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THE INCIDENCE OF MATING IN X-IRRADIATED FEMALE RATS

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Modification of the vaginal oestrous cycle is a well-documented sequel to irradiation of the common laboratory mammals. In general, irradiated females experience a period of fairly regular oestrous cycles, followed by a reduction or cessation of cycling (Parkes, 1927; Genther, 1931; Shapiro & Nuzdin, 1958; Slate & Bradbury, 1962). In less severely irradiated animals, an eventual return to normal cycling has been reported (Geist, Gaines & Escher, 1941; Bischoff, Ullmann & Ingraham, 1944). Oestrous cycle depression is characterized by prolongation of vaginal cornification, leading to constant cornification and finally permanent di-oestrus (Mandl, 1959a; Plagge & Krehbiel, 1962; Slate & Bradbury, 1962).

While X-ray-induced changes in vaginal oestrus have been described in considerable detail, very little information is available concerning the pattern of psychological and physiological oestrus in irradiated animals. Exposure of rats during metoestrus to 50 to 200 R X-rays has been found to induce typical signs of psychic oestrus—increased activity, lordosis, ear quivering—in 49% of the animals (Freed, Farris, Murphy & Pendergrass, 1948). The induced oestrus was of short duration (4 to 12 hr), but was accompanied by mating in fifteen of twenty-eight animals and by pregnancies in four.

Mandl (1959b) observed that, following the exposure of exteriorized rat ovaries to 4400 R X-ray, a dose sufficient to induce sterility, vaginal oestrous cycles persisted for up to 40 days. The irradiated females mated as long as cycling occurred. Of 187 matings, 138 were recorded during the first 4 weeks after exposure, and forty-nine at longer intervals. The presence of freshly formed corpora lutea indicated that ovulation had occurred, although none of the mated animals became pregnant. Those animals breeding within 3 days of irradiation occasionally became pseudopregnant.

In this study, mating (psychic oestrus) resulting in term pregnancy (indicating physiological oestrus) is used to characterize the oestrous cycles of an initially synchronous (metoestrus) population of rats for a period of 30 days following exposure to sublethal levels of whole-body X-irradiation.

Virgin female rats of Sprague-Dawley ancestry (Blue Spruce Farms, Alta-

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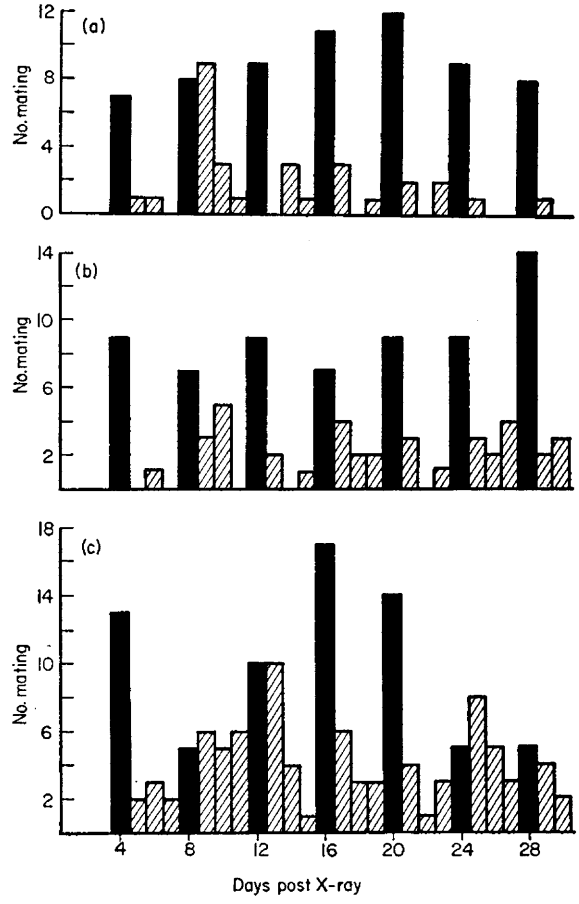
mont, N.Y.), weighing 190 to 210 g, were housed in groups of six in a controlled temperature environment (72±2° F) under 12-hr fluorescent light/24-hr period (06.00 to 18.00 hours). Daily vaginal smears were taken, and those animals which presented cheese-like clumps of cornified epithelial cells (defined as metoestrus in this laboratory) were subjected to single whole-body (W.B.) exposures of either 250 R or 500 R X-irradiation delivered at a dose rate of approximately 18·5 R/min. A detailed description of the irradiation procedure has been published previously (Hahn & Ward, 1967). Control mating incidence was obtained from comparably handled, unirradiated animals. The metoestrus at which groups of rats were irradiated was designated Metoestrus 1.

Following exposure, the females were again housed in groups of six for varying lengths of time (1 to 27 days) before being placed with an experienced male. Previous observations had indicated that 70 to 75% of metoestrous



TEXT-FIG. 1. All animals irradiated during metoestrus. Expected days of mating based on the *a priori* assumption that animals maintain regular 4-day oestrous cycles. (a) Incidence of mating in un-irradiated female rats. (b) Incidence of mating in female rats irradiated with 250 R W.B. X-ray. (c) Incidence of mating in female rats irradiated with 500 R W.B. X-ray. Solid columns: incidence of mating on expected day of mating; hatched columns: incidence of mating on days other than the expected day of mating.

females housed with males mated 4 days later. In the absence of males, it was assumed *a priori* that the females maintained regular 4-day oestrous cycles. Thus, an animal housed with a male 21 to 24 days after Metoestrus 1 should have completed five cycles, and would be expected to mate on the 24th day, or six cycles later. For breeding purposes, females were housed individually with a male 2 to 3 days before the next anticipated oestrus. The incidence of mating, recorded for 30 days after irradiation, was determined by the presence of copulatory plugs in the underlying litter trays. Only those matings resulting in term pregnancy were included in the results. Females remained with the male until mating occurred or until the 30-day observation period elapsed. Food and water were unrationed throughout the experiment. Chi-square analysis was used to validate comparisons.

As Text-fig. 1 indicates, the assumption that initially synchronous rats would maintain regular 4-day oestrous cycles in the absence of physical contact with males was not fully substantiated. Of ninety-three control animals, sixty-four (69%) mated on one of the anticipated days after Metoestrus 1. In all cycles, mating was infrequent on days other than the expected one, although there was an equal propensity for mating on the 8th and 9th days after Metoestrus 1. Either group-housed female rats are less susceptible than mice to oestrous cycle alteration in the absence of males, or the presence of male rats in the same room (but in different cages) provided sufficient stimulus for continuance of cycling in this experiment (Whitten, 1966).

Following 250 R whole-body X-irradiation of 102 animals on the day of Metoestrus 1 (Text-fig. 1), sixty-four (63%) mated on one of the expected days. As with unirradiated females, those receiving 250 R exhibited fairly regular cycles of psychic and physiological oestrus during the first 30 days after exposure.

Of 150 females receiving 500 R whole-body X-irradiation on Metoestrus 1, only sixty-nine (46%) mated on one of the expected days (Text-fig. 1). This is a significantly (Chi square = 41·4, $P = 0{\cdot}001$) smaller proportion than that observed in the control group. Mating occurred with essentially equal frequency on Days 8 to 13 and Days 24 to 29 after exposure, suggesting that the second and third and the sixth and seventh cycles after 500 R were irregular. On the other hand, the first cycle and the fourth and fifth cycles appear to be fairly regular in that mating occurred when expected. A similar alteration between normal and abnormal vaginal oestrous cycles in the irradiated rat has been reported by Levine & Witschi (1933). The equal frequency of mating on Days 8 to 13 suggests that 500 R can induce prolongation of the mating cycle. This would confirm the reports of Drips & Ford (1932), Levine & Witschi (1933) and Mandl (1959a) indicating a temporary prolongation of vaginal oestrus in the cycles immediately following irradiation in the rat.

While 500 R whole-body X-irradiation significantly influenced the particular day on which the animal mated, it had no effect on the total percentage of animals mating during the first 30 days after Metoestrus 1. Regardless of radiation dose, 90 to 95% of the animals mated during the first month, and of these 90 to 95% were pregnant on Day 20 of gestation. There appeared therefore, to be no dissociation of psychic and physiological oestrus in the irradiated animals.

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