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.

1. Woglom, W. H., *Cancer Review,* 1929, v4, 293.
2. Eisen, M. J., and Woglom, W. H., *Cancer Research,* 1941, v1, 629.
3. Sturm, E., *Cancer Research,* 1941, v1, 627.
4. Lewis, M. R., *Bull. Johns Hopkins Hosp.,* 1940, v67, 325.
5. Barrett, M. K., *J. Nat. Cancer Inst.,* 1940, v1, 387.
6. MacDowell, E. C., Taylor, M. J., and Potter, J. S., *Proc. Nat. Acad. Sci.,* 1935, v21, 507.
7. Rhoads, C. P., and Miller, D. K., PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 817.
8. Foley, E. J., and Silverstein, R., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 713.

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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.

cutaneously in the abdominal region. The mice were palpated daily beginning on the fifth day following tumor cell implantation, and observations were continued for a 45-day period. Additional details are given in connection with the various experiments.

Experimental. A-methopterin (4-amino-N-methyl folic acid) is known to retard the growth of lymphosarcoma 6-C3H-ED in C3H mice. As a preliminary step an experiment was set up to determine whether this compound influences the development of the resistance induced in C3H mice by implantation of ZBC spleen.

Two groups of 15 mice each were implanted subcutaneously in the left flank with ZBC spleen cell suspension. After 5 days these mice and 15 untreated controls were implanted on the right flank with approximately 5,000,000 lymphosarcoma cells. Drug treatment was begun on the day of tumor implantation as follows: Group 1, A-methopterin 2 mg/kg/day for 11 consecutive days; Group 2 received no drug; Group 3 served as untreated controls. Mice treated with spleen and A-methopterin failed to develop tumors, as did those treated with spleen alone. All untreated controls died with enormous tumors on the 21st to 23rd day.

The experiment showed that spleen cell induced resistance to lymphosarcoma developed in mice being treated with A-methopterin. An experiment was done in which 3 groups of C3H mice were implanted with different doses of lymphosarcoma cells 48 hours before starting treatments. One group (A) was implanted with 5,000,000, another (B) with 500,000, and the last (C) with 50,000 tumor cells. These 3 groups were then subdivided into 4 additional groups: one of which received A-methopterin, 2 mg/kg/day for 11 consecutive days; another group received one injection of ZBC spleen cell suspension; the third, one injection of ZBC spleen cell suspension, and A-methopterin 2 mg/kg/day for 11 consecutive days; the fourth group served as untreated controls. The results are shown in Experiment 1 in Table I. Other experiments were made in which groups of C3H mice were implanted with approximately 250,000 tumor cells; treatments were begun in one, 24 hours, and in the other 96 hours following tumor cell implantation. Results of this work are shown in Experiments 2 and 3 in Table I.

It is seen in Experiment 1 that among the mice implanted with 5,000,000 tumor cells 48 hours before treatments were begun, 3 of the 5 mice which survived the combination treatment with ZBC spleen and A-methopterin were protected as compared with 1 of 8 treated with A-methopterin alone. All of the ZBC spleen-treated mice and all controls died of progressive tumor growth. Of those mice implanted with 500,000 tumor cells and given the combination treatment 5 out of 8 were protected; of those treated with A-methopterin alone 2 of 8 were protected; one regression occurred among the 6 implanted with spleen tissue. Of those implanted with 50,000 tumor cells, all 5 mice receiving the combination treatment were protected, 2 out of 4 surviving the A-methopterin treatment were protected, and in 4 of the 5 receiving spleen implantation alone regression of palpable tumors occurred.

Untreated mice receiving larger numbers of tumor cells showed earlier appearance of tumors and died more quickly than those having received fewer cells. Those implanted with 5,000,000 tumor cells were palpable on the 5th day and all died on the 20th to 21st day; those implanted with 500,000 cells became palpable on the 7th day and died on the 24th day, and those implanted with 50,000 cells were palpable on the 10th day and all died on the 26th day. This experiment illustrates effective treatment of C3H mice with lymphosarcoma 6-C3H-ED.

In Experiment 2, similar results were obtained with mice in which treatment was delayed 24 hours after the implantation of 250,000 tumor cells. All 7 mice surviving the combination treatment with spleen cells and A-methopterin failed to develop palpable tumors; 6 of 8 treated with spleen cells alone were similarly protected; and 4 of 8 with treated A-methopterin alone were protected.

When mice were implanted with 250,000 tumor cells and treatment delayed for 4 days (Experiment 3), 5 of 8 mice treated with A-methopterin, and 4 of the 7 mice surviving the combination spleen cell and A-methopterin

TABLE I. Effect of Treatment with Alien Mouse Spleen, A-methopterin, and a Combination of Both on Growth of Lymphosarcoma 6-C3H-ED in C3H Mice.*

Experiment	Delay following tumor implantation before treatment was begun	No. of tumor cells implanted	Treatment	No. of mice in groups	No palpable tumors	Palpable tumors regressed	Progressive tumors	Died during treatment	No. of mice protected
1 (A)	48 hr	5000000	A-methopterin†	8		1 (23)‖	7		1
	,,	,,	ZBC spleen	8			8		
	,,	,,	A-methopterin† + ZBC spleen	8		3 (19)	2	3	3
	,,	,,	None	8			8		
(B)	48 hr	500000	A-methopterin†	8	2		6		2
	,,	,,	ZBC spleen	6		1 (17)	5		1
	,,	,,	A methopterin† + ZBC spleen	8	4	1 (35)	3		5
	,,	,,	None	8			8		
(C)	48 hr	50000	A-methopterin†	5		2 (31–35)	2	1	2
	,,	,,	ZBC spleen	5		4 (18–20)	1		4
	,,	,,	A-methopterin† + ZBC spleen	5	5				5
	,,	,,	None	5			5		
2	24 hr	250000	A-methopterin‡	8	3	1 (32)	4		4
	,,	,,	ZBC spleen	8	2	4 (16)	2		6
	,,	,,	A-methopterin‡ + ZBC spleen	8	7				7
	,,	,,	None	8			8		
3	96 hr	250000	A-methopterin§	8	3	2 (32–34)	3		5
	,,	,,	ZBC spleen	8			8		
	,,	,,	A-methopterin§ + ZBC spleen	8	2	2 (33–35)	3	1	4
	,,	,,	None	8			8		

* Tabulation 45 days after tumor implantation. † 2 mg/kg/day for 11 days. ‡ 2 mg/kg/day for 10 days. § 2 mg/kg/day for 7 days. ‖ Day on which regression was complete.

treatment were protected. The 8 control mice as well as the 8 receiving spleen cells alone all died of progressive tumor growth.

Discussion. The data show that C3H mice in which treatment was begun 24 or 48 hours subsequent to implantation of lymphosarcoma cells may be protected from progressive tumor growth by a combination treatment consisting of a single injection of spleen tissue and daily injection of A-methopterin. Treatment with either spleen cell suspension alone, or A-methopterin alone is less effective. When treatment is begun 4 days following implantation of the tumor, 5 of 8 mice treated with A-methopterin alone, and 4 of 7 mice (which survived combination treatment with A-methopterin and spleen cell suspension) were protected from progressive tumor growth. It would appear that under these conditions, tumor growth is retarded sufficiently to allow development of a state of resistance as a result of ZBC spleen cell implantation. This is shown by the greater number of protected mice among those treated with the combination than with either treatment alone (Experiments 1 and 2). Development of a resistant state is clearly indicated by the regression observed in mice implanted with 50,000 tumor cells and treated with a single implantation of ZBC spleen tissue in Experiment 1 and in those implanted with 250,000 tumor cells and similarly treated in Experiment 2.

The regressions observed in mice treated with A-methopterin alone, with the exception of one mouse (Experiment 1 (A)) all took place within 31 to 35 days following tumor cell implantation. This is in contrast to the regressions observed in mice treated with ZBC spleen cells alone (Experiment 1 and 2) which occurred 17 to 20 days after tumor cell implantation. In Experiment 3 (in which treatment was delayed 4 days) the regressions

observed took place 32 to 35 days after tumor cell implantation. The mechanism responsible for these regressions has not been determined and is the subject of further study.

Summary. Under certain conditions C3H mice implanted with lymphosarcoma 6-C3H-ED were protected either by injection of ZBC spleen tissue alone or by A-methopterin treatment alone. A more successful treatment consisted of a single subcutaneous injection of ZBC spleen tissue with daily doses of A-methopterin. Mice which had received tumor implantations 24, 48 or 96 hours preceding therapy were treated with this combination. Some were not protected, but in others tumors either failed to become palpable, or having become palpable appeared to have regressed completely.

ADDENDUM. The mice enumerated in the column, "Number of Mice Protected," shown in Table I, were challenged for immunity by injection of 5,000,000 lymphosarcoma cells subcutaneously into the left flank. The mice in Exp. 1 and 2 were challenged on the 42nd, and those in Exp. 3 on the 40th day of the experiment. Immune mice either developed small tumors which regressed, or no tumors at all; non-immune mice died within 30 days with progressive lymphosarcoma. Of the 14 mice which had been treated with A-methopterin alone in the three experiments, 6 were immune; of 11 which had been treated with ZBC spleen alone all were immune, while of the 24 which had received the combination treatment (A-methopterin and ZBC spleen) 20 were immune.

1. Foley, E. J., PROC. SOC. EXP. BIOL. AND MED., 1951, in press.

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Replacement of Protogen by Lipoic Acid in the Growth of *Tetrahymena*. (19306)

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The ciliate *Tetrahymena geleii* is readily grown in synthetic medium comprising, with only one exception, known chemicals. The one unknown factor is the liver fraction concentrate, protogen(1). Protogen has been identified(2) with the acetate(3) and the pyruvic oxidation(4) factors. Kidder and Dewey(5) found, as would thus be expected, that in *T. geleii,* protogen serves at least one other function than the replacement of acetate.

Reed and co-workers have recently(6,7) purified a series of factors from liver residues which show high activity in replacing both the acetate and pyruvic oxidation factors. One of these factors has been crystallized(8) and named, tentatively at least, lipoic acid. It was thus of interest to ascertain if this compound may replace protogen in the growth of *Tetrahymena geleii.*

Methods. Tetrahymena geleii S(9) was grown in the synthetic medium described by Elliott(10). Protogen was omitted from the medium and was replaced by varying amounts of lipoic acid.* Inoculations were made in all cases by loop transfer. Cells for inoculation into the first experimental culture were washed with 500 volumes of distilled water before transfer, to assure against a carry over of material. The data presented was obtained from cultures which had gone through three sub-cultures in the experimental medium. Growth was measured turbidimetrically after 96 hours growth at 25°C and is expressed as optical density.

Results and discussion. Fig. 1 shows that lipoic acid in concentrations of 1 unit†/ml and higher is capable of maintaining growth

* Protogen was generously supplied by Dr. E. L. R. Stokstad. Concentrates of lipoic acid were kindly supplied by Dr. L. J. Reed.

† One unit is equivalent to the manometric pyruvate oxidase response produced by 1 mg of yeast extract.