

# On the Role of Thymus, Spleen, and Gonads in the Development of Leukemia in a High-Leukemia Stock of Mice\*

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Little knowledge exists concerning the factors that lead to the development of spontaneous leukemia in a high-leukemia stock of mice. It is known that the occurrence of the disease is governed by factors of heredity (2), that the leukemic cells are not present as such in the preleukemic period (13), and that the malignant transformation, which takes place at about the fifth to eighth month of life, is postponed or prevented by underfeeding (13). Furthermore, genetic studies (4) have suggested that certain cells with high neoplastic potentialities may be present in a dormant form in mice with hereditary susceptibility to leukemia.

It has been noted that the thymus is almost always involved in leukemia affecting the high leukemia stock (Ak) under investigation. It is sometimes the sole organ involved, while the degree of leukemic infiltration in spleen and lymph nodes is variable and the bone marrow often appears normal. Thus the thymus may be the most common site of potentially neoplastic lymphocytes, and consequently it seemed advisable to ascertain what effect its removal at an early age would have on the incidence of leukemia. The spleen is another lymphoid organ, and if neoplasms originate in it from preformed cells destined to become malignant at a later age, then removal of the spleen during the preleukemic period might lower the incidence of this disease. It is also possible that these organs influence the occurrence of leukemia in some other way and that this would be revealed by their removal.

The relation of the sex organs to leukemia in mice is indicated by the greater frequency of this disease in female animals (2) and by the experimental production of leukemia by sex hormones. As early as 1937 Gardner (5) and Lacassagne (7) had noted the frequent occurrence of leukemia in estrogen-treated mice of stocks in which leukemia was uncommon.

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This lead was followed up by Gardner and his associates (6); Shimkin, Grady, and Andervont (15); and Bischoff, Long, Rupp, and Clarke (1), all of whom have shown under well-controlled experimental conditions that the incidence of leukemia can be greatly enhanced in most stocks of mice by prolonged administration of relatively large doses of natural or synthetic estrogenic hormones. Marine and Rosen (8) found that lymphomatosis was more common in castrated than in normal male fowls.

In order to learn more about the part played by sex hormones on the incidence of lymphoid neoplasms in the high leukemia stock Ak, both males and females of this stock were subjected to gonadectomy and observed until natural death.

## MATERIAL AND METHODS

All experiments were performed on mice of the high-leukemia stock Ak, inbred in this laboratory during the past 15 years. There is from time to time a slight variability in the percentage incidence of leukemia for different sublines of this stock, and therefore each group of experimental animals was matched with related mice of the same generation and subline as controls. The incidence of spontaneous leukemia in the various sublines carried is approximately the same.

All mice were kept under as nearly identical conditions as possible until natural death. All animals were autopsied; when the gross diagnosis was doubtful, microscopic sections were made from liver, spleen, lymph nodes, bone marrow, and sometimes thymus.

The thymectomies were performed on mice 31 to 71 days of age because the operative mortality was very high among younger mice. The technic used was essentially the same as that described by Segaloff for the rat (14). At autopsy the mediastinum was carefully explored.

The splenectomies were performed on mice 28 to 48 days of age. A lateral incision in the region of the spleen was made through the skin and ab-

dominal wall. The spleen was drawn through this opening, the vessels ligated, and the organ excised.

The ovariectomies were performed on animals 23 to 56 days old. The ovaries were exposed by a lumbar incision and removed, together with the oviducts and a portion of the fat body. The orchidectomies were performed on mice 20 to 56 days old. A scrotal incision was made, the vas deferens and testicular vessels were ligated with a single ligature, and the testis and epididymis removed.

Animals that died before the appearance of the first case of leukemia were discounted from the experiment. Accordingly, in the thymectomy and gonadectomy groups and their controls are included animals living 6 months or longer; while in the splenectomy group and its controls are included animals living 5 months or longer. The loss in each of the female groups was small (1 to 3 mice), while among the male animals it was relatively high (3 to 27 mice) because of fighting at 3 to 5 months of age.

#### EXPERIMENTAL RESULTS AND THEIR DISCUSSION

The effect of thymectomy is seen in Figs. 1 and 2, that of splenectomy in Figs. 3 and 4, that of ovariectomy in Fig. 5, and that of orchidectomy in Fig. 6. The results of all experiments are summarized in Table I.

TABLE I: INCIDENCE OF LEUKEMIA IN EXPERIMENTAL AND CONTROL MICE

Mice	Thymectomy				Splenectomy				Gonadectomy			
	Females		Males		Females		Males		Females		Males	
	Experi- mental	Con- trols	Experi- mental	Con- trols	Experi- mental	Con- trols	Experi- mental	Con- trols	Experi- mental	Con- trols	Experi- mental	Con- trols
No. in experiment	40	44	46	44	71	75	87	94	56	67	83	67
No. with leukemia	3	34	5	27	38	43	40	48	25	50	50	35
Percentage with leukemia	8	77	11	61	54	57	46	51	45	74	60	52

The reduction in the incidence of leukemia as the consequence of *thymectomy* is conspicuous in both male and female mice. Never have such low percentage figures for leukemia (11 per cent and 8 per cent) been observed in normal mice of this stock. Similar low figures (10.1 per cent) have been encountered in only one experimental series, that is, in mice that were underfed (13), and the question arises whether the effects of thymectomy are due to malnutrition. In the underfeeding experiments (13) caloric reduction of an adequate diet began at the age of 4 weeks, and the animals were definitely underweight and small in size as compared with the controls, whereas the animals of the present series were almost as large as the controls, though they appeared to be slightly underweight. The animals of this experimental series were not weighed. Precise data on the relation of thymus to body weight will be forthcoming from experiments

now in progress. The underfed animals remained sterile (13), whereas the thymectomized animals bred well. In both underfed and thymectomized animals mortality during the first few months of life was greater than in the controls. Among the underfed mice the excessive mortality occurred at 1 to 6 months of age, and among the thymectomized mice at 6 to 10 months. Figs. 1 and 2 indicate that a larger proportion of thymectomized animals reached an old age than among the controls, and this is also true for the underfed animals (13).

Retardation of development of rats by underfeeding causes involution of the thymus and an increase in the ratio of body weight to thymus weight; after about 250 days, however, the difference in these ratios is slight and the figures are even higher in normally fed than in retarded animals (12). It is thus conceivable that the decrease in the incidence of lymphoid neoplasms resulting from underfeeding is indirect and is the immediate consequence of involution of the thymus. However, neoplasms other than lymphomatosis are likewise infrequent among the underfed animals (11), and if the effects of underfeeding are related to atrophy of the thymus it must be supposed that this organ contains substances that influence neoplastic tendencies in general. Thus there are at least three possibilities to explain the results of thymectomy:

(a) removal of sites of origin of leukemia, (b) developmental disturbance not specifically due to absence of thymus, (c) lack of hypothetical thymic hormones. The last assumption seems unlikely, since the administration of estrogen brings about an increased incidence of leukemia with involution of the thymus (6). An interpretation of the consequences of thymectomy, here described, must therefore be postponed until data are forthcoming from experiments such as that on the effect of thymic extract in normal and thymectomized animals.

*Splenectomy* had no significant effect on the incidence of leukemia or on the longevity of the animals (Figs. 3 and 4). The incidence of leukemia was slightly lower among splenectomized animals than among the controls, but there was a slightly higher incidence of death from causes other than leukemia among the splenectomized animals 5 to 9 months

of age. The findings suggest that in the majority of cases the involvement of the spleen in leukemia is a secondary process. This conclusion is in harmony with the results of experiments aimed to determine the onset of leukemia by bioassays of various organs (13).

The spleen is generally not regarded as an organ essential for life, and we are not familiar with any data to indicate that the life span of animals and

on mammary gland carcinoma Cori (3), following up the observations of Leo Loeb, found that the incidence of the disease was reduced from 78.5 per cent to 10 per cent in animals ovariectomized at 2 to 6 months of age and to zero per cent in those ovariectomized at 15 to 22 days. In the experiments of Murray (9) the incidence of mammary tumors among female mice spayed at 4 to 6 weeks was 17.1 per cent, as compared with 11.5 per cent in normal non-

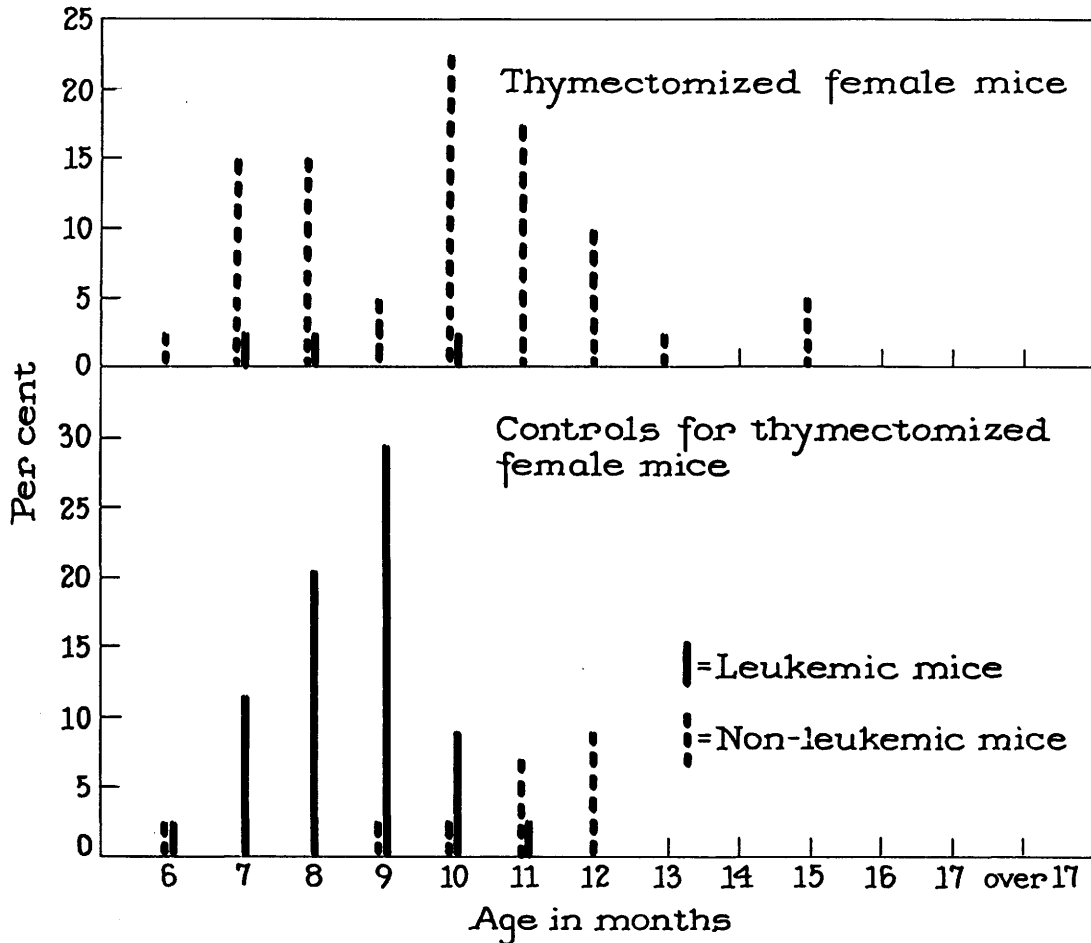


FIG. 1.—Incidence of leukemia and mortality from other causes among thymectomized female mice of the Ak stock and controls.

the incidence of disease are notably altered when splenectomy is performed in immature adult animals that are not carriers of latent infections. The present experiments failed to reveal any such effect resulting from splenectomy.

Fig. 5 shows that the incidence of spontaneous leukemia was reduced in mice by *ovariectomy*, and Fig. 6, that it was raised by *orchidectomy*. Gonadectomy was performed at 20 to 56 days, and it is probable that the animals had by this time been stimulated by some androgenic or estrogenic hormones. In studies

breeding females and 80 per cent in breeding females. The inference can be drawn from these studies that some hormones instrumental in the production of mammary cancer have already been secreted at 4 to 6 weeks of age, or slightly earlier, in sufficient quantities to influence the appearance of this neoplasm in highly susceptible animals.

The magnitude of the changes resulting from gonadectomy was not great in our experiments, but appeared definite. The incidence of leukemia in spayed females was 45 per cent as compared with 74 per

cent in the controls, while the reverse effect was noted among orchidectomized animals, the incidence of leukemia rising to 60 per cent in the experimental males as compared with 52 per cent in the controls. The results of orchidectomy alone are probably not statistically significant, but it is the only instance thus far known to us in which leukemia was higher in a group of mice that had been subjected to an operative procedure than among the corresponding controls. Moreover, the finding is in harmony with the ob-

ner, Dougherty, and Williams (6), and this could not be correlated with a concomitant tendency to acquire mammary, hypophyseal, or testicular tumors in response to estrogens. The lymphoma-producing action was inhibited by testosterone propionate (6).

## SUMMARY

Removal of the thymus from mice of a high-leukemia stock (Ak) at 31 to 71 days of age resulted in a reduction of the incidence of spontaneous leu-

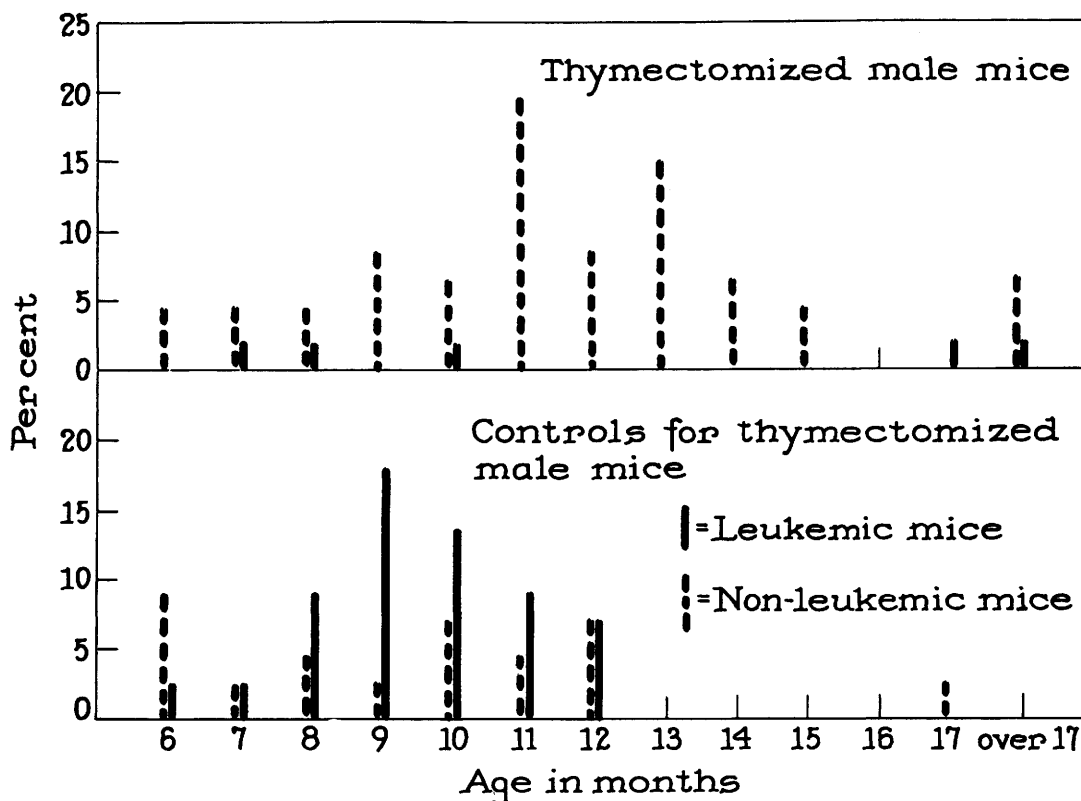


FIG. 2.—Incidence of leukemia and mortality from other causes among thymectomized male mice of the Ak stock and controls.

servations (a) that the incidence of leukemia is definitely higher among female than among male mice, and (b) that ovariectomy lowers considerably the incidence of leukemia.

Pybus and Miller (10) noted that castration delayed the onset of leukemia in one of their strains. In the experiments of Gardner and his associates (6) intact or breeding animals had no more lymphomas than did castrated or virgin females. However, the incidence of lymphoid tumors was not higher in female than in male mice of the stocks studied by them, indicating that in these stocks the role of sex hormones in the spontaneous occurrence of this disease is negligible. The tendency for estrogens to be more effective in increasing the incidence of lymphoid tumors in some strains than in others had been noted by Gard-

ner, Dougherty, and Williams (6), and this could not be correlated with a concomitant tendency to acquire mammary, hypophyseal, or testicular tumors in response to estrogens.

Leukemia is more common in female than in male mice. The incidence of this disease was lowered from 74 to 45 per cent by ovariectomy at 23 to 56 days. Among males subjected to orchidectomy at 20 to 56 days the incidence of leukemia was 60 per cent, as compared with 52 per cent among the controls of this experimental series.

Splenectomy at 28 to 48 days did not significantly alter the incidence of spontaneous leukemia.

The role of thymus, spleen, and gonads in the causation or evolution of spontaneous leukemia is discussed in the light of the data here presented.

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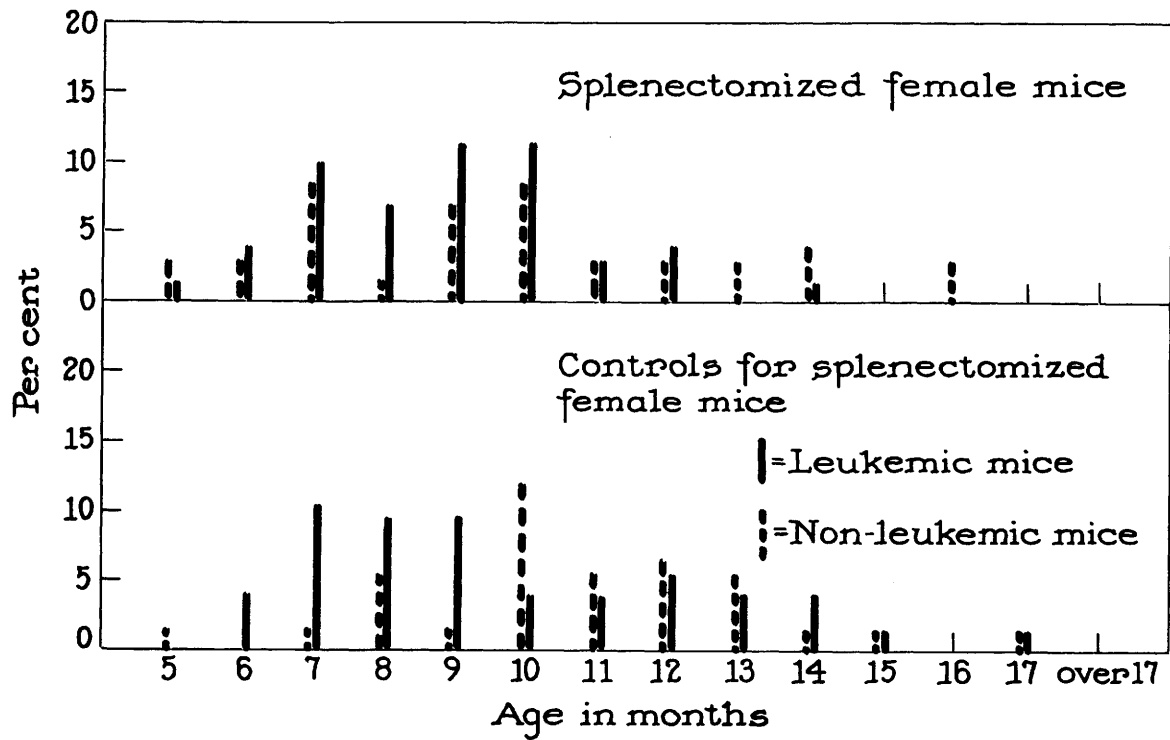


FIG. 3.—Incidence of leukemia and mortality from other causes among splenectomized female mice of the Ak stock and controls.

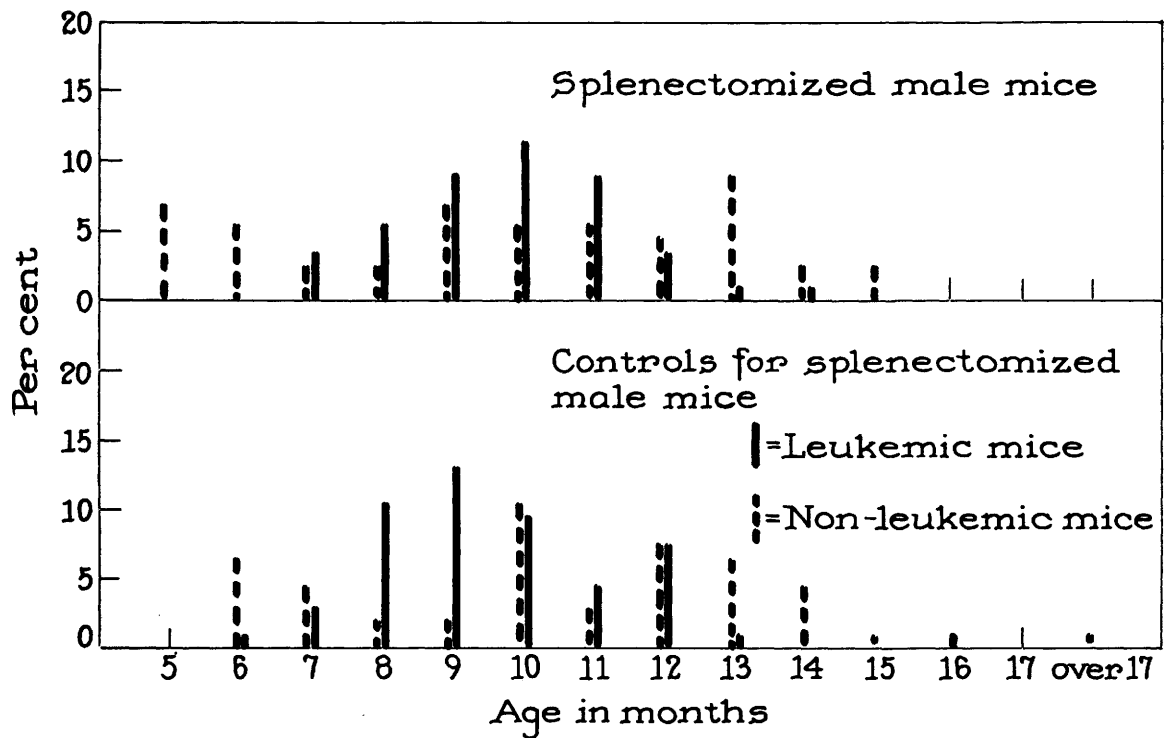


FIG. 4.—Incidence of leukemia and mortality from other causes among splenectomized male mice of the Ak stock and controls.

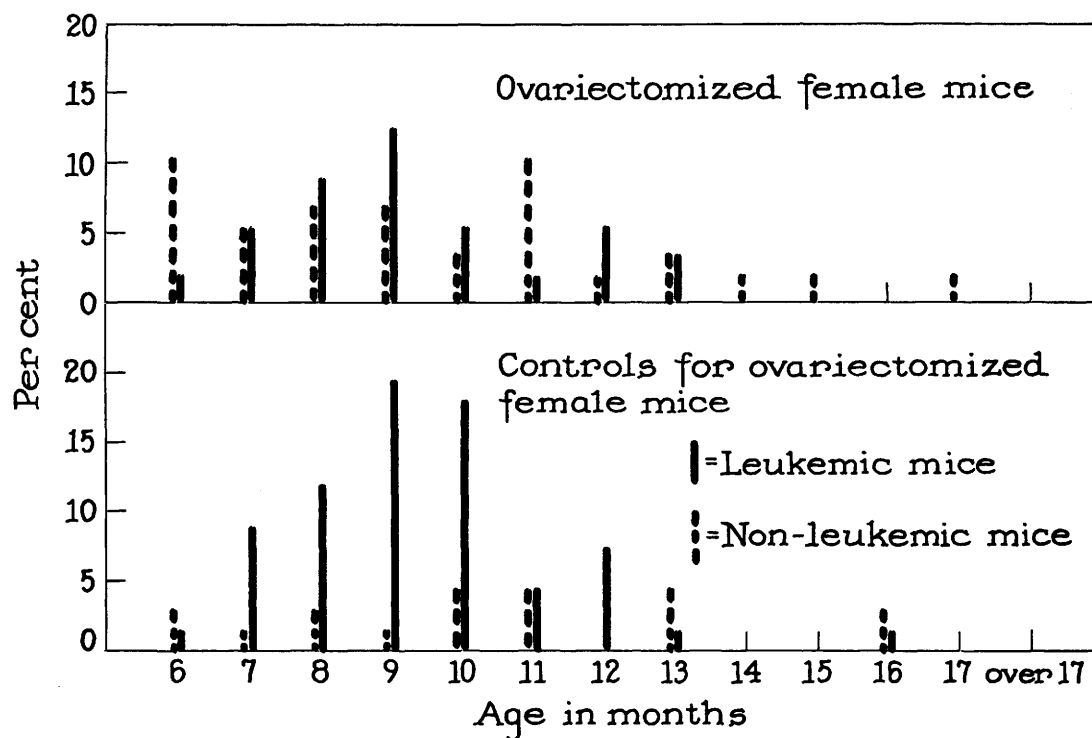


FIG. 5.—Incidence of leukemia and mortality from other causes among ovariectomized female mice of the Ak stock and controls.

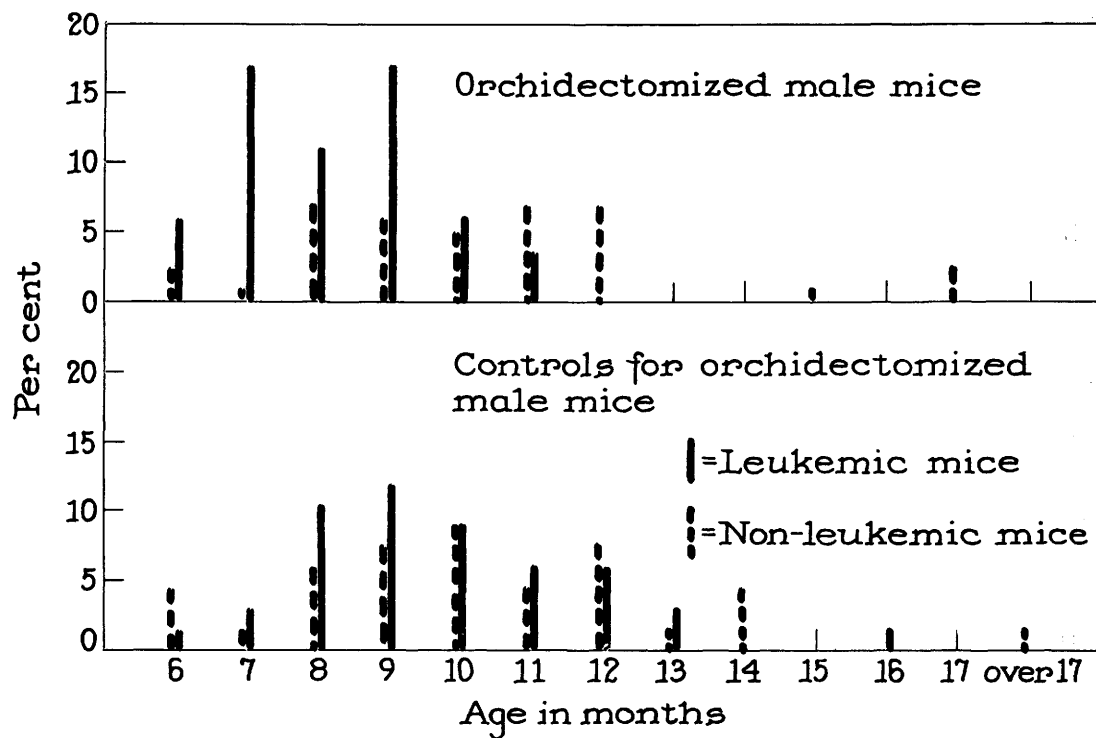


FIG. 6.—Incidence of leukemia and mortality from other causes among orchidectomized male mice of the Ak stock and controls.

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