PATTERNS OF EMBRYONIC MORTALITY IN MICE AFTER EXPOSURE TO ^{137}Cs GAMMA IRRADIATION

by

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INTRODUCTION

The effects of irradiation on embryonic development in mammals have been studied extensively in mice and rats, and reviewed by Russell and Russell, 1954a; Rugh and Wohlfromm, 1962; and Rugh, 1971, 1973. Most of this work has been done using partial or whole body irradiation to the maternal organism. In these investigations, the effect of irradiation on embryonic development was measured. However, the primary effects of irradiation on the embryo, and the secondary effects on the embryo resulting from physiological and biochemical changes in the dam were not separated. Present embryo transfer techniques make the assessment of these two effects possible.

It is imperative to separate the embryonic and maternal responses to varying environmental stresses if the complex interactions which exist between the dam and fetus are to be understood. It also becomes necessary to determine how much stress the maternal organism can absorb before embryonic development is affected. Radiation is a useful tool in studying the general problem of maternal stresses, since its primary effects are instantaneously distributed throughout the protoplasm of all cells in the organism (Russell and Russell, 1954b). Past experiments have failed to measure the separate response of the dam and embryo to irradiation and the interaction between them. This study was designed to measure these responses in terms of subsequent embryonic mortality.

The objectives of this study were:

(1) To investigate the viability of embryos after combinations

of whole body irradiation to the recipient and *in vitro* irradiation to the embryo.

(2) To determine the stage of gestation at which embryonic mortality occurs after exposure of the recipient or embryo to 200 R.

(3) To study the viability and structural changes occuring in blastocysts irradiated at 0, 100 and 200 R *in vitro* and cultured for 96 hours.

REVIEW OF RELATED LITERATURE

The Effects of Irradiation of the Preimplantation Embryo on Subsequent Embryonic Mortality

The high incidence of embryonic loss which results when animals are exposed to irradiation is primarily due to damage to the embryo (Rugh and Grupp, 1959, 1961; Harvey, 1964; Harvey and Chang, 1964). Several excellent review articles outline the deleterious effects of irradiation on the embryo (Butler, 1936; Russell, 1950; Russell and Russell, 1954a,b; Rugh, 1971, 1973). Russell and Russell (1954b) state that the developing embryo exhibits a changing response to irradiation. This variability may be due in part to the fact that the embryo is a mosaic of changing activity centers, and differs from moment to moment during its development (Rugh and Wohlfromm, 1962). The overall result of irradiation to the preimplantation embryo is either failure to implant, and death, or survival to term (Russell, 1957). Preimplantation embryos possess great regulatory powers, since a large proportion of the fetuses surviving irradiation during the preimplantation stages were probably some in which a considerable proportion of the blastomeres were killed (Russell and Russell, 1954a). Gardner and Edwards (1968) have shown that rabbit blastocysts can develop normally after removal of a large portion of the trophoblast. Gardner (1968) has shown that extensive mosaicism may occur in mice when as few as three foreign cells were injected into a mouse blastocyst.

Prenatal losses of mice within a litter, and entire litters,

occur during irradiation in the preimplantation period. As the embryo increases in age, preimplantation death decreases (Rugh and Grupp, 1961).

Five to twenty-five Roentgen (R) of irradiation have been shown to interfere considerably with the growth and development of mammalian embryos (Russell, 1957; Rugh and Grupp, 1959, 1961; Rugh, Wohlfromm and Varma, 1969). As gestation advances, it becomes increasingly difficult to terminate pregnancy with irradiation, and after 13 days gestation in the mouse, it is virtually impossible to terminate pregnancy with a dose less than the LD50/30 for the dam (Rugh and Wohlfromm, 1962).

The response of the mouse embryo to in vivo irradiation during various stages of gestation is clear. Irradiation on day 0 to 4 1/2 postcoitum (p.c.) causes high prenatal death, but no abnormalities. During the period of major organogenesis (day 4 1/2 to 13 1/2 p.c.) the susceptibility of the embryo to abnormalities and neonatal death peaks. During the final phase of gestation (day 13 1/2 p.c. to term), neonatal death, abnormalities and other gross changes are at their lowest levels (Russell and Russell, 1954b; Russell, 1957). Chang, Hunt and Harvey (1963) have found similar results in rabbits.

Rugh and Grupp (1959) found that in vivo irradiation at levels as low as 5 R to the precleavage mouse embryo, caused 11% more deaths during the first 18.5 days p.c. than controls. A dose of 25 R resulted in 38% more deaths. Rugh and Grupp (1959) recovered embryos irradiated in vivo with 5 R at 0.5 days p.c. at six and 24 hours after irradiation. They found 20% of the embryos to be abnormal,

while 2.5% of the unirradiated controls were abnormal.

Some of the abnormalities found in preimplantation embryos a short time after irradiation include: hyper-chromaticity of cytoplasm and nucleus, exudation of cytoplasm through ruptured membranes, congealing (sticky) chromosomes, complete dissolution of blastomeres with only polar bodies remaining, cytoplasmic damage appearing as a wave of necrosis over the egg (Rugh and Grupp, 1961), micronuclei (Russell and Russell, 1954b; Harvey and Chang, 1964), fragmentation of the egg and nuclear pyknosis (Rugh and Grupp, 1959).

Harvey and Chang (1964) working with golden hamsters, showed that whole body irradiation at 25, 50, 100, 150 and 200 R induced micronuclei in 15, 41, 46, 45 and 61% of the embryos respectively, and embryonic degeneration in 30, 58, 74, 70 and 90% following irradiation. They state that embryonic degeneration of these embryos following irradiation is clearly related to ova with micronuclei.

Besides increased preimplantation death, Ohzu (1965) found that 5, 15 and 25 R to mouse embryos at 0.5 and 1.5 days p.c. caused significantly higher resorptions than non-irradiated controls.

The Effect of Irradiation of the Maternal Organism on Subsequent Embryonic Development

In contrast to the well documented effects of irradiation on the embryo, the maternal response to irradiation as it affects subsequent embryonic development is unclear. This is due in part to the variety of parameters that have been measured.

Ketchel and Banik (1964) irradiated female rats with 800 R on

the morning sperm was detected in the vagina. The embryos were recovered three days later and transferred to the uteri of non-irradiated pseudopregnant rats. Implantations occurred in 13.5% of the embryos transferred, while 55% of the controls implanted. Implantation was not observed when non-irradiated embryos were transferred to recipients which had received 800 R. It was concluded that the loss of observed implantations was due to irradiation of the maternal organism, since some irradiated embryos were shown to be capable of further development.

Even though the dam has been shown to respond to irradiation, the levels of irradiation which will cause this effect are uncertain. Brent (1960) and Brent and McLaughlin (1960) showed that only high level irradiation (1000 to 1400 R) could elicit this effect, but later (Brent and Bolden, 1967) reported a small decline in the mean number of fetuses in rats whose uteri received 150 rad on day one of pregnancy. Chang and Hunt (1960) set a lower limit of 400 R for this maternal response to occur in rabbits. Glass and McClure (1964) state that maternal injury is not a critical factor in oocyte death with irradiation doses up to 250 R.

The widespread histological changes which occur in the ovary, oviducts and uterus following irradiation of 100 to 700 R are well documented (Brooksby, Sahinin and Soderwall, 1964). Glasser (1964) found that doses of 250 R and below, while causing the endometrium to change structurally and biochemically, caused no interference with implantation. He suggests placental dysfunction as the major cause of pregnancy wastage, possibly involving the ability of the

placenta to secrete progesterone, chorionic gonadotrophin and estrogens.

The hormonal requirements for pregnancy are not met in rats irradiated on the morning prior to mating (Ward and Hahn, 1968). Placental dysfunction may be responsible for 50% of the embryonic mortality in rats irradiated at that time (Ward and Hahn, 1968). Numerous workers have shown that both whole body and ovarian irradiation alter the maternal hormone balance required for normal reproductive function (Humphreys and Zuckerman, 1954; Spalding et al., 1957; Shapiro and Nujdin, 1958; Westman, 1958; Ely, 1960; Khrehbiel and Plagge, 1963; Andersen, 1964). This is supported by the evidence that exogenous progesterone will reduce embryonic mortality resulting from irradiation, although the mechanism of action is unknown (Glasser, 1964; Ward and Hahn, 1968). Brent (1960) found that direct irradiation of the placenta in rats, while shielding the dam and fetus, resulted in no difference in fetal mortality or fetal weights from controls.

The failure of transition from yolk to placental nutrition is believed to be a major cause of postimplantation death in rabbits between days 9 and 17 of gestation, and irradiation may intensify this condition (Inman and Markivee, 1963).

It is known that the uterine environment is important in embryonic development (Schultze, 1966). Gibbons and Chang (1973) were the first to study in detail the effects of irradiating the rat uterus while shielding the remainder of the animal before (0.5 to 3.5 days p.c.) and after (4.5 to 5.5 days p.c.) the entry of the

embryo. Irradiation on days 0.5 to 3.5 p.c. at 75, 150, 200 or 300 rad did not induce significant preimplantation losses, but 350, 450 or 600 rad did. They conclude that irradiation of the uterus impairs embryonic development, and mortality is dependent on the day and dose of irradiation. They suggest interference with normal processes in uterine decidualization as the cause of preimplantation losses due to irradiation prior to day 4.5 p.c.

Transfer of Irradiated and Non-Irradiated Embryos into Irradiated and Non-Irradiated Dams

Lin and Glass (1962) transferred eggs irradiated *in vitro* with 10 to 900 R into one uterine horn of recipient females which had been mated one hour previously. Upon examination at 17 to 19 days after transfer it was found that 33% of the transferred oocytes resulted in live fetuses after doses of 10 to 100 R. Transfer of oocytes irradiated at 200 to 300 R, resulted in 21% live fetuses 17 to 19 days after transfer, but only 7% after doses of 400 to 700 R.

Glass and Lin (1963) irradiated unfertilized oocytes *in vitro* with a 0 or 250 R. They were transferred into one uterine horn of recipient females who had received 0, 100, 250, 400 or 600 R. These females had been mated one hour previous to the transfer. Few females receiving 400 or 600 R became pregnant.

Glass and McClure (1964) irradiated oocytes *in vivo* and *in vitro*, with 100, 200 or 250 R and transferred them to non-irradiated mated recipients. No difference in mortality was found between *in vivo* and *in vitro* irradiation. Increasing doses of irradiation resulted

in decreasing numbers of living fetuses.

Lin and Glass (1962), Glass and Lin (1963) and Glass and McClure (1964) have found that irradiation in vivo or in vitro is harmful to embryos. Intra-uterine competition was shown to occur between donor and native embryos (Glass and Lin, 1963) making it difficult to clearly separate maternal injury from embryonic injury.

Chang, Hunt and Romanoff (1958) irradiated unfertilized rabbit ova in vitro with 45 to 32,000 R. These ova were transferred to mated rabbits, and recovered at various intervals up to six days. Normal cleavage and blastocyst development decreased inversely as the dose increased from 45 to 6500 R. Doses of 800 and 6500 R yielded 3% and 0% blastocysts, respectively.

Hunt and Bogart (1963) also utilizing embryo transfer techniques in rabbits found that 2 cell embryos irradiated at 0, 15.4, 61.2 and 91.8 rads resulted in 62, 41, 31, and 25% implantations on day 9 and 55, 36, and 6% survival at term for the first three treatments.

Fisher and Smithberg (1973a) transferred mouse embryos irradiated at 0 to 388 R into pseudopregnant, non-irradiated recipients, and found no fetal development at doses greater than 170 R.

Neyfakh (1964) studying the loach Misgurnus fossilis, has shown that these embryos exhibit a periodicity to irradiation. Not all embryonic stages are equally sensitive to irradiation. Irradiation during periods of active information transmission by the nucleus results in increased sensitivity of the cell. This periodicity may eventually explain the varying response of mammalian embryos to irradiation.

MATERIALS AND METHODS

Experimental Design. A total of 5418 mouse blastocysts were removed from donor mice and were subsequently transferred to the uteri of pseudopregnant recipient mice, or cultured. Random bred ICR albino mice were used as both donors and recipients. Three experiments were conducted.

Experiment 1. "Whole body irradiation of recipients and *in vitro* irradiation of embryos." This experiment was designed to measure embryonic mortality in terms of independent effects of irradiation to the embryo, the recipient, and the interaction of irradiation effects on both. The design of this experiment is given in Table 1.

TABLE 1

Treatment Combinations Used In Experiment 1

		In vitro irradiation of embryos in R		
		0	100	200
Whole body irradiation to recipient dams in R	0	1*	2	3
	100	4	5	6
	200	7	8	9

*Treatment numbers.

Experiment II. "Observation of embryonic resorption and viability on days 4, 5, 6, 7, 9, 9 and 10 after irradiation and transfer." This experiment employed treatments 1, 3 and 7 as given in Table 1. These treatments were selected in order to determine whether there were any differences in patterns of embryonic mortality between embryos irradiated

in vitro or recipients which had received whole body irradiation.

Experiment III. "Culture of embryos after *in vitro* irradiation."

1. Controls - no irradiation.
2. Embryos irradiated at 100 R.
3. Embryos irradiated at 200 R.

In Experiment I, the recipients and embryos were irradiated separately. Each of nine possible recipient-embryo treatments contained at least 25 recipient females and 242 transferred embryos. A total of 247 transfers involving 2242 embryos were used in this experiment.

In Experiment II, the recipients and embryos were irradiated separately. Each of three treatments contained 70 recipient females and at least 670 embryos. Ten females from each treatment were euthanized, and embryonic resorptions and viability were observed on days 4, 5, 6, 7, 8, 9 and 10 after transfer. There were 68 to 133 embryos studied on each of these days within each treatment.

The results in Experiments I and II were assessed as the ratio of live fetuses and resorptions to the total number of blastocysts transferred.

Experiment III was conducted to observe the viability, hatchability and morphological changes occurring in embryos irradiated and cultured *in vitro*. This experiment contained three treatments: non-irradiated controls, embryos irradiated at 100 R and embryos irradiated at 200 R.

A total of 289 blastocysts were evaluated by examining each individual blastocyst under the differential interference contrast (DIC) microscope, using Nomarski optics, for several morphological

traits. Embryos from each treatment were observed at 24, 48 and 72 hours after irradiation. The following criteria were used in evaluation of these blastocysts.

Indicators of Growth and Development:

(a) increase in total volume of blastocyst

(b) change in shape from round to ellipsoid

(c) thinning of zona pellucida

(d) increase in size and number of trophoblast cells

(e) hatching of the blastocyst from the zona pellucida

Indicators of degeneration:

(a) pyknosis of blastomeres

(b) contraction of blastocoele with darkening of blastomeres

(c) appearance of vacuoles

(d) rupture and fragmenting of zona pellucida

In addition, 686 blastocysts from these same treatments were evaluated using a stereomicroscope. Observations were made immediately after irradiation and at 12 hour intervals through 96 hours after irradiation. The same indicators of growth, development and degeneration described previously were used in stereoscopic evaluation of embryos.

Glassware and Materials. All glassware that came into contact with embryos or culture medium was soaked overnight in 7X anionic cleaner (tradename, Linbro Chemical), rinsed and sterilized according to the methods prescribed by Whittingham (1971).

Culture Medium. BMOC-2 culture medium (Brinster, 1963) was modified with 0.50 mM of sodium pyruvate, 5 mg/ml of BSA (bovine

serum albumin), 100 units/ml of potassium penicillin G, and 50 µg/ml of dihydrostreptomycin sulfate.

Embryo Culture. All embryos cultured in this study were placed in 35 X 10 mm culture dishes (Falcon Plastics) containing a 0.25 ml drop of modified BMOC-2 under liquid paraffin oil. Cultures were incubated at 37 °C in a chamber under a gas phase of 100% humidity in 5% CO_2 in air (Brinster, 1963). The incubator used was Model 3312, National Appliance Co.

Collection of Embryos. All mice used in this experiment were random-bred ICR albinos, kept on a cycle of 14 hours of light and 10 hours of dark. Donor females were killed and blastocyst stage embryos were flushed from the excised uteri at 3.5 days after detection of the vaginal plug. Excised uteri were flushed retrograde with 0.5 to 1 ml of warmed (37 °C) modified BMOC-2. Flushings were collected in a sterile watchglass and observed under the stereomicroscope at 40X. All embryos were transferred to sterile watchglasses containing warmed culture medium until all red blood cells and uterine debris was removed. The embryos were then pooled and placed in culture until irradiation (usually 6 hours).

Micropipets. Micropipets for the collection, handling, and transfer of embryos were made by drawing out flame heated capillary tubes (ID 1.1 mm). The drawn portions of the capillary tubes (ID 100µ) were then scored with a diamond stylus and broken perpendicular to the barrel.

Prior to use, micropipets were placed in 16 X 115 mm test tubes and heat sterilized (Whittingham, 1971).

Embryo Transfers. ICR albino females mated 2.5 or 3.5 days pre-

viously by vasectomized ICR albino males were used as recipients. Fisher and Smithberg (1973b) have shown that optimum results in mice are obtained when the donor and recipient are perfectly synchronous, or when the recipient females are one day asynchronous with the embryos. Recipient females were selected at random, and were either irradiated or not irradiated. After treatment they were anesthetized (Metofane), and the uterus was exposed by a mid-ventral incision. Pseudopregnant recipients which did not exhibit increased vascularity of the uterus were discarded. Embryo transfers were performed by puncturing the anti-mesometrial border of the uterine horn with a micropipet containing the embryos. Five embryos were usually transferred into each uterine horn. The total volume of the fluid, including the embryos, injected into each uterine horn was approximately .025μ 1.

During transfer, the uteri were kept moist with a gauze pad soaked in physiological saline. Following transfer, the uteri were replaced in the abdominal cavity, and the abdominal muscles were sutured with 5-0 chromic gut (Ethicon, Davis and Geck). The skin was closed with 11 mm wound clips. The average time from administration of anesthesia until suturing of the wound was six minutes. Recipient females recovered from surgery about 10 minutes after the skin was closed. Recipients were then killed according to the treatment schedule in which they were placed. All transfers were done at room temperature.

Irradiation of Embryos and Dams. The gamma-irradiation source used in this experiment was a Radiac Calibrator Set, AN/UDM-1A with a ^{137}Cs source. The source strength during the course of this experi-

ment averaged 87.87 Curies.

The embryos to be irradiated were removed from culture and placed in micropipets containing culture medium. The embryos were placed 15.3 cm from the center of the source and received 27.4 R/min. According to standard tables attenuation of gamma rays through glass the thickness of the micropipets would be considered negligible. After irradiation, embryos were placed in a sterile watchglass containing warmed (37°C) modified BMOC-2 under oil until transfer.

Recipient females were placed in a four-celled wooden cage measuring 11.5 X 10.5 X 4.0 cm. The dimensions for each cell were 5.0 X 4.5 X 4.0 cm. The mice used in this study fit snugly into each cell, and were able to turn around inside the cell. The recipient females within the cells were perpendicular to the beam of irradiation. The surface of each recipient closest to the source was 30.08 cm from the source and received 5.34 R/min. The midpoint irradiation dose within each cell was 5.13 R/min, only 88% of the surface dose. In addition, tissue attenuation to a depth of 1 cm was 88.6%. Recipients were irradiated in groups of one to four, and embryos were irradiated in groups of 5 to 40. Embryos were irradiated immediately after irradiation of recipient females. All transfers were completed within 30 minutes after irradiation of the embryos.

Autopsy of Animals. Recipient females were autopsied on day 14 following transfer in Experiment I, or on days 4 through 10 following transfer in Experiment II. Since the transfers were performed on day 3.5, the stage of gestation at autopsy was day 17.5 in Experiment I, and days 7.5 through 13.5 in Experiment II. Each mouse was anesthetized

(Metofane) and the uterus was excised. Each fetus or resorption site was carefully dissected, examined and the observations were recorded. The animals were then killed by cervical dislocation.

Microscopic Examination of Embryos. Embryos in Experiment III were irradiated at 0, 100 and 200 R. After irradiation, embryos within each treatment were pooled and divided approximately equally into three groups for study under the DIC microscope. Each of these groups were placed into separate drops of modified BMOC-2 in sterile culture dishes. The embryos within each of these drops were examined once at either 24, 48 or 72 hours after irradiation. This minimized the manipulation of the embryos. Control embryos were handled in the same manner. Embryos were randomly selected from one of three drops of medium at 24, 48 or 72 hours after irradiation, and placed in culture chamber slides (Lab-Tek, No. 4802) containing 0.1 ml of modified BMOC-2 under oil. They were then examined using Nomarski optics, and a 50X salt water immersion lens.

Embryos to be examined under the stereomicroscope were divided approximately equally into three groups, and irradiated. Embryos from each of the three treatments were handled simultaneously. After irradiation, embryos from each treatment were immediately placed separately into fresh drops of modified BMOC-2 under oil in sterile culture dishes. Therefore, each dish contained three drops of medium with a separate treatment in each drop. The embryos were observed for the effects of irradiation immediately after placement in culture and at 12 hour intervals for 96 hours. Embryos were cultured at 37°C. The culture dishes were removed from the culture chamber and placed

under the stereomicroscope for observation. Each observation period took approximately five minutes.

Statistical Analysis. All analyses were performed using the Statistical Analysis System (SAS). The means in Experiments I and II were compared using a Student-Newman-Kuels Test as described by Steel and Torrie (1960). The comparison of means in Experiment III was performed using a Chi-square Test as described by Steel and Torrie (1960).

RESULTS

1. Experiment I

Dams and embryos were irradiated separately at 0, 100 and 200 R, giving nine treatment combinations of recipient dams and embryos (Table 1). Control transfers resulted in 41.3% viable fetuses on day 14 following irradiation and transfer, and were significantly different from all other treatments ($P < 0.01$). Embryo irradiation at 200 R gave 8.8, 4.8 and 12.1% viable fetuses when transferred into recipients irradiated at 0, 100 and 200 R, respectively. For recipients irradiated at 200 R, the percentage of the fetuses viable were 25.3, 17.7 and 12.1%, respectively, for embryos irradiated at 0, 100 and 200 R. Results for all nine treatments within this experiment are presented in Figure 1.

The average percentage of viable fetuses at 14 days after irradiation and transfer for embryos irradiated at 0, 100 and 200 R, regardless of recipient irradiation, was 28.4, 18.2 and 8.6%, respectively. A regression analysis of variance showed that irradiation of the embryo had a significant effect ($P < 0.05$) on the percentage of fetuses alive 14 days after transfer. When dams were irradiated at 0, 100 and 200 R, regardless of irradiation to the embryo, 22.1, 15.0 and 18.4% of the fetuses were alive 14 days after irradiation and transfer. Irradiation of the recipient approached significance ($P = 0.052$) in terms of inducing embryonic mortality. The recipient-embryo interaction was not significant ($P > 0.05$). The regression equation showing the relationship between irradiation of the embryo and percent viability on day 14

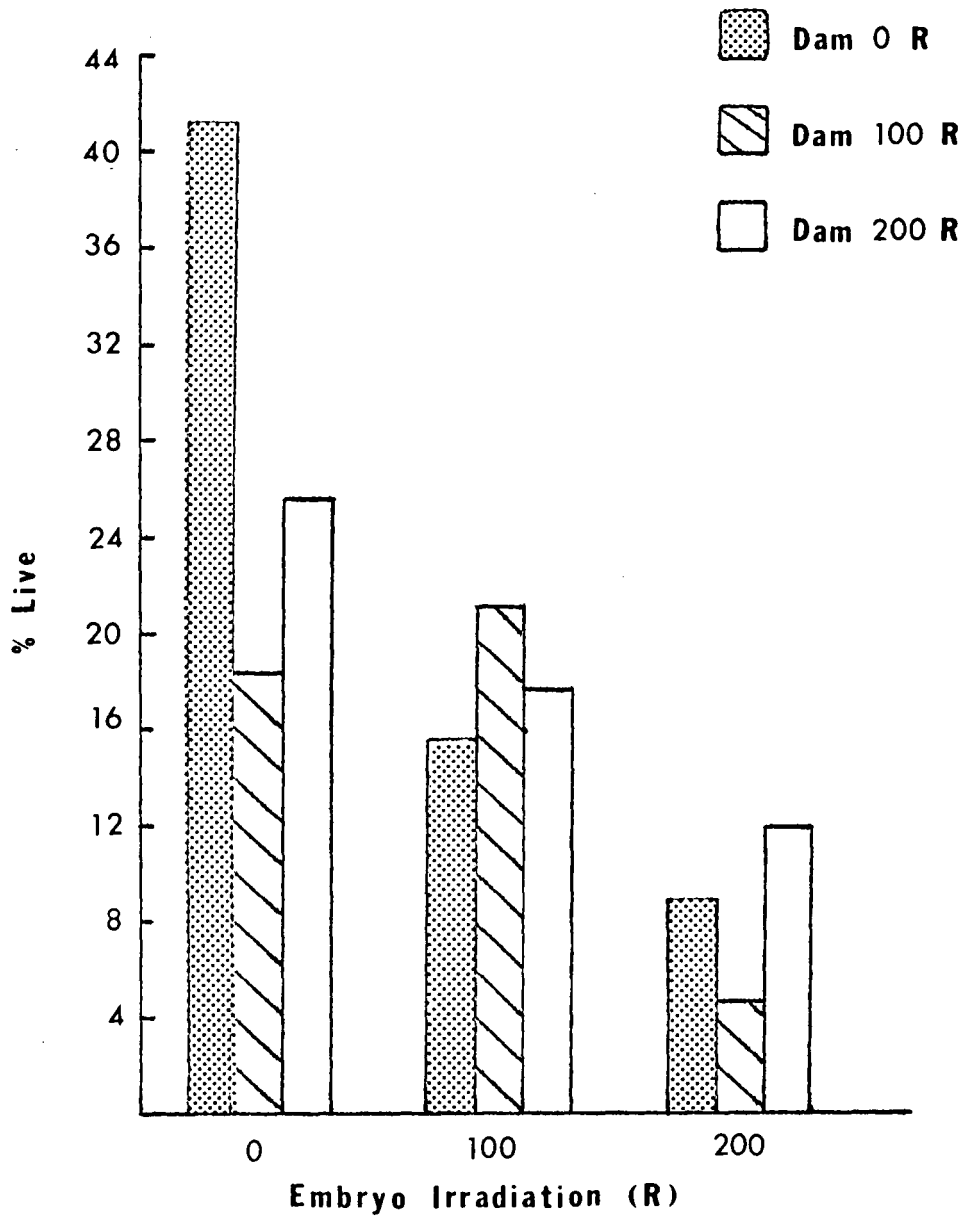

Fig. 1. Percentage of embryos transferred that were alive 14 days after separate irradiation of recipients and embryos.

after irradiation and transfer is presented in Figure 2. There was no difference in resorptions between treatments (Figure 3).

2. Experiment II

Recipient females were autopsied at 4, 5, 6, 7, 8, 9 and 10 days after irradiation and transfer. This experiment contained three treatments: Treatment 1 - non-irradiated controls, Treatment 2 - recipients irradiated at 200 R and embryos not irradiated, Treatment 3 - recipients not irradiated and embryos irradiated at 200 R. Each treatment contained from 70 to 72 recipients, and 670 to 725 embryos. There were no significant differences between the three treatments for percent viability or percent resorptions. There was insufficient data for each individual day of autopsy within each treatment to allow for statistical analysis. To facilitate statistical analysis for determining day of embryo death, observations were grouped into days 4 and 5, 6 and 7, and 8, 9 and 10. There were no differences between treatments 2 and 3, therefore they were combined and compared with controls. The average viability on days 4 and 5 was 30.8% for the controls (treatment 1) and 32.2% for treatments 2 and 3 combined. On day 6 and 7, 34.6% of the controls, and 32.8% of the irradiated dams and embryos in treatments 2 and 3 were viable. There were no significant differences between controls and irradiated dams and embryos for viability on days 4 and 5, or on days 6 and 7. The viability on days 8, 9 and 10 was significantly higher ($P > 0.05$) in the controls (29.0) than in the combined treatments 2 and 3 (15.6). The means for viability on days 8, 9 and 10 in combined treatments 2 and 3 were significantly different ($P > 0.01$) from the means on day 4 and 5, and 6 and 7, within that same

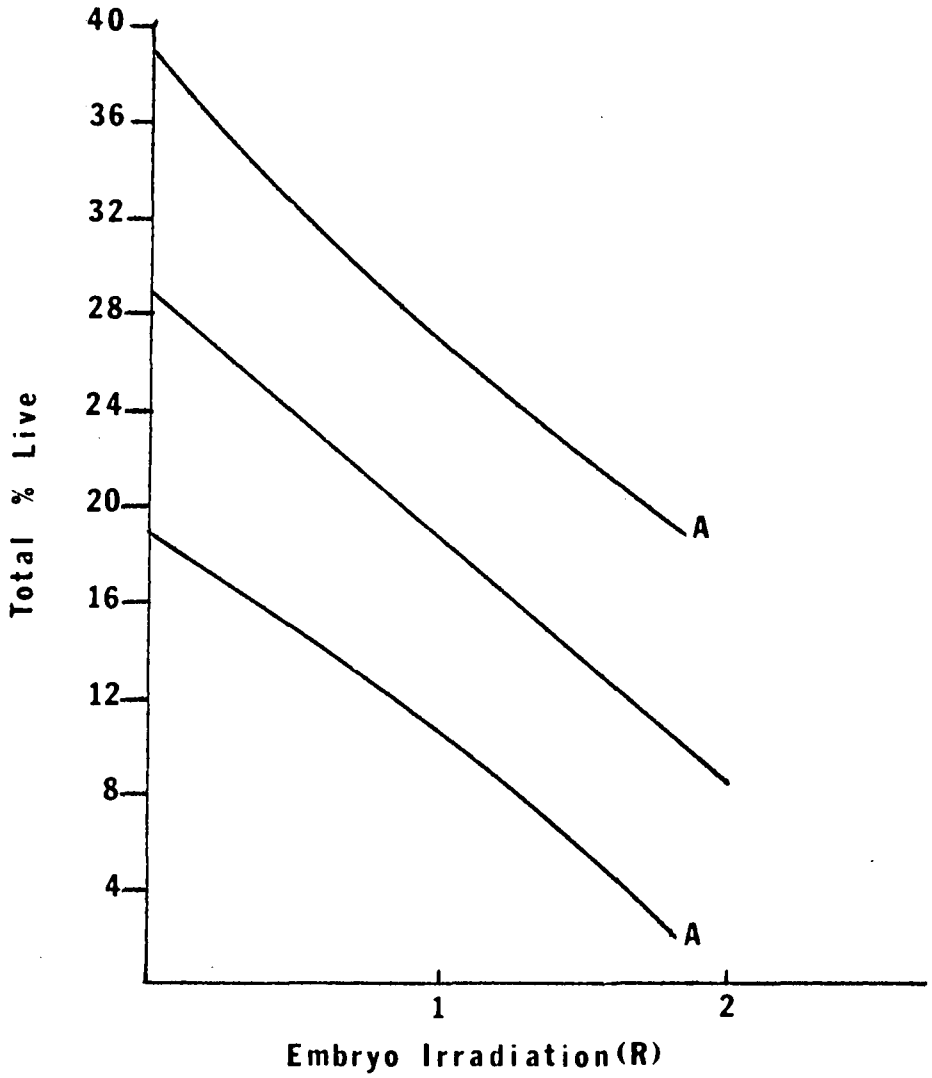

Fig. 2. Relationship between embryo irradiation and the percentage of viable fetuses 14 days after irradiation. Y = 28.33 − 9.90(R) where 1R = 100 R and 2R = 200 R. A : 95 % Confidence Limits

SE_{regr} : 1.39

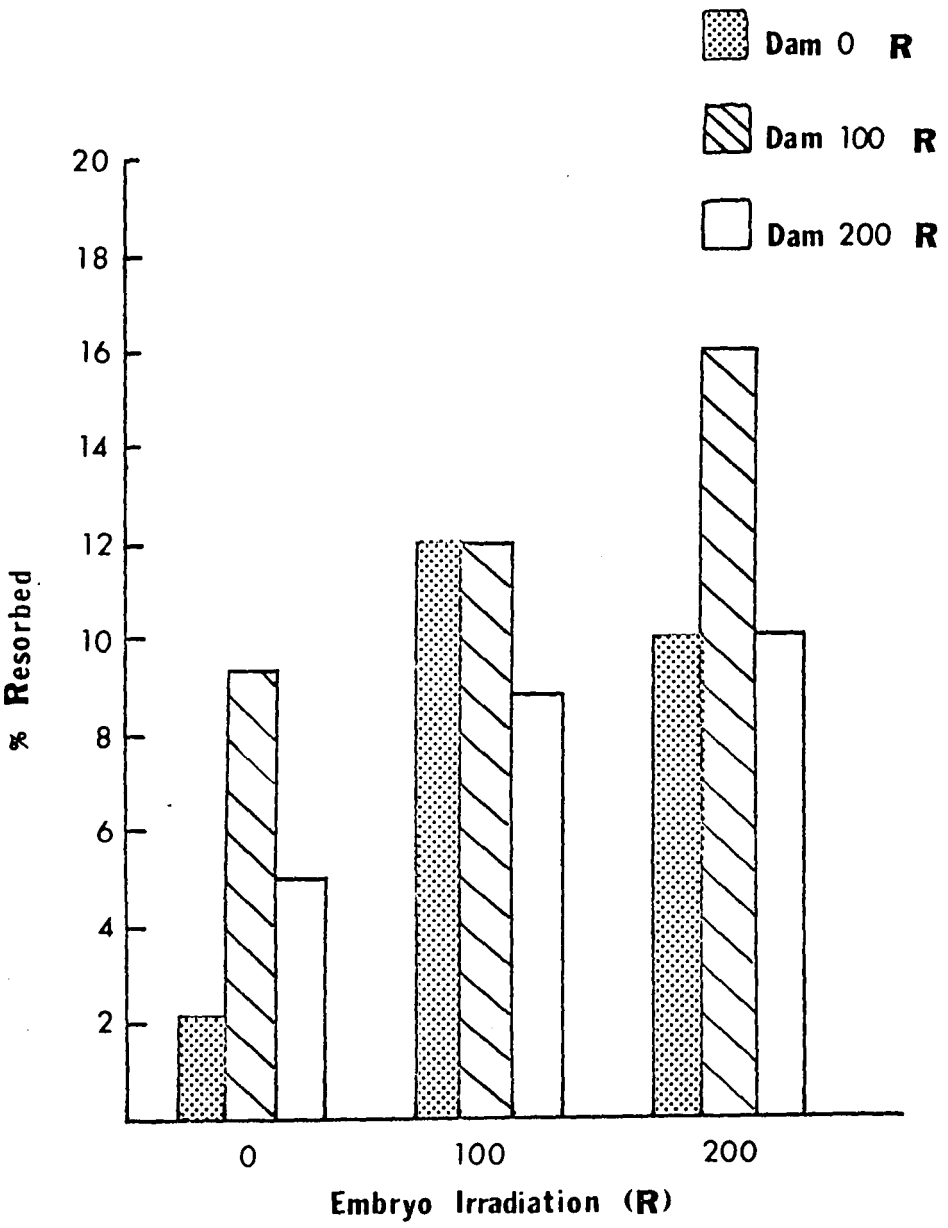

Fig. 3. Percentage of embryos transferred that were observed in a state of resorption on day 14 after separate irradiation of recipient and embryos

combined treatment. The control and combined treatment means for viability are presented in Table 2.

Data for resorptions were also evaluated by combining day of autopsy. The average percentage of embryos in the process of being resorbed on days 4 and 5 was 4.1% in controls and 7.7% in treatments 2 and 3. On day 6 and 7, resorptions were 5.9% in controls and 4.3% in treatments 2 and 3. There were no significant differences between controls and irradiated recipients and embryos for resorptions on days 4 and 5, or 6 and 7. Resorptions were significantly higher ($P < 0.05$) on days 8, 9 and 10 in treatments 2 and 3 (19.2%), than in controls (10.1%). The control and combined treatment means for percent resorptions are presented in Table 3.

3. Experiment III

(a) Stereomicroscopic Examination. To determine the stage at which embryos ceased development after irradiation, blastocysts were examined at 12 hour intervals following irradiation. Doses of 0, 100 and 200 R were administered to 314, 186 and 187 embryos in treatments 1, 2 and 3, respectively. Embryo hatching and degeneration were determined to be the best measures of treatment differences using the stereomicroscope. Treatments 1, 2 and 3 yielded 50.0, 72.6 and 78.5% degenerating embryos, respectively at 96 hours after irradiation. Hatching occurred 96 hours after irradiation in 19.4, 16.1 and 14.9% of the embryos irradiated at 0, 100 and 200 R, respectively. A Chi-square analysis detected significant differences in percent hatched ($P < 0.01$) and percent degenerating ($P < 0.01$) at 96 hours after irradiation between the control and irradiated embryos. The results

TABLE 2

CONTROL AND IRRADIATION TREATMENT MEANS FOR PERCENT VIABLE FETUSES ON DAYS 4 THROUGH 10 FOLLOWING IRRADIATION AND TRANSFER IN EXPERIMENT II

Day of Autopsy	Control	Treatment 2 and 3
4 and 5	30.8	32.2
6 and 7	34.6	32.8
8, 9 and 10	29.0	15.6[a,b]

[a] $P < 0.05$, Control compared to treatment 2 and 3 on days 8, 9 and 10.

[b] $P < 0.01$, Days 8, 9 and 10 compared to days 4 and 5 and 6 and 7.

TABLE 3

CONTROL AND IRRADIATION TREATMENT MEANS FOR PERCENT RESORBING FETUSES ON DAYS 4 THROUGH 10 FOLLOWING IRRADIATION AND TRANSFER IN EXPERIMENT II

Day of Autopsy	Control	Treatment 2 and 3
4 and 5	4.1	7.7
6 and 7	5.9	4.3
8, 9 and 10	10.1	19.2[a,b]

[a] $P < 0.05$, Control compared to treatment 2 and 3 on days 8, 9 and 10.

[b] $P < 0.01$, Days 8, 9 and 10 compared to days 4 and 5 and 6 and 7.

for percent degenerating and percent hatched after 96 hours in culture are presented in Figures 4 and 5, respectively.

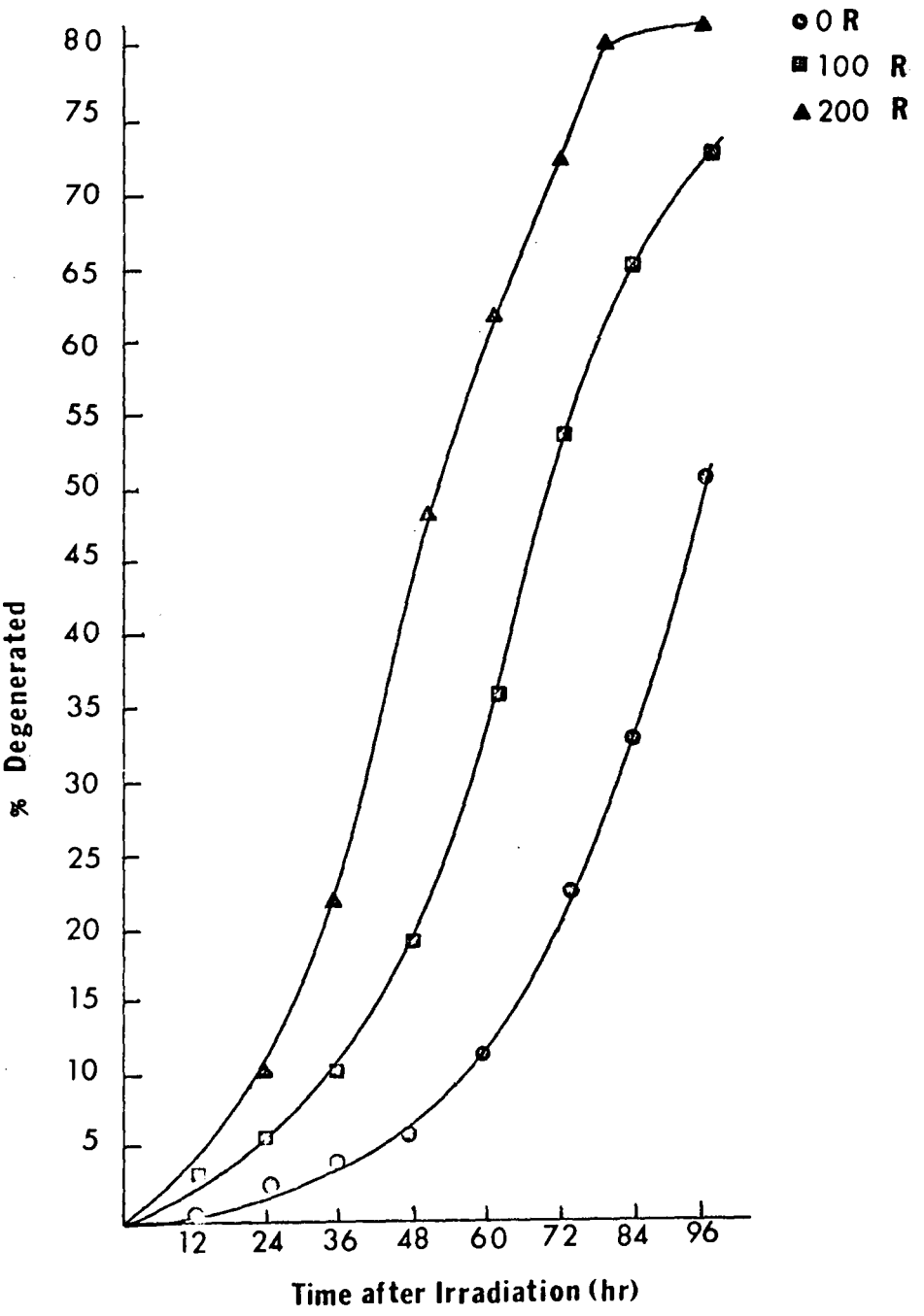

Fig. 4. Relationship between noticeable degenerative changes and lengtl of culture in mouse blastocysts irradiated at 0, 100 and 200 R

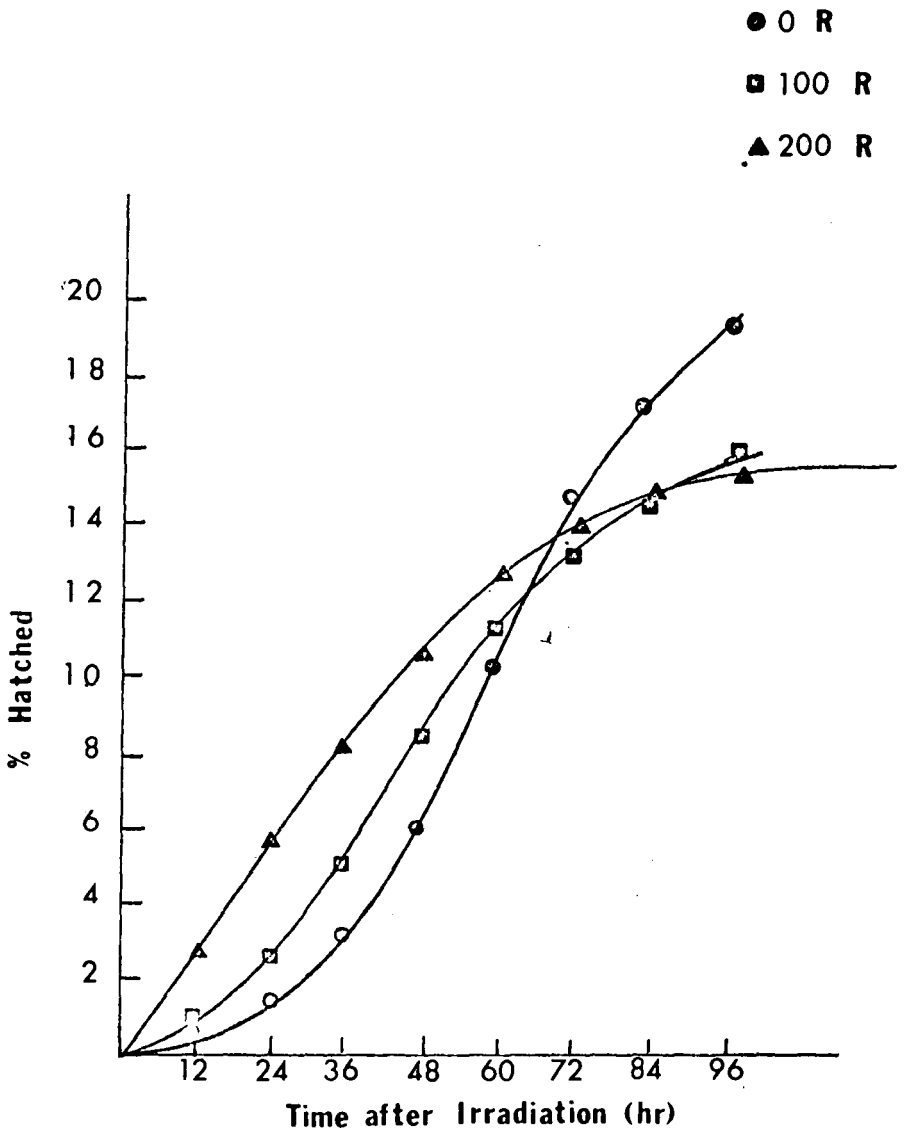

Fig. 5. Relationship between hatching and length of culture in mouse blastocysts irradiated at 0, 100 and 200 R

DISCUSSION

Experiment I demonstrates that irradiation of the embryo or recipient is detrimental to the survival of the conceptus. Irradiation of the embryo was more severe than irradiation of the recipient in inducing embryonic mortality. These results are in agreement with previous research in this area (Rugh and Grupp, 1959, 1961; Harvey, 1964; Harvey and Chang, 1964). In vitro irradiation of the embryo and subsequent transfer into non-irradiated recipients resulted in significantly fewer viable fetuses at 14 days after transfer as the level of irradiation was increased from 0 to 100 to 200 R. These results agree quite closely with the reports in the literature and the results expected. As dose level was increased, theoretically an increased percentage of the cells of the embryo were damaged sufficiently that embryogenesis could not continue. The time required to expose the embryos to irradiation was not considered to be a factor in this part of this study, since 3.68 and 7.37 minutes were required to irradiate at 100 and 200 R, respectively.

The primary response to irradiation is on the embryo (Russell, 1957; Chang and Hunt, 1960). The embryo responds in an all-or-none manner by either failing to implant and dying, or surviving to term. Irradiation of the preimplantation mouse embryo does not generally result in deformed or abnormal fetuses (Chang, Hunt and Harvey, 1963; Russell, 1950; Russell and Russell, 1954b). A total of 4356 mouse blastocysts were transferred in Experiments I and II, and no fetal abnormalities were observed.

There is much disagreement concerning the existence of a "maternal" effect to irradiation in terms of subsequent embryonic mortality. The results of maternal irradiation in Experiment I, while not statistically significant, indicate that irradiation of 100 or 200 R to the recipient increases embryonic mortality. Irradiation levels of 100 and 200 R are lower than those previously reported in the literature as sufficient to cause increased embryonic mortality. Glass and McClure (1964) suggest that 250 R to the recipient is the minimum dose needed for increasing embryonic mortality. Based on the structural and biochemical changes which occur as a result of ionizing radiations, even at low levels, it is quite possible that exposure of the recipient to very low levels of irradiation could impair reproductive function. This could be due to structural changes in the endometrium, interference with functional enzyme systems or direct or indirect actions on the pituitary and ovary. The actual dose of irradiation is the most important single factor affecting the biological response, however the rate at which the dose is delivered is also important. Relatively low dose rates possibly could permit the embryo recipient to repair the physiological and biochemical processes initially damaged by exposure to irradiation. The literature concerning maternal and embryonic response to irradiation does not stress dose rates as being important. Many authors have not reported dose rates, although dose rates from 4.55 R/min (Rugh and Grupp, 1961) to 100 R/min (Rugh and Wohlfromm, 1962) have been reported in mice.

There were no statistically detectable between treatment differences in percentage of fetuses resorbing in Experiment I. Experiment I

was initially designed to study changes in embryo viability and resorption after exposure of embryos and/or recipients to varying levels of irradiation. Significant decreases in viability associated with non-significant changes in fetal resorption in this experiment fostered the need for Experiment II. Some of the embryos could have implanted, survived for a few days, died and been completely resorbed by day 14 after transfer. Experiment II was designed to more accurately determine when embryonic death occurred. Due to the variation in response among individuals within treatments, the number of observations for each day of each treatment was not large enough for accurate analysis. Observations were pooled for day 4 and 5, 6 and 7 and 8, 9 and 10 and the analysis conducted.

Experiment II contained three of the treatments found in Experiment I. Even though the treatment responses were not numerically the same, the overall viability of fetuses at day 10 of Experiment II presented the same pattern as the same three treatments on day 14 of Experiment I. Resorptions also presented the same pattern in both experiments. Upon analysis, insignificant differences were found between the controls and irradiated embryos or recipients relative to percent viable embryos or percent resorbing embryos on days 4 and 5 and 6 and 7 after transfer. During days 8, 9 and 10 after transfer embryo viability was significantly lower ($P < 0.05$) and embryo resorption was significantly higher ($P < 0.05$) in the irradiation treatments. Therefore, irradiation damage in this experiment appears to have caused embryonic death during days 8, 9 and 10. These results indicate the effects of irradiation probably did not manifest themselves to a degree sufficient to cause

detectable embryo death until days 8, 9 and 10 after transfer. Rugh (1973) indicates that death and resorption are most likely to occur from 4 through 11 days of gestation in the mouse. Embryos which die shortly after implantation and resorb shortly thereafter would be difficult to detect. The occurrence of this phenomena in Experiment I would help explain why percent resorptions were significantly higher in irradiation treatments in Experiment II but not Experiment I.

No phenotypic abnormalities or malformations were found in any of the fetuses examined in Experiments I and II. This is in agreement with the literature. Only a few cases of exencephaly and cataracts have been reported to result from irradiation of the preimplantation mouse embryo (Rugh, 1973).

The in vitro culture of embryos irradiated at 0, 100 and 200 R resulted in distinct degenerative patterns. The time required for 10% of the embryos within each treatment to become degenerated after irradiation was quite different among treatments. While the controls required 60 hours to reach this level, embryos exposed to 100 and 200 R reached 10% degeneration in 36 and 24 hours, respectively. Once 20% of the embryos within each of the treatments became degenerated, the rates of degeneration paralleled one another in each of the treatments.

No consistent changes in the morphology of the blastocyst due to irradiation were detected with the DIC microscope. This does not eliminate the possibility that such changes did not occur. The blastocyst does not make a good model for DIC microscopy since the cells are small, and organelles difficult to observe. Many of the primary effects of irradiation occur at the nuclear level.

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APPENDIX

1. Composition of modified BMOC-2 used for embryo culture

Reagent		
Glucose		1.0 g
Sodium chloride	(NaCl)	5.546 g
Sodium pyruvate		0.056 g
Potassium chloride	(KCl)	0.356 g
Calcium chloride	($CaCl_2$)	0.189 g
Monopotassium phosphate	(KH_2PO_4)	0.162 g
Magnesium sulfate	($MgSO_4 7H_2O$)	0.294 g
Sodium bicarbonate	($NaHCO_3$)	2.106 g
Sodium lactate	(85% liquid)	2 ml in 100 ml of 3X distilled H_2O & adjusted to pH 7.4 with 1N NaOH
Potassium penicillin G		100 units/ml
Dihydrostreptomycin sulfate		50 ug/ml
Bovine serum albumin		5 mg/ml
3 X distilled water		to make 1 liter

2. Total number of embryos and recipients used and observed in Experiment I

	Treatment								
	1	2	3	4	5	6	7	8	9
Number of recipients	27	27	26	26	26	25	27	25	26
Number of embryos transferred	245	248	257	251	253	250	263	242	253
Number of fetuses alive at 14 days after transfer	102	39	23	47	56	12	66	43	31
Number of resorbing fetuses at 14 days after transfer	9	25	44	33	27	34	33	33	46
Number of embryos not accounted for at 14 days after transfer	134	184	190	171	170	204	164	166	176

3. Average percentage of viable fetuses within each treatment in Experiment I

		Embryo Irradiation (R)		
		0	100	200
Recipient Irradiation (R)	0	41.3	15.6	8.8
	100	18.4	21.4	4.8
	200	25.3	17.7	12.1

Error m.s. = 454.8

4. Total number of embryos and recipients used in Treatment 1 (Control) of Experiment II

	Day of Autopsy						
	4	5	6	7	8	9	10
Number of recipients	10	10	10	10	10	10	10
Number of embryos transferred	95	100	93	95	93	94	99
Number of fetuses alive after transfer	27	33	39	26	22	24	37
Number of resorbing fetuses after transfer	1	7	2	9	18	6	5
Number of embryos not accounted for after transfer	67	60	52	60	53	64	57

5. Total number of embryos and recipients used in Treatment 2 of Experiment II

	Day of Autopsy						
	4	5	6	7	8	9	10
Number of recipients	10	13	10	7	10	10	12
Number of embryos transferred	109	133	97	68	97	103	128
Number of fetuses alive after transfer	30	51	50	20	5	21	22
Number of resorbing fetuses after transfer	5	9	4	3	28	13	42
Number of embryos not accounted for after transfer	74	73	43	45	64	69	64

6. Total number of embryos and recipients used in Treatment 3 of Experiment II

	Day of Autopsy						
	4	5	6	7	8	9	10
Number of Recipients	10	10	10	10	11	10	10
Number of embryos transferred	97	102	103	101	112	89	106
Number of fetuses alive after transfer	36	25	30	21	19	11	21
Number of resorbing fetuses after transfer	6	14	2	7	14	11	14
Number of embryos not accounted for after transfer	55	63	71	73	89	67	71

7. Day and treatment means for percent fetal viability in Experiment II

Treatment	Day of Autopsy							Treatment Mean
	4	5	6	7	8	9	10	
Control	26.3	32.7	38.1	27.2	23.2	26.5	36.3	30.0
Dam 0 R Embryo 200 R	25.2	38.9	50.1	29.0	5.0	21.5	16.3	26.7
Dam 200 R Embryo 0 R	35.2	24.9	28.4	20.8	17.0	13.4	19.3	22.7
Day Mean	28.9	32.1	39.1	25.7	15.0	20.5	24.0	26.4

Error m.s. = 784.3

8. Day and treatment means for percent fetal resorptions in Experiment II

Treatment	Day of Autopsy							Treatment Mean
	4	5	6	7	8	9	10	
Control	0.8	6.8	2.5	9.5	18.4	6.0	5.0	7.0
Dam 0 R Embryo 200 R	5.2	6.6	4.1	4.3	30.4	13.0	33.3	13.8
Dam 200 R Embryo 0 R	5.8	15.4	2.0	6.8	12.0	12.6	12.7	9.6
Day Mean	3.9	9.7	2.9	6.9	20.3	10.6	11.3	10.1

Error m.s. = 331.3

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PATTERNS OF EMBRYONIC MORTALITY IN MICE AFTER EXPOSURE TO ^{137}Cs GAMMA IRRADIATION

by

Mark J. Manno

(ABSTRACT)

A series of experiments were designed to test the effects of ^{137}Cs irradiation on the viability of mouse embryos irradiated *in vitro* and subsequently transferred to recipient females or cultured *in vitro*.

The first experiment was designed to evaluate the viability of embryos after combinations of whole body irradiation to the recipient (0, 100 and 200 R) and *in vitro* irradiation to the embryo (0, 100 and 200 R). Nine treatment combinations were possible. Controls, in which neither recipient or embryo were irradiated, resulted in 41.3% viable fetuses 14 days after irradiation and transfer. Controls were significantly different from all other treatments ($P < 0.01$). Embryo irradiation at 200 R resulted in 8.8, 4.8 and 12.1% viable fetuses when the recipients received 0, 100 and 200 R, respectively. Embryo irradiation at 100 R resulted in 15.6, 21.4 and 17.7% viable fetuses when the recipient received 0, 100 and 200 R. Irradiation of the recipient at 100 and 200 R, while the embryo was unirradiated, resulted in 18.4 and 25.3% viable fetuses. Irradiation of the embryo had a significant effect ($P < 0.05$) on the percentage of the fetuses alive 14 days after transfer. Irradiation of the recipient and the dam embryo interaction were not significant in terms of inducing embryonic mortality. There

was no significant difference in resorption percentage between the nine treatments.

The second experiment was designed to measure the day of death of embryos placed into each of three treatments. The treatments consisted of controls, recipients receiving 200 R and embryos 0 R, and recipients receiving 0 R and embryos 200 R. Recipients were autopsied on days 4 through 10 following transfer. There was no significant difference between treatments, or on days of autopsy between or within treatments. The two treatments involving irradiation were combined and the days of autopsy grouped into 4 and 5, 6 and 7 and 8, 9 and 10 and compared with controls. Viability and resorptions were significantly different ($P < 0.05$) on days 8, 9 and 10 in the irradiated embryos when compared with controls.

The third experiment was designed to measure the response of mouse blastocysts irradiated at 0, 100 and 200 R and cultured in vitro. Irradiation at 100 and 200 R significantly increased degeneration of embryos (P 0.01) and decreased hatching ability ($P < 0.01$). Embryos were examined using the differential interference contrast microscope at 24, 48 and 72 hours after receiving 0, 100 or 200 R. No consistent morphological changes due to irradiation were observed.