Summary. A protective effect against both audiogenic and electroshock convulsions in mice has been demonstrated for the compound 2,4 dichlorophenoxyacetic acid, and for the vital dye trypan blue. The mechanism involved was not revealed but attention was directed to the finding that both substances are not depressants, and that they are probably centrally acting. The threshold to electroshock convulsions was compared in 2 sublines of the Swiss Webster strain of mice, selected for susceptibility and resistance to audiogenic seizures, respectively. No significant difference in electroshock threshold between the two lines was observed.

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Received September 26, 1963. P.S.E.B.M., 1963, v114.

Attempts to Interrupt Virus Tumorigenesis by Immunization Using Homologous "Bjorklund-Type" Antigen.* (28704)

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Investigations to date have established quite conclusively that certain malignant tissues may differ antigenically from those of corresponding normal host tissues(1-6). Such differences† may consist of apparent addition of a cellular antigen or of deletion of an antigen. Whether these differences represent appearance *de novo* of new antigen and total deletion of old antigen or whether they reflect only quantitative shifts in relative amounts of antigens present both in normal and corresponding malignant tissues is not clear. The possibility for existence of antigens unique to neoplastic tissue and against which a specific immunity may be induced provides a theoretical potential for eventual prophylaxis and therapy of cancer by immunologic procedures.

* Supported in part by grant from U. S. Public Health Service.

† This refers to cellular antigens resulting from malignant transformation and is not related to the specific antigens of viruses which might have been involved in induction of the malignancy.

There has been considerable interest in the studies of Bjorklund and his co-workers (7-10). Bjorklund formulated and applied the concept that if malignant tissues of man contain a cancer-specific antigen *in common,* then the pooling of a variety of tumors from a number of persons would provide a material in which the cancer antigen would not be diluted whereas the normal host antigens, unique to the individuals, would be diluted in proportion to the number of such individuals in the pool. Thus, a selective concentration of cancer-specific antigens would be effected. Horses were immunized over a long period with such pooled antigen and it was reported that the antiserum, following absorption with human plasma, caused lysis of human cancer cells in culture but did not damage normal human cells grown under similar conditions. Complete removal of such cytotoxic effect was reported to occur following absorption with cancer cells but not following absorption with normal tissues. The malignant antigen was found to be a heat

labile, water insoluble lipoprotein probably present in the cell membrane. Antigenically similar or related antigens were postulated to be distributed widely in human malignant tissues. During the recent period Bjorklund has initiated experiments, using such unique cancer antigen, to attempt immunization of normal human adults against the inevitable subsequent occurrence of spontaneous malignancies(11,12).

Studies were undertaken in our laboratory to ascertain whether homologous lipoprotein tumor antigen, prepared according to Bjorklund, could interrupt the appearance of tumors in animals infected at birth with oncogenic viruses. In the experiments, both the polyoma- and SV_{40}-hamster tumor systems were employed. "Bjorklund-type" lipoprotein antigen vaccine, prepared from corresponding virus-induced tumors, was given in incomplete Freund's adjuvant and in aqueous suspension during the time interval between virus inoculation and the first appearance of tumors. In addition, uninoculated adult hamsters were injected with such antigens prior to homologous transplant tumor or malignant cell culture challenge. Except for one example, no apparent protective effect was afforded. Instead, evidence for possible immunologic enhancement of tumorigenesis was obtained.

Materials and methods. Viruses and cell cultures. Prototype strain VA 45-54 of SV_{40} virus propagated in cell cultures of grivet monkey kidney (GMK) and LID-1 strain of mouse polyoma virus, obtained from Dr. W. Rowe and grown in mouse embryo cell culture, were used. Methods for virus propagation and for cell culture preparation were as described earlier(13,14). SV_{40} virus was employed as GMK passages 3 and 4 and LID-1 polyoma virus was used in passage 9. All virus preparations used were free of extraneous agents as shown in appropriate neutralization tests. Normal cell culture fluids for control purpose were prepared from uninfected cell cultures. *SV_{40} and polyoma tumors.* Primary SV_{40} virus tumors were induced in hamsters by methods described earlier(13) and were used to prepare "Bjorklund-type" antigen. Polyoma tumor, initially induced in a newborn animal by polyoma virus, was obtained from Dr. B. Eddy and was routinely transplanted in our laboratory. This served as a source of tissue for "Bjorklund-type" antigen and for tumor transplant challenge material. *SV_{40} tumor cell culture.* Minced tumor tissue from a hamster inoculated with 1000 TCD_{50} of Selas-filtered SV_{40} virus was prepared in monolayer cell cultures and was carried serially. The growth medium was medium 199 with 5% calf serum. SV_{40} virus was detected in the fluid of the primary passage culture but all fluids from subsequent passages were free of detectable virus. *Preparation of "Bjorklund-type" antigen.* The method was as described generally by Bjorklund and associates(7) and as described specifically by Dr. D. Blaney‡ who prepared such antigen in Dr. Bjorklund's laboratory in Sweden. Essentially, the fresh primary SV_{40} or transplant polyoma tumors grown in hamsters were trimmed free of normal tissue and necrotic areas and were stored frozen at −20°C until used to prepare antigen. The thawed malignant tissues were homogenized at top speed for 5 minutes with an equal weight of distilled water in an Omnimix cup immersed in an ice bath. After straining through gauze, the suspension was extracted 3 times at 0 to 5°C with an equal volume of diethyl ether using a mechanical shaker. The 3 serial extractions were carried out for two, one, and one hour, respectively, and the ether was removed each time following centrifugation at 2000 rpm for 30 minutes under refrigeration. The tissue layer was preserved after the final extraction and the residual ether was removed by bubbling nitrogen through the suspension. The ether extracted tissue was lyophilized by conventional means and the dried powder was ground for 2 to 3 hours in a stainless steel ball mill (as copied from Dr. Blaney's unit) in which the head rotated in an alcohol-dry ice bath. The powdered tissue was extracted twice with 2 volumes of diethyl ether. The residual ether was removed by passing nitrogen through the dried powder in a bottle immersed in an ice bath. The final dried powder was considered

‡ Personal communication, Dr. D. Blaney, Cancer Immunology Laboratory, Cincinnati, Ohio.

to be Bjorklund's sediment 1 and was stored at –20°C. Aqueous vaccine was prepared by blending one gram of sediment 1 with 20 ml of physiological saline solution at top speed in an Omnimix cup immersed in an ice bath. Adjuvant vaccine consisted of aqueous vaccine suspension emulsified with an equal volume of Freund's incomplete adjuvant (90% Drakeol, 10% Arlacel A). The adjuvant components were tested for chemical and physical properties and for toxicity according to the Tentative Draft of Minimum Requirements for Influenza Virus Vaccine Emulsified in Mineral Oil.§ All reagents passed the tests and the emulsions used to vaccinate animals were stable as indicated by retention of continuity after dropping into water. The vaccines were generally rehydrated just prior to use and employed fresh. In 2 instances, however, the antigen in mineral oil adjuvant was stored for as long as 12 days at 4°C. In certain experiments, fluids from appropriate SV_{40} virus- or polyoma virus-infected cell cultures were extracted in the same manner as the tumor tissues and were used for immunization purpose as controls. In other studies, saline solution was substituted for antigen to prepare adjuvant placebo for control. The SV_{40} tumors used to prepare vaccine generally contained a minimal but detectable amount of infectious SV_{40} virus while the polyoma tumors were free of detectable virus. In the preparations tested, no live virus was detectable after processing to prepare "Bjorklund-type" antigen.

Experimental design. Virus-induced tumors. Pregnant hamsters obtained from the Lakeview Hamster Colony, Newfield, N. J., were placed individually in isolation filter cages (15). The newborn hamsters less than 24 hours of age were inoculated subcutaneously into the area between the scapulae with 0.2 ml of SV_{40} or 0.1 ml of polyoma virus. The SV_{40} virus preparations used titered 10^{-6} or 10^{-7} per 0.2 ml when assayed in GMK culture and read for cytopathology for a minimum of 20 days' incubation. The polyoma virus titered $10^{-5.5}$ per 0.2 ml when assayed in mouse embryo cell culture and was diluted 10^{-2} for use in order to delay as long as possible the time of first appearance of tumors. At the appropriate time periods shown in Tables I, II or III, each litter of animals was divided into subgroups to receive vaccine or control material or to be held uninoculated. Vaccine or control material was given intramuscularly and/or subcutaneously in 1.0 or 2.0 ml amount. The vaccination regimen usually consisted of an adjuvant vaccine primary dose followed later by a second aqueous dose of vaccine given intraperitoneally. There were deviations from this as shown in the tables. *Transplant tumor challenge experiments.* Animals used in Table III were not given live virus but were vaccinated as adults in the manner described above and according to the regimen shown in the Table. At the appropriate time period, all animals were challenged subcutaneously with a trypsinized suspension of SV_{40} hamster tumor cell culture or were implanted subcutaneously with 7 pieces of fresh hamster polyoma tumor tissue in hamster passages 22 and 23. *Observations.* Animal care, clinical observations, and pathologic examinations were as described previously(13). Generally, all animals in an experiment were palpated at least once each week for presence of masses. The initial tumor-bearing animals were autopsied at death and the tumors were examined histopathologically. The experimental findings were analyzed in terms of time of appearance of the first tumor, rate of appearance, and final incidence of tumors in the comparable groups. In the tests, a portion of the animals died in the absence of palpable tumors and some were cannibalized. In the analyses, therefore, rates for tumor occurrence were adjusted for the non-specific deaths according to standard life table procedures.

Antibody response to "Bjorklund-type" vaccine. Hamster. Sera from hamsters which were immunized as adults for the SV_{40} transplant tumor challenge experiments (Table III) were stored frozen at –20°C and assayed for antibody by immuno-diffusion (Ouchterlony)‖ and by cytotoxicity procedures. The

§ U. S. Dept. of H.E.W., Public Health Service, Nat. Inst. Health, Bethesda, Md., Jan. 30, 1956.

‖ Immuno-diffusion plates purchased from Hyland Laboratories, Los Angeles, Calif.

antigens used in the immuno-diffusion tests were a saline extract(16) of a primary SV_{40} hamster tumor or the soluble fraction of a 1:10 suspension of "Bjorklund-type" SV_{40} hamster tumor in physiological saline solution. To measure cytotoxicity, the uninactivated sera diluted 1:2 were dispensed in triplicate into Leighton cell culture tubes in 0.1 ml amount and 1.1 ml of medium 199-5% calf serum containing 165,000 cells of an SV_{40} hamster tumor cell culture line was added to each. The cultures were incubated at 36°C and observed daily for cytopathic change. On the fifth day of incubation, each culture was washed with Earle's balanced salt solution and Lowry protein values(17) were measured for the cell substance. *Rabbit and guinea pig.* Adult animals of these heterologous species were each given a total of 1 ml of "Bjorklund-type" SV_{40} hamster tumor antigen in adjuvant by the intramuscular and subcutaneous routes. Two additional 1 ml doses of aqueous antigen of the same type were given 10 and 16 days later *via* the intraperitoneal route. All animals were bled prior to starting immunization and 21 days following the third dose of vaccine. The sera were tested by the immuno-diffusion procedure using as antigen saline extracts of primary SV_{40} hamster tumor, of transplant SV_{40} hamster tumor in passage 21, of normal hamster muscle, and of a 1:10 suspension of "Bjorklund-type" SV_{40} hamster tumor antigen. Tests for cytotoxicity were carried out as described above using second passage cell cultures of hamster embryo and adult hamster kidney in Eagle's minimal essential medium with 10% calf serum and fiftieth cell culture passage of SV_{40} hamster tumor.

Findings. SV_{40} virus-induced tumor system. Table I presents the findings in 3 experiments to ascertain the effect of vaccination on SV_{40} virus-induced tumor occurrence in hamsters. The results are expressed as cumulative rates for tumor occurrence at the time periods shown and adjusted for nonspecific deaths by standard life table procedures. The times were generally selected from the raw data to represent periods in which some notable change had occurred. Ninety-five per cent confidence limits for difference between test and control group were calculated with the results as shown. A difference was considered significant for a particular time only if it exceeded the accompanying $\pm$ value. There was no indication whatever for protection by the vaccine. Instead, there was indication in certain experiments for increased tumorigenesis in the vaccinated animals. Such difference was greatest in Exp. 9B and least in Exp. 3 and was referable, possibly, to the time schedule for vaccination. In Exp. 3, very few of the differences at a particular time were significant statistically; in Exp. 9A, the day 113 and 140 differences alone were significant; and in Exp. 9B, all differences except that for day 228 were significant. There was a consistent pattern in all experiments, however, for earlier tumor occurrence among vaccinated animals and this difference tended to be lost with time. In all experiments, and irrespective of vaccine regimen, this same trend was shown.

Polyoma virus-induced tumor system. The findings in Exp. 8A, B and C in the polyoma system shown in Table II were essentially the same as shown for the SV_{40} system. Thus, there was enhancement in rate of tumor occurrence resulting from vaccination. Except for the very early time periods, these differences were not significant statistically. However, the same consistent pattern for earlier tumor development was shown. Exp. 4 is unique in that it shows a slight trend in the opposite direction, *i.e.,* for possible slight protection against or delay in tumor appearance. It is perhaps worthy of note that this was the only experiment in which only a single dose of vaccine was given.

SV_{40} and polyoma tumor transplant system. Table III presents the findings in attempts to vaccinate adult hamsters against SV_{40} cultured tumor cells or polyoma tumor given by transplant. There was no indication for protection. Instead, there was generally a shortened induction period resulting from vaccination.

Tests for antibody-inducing capacity of "Bjorklund-type" SV_{40} hamster tumor antigens. Hamsters. The sera from hamsters im-

munized as adults with "Bjorklund-type" (Table III) antigen and tested as described in the section on materials and methods failed to show the presence of antibody as measured by immuno-diffusion or by cytotoxicity procedures. *Rabbits and guinea pigs.* Failure to demonstrate an antibody response to "Bjorklund-type" SV_{40} tumor antigen using the various components of the hamster species raised the question of whether the preparations were antigenic at all or whether they were antigenic but non-stimulating of detectable antibody in the homologous and histocompatible hamster model. To test the latter possibility, heterologous species, *i.e.*, rabbits and guinea pigs, were immunized with "Bjorklund-type" SV_{40} hamster tumor antigen along the lines employed for vaccinating hamsters and as described in the section on materials and methods. As shown in Table IV, most of the rabbits and guinea pigs developed precipitating antibody against primary and transplant SV_{40} hamster tumor antigen but only the rabbit antisera reacted with the extract of normal hamster muscle and only the guinea pig sera reacted with "Bjorklund-type" antigen. Sera from most of the animals were frankly cytotoxic or depressed the growth of normal hamster cells and SV_{40} hamster tumor cells in culture. These findings showed that "Bjorklund-type" vaccine, as prepared in these laboratories, was antigenic.

Discussion. The validity of the concept for

TABLE I. Tumor Occurrence in Hamsters Inoculated in the Neonatal Period with SV_{40} Virus and Vaccinated, Pre-Tumor Appearance, with Homologous Hamster Tumor Antigen of the "Bjorklund Type."

Exp. no.	Immunization schedule: Group	Day post-virus	Kind of antigen	Result. Cumulative percentage tumor occurrence: Day observed:			138	159	166	175	208	250	292
3	A (Test)	28 56	Bjork.-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals			4/22	8/22	10/22	13/22	17/22	19/22	19/21
				Cum. % with tumor:			18	36	45	59	77	86	87
	B (Control)	28 56	SV_{40} virus ext.-Adjuv. " " " -Aqueous	Cum. no. with tumor / Total animals			3/23	4/23	5/23	9/23	13/23	14/21	14/19
				Cum. % with tumor:			13	17	22	39	56	61	62
	C (Control)	28 56	Placebo-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals			0/25	3/25	9/24	10/24	16/22	18/21	18/21
				Cum. % with tumor:			0	12	37	41	67	79	79
			Analysis of difference*	Cum. % A - B:			5 ± 21	19 ± 26	23 ± 27	20 ± 29	21 ± 27	25 ± 24	25 ± 25
				A - C:			18 ± 16	24 ± 24	8	18 ± 29	10	7	8 ± 23
				B - C:			13 ± 14	5	15 ± 26	2 ± 29	11	18 ± 28	17 ± 28
9A				Day observed:	98	113	140	155	165	201	228		
	A (Test)	7 30	Bjork.-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals	3/33	10/33	14/33	15/33	15/33	22/31	28/31		
				Cum. % with tumor:	10	30	42	45	45	69	90		
	B (Control)		Untreated	Cum. no. with tumor / Total animals	0/33	1/33	4/33	11/33	12/33	20/33	29/33		
				Cum. % with tumor:	0	3	12	33	36	61	88		
			Analysis of difference*	Cum. % A - B:	10 ± 10	27 ± 17	30 ± 21	12 ± 24	9	8	2 ± 16		
9B				Day observed:	98	113	126	148	165	194	228		
	A (Test)	15 30	Bjork.-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals	8/32	19/32	23/32	26/32	29/32	30/32	31/32		
				Cum. % with tumor:	25	59	72	81	91	94	97		
	B (Control)		Untreated	Cum. no. with tumor / Total animals	0/33	2/33	3/33	15/33	18/33	22/32	27/32		
				Cum. % with tumor:	0	6	9	45	55	67	83		
			Analysis of difference*	Cum. % A - B:	25 ± 15	53 ± 19	63 ± 19	36 ± 22	36 ± 20	27 ± 19	14 ± 15		

* Difference and 95% confidence limits. A difference between a test and control group was considered significant only if the difference exceeded the accompanying ± value.

Differences for which no confidence limits are given were found, by inspection, not to be significant.

TABLE II. Tumor Occurrence in Hamsters Inoculated in the Neonatal Period with Polyoma Virus and Vaccinated, Pre-Tumor Appearance, with Homologous Hamster Tumor Antigen of the "Bjorklund-Type."

Exp. no.	Immunization schedule: Group	Day post-virus	Kind of antigen	Result. Cumulative percentage tumor occurrence: Day observed										
4	A	36	Bjork.-Adjuv.	Cum. no. with tumor / Total animals		1/36	8/28	11/26	15/26	17/23	18/23	19/23		
				Cum. % with tumor		3	27	38	55	64	69	75		
	B	36	Polyoma virus ext.-Adjuv.	Cum. no. with tumor / Total animals		1/37	8/34	20/34	25/34	29/33	30/32	30/32		
				Cum. % with tumor		3	23	58	73	86	92	92		
	C	36	Saline placebo-Adjuv.	Cum. no. with tumor / Total animals		9/31	11/21	14/19	14/19	15/19	17/19	18/19		
				Cum. % with tumor		26	35	57	65	65	83	91		
			Analysis of difference	Cum. % A - B		0	4*	20 ± 25	18 ± 25	22 ± 23	23 ± 21	17 ± 21		
				A - C		23 ± 16	8	19 ± 30	10	1	14 ± 29	16 ± 25		
				B - C		23 ± 16	12 ± 22	1	8	21 ± 27	9	1 ± 18		
8A				Day observed		48	62	69	76	104	111	250		
	A	7 28	Bjork.-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals		2/10	3/10	4/10	6/10	8/10	9/10	10/10		
				Cum. % with tumor		19	29	39	60	80	90	100		
	B		Untreated	Cum. no. with tumor / Total animals		3/12	3/12	3/12	4/10	6/10	8/10	10/10		
				Cum. % with tumor		25	25	26	34	56	78	100		
			Analysis of difference	Cum. % A - B		6 ± 35	4	13 ± 40	26 ± 42	24 ± 40	12 ± 33			
				Day observed		48	62	83	127	250				
	C	14 28	Bjork.-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals		11/22	14/21	15/21	16/21	17/21				
				Cum. % with tumor		48	63	68	73	79				
	D		Untreated	Cum. no. with tumor / Total animals		4/19	5/18	7/17	10/17	10/15				
				Cum. % with tumor		20	25	37	56	56				
			Analysis of difference	Cum. % C - D		28 ± 27	38 ± 29	31 ± 30	17 ± 31	23 ± 31				
8B				Day observed	34	42	56	73	77	84	91	121	155	228
	A	7 28	Bjork.-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals	5/27	8/27	10/26	14/26	20/26	22/26	24/26	24/26	25/26	26/26
				Cum. % with tumor	19	30	37	53	76	84	92	92	96	100
	B		Untreated	Cum. no. with tumor / Total animals	0/27	3/27	9/26	10/25	12/25	13/25	14/25	17/25	18/25	20/25
				Cum. % with tumor	0	11	34	38	46	50	54	67	71	79
			Analysis of difference	Cum. % A - B	19 ± 15	19 ± 21	3	15 ± 27	30 ± 26	34 ± 24	38 ± 23	25 ± 22	25 ± 20	21 ± 16
8C				Day observed	36	41	54	76	84	139				
	A	7 28	Bjork.-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals	2/18	10/17	11/17	11/17	12/17	16/17				
				Cum. % with tumor	11	57	63	63	69	94				
	B		Untreated	Cum. no. with tumor / Total animals	0/14	2/14	4/14	5/13	8/13	12/13				
				Cum. % with tumor	0	14	29	36	58	92				
			Analysis of difference	Cum. % A - B	11 ± 15	43 ± 30	34 ± 34	27 ± 35	11 ± 35	2 ± 20				
				Day observed	36	41	68	76	139					
	C	14 28	Bjork.-Adjuv. " -Aqueous	Cum. no. with tumor / Total animals	4/21	12/20	15/20	15/20	15/20					
				Cum. % with tumors	19	58	74	74	74					
	D		Untreated	Cum. no. with tumor / Total animals	0/12	2/11	5/10	6/9	7/9					
				Cum. % with tumor	0	17	47	58	72					
			Analysis of difference	Cum. % C - D	19 ± 17	41 ± 31	27 ± 36	16 ± 37	2 ± 37					

* Numbers without a ± value were found to be insignificant, by inspection.

prevention or cure of cancer by immunologic methods must necessarily be based on the existence in tumor of a unique or sufficiently different antigen to which the host can respond favorably. Bjorklund(7-9) claimed to have demonstrated such antigen in human tumors based on development in horses, following immunization, of cytolytic antibody which destroyed cells of human malignant and normal origin, the latter having been

TABLE III. Tumor Development in Adult Hamsters Implanted with Tumor Tissue Following Immunization with Homologous Hamster Tumor Antigen of the "Bjorklund-Type."

Exp. no.	Group	Immunization schedule: Day	Kind of antigen	Challenge: Day	Kind	No. cells per hamster	Result. Cumulative percentage tumor occurrence according to day post-challenge: Day observed						
5A	A	0 10 16	Bjork., SV_{40} tumor-Adjuv. " " " -Aqueous	44	SV_{40} tumor cell culture	10,000	Cum. no. with tumor / Total animals	7/8	8/8	8/8			
							Cum. % with tumor	87	100	100			
	B	Untreated		"	"	"	Cum. no. with tumor / Total animals	5/9	6/8	8/8			
							Cum. % with tumor	56	68	100			
		Analysis of difference					Cum. % A - B	31 ± 41	32 ± 32	-			
							Day observed	15	21	29	62		
5B	C	0 10 16	Bjork., SV_{40} tumor-Adjuv. " " " -Aqueous	46	"	15,000	Cum. no. with tumor / Total animals	1/15	11/15	15/15	15/15		
							Cum. % with tumor	7	73	100	100		
	D	Untreated		"	"	"	Cum. no. with tumor / Total animals	4/18	12/17	16/17	17/17		
							Cum. % with tumor	22	68	94	100		
		Analysis of difference					Cum. % C - D	15 ± 23	5 ± 32	6 ± 12	-		
							Day observed	6	20	27	34	51	
5C	E	0 10 16	Bjork., SV_{40} tumor-Adjuv. " " " -Aqueous	43	"	1,000	Cum. no. with tumor / Total animals	0/7	0/7	7/7	-	-	
							Cum. % with tumor	0	0	100			
	F	0 10 16	Bjork., SV_{40} tumor-Adjuv. " " " -Aqueous	"	"	500	Cum. no. with tumor / Total animals	1/7	3/7	4/7	5/7	6/7	
							Cum. % with tumor	14	43	57	71	86	
	G	0 10 16	Placebo-Adjuv. " -Aqueous	"	"	1,000	Cum. no. with tumor / Total animals	0/7	0/7	6/7	6/7	6/7	
							Cum. % with tumor	0	0	86	86	86	
	H	0 10 16	Placebo-Adjuv. " -Aqueous	"	"	500	Cum. no. with tumor / Total animals	0/7	4/7	6/7	7/7	-	
							Cum. % with tumor	0	57	86	100	-	
		Analysis of difference					Cum. % E - G F - H	0 14 ± 26	0 14 ± 53	14 ± 26 29 ± 46	29 ± 34	14 ± 26	
							Day observed	19	28	42	49	56	91
6A	A	0 7 14	Bjork., polyoma tumor-Adjuv. " " " -Aqueous	22	Polyoma transplant tumor	Graft	Cum. no. with tumor / Total animals	5/11	10/11	11/11			
							Cum. % with tumor	45	91	100			
	B	Untreated		"	"	"	Cum. no. with tumor / Total animals	1/9	3/9	5/9	6/9	7/9	9/9
							Cum. % with tumor	11	33	56	67	78	100
		Analysis of difference					Cum. % A - B	34 ± 37	58 ± 36	44 ± 33	33 ± 31	22 ± 28	-
							Day observed	19	26	47			
6B	C	0 7 14	Bjork., polyoma tumor-Adjuv. " " " -Aqueous	22	Polyoma transplant tumor	Graft	Cum. no. with tumor / Total animals	19/26	25/26	26/26			
							Cum. % with tumor	73	96	100			
	D	Untreated		"	"	"	Cum. no. with tumor / Total animals	15/18	16/18	18/18			
							Cum. % with tumor	83	89	100			
		Analysis of difference					Cum. % C - D	10 ± 25	7 ± 17	-			

TABLE IV. Tests for Antigenic Quality of "Bjorklund-Type" SV_{40} Hamster Tumor Antigens.

Animal serum		Results obtained in tests for									
		Immunoprecipitation with antigen*				Cytotoxicity for cell culture**					
						Normal				Malignant	
Species and no.	Specimen with respect to vaccination	Primary SV_{40} hamster tumor	Transplant SV_{40} hamster tumor	Normal hamster muscle	"Bjork.-type" SV_{40} hamster tumor (1:10)	Secondary hamster embryo		Secondary hamster kidney		SV_{40} hamster tumor, tissue culture passage 50****	
						Cytopath.	Protein***	Cytopath.	Protein	Cytopath.	Protein
Rabbit											
7251	Pre	0	0	0	0	0	50γ	0	24γ	0	51γ
"	Post	±	0	±	0	+	17	+	13	±	38
7252	Pre	0	0	0	0	0	57	0	26	0	59
"	Post	+	+	±	0	+	10	+	12	+	11
Control	Nil						73		35		92
Guinea pig											
7087	Pre	0	0	0	0	0	60γ	0	24γ	0	46γ
"	Post	+	0	0	0	±	48	±	18	0	54
7089	Pre	0	0	0	0	0	64	0	26	0	60
"	Post	+	+	0	+	+	23	±	26	±	39
7090	Pre	0	0	0	0	0	65	0	26	0	55
"	Post	+	+	0	+	±	57	±	30	0	57
7091	Pre	0	0	0	0	0	66	0	27	0	48
"	Post	±	+	0	0	+	32	+	31	±	45
Control	Nil						65		48		70

* + = Well-defined band; ± = light band.

** + = Major cytopathic change; ± = minor cytopathic change.

*** Average values, three tubes.

**** Essentially identical results were obtained when first passage cell culture of an SV_{40} transplant tumor was used.

altered after long-term passage *in vitro*. The sera, however, did not adversely affect normal human cells in primary cell culture. These experiments suffered the usual hazard of operating in a heterologous host-antigen system in which response to heterologous normal antigen would likely be achieved as readily as to any unique malignant antigen present. Bjorklund's findings might have been more convincing had homologous normal tissue been used and shown not to induce such antibody in horses. Goldstein and Hiramoto(18), using antiserum and antigen prepared by Bjorklund, were able to show lysis of normal as well as malignant human cells and absorption of the serum with Bjorklund's antigen prepared from malignant human cells removed the cytolytic antibody against normal as well as malignant cells of human origin. The fact, however, that Bjorklund failed to provide any convincing data to support his concept for a unique lipoprotein antigen in human malignant tissue pools does not negate the existence of such antigen. Indeed, data have been obtained in well-controlled systems to support the conviction that unique or unusual antigens may be present in human and in animal tumors(1-4,19-21). On theoretical bases, also, there is good reason to believe that there would be a plurality of "malignant" antigens in different kinds of tumors rather than a single unique malignant antigen as postulated by Bjorklund. Such antigens may well be located on the surface of cells and might be of lipoprotein chemical composition.

The present studies were designed to ascertain whether lipoprotein antigen of the "Bjorklund-type" derived from SV_{40} or polyoma virus-induced tumors could interrupt tumorigenesis caused by a virus given pre-vaccination into newborn hamsters or effected by homologous transplant given post-vaccination into adult hamsters. The virus-newborn animal systems employed were among those in which there is early age dependence for tumor induction. In this respect, the systems selected parallel those for other virus-induced tumors such as avian leucosis and Bittner mouse mammary carcinoma(22) and might even be equivalent to as yet hypothetical situations with regard to possible virus induced malignancies in man. The hamster system was considered to be immunologically isologous for all practical purposes since these animals are uniformly and completely histocompatible with respect to transplantation antigens(23). The immunization procedure employed consisted generally of an initial injection of antigen in adjuvant followed by a second aqueous dose, a procedure proved adequate for eliciting antibody against ordinary antigens in animals.

The isologous hamster system failed to demonstrate cytolytic antibody such as reported by Bjorklund for the heterologous horse-human system. Additionally, the isologous hamster system failed to show precipitating antibody by the immuno-diffusion method. Such negative results might well be expected in view of the equivocal findings, to date, to demonstrate specific antitumor antibody in any truly isologous or autologous system even though unique tumor antigen or tumor immunity has been demonstrated in other systems by other procedures(1-4,19).

The retention of antigenic quality of the "Bjorklund-type" lipoprotein antigen used in our study was proved in the heterologous rabbit and guinea pig systems in tests for induction of cytolytic and precipitating antibodies. Such antibodies were elicited against normal tissue as well as against malignant tissue and it is not possible to judge from present data whether there was antibody against a unique tumor antigen.

The "Bjorklund-type" antigens employed for protection tests failed, with one possible exception, to provide any measurable immunity against subsequent tumor development in the virus-inoculated newborn hamster or in adult animals challenged with homologous tumor transplant. Failure to induce immunity to tumorigenesis in newborn animals given virus might be explained on the basis that malignant transformation took place shortly following virus inoculation and that the animals developed immunologic tolerance against the possible unique tumor antigen when administered later as vaccine. Such explanation could not apply, however, to the situation in which adult hamsters failed

to develop immunity, by vaccination, against homograft tumor even though very small numbers of tumor cells were used as challenge.

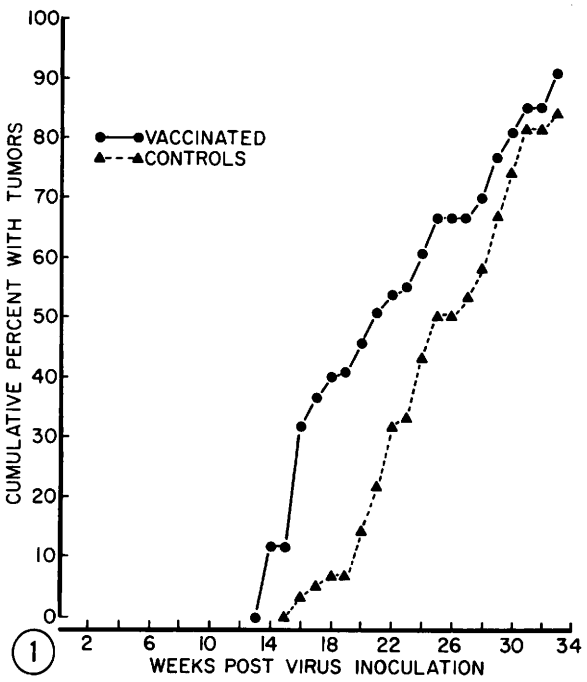
Instead of protection against virus-induced malignancy, vaccination appeared in most instances to effect an enhancement of tumorigenesis. The experiments did not, however, definitively exclude the possible role of the mineral oil adjuvant in enhancing tumorigenesis. The tumor enhancement was most often shown in terms of earlier appearance of tumors but included examples of increased final tumor incidence. To appraise further the overall significance of the findings, animals which were treated similarly in Tables I or II were combined. A summary analysis of the combined data is presented in Table V and in Fig. 1 and 2. The same general pattern of earlier tumor appearance shown in immunized animals in the individual experiments was clearly shown. This single instance of possible protection (Table II, Exp. 4) was different in that it was obtained using a single dose of tumor vaccine given relatively late following polyoma virus, *i.e.*, 36 days.

The phenomenon of enhanced tumor-take in animals, on an immunologic basis, is neither new nor unique in studies of oncogenesis. Working with the mouse sarcoma system, Kaliss(24-26) has shown that a humoral antibody response to tumor antigen may effect tumor enhancement in contrast to a cellular response which results in rejection. These phenomena have been demonstrated under proper circumstances following

TABLE V. Summary Analysis of Data from Tables I and II in Which Comparable Groups Were Combined.*

Time post-virus (wk)	Table I data (SV_{40}) Cumulative % with tumor Test	Control	Difference & 95% confidence limits	Table II data (polyoma) Cumulative % with tumor Test	Control	Difference & 95% confidence limits
4				0	0	0
5				12	0	12 ± 6
6				32	8	24 ± 11
7				45	16	29 ± 13
8				52	26	26 ± 14
9				57	28	29 ± 14
10				60	34	26 ± 14
11				69	41	28 ± 15
12				71	50	21 ± 15
13	0	0	0	77	51	26 ± 14
14	12	0	12 ± 7	78	55	23 ± 14
15	12	0	12 ± 7	80	58	22 ± 14
16	32	3	29 ± 11	81	61	20 ± 14
17	37	5	32 ± 11	81	67	14 ± 14
18	40	7	33 ± 12	84	68	16 ± 13
19	41	7	34 ± 12	85	70	15 ± 13
20	46	14	32 ± 13	85	74	11 ± 13
21	51	22	29 ± 14			
22	54	32	22 ± 14			
23	55	33	22 ± 14			
24	61	43	18 ± 15			
25	67	50	17 ± 15			
26	67	50	17 ± 15			
27	67	53	14 ± 15			
28	70	58	12 ± 14			
29	77	67	10 ± 14			
30	81	74	7 ± 13			
31	85	79	6 ± 12			
32	85	79	6 ± 12			
33	91	84	7 ± 10			

* Combinations.
Table I. Groups 3A, 9AA, 9BA; and 3C, 9AB, 9BB.
Table II. " 8AA, 8AC, 8BA, 8CA, 8CC; and 8AB, 8AD, 8BB, 8CB, 8CD.



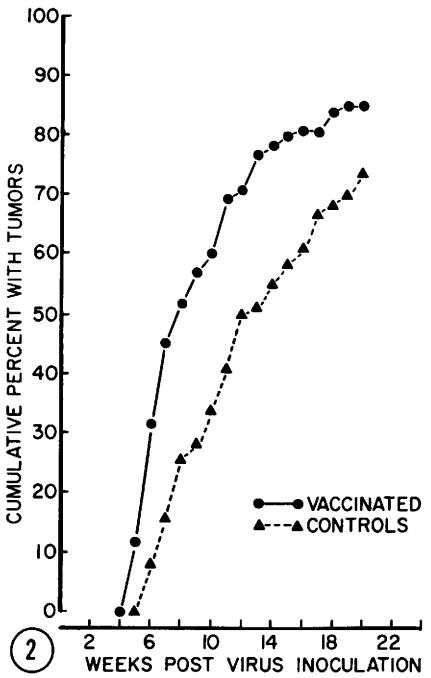


FIG. 1. Tumor appearance, according to time, in vaccinated and non-vaccinated animals in Table V, SV_{40} hamster model.

FIG. 2. Tumor appearance, according to time in vaccinated and non-vaccinated animals in Table V polyoma hamster model.

immunization with killed freeze-dried tumor tissue or normal tissue. Boyse *et al*(27) note that this phenomenon is not general for all tumor types and have reported that suppression of tumor growth by antibody may occur, especially if the antibody titer is high. The relationship between apparent immunologic enhancement in our studies compared with those of Kaliss *et al*(24-26) cannot now be stated but it may be worthy of note that the phenomenon was accomplished in our experiments in the absence of detectable antibody.

Attempts to vaccinate against malignancy in human beings using their own tumor as antigen have generally given negative or at best equivocal protection(28-30). In certain animal experiments, suggestive evidence for protection has been noted and failure was seen in others(31-38). Worthy of special attention was the failure by Prehn(38) to immunize against carcinogenesis by exposure of mice to a homologous tumor prior to a chemical carcinogen.

Our results represent the first attempt to our knowledge to interrupt virus-induced tumor by vaccination with tumor antigen during the latent period. This might bear some analogy to as yet hypothetical situations in man. While such distant extrapolation can hardly be justified on the basis of present experiments in animals, it is nevertheless true that the apparent immunologic enhancing effect was not expected. This might provide some theoretical basis, at least, for caution where immunization of the human subject is concerned.

Summary. Studies were carried out to determine whether "Bjorklund-type" lipoprotein tumor antigen could interrupt the appearance of tumors in animals infected at birth with oncogenic viruses. The polyoma and SV_{40} hamster systems were employed. "Bjorklund-type" antigen was prepared from corresponding virus-induced tumor and was made into aqueous and mineral oil adjuvant formulations. These vaccines were given during the time period between virus inoculation and first appearance of tumors. Also, adult animals, not previously exposed to

virus, were immunized prior to challenge with homologous tumor transplant. None of the adult hamsters given "Bjorklund-type" antigens developed detectable cytolytic or precipitating antibody against homologous tumor. The vaccine was antigenic, however, as proved by development of such antibody in the heterologous guinea pig and rabbit species. The experiments were designed to determine whether protection would be afforded by the vaccine and such was clearly not obtained. Instead, a small but statistically significant enhancement of tumor induction was noted. The implications of these findings are discussed and a word of caution is given, on theoretical bases, for studies of such vaccines in man.

The authors are indebted to W. Raupp, T. Beddow and R. Grady for technical assistance. Statistical evaluations were made by A. Itkin.

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Received July 12, 1963. P.S.E.B.M., 1963, v144.