Increased Litter Size in the Rat X-Irradiated during the Estrous Cycle before Mating

Abstract. *Average numbers of ovulations, implantations, and living fetuses obtained from rats x-irradiated during the estrous cycle before mating are a function of both the radiation dose and the time of exposure during the cycle. Animals irradiated during metestrus or the day thereafter exhibit significant increases in the factors studied, while those irradiated on the 3rd day after metestrus maintain fewer implantations after exposure to 300, 500, and 600 roentgens, and fewer living fetuses after exposure to 100 to 600 roentgens.*

Exposure of the female rat to x-rays induces a significant increase in the number of ova shed during the next estrous cycle (*1–4*). The degree of increase of ova shed is dependent on both the radiation dose (*2*) and the time of exposure during the estrous cycle (*3*). In rats exposed to 400 roentgens (r) x-irradiation during the metestrus or diestrus before mating, one also observes a significant increase in the number of implantations and living fetuses in the ensuing pregnancy (*3, 4*). Pregnant females carrying living fetuses have normal parturition and significantly larger litters (*5*). This study also characterizes the nature of the increase in the number of living fetuses induced with x-rays with respect to dosage of radiation and time of exposure during the estrous cycle before mating.

A total of 512 virgin female rats (Sprague-Dawley), weighing 175 to 190 g, were subjected to whole-body exposures of 50- to 600-r x-rays on the day of metestrus (M) or 1, 2, or 3 days after metestrus (M+1, M+2, M+3). Metestrus, as determined by daily vaginal smears, is characterized by cheezy clumping of cornified cells. The irradiated females were immediately placed with males, and the time of mating was ascertained by vaginal plugs or sperm in the vagina, or both. After mating, the females were housed individually until the 18th day of pregnancy, when they were killed; the contents of the uteri were examined for resorption sites and live and dead fetuses. The number of corpora lutea in each ovary indicated the number of ova shed.

All exposures were performed at 14:50 hours ± 1 hour. The females were irradiated in a lucite wheel radially divided into 16 individual compartments and rotated slowly under the x-ray beam to insure a more uniform exposure. The source of radiation was an industrial x-ray machine operated at 250 kv peak and 15 ma, 63.5 cm from the skin. The x-ray beam was passed through an aluminum parabolic filter and a 0.5-mm copper filter (half-value layer of 2.2 mm Cu). The resultant dose rate, 18.5 r/min, was corrected for barometric pressure and temperature. Statistical analyses were carried out according to Snedecor (*6*).

Of the 512 rats irradiated, 315 bred during the first anticipated estrus after exposure and were included in this experiment. There was no consistent trend indicating that any irradiation dose had an effect on the incidence of mating.

Animals irradiated during metestrus (M) and 1 day after metestrus (M+1) had significant increases in numbers of corpora lutea beginning at doses of 100 r, with average values of 13.5 and 14.4, respectively, and reaching a maximum value of 30 to 31 for metestrous females receiving 500 and 600 r (Fig. 1). Females irradiated on the day after metestrus exhibited a biphasic ovulation response with peaks of 20.8 and 24.0 ova shed after 300 and 600 r, respectively. Females irradiated on the 2nd day after metestrus also shed significantly more ova after exposure to 200 to 600 r. Unlike the M and M+1 females, however, the number of ovulations did not bear an increasing dose-response relation, but rather showed a maximum response of approximately 14 ova after 200 r which was maintained at the higher doses. Peculiarly, among females irradiated on the 3rd day after metestrus, only those receiving 400 r shed significantly more ova, an observation in agreement with previous data (*3*).

As with ovulations, there was a significant increase in numbers of implantations in females exposed on the day of metestrus to 100 to 600 r. The values range from 13.0 after 100 r to 19.8 after 600 r, and bear a linear relation with increasing dose (Fig. 1). Increased implantations were also observed in females exposed on the 1st day after metestrus to 200 to

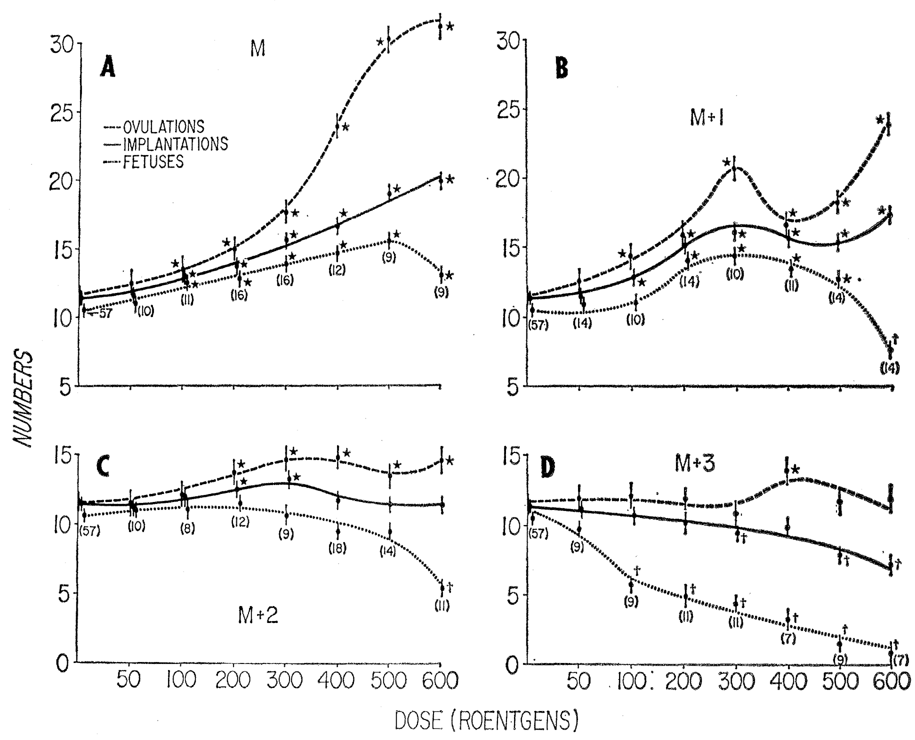

Fig. 1. Comparison of average numbers of ovulations, implantations, and living fetuses obtained from rats exposed to several doses of x-rays at different times during the estrous cycle before mating. Points represent the mean ± 95 percent confidence interval. Key: *, *P* is 0.05 greater than control; †, *P* is 0.05 less than control. Number of animals in group is indicated in parentheses. (A) Irradiation during metestrus (M). (B) Irradiation 1 day after metestrus (M + 1). (C) Irradiation 2 days after metestrus (M + 2). (D) Irradiation 3 days after metestrus (M + 3). Control values are 11.7 ± 0.4 ovulations, 11.4 ± 0.4 implantations, and 10.6 ± 0.4 living fetuses.

600 r, but the response, unlike females irradiated on the day of metestrus, remained fairly uniform in the groups that received 200 to 500 r. In animals irradiated on the 2d day after metestrus, only those receiving 200 and 300 r had significantly more implants. Numbers of implantations in females irradiated on the 2d day after metestrus were significantly below controls after exposure to 300, 500, and 600 r, while the value at 400 r remained at control levels and reflects the significant increase in numbers of ova shed at this dose.

There was no discernible increase in abnormally developed fetuses in irradiated females. Also the incidence of fetal death was extremely low during the latter trimester of pregnancy. In females irradiated on the day of metestrus there was, like ovulations and implantations, a significant increase in the number of living fetuses following 100 to 600 r (Fig. 1). The response paralleled the linear increase in implantations, reaching a maximum of 15.5 fetuses per female after 500 r, then declining to 13.0 per female after 600 r. Females irradiated on the day after metestrus responded with significantly more living fetuses following doses of 200 to 500 r. The maximum responses were observed after 200 and 300 r, followed by a slight decline after 400 and 500 r and a significant decrease to 7.7 fetuses after 600 r. Number of fetuses in females irradiated on the 2nd day after metestrus remained at control levels for all doses except 600 r when the number was significantly reduced to an average of 5.4 fetuses. Average number of fetuses found in females irradiated on the 3rd day after metestrus decreased in a linear fashion from 100 to 600 r with a range of 5.8 after 100 r to 0.9 after 600 r.

The data obtained from females irradiated during metestrus indicate that there exists a clear-cut dose-response relationship for ovulations, implantations, and numbers of fetuses. On the other hand, females irradiated on the day after metestrus (M+1) exhibit a biphasic response. At lower doses M+1 females actually have a greater response than do M females; for example, ovulations at 200 and 300 r for M+1 females are higher than for M females at the same doses. Likewise the number of implantations for M+1 females reach 15.2 after 200 r and remain essentially the same for 300, 400, and 500 r. Numbers of fetuses reach a maximum of 14.1 and 14.4 after 200 and 300 r for M+1 females while a comparable value in M females is not reached until 300 and 400 r (13.8 and 14.5). On the other hand, after 600 r the numbers of fetuses are 13.0 for M females, while they are significantly reduced to 7.7 for M+1 females.

The significant though uniform increase in ovulation rate obtained in M+2 females at doses of 200 to 600 r suggests that only those follicles that survived follicular atresia and attained a particular stage of development were affected by the irradiation. The maximum number of ovulations obtained for M females after exposure to 500 and 600 r (30.1 and 31.1) appears to be the upper limit of response. These numbers coincide with the 30 to 35 developing follicles, 250 μ or greater in diameter, found during metestrus (*7*).

Exposure to 50 r had no effect on the reproductive factors studied, regardless of the time of exposure.

Exogenous sources of gonadotrophins will also cause an increase in the number of ova shed with subsequent implantation, but the end result is generally a decrease in average litter size. This has been attributed to uterine overcrowding (*8*) and to hormonal inadequacy (*9*). The increase in litter size found in females irradiated during metestrus or the day after parallels the implantation rate and clearly suggests that a radiation-induced euhormonal balance exists.

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10. This paper is based on work performed under contract with the U.S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, and has been assigned publication No. UR-49-733. We thank F. Van Slyke and C. Gale for carrying out the irradiation of the animals.

27 February 1967; revised 22 June 1967

Pteridinium and the Precambrian-Cambrian Boundary

Cloud and Nelson (*1*) have discussed the vexed question of the Precambrian-Cambrian boundary (or the Phanerozoic-Cryptozoic boundary, as they seem to prefer) in the light of the discovery of a *Pteridinium* impression in the Deep Spring Formation of California. While remaining unconvinced that their interpretation of the impression is correct, I do not wish to carry this further. In their discussion of the philosophical aspects of the boundary problem they have overlooked the fact that the Ediacara and Nama Beds have been correlated by Glaessner (*2*) with the Precambrian rocks of Charnwood Forest, in Leicestershire, England. This area of Precambrian rocks is separated from the overlying Lower Cambrian by a major unconformity, representing a phase of orogeny during which the Charnian rocks were folded, cleaved, intruded by porphyry and diorite masses, and deeply eroded (*3*). A date of 684 ± 29 million years on one of the latter intrusives has been obtained by Meinesy and Miller (*4*), which indicates that the age of the sediments surrounding the diorite must be considerably older. If Cloud and Nelson's interpretation of Palaeozoic pre-Cambrian (or Phanerozoic) time is to be extended back to include all metazoan fossils it must thus be extended to include far more than the 2000 feet (600 m) of the Deep Spring Formation, to include from Charnwood Forest what is generally regarded as part of Precambrian time more than 680 million years ago. An alternative explanation of the occurrence of their *Pteridinium* in the Deep Spring Formation, which does not seem to have occurred to Cloud and Nelson, is that the chronological range of *Pteridinium* extends upward from the Precambrian well into the Cambrian, possibly much later. Indeed, the whole correlation of the Ediacara, Nama, and Charnwood beds by their fossil faunas is dubious, as what has really been correlated is their unusual state of preservation, and until the chronological range of the faunas is known, no more than a very broad Late Precambrian date can be accepted. Cloud and Nelson's discovery simply adds a range up into the Cambrian.

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