transplanted ovary resembled in size and histologic pattern that seen in those castrate animals that develop adhesions or receive estrogen. As noted in our experiments, not only did the transplants behave as if they were under estrogenic stimulation, but also the uterus and vagina in the castrated Group C had the histologic pattern that results from estrogenic stimulation, which indicates that parahydroxypropiophenone apparently exerted its influence by its estrogenic activity. Group B with added thyroid behaved identically to Group A.

Conclusion. Parahydroxypropiophenone, when fed to castrate animals at a level of 100 mg a day, behaved like an estrogen by producing cornification of the vagina and hyperplasia and metaplasia of the uterine endometrium. Exogenous thyroid had no added effect other than that produced by parahydroxypropiophenone alone. The evolution of experimental ovarian tumors obtained by transplantation of an ovary to the spleen of a castrated rat was inhibited by parahydroxypropiophenone similarly to that reported when estrogens were given or when splenic adhesions circumventing the portal circulation were present.

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Resistance Induced by Alien Strain Mouse Lymphoid Tissue to Lymphosarcoma 6-C3H-ED in C3H Mice. (19304)

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The growth of transplanted tumors as influenced by the previous implantation of tumor cells or other tissues from alien strains of mice or rats has been the subject of much investigation. The literature up to 1929 has been reviewed by Woglom(1). Eisen and Woglom(2) reported protection of rats of strain 990 against the August carcinoma by implantations of embryo tissue from August strain rats, and Sturm(3) showed protection of Wistar rats against transplantable lymphatic leukemia by implantation of embryo or injection of blood from Wistar rats or embryo from Rockefeller Institute hooded rats. Protection of pure strain mice against growth of strain specific tumors (with the exception of certain leukemias) by such procedures has not been reported. Lewis(4) has shown that spindle cell sarcomas arising in inbred mice would grow progressively and kill when implanted in mice of the strain in which they originated. However, when transplanted in mice of alien genetic constitution the tumors regressed after initial growth. The sojourn of such tumors in alien strain mice conferred immunity against subsequent implantation of the same tumor. Implantation of various organs of alien strain mice had no inhibiting effect on the growth of the strain specific tumors. Data of similar significance were presented by Barrett(5). However, MacDowell, *et al.*(6) by prior implantation of embryo tissue from foreign strain mice, found that C58 mice were protected against strain specific transplantable leukemia, and Rhoads and Miller(7) similarly found that AR mice are protected against transplantable leukemia by courses of injections of emulsions of spleen and lymph node cells from the Rockefeller Institute strain of mice given prior to tumor cell implantation.

It was found during the course of studies on the progressive growth of lymphosarcoma 6-C3H-ED in CF1 mice treated with cortisone

acetate(8) that not only are CF1 mice in which the tumor had regressed immune, but similar immunity is conferred by the implantation of lymph node or thymus tissue from C3H mice. This observation led to attempts to protect C3H mice by the implantation of lymphoid tissue from CF1 mice. The present report is concerned with these results and those obtained using other tissues from CF1 mice, and from other random bred and pure line strains of mice.

Materials and methods. The Gardner lymphosarcoma 6-C3H-ED obtained from the Jackson Memorial Laboratory and carried in JAX C3H mice was used for implantation in all experiments. Implantation of the tumor was carried out by subcutaneous injection into the right axillary region of 0.1 ml of a cell suspension obtained by withdrawing the top portion from a suspension of tumor cells minced in saline. Counts indicated that the dose implanted contained approximately 5 million tumor cells. C3H mice so treated regularly die with enormous tumors within 18 to 22 days following implantation. Implantation of lymphoid tissues from donor mice was carried out using 18-gauge trocars loaded with one axillary and one inguinal lymph node and a ¼ portion of thymus. This tissue mixture was discharged subcutaneously into the left axillary region of recipient C3H mice. Foreign strain tumors (EO771 carried in C57 black mice, and lymphoid leukemia P1534 carried in DBA-2 mice), and other tissues were implanted in the same way. In most experiments spleen cell suspensions in saline were implanted by injection. 140 to 160 mg of spleen tissue was minced with scissors in 2.25 ml of saline and worked until it would pass through an 18-gauge needle. 0.25 ml volumes were injected into recipient mice. Specified tissues from various strains of mice were implanted subcutaneously in young adult 18 to 22 g C3H mice. After an interval (in most experiments 5 to 7 days) these mice were challenged on the opposite flank with 0.1 ml of a saline suspension of tumor cells. The mice were palpated at 2- or 3-day intervals, beginning on the fifth day following tumor cell implantation to determine growth of the tumor. Experiments were terminated 7 to 10 days after all tumor bearing mice had died. All survivors were then again challenged with lymphosarcoma cells to determine whether they were immune. C3H, DBA-2, C57 Black and CAF1 mice were obtained from the Jackson Memorial Laboratory. ZBC mice (produced by mating mice of the A strain and C3H (called Z strain) to produce F1 hybrids, and mating F1 females with Z males), were obtained from the surplus stock of Dr. J. J. Bittner, University of Minnesota. Random bred mice, CF1 and CFW, were obtained from Carworth Farms, and Manor mice were obtained from Manor Farms. Embryo tissue was obtained from pregnant CF1 females near term and mammary tissue from nursing CF1 dams. Male C3H mice were used in most experiments, but similar results have been obtained in the few experiments made with females.

Results. It was shown in repeated experiments that a significant number of C3H mice are protected against growth of lymphosarcoma 6-C3H-ED as a result of previous implantation of lymph node-thymus tissue from CF1 mice. It was of interest to determine whether the ability to protect against this tumor was peculiar to lymphoid tissue of CF1 mice, or whether other CF1 tissue would elicit this response. Tissue from other strains of mice were also studied as the work progressed. The results are tabulated in Table I.

It was found that lymph node-thymus mixtures from CF1 mice implanted in C3H mice 4 to 21 days prior to challenge with lymphosarcoma protected 119 (63%) of the 188 mice so treated. From 60 to 70% of mice implanted at intervals between these two extremes were protected in various experiments. This protection was manifest either by failure of the tumor to grow at the site of implantation or by regression of well defined palpable tumors. When the interval between tissue implantation and tumor cell challenge was reduced to 2 days, only 3 of 10 mice were protected, and none of the mice were protected which received the tissue on the same day, or 2 or 4 days after the day of tumor cell implantation. Spleen tissue of CF1 mice implanted either by trocar or as cell suspension protected 9 of 25 mice used

TABLE I. Growth of Lymphosarcoma 6-C3H-ED in C3H Mice as Influenced by Implantation of Tissues from Alien Strain Mice.

Species of tissue donor	Tissue implanted	Interval between tissue and tumor implantation, days	No. of mice in tests	Tumors failed to develop	Palpable tumors regressed	Progressive tumors	No. of mice protected	% protected
CF_1	Lymph node and thymus*	4-21 before tumor implantation	188	51	68	69	119	63
		2 " " "	10		3	7	3	30
		Same day	8			8		
		2-4 after tumor implantation	16			16		
C57 black		7 before " "	8			8		
DBA-2		"	6			6		
ZBC		"	8		2	6	2	25
CAF_1		"	8			8		
A		"	8			8		
Manor		"	11		6	5	6	54
CFW		"	8	1	2	5	3	37
C3H		"	10			10		
C57 black	Mammary carcinoma EO771*	"	8			8		
DBA-2	Lymphoid leukemia*	"	8			8		
CF_1	Embryo*	"	10			10		
	Leg muscle*	"	18			18		
	Heart muscle*	"	10			10		
	Mammary tissue*	"	8			8		
	Spleen†	5 "	8		3	5	3	37
	" *	7 "	10		2	8	2	20
	" *	"	7	2	2	3	4	57
ZBC	" †	"	15	13	2		15	100
	" †	5 "	8	7		1	7	87
	" *	7 "	7	6	1		7	100
Manor	" †	5 "	8		3	5	3	37
DBA-2	" †	"	8		2	6	2	25
C57 black	" †	"	8		2	6	2	25
CFW	" †	"	8		4	4	4	50
CAF_1	" †	"	8			8		
A	" †	"	8			8		
C3H	" †	"	8			8		
Untreated controls			112			112		

* Implanted by trocar. † Implanted as cell suspension.

in 3 experiments, but embryo skin, leg muscle, heart muscle, and mammary tissue from CF1 mice were without effect. Neither spleen nor lymph node-thymus mixture from CAF1, A or C3H mice protected C3H mice. Lymph node-thymus mixtures from C57 and DBA-2 mice failed to protect, but spleen tissue from both strains protected 2 of 8 mice. Both spleen and lymph node-thymus mixtures from Manor, CFW and ZBC mice gave protection. The results obtained with ZBC spleen are of particular interest; 29 of 30 mice treated in 3 experiments were protected. Mammary carcinoma EO771 growing in C57 mice, and lymphoid leukemia P1534 growing as a subcutaneous tumor mass in DBA-2 mice, when transplanted to C3H mice failed to protect against lymphosarcoma 6-C3H-ED subsequently implanted. Spleen or lymph node-thymus tissue from C3H mice implanted in C3H mice failed to protect. All of 112 untreated C3H control mice implanted with lymphosarcoma died of progressive tumor growth.

The high incidence of regression of palpable tumors in treated mice is of particular interest. The regressions observed followed a definite pattern with respect to time of occurrence. In mice implanted with spleen or lymphoid tissue 5 or 7 days prior to tumor cell implantation, rapid disappearance of large masses (1000-3000 mm^3) occurred between the fourteenth and sixteenth day after tumor implantation. All of the mice in which regression occurred, as well as those in which no tumor developed, were found to be immune upon challenge with lymphosarcoma cells 30 to 35 days following the original tumor implantation.

In experiments not recorded it was shown that such lymphosarcoma immune mice were not immune to Sarcoma 180 or Mammary Carcinoma C3H-BA.

Discussion. It is known that resistance to strain specific transplantable leukemia in inbred mice may be induced by injection of certain tissues from alien strain mice prior to tumor inoculation. The experiments reported in this paper establish the same fact for transplantable lymphosarcoma in C3H mice.

Lymph node-thymus or spleen tissue from CF1 mice implanted into C3H mice protect against progressive growth of lymphosarcoma 6-C3H-ED subsequently implanted. This property appears to be specific for these tissues since CF1 embryo, mammary gland, leg or heart muscle failed to induce resistance in similar experiments. In this respect our results differ from those of MacDowell *et al.* (6) who found C58 mice to be protected by implanting embryo tissue, but resemble those of Rhodes and Miller(7) who reported AR mice to be protected against transplantable leukemia by prior injections of lymph node and spleen mixtures from an alien strain.

The ability of spleen or lymphoid tissue to protect C3H mice against the C3H lymphosarcoma varied with the strain of donor mouse. Neither lymph node-thymus nor spleen of A or CAF1 mice protected. Lymph node-thymus from C57 and DBA-2 mice were inactive, while spleen from these strains protected a few mice in the various experiments. Both lymph node-thymus mixtures and spleen tissue from the random bred mice (CF1, CFW and Manor) were active. CF1 lymph node-thymus mixtures, and ZBC spleen tissue appeared to be the most active for inducing protection. ZBC spleen tissue was highly active; only 1 of 30 mice implanted with this tissue failed to resist lymphosarcoma growth on subsequent challenge. The reason for our failure to find more than partial protection of the mice in various experiments remains obscure, and we continue to seek the conditions necessary to increase the percentage of mice protected.

An interval of from 4 to 7 days between tissue implantation and subsequent tumor cell challenge appears to be necessary for significant protection. This is shown in the experiments done with CF1 thymus-lymph node implantation wherein over 60% of the mice implanted 4 or more days before tumor challenge were protected. Mice implanted with CF1 lymph node-thymus mixtures on the day of tumor implantation, or 2 or 4 days thereafter were not protected.

Summary. Lymph node and thymus mixtures, or spleen tissue from certain strains of mice implanted in JAX-C3H mice, 4-7 days

prior to subcutaneous injection of lymphosarcoma cells, protect some of the mice so treated against progressive growth of the tumor. This protection is manifest either by failure of the tumor to grow at the site of implantation or by regression of palpable tumors. Such protected mice are immune to subsequent reimplantation of the tumor. Mice implanted 2-4 days after, or on the day of tumor implantation are not protected. Embryo skin, mammary tissue, leg or heart muscle, from mice whose lymphoid tissue is active failed to protect. Under the conditions of the experiments tissue from some strains of mice were more powerful in eliciting protection than those of other strains.

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Retardation and Regression of Lymphosarcoma in C3H Mice Treated with Alien Mouse Spleen and A-Methopterin. (19305)

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It was shown in a previous publication(1) that lymph node-thymus mixtures, or spleen tissue from certain alien strains of mice, implanted into C3H mice induced a state of resistance to progressive growth of lymphosarcoma 6-C3H-ED subsequently implanted. This resistance was well developed in mice implanted 4 to 21 days prior to tumor implantation, and less so in mice implanted 2 days before, but was not evident in mice implanted with lymphoid tissue on the same day or 2 to 4 days following the day on which tumor cells were introduced. Spleen tissue from ZBC mice was found to be particularly active in eliciting the resistant state. It was postulated that advantage could be taken of this to treat C3H mice bearing lymphosarcoma if the growth of the tumor could be retarded long enough for the resistant state to develop. The present paper is concerned with experiments in which delay of tumor growth was attempted by using A-methopterin, and by use of small numbers of malignant cells to induce tumors.

Materials and methods. Lymphosarcoma 6-C3H-ED obtained from the Jackson Memorial Laboratory and carried in JAX-C3H mice was used in all experiments. This tumor arose in C3H mice, and in our experience grows progressively and kills all C3H mice implanted with it. Eighteen to 22 g C3H mice were employed as test subjects. The tumor was induced by subcutaneous injection of 0.1 ml volumes of saline suspension of minced tumor cells from 10- to 14-day-old tumors in C3H mice. Tumor cell dosage was determined by hemocytometer counts. Spleens removed from ZBC mice were used. (These mice are a backcross generation to the C3H strain of Dr. J. J. Bittner, University of Minnesota).* Several spleens were pooled and minced with scissors and suspended in saline. The mice received one 0.25 ml injection of spleen cell suspension through an 18-gauge needle into the left flank. The dose represented approximately 20 mg of moist spleen tissue. A-methopterin was administered sub-

* ZBC mice are produced by mating mice of the A strain and C3H (called Z strain) to produce F_1 hybrids. The F_1 females are crossed with Z males and the resulting animals are ZBC's.