Differential Radiosensitivity of Rat Blastocysts During Delayed Implantation[1] (36555)

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The development of rat embryos can be interrupted at the blastocyst stage by bilateral ovariectomy before the fourth day of pregnancy, followed by daily injections of progesterone (1, 2). Blastocysts thus delayed remain unattached but viable in the uterine lumen. Implantation and resumption of normal development can be induced at will by the administration of estrogen.

On Day 5 of castration-induced delayed pregnancy (Day 1 = the day sperm are found in the vaginal smear) the rat blastocyst consists of approximately 30 cells, most of which synthesize nucleic acids (3–5) and undergo mitosis [(6); Ward and Miller, unpublished data]. There is a decrease in, then virtual cessation of, DNA synthesis and cell division by Day 9 of delayed pregnancy (3, 4, 6–8). Embryonic RNA and protein syntheses also decrease during this 4 day period, but persist at a rate greater than that of DNA (5, 7–10). Carbon dioxide production is reduced (11), but oxygen consumption apparently is not (12). By Day 9 the delayed rat blastocyst attains a maximum size of approximately 85 cells, then remains in a state of apparent developmental arrest until estrogen is administered (4–10, 13).

With few exceptions the vulnerability of living cells to ionizing radiation is directly proportional to their mitotic and metabolic activity (14). If the rat embryo conforms to the law of Bergonie and Tribondeau (14), the blastocyst on Day 5, being mitotically more active, should also be more radiosensitive than the essentially inactive blastocyst on Day 9 of delayed pregnancy. This experiment is designed to test this hypothesis, using the survival of the embryo after *in vivo* X-irradiation as an index of radiosensitivity. This parameter measures the embryo vulnerability to both direct and indirect (maternal) X-ray damage.

The survival of rat blastocysts X-irradiated *in vivo* on Day 5 of delayed pregnancy increases significantly if the period between irradiation and implantation is prolonged, *i.e.*, if implantation is delayed (15, 16). Apparently some portion of the potentially lethal X-ray damage in the embryo, or mother, or both is amenable to repair, if sufficient recovery time is provided before the demands of nidation must be met. Both the rate and the extent of recovery from potentially lethal X-ray damage during delayed implantation are functions of the radiation dose (16).

Hooverman, Meyer and Wolf (15) demonstrated that rat blastocysts exposed to 400 R X-irradiation either on Day 5 or 13 of delayed pregnancy exhibited similar survival rates when estrogen was administered 24 hr after exposure. They concluded that the blastocysts were equally radiosensitive throughout delayed implantation. In light of recent evidence concerning the rapidity with which recovery from X-ray damage can occur (16) however, it was felt that the survival of embryos given estrogen 24 hr after irradiation (*i.e.*, after a 24 hr recovery period) may not be an accurate index of their initial radiosensitivity. In addition to comparing the initial radiosensitivity of rat blastocysts of Days 5 and 9 of delayed pregnancy, we have determined the effect of a preimplantation recovery period on the survival of the active

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TABLE I. Effect of Preimplantation Recovery Period on Embryonic Survival and Development in Rats Sham-irradiated on Day 5 or 9 of Delayed Pregnancy.[a]

Estrone (day)	Recovery time (hr)	N	Av no.		Percentage survival[b]	Av wt (g)	
			Implantations	Live fetuses		Fetus	Placenta
				Sham X-ray Day 5			
5	0	10	7.1 ± 0.8	5.7 ± 0.9	79.0 ± 7.1	1.98 ± 0.05	1.14 ± 0.04
6	24	11	7.1 ± 0.8	5.4 ± 0.8	73.7 ± 5.3	2.09 ± 0.03	1.07 ± 0.03
7	48	9	6.3 ± 1.2	4.7 ± 0.9	72.5 ± 7.4	2.18 ± 0.03	1.03 ± 0.03
8	72	12	6.1 ± 0.9	4.3 ± 0.9	66.4 ± 10.3	2.15 ± 0.05	1.08 ± 0.03
				Sham X-ray Day 9			
9	0	12	4.8 ± 0.4	3.3 ± 0.5	70.7 ± 8.4	2.01 ± 0.05	1.24 ± 0.07
10	24	10	5.3 ± 1.0	4.1 ± 1.0	70.6 ± 10.6	1.94 ± 0.04	0.99 ± 0.03
11	48	12	6.3 ± 1.0	5.1 ± 0.8	79.9 ± 5.3	1.84 ± 0.03	0.98 ± 0.03
12	72	9	6.2 ± 0.8	5.2 ± 0.9	83.7 ± 7.1	2.04 ± 0.03	1.03 ± 0.03

[a] All values ± standard error of the mean.
[b] Number live fetuses/number implantations.

(Day 5) compared to the dormant (Day 9) blastocysts after irradiation.

Materials and Methods. Nulliparous female rats of Sprague-Dawley ancestry (Badger Research Corp., Madison, WI) weighing 180–220 g were placed with males, and the day sperm were detected in the vaginal smear was designated Day 1 of pregnancy. Pregnant rats were housed in groups of four in a room maintained at 80 ± 3°F with 14 hr fluorescent light/day (5 a.m. to 7 p.m.). Food and water were provided *ad libitum*.

On Day 3 of pregnancy (40 to 60 hr after fertilization), all animals were bilaterally ovariectomized as described previously (2). Care was taken to avoid trauma to the embryos, which are, at this time, in the four cell stage and located in the oviduct. All castrate rats were given daily subcutaneous (SC) injections of 4.0 mg progesterone (Upjohn Co., Kalamazoo, MI, Lot HR 855) dissolved in 0.2 ml of corn oil (USP, Magnus, Mabee, and Reynard, Inc., New York).

Between 10 and 11 a.m. on Day 5 or Day 9 of delayed pregnancy, rats were exposed to 500 R acute, whole-body X-irradiation. The animals were irradiated in a wire-bottomed Lucite wheel, radially divided into 12 individual compartments. The wheel was rotated slowly under the X-ray beam to insure uniform exposure. The source of radiation was a General Electric Maxitron 300 X-ray machine, operated at 250 kilovolt peak (kVp) and 20 mA, at a target–skin distance of 100 cm. The X-ray beam passed through 1.0 mm Al and 1.0 mm Cu filters (HVL = 2.05 mm Cu), and the resulting dose rate, approximately 21 R/min, was corrected for barometric pressure and temperature. Sham-irradiated control females were confined in the rotating wheel for a comparable period of time, but the X-ray machine was not turned on.

Following irradiation, groups of animals were given 0, 24, 48, or 72 hours to recover before implantation was induced by the administration of 1.0 μg estrone (USP, International Hormones, Brooklyn, NY, Control No. 5274) added to the daily progesterone injection. Five days after the administration of estrogen, the animals were laparotomized, and the number, position, and size of the implantation sites were recorded. Animals having no implantation sites or sites larger than 6.3 mm (indicating spontaneous implantation before estrogen treatment) were discarded. Fifteen days after estrogen administration, the animals were killed and examined for numbers of live and dead fetuses and resorbed implantation sites. Live fetuses were sexed, weighed, and examined for gross morphological abnormalities. Placental weights were also recorded. Embryonic sur-

TABLE II. Effect of Preimplantation Recovery Period on Embryonic Survival and Development in Rats X-irradiated[a] on Day 5 or 9 of Delayed Pregnancy.

Estrone (day)	Recovery time (hr)	N	Av no.		Percentage survival[c]	Av wt (g)	
			Implantations	Live fetuses		Fetus	Placenta
				500 R X-ray Day 5			
5	0	9	6.6 ± 0.7	0.1 ± 0.1[e]	1.9 ± 1.9[e,f]	1.78 ± 0.00	1.03 ± 0.00
6	24	11	7.1 ± 1.0	1.5 ± 0.3[e]	24.1 ± 4.7[e]	1.77 ± 0.09[e]	0.83 ± 0.06[e]
7	48	10	7.7 ± 1.0	3.4 ± 0.8	40.6 ± 5.7[d,g]	1.78 ± 0.03[e]	0.78 ± 0.04[e]
8	72	13	8.3 ± 0.8	3.0 ± 0.7	37.3 ± 7.4[d]	1.75 ± 0.04[e]	0.78 ± 0.04[e]
				500 R X-ray Day 9			
9	0	9	6.7 ± 1.0	1.4 ± 0.5	22.5 ± 7.2[e]	1.60 ± 0.06[e]	0.83 ± 0.10[e]
10	24	10	4.7 ± 0.6	1.1 ± 0.4[d]	25.0 ± 8.7[e]	1.49 ± 0.04[e]	0.77 ± 0.07[e]
11	48	12	6.5 ± 0.8	1.4 ± 0.4[e]	18.1 ± 4.2[e]	1.43 ± 0.06[e]	0.70 ± 0.04[e]
12	72	8	4.8 ± 0.8	0.9 ± 0.3[e]	21.7 ± 7.4[e]	1.71 ± 0.04[e]	1.00 ± 0.10[g]

[a] 500 R whole-body X-irradiation 10–11 a.m. Day 5 or 9.
[b] All values ± standard error of the mean.
[c] Number live fetuses/number implantations.
[d] $p < .05$ less than sham-irradiated control.
[e] $p < .01$ less than sham-irradiated control.
[f] $p < .05$ less than 500 R X-ray, Day 9, 0 hr recovery.
[g] $p < .05$ greater than 500 R X-ray, Day 9, 48 hr recovery.

vival was expressed as the percentage of implanted embryos, observed at laparotomy, surviving to autopsy.

The data were analyzed by Duncan's new multiple range test (17) to determine the significances of differences between group means.

Results. There were no significant differences in average numbers of implantations in the irradiated and control groups (Tables I and II), indicating that under these conditions irradiation had no effect on the survival of the embryo before implantation. The slightly smaller litter sizes apparently were the result of trauma to some embryos in each rat the time of castration.

Embryonic survival: Initial radiosensitivity. Embryonic survival in groups receiving estrogen immediately after irradiation is an index of the initial radiosensitivity of active (Day 5) and inactive (Day 9) blastocysts. In 9 animals exposed to 500 R whole-body X-irradiation on Day 5 of delayed pregnancy, and given estrogen immediately, only 1 of 63 (1.9%) implanted embryos survived to autopsy. In 9 animals given estrogen immediately after a comparable exposure on Day 9, 13 of 61 (22.5%) embryos survived, a significantly higher percentage ($p < .05$) than that observed in animals irradiated on Day 5. In both cases, however, the survival of the irradiated blastocysts was significantly ($p < .01$) lower than that observed in the comparable sham-irradiated control groups given estrogen on Days 5 or 9 (79.0 and 70.7%, respectively).

Embryonic survival: Recovery. Embryonic survival in sham-irradiated control rats ranged from 66 to 83% regardless of when estrogen was administered.

Embryonic survival in animals irradiated on Day 5 of delayed pregnancy increased linearally with recovery time for approximately 48 hr. With each 24 hr increment in recovery time, the increase in embryonic survival was significant at the 95% confidence level. Maximum recovery apparently was attained after 48 hr, since an additional 24 hr (estrone Day 8, recovery time 72 hr) resulted in no further increase in embryonic survival.

In animals exposed to 500 R on Day 9 of delayed pregnancy, embryonic survival did not increase as a result of increased time

between irradiation and implantation (Table II). Survival ranged between 18 and 25%, and was independent of recovery time. In all irradiated groups, including those exhibiting significant recovery from potentially lethal X-ray damage, embryonic survival was significantly ($p < .05$) below that of the corresponding sham-irradiated control groups.

Fetal development. All irradiated fetuses were significantly smaller than controls at autopsy. Placental weights were significantly reduced in all irradiated animals except those given estrogen 4 days after 500 R on Day 9 (Table II). The sex ratio of control and irradiated fetuses, 97.4 and 102.9, respectively, was not significantly different. In the control rats, 18 of 422 fetuses were dead at autopsy, an average of one dead for each 22 live fetuses. In the irradiated animals, 13 of 153 fetuses were dead at autopsy, an average of one dead for each 11 live fetuses. Most of the embryonic mortality occurred shortly after implantation, as indicated by resorption sites at autopsy. None of the 404 living fetuses from sham-irradiated females were grossly abnormal, whereas 4 of the 140 living fetuses from irradiated females presented abnormalities, including 3 with umbilical hernias and one with marked edema.

Discussion. The data demonstrate that the rat blastocyst on Day 5 is significantly more sensitive to X-irradiation *in vivo* than is the embryo on Day 9 of delayed pregnancy. The blastocyst is mitotically active on Day 5, and is mitotically quiescent on Day 9 [(6); Ward and Miller, unpublished data). The response of rat embryo to X-irradiation *in vivo*, therefore, appears to obey the law of Bergonie and Tribondeau (14). While the embryo's vulnerability to direct radiation damage may be a function of its rate of mitosis, factors influencing its susceptibility to indirect damage are not clear. Changes in uterine function may occur during delayed implantation, and may affect the embryo response to ionizing radiation.

Although the rat blastocyst on Day 5 is initially more radiosensitive, it possesses the capacity to repair potentially lethal X-ray damage for approximately 48 hr, provided implantation (estrogen administration) is postponed. Embryos irradiated on Day 9 of delayed pregnancy, while initially more resistant, exhibit no capacity to repair lethal damage. After 48 hr of recovery, the survival of embryos irradiated on Day 5 increased to a level significantly greater ($p < .05$) than that of embryos irradiated on Day 9, a reversal of the initial radiosensitivities of the two groups.

It is tempting to speculate that the repair mechanism, as yet unknown, is one of the metabolic processes (in addition to mitosis, DNA, RNA and protein synthesis) which is arrested during delayed implantation. The generally higher metabolic rate on Day 5 could then account for both the greater initial radiosensitivity and the capacity to repair X-ray damage observed in blastocysts irradiated at that time.

X-irradiation on Day 5 of delayed pregnancy inhibits mitosis in the blastocysts for approximately 12 hr (Ward, Meyer, and Wolf, unpublished data). This radiation effect cannot be detected in blastocysts irradiated on Day 9, since mitosis does not occure at this time, even in nonirradiated controls. This differential response to X-irradiation may account, in part, for the differential radiosensitivities of embryos irradiated on Days 5 and 9.

The present data support Hooverman, Meyer and Wolf (15) report of equal embryonic survival in rats given estrogen 24 hr after 400 X-irradiation on Day 5 or 13 of delayed pregnancy. The data indicate, however, that this observation should not be interpreted as evidence of equal radiosensitivity in the active and dormant blastocysts, since the 24 hr recovery period is the only time at which their survival rates are similar.

Summary. Mitotically active rat blastocysts on Day 5 of castration-induced delayed pregnancy are initially more sensitive to the lethal effects of X-irradiation *in vivo* than are mitotically dormant blastocysts on Day 9. However, embryonic survival in animals irradiated on Day 5 increases significantly if implantation is postponed for 24 or 48 hr, indicating that recovery from potentially lethal X-ray damage can occur during early delayed implantation. Although the quiescent

blastocyst on Day 9 of delayed pregnancy is initially more resistant to radiation insult than is the embryo on Day 5, the former exhibits no capacity to repair lethal X-ray damage.

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