

Effects of 0.6-Gy Prenatal X Irradiation on Postnatal Neurophysiologic Development in the Wistar Rat (42299)

RONALD P. JENSH AND ROBERT L. BRENT

Departments of Anatomy, Pediatrics, and Radiology, Jefferson Medical College of Thomas Jefferson University, 1020 Locust Street, Philadelphia, Pennsylvania 19107

Abstract. Forty-one pregnant Wistar strain rats were irradiated with 0.6-Gy X rays or were sham irradiated on the 9th or 17th days of gestation to determine if this dosage level would result in alterations in postnatal neurophysiologic development. Half of the mothers were sacrificed at term, and the developmental status of 221 newborns was evaluated. The remaining mothers delivered and raised their litters. The 161 offspring were observed for the age of attainment of the following physiologic parameters: pinna detachment, eye opening, testes descent, and vaginal opening. Offspring were also tested for the acquisition of the following selected reflexes: surface righting, negative geotaxis, auditory startle, air righting, and visual placing. Term fetal weight was lower than the controls in the group irradiated on the 9th day but was recuperable postnatally. None of the 9 developmental tests performed postnatally were abnormal in the animals irradiated on the 9th day. Thus, at least with regard to these measures, the surviving embryos exposed during the all-or-none period could not be differentiated from the controls. Offspring irradiated on the 17th day exhibited retarded growth which persisted during neonatal life. The three-day-mean neonatal weight was significantly lower in the group irradiated on the 17th day compared to controls. There were no significant maternal body weight or organ/weight differences between the groups. Rats exposed *in utero* on the 17th day had a significantly delayed acquisition of air righting. These results demonstrate that 0.6-Gy *in utero* irradiation on the 17th day of gestation can cause subtle alterations in growth and development of the Wistar strain rat during postnatal life. © 1986 Society for Experimental Biology and Medicine.

X radiation is an established teratogen as has been shown by numerous investigators (1-11). Several investigators have been concerned specifically with the effects of X irradiation on the central nervous system (12-15), demonstrating that high levels of prenatal X irradiation can result in morphologic cytologic alterations in this organ-system.

A number of studies have been concerned with the effects of low level prenatal X irradiation (16-24). Although several investigations have been completed concerning postnatal behavioral effects of prenatal exposure, most of the studies have been concerned with exposure in excess of 0.75 Gy (25-38). A Gray (Gy), a newly adopted term, is equal to 100 rad (1.0 Gy = 100 rad). A rad refers to the absorbed dose in ergs per gram of tissue, and a roentgen (R) refers to exposure to an equivalent dose standardized by the number of ionizations produced in air. Generally a rad and a roentgen vary only slightly (1.0 rad = 1.0 R). Several studies have been completed in which behavioral alterations were observed following low dose prenatal exposure (39-46).

No previous investigation, however, has been completed in which the effects of low dose irradiation on term morphology and neonatal and early postnatal growth and development were analyzed together. The objectives of this study were (a) to determine if prenatal exposure to 0.6-Gy X irradiation on the 9th or 17th day of rat gestation would result in alterations in postnatal growth and neurophysiologic development; (b) to determine which postnatal parameters are the most sensitive indicators of the effects of *in utero* radiation exposure; and (c) to determine if an all or none phenomenon applies to postnatal neurophysiologic development. The 9th day of rat gestation was chosen because it is at the end of the all-or-none period of development (2) and would be a type of control since survivors should not exhibit anatomic malformations at term in spite of their X-ray exposure. The neurophysiologic tests are part of a standard battery of tests which has been used routinely in our laboratories for 10 years. The tests used in the present study were chosen for the following reasons: (i) the type of response

which is required by the animal is different in each test; (ii) the age at which the response (acquisition of reflex) would normally appear is different for each test, that is, all test responses are not clustered at one or two days of age; (iii) the time necessary to train observers and to complete testing is minimal; (iv) the acquisition of the response is easily observed; and (v) the results are amenable to statistical analysis.

Methods and Materials. Irradiation of animals was completed using a Siemens 250 kVp unit which was operated at 15 MA. The X-ray beam was filtered with a Thoreus III filter, 1.94 mm copper, and 3.0 mm aluminum and the HVL was 3.0 mm copper. The animals were exposed at a dose rate of 0.12–0.14 Gy/min at an average target–object distance of 82.25 cm. A Victoreen chamber was used to monitor exposure levels which were corrected for atmospheric pressure and ambient temperature. Rats were placed in a plexiglass wheel which was rotated within the field of radiation to assure exposure homogeneity.

Forty-one Wistar strain female rats were maintained in a temperature and humidity controlled animal facility with a 12:12-hour light:dark cycle. Animals were given Purina brand laboratory chow and water *ad libitum* throughout the test period. Males and females were housed together overnight, and 9:00 AM on the morning when sperm were found in a vaginal smear was considered to be 0 hr, 0 days of pregnancy. The females were randomly assigned to 1 of 4 groups: (a) 0.6-Gy irradiation on the 9th day of gestation, (b) 0.6-Gy irradiation on the 17th day of gestation, (c) sham irradiation on the 9th day of gestation, and (d) sham irradiation on the 17th day of gestation.

At term, half of the females in the four groups were killed and a conventional teratologic evaluation was completed. Although overt gross organ–system malformation would not be expected due to irradiation on the 17th day, exposure on this day of gestation could result in growth retardation. Maternal brain, liver, kidneys, and ovaries were removed, weighed, fixed in 10% formalin or Bouin's fixative, and later stored in 70% ethanol. The uterine horns were removed after the position and viability of the fetuses and the location of

implantation sites and resorptions were observed and recorded. Each fetus and its placenta was removed, weighed, labeled, fixed in Bouin's fixative, and, after 3 weeks, stored in 70% ethanol. The 221 fetuses were dissected using Wilson's (47) cross-sectional dissection technique.

The remaining mothers were allowed to deliver and raise their young. At 2 days of postnatal age litters were reduced to produce a maximum of 8 offspring per litter. Mothers were weighed daily throughout the test period. Beginning on Postnatal Day 1 (PN 1) the offspring were examined for the development of the following specific physiological landmarks.

(a) *Pinna detachment.* The appearance of pinna detachment was recorded as the age in days when the pinnae of both ears unfolded to a fully erect position. Ears were inspected from Day 1 until the entire litter exhibited complete unfolding. The mean age of achievement of 17 colony control litters was 3.2 days of age.

(b) *Eye opening.* Eye opening was defined as any visible break in the membrane covering the eye. The eyes of each animal were examined from Day 12 until both eyes of every animal in the litter were open. The mean age of achievement of 30 colony control litters was 16.2 days of age.

(c) *Testes descent.* Testes descent was defined as the age in days when the testes descended to lie in the scrotal sac. The males were held vertically under their forelimbs. The testes were observed to immediately descend into the sac and could be palpated. The average day of achievement among colony control males was 25 days.

(d) *Vaginal opening.* Vaginal opening was defined as any visible break in the membrane when the vaginal lips were gently pulled laterally. This examination was initiated on Day 25, with average age of positive response of colony control females being 30 days of age.

Reflex development was examined using the following tests.

(a) *Surface righting.* This is a test of integration of the motor and vestibular systems. The rat was placed on its back, held momentarily, and then released. Success was indicated when the rat was able to attain a fully prone position within 2 sec, 3 successive times. This

test was initiated on Day 5. The age of achievement of 30 colony control litters was 9.2 days.

(b) *Negative geotaxis*. This is a test of the ability of an animal to react to gravitational positioning. The animal was placed on a 30° incline with its head directed downward. A positive response was recorded when the animal was able to turn and climb up the incline against gravity within 15 sec. This test was administered to the entire litter until each neonate achieved 3 out of 3 positive responses in a daily test session. This test was initiated on Day 6, the average age of achievement of 22 colony control litters being 9.3 days.

(c) *Auditory startle*. The rats were exposed to a single loud sound presented suddenly without visual cuing. A positive response was considered to be a quick jerk of the entire body in response to the sound. Two successive positive responses constituted achievement of criterion. This test was initiated on Day 9. The average age of achievement of 30 colony control litters was 14.0 days.

(d) *Air righting*. This tests motor and vestibular integration. The rat was held in an inverted position, horizontally, 18 in above a padded surface and then released. Success criterion constituted landing in a fully prone position in 3 successive trials. Age of achievement of 30 colony control litters was 19.0 days of age. This test was initiated on Day 13.

(e) *Visual placing*. In this test of visual acuity the rat was lowered head-first toward a horizontally directed rope placed 18 in above a padded surface, care being taken that vibrissae did not contact the rope. Criterion was achieved when the rat extended its head and

forelimbs toward the rope in 2 successive trials. Age of achievement of 30 colony control litters was 23.7 days. This test was initiated on Day 17.

A total of 161 offspring were given each of these 9 tests. Statistical analyses of the maternal, fetal, and neonatal data were completed using the Student *t* test, ANOVA, and, when appropriate, the outlier's and Mann-Whitney *U* tests.

Results. The results of the term teratologic evaluation are presented in Table I. As expected from exposure at these stages of pregnancy, no significant differences between the irradiated and control groups were observed for maternal weight or weight gain, mean litter size, resorption rate, or abnormality rate or type, based on fetal dissection. Term fetal weights were significantly lower ($P < 0.05$) only in the group irradiated on the 9th day compared with those of their corresponding control group.

The results of the evaluation on PN 3 of liveborn offspring are shown in Table II. The 3-day neonatal weights of offspring exposed to 0.6 Gy on the 17th day were significantly less ($P < 0.05$) than those of the 17th day sham irradiated offspring, indicating nonrecuperable growth retardation. There were no significant differences ($P > 0.05$) between the control and irradiated groups for maternal weight or weight gain or mean litter size.

Table III summarizes the maternal organ weight and organ:body weight ratio data. None of these parameters were significantly ($P > 0.05$) affected by exposure to X radiation. Although mothers were subjected to acute whole-body X radiation, significant changes

TABLE I. RESULTS OF THE TERATOLOGIC EVALUATION

Group	Litter sample size	Maternal weight (+1 SD)		Weight change	Number of resorptions/total	Mean litter size ±1 SD	Mean fetal weight ±1 SD
		Day 0	Day 21				
0 Gy, 9 day (sham)	4	194 ± 11	327 ± 24	134 ± 14	5/44 (11.4%)	9.8 ± 1.7	5.79 ± 0.45
0.6 Gy, 9 day	5	202 ± 5	377 ± 17	175 ± 16	6/67 (9.0%)	12.2 ± 1.9	5.36 ± 0.40 ^a
0 Gy, 17 day (sham)	6	223 ± 26	392 ± 47	168 ± 31	1/81 (1.2%)	13.3 ± 2.3	5.74 ± 0.51
0.6 Gy, 17 day	5	220 ± 28	357 ± 27	137 ± 22	5/46 (10.9%) ^b	10.3 ± 1.7 ^b	5.59 ± 0.48

^a Significantly different from controls at $P \leq 0.05$.

^b Based on four litters; one litter was a statistical outlier with a 40% resorption rate.

TABLE II. SUMMARY OF RESULTS ON POSTNATAL DAY 3

Group	Litter sample size	Maternal weight (+1 SD)			Number of		Mean litter size (± 1 SD)	Mean 3-day weight (± 1 SD)
		Day 0	Day 21	Change	F1 off-spring	3-Day neonates		
0 Gy, 9 day (sham)	4	206 \pm 23	368 \pm 24	162 \pm 13	50	32	12.5 \pm 0.6	8.06 \pm 0.92
0.6 Gy, 9 day	6	201 \pm 10	359 \pm 38	158 \pm 30	63	45	10.5 \pm 3.3	8.68 \pm 0.84
0 Gy, 17 day (sham)	6	221 \pm 16	362 \pm 22	141 \pm 12	66	48	11.0 \pm 2.8	8.26 \pm 0.97
0.6 Gy, 17 day	5	198 \pm 9	352 \pm 29	154 \pm 31	49	36	9.8 \pm 3.8	7.18 \pm 0.63*

* Significantly different from controls at $P \leq 0.05$.

would not be expected since dosage levels were low and of short duration. These analyses were completed to monitor maternal health during pregnancy.

The neonatal reflex test results are summarized in Table IV. Rats irradiated on the 17th day exhibited a statistically significant ($P < 0.05$) retardation in acquisition of the air righting reflex compared to their control counterparts.

Table V summarizes the results of the evaluation of attainment of the 4 physiological parameters. No significant differences were observed between the control offspring and the offspring irradiated *in utero* on either day.

Discussion. X irradiation at high dosage levels, during specific developmental stages, causes an increased incidence of malformations, *in utero* death, and growth retardation. Brent (4, 6) stated that the greatest teratogenic effect would result from irradiation during the period of major organogenesis, but that irradiation later in gestation could affect the central nervous system and other organ-systems which continue to differentiate later in gesta-

tion. High dosage levels of X irradiation are known to specifically affect central nervous system growth and differentiation during an extensive period of development. Brizzee *et al.* (12) observed damage in the olfactory region, striatum and ependymal primordia, and cortical layers by Day 19.5, with the most severe results being obtained from a single acute 1.5-Gy exposure. D'Amato (14) observed cellular damage in the central nervous system within 5 to 8 hr after rats were exposed to 1.5-Gy X radiation *in utero* on Day 12. There was a high level of cellular necrosis which disappeared within 48 hr after irradiation with the exception of some minute rosette formations.

Behavioral effects of X irradiation at high dosage levels have also been well documented. Furchtgott and Echols (25) observed changes in general activity in rats prenatally exposed to a 1.0–3.0-Gy dosage level on Days 14–18. The offspring were hyperactive and exhibited increased locomotion in an open field. Semagin (40) irradiated rats throughout gestation with 0.1 Gy daily (total = 2.0 Gy) and tested the offspring postnatally. The irradiated off-

TABLE III. THE ORGAN WEIGHTS AND ORGAN:BODY WEIGHT RATIOS

Group	Sample size n	Body wt	Brain	Liver	Actual weights of organs (g)	
					Kidneys	
					Left	Right
0 Gy, 9 day	4	327 \pm 24	1.87 \pm 0.04	12.21 \pm 1.33	0.98 \pm 0.21	0.98 \pm 0.14
0.6 Gy, 9 day	5	377 \pm 17	1.80 \pm 0.06	13.69 \pm 1.25	0.94 \pm 0.10	1.10 \pm 0.05
0 Gy, 17 day	6	392 \pm 47	1.92 \pm 0.12	13.21 \pm 1.20	0.98 \pm 0.06	1.05 \pm 0.11
0.6 Gy, 17 day	6	372 \pm 43	2.02 \pm 0.15	13.14 \pm 1.70	1.11 \pm 0.16	0.98 \pm 0.09

* 1×10^4 .

spring took significantly longer to acquire conditioned responses than did the control offspring. Sharp (30) tested rats postnatally at 185 days of age in a water T-maze. Rats had been exposed to a 2.8-Gy dose of X radiation between Days 10 and 17 of gestation. The irradiated rats exhibited altered behavior at this age. Mullenix *et al.* (32) demonstrated that prenatal irradiation at a 1.25-Gy dosage level on Day 14 could cause an abnormal hopping-type gait in rats 5 to 6 months later. Tamaki and Inouye (33) showed that male rats irradiated *in utero* on Day 17 with 2.0 Gy X radiation had a higher degree of avoidance behavior in a shuttle box test than did the controls. In a similar study (34) they showed that the irradiated rats had an increased incidence of anticipatory response to shock as well as significant cellular damage in the hippocampus. Kiyono *et al.* (37) observed that prenatal irradiation at 2.0 Gy on Day 17 of rat gestation caused increased error rates and longer running times in a Hebb-Williams maze. These rats were also microcephalic.

Low level X irradiation has also been shown to cause morphologic and behavioral alterations. Wilson *et al.* (2) produced a low incidence of microphthalmia, Russell (16) observed skeletal malformations, Hicks and D'Amato (18) observed permanent nerve cell damage and disorganization of the cerebral cortex, and Brizze and Brannon (13) observed a slight decrease in the cerebral cortical mantle. Locomotor skill was impaired in rats prenatally irradiated on Day 14 with 0.5 Gy (26). A dose of 1.0 Gy on Day 15 of rat gestation caused an abnormal gait, decreased motor maturation and strength, and retarded loco-

motor learning in rats (42). Exposure at the 0.25 Gy or 0.50 Gy level on Day 10 or 20 significantly facilitated performance in a Lashley III maze (43), and similar exposure levels on Days 15 or 20 caused increased audiogenic seizures (44).

Blot and Miller (48), in their analysis of humans exposed to radiation in Hiroshima and Nagasaki, have indicated that human risk of radiation-induced effects was maximal from 8 to 15 weeks of pregnancy. Mental retardation was observed in young adults exposed *in utero*. Their data indicate that there is a dose-response effect and a threshold below which mental retardation is not evidenced. Otake and Shull (49) suggest, however, that there may not be a threshold for radiation-induced mental retardation.

The results of the term morphologic analyses in the present study showed that exposure to a 0.6-Gy dose of X radiation on the 17th day did not result in statistically significant growth retardation of the fetuses at term, but 3-day neonatal weight was significantly reduced. Animals irradiated on the 9th day exhibited reduced term fetal weights, which is in agreement with the work of Rugh *et al.* (50-52) who observed growth retardation in mice following prenatal X irradiation at 1.0 Gy. Jensh *et al.* (53) have shown that there is an inverse relationship between litter size and fetal weight at the extremes of the litter size range, but a significant difference in term fetal weight was also observed following analysis of the data after exclusion of one unusually large (statistical outlier) litter. Term fetal weight was reduced in the group irradiated on the 17th day, approaching the 0.05 level of significance,

(±1 SD) OF THE EXPOSED MATERNAL RATS ARE SUMMARIZED

Organ weight ratios (Organ wt × 1000)/(Body wt)							
Ovaries						Ovaries*	
Left	Right	Brain	Liver	Left	Right	Left	Right
0.06 ± 0.01	0.05 ± 0.01	5.75 ± 0.49	37.27 ± 2.52	2.97 ± 0.45	3.00 ± 0.32	1.71 ± 0.32	1.60 ± 0.22
0.05 ± 0.01	0.11 ± 0.02	4.86 ± 0.32	36.36 ± 3.05	2.48 ± 0.17	2.64 ± 0.09	1.35 ± 0.31	1.51 ± 0.44
0.08 ± 0.03	0.06 ± 0.04	4.98 ± 0.77	33.86 ± 2.23	2.53 ± 0.29	2.71 ± 0.41	2.07 ± 0.92	1.70 ± 1.24
0.11 ± 0.01	0.06 ± 0.01	5.48 ± 0.72	35.72 ± 5.56	2.69 ± 0.42	2.68 ± 0.41	1.52 ± 0.26	1.55 ± 0.35

TABLE IV. RESULTS OF THE FIVE NEONATAL REFLEX TESTS FOR THE F1 OFFSPRING ARE PRESENTED (MEAN DAY OF AGE AT ACHIEVEMENT OF CRITERION \pm 1 SD)

Group	Number of		Surface righting	Negative geotaxis	Auditory startle	Air righting	Visual placing
	Litters	Offspring					
0 Gy, 9 day (sham)	4	32	5.9 \pm 0.9	9.4 \pm 1.5	14.1 \pm 1.7	19.8 \pm 1.4	24.6 \pm 1.2
0.6 Gy, 9 day	6	45	5.5 \pm 1.2	9.2 \pm 0.8	15.2 \pm 0.8	18.4 \pm 0.6	24.2 \pm 1.6
0 Gy, 17 day (sham)	6	48	5.5 \pm 0.9	9.9 \pm 2.7	14.7 \pm 1.9	18.6 \pm 0.9	24.0 \pm 1.2
0.6 Gy, 17 day	7 ^a	37	5.2 \pm 0.6	9.5 \pm 2.6	13.9 \pm 0.9	20.7 \pm 0.5*	23.8 \pm 2.1

^a Sample number at initiation of reflex test (one litter dead by Day 30).

* Significantly different from controls at $P \leq 0.001$.

due to the difference in mean litter size between the 17th day irradiated and control groups. However, the 3-day neonatal weight was also significantly depressed in this group, indicating possible nonrecuperable growth retardation due to irradiation during the fetal period of gestation.

Statistical analyses of these data, based on the mean time of reflex acquisition for the litter, may not provide the full picture of possible radiation-induced effects. None of the physiologic parameters were significantly altered, and only the air righting reflex was significantly affected by exposure to X radiation. The acquisition of this reflex was delayed due to exposure on the 17th day. Population trends and distribution may also provide helpful approaches in data analysis. Although sample sizes are small in the present study, some observations are provocative. For example, only 57% of the control males acquired the air righting reflex by PN 19, but 100% of the 9th day irradiated males achieved this reflex, although females were unaffected. Irradiation on

the 17th day resulted in similar accelerated acquisition of this reflex; 46% of the irradiated males and 56% of irradiated females versus 11% of control males and 19% of control females attained this reflex by PN 18. Similar population distribution shifts were observed for several of the other reflexes and physiologic parameters. These alterations in reflex acquisition may be indicative of changes in timing, or the orderly sequence of events in maturation, or in the developmental continuum of systems within the organism. The present protocol was not appropriate for an in-depth analysis of this type. Such analyses of population distributions, however, may prove to be more sensitive than analyses of population means.

Alterations in the age of acquisition of reflexes may be indicative of developmental delays or accelerations. Coyle *et al.* (54) have stated that not only deviations in behavior but also delays (or accelerations) in the attainment of some types of behavior early in life may be sensitive indicators of toxicity. Normal growth

TABLE V. THE RESULTS OF THE ANALYSES OF FOUR F1 PHYSIOLOGIC PARAMETERS ARE SUMMARIZED (MEAN DAY OF AGE OF CRITERION ACHIEVEMENT, \pm 1 SD)

Group	Number of		Pinna detachment	Eye opening	Testes descent	Vaginal opening
	Litters	Offspring				
0 Gy, 9 day (sham)	4	32	3.8 \pm 0.6	16.6 \pm 0.8	23.8 \pm 1.6	42.6 \pm 1.7
0.6 Gy, 9 day	6	45	3.4 \pm 0.9	15.8 \pm 0.9	25.0 \pm 3.5	38.6 \pm 7.5
0 Gy, 17 day (sham)	6	48	3.3 \pm 0.5	15.9 \pm 1.1	23.2 \pm 1.7	36.2 \pm 5.8
0.6 Gy, 17 day	7 ^a	37	3.6 \pm 0.4	15.8 \pm 0.8	25.0 \pm 2.0	42.2 \pm 4.5

^a Sample number at initiation of reflex test (one litter dead by Day 30).

and development depends on an ordered and timely sequence of cellular events, and alterations in behavior may indicate that such sequencing has been disturbed. The results of one reflex test were altered due to prenatal irradiation on the 17th day. The absence of radiation-induced changes in the acquisition of other reflexes due to exposure at the 0.6-Gy level on the 17th day of gestation may be because (a) the tests chosen were inappropriate, or (b) the recuperative powers of the rat were sufficient to correct any temporary deficits which may have adversely affected the test results. Further experiments at lower dosage levels are necessary to determine when the radiation-induced effects on air righting and retardation of growth disappear.

The results of this study indicate that X irradiation on the 9th or 17th day of rat gestation at a dosage level of 0.6 Gy does not cause significant alterations in postnatal physiologic development. Exposure to X radiation on the 9th day of rat gestation would not be expected to result in anatomic malformations, nor would major central nervous system effects be expected since (a) the central nervous system is not yet organized at this stage, and (b) the central nervous system has great recuperative powers during this stage. Exposure on the 17th day would not be expected to produce major anatomic malformations since this stage is well beyond the period of major organogenesis. However, growth retardation, cellular changes, and neurophysiologic alterations could be expected from exposure during this period of rat gestation. Growth retardation due to *in utero* irradiation on the 9th day did not persist neonatally, but irradiation on the 17th day did result in postnatal growth retardation. There were also no radiation-induced alterations in reflex acquisition due to exposure on the 9th day of gestation. The acquisition of one reflex was delayed in the group irradiated on the 17th day. These results indicate that exposure to ionizing radiation during the fetal period of rat gestation can cause alterations in postnatal growth and psychophysiological development. Studies, including neuropathologic correlation with functional alterations, are needed to further elucidate the biologic significance of these alterations in postnatal growth and development.

The authors express their thanks to Jeanne Ludlow and Eileen Piasecki-Couch for their technical assistance and to Sandra Parsons and Ruth-Eleanor Jensh for typing the manuscript. This study was supported by National Institutes of Health Grants HDHS-14840 and HD-19165.

1. Job TT, Leibold GJ, Fitzmaurice HA. Biological effects of roentgen rays: The determination of critical periods in mammalian development with x-rays. *Amer J Anat* 56:97-117, 1935.
2. Wilson JG, Jordan HC, Brent RL. Effects of irradiation on embryonic development. II. X-rays on the 9th day of gestation in the rat. *Amer J Anat* 92:153-188, 1953.
3. Russell LB. The effect of radiation on mammalian development. In: Hollaender A, ed. *Radiation Biology*. New York, McGraw-Hill, No I, Part 2, Chapt 13, pp861-918, 1954.
4. Brent RL. The effect of irradiation on the mammalian fetus. *Clin Obstet Gynecol* 3:928-950, 1960.
5. Brent RL. The long term effects of embryonic and fetal irradiation. *Pediatr Res* 2:291-292, 1968.
6. Brent RL. Radiations and other physical agents. In: Wilson JG, Frazer RD, eds. *Handbook of Teratology*. New York, Plenum, Chapt 5, pp153-223, 1977.
7. Kimmeldorf DJ, Hunt EL. *Ionizing Radiation: Neural Function and Behavior*. New York, Academic Press, pp1-302, 1965.
8. Dekaban A. Abnormalities in children exposed to x-radiation during various stages of gestation: Tentative timetable of radiation injury to the human fetus, Part I. *J Nucl Med* 9:471-477, 1968.
9. Rugh R, Wohlfromm M, Varma A. Low-dose x-ray effects on the precleavage mammalian zygote. *Radiat Res* 37:401-414, 1969.
10. Brent RL, Gorson RO. Radiation exposure in pregnancy. In: Moseley RD Jr, Baker DH, Gorson RO, Lalli A, Latuarette HB, Quinn J, III, eds. *Current Problems in Radiology*. Chicago, Medical Publishers, Vol 2:pp1-46, 1972.
11. Kameyama Y. Low-dose radiation as an environmental agent affecting intrauterine development. *Environ Med* 26:1-15, 1982.
12. Brizzee KR, Jacobs LA, Bench CJ. Histologic effects of total-body x-irradiation in various dose fractionation patterns on fetal cerebral hemisphere. *Radiat Res* 31:415-429, 1967.
13. Brizzee KR, Brannon RB. Cell recovery in foetal brain after ionizing radiation. *Int J Radiat Biol* 21:374-388, 1972.
14. D'Amato CJ. Regeneration and recovery in the fetal nervous system after radiation injury. *Exp Neurol* 76:457-467, 1982.
15. Heinzmann U. X-ray induced dysplasia in the developing telencephalic choroid plexus of mice exposed in utero. *Teratology* 26:39-52, 1982.
16. Russell LB. Effects of low doses of x-rays on embryonic

- development in the mouse. *Proc Soc Exp Biol Med* **95**:174-178, 1957.
17. Rugh R, Grupp F. Response of the very early mouse embryo to low levels of ionizing radiation. *J Exp Zool* **141**:571-587, 1959.
 18. Hicks SP, D'Amato CJ. Low-dose radiation of developing brain. *Science* **141**:903-905, 1963.
 19. Brown SO. Effects of continuous low intensity radiation on successive generations of the albino rat. *Genetics* **50**:1101-1113, 1964.
 20. Stadler J, Gowen JW. Observations of the effects of continuous radiation over 10 generations on reproductivities of different strains of mice. In: Carlson WD, Gassner FX, eds. *Proceedings of an International Symposium on the Effects of Ionizing Radiation on Reproductive Systems*. New York, Pergamon, 1964.
 21. Coppenger CJ, Brown SO. Postnatal manifestations in albino rats continuously irradiated during prenatal development. *Texas Rep Biol Med* **23**:45-55, 1965.
 22. Brizzee KR. Quantitative histological studies on delayed effects of prenatal x-irradiation in rat cerebral cortex. *J Neuropathol Exp Neurol* **26**:584-600, 1967.
 23. Kameyama Y, Hoshino K, Hayashi Y. Effects of low-dose x-radiation on the matrix cells in the telencephalon of mouse embryos. In: *Developmental Toxicology of Energy-related Pollutants*. Oak Ridge, Tenn., US Dept of Energy, Technical Information Center, pp228-236, 1978.
 24. Brent RL. The effect of low dose x-irradiation during early organogenesis in the mammal. *Teratology* **19**:20A, 1979.
 25. Furchtgott E, Echols M. Activity and emotionality in pre- and neonatally x-irradiated rats. *J Comp Physiol Psychol* **51**:541-545, 1958.
 26. Furchtgott E, Echols M. Locomotor coordination following pre- and neonatal x-irradiation. *J Comp Physiol Psychol* **51**:292-294, 1958.
 27. Furchtgott E, Echols M, Openshaw JW. Maze learning in pre- and neonatally x-irradiated rats. *J Comp Physiol Psychol* **51**:178-180, 1958.
 28. Furchtgott E, Jones JR, Tacker RS, Deagle J. Aversive conditioning in prenatally x-irradiated rats. *Physiol Behav* **5**:571-576, 1970.
 29. Graham TM, Marks A, Ershoff B. Effects of prenatal x-irradiation on discrimination learning in the rat. *Proc Soc Exp Biol Med* **100**:78-81, 1959.
 30. Sharp JC. Effects of fetal x-irradiation on maze learning ability and motor coordination in albino rats. *J Comp Physiol Psychol* **54**:127-129, 1961.
 31. Sharp JC. The effects of prenatal x-irradiation on acquisition, retention, and extinction of a conditioned emotional response. *Radiat Res* **24**:154-157, 1965.
 32. Mullenix P, Norton S, Culver B. Locomotor damage in rats after x-irradiation in utero. *Exp Neurol* **48**:310-324, 1975.
 33. Tamaki Y, Inouye M. Avoidance learning under delayed shock termination in prenatally x-irradiated rats. *Dev Psychobiol* **14**(2):95-99, 1979.
 34. Tamaki Y, Inouye M. Avoidance of and anticipatory responses to shock in prenatally x-irradiated rats. *Physiol Behav* **22**:701-705, 1979.
 35. D'Amato CJ, Hicks SP. Development of the motor system: Effects of radiation on developing corticospinal neurons and motor function. *Exp Neurol* **70**:1-23, 1980.
 36. Hicks SP, D'Amato CJ. Development of the motor system: Hopping rats produced by prenatal irradiation. *Exp Neurol* **70**:24-39, 1980.
 37. Kiyono S, Seo M, Shibagaki M. Effects of environmental enrichment upon maze performance in rats with microcephaly induced by prenatal x-irradiation. *Jpn J Physiol* **31**:769-773, 1981.
 38. Seo ML, Inouye M, Kiyono S, Shibagaki M. Effects of environmentally differential rearing upon maze performance in prenatally irradiated microcephalic rats. *Teratology* **26**:221-227, 1982.
 39. Piontkovskii IA. Certain properties of the higher nervous activity in adult animals irradiated prenatally by ionizing radiations: The problem of the effect of ionizing radiation on the offspring. *Byull Eksp Biol Med* **46**:77-80, 1958.
 40. Semagin VH. The state of the higher nervous activity in rats subjected to daily x-ray irradiation at the stage of embryonic development. *Med Radiol* **4**(6):16-21, 1959.
 41. Piontkovskii IA. Some peculiarities of higher nervous activity in adult animals subjected to radiation in utero. Part III. *Byull Eksp Biol Med* **51**:27-31, 1961.
 42. Werboff J, Goodman I, Havlena J, Sikov M. Effects of prenatal x-irradiation on motor performance in the rat. *Amer J Physiol* **201**:703-706, 1961.
 43. Werboff J, Havlena J, Sikov MR. Effects of prenatal x-irradiation on activity, emotionality, and maze learning ability in the rat. *Radiat Res* **16**:441-452, 1962.
 44. Werboff J, Havlena J, Sikov MR. Behavioral effects of small doses of acute x-irradiation administered prenatally. *Atompraxis* **9**:103-105, 1963.
 45. Kaplan SJ. Learning behavior of rats given low-dose x-irradiation in utero on various gestation days. In: Harley TJ, Snider RS, eds. *Response of the Nervous System to Ionizing Radiation*. New York, Academic Press, pp645-657, 1962.
 46. Sikov MR, Resta CF, Loftrom JE, Meyer JS. Neurological deficits in the rat resulting from x-irradiation in utero. *Exp Neurol* **5**:131-138, 1962.
 47. Wilson JG. Embryological considerations in teratology. In: Wilson JG, Warkany J, eds. *Teratology, Principles and Techniques*. Chicago, Univ. of Chicago Press, pp251-278, 1965.
 48. Blot WJ, Miller RW. Mental retardation following in

- utero exposure to the atomic bombs of Hiroshima and Nagasaki. *Radiology* **106**:617-619, 1973.
49. Otake J, Shull WJ. In utero exposure to a-bomb radiation and mental retardation; a reassessment. *Brit J Radiol* **57**:409-414, 1984.
50. Rugh R, Duhamel L, Chandler A, Varma A. Cataract development after embryonic and fetal x-irradiation. *Radiat Res* **22**:519-534, 1964.
51. Rugh R, Duhamel L, Osborne AW, Varma A. Persistent stunting following x-irradiation of the fetus. *Amer J Anat* **115**:185-197, 1964.
52. Rugh R, Duhamel L, Skaredoff L. Relation of embryonic and fetal x-irradiation to lifetime average weights and tumor incidence in mice. *Proc Soc Biol Med* **121**:714-718, 1966.
53. Jensch RP, Brent RL, Barr M. The litter effect as a variable in teratologic studies of the albino rat. *Amer J Anat* **128**:185-191, 1970.
54. Coyle I, Wayner MJ, Singer A. Behavioral teratogenesis: A critical evaluation. *Pharmacol Biochem Behav* **4**:191-200, 1976.
-

Received September 3, 1985. P.S.E.B.M. 1986, Vol. 181.

Accepted January 7, 1986.