Influence of Age, Body Weight, and Sex on Susceptibility of Mice to the Lethal Effects of X-radiation.* (18610)

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The effects of age, body weight, and sex on susceptibility to radiation mortality have not been thoroughly explored. Furth(1) mentioned that, among a series of mice exposed to 300-400 r, mortality was greatest in young animals aged about 5 weeks at the time of irradiation. Quastler(2) drew a hypothetical curve of the age-survival time relationship based upon his experimental age-weight and weight-survival time relationships. This curve indicated that among mice 25 days or older, increasing age was accompanied by increased resistance to irradiation. He suggested that this was a reflection of the increasing body weight found in older age groups. His actual observations, however, were too few and too scattered to delineate a definitive age-survival time relationship. He also stated that "infant mice survive a given dose considerably longer than adult mice."

In the present experiments, the effects of age, weight and sex on mortality and survival time in irradiated mice were investigated.

Methods. Strain C57 black mice received a single exposure of 550 r measured in air.‡ They were divided into 6 groups of about 50 mice each, one group being irradiated at an average age of 1, 15, 30, 45, 60 and 90 days, respectively. Litters were distributed among all groups. The over-all sex distribution was approximately equal, although there were variations among the groups. All mice were weaned at the age of 30 days and subsequently maintained on Purina laboratory chow and water *ad libitum* under identical laboratory conditions. Body weight was determined on the day of irradiation.

Results. (1) *Age.* Susceptibility of mice to the lethal effects of irradiation is strikingly influenced by age at the time of exposure (Fig. 1, Table I). During a 30-day observation period, mortality was low in the 1- and 15-day-old groups, very high in the 30-day-old group, and decreased progressively with age thereafter. When the observation period was extended to 60 days, the mortality of the 1-day-old mice increased significantly (Fig. 1). In general, survival time was inversely related to mortality. Among the mice irradiated at the age of 1 and 15 days, a majority of deaths occurred within 2 weeks after weaning (Fig. 2). Surviving mice of the 1-day-old group exhibited marked stunt-

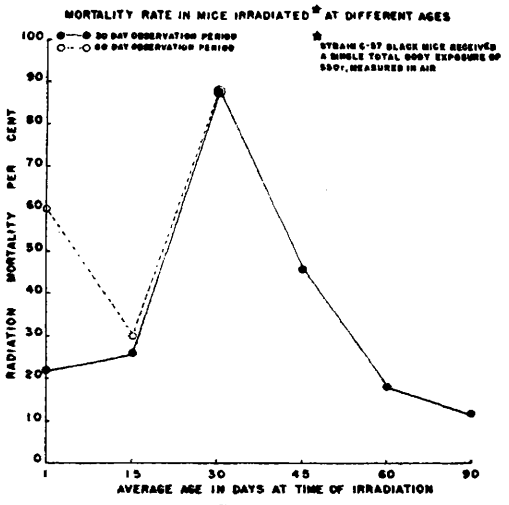

FIG. 1.

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‡ Physical factors: 120 KVP, 9 ma, 30 cm focus skin distance, 0.25 mm Cu and 1.0 mm Al added filtration. HVL 0.36 mm Cu, output 32.2r per minute. Groups of 8 mice were irradiated in a flat, round lucite container. In an effort to ascertain whether increased scatter might alter the results among heavier mice, readings were made with an ionization chamber under conditions duplicating those of the experiment. No significant difference in dose within the lucite container could be found among the various age groups.

TABLE I. Mortality and Average Survival Time in Mice Irradiated at Different Ages.

Avg age at time of irradiation, days	No. of mice irradiated	Deaths				Avg survival time (days)	
		30-day observation period		60-day observation period		30-day observation period	60-day observation period
		No.	%	No.	%		
1	58	13	22.5	35	60	26.5	40
15	57	15	26.5	17	30	26.5	47
30	59	53	90	53	90	12	15
45	65	30	46	30	46	22	37.5
60	52	10	19	10	19	27	51
90	45	6	13.5	6	13.5	28	54

ing, physiological immaturity, and neuromuscular incoordination.

(2) *Weight.* Mortality is plotted against body weight in Fig. 3. A total of 316 mice were used, each point representing at least 5 mice. No correlation between mortality and body weight was found. Mortality tended to vary with age rather than with body weight. Striking evidence of the absence of a mortality-weight correlation is seen in the group of mice weighing 17 to 18 g. Among 30-day-old mice of this weight, mortality was 83%, while 45-day-old mice of the same weight had a mortality of only 22%. Analysis of unpublished data for a large single age group (255 mice aged 30 days at time of irradiation) likewise revealed an absence of relationship between weight and mortality.

(3) *Sex.* With the possible exception of 90-day-old mice, in which the mortality among females was suggestively higher than among males, sex did not influence susceptibility significantly.

Discussion. In general, young, rapidly growing tissues are thought to be most susceptible to the effects of irradiation. Yet many mice irradiated at 1 and 15 days of age were capable of surviving a 30-day post-irradiation period, while 30-day-old mice suffered a high mortality. This result is contrary to what might be expected on the basis of a simple relationship to general tissue maturity or to rate of body growth.

Some other explanation must therefore be sought for this age effect. That nutritional disturbances, physiological state, and other factors associated with weaning may be operative is suggested by the high incidence of deaths soon after weaning in the 1- and 15-day-old groups. In general, mice subjected to a single whole body exposure of 550 r develop anorexia and diarrhea and lose weight prior to death. Within 3 days after irradiation, many mice of the 30-day-old group were too weak to obtain food or water; when food was presented, they ate little. Attempts to substitute bread and milk for Purina laboratory chow met with the same response. In contrast, mice irradiated at the age of 1 day continued to suckle until the time of weaning. Adequate protein intake has been shown to result in diminished susceptibility to total body irradiation in rats(3). Thus, their relative nutritional state may explain in part the difference in response to irradiation of 1- and 30-day-old mice. Other hypothetical protective influences associated with suckling include: (a) preservation of fluid balance; (b) an anti-hemorrhagic factor in mouse milk to counteract increased plasma concentrations of anti-coagulant substances after irradiation (4); (c) a factor stimulating bone marrow recovery(5); (d) factors increasing resistance to infection, and (e) hormonal factors. Proximity of suckling mice to the mother affords additional warmth which may also be supportive(6). These possibilities will require further experimental investigation.

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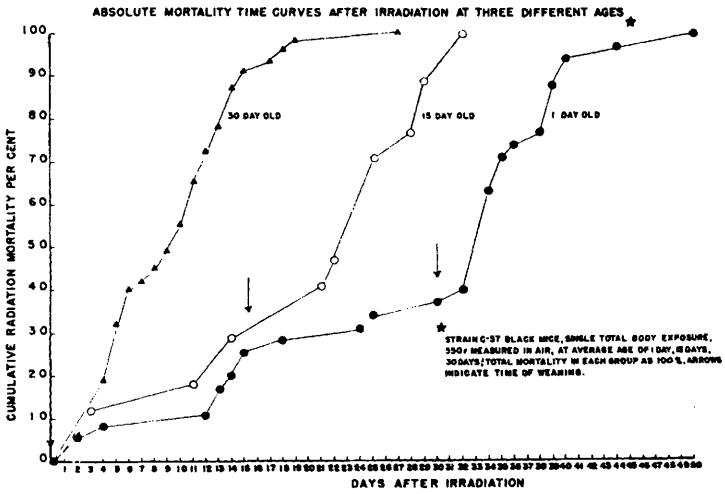

FIG. 2.

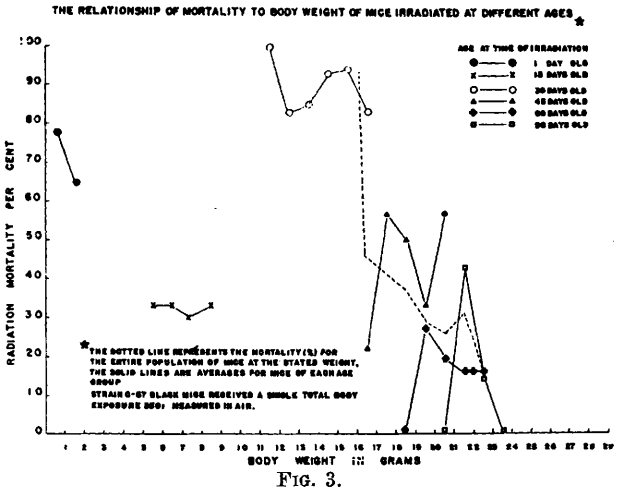

FIG. 3.

Conversely, emphasis may be placed upon the heightened susceptibility of the 30-45-day-old groups. It is noteworthy that this is the age of puberty in mice, and the endocrine upheaval which takes place during this period may well be an important factor. Support for this hypothesis may be found in the fact that this is also the age period of maximal susceptibility to radiation-induced lymphoid tumors in mice(7), and recent studies have confirmed the suspected operation of hormonal and indirect factors in the induction process(8,9). In addition administration of estradiol benzoate(10,11) or testosterone(12) at the time of irradiation has been reported to increase the mortality rate in mice following irradiation. Further studies will be necessary to determine whether this effect is related to physiologic or direct toxic properties

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of these substances.

The relative resistance of mice irradiated at the age of one day is not shared by mice irradiated before birth. A relatively high mortality has been observed among a small group of mice exposed to intrauterine irradiation. Mice irradiated one day before birth survive considerably longer than mice irradiated 2 or 3 days before birth. These findings suggest that profound alterations occur shortly before, during or immediately after birth which render mice less susceptible to irradiation.

Sex does not appear to affect susceptibility of mice to whole-body irradiation. A possible exception is the 90 day age group in which a suggestively greater proportion of females than males died. It is of interest that a high mortality rate (50 per cent) has been observed in a small group of pregnant female mice 90 days of age at the time of irradiation. Further investigation of the influence of pregnancy on susceptibility to whole-body irradiation is in progress.

Some of the results reported here may be helpful in the design of future experiments concerned with radiation mortality in mice (and presumably also in other species). Attention is particularly directed to the need for controlling age as a variable, and to the desirability of selecting an age such that the anticipated mortality may facilitate statistical analysis of the experimental results. The usual 30 day observation period following irradiation, which is adequate for older mice, should probably be extended to 60 days when suckling mice are being studied, and weaning time must be suitably controlled among different experimental groups.

Summary and conclusions. (1) The age at which mice are irradiated profoundly alters their resistance to a single exposure of whole body irradiation. Mortality is low after irradiation at 1 and 15 days of age, very high at 30 days, and decreases rapidly with increasing age thereafter. The possible mechanisms of this effect are discussed. (2) The response of mice to whole body irradiation is not related to body weight except insofar as weight reflects a particular age range. (3) In general, sex appears to exert no significant influence on the capacity of mice to withstand whole body irradiation.

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Influence of Concentration of Thromboplastin on Prothrombin Time of Human and Dog Plasma.* (18611)

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In the development of the one-stage prothrombin test, it was observed that on adding increasing amounts of thromboplastin, a minimum clotting time was obtained which remained constant irrespective of the excess of thromboplastin(1). In the present study, an attempt is made to correlate quantitatively the concentration of thromboplastin required for obtaining minimal clotting values when the prothrombin activity of the plasma is varied by different experimental means.

Methods. As the source of thromboplastin rabbit brain dehydrated with acetone as previously described(2) was employed. To test the potency of the material, various concentrations were prepared by mixing weighed amounts with 5 cc physiological saline and incubating the mixture at 50°C for 20 minutes. The various concentrations of thromboplastin which ranged from 1 to 250 mg in 5 cc of saline (0.02 to 5.0%) were tested on

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