 ± 5.6	8	7.0	†
	PW-13	PW-13	90.0 ± 5.3	8	4.7‡	†
Fibroblasts	Normal		10.0 ± 1.6	8		
	6207 (C)		8.5 ± 1.1	8	15.0	†
	PW-13		10.9 ± 0.8	8	− 9.0	†
			Experiment 8			
6207 (C)	Normal	Normal	20.6 ± 1.8	8		
	6207 (C)	Normal	6.0 ± 0.6	8	70.9	< .001
	PW-13	Normal	14.9 ± 1.2	8	27.7	†
	Normal	4909 (C)	17.6 ± 1.9	8		
	6207 (C)	4909 (C)	12.4 ± 1.0	8	29.5‡	< .001
	PW-13	4909 (C)	16.3 ± 1.4	8	7.4	†
	Normal	PW-13	17.8 ± 1.1	8		
	6207 (C)	PW-13	5.5 ± 0.7	8	69.1	< .001
	PW-13	PW-13	15.9 ± 0.8	8	10.7	†
Fibroblasts	Normal		13.3 ± 1.0	8		
	6207 (C)		12.8 ± 0.9	8	3.8	†
	PW-13		13.8 ± 1.6	8	− 3.8	†

* This animal had a very large tumor and was debilitated at the time of the test. † Not significant ($P > .05$). ‡ Significant blocking activity demonstrated.

sarcoma target cells under similar circumstances. Neither sarcoma cells nor normal skin fibroblasts were affected by LNC from mice with bladder carcinomas. In contrast, LNC from mice sensitized in vivo against sarcomas are cytotoxic in vitro against sarcoma target cells, but not carcinoma. These results in vitro demonstrate the specificity of the cytotoxic potential of LNC which are sensitized in vivo.

Table 2 presents results obtained in eight experiments in vitro with rat bladder tumors being used as target cells and peripheral blood lymphocytes as potential killer cells manifesting cell mediated immunity. Bladder carcinoma target cells were destroyed by lymphocytes taken from rats bearing either papillomas or carcinomas (experiments 2 and 3). Target cells from two papillomas were similarly affected by lymphocytes from rats bearing either one of two other papillomas or bearing a carcinoma (experiments 1 and 4). The only exception to this reactivity was in the case of lymphocytes obtained from a rat debilitated by a large carcinoma (experiment 4). Target cells from two papillomas were destroyed by lymphocytes from rats bearing either one of two other papillomas, while these lymphocytes had no adverse effect on normal fibroblasts from rat skin in the same experiments (experiments 5 and 6). Polyoma virus–induced sarcoma cells were not destroyed in vitro by lymphocytes immune to bladder carcinoma (experiment 7). The reverse situation was also true when the same lymphocytes were tested in a reciprocal pattern (experiment 8). Serums taken from animals with either a growing bladder carcinoma or polyoma tumor appeared to specifically block the cytotoxic potential of the added lymphocytes (experiments 7 and 8).

Based on our findings of common TTS antigens in murine bladder tumors, we have developed an immunization procedure aimed at preventing chemically induced bladder papillomas in rats, similar to the prevention of certain DNA virus induced tumors (*8*). Weanling Fisher male rats were immunized by injection in the right hind foot pad. Experimental animals received 0.2 ml of a crude suspension of rat papilloma cells combined either with Waymouth's medium or Freund's complete adjuvant. Controls included animals that received minced normal rat bladder with Waymouth's medium or Freund's adjuvant and animals that were either unimmunized or that received only Freund's adjuvant. After immunization, a 6-week period was allowed for tumor outgrowth. The right hind foot of these animals was then removed, with the same procedure being followed in comparable control animals. The animals were anesthetized and a 30-mg pellet containing 20 percent methylcholanthrene in paraffin was implanted in each bladder.

Table 3. Immunization of rats by intradermal injection of syngeneic urinary bladder tumor cells.

Immunization procedure	Score (No.) at 4.5 months		Score (No.) at 6.5 months	
	Animals	Tumors	Animals	Tumors
*Group A**				
None	8	5	8	5
Freund's adjuvant	7	4	7	6
Rat bladder in Freund's adjuvant	4	3	4	3
Rat bladder in Waymouth's medium	7	4	7	5
*Group B**				
Rat tumor in Freund's adjuvant	17	2	17	8
Rat tumor in Waymouth's medium	7	2	7	3

* The P value for difference between A and B: $P < .005$ at 4.5 months and $P > .08$ at 6.5 months.

The rat bladders were examined for tumors 4.5 and 6.5 months after the pellet was implanted by a transillumination technique that permitted detection of tumors as small as 1 to 2 mm in diameter (*9*). Tumors that had developed at 4.5 months were shown, on excisional biopsy, to be papillomas.

The suppression of tumor growth in vivo by immunization prior to the administration of carcinogenic stimuli was demonstrated by the data summarized in Table 3. The two experimental groups that received papilloma cells alone or papilloma cells together with Freund's adjuvant had significantly ($P < .005$) fewer (4 of 24) papillomas than the control groups (16 of 26) when observed at 4.5 months after insertion of the methylcholanthrene pellets. The difference between the experimental and control groups was not significant 6.5 months after insertion of the pellets (11 of 24 as compared to 19 of 26). No carcinomas developed in any of the rats during the 6.5-month observation period.

Our results indicate that the murine urinary bladder tumor is a good model system for immunological studies of TTS antigens since the antigens associated with different bladder papillomas and carcinomas of rats cross-react and the antigens of different bladder carcinomas of mice are also cross-reactive. Serums from rats with growing bladder tumors could specifically block in vitro cell mediated immunity to these neoplasms. This is in agreement with findings in other systems (*6*) and further strengthens the view that rat bladder tumors possess a common TTS antigen. Further, rats immunized in vivo with bladder papilloma cells were significantly more resistant to the chemical induction of bladder papillomas when tested at 4.5 months after the first exposure to the carcinogen. The difference between the two groups was less significant at 6.5 months. Whether the delayed tumor appearance was due to the development of specific cell mediated immunity or to the formation of cytotoxic or antiviral antibodies or whether it had some other cause remains unknown.

The demonstration of common antigens in chemically induced papillomas and carcinomas of the bladder is at variance with most findings made on chemically induced murine sarcomas and hepatomas (*1, 10*). It does, however, agree with observations made on human neoplasms (*2*). This model of a murine bladder tumor may thus provide a better system for the study of the human problem than animal tumors previously studied. It is unknown why most chemically induced animal tumors do not have any detectable common antigens while murine bladder carcinomas, like the human ones, do. Perhaps neoplasms with strong and individually unique antigens do not express detectable amounts of the common TTS antigens (*11*), and it is possible that procedures used for induction of sarcomas and hepatomas may favor the expression of individually unique antigens rather than the common ones, with the situation reversed for the bladder tumors. Finally, it should be pointed out that there are occasional reports of common antigens in chemically induced murine sarcomas (*11*).

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Tumor Etiology and Chromosome Pattern

Abstract. *Fibrosarcomas induced in Chinese hamsters and rats by Rous sarcoma virus and 7,12-dimethylbenz(a)anthracene are associated with nonrandom chromosome variation. Although histologically indistinguishable, the tumors induced by the virus or chemical in each host species are characterized by completely different karyotypic patterns.*

Nonrandom karyotypic patterns in tumors may be largely dependent on the inducing agent, with different agents predisposing to different patterns (*1*). This has been observed in tumors of two host species, Chinese hamsters and rats, after induction with two oncogenic agents, Rous sarcoma virus (RSV) and 7,12-dimethylbenz(a)anthracene (DMBA).

In 42 primary sarcomas induced by RSV in an inbred strain of Chinese hamsters, Kato analyzed 32 hyperdiploid stem- and sidelines (*2*). Among them, 29 had one or more additional chromosomes of chromosome pairs No. 5, 6, or 10. In 20 of the 24 trisomic stem- and sidelines, the only abnormality was the addition of a chromosome of one of these three pairs; this is obviously a nonrandom pattern.

In five out of six primary sarcomas induced by DMBA in the same inbred Chinese hamster strain, we found a different chromosomal pattern, characterized primarily by additional chromosomes of pair No. 11. This was found in four diploid or near-diploid tumors and in one near-triploid, with one diploid tumor showing no deviation from the normal Chinese hamster karyotype (*3*).

In the rat, the RSV-induced sarcomas exhibited one of the most striking nonrandom patterns ever observed, involving a characteristic three-step karyotypic evolution with additions, in turn, of one medium-sized t (*4*), one st_3, and one st_5 chromosome (Fig. 1). The establishment of this pattern was based on the analysis of stemlines, sidelines, and single deviating cells in 80 primary and 20 metastatic tumors, including sequential passages of a number of parallel sublines from two of the primary tumors (*1*, *5*).

Chromosome analysis of 12 primary DMBA-induced sarcomas in the same inbred rat strain revealed a completely different karyotypic pattern (*6*). Among these sarcomas were ten diploid or near-diploid tumors, in which trisomy for the longest t chromosome, t_1, was the first characteristic feature, the second being the addition of an m chromosome (Fig. 1). The chromosomes most often involved in the evolution of the RSV tumors were participating only exceptionally in the variation of the DMBA tumors. Gains of the t_1 chromosome and of one m chromosome were leading features also in rat leukemias induced by DMBA and by the closely related hydrocarbons 6,8,12- and 7,8,12-trimethylbenz(a)anthracene (*7*).

These results throw light on the interaction during oncogenesis of the inducing agents with the hereditary apparatus of the cell. If the chromosomal changes are indicators of the underlying variation in the genic material on the molecular level, it appears that with different oncogenic agents essentially different pathways must be involved. Our observations may explain the ap-

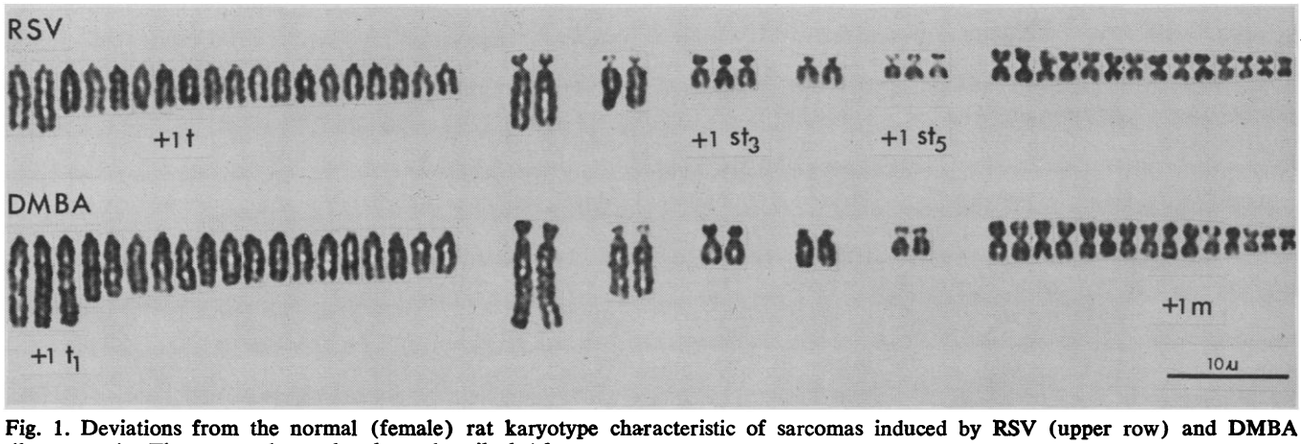

Fig. 1. Deviations from the normal (female) rat karyotype characteristic of sarcomas induced by RSV (upper row) and DMBA (lower row). The nomenclature has been described (*4*).