14949

Significance of the Age Factor and Sex Glands in Experimental Leukemia of Mice.*

MARTIN SILBERBERG AND RUTH SILBERBERG.

From the Snodgras Laboratory of Pathology, City Hospital and the Laboratory of the Jewish Hospital, Saint Louis, Mo.

In the course of experiments concerning the effect of the age factor on the development of estrogen cancers in the mammary glands of mice of strain D,[1] leukemia occurred in a large number of animals. The following report deals with our observations on the influence of age and sex glands on the development of leukemia in these mice.

Material and Methods. 258 mice (141 males and 117 females) of the closely inbred strain D were examined. The incidence of spontaneous leukemia in this strain was 2%. Seventy-three males and 57 females were castrated at the age of 2 to 3 weeks, 68 males and 60 females remained as non-castrated controls. All mice were injected subcutaneously with 200 Rat Units of estradiol benzoate in sesame oil† once a week for 5 months. The animals were divided into 2 groups: In Group A, estrogen was injected about a week after operation, *i.e.* at the age of 3 or 4 weeks; in Group B, the administration of estrogen was begun only 6 weeks or 3 months after castration. After discontinuation of the estrogen treatment, the animals were allowed to live until their natural death. Liver, spleen, lymphnodes and, if necessary, additional tissues were studied histologically, and in many cases suspect of leukemia blood smears were made at autopsy.

In the following table, columns 3 to 6 show results in age group A, columns 6 to 9 those in age group B, column 11 the percentage of leukemia irrespective of the age at which the treatment was started. Columns 3 and 7 give the number of animals in each group, columns 4 and 8 the absolute number of cases, columns 5 and 9 the percentage of cases of leukemia, and columns 6 and 10 the mean age of the animals at the time of death.

Observations. In Group A, the incidence of leukemia in intact males receiving estrogen was 30%; in castrated males treated with estrogen it rose to 50%. In the corresponding females, the difference was less conspicuous and probably not of significance. The trend seemed reversed, intact females developing leukemia in 45.8% as compared with a 40% incidence in ovariectomized estrogen-treated animals.

A comparison of age groups A and B gives the following results: Neither in castrated males nor in ovariectomized females was the incidence of leukemia affected by the age factor; the same number of castrated animals developed leukemia, whether they were injected immediately after castration or at a later date. Likewise in females with intact ovaries, the difference in the two age groups was less marked and perhaps not significant: Leukemia was somewhat more frequent in females injected at 3 or 4 weeks of age (45.8%) than in females injected at the age of 2 to 3 months (36.1%). However, the age at the beginning of the treatment decidedly influenced the incidence of leukemia in non-castrated males. In this group, leukemia developed almost twice as often (57.9%), if estrogen was administered after sexual maturity than if it was given at an earlier age (30%).

Seven animals of group B developed lymphosarcomas, whereas none occurred in group A. Five of these tumors were seen in intact males and only one each in an ovariectomized and

* The experiments were carried out in the Department of Pathology, New York University, College of Medicine, New York City. Aided by the David May-Florence G. May Fund.

1 Loeb, L., Suntzeff, V., Burns, E. L., and Schenken, I. R., *Arch. Pathol.*, 1944, **38**, 52.

† We are indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J., for the generous supply of Progynon B.

TABLE I.
Incidence of Leukemia in the Two Age Groups.

	Group A				Group B				Total group A and B
	No. of animals	No. of animals	No. of cases of leukemia	% leukemia	Age at death mo	No. of animals	No. of cases of leukemia	% leukemia	Age at death mo	% irrespective of age
♂ controls	68	30	9	30.0	11.6	38	22	57.9	12.6	45.6
♂ castrates	73	30	15	50.0	11.3	43	22	51.2	11.7	50.7
♀ controls	60	24	11	45.8	11.3	36	13	36.1	13.3	40.0
♀ ovariectomized	57	20	8	40.0	12.0	37	14	37.8	11.7	38.6

in an intact female.

Our observations in the two age groups suggest that (1) Orchidectomy increases, (2) ovariectomy perhaps slightly decreases the incidence of leukemia, if estrogen treatment is begun before the age at which sexual maturity occurs, (3) these differences in the incidence of leukemia do not exist, if estrogen is administered at or after the time of sexual maturity. The presence of the testicle in sexually immature males seems somehow to inhibit the development of leukemia which would occur under the influence of estrogen.

In spite of the limited number of animals in the various groups, our results agree with those of other investigators and seem to clear up some discrepancies between their conclusions. Whereas McEndy, Boon and Furth[2] found ovariectomy to decrease and orchidectomy slightly to increase the incidence of leukemia in the high leukemia stock Ak, Murphy[3] reported that ovariectomy hardly affected but that orchidectomy markedly increased the incidence of leukemia in the high leukemia strain RIL. In discussing his findings, Murphy suggested that the age of the animal at which castration was performed might account for the differences between his results and those of McEndy, Boon and Furth.

In our experiments, a low leukemia strain was used; the animals were castrated at the same age, but estrogen injections were begun at different ages. The results obtained in the two different age groups seem to support Murphy's suggestion. If the age factor is not considered and the incidence of leukemia computed with disregard of the age groups (Col. 11), the effect of castration is obscured, the differences between castrates and noncastrates being as low as 5.1% in males and 1.4% in females.

Conclusions. Male mice of strain D treated with estrogen after sexual maturity developed leukemia more readily than those injected from an earlier age on. In castrated males, the incidence of estrogen induced Leukemia was significantly increased over that in noncastrated animals, if the treatment was started at an early age. In females receiving estrogen from an early age on, ovariectomy slightly decreased the incidence of leukemia, and in those treated after sexual maturity, no effect of ovariectomy was noted.

2 McEndy, D. P., Boon, M. C., and Furth, J., *Cancer Research*, 1944, **4**, 377.

3 Murphy, J. B., *Cancer Research*, 1944, **4**, 622.