lated to the observed immunity in the nursing mother.

8. Milk collected from normal mice contained no antiviral substances while milk from immune mice was capable of neutralizing the virus *in vitro*.

9. The data indicate that the protection of young mice is passive in nature and probably brought about by the ingestion of antiviral substances (antibodies?) in the milk of immune mothers. There is no evidence in this work to suggest that the offspring of infected mothers may acquire a state of active immunity through inapparent infection.

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Effect of Purified Hyaluronidase on Growth of Sarcoma 37 in the Mouse. (18052)

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Several studies of the effect of hyaluronidase on the growth and invasiveness of experimental tumors have been reported with contradictory results ranging from enhancement (1,2) to inhibition(3,4) and no effect(5,6,7). In all these studies crude preparations and undetermined doses of enzyme were administered. It seemed desirable, therefore, to carry out further studies with massive doses of a highly purified hyaluronidase of the quality used as a therapeutic and diagnostic agent.*

Materials and methods. One hundred and sixty-three male Swiss mice weighing between 17 and 20 g were implanted subcutaneously near the axilla with fragments of sarcoma 37 measuring approximately 2 sq mm.† The animals were injected daily either intravenously or subcutaneously with physiological salt solution (PSS), hyaluronidase dissolved in PSS, or heat inactivated hyaluronidase in PSS, as outlined in Table I. In Group VII the tumor implant consisted of a standardized macerated suspension of a 7 days old tumor mixed with 20 mg hyaluronidase. The subcutaneous injections were administered apart from the immediate site of tumor implantation. The hyaluronidase* was prepared from bull testes and assayed 6300 turbidity reducing units per mg of nitrogen. Inactivated hyaluronidase was prepared by heating the enzyme at 60°C for 2 to 6 hours. All injections were begun either on the day of implantation or on the seventh day following implantation. Thirteen to 16 days after implantation the mice were killed and the tumors dissected out and wet weighed. Sections of the following organs and tissues were examined for metastases by Dr. W. E. Ehrich of the Philadelphia General Hospital: liver, lungs, thymus gland, kidneys, salivary and mesenteric lymph nodes, adrenal glands, thyroid gland, salivary glands, pituitary gland, and bone marrow.

Results. Intravenous administration. In Group I in the control series of mice, which received PSS only, the tumors grew to a size which was significantly larger than those in mice which had received 10 mg hyaluronidase daily from the day of implantation. In Group

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* Wydase, Wyeth.

† A transplant of mouse sarcoma 37 was kindly supplied by Dr. H. J. Creech of the Lankenau Hospital Research Institute.

TABLE I.
Effect of Hyaluronidase on the Growth and Invasibility of Mouse Sarcoma 37.

Group	No. of mice	Material injected	Amt inj. daily (mg)	Period of inj. (days)	Total dose inj. (mg)	Route of inj.	Tumor wt* (g)	Lymph node metastasis
I	4	Hyaluronidase	10	10	100	Intrav.	.21	No exam.
	4	"	10	6‡	60	"	.32	" "
	4	PSS†	—	10	—	"	1.12	" "
II	10	Hyaluronidase	30	11	330	"	.77	1
	9	"	30	7‡	210	"	.42	3
	8	PSS	—	11	—	"	.70	1
III	11	Hyaluronidase	20	11	220	Subcut.	.089	0
	13	PSS	—	11	—	"	.34	0
IV	8	Hyaluronidase	10	11	110	"	.25	1
	8	Heat inactiv. hyaluronidase	10	11	110	"	.44	0
	8	PSS	—	11	—	"	.44	1
V	10	Hyaluronidase	2	9	18	"	.86	5
	10	Heat inactiv. hyaluronidase	2	9	18	"	.40	5
	9	PSS	—	9	—	"	.45	1
VI	7	Hyaluronidase	2	9	18	"	.27	1
	6	Heat inactiv. hyaluronidase	2	9	18	"	.32	1
	8	PSS	—	9	—	"	.67	0
VII	12	Hyaluronidase	2	10	40§	"	.56	0
	14	PSS	—	10	—	"	.42	0

* Log geometric mean.
† Physiological salt solution.
‡ Initial injection 7 days following tumor implantation.
§ Includes 20 mg hyaluronidase injected with tumor cell suspension.

II, where the mice received more hyaluronidase than in any one of the other groups (30 mg daily), two experiments carried out concurrently (except that the initial injection of hyaluronidase in one experiment was 7 days following the tumor implantation rather than on the same day) indicate that there was no striking effect on the growth or invasibility of sarcoma 37 (Table I).

Subcutaneous administration. A striking inhibitory effect on the growth of sarcoma 37 was shown by mice which received 20 mg hyaluronidase daily from the day of implantation (Group III). Where one-half this amount of enzyme was injected (Group IV) the tumors were likewise smaller than those in the PSS or inactivated hyaluronidase series. Of 2 groups of mice receiving 2 mg hyaluronidase daily (Groups V and VI), enhancement of tumor growth occurred in only one group. Mice injected with tumor cell suspension mixed with 20 mg hyaluronidase and then treated daily with 2 mg hyaluronidase (Group VII) did not show an increase in size of tumors as compared with the saline controls.

Microscopic examination of the liver, lungs, thymus gland, kidneys, thyroid gland, adrenal glands, salivary glands, pituitary gland, and bone marrow did not reveal metastasis.

Discussion. Because of the spreading action of hyaluronidase it might be assumed that the enzyme enhances the growth of tumors and encourages metastasis. However, a tumor probably requires the ground substance of connective tissue for growth. The intercellular ground substance cement is rich in hyaluronic acid, a mucopolysaccharide which is depolymerized by hyaluronidase. This enzyme, therefore, might conceivably even interfere with tumor growth, at least

until a sufficient antihyaluronidase titer had developed to prevent the enzyme from reacting with the mucopolysaccharide.

Although the data obtained are not altogether consistent, hyaluronidase did not appear to enhance the growth or invasibility of the tumors. The average weight of all the tumors of mice which received hyaluronidase is 18% lower than the average for the tumors in the PSS control series. Neither the concentration of enzyme nor the route of administration was a determining factor in the rate of tumor growth.

Summary and conclusions. 1. One hundred and sixty-three male Swiss mice weighing between 17 and 20 g were implanted subcutaneously with fragments of sarcoma 37 and then injected subcutaneously or intravenously beginning on the day of implantation or 7 days thereafter with hyaluronidase, heat inactivated hyaluronidase, or physiological salt solution.

2. The results on the effect of hyaluronidase administered subcutaneously or intravenously were somewhat variable, from which it is concluded that there was no effect on the growth or invasibility of implanted sarcoma 37. Neither was there metastasis to distant organs.

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Growth Retardation of Lymphosarcoma Implants in Pyridoxine-Deficient Rats by Testosterone and Cortisone. (18053)

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Previously it was shown that pyridoxine is more important than other dietary essentials for the maintenance of normal(1) and of neoplastic(2) lymphoid tissue. The administration of "glucocorticoids" as well as of androgenic steroids, like pyridoxine deprivation, produces a striking loss of normal lymphoid tissue(3). An influence of adrenal cortical secretion upon neoplastic lymphocytes was first demonstrated by Murphy and Sturm(4). Actual regression of lymphosarcoma transplants in mice, though only temporary, was observed by Heilman and Kendall (5) following the administration of cortisone. Androgens have so far not been reported to affect neoplastic lymphoid tissue and it has been concluded by Biesele and Gasic(6) that testosterone propionate does not diminish the chromosome size of neoplastic lymphocytes as it does that of normal lymphocytes. In the present experiments, the effect of cortisone was studied in lymphosarcoma-bearing rats, rendered deficient in vitamin B_6 and was compared to that of methyl testosterone.

Fifty male rats of the Sprague-Dawley strain of about 200 g body weight were divided into 7 groups as indicated in Table I. They were fed a diet composed of Casein Labco, 30%; Dextrose, 56%; Crisco, 10%; U.S.P. Salt Mixture No. 1, 4%; Thiamine, .001%; Riboflavin, .002%; Pyridoxine, .001%; Ca Pantothenate, .01%; Nicotinamide, .01%; Choline, 0.1%; Inositol, .05% except as follows: for Groups V, VI and VII, Pyridoxine HCl was omitted from the ration as indicated in the table. Cortisone acetate and methyl testosterone, suspended in saline, were ad-

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