

# A SEX DIFFERENCE IN THE MORTALITY PATTERN OF L 1210 LEUKEMIA IN DBA/2 MICE<sup>1</sup>

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**Abstract.** Female DBA/2 mice inoculated with  $1 \times 10^6$  L 1210 leukemia cells died more rapidly than simultaneously inoculated male mice with a mean survival of  $6.1 \pm 0.2$  days vs.  $7.2 \pm 0.4$  days. For males, this represents an average of 18% longer survival than females. Trials involving gonadectomy or treatment with exogenous sex hormones suggest that the sex difference in mortality was due to exacerbation of L 1210 leukemia by estrogen.

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This report describes the effect of sex, gonadectomy and sex hormone treatment on mortality of DBA/2 mice carrying L 1210 transplantable murine leukemia. L 1210 is widely used as a screening entity in leukemia (Schabel *et al* 1966; Hofer and Hofer 1971). Recognition of a sex difference in the mortality pattern may be of value in improving the reliability of the test. A sex difference in susceptibility and resistance has been seen in a number of other diseases, including cancer (Gobe and Konopka 1973; Weiss *et al* 1973; Yohn 1973; Yohn *et al* 1967; Metcalf 1971).

## METHODS AND MATERIALS

**L 1210 Leukemia.** L 1210 cells were maintained in DBA/2 mice using a 7 day transfer cycle. Mean weight of mice at time of use was  $23.9 \pm 1.2$  g. One ml of ascites fluid was drawn from a donor mouse, diluted, and the white cells counted. The cell count was adjusted, if necessary, with sterile Lockes solution so that 0.1 ml contained approximately  $1 \times 10^6$  cells. For experimental procedures, 0.1 ml of the adjusted solution was injected intraperitoneally. Sham inoculated animals (controls) were injected intraperitoneally with 0.1 ml of sterile Lockes solution. There were no deaths in any sham group. The mean survival time of the several groups of DBA/2 mice inoculated with  $1 \times 10^6$  cells in this study varied between 6.1 and 7.2 days with an overall mean of  $6.6 \pm 0.3$  days, in agreement with the known dynamics of L 1210 leukemia (Skipper *et al* 1964).

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**Sex Differences.** Three procedures were used to explore the sex differences. The first simply involved simultaneously inoculating male and female DBA/2 mice with  $1 \times 10^6$  L 1210 cells and observing the mortality patterns. Four such trials were conducted. In the second procedure, male and female mice were gonadectomized several weeks before L 1210 inoculation. Mice were anesthetized with sodium pentobarbital (about 27  $\mu$ g/g mouse). For orchidectomy, the scrotum and tunica were cut and the testes expelled with slight abdominal pressure. Blood vessels were ligated before the testes were cut free and separate sutures were used to close the tunica and the scrotum. A topical antibiotic (nitrofurazone) was sprinkled on the wound. For ovariectomy, bilateral incisions 0.5 cm long were made 0.5 cm on each side of the spine, extending posteriorly from the last rib. The ovaries were exteriorized, isolated by ligature and cut free. Separate sutures were used to close muscle and skin, and the antibiotic was sprinkled on the wound. Approximately 2 weeks recovery was allowed prior to L 1210 inoculation.

The third procedure involved treatment with hormones one day before L 1210 inoculation. Males were given 125  $\mu$ g Delestrogen (estradiol valerate) in peanut oil, subcutaneously. Females were injected with 4 mg Delatestryl (testosterone enanthate) in peanut oil, subcutaneously. The dose for long acting hormones was derived from Drug Dose in Laboratory Animals (Barns and Eltherington 1964). Controls received peanut oil alone before the L 1210 cells. Two gonadectomy and 2 hormone treatment trials were carried out.

## RESULTS

The cumulative percent mortality plot shows that female DBA/2 mice died significantly more rapidly than the males following inoculation with L 1210 cells (fig. 1). While mortality appeared similar on day 5, some 70% of the females,

as compared to 35% of the males, were dead by day 6. The female mean survival was  $6.1 \pm 0.2$  (SE) days while the male mean survival was  $7.2 \pm 0.4$  days (table 1). This difference was significant at the  $p < 0.01$  level using a *t*-test. Using a probit plot of the pooled data, the

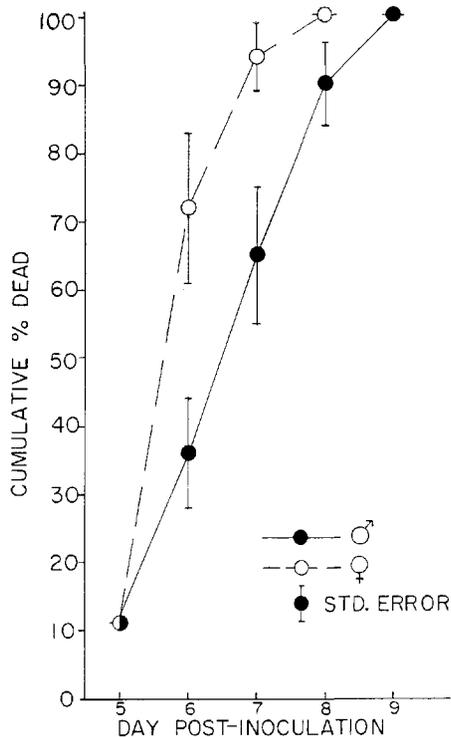


FIGURE 1. Sex difference in survival of DBA/2 mice following inoculation with  $1 \times 10^6$  L 1210 leukemia cells. (Avg. of 4 trials, involving a total of 36 male and 42 female mice.)

male  $LT_{50}$  was estimated as day 6.7 post-inoculation and the female  $LT_{50}$  as day 5.5. This difference was also significantly different ( $p < 0.01$ ) using a Chi Square Test. While males lived only 1.1 to 1.2 days longer than females, on the average, this amounts to an 18% increase in survival time.

After gonadectomy, the sex difference in mortality was absent (table 1). The mean survival time for both gonadectomized males and females was 7.0 days and the  $LT_{50}$  was 6.5 days, similar to normal males. After treatment with exogenous hormones of the opposite sex, the sex difference in mortality was again absent (table 1). In this case, however, the mean survival of 6.2 days and the  $LT_{50}$  of 5.5 days were similar to those of normal females. Peanut oil alone did not alter the mortality pattern.

#### DISCUSSION

These results suggest that the more rapid mortality in female DBA/2 mice inoculated with L 1210 leukemia cells was due to an estrogenic effect. With hormones lacking due to gonadectomy, both males and females responded as normal males. Thus, gonadectomized females survived longer, but gonadectomy did not affect mortality in the males. In the procedure where mice were treated with hormones of the opposite sex, both sexes responded as normal females. Thus, estrogen treated males died sooner than normal males, but androgen did not affect the mortality pattern in the females. Estrogen thus appeared to exacerbate (to some extent) the

TABLE 1

*Effect of sex and sex hormone treatment on survival of DBA/2 mice inoculated with  $10^6$  L 1210 leukemia cells.*

Treatment	No. of trials	Total number of mice	Mean Survival (days $\pm$ SE)	$LT_{50}$
MALES				
L 1210 only	4	36	$7.2 \pm 0.4^*$	6.7*
Gonadectomy + L 1210	2	8	$7.0 \pm 0.4$	6.5
Estrogen + L 1210**	2	10	$6.2 \pm 0.2$	5.5
FEMALES				
L 1210 only	4	43	$6.1 \pm 0.2$	5.5
Gonadectomy + L 1210	2	8	$7.0 \pm 0.4$	6.5
Androgen + L 1210†	2	10	$6.2 \pm 0.3$	5.5

\*Significantly different from similarly treated females ( $p < 0.01$ ).

\*\*Intraperitoneal injection of 125  $\mu$ g Delestrogen (estradiol valerate), in peanut oil, S.C.

†Intraperitoneal injection of 5 mg Delatestryl (testosterone enanthate), in peanut oil, S.C.

L1210 leukemia disease process while androgens had little effect.

Estrogen has been implicated in other experimental cancers. Females are more susceptible to avian adenovirus, due to estrogenic enhancement of adenovirus oncogenesis (Jones *et al* 1970) and female hamsters develop tumors more readily, have a shorter survival time and show regression less often than male hamsters (Yohn 1973; Yohn *et al* 1967; Hatch *et al* 1970). In cell cultures, female hamster cells were transformed with adenovirus with a higher frequency than male cells, a result which was enhanced by the addition of estrogen (Fong and Ledinko 1970; Milo *et al* 1972). Enhancement of disease process in the female however, is far from universal. In infectious diseases caused by pneumococcus, the female was shown to be more resistant than the male (Weiss *et al* 1973). The sex difference in susceptibility to tumors induced with benzo(a)pyrene was shown to depend on the tissue involved (Vesselinovitch *et al* 1975) and in leukemias involving AKR, RF and C58 mice, a sex difference exists but which sex is more susceptible depends upon the specific type of leukemia (Metcalf 1971).

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