PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 256–263 (1987)

# The Effect of Low-Level Prenatal X-Irradiation on Postnatal Development in the Wistar Rat (42476)

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*Abstract.* The objective of this investigation was to determine the effect of low-dose prenatal X-irradiation on postnatal growth and neurobehavioral development, and whether alterations would manifest at dosages lower than those which produce anatomic malformations from exposure at the most sensitive period of organogenesis. Ninety-eight Wistar strain rats were exposed to 0.1, 0.2, or 0.4 Gy X-radiation of were sham irradiated on the 9th or 17th day of gestation. A conventional teratologic evaluation was completed on half of the animals (572 fetuses). The age of appearance of four physiologic markers and of acquistion of six reflexes was observed in 372 offspring. Exposure during early organogenesis at these levels had no effect on any of these parameters. Prenatal exposure to X-radiation on the 17th day of gestation at dosage levels greater than 0.1 Gy resulted in alterations in the appearance of three postnatal neurophysiologic parameters. Growth retardation throughout the postpartum period also was observed in the offspring. The induction of developmental and reflex alterations had a comparable threshold to the known threshold for anatomic malformations on the 9th day. These results indicate that all of the parameters studied had thresholds either at or above 0.2 Gy acute radiation, and that the postpartum developmental and reflex acquisition measures were not more sensitive indicators of exposure to X-radiation than growth parameters. 

Ionizing radiation is an established teratogen (1–7) which causes classic morphologic alterations in development. Exposure of pregnant mice or rats to X-radiation in excess of 1.0 Gy will result in embryopathology in the offspring (1, 8–13). The results of several studies also indicated that exposure to low levels of X-radiation can result in increased resorption rates and intrauterine growth retardation (14, 15). Russell (16) observed skeletal malformations in mice exposed to X-radiation on Days 7.5, and 8.5 of gestation utilizing 0.25 Gy (1 Gy = 100 rad).

A number of investigators have been concerned with the effects of prenatal X-irradiation on postnatal growth and development. The majority of these studies described the effects of X-radiation on central nervous system morphology (17–22), but postnatal effects on other systems also have been described (23–25). Rugh *et al.* (9–11) have demonstrated persistent postnatal growth retardation due to prenatal exposure to 1.0 Gy X-radiation.

Several studies have been concerned with postnatal behavioral sequelae of prenatal X-irradiation. Exposure levels were generally in excess of 0.75 Gy (26, 27). Subtle central nervous system damage due to exposure to low levels of X-rays has been observed (28–31).

The present study was concerned with the effects of low-level prenatal ionizing radiation on postnatal growth and development. The study was designed to determine (a) if low-dose prenatal X-irradiation would induce changes in postnatal growth and neonatal reflex and physiologic development, and (b) whether such neurobehavioral and developmental parameters would be altered by lower doses than those which produce anatomic malformations at the most sensitive period of organogenesis. Blot and Miller (32) observed a dose-response effect in children exposed *in utero* to radiation from the Hiroshima and Nagasaki atomic bomb explosions. Their analysis of the data indicated that there was a threshold below which mental retardation was not observed. Otake and Shull (33), using different analytical technique, have maintained, however, that there may not be a threshold level for behavioral effects. The objective of this study was to determine if exposure to 0.1, 0.2, or 0.4 Gy X-radiation on the 9th or 17th



0037-9727/87 $1.50

day of gestation would cause alterations in term and postnatal development as determined by analysis of physiologic markers and the attainment of specific reflexes. The 9th day of rat gestation was chosen because it is at the end of the all-or-none period of development, that is, the time in pregnancy during which exposure to X-radiation would not affect normal development adversely or would result in intrauterine death (8). Exposure at this time would be a type of control for morphologic abnormalities since survivors should exhibit no anatomic malformations in spite of their X-ray exposure. This investigation also was designed to determine whether the all-or-none phenomenon applies to postnatal neurophysiologic development. The 17th day in the rat is a fetal stage during which the cells of the central nervous system are actively proliferating, differentiating, and migrating.

**Materials and Methods.** Female Wistar strain rats were maintained in a temperature- and humidity-controlled animal facility with a 12-hr light–dark cycle. All animals were given Purina laboratory chow and water *ad libitum.* Males and females were placed together 1 night for breeding. The zygote of the pregnant rat was considered to be 0 hr, 0 days old, or it was considered to be from the beginning of the first day of gestation, at 9:00 AM, when sperm were observed, using a standard vaginal smear technique.

All irradiation procedures were completed using a Siemens 250-kVp irradiation unit operating at 15 mA. The rats were placed in a rotating Plexiglas wheel, one in each compartment. The wheel rotated within the irradiation field to assure homogeneity of exposure. The beam was filtered with a Thoreus III filter plus 1.94 mm copper and 3.0 mm aluminum. The HVL ws 3.0 mm of copper. The average target–object distance ws 82.25 cm, and the dose rate was 0.12–0.14 Gy/min. Calibrations were completed using a Victoreen chamber and exposure was corrected for atmospheric pressure and environmental temperature.

The 62 pregnant rats were irradiated on the 9th or 17th day of gestation. They were randomly assigned to one of three groups: 0.1 Gy, 0.2 Gy, or 0.4 Gy on 1 of the 2 days. Thirty-six animals were sham irradiated (0 Gy) at the same time. Approximately half of the animals in each group were sacrificed at term (22nd day) and the fetuses were examined, and live, dead, and resorbed fetuses and implantation sites were recorded. After the uterine position of each fetus was recorded, the umbilical cord was cauterized, and each fetus and placenta was individually weighed. Each fetus was labeled for later identification, fixed in Bouin's fixative for 3 weeks, and stored until dissection in 70% ethanol. After at least 5 weeks all fetuses were dissected using the Wilson (34) technique. At autopsy, maternal brain, liver, kidney, and ovaries also were removed, weighed, fixed in Bouin's fixative or 10% Formalin, and stored in 70% ethanol.

The other half of the pregnant rats in each group were allowed to deliver their offspring. At 2 days of age the litters were randomly culled to a maximum of 8 offspring per litter, resulting in 372 offspring at 3 days of age. The mothers were weighed daily. The offspring were subjected to a series of physiologic marker examinations and reflex tests. Colony control data results were compared with control data in the present study to determine if response drift over a period of time had occurred. The mean age at which the last member of the litter achieved criterion in each test was recorded. The offspring were examined for development of the following physical features:

(i) *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.

(ii) *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.

(iii) *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 in a vertical position by their forelimbs. The testes were observed to immediately descend into the sac and could be palpated. The average time of achievement of this feature among colony control males was at 25 days.

(iv) *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 the average age of positive response among colony females being 30 days. Methodologic alterations during the present study resulted in an increase in observed acquisition time of about 1 week in the concurrent control animals.

The following neonatal reflex tests were completed:

(i) *Surface righting.* This is a test of the integration of the motor and vestibular system. 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, three successive times. This test was initiated on Day 5. Age of achievement of 30 colony control litters was 9.2 days.

(ii) *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 three out of three 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.

(iii) *Auditory startle.* The rats were exposed to a single loud sound presented suddenly without visual cuing. A positive startle 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 among 30 colony control litters was 14.0 days.

(iv) *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 three successive trials. Age of achievement of 30 colony control litters was 19.0 days. This test was initiated on Day 13.

(v) *Visual placing.* In this test of visual acuity the rat was lowered headfirst 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 two successive trials. Age of achievement of 30 colony control litters was 23.7 days. This test was initiated on Day 17.

Statistical analyses were completed using the Student *t*-test, ANOVA, and, where considered appropriate, the Mann–Whitney *U*-test. In the analysis of neonatal data, the number of litters per treatment group was used as the sample size, rather than the number of neonates, since testing occurred primarily before weaning, and litter effects would still be prevalent.

**Results.** The results of a previous study (35) in which a similar protocol was used to determine the effect of 0.6 Gy prenatal X-radiation are included in the tables. Although the study was completed at a different time, the methodology was the same, and the inclusion of these data will aid in the understanding of the effects of prenatal low-dose X-radiation on postnatal growth and development.

A conventional teratologic evaluation was completed on half of the animals in each group on the 21st day of gestation. The results of that analysis are presented in Table I. There was no radiation-induced growth retardation at term in animals irradiated on the 9th day with dosages up to and including 0.4 Gy, nor was there any dose-response relationship evident for this developmental parameter. The 17th day, the 0.4 Gy irradiated group was significantly growth retarded ($P < 0.05$) compared to the control group, due to one litter of 16 which had a mean term fetal weight of 4.161 g. When this litter was omitted, based on the Outlier's test, mean litter size and fetal weight of this group were not significantly different ($P > 0.05$) from the 17th day control group. None of the irradiated organ weights (brain, liver, kidneys, gonads) or organ weight/body weight ratios in any group differed significantly ($P > 0.05$) from the control group.

TABLE I. MATERNAL AND FETAL DATA OF ANIMALS EXAMINED AT TERM

| Group | Sample size | Maternal weight (±1 SD) 0 days | 21 days | Change | Number of resorptions/ total (%) | Mean litter size ±1 SD | Mean fetal weight ±1 SD (g) |
|---|---|---|---|---|---|---|---|
| 0 Gy, 9 days (sham) | 8 | 202 ± 19 | 343 ± 31 | 141 ± 24 | 6/90 (6.7) | 10.9 ± 1.9 | 5.78 ± 0.44 |
| 0.1 Gy, 9 days | 3 | 199 ± 12 | 380 ± 36 | 181 ± 34 | 0/40 | 13.3 ± 2.1 | 5.49 ± 0.31 |
| 0.2 Gy, 9 days | 5 | 198 ± 15 | 361 ± 23 | 163 ± 18 | 5/61 (8.2) | 11.2 ± 2.2 | 5.74 ± 0.58 |
| 0.4 Gy, 9 days | 5 | 162 ± 78 | 346 ± 24 | 146 ± 12 | 1/54 (1.9) | 10.8 ± 1.8 | 5.65 ± 0.41 |
| 0.6 Gy[a] 9 days | 5 | 202 ± 5 | 377 ± 17 | 175 ± 16 | 6/67 (9.0) | 12.2 ± 1.9 | 5.36 ± 0.40 |
| 0 Gy, 17 days (sham) | 14 | 218 ± 20 | 344 ± 98 | 148 ± 31 | 4/161 (2.5) | 10.9 ± 3.3 | 5.72 ± 0.49 |
| 0.1 Gy, 17 days | 5 | 201 ± 4 | 365 ± 13 | 164 ± 15 | 4/59 (6.8) | 11.0 ± 1.6 | 6.11 ± 0.44 |
| 0.2 Gy, 17 days | 5 | 214 ± 18 | 357 ± 23 | 139 ± 15 | 1/67 (1.5) | 13.2 ± 2.2 | 5.36 ± 0.25 |
| 0.4 Gy, 17 days | 5 | 221 ± 29 | 363 ± 31 | 142 ± 14 | 2/63 (2.2) | 11.8 ± 1.3 | 5.27 ± 0.43 |
| 0.6 Gy[a], 17 days | 5 | 220 ± 28 | 357 ± 27 | 137 ± 22 | 5/46 (10.9) | 10.3 ± 1.7 | 5.59 ± 0.48 |

[a] Previous study (35): data included for comparison.

Data from those mothers which were allowed to deliver and raise their offspring are shown in Table II. Three-day neonatal weights were analyzed using the number of litters per treatment group as the sample size. Weights were significantly lower ($P < 0.05$) in the 0.2 and 0.6 Gy, 17th day irradiated groups when compared to the control group. The 0.4 Gy group approached but did not attain significance due to several fetuses in one litter which were outliers. When these animals were eliminated from the statistical analysis, this dosage group was also significantly different ($P < 0.05$) from the control group.

*Reflex tests.* Animals irradiated with 0.2 or 0.6 Gy on the 17th day were significantly de-

TABLE II. SUMMARY OF MATERNAL AND OFFSPRING DATA OF LIVE-BORN LITTERS

| Group | Sample size | Maternal weight (+1 SD) 0 days | 21 days | Change | Off-spring | No. of 3-day neonates | Mean litter size (±1 SD) | Mean 3-day weight (±1 SD) (g) |
|---|---|---|---|---|---|---|---|---|
| 0 Gy, 9 days (sham) | 6 | 214 ± 28 | 319 ± 22 | 155 ± 16 | 74 | 48 | 12.3 ± 0.6 | 8.48 ± 1.07 |
| 0.1 Gy, 9 days | 5 | 203 ± 9 | 368 ± 5 | 165 ± 10 | 54 | 40 | 10.8 ± 0.8 | 7.45 ± 0.68 |
| 0.2 Gy, 9 days | 6 | 211 ± 20 | 366 ± 40 | 154 ± 33 | 72 | 48 | 12.0 ± 1.3 | 7.63 ± 0.95 |
| 0.4 Gy, 9 days | 7 | 291 ± 31 | 364 ± 33 | 146 ± 30 | 82 | 48 | 11.7 ± 1.0 | 7.61 ± 1.04 |
| 0.6 Gy, 9 days[a] | 6 | 201 ± 10 | 359 ± 38 | 158 ± 30 | 63 | 45 | 10.5 ± 3.3 | 8.68 ± 0.84 |
| 0 Gy, 17 days (sham) | 8 | 219 ± 16 | 362 ± 20 | 143 ± 11 | 88 | 64 | 11.0 ± 2.4 | 8.32 ± 0.95 |
| 0.1 Gy, 17 days | 6 | 199 ± 25 | 355 ± 37 | 156 ± 23 | 56 | 45 | 9.3 ± 2.4* | 8.77 ± 1.17 |
| 0.2 Gy, 17 days | 5 | 209 ± 13 | 351 ± 23 | 142 ± 23 | 53 | 39 | 10.6 ± 2.2 | 7.07 ± 0.41* |
| 0.4 Gy, 17 days | 5 | 224 ± 23 | 363 ± 16 | 139 ± 9 | 61 | 40 | 12.2 ± 0.7 | 7.19 ± 1.19 |
| 0.6 Gy, 9 days[a] | 5 | 198 ± 9 | 352 ± 29 | 154 ± 31 | 49 | 36 | 9.8 ± 3.6 | 7.18 ± 0.63* |

[a] Previous study (35): data included for comparison.

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

TABLE III. RESULTS OF THE FIVE NEONATAL REFLEX TESTS FOR THE OFFSPRING ARE PRESENTED

| Group | Number of | | Surface righting | Negative geotaxis | Auditory startle | Air righting | Visual placing |
|---|---|---|---|---|---|---|---|
| | Litters | Offspring | | | | | |
| 0 Gy, 9 days (sham) | 6 | 43 | 5.3 ± 1.3[a] | 9.6 ± 1.7 | 13.8 ± 1.5 | 20.0 ± 1.3 | 24.8 ± 1.1 |
| 0.1 Gy, 9 days | 5 | 40 | 6.3 ± 1.2 | 8.4 ± 1.1 | 14.0 ± 1.4 | 20.4 ± 0.6 | 25.9 ± 1.4 |
| 0.2 Gy, 9 days | 6 | 48 | 6.2 ± 1.8 | 8.7 ± 2.0 | 15.2 ± 1.3 | 19.8 ± 1.7 | 25.2 ± 2.3 |
| 0.4 Gy, 9 days | 6 | 48 | 6.0 ± 0.6 | 11.4 ± 3.3 | 13.8 ± 0.8 | 19.2 ± 0.8 | 25.6 ± 1.5 |
| 0.6 Gy, 9 days[b] | 6 | 45 | 5.5 ± 1.2 | 9.2 ± 0.8 | 15.2 ± 0.8 | 18.4 ± 0.6 | 24.2 ± 1.6 |
| 0 Gy, 17 days (sham) | 8 | 60 | 5.6 ± 0.8 | 9.9 ± 2.4 | 14.4 ± 1.7 | 18.7 ± 0.9 | 23.8 ± 1.0 |
| 0.1 Gy, 17 days | 6 | 45 | 6.4 ± 2.2 | 10.6 ± 2.2 | 14.5 ± 1.6 | 19.6 ± 1.8 | 25.4 ± 1.7 |
| 0.2 Gy, 17 days | 5 | 39 | 5.7 ± 0.6 | 8.6 ± 1.3 | 13.7 ± 0.5 | 19.9 ± 0.4* | 25.2 ± 1.8 |
| 0.4 Gy, 17 days | 5 | 40 | 5.6 ± 0.8 | 12.0 ± 3.0 | 14.6 ± 0.6 | 19.4 ± 0.8 | 25.1 ± 2.4 |
| 0.6 Gy, 17 days[b] | 7 | 37 | 5.2 ± 0.6 | 9.5 ± 2.6 | 13.9 ± 0.9 | 20.7 ± 0.5* | 23.8 ± 2.1 |

[a] Mean day of age at achievement of criterion ±1 SD.
[b] Previous study (35): data included for comparison.
* Significantly different from controls at $P \leqslant 0.05$.

layed ($P < 0.05$) in acquiring the air-righting reflex. Animals irradiated at the 0.4 Gy level did not achieve statistical significance due to larger intragroup variability. Elimination of outliers resulted in a statistically significant level of delay ($P < 0.05$) in this group. The results of the neonatal reflex tests are shown in Table III. There is no significant differences ($P > 0.05$) between the 9th day irradiated animals and their control counterparts for any of the reflex tests.

*Developmental markers.* The results of the analyses of the four physiologic parameters are summarized in Table IV. Testes descent was significantly delayed ($P < 0.05$) in offspring irradiated with 0.4 Gy on the 17th day. Testes descent was delayed in the 0.6 Gy groups, approximating but not achieving statistical significance due to a larger intragroup variability. Vaginal opening was accelerated in those offspring irradiated with 0.1 or 0.2 Gy on the 9th day. However, in the absence of a significant alteration in the age of acquisition of this developmental marker at higher dosage levels, these results do not appear to be biologically significant. Significantly delayed ($P < 0.05$)

TABLE IV. THE RESULTS OF THE ANALYSES OF FOUR $F_1$ DEVELOPMENTAL MARKERS ARE SUMMARIZED

| Group | Number of | | Pinna detachment | Eye opening | Testes descent | Vaginal opening |
|---|---|---|---|---|---|---|
| | Litters | Offspring | | | | |
| 0 Gy, 9 days (sham) | 6 | 43 | 3.6 ± 0.6[a] | 16.8 ± 0.6 | 24.0 ± 1.3 | 41.3 ± 2.5 |
| 0.1 Gy, 9 days | 5 | 40 | 4.3 ± 1.6 | 16.2 ± 0.8 | 23.5 ± 1.3 | 37.2 ± 1.6* |
| 0.2 Gy, 9 days | 6 | 48 | 3.7 ± 0.4 | 16.5 ± 0.8 | 24.3 ± 1.9 | 37.8 ± 1.4* |
| 0.4 Gy, 9 days | 6 | 48 | 3.6 ± 0.6 | 15.7 ± 0.5 | 24.8 ± 1.4 | 45.3 ± 8.0 |
| 0.6 Gy, 9 days | 6 | 45 | 3.4 ± 0.9 | 15.8 ± 0.9 | 25.0 ± 3.5 | 38.6 ± 7.5 |
| 0 Gy, 17 days (sham) | 8 | 60 | 3.6 ± 0.7 | 15.9 ± 0.9 | 23.1 ± 1.5 | 36.4 ± 4.9 |
| 0.1 Gy, 17 days | 6 | 45 | 3.4 ± 0.5 | 16.2 ± 1.0 | 22.6 ± 3.4 | 36.1 ± 7.0 |
| 0.2 Gy, 17 days | 5 | 39 | 4.0 ± 0.6 | 16.4 ± 0.9 | 24.4 ± 2.6 | 41.2 ± 4.8 |
| 0.4 Gy, 17 days | 5 | 40 | 4.2 ± 0.6 | 16.4 ± 0.6 | 25.6 ± 1.4* | 48.9 ± 6.9* |
| 0.6 Gy, 17 day[b] | 7 | 37 | 3.6 ± 0.4 | 15.8 ± 0.8 | 25.0 ± 2.0 | 42.2 ± 4.5* |

[a] Mean day of age of criterion achievement, ±1 SD.
[b] Previous study (35): data included for comparison.
* Significantly different from controls at $P \leqslant 0.05$.

vaginal opening was observed in those offspring irradiated with 0.4 or 0.6 Gy X-rays on the 17th day of gestation.

**Discussion.** X-radiation is a well-established teratogen, inducing congenital malformations, intrauterine death, and irreversible growth retardation. Brizzee *et al.* (18) subjected pregnant rats to 0.6 Gy/day X-radiation on Days 10–14, accumulating a total dose of 3.0 Gy, and observed a marked decrease in cortical thickness and total brain weight. Cowen and Geller (17) also have shown that the cerebellum was significantly reduced in size from exposure on Day 15. Brizzee *et al.* (12) have shown that a 12-hr fractionated exposure to 1.5 Gy on Days 13.5 or 14 of rat gestation will result in histologic central nervous system alterations which can be observed on Day 19.5. Progressive dose protraction reduced the degree of damage, the most severe damage being observed from a single acute exposure. Das (20, 21) irradiated pregnant Wistar rats on Day 18 with 1.7 Gy X-rays. He demonstrated a timeline of degeneration which included initial migratory arrest and death of granular cells followed by recovery, permanent abnormal clustering of neuroblasts, and, postnatally, abnormal patterns in the Purkinje cell layer, including nonreplaced cell death.

Norton (13) observed delayed damage to the rat central nervous system due to irradiation on Day 15 with 1.25 Gy. Rugh and co-workers (9–11) have demonstrated that exposure to levels of X-radiation in excess of 0.8 Gy can cause malformations, particularly of the central nervous system, and postnatal growth retardation. Exposure levels as low as 0.2–0.4 Gy on the 16th day of rat gestation can result in a permanent alteration of individual nerve cells and a disorganization of the cellular components of the cerebral cortex (28). Hayashi and Kameyana (29) observed cytoplasmic alterations in the neuroepithelium of the mouse telencephalon from irradiation at 0.25 Gy on Days 10 and 13 of gestation, and Brizzee and Brannon (19) demonstrated hypoplasia of the cerebral cortex from the same dosage level on Day 13 of gestation. Kameyama (31) suggests that acute cytologic changes may be observed from exposure levels of 0.05–0.1 Gy and that functional changes occur from exposure to 0.2–0.25 Gy. A dosage level of 1.0 Gy between Days 8 and 18 of mouse gestation caused growth retardation which persisted and was most severe in the offspring at 4 months of age. Irradiation on Days 12 to 13 caused the greatest permanent postnatal retardation. Brent (36) observed similar growth retardation at term from exposure to less than 0.6 Gy and postulated that such an effect may occur from exposure to doses between 0.3 and 0.6 Gy.

Although in the present study term fetal weight was statistically significantly reduced in the 0.1 Gy, 9th day irradiated animals, neither of the two higher dosage groups exhibited any significant term growth retardation. Furthermore, the very large litter size in this group, which is unrelated to the radiation exposure, can explain the lower weight in the newborns (37, 38). Therefore, no biologic significance can be inferred from this observation. However, analysis of 3-day neonatal weights indicated that slight growth retardation approaching statistical significance was exhibited in all three groups irradiated on the 9th day. Exposure on the 17th day at the 0.2 Gy or greater dosage level resulted in significant term fetal growth retardation which persisted in the 3-day neonates. The 0.2 and 0.4 Gy, 17th day irradiated neonates did exhibit growth retardation similar to the data obtained at term which supports the work of Rugh *et al.* (9–11) and Brent (36).

Postnatal behavioral alterations have been induced by exposure to high dosage levels of X-radiation prenatally. Hicks (26) demonstrated that exposure to 1.5 Gy on Day 11, 12, or 13 of gestation resulted in an irregular waddling gait in the offspring. If the irradiation occurred near term or during the first week after parturition the offspring were ataxic.

The reflex tests used in this study were chosen for several reasons: (a) a different response mechanism is necessary for each reflex; (b) the elicited body action for each is distinct; (c) the normal appearance of each reflex is highly specific; (d) the reflexes occur throughout a wide age span in the offspring; (e) the tests require minimal time to learn and to complete; (f) the results are either positive or negative rather than continuous, requiring minimal judgmental evaluations; (g) the data generated are amenable to statistical analyses. Four physiologic parameters were chosen for the present study because (a) the tests were easy to learn and to complete, (b) the tests were not time consuming, (c) presence or absence of the parameters is obvious, (d) the appearance of each is normally very time specific, and (e) they appear over a wide age span in the offspring. It should not be interpreted, however, that these are the only tests that could be used.

Exposure to X-rays on the 9th day did not result in biologically significant changes in the onset of the physiologic markers. Vaginal opening was statistically significantly accelerated only in the two low-dose groups and, therefore, does not appear to be biologically significant. Exposure on the 17th day did cause significant delays in the acquisition of two parameters, vaginal opening and testes descent, at dosage levels in excess of 0.2 Gy.

There were no biologically or statistically significant changes in reflex activity in any of the five reflexes tested in the 9th day group. Air righting was significantly delayed in the 0.2 Gy, 17th day group, but in the absence of any discernable dose-response trend, the biologic significance of this is questionable.

Previous studies have indicated that low-dose X-irradiation can induce postnatal alterations in morphology and function. The present study has shown, as expected, that a single exposure to X-radiation on the 9th or 17th day of gestation, at a dosage level of 0.1 to 0.4 Gy, does not produce a statistically significant increase in the incidence of malformations at term or intrauterine death. However, exposure at dosage levels greater than 0.2 Gy on the 17th day can result in growth retardation and an alteration in physiologic development. These data indicate that using the present paradigm, the reflex acquisition parameter was no more sensitive than the growth parameter in detecting subtle low-level prenatal radiation-induced alterations. The results suggest that growth measures may be as sensitive (or more so) an indicator as reflex or physiologic measures. These indices, however, may be strongly related to the time in pregnancy during which the radiation occurs. The results of the present study indicate that a dosage level as low as 0.2 Gy, administered on the 17th day of pregnancy, causes subtle alterations in growth and development, and exposure on the 17th day to dosage levels in excess of 0.2 Gy causes alterations in the acquisition of one reflex and the appearance of selected physiologic markers.

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.

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Received June 3, 1986. P.S.E.B.M. 1987, Vol. 184.
Accepted November 7, 1986.