

# EFFECTS OF X-RADIATION ON MOUSE FETUS DURING GESTATION: EMPHASIS ON DISTRIBUTION OF CEREBRAL LESIONS, PART II

Anatole S. Dekaban

*National Institute of Neurological Diseases and Blindness,  
National Institutes of Health, Bethesda, Maryland*

Exposures to ionizing radiation can threaten health and life, increase mutations and damage the fetus. The type and extent of lesions produced in the fetus depend greatly on the gestational stage and the dose of ionizing radiation received (1-4). Most tissues show greatest sensitivity to irradiation during the early stage of organogenesis, but the brain continues to be very susceptible to damage during its prolonged period of differentiation and growth. For understandable reasons interest in irradiation effects on living organisms has increased greatly in recent decades, and many pertinent papers have been published. It would be beyond the scope of this article to review the vast literature on the subject; interested readers are referred to original articles and special reviews (1-13).

The purpose of this investigation is twofold: First, it is a complementary study to Part I, "Abnormalities in children exposed to x-radiation during various stages of gestation: tentative timetable of radiation injury to the human fetus" (8). The existing publications could not fill the particular need of providing time-specific pathological correlates for interpretation of the findings in these 26 children exposed to irradiation *in utero*. Second, this investigation is intended to provide semiquantitative data on differential sensitivity to irradiation of phylogenetically different cerebral structures. These particular findings are important in interpreting one of the most common features in surviving children after irradiation *in utero*: type and degree of mental retardation (8).

## MATERIAL AND METHODS

The experiments were carried out on the Webster Swiss albino strain of mice. Five to six-months-old fertile females were mated between 7 pm and 9 am the next morning. The fetal age on that morning was

considered as zero day although the actual age was in a range between 0 and 14 hr. Twelve groups of pregnant females totaling 152 were irradiated on each consecutive day between the 7th and 18th day of gestation. To prevent loss of some abnormal newborns due to cannibalism, Caesarean section deliveries were carried out in all instances on the 19th day of gestation (spontaneous birth in mice occurs on the 19th and 20th day of pregnancy). The litter and contents of the uterus were examined with low-power magnification for gross abnormalities. Four transverse blocks from the head and two from the thorax were processed for histological studies. The sections were cut at 8 microns and stained with hemotoxylin-eosin. In selected sections, trichrome and silver stains were also used. To obtain some information on acute cell damage, a few of the irradiated mice were sacrificed 12 hr after irradiation and fetuses processed for microscopic studies.

All mice were exposed to 200 R. The parameters of irradiation were: 200 kv constant potential, 0.25 mm Cu + 0.55 mm Al filter, HVL 0.9 mm Cu, source distance to midline of animals 54 cm, exposure rate in air 60 R/min. Irradiation exposure rate was measured in air with a Victoreen dosimeter next to the mouse uterus before, during and after the animals were exposed. The mice were maintained in specially designed Lucite box containers with a lead plate to shield the head and upper one fourth of the thorax of the pregnant mice. Stained serial sections of the brain of normal control animals of 17, 18, 19 and 20 days of gestation were available at the time microscopic examination of the brains

Received Feb. 27, 1968; revision accepted July 18, 1968.

For reprints contact: A. S. Dekaban, Section of Child Neurology, National Institute of Neurological Diseases and Blindness, Building 10, Room 4N-250, National Institutes of Health, Bethesda, Maryland 20014.

of irradiated animals was made. This allowed us to compare the stage of development of various structures of the brain in irradiated and nonirradiated mice.

### RESULTS

Because it has been shown previously that irradiation of pregnant mice up to 7 days of gestation leads to a high mortality rate among fetuses while the few survivors are apparently normal (2,13), we began irradiation in this series with the 7th day of pregnancy.

Table 1 shows the numbers of pregnant mice irradiated in each group, the total number of litters and the major abnormalities seen by gross examination. The mean number per litter after irradiation (200 R) on the 7th gestation day (g.d.) was 2.2, on the 8th g.d. 3.6 and on the 9th g.d. 4.6. The number of animals in the litter increased as the fetuses became older at the time of irradiation and after the 12 g.d. the litter size was between 7 and 8 which is close to the normal range for this strain of mice. Early resorption of selectively damaged fetuses was responsible for the small size of the litter when irradiation occurred during early stages of gestation. In many of these pregnant females either several placentas alone or placentas with disintegrated fetal tissues were observed.

In contrast to the small litter number in pregnancies irradiated early, the percentage of normal animals at delivery was relatively high; the percentage, however, decreased as progressively older fetuses were irradiated. Irradiation at 7 and 8 g.d. was associated with 89 and 73%, respectively, of apparently normal animals, but the percentage fell to 41 at 9, 10 at 10 and 3 at 11 g.d. From the 13th g.d. onward the percentages of normal animals determined by external examination alone at the time of birth began to rise again.

Among the abnormalities seen by gross examination (Table 1) when pregnant mice were irradiated at 7 g.d. (more exactly between 7 and 7½ days) one animal had exencephaly (anencephaly) and one hydrocephalus. The main abnormalities at 8 and 9 g.d. were stunted size, abnormal head size and dysraphism. Grossly observed abnormalities were at the peak when irradiation occurred between 10 and 12 g.d. Stunted size, tail kinking and head and skeletal deformities were among the most common abnormalities; these gradually decreased in frequency with age at irradiation, and none were seen on gross examination after irradiation at 16–18 g.d. However, assessment of the head size and less severe skeletal changes at birth is difficult and only definite deviations from the normal litter of the same age were recorded.

TABLE 1. ABNORMALITIES DETECTED BY EXTERNAL EXAMINATION OF NEWBORN MICE

Irradiated gest. days	No. pregnant mice	Total no. animals	Mean litter size	No. animals alive & apparently normal	No. grossly apparent abnormalities					
					Stunted	Dysraphism	Small or deformed head	Large head	Kinked or short tail	Others
7	12	27*	(2.2)	24 (89%)	—	1 ex (4%)	—	1 (4%)	—	—
8	18	64*	(3.6)	47 (73%)	4 (6%)	3 (4%) (2 ex 1 en)	6 (9%)	2 (3%)	2 (3%)	1 ch (1.5%)
9	15	69†	(4.6)	28 (41%)	12 (17%)	3 en (4%)	9 (13%)	11 (16%)	10 (15%)	2 ch (3%)
10	29	170†	(5.8)	17 (10%)	128 (75%)	11 (7%) (8 en 3 m)	97 (57%)	15 (9%)	89 (52%)	10 sk (6%)
11	30	189†	(6.3)	7 (3%)	150 (79%)	3 en (2%)	89 (47%)	14 (7%)	163 (86%)	19 sk (10%)
12	9	69	(7.6)	5 (7%)	29 (42%)	—	24 (35%)	2 (3%)	25 (36%)	5 sk (7%)
13	8	61	(7.6)	22 (36%)	18 (30%)	—	16 (26%)	1 (2%)	12 (20%)	—
14	8	57	(7.4)	41 (72%)	12 (21%)	—	8 (14%)	—	—	—
15	7	53	(7.6)	49 (92%)	1 (2%)	—	2 (4%)	—	—	—
16	6	42	(7.0)	40 (95%)	—	—	—	—	—	—
17	5	39	(7.8)	39 (100%)	—	—	—	—	—	—
18	5	36	(7.2)	35 (97%)	—	—	—	—	—	—
Total	152	876								

\* Over 30 degenerating placentas.

† 8–30 degenerating placentas.

ex = exencephaly

en = encephalocele or protrusion at the vertex of the head (see HTV in Table 2).

m = myelocele

ch = cardiac hernia

sk = skeletal malformation

Table 2 shows results of microscopic examination of stained sections of brains following irradiation at various stages of pregnancy. A small number of the animals were dead at delivery; those that appeared less autolyzed were processed for histological sections but a proportion of these brains was found to be macerated and therefore was not rated although it is recorded in column 2 of Table 2 for the sake of completeness. Microscopic examination of the brains allows infinitely more accurate assessment of abnormality than gross examination. This is clearly reflected in a high percentage of abnormal brains in mice who received radiation between 10 and 16 days of gestation.

Microscopic studies of mice irradiated on the 7th g.d. showed the same types of abnormal brains as on gross examination: exencephaly and hydrocephalus. Mice irradiated at the 8th g.d. showed in addition to three cases of dysraphism seen grossly, two brains with a single ventricle (arrhinencephaly—Fig. 1C) and 4 with hydrocephalus. Irradiation on the 9th g.d. resulted in three brains with encephalocoele, three with a single cerebral ventricle, 11 with hydrocephalus without obvious evidence of parenchymatous lesions (Fig. 1B) and seven with mild abnormalities in the diencephalon, olfactory and limbic systems. In addition seven more brains were judged as small (the transverse sections passing

through the anterior commissure were at most  $\frac{2}{3}$  size of those of control animals of the same age) although without evident structural or cellular abnormality. Following irradiation between 10 and 14 g.d. the malformations seen at the preceding stages of gestation were very rare. Instead, a high incidence of brains (98–100%) showed structural abnormalities and an apparent attempt at regeneration to replenish massive damage to cells which occurred at the time of irradiation.

Table 3 shows detailed distribution of various types of cerebral lesions in those brains that are listed as abnormal in columns 4 and 5 of Table 2. Out of 11 cases of hydrocephalus following irradiation at the 9th g.d., two were of mild, four of moderate and five of marked degree. The pathogenesis of the hydrocephalus was heterogenous. Aqueductal stenosis associated with large lateral and third ventricles but with fourth ventricle normal in size was present in three of these brains. In the remaining eight mice the fourth ventricle was also dilated although to a variable extent and frequently only slightly; here, the cause of hydrocephalus was not entirely clear, and the hydrocephalus has to be differentiated between obstructive, hypersecretory and impaired absorption types. The present experimental material did not permit clarification of this particular problem.

TABLE 2. RESULTS OF MICROSCOPIC EXAMINATION

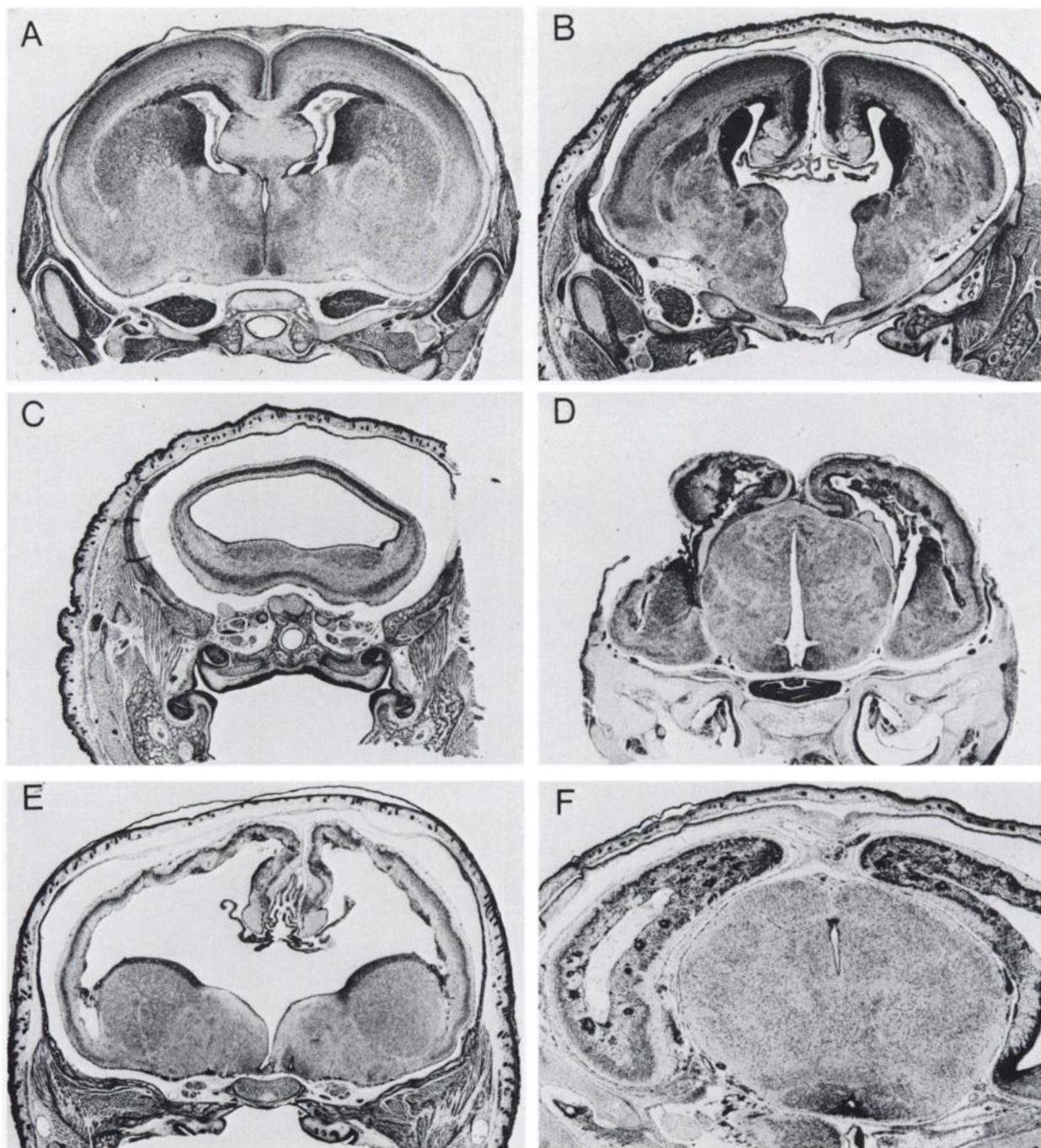
Irradiated gest. days	Total no. brains	Macer. & not studied	No. abnorm. brains†	Malform. & other lesions	Hydroceph.	Small brain, otherwise normal structure
7	27	1	2 (8%)	1 (exencephaly)	1	—
8	64	7	11 (19%)	5 { 2 exencephaly 2 single ventr. 1 encephalocoele	4	2
9	69	5	31 (49%)	13 { 3 encephalocoele 3 single ventr. 7*	11	7
10	151	8	141 (98%)	138 { 8 HTV + 130°	3	—
11	168	6	162 (100%)	162 { 3 HTV + 159°	—	—
12	69	2	67 (100%)	67°	—	—
13	60	1	59 (100%)	59°	—	—
14	57	—	56 (98%)	55°	1	—
15	53	1	26 (50%)	24°	—	2
16	42	1	8 (19%)	7°	—	1
17	39	—	2 (5%)	2°	—	—
18	36	—	‡	‡	—	—
Total	835	32				

\* Heterotopias, deformities, dysgenetic architectonics, etc.; for details see Table 3.

† Macerated brains were not assessed. Percentages in third column were calculated from number of brains minus macerated.

‡ Rare cells with signs of acute necrosis in neopallium.

HTV—Abnormally high situation of third ventricle; this was always associated with the absence of the corpus callosum.



**FIG. 1.** Photomicrograph of H & E-stained brain showing transverse sections; all mice were 19 g.d. old. Enlargement in A-E is 15 times; in F it is 22 times. A is normal control mouse of 19 g.d.; B is mouse irradiated on 8th g.d. Moderate degree of hydrocephalus is seen with unusual feature of greatly enlarged third ventricle (possibly related to some cellular deficiency in diencephalon?); C is mouse irradiated on the 9th g.d. Brain is small and grossly malformed consisting of single prosencephalic cavity (arrhinencephaly); D is mouse irradiated on 10th g.d. Large

heterotopic masses of cells are present in subependymal region; pallium is narrowed, and there is porencephalic defect on left side. Brain is generally small; E is mouse irradiated on the 10th g.d. Marked deficiency occurs and thinning of pallium with compensatory enlargement of the ventricles. Heterotopic cell masses are present in subependymal region; F is mouse irradiated on 11th g.d. Heterotopic cell masses largely in form of rosettes are located in both subependymal region and in white matter. Pallium is thin and ventricles are secondarily enlarged.

Between 10 and 14 days of fetal life, irradiation produced specific types of cerebral abnormality. Dense groups of cells of various sizes were present in atypical regions (heterotopias), disorganizing nor-

mal structures and affecting their relationship (Figs. 1D-F and 2A-C). Also affected were morphogenesis and emergence of new structures such as corpus callosum (Fig. 1D, E). In addition, the cellular

TABLE 3. DETAILED DISTRIBUTION OF TYPES OF ABNORMALITIES FROM TABLE 2

Irradiated gest. days	No. abnor. brains from col. 3, Table 2	Malform. of early organogenesis	Damage, repair, regen., malform. & others		
			Heterotopic cell masses: rosettes, cords, clumps; damage & repair	Second ventr. dilatation	Small groups of atypical cells; min. archit. imperf.
7	2 (8%)	2 { 1 exencephaly 1 hydroceph.	—	—	—
8	9 (16%)	9 { 2 exencephaly 2 single ventr. 1 encephalocele 4 hydroceph.	—	—	—
9	24 (37%)	17 { 3 encephaloceles 3 single ventr. 11 hydroceph. mild 2 mod. 4 mark. 5	2 few (dienc. & olf.)	—	5 (dienc. & olf.)
10	141 (98%)	11 { 3 hydroceph. mod. 8 HTV*	133 { small am't. 19 mod. am't. 31 many 83	121 { mild 18 mod. 33 mark. 70	—
11	162 (100%)	3 HTV*	161 { small am't. 12 mod. am't. 38 many 111	159 { mild 21 mod. 42 mark. 96	—
12	67 (100%)	—	67 { small am't. 42 mod. am't. 16 many 9	67 { mild 42 mod. 16 mark. 9	—
13	59 (100%)	—	59 { small am't. 24 mod. am't. 29 many 6	53 { mild 18 mod. 29 mark. 6	—
14	56 (98%)	—	55 { few 39 small am't. 14 mod. am't. 2	7 { min. 3 mild 2 mod. 2	—
15	26 (50%)	—	—	—	26 { 24 min. imperf. neopal. 2 mild imperf. neopal.
16	8 (19%)	—	—	—	8 min. imperf. neopal.
17	2 (5%)	—	—	—	2 min. imperf. neopal.
18	†	—	—	—	—

\* HTV—High situation of the third ventricle.  
† Rare necrotic cells.

complement of many structures was deficient, the degree of low cellularity being greatest when irradiation occurred on 10–12 g.d.

The heterotopias present in fetuses irradiated on the 10th day consisted of large, solid islands of immature cells located mainly in the subependymal region (Fig. 1D, E). In fetuses irradiated on the 11th and 12th g.d. heterotopias were extensive and consisted of numerous cones of cells with central lumen and radially arranged cells imitating the neural tube (Fig. 1F, Fig. 2A). In addition, clusters of cells and large groups of cells in a form of solid cords were seen. When heterotopic cell masses were present in the cortex and basal ganglia, they produced distortion of these structures. In many brains the presence of heterotopias, which can be considered an attempt at regeneration, was not sufficient to supplement destroyed cellular elements at the time of irradiation and as a result of this compensatory ventricular enlargement occurred (Fig. 1E, F).

Irradiation during the 13th and 14th g.d. also led to a high incidence of cerebral abnormality but the heterotopias were much smaller and discrete and the nervous parenchyma less deficient (Fig. 2B); here ventricular dilatation, if present, was mild. Irradiation during 15–17 g.d. was associated only with isolated minor architectonic imperfections in the neocortex and rarely with a small nest of cells (Fig. 2C). Irradiated fetuses on the 18th g.d. were sacrificed 24 hr later. Here, the microscopic lesions were very rare and consisted of scattered necrotic cells in the neopallium. It must be stressed, however, that present experimental data are derived from the microscopic examination at the time of birth when certain cerebral structures have not yet reached their mature condition. Cowen and Geller (9) and Hicks (10) have found abnormalities in the brain of adult rats which were irradiated during last days of fetal life.

The degree of compensatory ventricular dilata-

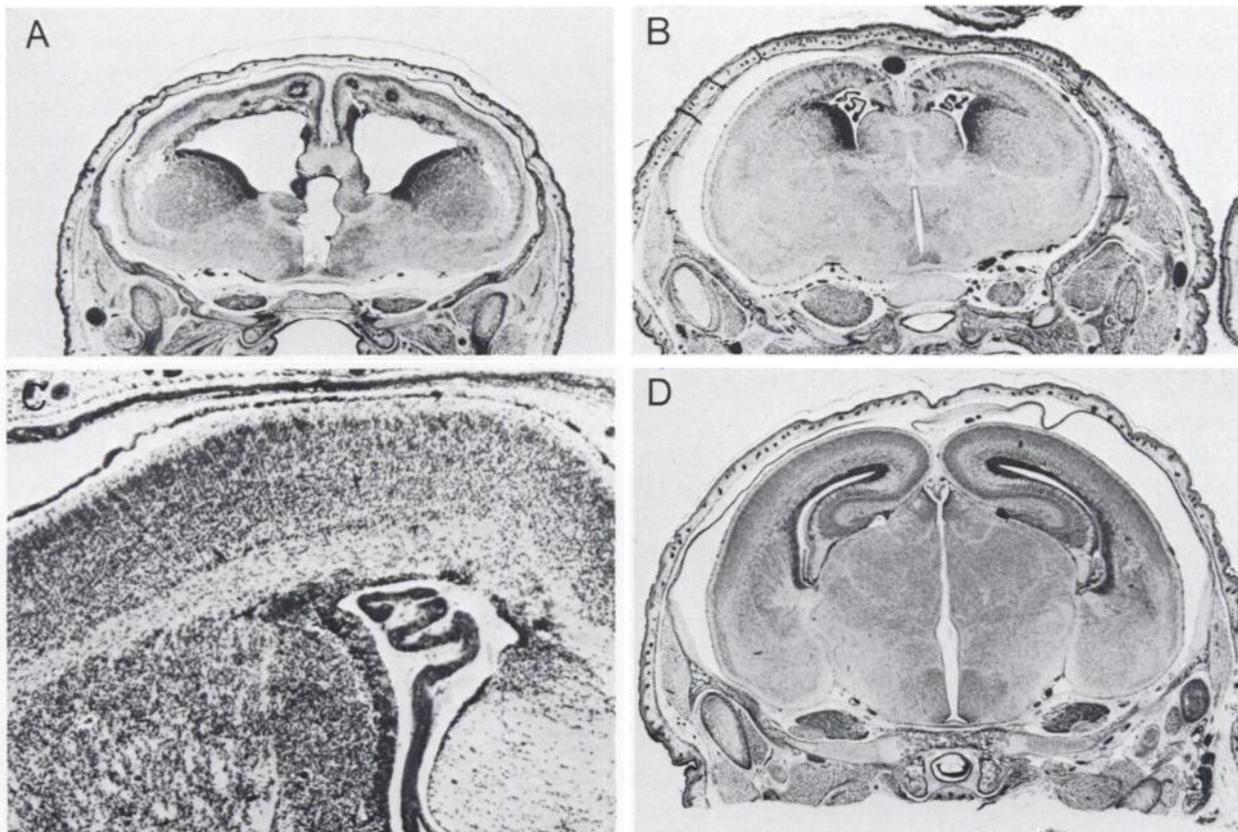
tion corresponded to the amount of initial tissue damage, extent of regeneration and stage of gestation when ionizing radiation was given. The details can be seen by comparing the data in column 4 and 5 of Table 3 in the corresponding gestational days.

A preliminary survey of the brain sections revealed that many of the observed lesions tended to group in a rather characteristic topographic distribution which changed depending on the gestational stage at the time irradiation took place. It was therefore decided to record the distribution of various types of lesions in the major brain subdivisions, noting also predominant involvement of the subependymal region, white matter and cortex. In general, the brain can be conveniently subdivided into the following functional systems: (1) neopallium (neocortex—all dorsolateral cortex above the rhinal fissure; underlying white matter including corpus callosum), (2) mesocortex (cingulate and retrosplenial region), (3) diencephalon and basal ganglia, (4) limbic system (hippocampal formation, entorhinal and prepyriform cortex, amygdala and septal

nuclei), (5) olfactory system (olfactory bulbs, tracts and tubercle; pyriform lobe) and (6) brainstem including cerebellum.

The last of the six subdivisions received little attention because the cerebellum begins to develop late in the fetal stage and its differentiation continues through the early postnatal life whereas for the purpose of this study the animals were sacrificed on the 19th day of gestation. Moreover, to properly assess brainstem lesions, it would have been necessary to include many intermediary stages between the day of irradiation and delivery at 19 days. Nevertheless, brief reference to early cerebellar lesions will be made.

Table 4 contains semiquantitative data on the severity and distribution of abnormalities in the main subdivisions of the brain after irradiation at various stages of pregnancy. In a small proportion of brains, irradiation up to the 9th day of gestation caused organ-type malformation but few focal lesions; these were located only in the diencephalon, limbic and olfactory systems.



**FIG. 2.** Photomicrographs in A, B and D are enlarged 15 times. C is enlarged 80 times. All mice were 19 g.d. A is mouse irradiated on 12th g.d. Heterotopic cell masses (rosettes) are predominantly located in white matter; moderate compensatory enlargement of ventricles and general smallness of brain occurs. B is mouse irradiated on 13th g.d. In parasagittal region small compact clumps of cells are present in white matter and in cere-

bral cortex; ventricles are only slightly enlarged but brain is generally small. C is mouse irradiated on 15th g.d. Note decrease of neural elements and disturbance of architectonics in parasagittal region. D is mouse irradiated on 17th g.d. Configuration of brain, size of ventricles and microscopic findings are within normal limits as assessed at birth (19 g.d.). Also brain is of expected size (compare with Fig. 1A).

TABLE 4. SEMIQUANTITATIVE DATA ON DISTRIBUTION OF LESIONS IN MOUSE BRAINS

Irradiated gest. days	Neopallium			Mesocortex			Dienceph. & Basal Gang.		Limbic System			Olfact. Syst.	
	Neocort.	Subcort. white M.	Subep. zone	Corp. call. absent.	Cort.	Subcort. white M.	Subep. zone	Dienceph.	Striatum pallidum caudate	Sept. amygd.	Entorh. cort.	Hipp. syst.	Bulbs, tracts, pyr. cort.
9	—	—	—	—	—	—	—	+	±	±	+	±	±
10	++	++	+++	+	+	+	+	+	++	+	+	++	+
11	+++	+++	++++	++	+	+	++	±	++	±	±	++	±
12	+++	+++	+++	+++	++	++	++	—	+	—	—	++	—
13	+++	+++	+	+++	++	+++	++	—	±	—	—	+	—
14	++	++	+	+	+	++	+	—	—	—	—	±	—
15	+	+	±	±	±	±	—	—	—	—	—	—	—
16	±	±	—	—	—	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	—

++++ Very severe abnormalities—more than ¾ of structure affected.  
 +++ Severe abnormalities—about ½ of structure affected.  
 ++ Moderate—about ⅓ of structure affected.  
 + Mild—about ¼ of structure affected.  
 ± Minimal—less than 1/10 of the structure affected.

Beginning with irradiation on the 10th g.d. the neopallium was the most severely affected part of the brain with the peak of abnormality occurring between the 10th and 13th g.d. The neopallial heterotopias were most frequent in the subependymal zone during 10 and 11 g.d. (Fig. 1C, D) whereas during 12 and 13 g.d. they were more prevalent in the white matter (Fig. 2A). Occasional dense nests of cells were present in the cortex of 13 and 14 g.d. fetuses (Fig. 2B). A marked decrease in the cell population of the neocortex was observed following irradiation between 11 and 13 g.d.; thereafter the cell deficiency in the cortex was progressively less noticeable. The peak incidence of the absence of the corpus callosum occurred following irradiation on 11 through 13 g.d.

In general, the mesocortex was affected the same way as the neocortex but possibly at slightly later stages of gestation. Irradiation lesions in the diencephalon were rather mild and their peak was during 9 and 10 g.d. The basal ganglia were affected with moderate frequency, and their sensitive stage extended through 10–12 g.d.

Of the limbic system, the hippocampus was most commonly involved, the abnormality being of moderate degree with a peak at 10–12 g.d. and then decreasing. Because of the natural tendency for cell grouping in amygdala and entorhinal cortex, the scoring of abnormality here was conservative. Genuine heterotopias in these structures were rare and usually following irradiation on the 10th g.d. The olfactory system was infrequently affected by the irradiation dose used. When the lesions were seen, they were confined to 9–10 g.d.

In cases of marked deformity of the entire brain approaching hydranencephaly, judgment regarding damage to specific structures was withheld. It has been repeatedly observed that cerebral abnormalities in the members of the same litter can vary to a marked degree. This has been true for the litter irradiated at all stages of gestation which led to malformation or other lesions. The variability of the organ response to ionizing radiation among the same litter was previously stressed by Russell (2) and Cowen and Geller (9).

The present material permitted only partial assessment of the cerebellar abnormalities caused by irradiation of the fetus. The abnormalities seen when animals were sacrificed on the 19th g.d. consisted of rudimentary or otherwise defective median portion of the cerebellum or cerebellar hypoplasia. These were observed in several animals following irradiation between 14 and 17 g.d.

Survey of brain sections 12 hr after irradiation of fetuses 13–15 days old showed scattered pycnotic cells among the migrating neuroblasts in the white matter and lower strata of the neocortex. The blood vessels were considerably dilated but otherwise intact.

#### DISCUSSION

Organ and tissue damage in animals exposed to ionizing radiation during fetal life has been previously reported (1,9,12,13). However, most of these experiments were conducted for a specific purpose and crucial parameters were variable. In this investigation a sufficiently large number of pregnant mice was used to permit the assessment of patho-

logical lesions in 12 groups of litters between 7 and 18 g.d. In this study, the gestational age at the time of irradiation was the main variable. Duration of gestation after irradiation was dependent on the main variable since all 12 groups of animals were delivered on the 19th day of pregnancy. The study was designed to provide as closely comparable data as possible to the available human material, especially for help in interpretation of the most common and serious condition: mental retardation associated with microcephaly resulting from irradiation of the pregnant mothers (8).

Observations of Russell (2) that a medium dose (100–300 R) of irradiation during earlier stages of gestation led to apparently normal newborn animals but a small size of litter were confirmed. The reason for this state is not clear but it is possible that the damaging effect of irradiation is sufficiently great for certain fetuses of the same litter to cause their death (in multiple pregnancies slight asynchrony of development is to be expected), whereas the others who survive the insult go on to develop normally. It may be recalled that the primitive streak stage in the mouse embryo occurs at 6½ g.d. (14); the neural plate appears at 7½ and the neural tube begins to form at 8 g.d. At 9½ days after conception the mouse brain is at one cerebral vesicle stage. Thus, organ formation in the mouse embryo does not commence until about the 8th gestational day and the primitive embryonic layers (still pluripotential) are probably more able to regenerate. Gross organ-type malformation of the brain without evident secondary lesions from the initial damage occurred when irradiation was given at 8 and 9 days of gestation. Here, the abnormalities included exencephaly, arrhinencephaly, grossly hypoplastic brain and hydrocephalus. Thus irradiation at this stage caused sufficient damage to some animals to cause fetal death (see Table 1), the others escaped without apparent abnormality of the brain, and a proportion suffered damage to the cephalic neural folds (8 days of gestation) or neural tube (9 days of gestation) which led to dysraphism or prevented formation of two cerebral hemispheres from the stage of a single prosencephalic vesicle. In a small proportion of brains, minimal abnormalities were also seen in the diencephalon and the primary olfactory area.

Irradiation between 10 and 13 days of gestation produced startling abnormalities. Here, predominant lesions, as seen at the time of birth, consisted of a greatly reduced amount of cerebral tissue with compensatory enlargement of the ventricles, presence of heterotopias and disorganization of architectonic patterns in various structures. As can be seen in

Table 2, columns 3, 4 and 5, the total incidence of 10–13 g.d. irradiated fetuses whose brains show such abnormalities is nearly 100%. At this gestational age the six main subdivisions of the brain were present—but in a primitive stage—and their further differentiation was in progress. The least advanced were neopallium, mesocortex and basal ganglia, whereas the brainstem, primary olfactory system and most of the hippocampal formation were considerably more developed. As recorded in the results (see also Table 4) the early differentiating structures such as the olfactory system, diencephalon, septum and entorhinal cortex showed greatest resistance to irradiation injury. The basal ganglia and hippocampus were moderately sensitive during 10–12 g.d. The neopallium and also mesocortex were the most sensitive structures to irradiation with the peak between 10 and 14 g.d. It is probable that greater susceptibility to irradiation damage of certain brain structures may depend (among others) on their long proliferative phase and faster cell divisions. The mechanism of production of heterotopias may be related to the disruption of the long fibrous elements which act as “pathfinders” for migrating neuroblasts to the cerebral cortex.

Comparison of gestational stages of the mouse and man can be made only with great approximation. The following is based on available studies of fetal brain development in man (15,16) and on our unpublished material on mice. The brain development of a 7-day-old mouse fetus is roughly comparable to the brain of a 3-week-old human fetus. The brain of an 8–9-day-old mouse fetus corresponds in its development to the human fetus brain of about 3–6 weeks of gestation. The brain development in a 10–13-day-old mouse fetus is comparable to the human fetal brain of about 7–16 weeks. The stage of brain differentiation in a 14–17-day-old mouse fetus corresponds roughly to the human fetus brain of 17–26 weeks of gestation. The brain of the mouse fetus from 17 to 20 gestation days approaches and finally reaches term; at this stage the neopallium and cerebellum of the mouse appear less advanced in development than the same structures in a full-term child.

Using informations obtained from the present experimental study in the mouse, a tentative prediction of the type of brain lesion in the human fetus following irradiation at various stages of gestation is offered. Since little is known about the relationship between the x-ray dose range and the resulting brain damage in the fetus of man, the predictions made in this paragraph refer to “a moderate to high dose.” The scant informations available (8) indicates that a dose in man defined in this way

would be over 200 R but its upper limit is difficult to estimate; perhaps it would be in the vicinity of 600–800 R. The high dose of total-body irradiation of the fetus must be contrasted with the maternal dose to a relatively small portion of total body. Because of the transplacental exchanges, the fetus is in a better survival situation than those exposed to ionizing radiation postnatally. Thus, if an irradiation dose defined in this way was given to a human fetus during the first 3 weeks or after 6 months of gestation, the probability that the child would be grossly mentally abnormal (if he survived) could be considered not very great. If irradiation of the human fetus occurred between 3 and 6 weeks of gestation, a considerable proportion of the children born would be expected to have severe organ-type cerebral malformations. These would include various degrees of dysraphism, single ventricle brain, microencephaly and hydrocephalus. Irradiation of the human fetus between 7 and 16 weeks of gestation would probably lead to severe cerebral lesions in all exposed children; the lesions would be of the secondary type such as heterotopias, deficient and deformed cerebral structures, cell depletion and compensatory dilatation of the ventricles. On the whole, the amount of nervous tissue would be reduced, and the brain would be small. The extent of abnormality would be greater between 8–12 weeks of gestation than between 13 and 16 weeks. Irradiation of the human fetus between 17 and 24 weeks of gestation could be expected to produce relatively mild cerebral abnormalities involving predominantly the neopallium. Such abnormalities would probably include slight depletion of the neural elements and disturbance of cortical architecture. In 17–20 weeks of gestation possibly small heterotopic cell nests may also be present in the subcortical white matter. At this stage the cerebral ventricles would be expected to be normal or only slightly dilated. The cerebellar abnormality, however, could be still considerable owing to the late differentiation of this organ.

It must be admitted that very little human pathological material is available to validate these assumptions. However, a few interesting examples can be cited. The child described by Johnson (17) received over 400 R at 5–6 weeks of gestation and died at 1 year of age. The pathological findings consisted of hydrocephalus from aqueductal stenosis and deficient frontal lobes. Glass (18) reported postmortem findings in a child whose mother received a therapeutic abortion dose to her pelvis at the 3rd and subsequent months of pregnancy. The main findings were stunted stature and hypoplasia of the brain with “warty” and small cerebellar folia. The patient of Hardouin (19) received an enormous irradiation

dose (about 22,000 R were delivered to the lower abdomen and pubic region of the mother) at 6 months of gestation. The infant died 24 hr after birth; the autopsy findings consisted of anemia, generalized hemorrhages and atrophy of the lymphoid tissue. The patient reported by Dekaban (8) received over 900 R at about 3 months of gestation. The main findings included hypoplasia of the cerebellar vermis as evidenced by pneumoencephalogram, microcephaly, stunted growth and mental retardation. The findings in these four cases seem to fit fairly well in the predicted categories of cerebral lesions, which were anticipated from the study of brain abnormalities in irradiated mouse fetuses at various stages of gestation.

#### SUMMARY

A total of 152 female mice were irradiated on consecutive days of pregnancy between the 7th and 18th gestational day (g.d.). This resulted in 876 irradiated newborns. Exposure to irradiation on the 7th and 8th g.d. led to death and absorption of the majority of the fetuses; however, those which survived were largely normal at birth and only a small proportion had organ-type brain abnormality. Irradiation on 9 g.d. produced an increased number of cerebral abnormalities which included dysraphism, hydrocephalus, microencephaly and arrhinencephaly. Following irradiation between 10 and 12 g.d. entirely different cerebral lesions were found to occur; they included heterotopias, deformities of various structures, cellular deficiency and compensatory dilatation of the ventricles. From the 13th g.d. onward these lesions became smaller and less frequent, and after the 15th g.d. only mild architectonic imperfections were present. The heterotopias were most prominent in the subependymal region when irradiation occurred on the 10th and 11th g.d., in the white matter after exposure on the 12th g.d. and in the cerebral cortex following irradiation on the 13th and 14th g.d.

Different functional regions (subdivisions) of the brain showed a varied degree of sensitivity to irradiation in general and especially in different gestational stages. Diencephalon, olfactory system and a part of the limbic system had a low sensitivity in the range of irradiation used; the hippocampus and basal ganglia were moderately sensitive between 10 and 12 g.d. The neopallium and mesocortex were the most sensitive structures and severe damage was caused by irradiation between 10 and 13 g.d. while milder abnormalities occurred after exposure between 14 and 16 g.d. Most of the mice with cerebral lesions had small heads and their bodies were stunted. Tentative correlation of the stages of brain development

in the mouse and man has been offered. Also a prediction of the type of cerebral lesions that may result from irradiation of the human fetus at various stages of pregnancy is suggested.

## ACKNOWLEDGMENT

The author wishes to express his appreciation to Miss Marie Kendall for her efficient assistance in preparation of animals and carrying out histological procedures and to R. W. Swain for irradiation of the experimental animals.

## REFERENCES

1. KAVEN, A.: Röntgenmodifikationen bei Mäusen. *Ztschr. f. menschl. Vererb.-u. Konstitutionslehre* **22**:238, 1938.
2. RUSSELL, L. B.: X-ray induced developmental abnormalities in the mouse and their use in the analysis of embryological patterns. I. External and gross visceral changes. *J. Exp. Zool.* **114**:545, 1950.
3. HICKS, S. P.: Acute necrosis and malformations of developing mammalian brain caused by x-ray. *Proc. Soc. Exp. Biol. Med.* **75**:485, 1950.
4. KRIEDEL, H., LANGENDORFF, H. AND SHIBATA, K.: Die Beeinflussung der Embryonalentwicklung bei der Maus nach einer Röntgenbestrahlung. *Strahlentherapie* **119**:349, 1962.
5. MURPHY, D. P.: The outcome of 625 pregnancies in women subjected to pelvic radium or roentgen irradiation. *Am. J. Obstet. Gynecol.* **18**:179, 1929.
6. GOLDSTEIN, L.: Radiogenic microcephaly—a survey of nineteen recorded cases, with special reference to ophthalmic defects. *Arch. Neurol. Psychiat.* **24**:102, 1930.
7. PLUMMER, G.: Anomalies occurring in children exposed in utero to the atomic bomb in Hiroshima. *Pediatrics* **10**:687, 1952.
8. DEKABAN, A. S.: Abnormalities in children exposed to x-radiation during various stages of gestation; tentative timetable of radiation injury to the human fetus, Part 1. *J. Nucl. Med.* **9**:471, 1968.
9. COWEN, D. AND GELLER, L. M.: Long-term pathological effects of prenatal x-irradiation on the central nervous system of the rat. *J. Neuropathol. Exp. Neurol.* **19**:488, 1960.
10. D'AMATO, C. J. AND HICKS, S. P.: Effects of low levels of ionizing radiation on the developing cerebral cortex of the rat. *Neurology* **15**:1,104, 1965.
11. WERBOFF, J.: *Conference Proceedings: Prenatal Irradiation Effects on CNS Development and Postnatal Behavior*. The Jackson Laboratory, Bar Harbor, Maine, 1963.
12. ROIZIN, L., RUGH, R. AND KAUFMAN, M. D.: Neuro-pathologic investigations of the x-irradiated embryo rat brain. *J. Neuropath. Exp. Neurol.* **21**:219, 1962.
13. RUSSELL, L. B.: X-ray-induced developmental abnormalities in the mouse and their use in the analysis of embryological patterns. *J. Exp. Zool.* **131**:329, 1956.
14. SNELL, G. D.: *Biology of the Laboratory Mouse*. Dover Publications, New York, 1941.
15. HOCHSTETTER, F.: Beiträge zur Entwicklungsgeschichte des menschlichen Gehirns, Franz Deuticke, Wien und Leipzig, 1919.
16. BARTELMÉZ, G. W. AND DEKABAN, A. S.: The early development of the human brain. *Carn. Inst. Wash. Publ.* **621**, *Contribs. Embryol.* **37**:13, 1962.
17. JOHNSON, F. E.: Injury of the child by roentgen ray during pregnancy. *J. Pediat.* **13**:894, 1938.
18. GLASS, S. J.: Dwarfism associated with microcephalic idiocy and renal rickets. *J. Clin. Endocrinol. Metab.* **4**:47, 1944.
19. HARDOÛIN, M. D. AND BRAULT, M.: Tumeur sarcomateuse du bassin chez une secondipare de 29 ans; radiothérapie profonde; césarienne à sept mois et demi suivie de Porro; mort rapide de l'enfant avec graves lésions viscérales dues aux rayons X. *Bull. Soc. d'Obstet. et de Gynec.* **16**:105, 1927.

## STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION (Act of October 23, 1962; Section 4369, Title 39, United States Code).

1. Date of filing: October 1, 1968.
2. Title of publication: Journal of Nuclear Medicine.
3. Frequency of issue: Monthly.
4. Location of known office of publication (Street, city, county, state, zip code): 211 E. 43rd St., New York, N.Y. 10017.
5. Location of headquarters of general business offices of the publishers (not printers): 211 E. 43rd St., New York, N.Y. 10017.
6. Names and addresses of publisher, editor and managing editor: Publisher—The Society of Nuclear Medicine, 211 E. 43rd St., New York, N.Y. 10017. Editor—George Thoma, M.D., St. Louis Univ., 1504 S. Grand Blvd., St. Louis, Mo. 63104. Managing editor—Margaret Glos, 211 E. 43rd St., New York, N.Y. 10017.
7. Owner (if owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given): If owned by a partnership or other unincorporated firm, its name and address as well as that of each individual must be given: The Society of Nuclear Medicine, 211 E. 43rd St., New York, N.Y. The Journal of Nuclear Medicine is the official publication of the Society of Nuclear Medicine. The corporation is nonprofit and there are no stockholders.
8. Known bondholders, mortgagees and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages or other securities: None.
9. For completion by nonprofit organizations authorized to mail at special rates: The purpose, function and nonprofit status of this organization and the exempt status for Federal income tax purposes have not changed during the preceding 12 months.
10. Extent and nature of circulation. (A) total number of copies printed: average during preceding 12 months—5,425; actual number of copies printed in October 1968—5,750. (B) Paid circulation: None. Mail subscriptions: average number—5,265; actual number in October—5,499. (C) Total paid circulation: average number—5,265; actual number in October—5,499. (D) Free distribution: average number—91; actual number in October—91. (E) Total distribution: average number 5,356; actual number in October—5,590. (F) Office use, left-over, unaccounted, spoiled after printing: average number—69; actual number in October—160. (G) Total: average number—5,425; actual number in October—5,750.