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)

HERBERT C. STOERK

From the Merck Institute for Therapeutic Research, Rahway, N. J.

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-

1. Stoerk, H. C., PROC. SOC. EXP. BIOL. AND MED., 1946, v62, 90.

2. Stoerk, H. C., *J. Biol. Chem.*, 1947, v17, 437.

3. Hammar, J. A., *Die Normal Morphologische Thymusforschung*, Barth Verlag, Leipzig, 1936.

4. Murphy, J. B., and Sturm, E., *Science*, 1944, v99, 303.

5. Heilman, F. R., and Kendall, E. C., *Endocrinology*, 1944, v34, 416.

6. Biesele, J. J., and Gasic, G., *Cancer Research*, 1947, v7, 65.

TABLE I.
Effect of Cortisone and of Methyl Testosterone Upon Growth of Lymphosarcoma Implants in Pyridoxine Deficient Rats.

Exp.	Group	Diet	Injection	Tumor wt, g (estimated) 1 wk	2 wk	3 wk	Days avg survival	No. of rats (male)
A	I	Complete	None	8	53	71	—*	6
	II	"	Cortisone (2 mg)	6	42	55	—	6
	III	"	Meth. test. (2 mg)	7	23	46	—	6
B	IV	Complete	None	5	31	61	20	8
	V	B_6 defic.†	"	4	18	25	23	8
	VI	" "	Cortisone (2 mg)	3	7	13	18	8
	VII	" "	Meth. test. (2 mg)	3	8	15	26	8

* Not determined.
† B_6 deficient diet + 2 mg of desoxypyridoxine daily.

ministered daily by subcutaneous injection. Two mg of desoxypyridoxine was fed daily by stomach tube to the animals of Groups V, VI and VII. The rats in Groups I to VII were inoculated under sterile precautions with fragments of the Murphy rat lymphosarcoma*. The implants were placed under the skin of the lower back. Twice weekly the animals were weighed and measurements taken of 3 cross diameters of the tumor implants. The approximate weight of the tumors was derived from a standard curve obtained from a control series in which the calculated volume was compared with the actual weight of dissected tumors. The dietary regimen and the injections in experiments A and B were begun the day after inoculation.

The findings summarized in Table I show that daily injections of cortisone and of methyl testosterone cause moderate but definite retardation of growth of the lymphosarcoma implants (Exp. A, Groups I, II and III). Comparing the 4 groups in experiment B, it is seen that "acute" pyridoxine deficiency (Group V) causes a depression of lymphosarcoma growth which was greater than that produced by either one of the two steroids (Groups II and III). However, the administration of cortisone or of methyl testosterone to animals deprived of vitamin B_6 (Groups VI and VII) resulted in marked suppression of the growth of the tumor implants. As in the animals on the complete diet, this action of cortisone was no greater than that of methyl testosterone.

In mice inoculated with lymphosarcoma (6C3H-ED) we have observed, in accordance with previous reports(5), that treatment with cortisone produced actual regression of this neoplasm. If cortisone treatment was combined with administration of the antivitamin, desoxypyridoxine, very rapid disappearance of the lymphosarcoma implants occurred; well established tumors disappeared within 2 days. This rapid regression, however, was invariably followed by death of the mice. From the present observations on a rat lymphosarcoma, reduction of survival time (Group VI) is less drastic but appeared significant from the fact that 3 rats in this group died much sooner than any animal in the other groups. Experiments, to be reported in detail elsewhere, have shown that early death in pyridoxine-deficient, cortisone-treated animals occurs similarly in non-tumor bearing rats and that the prolongation of life of rats treated with testosterone was much more striking in pyridoxine deficiency uncomplicated by neoplastic disease.

The augmentation of dietary protein is known to increase pyridoxine requirements and it appears that enhanced utilization of amino acids is associated with a greater requirement for vitamin B_6(7). The action of

* Tumor obtained through the courtesy of Dr. J. B. Murphy.

7. Cerecedo, L. R., and Foy, J. R., *Arch. Biochem.*, 1944, v5, 207.

"glucocorticoids" presumably causes an increased utilization of amino acids which is associated either with inhibition of protein anabolism or with enhancement of protein catabolism. Consequently, an excessive demand for pyridoxine may be expected to result from the administration of 11-oxygenated adrenal steroids or of substances with similar activity. Conversely, androgenic steroids having a metabolic effect opposite to that of "glucocorticoids" may exert a sparing action upon vitamin B_6. Evidence supporting these possibilities has been obtained from the present experiments. Furthermore, both cortisone and testosterone were found to accentuate the retardation of lymphosarcoma growth associated with lack of pyridoxine. This effect in the case of testosterone was accompanied by an alleviation of the nutritional disease.

Summary. Marked retardation of growth of lymphosarcoma implants was observed in pyridoxine-deficient rats injected with 2 mg daily of cortisone or of methyl testosterone. Survival of pyridoxine-deficient, tumor-bearing rats was prolonged by testosterone and shortened by cortisone.

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Studies on the Early Phase of Induced Fibrosarcoma in the Hamster. (18054)

A. NETTLESHIP AND ALBERT G. SMITH*

From Department of Pathology, University of Arkansas, School of Medicine, Little Rock, Ark.†

We have been able to produce fibrosarcomas in the golden hamster by injection of 20-methylcholanthrene in tricaprylin or olive oil. Similar sarcomas have been produced in the same species by the injection of 9,10-dimethyl-1,2 benzanthracene(1). The incidence of tumors was high, and the true malignant nature of the new growths was indicated by their morphology and transplantability(1). The sarcomas induced by 20-methyl-cholanthrene were similar to those reported with benzanthracene; it was noted, however, that often they originated as early as the 60th day. This rapid induction was a favorable condition in which the earliest stages of sarcoma inception might be studied.

Experimental. Tumors were produced in 6 hamsters by the injection subcutaneously into the right median thigh area of 0.5 mg of 20-methylcholanthrene in 0.25 ml of tricaprylin. As soon as the first tumor appeared, trocar transplants were made in 7 animals. Thirteen days later all of the injected animals showed small tumors. Transplants were carried through 5 generations. Twelve animals were used in each generation of transplants. In the first generation, 11 takes were recorded; 10 in the second generation, 7 in the third, 9 in the fourth and 9 in the fifth. Growth of the transplants in the last series was evident within one week following transplantation.

Following this pilot experiment, groups of animals were injected subcutaneously with 1 mg 20-methylcholanthrene in: (1) olive oil plus carbon black, (2) saline suspension plus carbon black, (3) saline suspension. Twenty animals were injected in each group. In Group I, there were 19 animals which subsequently showed tumors. In Groups II and III, no tumors were found. Following in-

* Doctor Smith's present address is Department of Pathology, Duke University, School of Medicine, Durham, N.C.

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1. Crabb, Edward D., *Cancer Research*, 1946, v6, 627.