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Gamma-Ray Sensitivity of Oocytes of Immature Mice. (27330)

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The high radiation sensitivity of oocytes in early stages of follicular development in the adult female has been known since the 1907 report by Bergonié and Tribondeau(1) on ovarian changes in irradiated rabbits. These early observations have been confirmed histologically in both the mouse(2-7) and rat(8). The reduced fertility of irradiated female mice likewise indicates high sensitivity of early oocytes, since 1.4 litters are obtained after 300 r owing to high resistance of late follicle stages, yet killing of early oocyte stages with 50 r reduces the number of litters to 4 as compared to the 13 or 14 obtained from controls(9). Fractionation of radiation doses at high intensity, and especially reduction of the dose rate, markedly reduces the effectiveness of a given total dose as regards effects on the fertility of irradiated adult females(9).

All of the above results were obtained with adult mice. Russell *et al.*(10) found recently that the effect on fertility is much greater in immature mice of certain ages, even at low dose rates. Thus, whereas 85 r given at a rate of 90 r/week had no detectable effect on fertility of adult mice, the same dose given during the second week after birth induced permanent sterility either after a single litter or after a second litter of reduced size. Possible explanations of these results are: (*a*) newly formed dictyate oocytes are more sensitive to radiation than are comparable oocyte stages in the adult, (*b*) radiation sensitivity in young and adult mice is the same for equivalent oocyte stages, but fertility is less disturbed in the adult owing to the presence of more oocytes in later, more resistant stages of follicular development, (*c*) dose rate is unimportant in young mice, chronic and acute irradiation being equally effective, and (*d*) oocytes in young mice pass through an extremely sensitive stage of short duration, chronic irradiation therefore being highly effective since all young oocytes are exposed in this sensitive period. The experiments reported here indicate that early oocyte stages in 10-day-old mice are extremely sensitive to acute gamma-ray exposure, and that they are more sensitive to chronic irradiation than are similar stages in the adult.

Materials and methods. Pair matings of 101 strain females with C3H strain males were checked each morning, and any new litters recorded. When 9 days old, females were toe-marked, and assigned to the different dose groups, with only one mouse per litter being used for any treatment. Mice were irradiated between 10:00 and 11:05 a.m. on the tenth day after birth (day of birth being

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TABLE I. Frequencies of Early Oocyte Stages 72 hr After Irradiation of 10-Day-Old Female Mice.

Dose (r)	Oocyte Stage 1 Normal cells	1 Exp./ control	2 Normal cells	2 Exp./ control
0	3110*		241*	
1	1423	.915	115	.954
3	1305	.839	102	.846
5	1155	.743	107	.888
7	823	.529	53	.440
10	494	.318	47	.390
15	257	.165	31	.257
25	47	.030	11	.091

* 6 controls; all other counts based on 3 animals per group.

TABLE II. Radiation Sensitivity of Stages 1 and 2 Oocytes of 10-Day-Old Mice.

Oocyte stage	Point estimate	LD_{50} values* 95% confidence limits Lower	Upper
1	8.4	7.2	9.7
2	9.4	6.7	14.9

* Computed from arc sine transformations of exp./control ratios of Table I.

designated day 0), and were returned to their mothers as quickly as possible. Doses used were 0, 1, 3, 5, 7, 10, 15 and 25 r of gamma rays from a Co^{60} source described by Kirby-Smith and Darden(11). During exposure, the mice were contained in Lucite tubes with an inside diameter of 1 inch and a wall thickness of 2.5 mm. Distance from the source was 36 cm, giving a dose rate of 2.85 r/min.

Continuous irradiation from a Cs^{137} source, dose rate 0.543 r/hr (~91 r/week) was used for chronic exposures. Young mice were irradiated with their mothers in plastic mouse cages. For a total dose of 26 r, exposure was started on day 10 and completed on day 13; for 91 r, exposure began on day 7 and was completed on day 14. Adult females were exposed to 44 and 87 r at the same dose rate. Mice of all ages were killed 72 hr after the end of exposure.

Ovaries were fixed in Zenker-formol, embedded in paraffin, and sectioned serially at 7 μ. Data in Table I were obtained by counting all oocytes in 8 sections from the middle region of the series for each ovary; adjacent sections were not counted, but the space between sections counted was not regular owing to avoidance of imperfect sections. Data in Table III were obtained by counting the entire series of sections for each ovary. Only those oocytes with the large nucleolus appearing in the section were counted. For smaller oocytes, this procedure avoids counting the same oocyte in 2 sections. However, as the oocyte enlarges, the number of nucleoli increases, and they frequently appear in more than one section. Therefore, for larger follicles, adjacent sections were checked to avoid counting the same oocyte twice.

Oocytes were classified into the following 7 stages on the basis of follicular development: 1—one to 3 follicle cells, no complete follicular later; 2—one complete later of flattened follicular cells; 3—one layer of cuboidal follicle cells; 4—follicle comprised of 2 layers of cuboidal cells; 5—more than 2 layers of cells, no antrum present; 6—antrum in

TABLE III. Survival of Stage 1 Oocytes in Adult and Young Mice.

Age at exposure* (days)	Number of Mice	Dose (r)	Radiation	r/hr	Number of cells	Exp./ Control
10	1	0	—	—	4192	
10	1	25	$Co^{60}\gamma$	171	82	.020
10–12	1	0	—	—	5090	
10–12	1	26	$Cs^{137}\gamma$	.543	672	.132
7–14	1	0	—	—	4819	
7–14	1	91	$Cs^{137}\gamma$	.543	2	.0004
70	3	0	—	—	9121	
59	3	50	X-rays†	5220	12	.0013
137	1	0	—	—	1303	
133–136	1	44	$Cs^{137}\gamma$	.543	407	.312
125–131	1	87	$Cs^{137}\gamma$	.543	364	.279
119–132	1	164	$Cs^{137}\gamma$	.543	24	.074

* All mice killed 72 hr after termination of radiation exposure.
† Head shielded with lead during irradiation.

process of formation; 7—mature or nearly mature follicles, with a well-formed antrum. A few follicles as advanced as stage 6 were observed in 13-day-old mice, but the number in all stages above 2 was so low that no analysis could be made of the data. Estimation of the LD_{50} in young mice was, therefore, made only for oocytes in follicle stages 1 and 2, and comparison of young and old mice has been limited to stage 1.

Experimental results. The counts and experimental/control ratios for stages 1 and 2 oocytes in 10-day-old females (Table I) suggest a reduction with the lowest dose, which was 1 r. Additional data will be required before this response can be considered established. After 3 r, however, a statistically significant reduction in oocyte numbers was observed, and the increase in frequency of "empty" follicles was so marked that qualitative identification of irradiated mice could readily be made at this dose.

The experimental/control ratios of Table I were converted to angles by the arc sine transformation, and LD_{50}'s estimated (Table II). The LD_{50} of 8.4 r for stage 1 and 9.4 r for stage 2 oocytes are, so far as we are aware, the lowest yet observed for any mammalian cell *in vivo*. No significant difference in sensitivity between stages 1 and 2 has been demonstrated. The number of oocytes in follicles more advanced than stage 2 was too low for statistical analysis, but the data suggested a higher resistance to radiation, paralleling the findings in adult females(4-8).

The difference in survival rate after 25 r acute Co^{60} gamma rays and 26 r chronic Cs^{137} gamma rays (2% and 13%, respectively, Table III) indicates that the lower effectiveness of chronic exposure previously observed in a variety of tissues also applies to the immature ovary. This difference also was obtained in adult females, where even 164 r of Cs^{137} gamma rays at 0.543 r/hr was less effective than 50 r of acute X rays. Although a difference in radiation quality enters into both the above comparisons, and particularly in the one involving X rays and Cs^{137} gamma rays in adult females, a difference in biological effect of the magnitude observed would not be expected on the basis of radiation quality alone.

The most interesting comparisons in Table III are those involving response of oocytes of young and adult females to the same dose rate of Cs^{137} gamma rays. Survival of stage 1 oocytes after 87 r in the adult (28%) is 700 times that observed for the closely similar dose of 91 r to young females (0.04%). The exact value of this comparison should not be given undue weight, since the 0.04% survival for 91 r to young females naturally has very wide confidence limits. Other comparisons also show higher sensitivity of oocytes of young females, with survival after 87 r in the adult twice that observed after 26 r in young females, and while 0.04% survival was obtained after 91 r in young females, 164 r to adults reduced oocyte numbers to only 7% of control. Thus the survival of early oocyte stages in young females is much less than that observed for morphologically similar stages in the adult.

Discussion. The marked reduction induced in fertility of female mice by an exposure to 86 r of Cs^{137} gamma rays ($\sim$90 r/wk) during the second week of life(10), as compared with no effect of 86 r at the same dose rate on adult females(9), could be explained by: (*a*) a difference in the frequencies of resistant follicle stages in young and old mice (*i.e.*, the presence of more follicles in later, more resistant stages in adult females), (*b*) different radiation sensitivity of comparable oocyte stages in young and old mice, (*c*) reduced activity of repair processes in young females, or (*d*) the presence, in the young mouse, of a short but very sensitive oocyte stage. An LD_{50} experiment on acute exposure of stage 1 oocytes in adults will be required for a precise estimate of the difference in sensitivity between oocytes of young and adult mice. The extremely low LD_{50} values obtained (Table II) and the homogeneous population indicated by survival curves obviate the need for postulating periods during which sensitivity is higher than in stages 1 and 2. The higher survival after chronic irradiation with 26 r than after acute irradiation (25 r) indicates that repair can take place in young oocytes of 10-12-day-old mice.

Comparison with survival values for adult females suggests that repair processes in young females, however, are not as efficient as in the adult. These data suggest, therefore, that the newly attained dictyate stage is particularly sensitive to radiation. This is in agreement with the results of Peters(12), in which 20 r destroyed most of the young oocytes if irradiation occurred at ages of 7, 14, and 21 days.

The progression of developmental stages of the germ cells during embryonic and early postnatal life are correlated with marked changes in radiation sensitivity. Beaumont (13) recently reported the highest sensitivity of the fetal rat ovary to be on day 15 postfertilization, or at the time of maximum mitotic rate of oogonia(13), but in the male, sensitivity increased with age of the fetus, being highest on day 19(14). This sex difference also has been observed by Rugh and Jackson(15) for the mouse. The above results obviously are correlated with the sexual dimorphism in development of the mammalian germ cells. Male and female gametogenesis is similar until the age at which meiotic prophase begins in the female [day 14 in the mouse(16), day 17 in the rat(13)]. With the initiation of leptotene, oocytes become more resistant, and from this time until birth, most oocytes are in meiotic prophase stages that have been found resistant in a variety of organisms. Primordial germ cells in the male do not develop into type A spermatogonia until after birth in both the rat(17) and mouse(18), and thus remain in a sensitive stage during the time that oocytes of the female are in relatively resistant meiotic prophase stages.

The data of Russell *et al.*(10) indicate that oocytes which have recently reached the dictyate stage are the most sensitive, while in a recent histological study of mice irradiated on the day of birth, Peters and Borum (19) concluded that late pachytene is the more sensitive stage. A more recent paper by Peters(12) indicates a high sensitivity of dictyate oocytes at 7, 14, and 21 days, which is compatible with the results obtained in our experiment. The data reported here involve only dictyate oocytes, since by day 7, all oocytes should have reached this stage(16,19, 20). Dictyate oocytes in early follicle stages remain sensitive to radiation in the adult(5-7), and consequently, the adult female mouse is more easily sterilized than the male. The parallelism between the high radiation sensitivity of oocytes in immature mice observed here, and Mandl's(8) observation that oocytes in 20-23-day rats are more sensitive than those of the adult, together with the results of Peters(12) for mice, raises the possibiltiy of similar periods of high sensitivity at certain developmental stages in the oocyte of other species.

The fertility of irradiated adult females is known to vary widely between species, and the mouse often is considered atypical because sterility can be so easily induced. Possible variations within species, which have long been recognized(6), have not been considered in comparative estimates of radiation damage that have been made(21). The recent demonstration of highly significant strain differences in fertility of irradiated female mice by Ehling(22) reveals the danger of basing the species response on results from a single inbred strain, or from a small number of random-bred animals. The causes for the above species and strain differences are not known, but the meiotic stage at which oocyte development is arrested(23), the meiotic stages present at specific ages, relative frequency of oocytes in follicle stages known to be resistant, rate of development and utilization of oocytes, and intrinsic differences in radiation sensitivity of identical cell stages, all could be important. It should be expected that certain of these variables will apply primarily to species differences, and others principally to within-species variation. Therefore, before valid comparisons on the intrinsic radiation sensitivity of the mammalian oocyte can be made, detailed information on comparative cytology and radiation response at various ages will be required.

Morphologically, stage 1 oocytes in young and old mice are indistinguishable, and with the exception of such obvious events as chromosome breakage, the light microscope is not adequate for study of cellular changes leading to cell death. Although the appearance

of both nucleoli and chromosomes changes with development of the oocyte, all stages during the growth of the follicle in the mouse can be classified as dictyate. During this development, however, the oocyte changes from high sensitivity to high resistance to radiation. It is not now possible to determine whether the morphological changes in the nucleus are correlated with this response, or if, as Mandl and Zuckerman(24) have claimed, systems other than those related to the nucleus are most important. In view of both the difficulties of oocyte cytology and the paucity of information on oocyte metabolism, it would be premature to designate either the nucleus or cytoplasm as the primary factor in radiation-induced cell death, nor can effects on the follicle cells and endocrine system be excluded. Cell division and DNA synthesis, 2 interrelated systems frequently involved in radiation effects, can be ruled out as contributing factors in oocyte response. The mammalian oocyte, therefore, is unique material for the study of radiation-induced cell death.

Summary. Sensitivity of oocytes of 10-day-old mice to radiation-induced cell death has been estimated by counting surviving cells 72 hr after Co^{60} gamma-ray exposures of 0, 1, 3, 5, 7, 10, 15, and 25 r. An LD_{50} of 8.4 r, with 95% confidence limits of 7.2 and 9.7 r has been estimated for stage 1, and an LD_{50} of 9.4 r, 95% confidence limits of 6.7 and 14.9 r, was obtained for stage 2 oocytes. Acute irradiation was more effective than chronic exposure in both young and adult females. After chronic exposure, survival is higher in the adult than for equivalent oocyte stages of young mice. This could result either from higher radiation sensitivity of oocytes of 7-14-day-old females, or from more efficient repair mechanisms in adult females. Oocytes in later stages of follicular development appeared to be more resistant in both immature and adult females. This result is in agreement with the fertility pattern of irradiated female mice and indicates highest resistance for more mature follicle stages. These data are discussed in relation to changes in fertility response of the mouse at different fetal and early postnatal oocyte stages. The possibility that radiation might have extremely severe effects on the ovary at particularly sensitive periods of development in other species is emphasized.

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