Conditioning of the Mouse Fetus Against X-Irradiation Death. (23424)

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Bisgard *et al.*(1) found that rabbits which had been exposed to x-rays acquired an increased resistance to bacterial toxins. Raper (2) increased the LD/50/30 of mice following total body beta irradiation by giving a prior sub-lethal cutaneous exposure of the same type of irradiation. Cronkite *et al.*(3) increased the radio-tolerance of adult mice by prior exposure to 144 r given at weekly intervals for 3 weeks and followed by a 30-day rest before exposure to 730 r whole body x-irradiation. Thus, a response in terms of radioresistance has been demonstrated to follow low level x-irradiation. It was conjectured that the mouse fetus, while demonstrably radioresponsive, might also acquire some degree of radiation resistance by very low exposure to x-rays during intra-uterine life. The following data are from such a study.

Materials and method. To obtain fetuses of known age, time-matings were made by placing males with non-pregnant females at 5 p. m. and removing them at 9 a. m. the following day. Matings generally occurred toward midnight. Since the youngest fetuses to be x-rayed were 13.5 days it was not necessary to make vaginal smears or to look for vaginal plugs in order to identify mated females. By 13.5 days pregnancies were obvious. Pregnant mice with fetuses ranging from 13.5 to 18.5 days were exposed to x-rays. In the preliminary experiments single exposures of 25 r to 300 r were given to the fetuses. In some cases, there was x-irradiation on 3 or 4 consecutive days of gestation or during early post-parturition so that a higher cumulative dose was received. In later experiments the dose was reduced to 10 r single exposure. The gravid uterus was measured at 50 cm from the target of a Quadrocondex constant potential x-ray tube run at 210 KVP, 15 MA and with 0.28 mm Cu and 0.51 mm Al filtration. The dose rate measured in air but at the level of the gravid uterus was 97.7 r/min. so that 300 r was delivered in 3.1 minutes. At 2 to 4 months of age, fetal-irradiated mice and their parallel unirradiated controls (of the same age) were exposed to doses of x-irradiation ranging from 525 to 625 r. The dose which approximates the LD/50/30 for males is somewhat lower than that for females. For the final experiment a single exposure of 525 r was used, a dose which proved to be 100% lethal for the control males and an LD/50/30 for the females. The lethality data were collected for all mice for a period of 30 days.

Results. Since there were no data for an immunizing type of reaction on the part of the fetus, preliminary experiments included ages from 14.5 days to 3 days post-parturition and the exposure levels from 25 r to 300 r. While these exploratory experiments were instructive, they will be reported here only in summary form. a. Exposure of the mouse fetus to 300 r at 16.5 days was not lethal but increased its radiosensitivity greatly when adult. b. An exposure of 25 r to 100 r gave variable results in tolerance of x-rays when adult, largely deleterious except in those cases where the lower doses were fractionated over several fetal days. (*e.g.* 16.5-19.5). In these latter cases some females appeared to show slight benefit.

The over-all impression from these preliminary data involving some 42 pregnancies and exposures from 25 r to 300 r at different fetal ages was that x-irradiation had a permanent and deleterious effect on the later survival value when the mice were exposed to an LD/50/30 level of x-rays. It was then decided to use a much lower level of single exposure of 10 r given at an earlier fetal age. These data are given in Table I.

The above data indicate that in almost every case there is an appreciable improvement of the tolerance of x-irradiation by mice at 4 months of age by virtue of their exposure to 10 r while *in utero*. Unfortunately, 525 r under the conditions of the experiment proved to be lethal for all control male mice but since

TABLE I. 30-Day Survival % of Mice Exposed at 4 Months of Age to 525 r X-Irradiation, following Fetal Exposure to 10 r.

	Fetal age exposed		30 days, % survival	Increment
♀	Controls (none)		30.4 (23)	—
	13.5 days	+ 10 r all	31.0 (29)	.6%
	14.5		40.6 (32)	10.2
	15.5		54.8 (32)	24.4
	16.5		45.8 (24)	15.4
♂	Controls (none)		0.0 (11)	—
	13.5 days	+ 10 r all	0.9 (29)	.9+
	14.5		14.8 (54)	14.8+
	15.5		17.4 (46)	17.4+
	16.5		9.1 (44)	9.1+

Numbers in parentheses indicate actual No. of mice tested. All are CF_1 white mice.

some experimental males survived in every instance, there is indication of improvement at least to the degree of survival. For the female the LD value was 30% so that the absolute changes could be given.

It is apparent that exposure to 10 r on gestation day 15.5 gave the best results. The male offspring so irradiated showed at least a 17.4% improvement in survival over the controls while the females showed a 24.4% improvement in survival (from 30.4 to 54.8%). The smallest number of experimental animals of either sex used was 24 females exposed to 10 r at 16.5 days so that the data can be considered significant.

Discussion. The causes of x-irradiation death may be numerous and may vary from animal to animal within the same experiment. Some animals die quickly (3-4 days), others in from 10 to 15 days, and some not for 3 or 4 weeks. The causes of death may be quite different at the 3 peaks. In the final experiment reported above most deaths occurred between 8th and 16th days following exposure. There were a few deaths about 24 days, but none during the first 4 days. It is suggested that such radio-resistance as is given to these mice by fetal irradiation may have protected those which would have normally died early within the 30 days following exposure, and a few which might have died late. The cause of death for those dying between 8 and 16 days seems to have been unaltered.

The data reported by Cronkite(3) indicate that most deaths occurred between 9 and 21 days. The resistance provided his adult animals seems to have been somewhat similar to that provided these fetal mice, subsequently exposed to the lethal dose when mature. However, the conditioning of the fetal mice was achieved by a very much lower and single exposure to x-rays.

The mechanism of this increased resistance to x-irradiation death is still completely obscure. It is certainly not due to the selective elimination of susceptible fetuses, for there appeared to be no reduction in litters nor in the normal viability of those exposed to the very low dose of 10 r. It would be extremely difficult to detect any changes in the whole blood, the serum, or in any of the highly radio-sensitive organs such as the spleen, lymph nodes or hematopoietic system following only 10 r exposure. Kiseljov *et al.*(4) recently found that 130 r to adult mice provided increased resistance to ionic emissions, but that subsequent exposures did not have this effect. They attribute this to antibody formation within the mice due to the denaturation of proteins. The data of the current experiment indicate definite improvement of radiation tolerance due to exposure of the fetus, particularly if it occurs on day 15.5.

Conclusions. Irradiation of the mouse fetus at 15.5 days gestation to only 10 r x-rays will give it a considerably better chance of survival if it is subjected to the LD/50/30 range of exposure when adult. Other fetal ages from 14.5 to 16.5 days show some benefit of 10 r exposure, but higher exposures (25 r to 300 r) to any age fetus will have a deleterious effect in later life if the mouse is exposed to x-rays.

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