Histol Histopath (1994) 9: 27-33

Histology and Histopathology

Effects of *in utero* exposure to low dose ionizing radiation on development in the rat

J.E. Bruni[1], T.V.N. Persaud[1], G. Froese[2] and W. Huang[3]

[1]Department of Anatomy, The University of Manitoba, Winnipeg, Canada, [2]Health Sciences Center, Manitoba Cancer Treatment and Research Foundation, Winnipeg, Canada and [3]Department of Anatomy, Hubei Medical University, Wuhan, Peoples Republic of China

Summary. Most studies of *in utero* effects of ionizing irradiation involve high doses and examination at postnatal intervals. Little information is available on the effects of low levels of ionizing radiation on embryogenesis. The developmental effects of *in utero* exposure to 50 cGy gamma radiation on gestational day-9.5 was investigated using Sprague-Dawley rats. Irradiated rats and appropriate controls were killed at prenatal intervals of 4h, 48h and 10 days after exposure. Fetuses were examined for abnormalities and random samples of tissues were prepared for microscopic study. With the exception of the neuro-epithelium, no histopathological changes were observed in embryos 4h after exposure to 50 cGy. In irradiated embryos, mitoses were reduced within the neuro-epithelium; pyknosis and some necrosis of cells were apparent at this gestational interval. Among the gross developmental abnormalities observed in embryos 48h after irradiation, excessive flexion of the embryo and abnormal flexion of the head were the only ones that appeared to be radiation-induced. The mean numerical score (42.3±0.2, controls; 42.4±0.1, irradiated) for 17 morphological parameters examined in fetuses at this gestational period compares favorably with other studies. Controls, however, showed greater variability in the extent of development of their forebrain, olfactory system, midbrain, hindbrain, and caudal neural tube. In all cases, there was evidence of slower development in these regions compared to their irradiated counterparts. At term, no significant differences in litter size or resorption rates were observed in irradiated animals compared to the controls, but there was a higher incidence of defective eye development, spinal curvature and visceral anomalies. *In utero* exposure to 50 cGY gamma-radiation during the period of early organogenesis can produce some irreversible defects that are discernible at term.

Offprint requests to: Dr. J.E. Bruni, Professor of Anatomy, Department of Anatomy, Faculties of Medicine and Dentristry, The University of Manitoba, 730 William Avenue, Winnipeg, MB, Canada, R3E OW3

Key words: Gamma-radiation, *In utero*, CNS, Histopathology, Prenatal development, Rats

Introduction

There is much interest in the potential health effects of *in utero* exposure to ionizing radiation because of the practical implications for risk estimation and radiation protection (UN Report, 1986; Persaud, 1990; Bentur et al., 1991). Studies in laboratory animals have shown that prenatal exposure to high levels of ionizing radiation causes resorptions, intrauterine growth retardation and a wide spectrum of developmental defects (Hicks and D'Amato, 1966; Hoffman et al., 1981; Ritenour, 1986; UN Report, 1986).

Central nervous system (CNS) lesions are amongst the most consistent pathological findings in the offspring following maternal exposure to radiation during gestation (Hicks and D'Amato, 1966; Yamazaki, 1966; Brent, 1980; Norton and Donoso, 1985; ICRP, 1986; UN Report, 1986; Schull et al., 1990; Bruni et al., 1993). Indeed, the sensitivity of the human CNS to radiation damage is evident from the follow-up studies on survivors of the atomic bombs in Hiroshima and Nagasaki (Blot and Miller, 1973; Otake and Schull, 1984).

Both the severity of damage and vulnerability to radiation are a function of dose, dose-rate, and developmental stage at the time of exposure (Hicks and D'Amato, 1966; Jacobson, 1968; Persaud, 1979; Hoffman et al., 1981; Kameyama and Hoshino, 1986). Prenatal exposure to large doses of radiation (≥ 1 Gy) produces a variety of well documented behavioral, mental and physical impairments in both experimental animals (Mullenix et al., 1975; Brent, 1980; Hicks and D'Amato, 1980; Ordy et al., 1982; Brent et al., 1986; Jensh and Brent, 1987; Norton and Kimler, 1988; Norton et al., 1991), and humans (Blot and Miller, 1973; Brent, 1980; Otake and Schull, 1984; Brent et al., 1986; Schull et al., 1990).

The consequences of prenatal exposure to low-level (≤ 1 Gy) radiation, however, are less familiar and more controversial. For these reasons, we have investigated the effects of *in utero* exposure at different gestational intervals, to a single dose of 50 cGy gamma-radiation on development in the rat. With the exception of significant cytopathological changes in the nervous system, no appreciable differences in gross morphological appearance or in behavior was observed in rats exposed to 50 cGy radiation on gestational day (GD) 15 and killed on either postnatal days 7 or 26 (Bruni et al., 1993). In non-human primates prenatal exposure on GD 80-90, corresponding to late fetal period in the rat, to 50 r gamma-radiation has been reported to impair postnatal development of reflexes, neuromuscular coordination, cognitive learning, spontaneous light-dark activity as well as head growth and crown-rump length (Ordy et al., 1982). In the present report, we reveal the observations obtained in rats 4h, 48h, and 10 days following exposure to 50 cGy on GD-9.5.

Materials and methods

Sexually mature female Sprague-Dawley rats, 8-10 weeks of age, were used in this study. They were maintained under conditions of controlled temperature (20±2 °C) and illumination (12h light/dark cycles, 20:00-08:00 dark) and allowed access to food and water *ad libitum*. All animals were cared for in accordance with the Canadian Council on Animal Care guidelines. Timed pregnancies were obtained by housing one male with three females overnight. The following day females were checked for vaginal plugs and the presence of spermatozoa. If present, the animals were designated as day 1 of gestation (GD-1) and randomly allocated to either a treatment or a control group.

Pregnant rats (1-3/cage) were then placed in specially constructed clear plexiglass cages and exposed to 50 cGy of ^{60}Co radiation from a Theratron F, cobalt radiotherapy unit. The animals were exposed to two parallel opposed radiation fields positioned 75 cm above and below the treatment table so that the dose was uniformly distributed throughout the irradiated volume. Irradiation time varied from 14-17 seconds and the total time of confinement amounted to 20-30 min. Control rats were identically handled except that they were not irradiated.

Groups of pregnant rats (N=12-15/group) were irradiated on GD-9.5 and killed prenatally at 4h, 48h, and 10 days (term) post-irradiation. At the time of sacrifice, all fetuses were recovered and fixed by immersion in 2.0% paraformaldehyde - 2.5% glutaraldehyde in 0.12M phosphate buffer with 0.02mM CaCl added.

The 11.5 and 20 day old fetuses were weighed, crown-rump length measured and scored for developmental abnormalities using the method described by Brown and Fabro (1981) or according to criteria for near term fetuses (Persaud et al., 1985), respectively. All measurements were made under a binocular dissecting microscope using a calibrated ocular graticule.

In addition, a random sample (47 irradiated and 33 controls) of term fetuses were also examined for visceral anomalies (Wilson, 1965; Persaud et al., 1985).

Results

A total of 174 embryos were recovered from controls and 159 from rats irradiated on GD-9.5 and killed 4h later. There was no statistically significant difference in the mean numbers recovered per littre from controls (14.5) or irradiated (13.3) dams at this gestational interval. The embryo at GD-9.5 is at the early neurula and headfold stage of development (Figs. 1, 2). It has only a few (0-3) somites. The nervous system consists of the prominent cranial brain plates continuous with the two caudal neural folds (Figs. 1,2). A single pseudo-stratified layer of mitotic and intermitotic columnar neuroepithelial cells (Figs. 2, 3) form the walls. At this stage of rudimentary development, no morphological differences were discerned between control and irradiated embryos 4h after exposure to 50 cGy except within the neuroepithelium itself. In irradiated embryos, the number of mitoses were reduced within this neuroepithelium and pyknosis, as well as some necrosis of cells, was also apparent at this time (see Bruni et al., 1993).

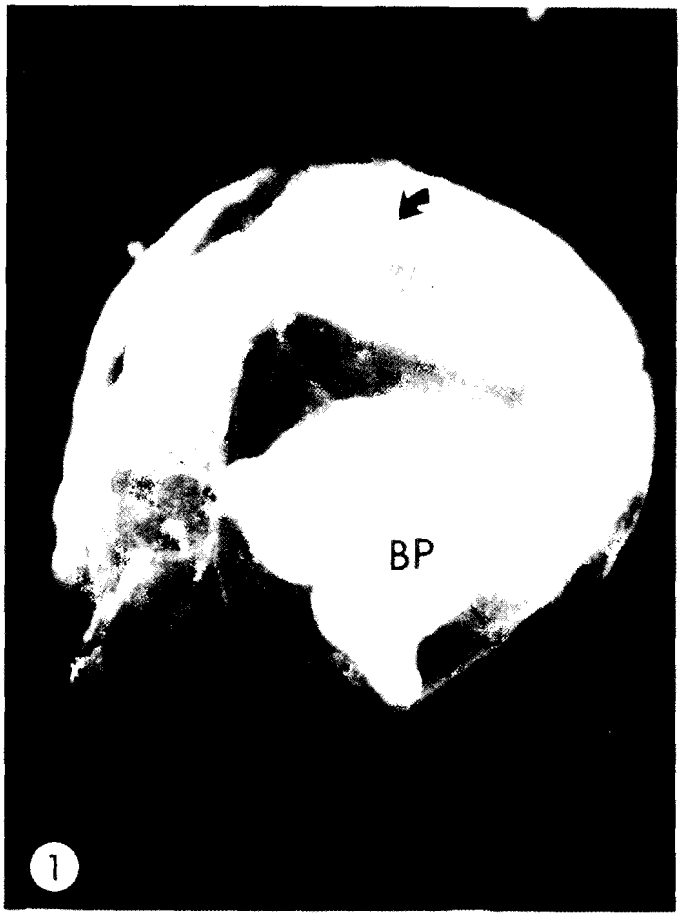
Fig. 1. Embryo removed from a control rat showing the extent of development of the nervous system on GD-9.5. BP, cranial brain plate; arrow, caudal neural folds. x 105

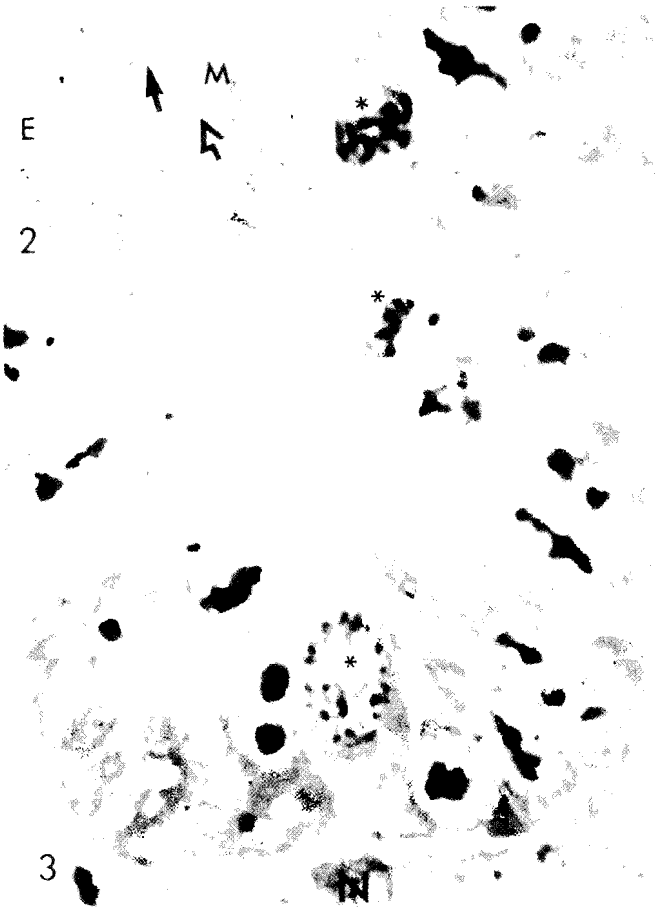
Fig. 2. Transverse section through a control rat embryo showing the stage of rudimentary development attained on GD-9.5. The walls of the primitive nervous system consist of only a single pseudostratified layer of neuroepithelial cells. Solid arrow, caudal neural folds; open arrows, cephalic neural folds; M, mesoderm; E, entoderm. Methylene blue - Azure II. x 80

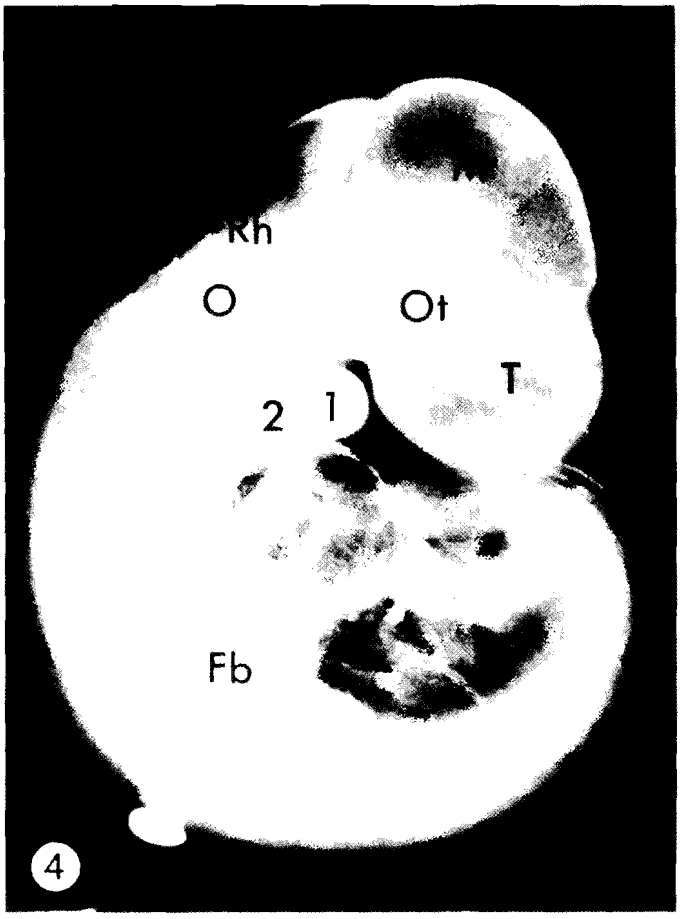
Fig. 3. Higher magnification of the cephalic neural folds (as in Fig. 2) from a control rat embryo on GD-9.5. A single pseudostratified layer of mitotic (*) and intermitotic columnar neuroepithelial cells form the walls of the primitive nervous system. N, notocord. Methylene blue - Azure II. x 1.680

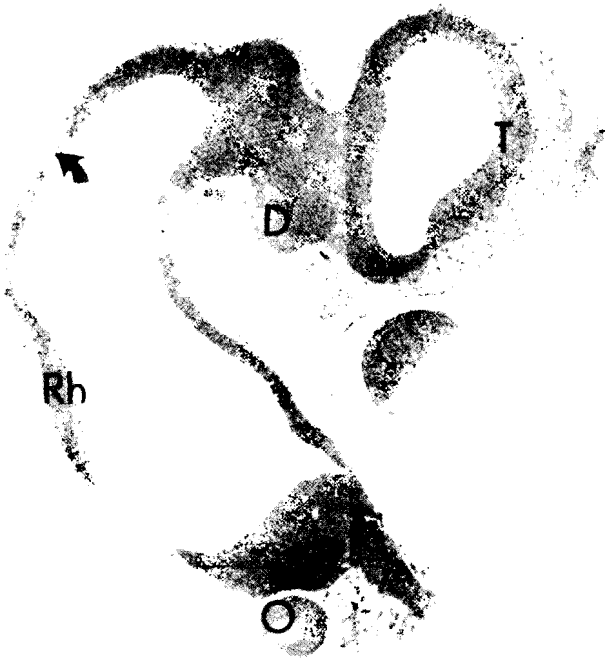
Fig. 4. Whole embryo removed from a control rat showing the extent of development on GD-11.5. Ot, optic vesicle; O, otic vesicle; Fb, forelimb bud; 1, 2 first and second branchial bars; Rh, rhombencephalon; T, telencephalon; M, mesencephalon. x 18

Fig. 5. Sagittal section of a control rat embryo as in Fig. 4 showing the extent of nervous system development attained at GD 11.5. O, otic vesicle; Rh, rhombencephalon; T, telencephalon; D, diencephalon; arrow, neuroepithelium. x 30


BP
1


M
E
2
3
TN


Rh
O
Ot
T
2
1
Fb
4


T
D
Rh
O
5

No statistically significant differences were observed in the number of embryos recovered per litter, the crown-rump length or head length of control and irradiated embryos killed 48h after irradiation (Table 1). In this group, 22 (13.5%) abortuses were recovered from control and 23 (16.4%) from irradiated embryos. A typical embryo on GD-11.5 is at the tailbud stage of development with the posterior neuropore closed and the cranial part of the neural tube differentiated into telencephalon, mesencephalon and rhombecephalon (Figs. 4, 5). The first and second branchial arches, as well as forelimb buds, are evident. Developmental abnormalities were observed in 12.9% of embryos examined 48h after 50 cGy irradiation and in 10% of controls. A summary of these along with their incidence is provided in Table 2. With the possible exception of excessive flexion of the embryo (3.7%) and abnormal flexion of the head (1.2%), none of the other observed anomalies, however, appeared to be radiation-induced.

Quantitation of the end points selected to assess embryonic development according to the objective scoring system of Brown and Fabro (1981) also failed to reveal any significant developmental differences between irradiated and control rats at this gestational period. The overall mean numerical score for the 17 morphological parameters evaluated (which in addition to the nervous system included the otic system, yolk sac/heart-circulatory system, allantois, branchial bars, maxilla and mandible, fore and hind limbs and somites) was 42.3±0.2 for control embryos and 42.4±0.1 for the irradiated embryos.

While the mean developmental stage attained by controls and irradiated embryos was not different for any of the 17 parameters examined, variation in the score of several parameters, including the nervous system was observed. At this gestational period, the nervous system has reached the 5 brain vesicle stage of development (Fig. 5). An inner cell layer of actively mitotic cells, an intermediate primitive differentiating zone and an acellular marginal zone constitute its walls. The nervous system of embryos irradiated on day 9.5 and examined 48h later was indistinguishable from controls. Neuropathological changes such as deformities, ectopias, retarded development, arrest of proliferation and/or migration of cells were not seen. Controls, however, did exhibit a greater range in the extent of development achieved of their forebrain, olfactory system, midbrain, hindbrain, and a caudal neural tube compared to their irradiated counterparts. In all cases they showed evidence of slower development of these regions than those irradiated. The same was true with the degree of flexion attained by the embryos i.e. irradiated embryos were more likely to reveal the degree of cephalocaudal flexion natural to GD-11.5 of development.

The number of fetuses born to control and irradiated rats killed at GD-19.5 is shown in Table 3. None of the values was significantly different statistically. There were also 0.6±0.2 ($\bar{X}$±SEM) and 0.8±0.2 fetal resorptions/litter in the control and irradiated groups, respectively. Mean fetal and placental weights of controls were 2.3±0.02 g and 0.6±0.01 g compared to

Table 1. Embryos that received 50 cGy gamma-radiation on GD-9.5 and were killed 48h later.

GROUPS OF RATS	No. OF EMBRYOS	EMBRYOS/ LITTER	CROWN-RUMP LENGTH (mm)	HEAD LENGTH (mm)
Controls (12)	140	13.6±2.2*	4.0±0.06	2.0±0.02
50 cGy (13)	163	14.3±1.1	4.0±0.03	2.0±0.02

*: $\bar{X}$± SEM.
( ): number of animals.
Not significantly different from controls (Student's t test).

Table 2. Morphological defects found in controls and embryos exposed to 50 cGy gamma-radiation on GD-9.5 and killed 48h later.

MORPHOLOGICAL DEFECT	IRRADIATED 50 cGy (N= 163) No. embryos (%)	CONTROLS (N=140) No. embryos (%)
Stunted	2 (1)	3 (2)
Excessive torsion	6 (4)	4 (3)
Excessive flexion (a)	6 (4)	1 (0.7)
Abnormal head flexion (b)	2 (1)	-
Misshapen head	2 (1)	2 (1)
Combination of a + b	3 (2)	4 (3)
TOTAL	21 (13)	14 (10)

Table 3. Fetuses exposed to 50 cGy gamma-radiation on GD-9.5 and killed 10 days post-irradiation.

	MALES	FEMALES	FETUSES/ LITTER	RESORPTIONS (%)
Control (12/151)	5.4±0.5*	7.2±0.7	12.6±0.8	7 (4.6)
50 cGy (15/206)	6.4±0.9	7.3±0.7	13.7±0.6	12 (5.8)

*: $\bar{X}$± SEM.
( ): No. of dams/total No. of fetuses.
Not significantly different from controls (Student's t test)

Table 4. Abnormalities in fetuses that received 50 cGy gamma-radiation on GD-9.5 and were killed at term.

MORPHOLOGICAL ANOMALIES	FETUSES	
	50 cGy (%)	Control (%)
Microphthalmia/anophthalmia	9 (4.4)	1 (0.7)
Kinked, rudimentary missing tail	3 (1.5)	2 (1)
Scoliosis	2 (1)	0
Eventration of abdominal viscera	1 (0.5)	0
Club foot	1 (0.5)	0
Genitalia	0	0
Cleft palate/lip	0	0
Small embryo	2 (1)	1 (0.7)
Hemorrhages	2 (1)	2 (1)
TOTAL	20 (9.7)	6 (4)

2.3±0.02 g and 0.6±0.01 g for irradiated fetuses, respectively.

A higher incidence of developmental abnormalities, however, was observed in irradiated (N= 20 or 9.7%) versus control (N= 6 or 4%) fetuses at term (Table 4). The most common anomalies were defects of eye development (microphthalmia, N= 6 or 3%; anophthalmia, N= 3 or 1.5%) with a frequency of occurrence of 4.4% and of spinal curvature (scoliosis, N= 2) with prevalence of 1% (Table 4). Using the Wilson (1965) sectioning technique to detect visceral anomalies, abnormally positioned kidneys were found in 5.8% (3/52) of irradiated fetuses and 7.1% (2/28) of controls. A higher incidence of ureteric anomalies (1/52 or 2%) and hemorrhagic liver lesions (6/52 or 11.5%) were encountered in irradiated fetuses but the differences were not significant statistically from controls (0/28 and 1/28 or 3.6%, respectively).

The nervous system at GD-19.5 has attained a more advanced stage of development than the primitive 5 vesicle stage of GD-11.5 of embryogenesis. Olfactory components within each cerebral hemisphere are clearly discernible as are many nuclei of the telencephalon and diencephalon. In the cerebrum and cerebellum migrating neuroblasts have invaded the peripheral marginal zone to establish the rudiments of an outer gray cortex. Development of the cerebrum is more advanced than the cerebellum at this stage; the latter only having evolved an external granular and a discernible Purkinje cell layer. The hippocampal formation has not yet achieved the adult laminar organization. Despite the higher incidence of developmental anomalies, however, no significant developmental differences were observed in the nervous system of control compared to irradiated fetuses at term.

Discussion

In this study, no statistically significant differences were observed in litter size or incidence of prenatal death in controls compared to animals irradiated with 50 r on GD-9.5. This is consistent with reports that exposure of rats to 50 r x-rays on GD-10 had no appreciable effect on mortality, body weight or growth of offspring unlike the increased intrauterine death and reduced growth rate observed with doses ≥ 100 r (Wilson and Karr, 1951). Similarly doses of 35-50 r of x-irradiation did not modify litter size but did alter the sex ratio in rats when administered on GD-9-11 (Warkany and Schraffenberger, 1947). Jensh and Brent (1986, 1987) observed no differences in mean litter size, resorption rate or abnormalities in rats exposed to 0.1-0.4 Gy x-rays on GD-9. Fetal weight of rats irradiated with 0.4-0.6 Gy on GD-9, however, were reduced at term but recovered postnatally.

In only a few studies, embryo lethality has been observed in animals following exposure to levels as low as 10 r during organogenesis (Hoffman et al., 1981) and larger doses of x-rays (70-95 r) have also been seen to reduce the size of litters (Warkany and Schraffenberger, 1947).

In this study, mean crown-rump and head lengths recorded in control embryos on GD-11.5 were 4.0±0.06 mm and 2.0±0.02 mm, respectively. These values did not differ from those obtained in their irradiated counterparts and is in agreement with the values of 3.6±0.06 mm and 1.9±0.05 mm reported by Brown and Fabro (1981) for conceptuses of comparable embryonic age (GD-11.5). The overall morphological scores that we recorded for both controls and irradiated embryos also compare favorably with 39.9±0.3 and 41.8±0.2 reported for the developmental stages of 11.5 and 11.7 days of embryonic age, respectively.

Although the mean developmental stage attained by controls and irradiated embryos was found not to differ in our study, controls did exhibit evidence of slower development of certain regions, mainly in the nervous system, compared to their irradiated counterparts. The significance if any, of this observation is unknown. Reports alluding to a similar phenomenon, however, can be found in the literature. Altman et al. (1968) noted that exposure of the cerebellum of rats at birth to one or two doses of 200 r paradoxically appeared to enhance cerebellar growth. Norton and Donoso (1985) found that at 14 days after birth the number of cortical and caudate neurons with dendritic spines was unexpectedly greater in rats exposed prenatally on GD-15 to 125 r of radiation than controls. Jensh and Brent (1986) observed an accelerated acquisition of air righting reflex behavior in rats exposed to 0.6 Gy x-radiation on G-9 or G-17 compared to controls. Rats exposed to ETOH *in utero* on GD 13-19, when generation is most active, experience a delay of 2 days in the generation of cortical neurons. Paradoxically, however, they are observed to undergo an anomalous late generation surge after GD-19 to compensate for the earlier reduction (Miller, 1986).

The embryo is more vulnerable to ionizing radiation than the adult because mitotically active cells, undifferentiated, and differentiated cells are considered to be most sensitive to radiation (Hicks and D'Amato, 1966; Ritenour, 1986; UN Report, 1986). An important determinant of sensitivity to radiation and its effect is the stage of gestational development attained at the time of exposure (Job et al., 1935; Hicks and D'Amato, 1966; Brent, 1980; Hoffman et al., 1981; Ritenour, 1986; UN Report, 1986). Within each period, of course, there is also a critical dose and dose rate of radiation that is also needed (Warkany and Schraffenberger, 1947; UN Report, 1986).

Irradiation during the pre-implantation period of development (GD 0-8 in the rat) is characterized by a high incidence of prenatal death but few if any abnormalities (Fields and Desmond, 1978; Brent, 1980; Hoffman et al., 1981; Ritenour, 1986; UN Report, 1986). Significant excesses of mortality have been reported following exposures as low as 5 r during this period (Hoffman et al., 1981). Pre-implantation exposure of mouse embryos to 50 r (in air) x-rays from GD 0-6.5 resulted in average 13% (range 0-42%) probability of

death as opposed to only 2% (range 0-6%) of developing gross anomalies. With each succeeding day of embryonic exposure there was less resorption and the incidence of developmental anomaly (exencephalia) was unchanged (Rugh and Grupp, 1959).

Irradiation during the fetal period (GD 13 or 15-22 in the rat) most often results in maldevelopment of tissues, maturation deficits, functional disabilities as well as permanent growth retardation (Fields and Desmond, 1978; UN Report, 1986; Jensh et al., 1988) sometimes with doses as low as 25-50 r (Konermann, 1982).

Irradiation during the period of organogenesis (GD 8-13 or 15 in the rat) which encompasses the time of irradiation used in our study (early organogenesis) generally results in fewer prenatal deaths, a prevalence of intrauterine growth retardation, and the greatest incidence of maldevelopment (Fields and Desmond, 1978; UN Report, 1986). Maximum sensitivity and production of specific defects coincide with the time particular organ system are experiencing their most active proliferation and differentiation. The nervous system has an extended vulnerability because of its prolonged development (Brent et al., 1986; ICRP, 1986; UN Report, 1986). In mice, exposure to 5 r during early organogenesis (GD-7) resulted in anomalies of the skull and vertebral skeleton at three times the spontaneous incidence whereas exposure to 25 r on GD-10 (late organogenesis in the mouse) produced only twice the spontaneous incidence. The incidence, however, did not differ from spontaneous levels after exposure to a lesser dose (12.5 r) at the same gestational interval (Konermann, 1982). Doses of 35-90 roentgen when administered on GD-9 have produced in offspring defects of the brain-hydrocephalus whereas developmental anomalies of the eye and jaw were typically encountered with radiation on GD-10 and GD-11, respectively (Job et al., 1935).

Wilson et al. (1953) and Wilson (1954) concluded that although the sensitivity of the rat fetus to x-irradiation induced mortality, retarded growth, and maldevelopment diminished as the embryo grew from GD-8 to GD-11 the radiation induced effects increased with dosage.

In our study, although a higher incidence of abortuses and developmental abnormalities were recorded in irradiated rats compared to controls, the differences were not significant statistically. The most common defects that we encountered were microphthalmia (3%), anophthalmia (1.5%), defects of spinal curvature (1%), excessive flexion of the embryo (3.7%), and abnormal head flexion (1.2%). Interestingly, confinement of pregnant female mice on GD-8 for from 20-36 minutes resulted on day 13 in a significantly higher incidence of anomalies (19-20%) of various types and growth retardation in 13-15% of fetuses but the latter was not significant statistically (Michel and Fritz-Niggly, 1978).

Wilson et al. (1953) and Wilson (1954) reported that no defects were produced in rats when exposed to any dose of radiation from 25-200 r on GD-8. The eye was the only site of abnormality with exposure to 25 r on GD-9. Exposure to 50 r on GD-9 caused a slight retardation in rate of growth and as in the case after irradiation on GD-10 resulted in ocular maldevelopment in 28%, anophthalmia in 6% and microphthalmia in 38% of animals (Wilson and Karr, 1951; Wilson et al., 1953; Wilson, 1954). The only other adverse effects noted at this dosage and gestational interval were malformations of the brain (herniation, hydrocephalus) and of the spinal cord (growths, irregularities of curvature) in 11% of animals (Wilson et al., 1953; Wilson, 1954). Of all organs, the eyes were most consistently affected by exposure to a dose of radiation equal to or exceeding twice the amount used in our study, on the 10th day of development. Microphthalmia was the most prevalent ocular defect. Defective brain development was next in order of prevalence being limited to abnormalities of size, shape, symmetry and hypoplasia of the forebrain (Wilson and Karr, 1951).

Wilson et al. (1951, 1953) also reported faulty development of the urinary organs and liver damage in rats (Wilson and Karr, 1951) exposed to ≥ 100 r x-radiation on GD 9-10. The former ranged from abnormal location of the kidneys to renal agenesis. Irradiation damage of the latter was manifested by sparsity of liver cords, lack of diversity in cell types, smallness of the organ to derangement in structure with larger dosages. With only 50 r, we have found only a higher but non-significant incidence of ureteric malformations (2%) and hemorrhagic liver lesions (11.5%) in our irradiated fetuses at term compared to controls.

Acknowledgements. The authors acknowledge with gratitude the excellent technical assistance provided by Mr. G. Seyoum, P.M. Perumal, R. Simpson, P. Daenick and Ms. L. Snell. We also thank the Unsolicited Proposals Fund, Department of Supply and Services; Atomic Energy Control Board and Health and Welfare, Canada for funding this investigation.

References

Altman J., Anderson W.J. and Wright K.A. (1968). Differential radiosensitivity of stationary and migratory primitive cells in the brains of infant rats. Exp. Neurol. 22, 52-74.

Bentur Y., Horlatsch N. and Koren G. (1991). Exposure to ionizing radiation during pregnancy: perception of teratogenic risk and outcome. Teratology 43, 109-112.

Blot W.J. and Miller R.W. (1973). Mental retardation following in utero exposure to the atomic bombs of Hiroshima and Nagasaki. Radiology 106, 617-619.

Brent R.L. (1980). Radiation teratogenesis. Teratology 21, 281-298.

Brent R.L., Beckmann D.A. and Jensh R.P. (1986). The relationship of animal experiments in predicting the effects of intrauterine radiation effects in the human. In: Radiation risks to the developing nervous system. Kriegel H. (ed). Gustav Fischer Verlag. Stuttgart. pp 367-397.

Brown N.A. and Fabro S. (1981). Quantitation of rat embryonic development in vitro: a morphological scoring system. Teratology

24, 65-78.

Bruni J.E., Persaud T.V.N., Huang W. and Froese G. (1993). Postnatal development of the rat CNS following *in utero* exposure to a low dose of ionizing radiation. Exp. Toxicol. Pathol. 45 (in press).

Fields W.S. and Desmond M.M. (1978). Effects of radiation on the developing embryo and fetus. In: Radiobiology for the radiologist. 2nd ed. Hall E.J. (ed). Harper and Row. Hagerstown. pp 397-410.

Hicks S.P. and D'Amato C.J. (1966). Advances of ionizing radiations on mammalian development. In: Advances in teratology. Woollam D.H.M. (ed). Logos Press. London. pp 196-250.

Hicks S.P. and D'Amato C.J. (1980). Development of the motor system: hopping rats produced by prenatal irradiation. Exp. Neurol. 70, 24-39.

Hoffman D.A., Felten R.P. and Cyr H.W. (1981). Effects of ionizing radiation on the developing embryo and fetus: a review. U.S. Dept. of Health and Human Services Publication (F.D.A.) 81-8170. pp 1-138.

International Commission on Radiological Protection. (1986). Developmental effects of irradiation on the brain of the embryo and fetus. ICRP Publication 49. Thorne M.C. (ed). Pergamon Press. Oxford. pp 1-43.

Jacobson L. (1968). Low dose X-irradiation and teratogenesis. Copenhagen. Munksgaard.

Jensh R.P. and Brent R.L. (1986). Effects of 0.6 Gy prenatal x-irradiation on postnatal neurophysiologic development in the Wistar rat (42299). Proc. Soc. Exp. Biol. Med. 181, 611-619.

Jensh R.P. and Brent R.L. (1987). The effect of low-level prenatal x-irradiation on postnatal development in the Wistar rat (42476). Proc. Soc. Exp. Biol. Med. 184, 256-263.

Jensh R.P., Brent R.L. and Bannon C.H. (1988). Effects of prenatal x-irradiation on the 14th-18th day on adult behavior in the Wistar rat. Teratology 37, 467.

Job T.T., Leibold G.J. and Fitzmaurice H.A. (1935). Biological effects of roentgen rays: the determination of critical periods in mammalian development with X-rays. Am. J. Anat. 56, 97-117.

Kameyama Y. and Hoshino K. (1986). Sensitive phases of CNS development. In: Radiation risks to developing nervous system. Kriegel H., Schmahl W., Gerber G.B. and Stieve F.E. (eds). Gustav Fischer. Stuttgart. pp 75-92.

Konermann G. (1982). Consequences of prenatal radiation exposure on perinatal and postnatal development: morphological, biochemical and histochemical aspects. In: Developmental effects of prenatal irradiation. Kriegel H. (ed). Gustav Fischer. Stuttgart. pp 237-250.

Michel C. and Fritz-Niggli H. (1978). Induction of developmental anomalies in mice by maternal stress. Experientia 34, 105-106.

Miller M.W. (1986). Effects of alcohol on the generation and migration of cerebral cortical neurons. Science 233, 1308-1311.

Mullenix P., Norton S. and Culver B. (1975). Locomotor damage in rats after X-irradiation *in utero*. Exp. Neurol. 48, 312-324.

Norton S. and Donoso J.A. (1985). Forebrain damage following prenatal exposure to low dose x-irradiation. Exp. Neurol. 87, 185-197.

Norton S. and Kimler B.F. (1988). Comparison of functional and morphological deficits in the rat after gestational exposure to ionizing radiation. Neurotoxicol. Teratol. 10, 363-371.

Norton S., Kimler B.F. and Mullenix P.J. (1991). Progressive behavioral changes in rats after exposure to low levels of ionizing radiation *in utero*. Neurotoxicol. Teratol. 13, 181-188.

Ordy J.M., Brizzee K.R., Dunlap W.P. and Knight C. (1982). Effects of prenatal ^{60}Co irradiation on postnatal neural, learning, and hormonal development of the squirrel monkey. Radiat. Res. 89, 309-324.

Otake M. and Schull W.J. (1984). *In utero* exposure to A-bomb radiation and mental retardation; a reassessment. Brit. J. Radiol. 57, 409-414.

Persaud T.V.N. (1979). Teratogenesis. Experimental aspects and clinical implications. Gustav Fischer Verlag. Jena. pp 28-30.

Persaud T.V.N. (1990). Environmental causes of human birth defects. Charles C. Thomas. Springfield. pp 23-33.

Persaud T.V.N., Chudley A.E. and Skalko R.G. (1985). Basic concepts in teratology. Alan R. Liss Inc. New York. pp 155-181.

Ritenour E.R. (1986). Health effects of low level radiation; carcinogenesis, teratogenesis, and mutagenesis. Semin. Nucl. Med. 16, 106-117.

Rugh R. and Grupp E. (1959). Exencephalia following X-irradiation of the pre-implantation mammalian embryo. J. Neuropathol. Exp. Neurol. 18, 468-481.

Schull W.J., Norton S. and Jensh R.P. (1990). Ionizing radiation and the developing brain. Neurotoxicol. Teratol. 12, 249-260.

United Nations (1986). Genetic and somatic effects of ionizing radiation. Report of the United Nations Scientific Committee on the effects of atomic radiation to the General Asembly. With Annexes. United Nations. New York. pp 1-365.

Warkany J. and Schraffenberger A.E. (1947). Congenital malformations induced in rats by roentgen rays: skeletal changes in the offspring following a single irradiation of the mother. Am. J. Roentgen Radiat. Ther. Nucl. Med. 57, 455-463.

Wilson J.G. (1954). Differentiation and the reaction of rat embryos to radiation. J. Cell Comp. Physiol. 43, 11-37.

Wilson J.G. (1965). Methods for administering agents and detecting malformations in experimental animals. In: Teratology: principels and techniques. Wilson J.G. and Warkany J. (eds). Univ. of Chicago Press. Chicago. pp 262-277.

Wilson J.G. and Karr J.W. (1951). Effects of irradiation on embryonic development. I. X-rays on the 10th day of gestation in the rat. Am. J. Anat. 88, 1-29.

Wilson J.G., Jordan C.H. and Brent R.L. (1953). Effects of irradiation on embryonic development. II. X-rays on the ninth day of gestation in the rat. Am. J. Anat. 92, 153-177.

Yamazaki J.N. (1966). A review of the literature on the radiation dosage required to cause manifest central nervous system disturbances from *in utero* and postnatal exposure. Pediatrics 37, 877-903.

Accepted July 30, 1993