of culture and with a maximum of 7 to 8 percent of the lymphocytes in active mitosis at any one time, the 10 to 15 percent of lymphocytes which appeared as transformed cells before, and after, 48 hours was doubtless metabolically significant. However, since the isozyme pattern changed maximally with the burst in actively dividing cells between 48 and 72 hours, it seems likely that mitosis rather than the period before mitosis, or transformation, was largely responsible for the activation, or de-repression, of the genetic locus controlling synthesis of M subunits. With the observed decline in cell division at 168 hours, this locus may have again become inactive, the isozymes tending to return to the pattern seen before culture and during early transformation.

Cultures of leukocytes are heterogeneous with respect to cell type and the relationship of cells to division. While more than 90 to 95 percent of the cells are lymphocytic in origin, the contribution of the remaining 5 to 10 percent of cells to both isozyme synthesis and energy metabolism is unclear. Further, at any given time beyond 48 hours, the cultures consist of nontransformed, transformed, and actively dividing cells. This heterogeneity and the asynchronous cell division preclude a definitive statement of when in interphase (before, during, or after DNA synthesis) or mitosis the isozyme shift took place.

The application of this study to cell functioning in vivo is unclear. The activity of genes under the stimulus of a potent mitogenic agent and in the presence of a synthetic medium for support of cell division precludes direct comparison with cells in the soma. However, it seems reasonable to conclude that isozyme synthesis does not simply depend on an adaptation of cells to a changing biochemical environment or to a changing metabolic pathway.

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12. From density tracings of the electrophoretic patterns obtained on the gel, the percent of each LDH band was calculated, and the distribution of H and M subunits was estimated. The proportions of H and M were assumed to be 4 : 0, 3 : 1, 2 : 2, 1 : 3, and 0 : 4 in LDH bands 1 to 5, respectively.
13. A.D.B. is a Surgeon, U.S. Public Health Service, National Center for Radiological Health, on assignment to the Atomic Bomb Casualty Commission, an agency of the U.S. National Academy of Sciences–National Research Council and the Japanese National Institute of Health.

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Cell-Bound Immunity to Autologous and Syngeneic Mouse Tumors Induced by Methylcholanthrene and Plastic Discs

Abstract. *A colony-inhibition assay was used to demonstrate specific immunity in vitro against syngeneic mouse sarcomas induced by methylcholanthrene or implanted Dictabelt plastic discs. An immunity to methylcholanthrene-induced tumors could also be demonstrated with lymphocytes derived from the autologous primary tumor-bearing host 3 to 8 days after removal of the primary tumor.*

Most experimentally induced neoplasms have specific tumor antigens which can be detected with the isograft-rejection technique (*1*). Tumors induced by methylcholanthrene (MCA) and other chemical carcinogens or by implantation of plastic films contain antigens which are individually distinct for each tumor when the tumors are also induced by the same agent (*2*). They differ in this respect from virus-induced tumors, which have specific antigens common to all neoplasms induced by the same virus (*1*).

Many methods have been used to demonstrate tumor specific antigenicity in vitro (*1*). We consider the recently introduced colony-inhibition (CI) assay to be of particular interest (*3*), since it probably reveals the same antigens as the isograft-rejection technique. It has been used to detect both cell-bound and humoral immunity to neoplasms induced by adenovirus 12 (*4*) and by the polyoma virus (*3*). We now report on data from a colony-inhibition assay of cell-bound immunity to specific antigens in mouse sarcomas induced by MCA and by plastic discs. These types of tumors were chosen, since they originate only at the place of carcinogen implantation and can therefore be easily removed. This makes it possible to investigate immunity to such tumors in the autologous host which may provide a model for similar studies with human material.

Sarcomas were induced in C3H male mice implanted with 3-methylcholanthrene pellets; they were individually numbered (Table 1). Three sarcomas were induced in BALB/c females, implanted with blue Dictabelt plastic discs (*5*). All mice were derived from a single line inbred colony and were skin-compatible.

Primary tumors were removed and used for explantation in vitro and transplantation to syngeneic mice. The explanted cells were propagated in vitro for 2 to 60 days before CI assays which were performed on tumor cells plated in 50-mm Falcon plastic petri dishes (*3*). Approximately 24 hours after plating of the tumors when the cells had attached to the petri dish surfaces, the medium was decanted, and 0.3 ml of either Difco phytohemagglutinin (PHA), diluted 1:15, or phosphate-buffered saline (PBS) was added to the petri dishes. Immediately afterward, 0.5 ml of a suspension of lymph node cells in Eagle's minimal essential medium (MEM) was also added. Two or three doses of lymphocytes were tested: 5×10^6, 2×10^6, and 10^6 cells per petri dish. After the cells were incubated for 45 minutes, each petri dish received 4 ml of MEM containing heat-inactivated fetal calf serum (15 percent). The dishes were then incubated at 37°C for 3 to 4 days in an atmosphere of 5 percent CO_2 and air, the colonies were stained with 0.1 percent crystal violet, and the number of colonies formed by the target cells was counted. The lymphoid cells did not form any colonies under the conditions of the experiments.

The lymphoid cells tested on the target cells were derived from axillary, cervical, inguinal, and mesenteric nodes.

Three types of lymphocytes were used, namely, normal syngeneic lymphocytes, lymphocytes derived from mice immunized against the specific target tumor, and lymphocytes harvested from animals similarly immunized against another syngeneic tumor line induced by the same agent. Such lymphocytes were used as controls, since the specific antigens of individual tumors induced by MCA or plastic films do not cross-react in isograft-rejection tests (*2*). Immunization of lymphocyte donors was accomplished in one of three ways: (i) by inoculating them with living primary tumor cells and harvesting the lymphocytes before palpable tumors had formed, (ii) by removing trans-

Table 1. Data from colony inhibition tests with 16 different MCA-induced C3H sarcomas. Primary tumors were explanted, plated in petri dishes, and exposed to phytohemagglutinin (+PHA) or saline (−PHA), and then to syngeneic lymphocytes of different origin. The number of colonies formed in individual petri dishes is given. The following types of syngeneic lymphocyte donors were used: untreated mice; mice transplanted with the target tumor (another syngeneic MCA tumor in controls) but yet negative; mice transplanted with the target tumor, which was later surgically removed; mice transplanted with heavily x-irradiated tumor cells; primary autologous hosts, whose tumors had been surgically removed and explanted. In one experiment (with tumor 40) a mouse still bearing a transplanted 40 tumor was used. C, control; S, specifically immunized; trspl, transplanted; tum, tumor; remov, removed; prim, primary; irr, irradiation.

Target tumor number	Group	Immunization of lymphocyte donors	Dose to test (days)	Colony numbers							
				5×10^6 lymphocytes		2×10^6 lymphocytes		10^6 lymphocytes		No lymphocytes	
				+PHA	−PHA	+PHA	−PHA	+PHA	−PHA	+PHA	−PHA
37	C	None		8,6	23,23	114,124	96,130				
	S	Trspl 37 tum	70	0,0	2,1	2,4	6,1				
38	C	None		9,5	13,12			4,5	10,10		
	S	Trspl 38 tum	26	3,1	3,3			3,4	10,8,8		
39	C	None		106,105	76,84			83,85	74,66		
	S	Trspl and remov 39 tum	7*	58,48	58,59			88,78	102,98		
39	C	None				100,130	110,100				
	S	X-irr 39 tum	20			3,3	52				
40	C	X-irr 47 tum	12	76,84	86,76	110,100	96,100	90,110	102,108	96,98	
	S	X-irr 40 tum	12	26,32	24,34	64,52	64,56	76,94	76,96		
40	C	None		77,63	88,62			50,74	60,64		
	S	Carries trspl 40 tum		43,36	50,52			42,48	58,56		
44	C	X-irr 48 tum	53		21,20		18,19		22,17	18,22	
	S	X-irr 40 tum	53		11,8		15,12		12,11		
56	C	Trspl 53 tum	5	56,44				58,50			
	S	Trspl 56 tum	5	28,28				32			
56	C	None		13,7	10,16			42,68	35,35		
	S	Trspl 56 tum	28	2,1	3,3			46,58	38,31		
65	C	None				44,30	38,58				
	S	Trspl 65 tum	40			10,8	7,5				
65	C	None		66,68	104,96	80,82	98,106			84,104	
	S	Trspl and remov 65 tum	13*	3,1	4,3	8,12	13,12				
76	C	Trspl 77 tum	16	60,66	98,92	98,84	96,94			90,92	
	S	Trspl 76 tum	16	48,44	74,84	64,68	98				
78	C	Prim 76 tum	8*	36,42	54,46	62,48					
	S	Prim 78 tum	8*	1,0	0,0	12,30					
82	C	Trspl and remov 9 tum	5*	40,41	48,38	40,50				54,52	
	C	X-irr 52 tum	83	38,31	54,46						
	S	Prim 82 tum	5*	22,23	24,34	36,48					
88	C	Trspl 32 tum	3	8,12	24,24					27,26	26,26
										22,24	24,26
	S	Prim 88 tum	3*	4,7	12,10						
	S	Trspl 88 tum	3	5,1	0,2	9,11	14,12				
89	C	Trspl 55 tum	4	36,37	44,40	34,28	28,32			22,22	30,22
	C	Trspl 34 tum	4	30,30	28,35						
	S	Prim 89 tum	4*	12,6	11,15						
	S	Trspl 89 tum	4	28,30	46,30						
90	C	Prim 91 tum	5*		22,28						
	C	Trspl 91 tum	5		24,20					24,26	38,34
	C	Trspl 76 tum	11	32,33	26,21	45,32	21,29				
	S	Prim 90 tum	5*	12,13	13,7						
	S	Trspl 90 tum	5	21,17	14,18	32,32	26,30				
93	C	Trspl 69 tum	6	21,15	27,19	15,9					
	S	Prim 93 tum	3*	7,4	8,8						
	S	Trspl 93 tum	3	0,0	1,2	7,8	7,5				
96	C	Trspl 78 tum	4	56,56	58,58	50,50				75,70	82,84
										70	80
	S	Prim 96 tum	5*	35,33	20,22						
	S	Trspl 96 tum	5	52,48	68,58						
97	C	Trspl 47 tum	6	32,38	18,22					32,30	30,36
	S	Prim 97 tum	3*	0,0	0,0						
	S	Trspl 97 tum	3	11,12	7,13	30,32	30,28				

* From removal.

planted tumors surgically and harvesting lymphocytes 7 to 14 days afterwards, or (iii) by inoculating the mice once with tumor cells that had been given 12,000 to 24,000 roentgens of x-irradiation and harvesting the lymphocytes from 10 to 80 days afterward. The irradiated cells were derived from the primary tumors or from their first or second transplant generations. Finally, in eight experiments, primary MCA-induced tumors were surgically removed, their cells were explanted, and the lymphocytes were tested on the autologous target cells 3 to 8 days afterward.

Table 1 shows that there was a lower colony number in groups receiving lymphocytes from donors immunized to the target tumor than in groups given lymphocytes from either untreated donors or from donors immunized to another syngeneic MCA sarcoma. The results with donors immunized to the syngeneic tumors agree with the demonstration in vivo that individual MCA tumors have specific antigens which do not cross-react (*2*). They also agree with a report by Rosenau and Morton (*6*) who found approximately 30 percent fewer living cells in cultures of transplanted MCA tumors which had been treated with specifically immune lymphocytes than in controls treated with lymphocytes immunized against the antigens of another MCA tumor. However, the CI assay appears to be more sensitive, since differences of 40 to 90 percent were generally observed in our study.

Autologous lymphocytes were strongly inhibitory when derived from operated mice that had carried MCA tumors (Table 1), as was seen in all of eight experiments performed. The demonstration of immunity to the tumors in the autologous host shows that either the tumor exists in vivo in spite of a capacity of the lymphoid cells to kill it in vitro or such a capacity is gained during the 3- to 8-day interval between tumor removal and harvesting of lymphocytes. This may be used as a starting point to analyze host immune mechanisms against autologous tumor cells.

Two of three tumors induced by Dictabelt plastic film were similar to the MCA sarcomas in being sensitive to the CI effect of specifically immunized lymphocytes. The immunization had been performed by inoculating the lymphocyte donors with either heavily x-irradiated or untreated tumor cells; in the latter case, the lymphocytes had been harvested before tumor appearance. The CI effect with these film-induced tumors was, however, smaller than that obtained with MCA sarcomas. This agrees with the lower antigenicity of the film-induced tumors in vivo (*1*).

The addition of phytohemagglutinin was not necessary to demonstrate colony inhibition with immune lymphocytes sensitized against target-cell histocompatibility antigens. On the other hand, PHA is needed to demonstrate cytotoxicity with untreated lymphocytes (*7*).

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Food and Water Intake after Intrahypothalamic Injections of Carbachol in the Rabbit

Abstract. *Direct chemical stimulation with carbachol in the hypothalamus of the rabbit caused a significant increase in food intake, as a function of place stimulated and concentration. Lower doses of carbachol injected near the supraoptic nucleus produced an increase in drinking without an increase in eating.*

Direct injection of carbamylcholine chloride (carbachol) in certain areas of the brains of rats increases intake of water. Application of levarterenol bitartrate (*l*-norepinephrine) to identical sites induces increased food intake. These results have led to the hypothesis that feeding is mediated by an adrenergic mechanism, and that drinking is mediated by a cholinergic mechanism (*1*).

We tested the generality of effects of direct stimulation by these substances in the New Zealand albino rabbit. Chemicals dissolved in mammalian Ringer-Locke solution were used to control doses and *p*H, and to reduce possible damage at the injection site. Crystalline carbachol has the same effect on rats as solutions have (*2*).

Rabbits were implanted with cannulas that were made from tubing (22-gauge) cut to 24-mm lengths and held by a piece of Amphenol connector strip (*3*). A Trent-Wells stereotaxic instrument adapted for rabbits was used, and the cannulas were localized by use of an atlas for the rabbit brain (*4*). Placements were verified histologically. Injections were made by inserting an inner cannula (28-gauge tubing) into the outer cannula. Test solutions were delivered with a microsyringe attached to the inner cannula with polyethylene tubing. The rabbits had free access to water and dry food pellets. Beginning 1 week after the operation, we tested all animals every other day.

Various doses of carbachol were administered to each animal at least twice in different sequences (Fig. 1). Eating began 30 seconds to 3 minutes after

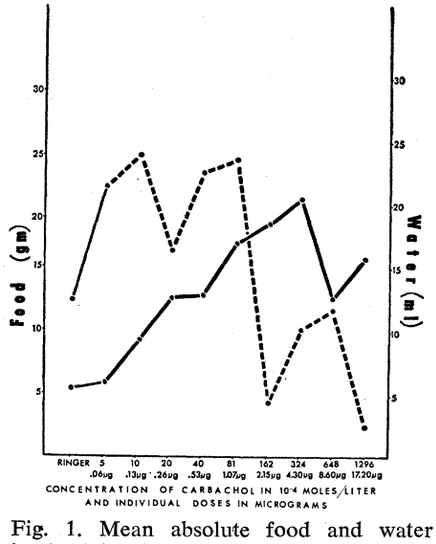

Fig. 1. Mean absolute food and water intake following injections of various concentrations of carbachol ($5 \times 10^{-4}M$ to $1296 \times 10^{-4}M$) and Ringer's solution. These data were obtained from eight animals. The solid line indicates food intake, and the dashed line indicates water intake.