hand, both estrogen and progesterone decrease the activity below that present in the uterus of the castrate and the enzyme does not appear in the endometrial stroma unless the latter is first traumatized. It is evident that there is a considerable species difference both in distribution of phosphamidase in the endometrium and in its hormonal regulation.

Meyer and Weinmann(9,10,11) have studied the distribution of phosphamidase in a number of rat organs and have correlated the enzyme activity with the physiological processes known to occur in the same sites. They have found phosphamidase to be associated with (1) active transport and other types of physicochemical work, (2) synthesis of nonprotein substances in both exocrine and endocrine glands, and (3) cellular differentiation. These processes are known to take place in various component tissues of rat endometrium and are largely dependent upon stimulation by estrogen and progesterone. Since the present experiments have demonstrated that both these hormones depress phosphamidase in the castrate rat uterus, the hypothesis of Meyer and Weinmann cannot be supported in this instance. In the deciduomal reaction, on the other hand, where there is a centrifugally progressive differentiation of stromal cells into decidual-like cells, phosphamidase activity approximates that which would be expected on the basis of the experience of Meyer and Weinmann. The present observations reemphasize the paucity of information concerning the metabolic role of phosphamidase and the need for further study of its activity in other tissues and organs.

Summary. The sites of phosphamidase were localized histochemically and amount of enzyme activity measured quantitatively in the uteri of untreated, estrogen- and progesterone-treated, and deciduomata-bearing ovariectomized rats. Enzyme activity was found in the lumenal and glandular epithelia of the endometria of all the animals studied. In addition, phosphamidase was present in the differentiating stromal cells in the uteri in which the deciduomal response had been elicited. Estrogen in high dosage and progesterone depressed the enzyme activity compared with that in untreated castrates. During the development of deciduomata the enzyme level rose to over 5 times that in animals treated with progesterone but not traumatized.

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Antitumor Activity of 1-β-D-Arabinofuranosylcytosine Hydrochloride. (26335)

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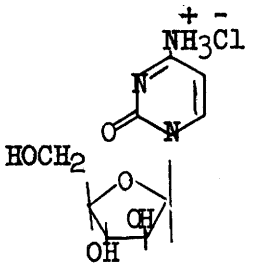
In this laboratory as well as others, investigations of purine and pyrimidine derivatives as potential antimetabolites which might inhibit *in vivo* biosynthesis of DNA and/or RNA have lead to development and clinical study of such compounds as 6-mer-

captopurine(1), psicofuranine(2), 5-fluoro-2′-deoxy-uridine(3), 5′-iodo-2′-deoxyuridine (4), etc.

Preliminary assays in this laboratory revealed that 1-β-D-arabinofuranosylcytosine hydrochloride [I]* exhibited activity against Sarcoma 180. Walwick, Roberts and Dekker(5) reported the preparation of the free base, "3-β-D-arabinofuranosylcytosine." Pizer and Cohen(6,7) investigated the metabolic activity of this base ("Spongocytidine") and related compounds in mutants of 2 strains of *Escherichia coli*. Evidence for the antitumor activity of I against Sarcoma 180, Ehrlich's carcinoma, and L-1210 leukemia is here presented.



(I)

Experimental. 1-(2′,3′,5′-Tri-O-acetyl-β-D-arabinosyl)-uracil(8) was converted into the base of I by a process essentially identical with that of Fox, *et al.*(9), and purified as the hydrochloride (I) which was used in the biological studies described below. The structure of I, in addition to its method of synthesis, elementary analysis, ultraviolet and infrared absorption spectra, was shown by its deamination with nitrous acid to 1-β-D-arabinofuranosyl uracil(8), by paper chromatographic detection of D-arabinose as the sole sugar moiety following the degradation of I according to the procedure of Burke (10), and by conversion of I into a crystalline product the properties of which were in good agreement with those described by Walwick, *et al.*(5), for "3-β-D-arabinofuranosylcytosine."

Female Swiss mice, weighing 18-20 g each, from Upjohn pathogen-free stock were used for Sarcoma 180 and Ehrlich carcinoma studies. L-1210 leukemia was carried in female BDF_1 mice from Diablo Labs., Berkeley, Calif. Sarcoma 180 and Ehrlich carcinoma were transplanted by subcutaneous injection of 500,000 to 650,000 cells into the groin. The drug (I) was given intraperitoneally once daily as indicated. The solid tumors were measured in 2 diameters periodically. Survival and complete regressions of the tumors were recorded. In studies using L-1210 leukemia comparison of mean survival time between treated and control groups was the criterion of activity.

The significance of the number of regressions and no takes was determined using the tables of Mainland and Murray(11). Standard errors were calculated.

Results. The acute LD_{50} of 1-β-D-arabinofuranosylcytosine hydrochloride (I), in either mice or rats, was greater than 1000 mg/kg. The animals were observed for 7 days for evidence of delayed toxicity. The results of tests of I against recently transplanted and established Sarcoma 180 are given in Tables I and II. It was observed that when dosage was begun the day after transplanting the tumor (T_1) and continued for 7 days, a high percentage of the mice showed no tumors 7 days after discontinuing the drug. Continued observation of the mice over a 25-30 day period showed that many of the tumors which did develop regressed completely without becoming necrotic and perforating.

When treatment was delayed until 5 days after transplanting the tumor, although there was no significant difference in number of tumor takes between treated and control groups, a significantly greater number of regressions occurred in the treated group. Preliminary studies showed that the activity of I orally in mice bearing Sarcoma 180 was one-fifth that found by the intraperitoneal route.

Results of studies on mice bearing the solid form of Ehrlich carcinoma (Tables I and II) indicate a similar type of activity. When treatment was delayed for 7 days after transplanting the tumor, the size of the tumors in the treated group was significantly

* Details of the synthesis of I will be reported elsewhere by one of us (J.H.H.).

TABLE I. Effect of 1-β-D-Arabinofuranosylcytosine Hydrochloride on Sarcoma 180 and Ehrlich Carcinoma.

Tumor	Dosage,* mg/kg/day	No. mice	Day of reading†	No. of tumors	Avg T. meas. ± stand. error, mm
S-180	5	10	8	10	9.3 ± .35
			17	10	15.3 ± 1.07
"	10	10	8	5	3.7 ± 1.23
			17	9	10.8 ± 1.41
"	20	10	8	2	1.4 ± .93
			17	4	4.2 ± 1.74
"	50	20	8	0	.0
			17	3	1.4 ± .78
"	—	20	8	20	10.8 ± .30
			17	20	16.4 ± .41
E. carc.	25	20	8	4	1.6 ± .72
			14	10	4.5 ± 1.08
"	—	20	8	20	11.8 ± .39
			14	20	15.9 ± .62

* Treatment for 7 days starting 24 hr after transplanting tumor.
† Days after transplanting tumor.

TABLE II. Effect of 1-β-D-Arabinofuranosylcytosine Hydrochloride on Established Sarcoma 180 and Ehrlich Carcinoma.

Tumor	Dosage, mg/kg/day	Dosage schedule*	No. mice	Day of reading*	No. of survivors	No. of tumors†	Avg T. meas. ± stand. error, mm
S-180	50	$T_5 \rightarrow T_{12}$	20	T_5	20	11	4.48 ± .95
				T_{12}	20	12	4.98 ± .94
				T_{35}	19	4 (11)	3.47 ± 1.68
"	—	—	20	T_5	20	16	7.28 ± .90
				T_{12}	20	20	12.70 ± .60
				T_{35}	19	16 (3)	19.08 ± 2.28
"	50	$T_7 \rightarrow T_{12}$	20	T_8	20	20	10.72 ± .42
				T_{10}	20	19	9.97 ± .68
				T_{33}	18	8 (10)	7.50 ± 2.25
"	—	—	20	T_5	20	20	10.77 ± .30
				T_{10}	20	20	13.25 ± .38
				T_{33}	20	20 (0)	22.23 ± 1.03
E. carc.	50	$T_5 \rightarrow T_{12}$	20	T_5	20	15	7.50 ± 1.04
				T_{14}	20	13	6.40 ± 1.12
				T_{33}	19	10 (6)	8.47 ± 2.23
"	50	$T_7 \rightarrow T_{14}$	20	T_5	20	17	8.98 ± .93
				T_{14}	20	19	11.58 ± .80
				T_{33}	19	16 (3)	12.50 ± 1.71
"	—	—	20	T_5	20	20	11.75 ± .39
				T_{14}	20	20	15.92 ± .62
				T_{33}	19	18 (1)	23.84 ± 2.21

* Subscripts indicate days after tumor implantation.
† No. of complete tumor regressions is given in parentheses.

smaller than the controls ($P = 0.01$).

1 - β - D - Arabinofuranosylcytosine hydrochloride when given to BDF_1 hybrid mice bearing the ascitic form of L-1210 leukemia produced a 260% increase in mean survival time when treatment was started on T_1. When start of treatment was delayed to the fourth day (T_4), a 12.5% increase in survival time was noted (Table III). The mice treated with the nucleoside starting on the fourth day showed complete absence of the ascitic fluid usually found in the controls and no gross evidence of solid tumors in and around the mesentery. Preliminary studies of the leukocyte and differential counts of treated L-1210 leukemic mice indicate that 1-β-D-Arabinofuranosylcytosine hydrochloride caused a leukopenia which was not pres-

TABLE III. Effect of 1-β-D-Arabinofuranosylcytosine Hydrochloride on L-1210 Leukemia.

Dose	Dosage schedule	No. mice	Avg body wt, g	50% surv., days	Time + range, days
50 mg/kg	$T_1 \rightarrow T_7$	10	19.1	18.0	18– *
Saline controls		10	18.2	7.0	7– †
50 mg/kg	$T_4 \rightarrow T_{11}$	10	19.7	9.0	8–15
Saline controls		10	19.9	8.0	7–12

* 2 mice survived for 28 days. All deaths on either 18th or 19th days.
† *Idem* . All deaths on either 7th or 8th days.

ent in treated nonleukemic mice. In L-1210 leukemic mice, average leukocyte counts were 15,500 cells per cu mm (range 9,750-23,750) when treatment was initiated on T_4. On T_{11}, the average leukocyte counts of 4 treated mice were 1,810 cells per cu mm (range 750-3,750) and that of 5 controls 52,000 cells per cu mm (range 18,250-88,000). In contrast, in 5 normal treated mice the leukocyte counts averaged 17,400 cells per cu mm (range 9,000-24,500) on the initial day and 12,600 cells per cu mm (range 6,500-17,300) after 7 days of treatment. In both leukemic and normal mice, number of polymorphonuclear neutrophils decreased more than other cells.

Twenty-five or 50 mg/kg of I given to rats bearing Walker 256, Murphy-Sturm and Guerin tumor for 10 days starting 24 hours after transplanting the tumor produced no tumor inhibition and did not affect normal growth of the rats.

The nucleoside (I) was relatively inactive as a cytotoxic agent against KB cells in tissue culture or when Sarcoma 180 or Ehrlich carcinoma cells were held at room temperature with the compound in the medium for 30 minutes prior to implantation.

Discussion. 1-β-D-Arabinofuranosylcytosine hydrochloride has been shown to be active against Sarcoma 180, E. carcinoma and L-1210 leukemia in experimental mice. The supply of material has limited testing of the compound at higher dosage levels in the tumor-bearing rat. The results in the 2 species are similar to those observed by Welch(12) using 5-iodo-2′-deoxyuridine.

It was considered possible that the antitumor activity of I might have resulted from deamination *in vivo* to 1-β-D-arabinofuranosyluracil. However, a preliminary test using the latter compound at 100 mg/kg/day for 7 days against a recently transplanted S-180 showed only weak activity. Studies on the mechanism of action of I are in progress.

Summary. 1-β-D-Arabinofuranosylcytosine hydrochloride has been shown to be active against recently transplanted and established Sarcoma 180, Ehrlich carcinoma and L-1210 leukemia in mice. This nucleoside was inactive at the same dose per kilogram of body weight in tumor-bearing rats.

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