

Indirect Effect of Irradiation on Embryonic Development. IV. Lethal Effects of Maternal Irradiation on First Day of Gestation In the Rat.* (32186)

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The concept of a maternal or indirect effect of irradiation to the zygote or embryo has interested several investigators(1-7). There is still some disagreement among authors as to whether a "maternal" effect exists. Some of the discrepancies in experimental results may have arisen from the utilization of different species of experimental animals, dose of irradiation, or stage of gestation. The most difficult period of gestation to prove or disprove a maternal effect is during the pre-implantation period, especially on the first and second day of gestation.

The results from our laboratory agree most closely with the data and interpretations of Russell(1,2,8). A summary of these conclusions is as follows: (1) The early embryo varies in its sensitivity to the lethal effects of irradiation at different times during the first and second day of gestation. (2) The very young preimplanted embryo has a high resistance to the teratogenic effects of irradiation but a low resistance to the lethal effects of irradiation. (3) Most of the deaths from irradiation of the early embryos may have resulted from chromosome breakage and it appears that there is a minimal or no maternal effect. It is this last point which is open to question since investigators have reported a maternal effect from experiments in rabbits dealing with the transfer of normal rabbit ova into irradiated recipients(7).

Our laboratory has investigated the relative importance of uterine, ovarian, oviduct and zygote irradiation in the rat using special shielding techniques. The experiments indicated that uterine and ovarian irradiation did not prevent embryonic death. Oviduct irradiation on one-half day of gestation resulted in the same mortality as simultaneous ovarian, uterine and oviduct irradiation(9). Thus, the

effects of abdominal reproductive organ irradiation were wholly due to oviduct irradiation. Since the ova were contained within the irradiated oviduct these experiments were not able to separate the effects of oviduct and ova irradiation.

It is the purpose of this report to extend these studies to include the effect of whole-body maternal irradiation, thus determining whether oviduct irradiation can account for all the embryonic mortality following irradiation of the pregnant rat on the first day of gestation. If the embryonic mortality is the same in pregnant mothers receiving whole-body irradiation as in pregnant mothers receiving only reproductive organ irradiation, then no extra-reproductive organ maternal effect would have been demonstrated in the rat. If the mortality is higher in pregnant mothers receiving whole-body irradiation, then a maternal effect will have been demonstrated.

Materials and methods. Wistar rats randomly inbred in our colony were mated overnight and the females were examined for vaginal sperm the next morning. Pregnancy was considered to be initiated in the inseminated females at 9 a.m. that morning, and the embryos were considered to be 0 hours and 0 days old at that time. Four groups of experimental control animals were utilized. Each of the 4 groups was either sham irradiated, irradiated, sham laparotomized or laparotomized at 4 p.m. on the first day of pregnancy, 7 hours after the estimated beginning of pregnancy.

1. The first group consisted of 19 inseminated females that were neither laparotomized nor irradiated (Table I).

2. The second group of 21 inseminated females was anesthetized, (30 mg sodium pentobarbital/kg), laparotomized and lead shields were placed over the uterus, oviduct

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TABLE I. Effect of 150R X-Irradiation to the Rat Zygote During First Day of Gestation. Evaluation of maternal effect.

Dose	0R Non-operated	0R Laparotomized shielding of ovary, oviduct, uterus	150R Non-laparotomized whole body irradiation of maternal rat	150R Laparotomized local irradiation of uterus, oviduct, and ovary
No. females inseminated	19	21	13	55
% Pregnant	79%	71%	69%	80%
Total implants	137	137	101	348
Mean implants/litter \pm SD	9.13 \pm 2.54	9.33 \pm 2.70	10.22 \pm 3.30	8.29 \pm 2.93
Total live term fetuses	134	131	32	109
Mean term litter size \pm SD	8.93 \pm 2.71	8.73 \pm 1.49	3.55 \pm 1.49	2.59 \pm 1.52
Mean term fetal wt in g \pm SD	4.82 \pm .56	4.71 \pm .67	5.04 \pm .24	4.84 \pm .79
Total resorptions	3	6	69	239
Mean resorptions/litter \pm SD	.20 \pm .15	.40 \pm .91	6.67 \pm 3.61	5.69 \pm 3.10
% Resorbed	2.1%	4.3%	63.7%	68.7%

and ovary on each side. No irradiation was given.

3. The third group of 13 inseminated females was anesthetized and received 150 R whole-body irradiation. The radiation factors were 100 KVP; 10 m.a.; dose rate 34 R/min; T.O.D. 40 cm; filtration 1 mm Al; half value layer 3.4 mm Al. A phantom was made with fresh intestine, skin, muscle and subcutaneous tissue to determine what dose the uterus ovary and oviduct received in the intact animal. In all determinations the uterus, ovary and oviduct received 98% or more of the dose delivered to the skin.

4. The fourth group of 55 inseminated females was anesthetized, laparotomized and received 150R to the ovary, oviduct and part of the uterus. The remainder of the rat was shielded from irradiation. Previous experiments had demonstrated that irradiation of the oviduct resulted in the same mortality as irradiation of oviduct, ovary and uterus(9). Furthermore, irradiation of the oviduct alone would have involved mechanical manipulation of the oviduct, which we wished to avoid. Finally, the primary purpose of this experiment was to determine whether there was an extra-reproductive organ maternal effect. Therefore, it was felt that the conditions chosen for the fourth experimental group were appropriate.

Following the irradiation or sham procedures the animals were housed in stainless steel cages with ample food and water. After 21 days of pregnancy the mothers were sac-

rificed by decapitation and the fetuses were delivered by cesarian section. The fetuses were examined, weighed and fixed in Bouin's solution for future dissection. The numbers of viable and non-viable fetuses were recorded along with the numbers of resorption sites. The corpora lutea were also counted. Various statistical measurements were calculated for the 4 experimental and control groups. These are tabulated in Table I.

Results. The term fetal weights of all experimental and control groups were not significantly different at the $p < .01$ level of significance. These results corroborated previous experiments which indicate that irradiation on the first day of gestation does not produce growth retardation in the surviving fetuses at term.

The data dealing with mortality include several parameters (Table I): (1) The number of implantation sites at term; (2) The number of live fetuses at term; (3) The number of resorptions observed at term. There was no difference in these parameters between the 2 non-irradiated groups. Similarly, the 2 irradiated groups had resorption rates which were not significantly different. The pregnant mothers receiving 150R whole-body irradiation had a resorption rate of 63.7% while the mothers receiving 150R local irradiation had a resorption rate of 68.7%. These were not significantly different at the $p < 0.1$ level of significance.

There was no drastic change in the percent of mothers coming to term in the 4 experi-

mental groups. Neither whole-body irradiation nor laparotomy increased the incidence of whole-litter resorptions.

There were no animals with gross external central nervous system malformations observed in either irradiated group.

Discussion. The results of the experiment demonstrate that the effect of 150R whole-body irradiation in the pregnant rat is not more deleterious than irradiation of only the abdominal reproductive organs on the first day of gestation. This would indicate that if there is a maternal effect following a dose of 150R it is not due to irradiation of non-reproductive organ structures. Since previous studies have reported a maternal effect with doses of 200R or above, it would appear that either the maternal effect does not appear at lower doses of irradiation or that it is due to irradiation of genital organ structures(9). Since a previous report from this laboratory indicated that the entire effect of local reproductive organ irradiation on the first day of gestation was due to irradiation of the oviduct and the ova therein, it would appear that if a maternal effect exists at this stage and dose of irradiation, then it is due to oviduct irradiation(9). The concept can be readily proved or disproved by ova transplantation experiments in which the irradiated recipient has the oviduct irradiated or shielded. Although this concept has not been proved, it is not improbable since there is ample evidence in the literature to indicate that the premature discharge of ova into the uterus can contribute to embryonic mortality (10-13).

These data reinforce our previous study which indicated that the incidence of exencephaly was very low in rat embryos irradiated on the first day of gestation(9) in comparison to the irradiated mouse embryo (14). A discussion of these results is included in the previous reports and these additional data do not change any of the previously published concepts.

It is interesting that the incidence of whole-litter resorptions was not increased in the pregnant rats irradiated on the first day of pregnancy. Previous reports from our laboratory also indicated that the pregnant rat

did not respond to irradiation with an all or none-phenomenon during several stages of the preimplantation period(15). On the other hand, both Russell(1) and Brent and Bolden (15,16) report that the mouse is quite susceptible to irradiation-induced whole-litter resorptions. Russell reported that 100R given to pregnant mice during the preimplantation period reduced the incidence of positive pregnancies from 72.2% in the controls to 46.7% in the irradiated mothers. Brent and Bolden reported that 30R reduced the incidence of positive pregnancies from 75% in the controls to 44.7% in the irradiated mothers. Thus, there appears to be no question that whole-body irradiation administered to pregnant mice causes a significant number of mothers to resorb their entire litter. These resorptions are not in a continuum with the increased incidence of resorptions observed in the litters that come to term, and it appears that the whole-litter resorptions in the mouse are an altogether different phenomenon than the increased radiation deaths observed in the surviving litters. Progesterone was administered to the pregnant mice in an attempt to abolish the whole-litter resorption effect but this was not successful(14). Russell recently reported that shielding the head and chest of pregnant mice reduced the increased incidence of whole-litter resorptions. She indicated that this might indicate that the whole-litter resorption effect might be the result of pituitary irradiation(1). On the other hand, all the pregnant mice irradiated in our laboratory have had the head and thorax shielded. With this radiation technique we observe the phenomenon of whole-litter resorptions in the mouse. Thus, we have observed the phenomenon without pituitary irradiation. The fact remains that during the process of irradiating pregnant mice early in gestation, whole-litter resorptions occur. There appears to be no proven explanation. The pregnant rat does not demonstrate this phenomenon. This difference is just one more variation in the response of the pregnant mouse and rat to x-irradiation.

Summary. 1. An attempt was made to determine whether there is a maternal influence involved in the lethality of rat ova

irradiated on the first day of pregnancy. It was demonstrated that 150R wholebody irradiation was no more deleterious than irradiation of the pregnant abdominal reproductive organs in rats. Therefore, it was concluded that there is no maternal effect due to irradiation of maternal structures other than abdominal reproductive organs at this stage of gestation and dose of irradiation. If a maternal effect exists it may be due to oviduct irradiation or it may be present with higher doses of irradiation. 2. One hundred fifty Roentgens irradiation on the first day of gestation in the rat did not increase the incidence of exencephaly or gross congenital malformations and did not result in fetal growth retardation. These facts and their significance, reported previously reinforce the discrepancy in the incidence and type of malformations induced in the mouse and rat following irradiation on the first day of gestation. 3. Although irradiation of the mouse during the preimplantation period produces a phenomenon referred to as whole-litter resorptions, this does not occur in the rat. Thus, the exencephaly reported by Rugh and the whole-litter resorptions reported by Russell and Brent are produced by irradiating the preimplanted mouse embryo, but do not occur following irradiation of the preimplanted rat embryo. These variations in species response must be explained before attempting to apply any of these data to the human.

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Synodic Monthly Modulation of the Diurnal Rhythm of Hamsters.* (32187)

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Biological rhythms and clocks appear to be very widespread, if not of universal occurrence, in animals, plants, and microorganisms, and probably most physiological processes

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reflect to some degree their presence. This indicates a very ancient origin, long prior to when living creatures left their ancestral marine habitat. A great deal of evidence has established the existence of a fundamental solar-lunar timing system for rhythmic physiological variations in marine organisms, adjusting the