Acute Necrosis and Malformation of Developing Mammalian Brain Caused by X-Ray.* (18240)

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The nervous system of the adult in contrast to that of the embryo is considered to be fairly resistant to ionizing radiation except in so far as it may be damaged secondarily by changes in its blood vessels(1). Lymphoid and hemopoietic tissues and cells in mitosis are usually classed as the most susceptible elements but there is little specific information concerning the vulnerability of developing mammalian nervous tissue. That neuroblasts might be radiosensitive has been indirectly suggested by the therapeutic effects on neurogenic embryomas such as neuroblastoma and medulloblastoma; also, it is widely believed that microcephaly and other failures of development of the human nervous system may follow irradiation of the embryo *in utero*(1). A more concrete form of evidence is given by the experiments of Rugh(2) who showed that the neuroblasts in developing lower animal forms such as Amblystoma, are the most sensitive cells to irradiation. The retinas in newborn mice and humans have been shown to be damaged by x-radiation(3), and gross malformations of skeletons of rats irradiated *in utero* have been observed by Russell(4).

In the course of an investigation of the pathologic effects of ionizing radiation upon the development of mammalian nervous system it was found that growing central nervous tissues in fetal rats and mice could be selectively and extensively destroyed by x-radiation with virtually no destructive effect on other organs. When the pregnant animal was given total body irradiation of 200-600 r her fetuses developed severe destruction of the developing nervous tissue in a few hours. After 150 r damage was less and fetuses allowed to come to term and grow up showed severe malformations of the brain.

Methods. Essentially the experiments consisted in administering total body radiation to pregnant white rats, C3H and C57 mice at varying stages of gestation. Usually this was done in the second and third week so that the fetuses were already fairly well developed. Irradiation was carried out at 35, 50, 75, 100, 150, 200, 400 and 600 r in single exposures. The fetuses were removed and sacrificed for pathologic study at intervals of 30 minutes, 2, 4, 24, 48 and 96 hours after irradiation, or they were allowed to go to term when they were studied at ages of one day to several months. To date, about 20 litters have been obtained for study from animals irradiated at 100 r or below, 10 litters at 150 r, 4 litters at 200 r, 15 litters at 400 r, and one litter at 600 r. In addition several litters of rats were irradiated (total body) one to 4 days after birth at 100, 150 and 200 r and studied at varying intervals after the radiation. Fixation of the freshly sectioned tissues was done in Bouin's fluid, embedded in paraffin, and examination chiefly with hematoxylin and eosin stains. Bodian protargol and phosphotungstic hematoxylin

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1. Warren, Shields, *Arch. Path.*, 1943, v35, 127; Goldstein, L., *Arch. Neurol. Psychiat.*, 1930, v24, 102; Hicks, S. P., and Warren, Shields, Introduction to Neuropathology. Chap. 1, 8. McGraw-Hill, 1950.

2. Rugh, R., Histologic effects on the embryo following X-irradiation. AECD-2596. Technical information division, Oak Ridge Extension, AEC, Oak Ridge, Tennessee. 10, 31, 49, 650-A9338.

3. Lorenz, E., and Dunn, T. B., *Arch. Ophthalmol.*, 1950, v43, 742; Goldstein, I., and Wexler, D., *Arch. Ophthalm.*, 1931, v5, 591.

4. Russell, L. B., *J. Exp. Zool.*, 1950, v114, 3.

stains were sometimes used. Fetuses were examined whole by interrupted serial frontal or lateral sections; older animals were completely autopsied and all organs examined histologically. The brain was studied in interrupted serial frontal sections.

Irradiation was delivered from General Electric Maximar machines at 200 Kv, 10 milliamperes and without added filter. The machines had an inherent filter of 3 mm of aluminum. The rats were irradiated in thin-walled flat cardboard containers, on a wood table. Mice were irradiated in a fiberboard flat cage with a copper screen top. The target distance was measured to the table, that is to the lowermost part of the animals which were obliged to lie flat by the narrow height of the respective containers. The target distance was 64 cm for rats and 44 cm for mice. The amount of radiation under these circumstances was estimated in air and checked at frequent intervals with a Victoreen condensor type ionization chamber placed in the empty containers. This measurement included the back-scatter from the containers and table. As checked with the Victoreen, variations of only a few r occurred from one experiment to another. The true error, as in any modern radiation technics, cannot be exactly measured, so that the figures 150 r, 400 r, etc., are approximations based on the Victoreen readings.

Results. Irradiation at 200, 400 and 600 r produced severe and often widespread destruction of the rapidly growing regions of the brain in all fetuses and usually of the retina, cord and some ganglia. This was complete in 24 hours, well developed in 6 hours, and clearly beginning in two hours after irradiation. *No difference in sensitivity between mouse and rat fetuses was apparent.* At 600 r the thymus showed severe necrosis, and there were rare foci of necrosis in other tissues as noted below. At 400 r there was rarely scant damage outside of the nervous system and at 200 r none was seen. At 150 r the brain and usually the cord and retina were damaged but to a lesser degree than at the higher doses. At 35 to 100 r necrosis did not occur except in slight degree in a single animal (100 r). In a given litter the neural lesions were remarkably uniform in pattern and such variations as were seen among different litters appeared to be related to the age of the fetuses; for example fetuses near term usually showed minimal cord lesions.

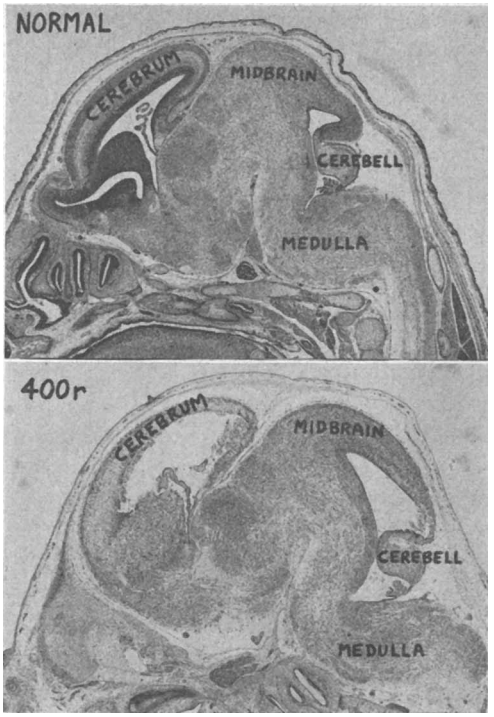

FIG. 1.
Acute radiation necrosis of fetal mouse brain. Above, lateral view of normal in third week of gestation, to show dark-stained zones of densely packed periventricular neuroblasts. Below, necrosis with edema of these zones 24 hr after 400r. (H & E, low power).

Description of the lesions may be conveniently put into arbitrary categories as they were studied: (1) the acute stage of necrosis during the first day following radiation, (2) proliferative and beginning malformative changes two to several days after radiation, (3) late maldevelopments seen in the postnatal period, and (4) extraneural lesions.

Acute stage of necrosis: The acute destructive lesions affected principally the periventricular masses of germinal cells and neuroblasts which are forming the striatal and thalamic masses during the latter two weeks of gestation. Similar regions of rapid growth

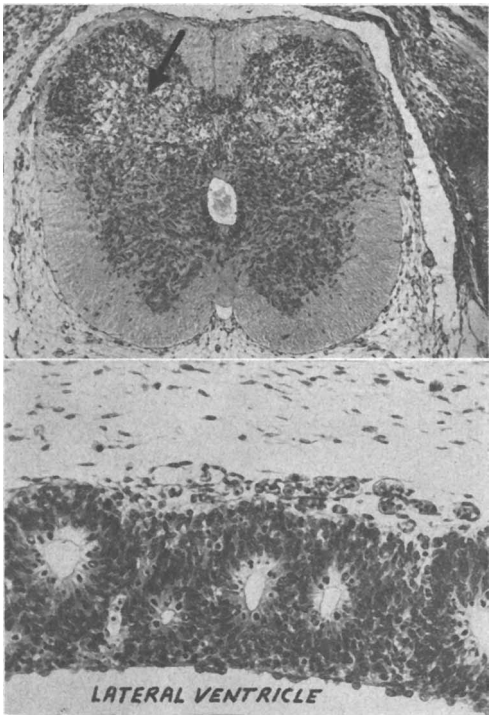

FIG. 2. Top.
Acute radiation necrosis of fetal mouse spinal cord, 24 hr after 400r. (H & E, ×45).

FIG. 3. Bottom.
Early malformation of neuroblast layer following radiation injury. Fetal rat cerebrum 48 hr after 150r, showing "rosette" proliferation of neuroblasts. Mitotic figures are abundant. (H & E, ×133).

were also severely affected in the middle cerebral cortical zones, olfactory lobes, the hippocampus, anterior hypothalamus, and dorsal midbrain just anterior to the cerebellum (Fig. 1). Scattered necrotic cells occurred also in the midbrain and medulla. In younger animals the dorsal gray columns of the spinal cord were damaged often severely (Fig. 2), while near term the cord was largely spared even at 400 r. In older fetuses and those irradiated just after birth where the cerebellum was developing rapidly the outermost cortical layer of neuroblasts was nearly completely destroyed. In a few instances necrosis of developing ganglia at the neuroblastic stage were seen. Mature cells of ganglia, cerebral cortex, cerebellum, etc., were unaffected. Ependymal cells were often spared but sometimes they disappeared with the liquefaction necrosis that was so severe in the subependymal periventricular zones. In the retina the neuroblastic layer was consistently damaged at 200 r and sometimes completely destroyed at 400 r. At 150 r there was usually damage but occasionally it was absent; thus the retina was slightly more radioresistant than the periventricular cerebral regions. In animals that happened to be born hours after irradiation and those irradiated soon after birth at 150 r or more, necrosis occurred in the periventricular subependymal forebrain zones where neuroblasts were still abundant. The outer cerebellar cortical layer was similarly damaged, the cord spared and retina moderately damaged. In the few early fetuses—probably 8 to 10 days old—necrosis of the germinal cells lining the primitive ventricles and "neural tube" was seen.

The earliest evidence of necrosis developed 2 hours after irradiation. There was eosinophilia of the cytoplasm and smudging of the chromatin pattern of those neuroblasts that were in mitosis. In addition there was a scattering of polymorphonuclear leukocytes in the damaged zones and slight capillary congestion. Scant patchy edema had also developed. In 4 to 6 hours necrosis of many of the cells was evident, and by 24 hours whole areas of liquefaction necrosis had developed in which floated cellular detritus and polymorphonuclear leukocytes. By this time there were also a fair number of large phagocytes containing cellular fragments, and sometimes red cells when minute hemorrhages occasionally had developed. The phagocytes seemed, in some instances, to originate from interstitial subependymal and vascular adventitial cells. Mitotic activity in surviving neuroblastic and germinal cells was not stopped. Choroid plexus was undamaged. The striatal white matter adjacent (dorsal and rostral) to the developing zone of neuroblasts was usually partially or completely necrotic. Whether this was death of fibers resulting secondarily from dead young neurons or due to a direct action on the fibers remains to be determined.

Stage of proliferation and early malformation: Animals from a number of litters were

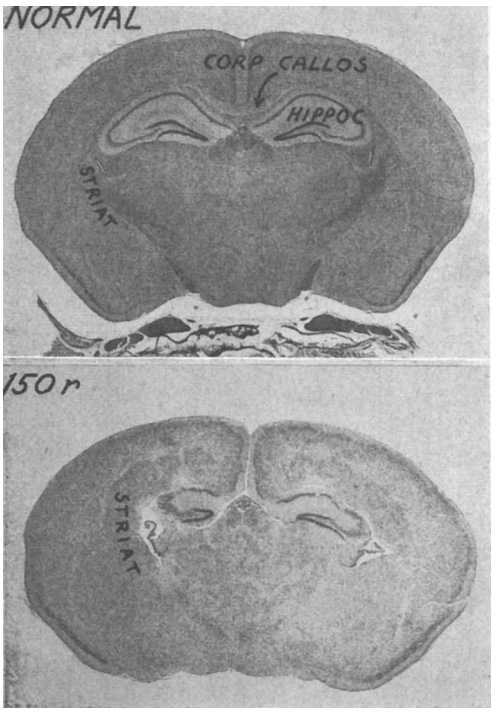

FIG. 1.
Late malformation of brain of mouse, about 3 weeks old, resulting from 150r irradiation about a week before birth. Above, brain of a comparable normal mouse, 3 weeks old. (H & E, low power).

studied 48 hours to several days after irradiation, especially at 150 r. In addition to the patchy necrosis, within 48 hours a bizarre proliferation of the remaining neuroblasts and germinal cells developed characterized by the formation of rosettes and small primitive ependymal canals (Fig. 3). This occurred in brain and cord, especially after 150 r, and was frequently the residual picture in the retina at 200 and 400 r. The pattern resembled that seen in some retinoblastomas and neuroepitheliomas.

Late maldevelopments: Animals from litters exposed *in utero* to 150 r and 200 r in the third week of gestation were found to have severe malformations of the brain when studied from a few days after birth up to the age of 3 weeks (Fig. 4 and 5). Despite their rather grotesque brains they were able to nurse, crawl, then eat, climb, run and bite, but their motor patterns seemed more jerky than normal controls. Detailed neurologic studies were not carried out. Also the animals were sometimes slightly smaller than comparable normal animals. Detailed studies of the skeleton were not made. The malformations seen thus far have been of the following general pattern, and need only be superficially described at present. The corpus callosum is virtually absent with an associated jumbling of the junction of the pyramidal layer of the hippocampus with the medial cerebral cortex. Spotty defects in the outer cerebral cortical neuron layers are seen. The hippocampus is small, its cells patchily jumbled. The striatum—caudate, putamen, globus pallidus and the incorporated bundles of fibers—is incompletely formed; the fiber bundles are nearly absent, the gray masses small and displaced upward. The ventricles are irregular, malformed and dilated. The white matter of the cerebellum is smaller in bulk than normal, and in animals irradiated just after birth the granular layer of the

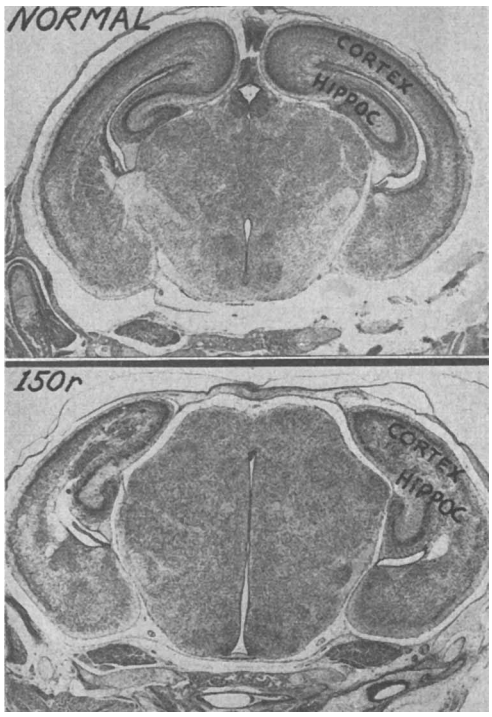

FIG. 5.
Late malformation of brain of mouse about 10 days old, resulting from 150r irradiation about a week before birth. Above, a comparable normal brain (H & E, low power.)

cerebellar folia is absent or reduced in volume. Retinal malformations (after 150 r) are limited to occasional "rosettes" in the rod cell layer. Details of midbrain and cord damage have not been worked out as yet.

Extraneural lesions: As noted, at 600 r a litter of fetuses showed thymus necrosis after 24 hours. In addition rare scattered foci of necrosis were found in the liver, kidney, adrenal and bone marrow. Osteoclasts showed pyknosis of nuclei and some cytoplasmic vacuolization. The blood-forming cells in the liver showed somewhat pyknotic nuclei but destruction was not evident. At 400 r, the thymus was unremarkable, rare foci of necrosis occurred in the marrow in some fetuses, osteoclastic changes appeared rarely as noted above, and in some younger animals necrosis of the outer layer of cells on the tips of the toes and the tip of the genital tubercle were occasionally seen. In one animal rare necrotic muscle fibers in the tongue were seen. After 200 r or less, extraneural lesions were absent.

Discussion. The reasons for the acute radionecrosis in the fetal central nervous system are obscure, but the cells in the areas affected are not only rapidly multiplying but also rapidly growing and differentiating. Protein synthesis is especially active in developing nerve cells(5). It remains to be determined how much of this susceptibility may be influenced by the type of metabolism obtaining at this period of development(6).

In regard to the malformations of the brain evident after birth the important fact is that they occur, and that the developing brain is by far the most radiosensitive tissue in the rat and mouse fetus in the latter half of gestation. At this early stage of the study descriptions of the malformations must be superficial and limited. Additional experiments with radiation given at more definitely known periods of gestation should make the malformations more susceptible to analysis in terms of stages of embryonic development.

The results of these experiments may provide useful tools for the histochemical and biochemical studies of the nature of radiation reaction and neuropathologic developmental anomalies. Investigation of the first and third of these is in progress.

Summary. Irradiation of pregnant rats and mice with 200 to 600 r to the whole body resulted in acute necrosis of the rapidly growing parts of the brain, spinal cord and retinas of their fetuses. This developed within a few hours, was highly selective for the central nervous system, and was often severe and extensive at 200 to 600 r. At 150 r damage was less, and was followed in two days by malformations in the neuroblastic zones. Fetuses allowed to go to term and sacrificed after days or weeks showed severe maldevelopments of corpus callosum, hippocampus, striatum and cerebral cortex. Extraneural lesions were absent below 200 r, rare at 400 r. Below 100 r no destructive lesions occurred.

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