THE EFFECTS OF RADIATION ON DEVELOPMENT
OF THE NERVOUS SYSTEM.*

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It has long been known that ionizing radiations can alter the development of living organisms but only recently have the mechanisms begun to be understood, especially as they relate to the nervous system. As a result of studies in several areas of biology including experimental mammalian neuroembryology these mechanisms are beginning to take shape. Radiation may modify vertebrate development in three ways. It may produce changes in the genes of the chromosomes of the reproductive cells, that is cause mutations. This is a "genetic effect." (2) It may alter the milieu of the embryo and disturb pathways of development that have already been initiated by normal chromosomes. The result may of these disturbances be a copy of what would have happened if the initiating gene were mutated. For such abnormalities Goldschmidt¹ coined the term phenocopy. (3) Radiation has a direct, highly selective, destructive ("extirpative") effect on cells of a certain stage of differentiation in the embryo, and the malformations that result are analogous to those that follow mechanical extirpations performed on embryos by experimental embryologists. These tend to reflect reaction patterns common to vertebrates rather than individual genetic differences among individuals. These three categories overlap but they provide a practical approach for medical and experimental purposes.

Genetic Effects: Mutations

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From the long range viewpoint, the genetic effects of radiation are the

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single most important aspect of radiation in relation to development, and this applies as much to the nervous system as the organism as a whole. We know that ionizing radiations produce changes in chromosomes in all living things that possess them. The changes may be thought of as alterations (mutations) in the chemical configuration of the desoxyribose nucleic acid (DNA) chains of the chromosomes that act as the templates or blueprints for development. These blueprints are the prime determinants of how the organism will develop in, and respond to, its environment and when they are mutated they may do a variety of things. They may have very little effect or it may be so extreme as to lead to a structural or biochemical deficiency that makes the early embryo incapable of further development. They may make a mammal so defective that it cannot survive extra-uterine existence. Some mutational abnormalities like anophthalmia (Fig. 1) are compatible with a sheltered existence like the laboratory. Many mutations, perhaps most, lead to what we call biologic variation. These are constantly being formed from "normal" background radiations and other agents. The great difficulty with applying radiation genetics to development of the nervous system, even in simpler animal forms, is that we cannot yet predict what mutations will occur, nor can we determine whether some patterns of development will be more common than others. We probably would not even all agree on whether some changes are abnormalities or whether they are biologic variants. It is known however that even minute amounts of radiation can alter DNA and that the alterations increase with the dosage. The best available evidence is that as we expose ourselves and other living things to greater amounts of radiation that we will increase for future generations the incidence of presently known abnormalities and variations, and undoubtedly create some new ones along the way.

Phenocopies

Development is the product of genetically determined ontogenetic pathways operating in an environment. "Normal" is the result of the gene configuration average for a species acting in the ordinary environment. Both factors can vary to cause biologic variation or frank abnormality and it is often hard to say that one or the other is solely responsible. It has been suggested that the sporadic abnormalities that can be produced by a great variety of teratogenic agents in the laboratory reflect mutations that usually are not expressed in the normal environment. Radiation in low doses, as little as 25 r of conventional x-rays in mice, may produce variations and abnormalities in embryonic development that are of this type.² The precise mechanism of this radiation effect is unknown and data on specific patterns of abnormal neural development are not available. It undoubtedly varies with the stage of development and the genetic constellation of the individual.

Extirpative Effects

The direct effects of radiation on the developing nervous system have been principally studied in rats and mice,^{3,4,5} and quantitative data are available.

Radiation in moderate doses (around 200 r of conventional x-rays) selectively destroys primitive non-mitotic differentiating precursors of spongioblasts and neuroblasts in the embryonic and fetal nervous system in a matter of hours. Destruction of cells at a similar stage of differentiation in the mesenchyme, somites and other organ anlagen at appropriate stages of development also occurs. The malformations that result are not simply the result of cell destruction but they depend on what critical differentiating and inductive processes are interfered with. In mammals the effects are

essentially comparable to what occur when analogous mechanical extirpations are made in simpler vertebrates by the experimental embryologist.

Larger doses destroy more members of the radiosensitive cell population, less radiation damages fewer cells, and malformations are correspondingly more or less severe. From the experimental viewpoint, 200 r is a useful selective extirpative tool for studying the growth of the nervous system as well as other organs. Four hundred roentgens destroys most of the non-mitotic primitive cells and begins to damage mitotic cells in the neurectoderm. Six hundred roentgens is extremely destructive to the embryo, and 1000 r is overwhelming. Below 40 r cellular damage is scant and malformations are sporadic instead of being uniform throughout a litter of animals as they are after 200 r. Such abnormalities may fall into the range of phenocopy effects, that is, subtle genetic differences among individuals may be revealed. The effects of the larger doses (200 r) reflect similarities of response to injury in vertebrate embryos.

Variation in sensitivity to radiation damage among various vertebrates is known. Although the non-mitotic differentiating embryonic cells can be selectively affected, some amphibians require much larger doses than mammals, and chick embryos seem to require more than mammals. Inbred strains of mice show some differences.

The patterns of malformations that result in rats from 200 r given to the gravid female at specific periods of gestation (checked by sampling and direct study of representative embryos removed by caesarian section four hours after radiation) are summarized in the timetable, Fig. 2. The inductive processes involving the formation of the forebrain, then the eyes and later certain viscera and skeletal structures are especially vulnerable and coincide

with damage to the corresponding mesenchyme. The malformations of the brain and spinal cord are extremely complex and result from a balance of specific factors. The stage of the proliferating germinal cells at the moment, the position of vulnerable migrating cells, and the local capacity to regenerate interact according to certain laws. Malformation or complete regeneration of a given injured part may be the result depending on these and other factors. The virtually complete restitution that occurs after the severe injury to rat embryos of 10 to 18 somites is comparable to that in simpler vertebrates after mechanical injury.

The pathological changes and the processes involved in the radiation malformations shown in the timetable may be illustrated by outlining some of the experimental work, based on several hundreds of litters of rats and mice and some other animals. A method much employed is multiple sampling of the same litters of embryos starting four hours after injury and continuing at successive intervals in gestation and into adult life. A really good estimate of the age of the embryos at the time of injury is obtained and the site of initial injury, and several stages of malformative process are seen.

In rat embryos (about 9 days) whose neural folds are just forming, and whose first two somites are not quite evident, 150 to 200 r produces a severe deformity of the forebrain, head, and face. This has been summarily designated "anencephaly." It is assumed by analogy with lower animals that inductive processes involving the mesoderm corresponding to the amphibian archenteron roof are primarily involved. A little later the embryo of some 4 somites can recover so much from the radiation damage that only degrees of anophthalmia result (Fig. 3). At both stages, destruction of the radiosensitive cells is relatively massive, but the deformity seems to be determined chiefly by what

inductive processes are going on, not by the number of cells destroyed. At 14 somites the inductive relationships between the optic cups and the overlying ectoderm and mesenchyme are undoubtedly affected by the frank destruction of the cells involved. The neural folds regenerate from the residual neurectoderm which is not destroyed by 200 r and a virtually normal brain, except for the anterior neuroptic apparatus, develops. Irradiation of embryos about 6 to 14 somites of age results in animals that grow up to have as their only defect smaller than normal eyes and even this is sometimes scarcely measurable.

Recovery is almost complete from the severe damage inflicted from 10 somites until the age of 18 somites is approached. The next malformative pattern that appears, in the neighborhood of 20 somites, is a defective outpocketing of the postero-medial part of the early cerebral vesicles where the choroid plexus will develop later, and the eyes again get into trouble. Eye defects ranging from moderate to complete anophthalmia with a rudimentary orbit result. The damage this time, in contrast to that of the earlier somite stage, is destruction of large numbers of cells in a very early lens and a retina already forming. The outpocketing of the choroid plexus is complicated and the deformity is compounded by a defective skull vault, a thin cerebral mantle, and sometimes general edema perhaps due to the malformed choroid. (The tail is short and urogenital imperfections are present. These seem to be related to damage to regional mesenchyme, for epithelial elements are spared.)

The restitution in the cerebral vesicles, brain stem, eyes and spinal cord is often considerable after radiation. In the retina after radiation on the 12th or 13th days (30 to 40 somites) the mitotic neurectoderm layer is preserved in contrast to the marked destruction of the primitive differentiating cells. Many cells in the lens are also destroyed. However, recovery is rapid in both structures from the remaining unaffected cells, and in the retina the

neurectodermal continuity is seldom disturbed enough to result in neurectodermal rosettes at this stage. Rosettes of neurectoderm are indicators that the integrity of the neurectoderm as a layer or "membrane" has been badly disturbed by the destruction of the adjacent differentiating cells which set as a tissue support. Evidence of rosettes, as such, following injury in the spinal cord and brain stem gradually lessens as the embryo grows, partly because their cellular constituents resolve into fairly normal positions as they mature into adult cells, and partly because their actual volume in embryonic life is so small that subsequent growth may dilute them. Evidence of them does persist into later development and adult life in some circumstances. This seems to occur when they are severe or when they develop in zones that are going to contribute large numbers of cells to local development or by migration to more remote regions. Irradiation at 40 somites results in enough rosette deformation that anomalous clusters of neural cells persist and mature as cell masses in subcortical regions while the outer cortex develops rather well. Although the source of migratory cells that form the cortex is disturbed, migration does occur and a cortex develops.

The details of how brain malformations come about in these circumstances are only beginning to be learned and for the later stages the results are known but the mechanics are still to be worked out. Of course there are correlations between damage to parts of the brain just forming and patterns of malformation that result, but they are not simply a matter of removing building blocks and producing simple defects. Development of the cerebral choroid can be disturbed as early as 20 somites and as late as 35 somites. Abnormalities of the layers of the cortex, the striatum, the hippocampus and parts of the cerebellum coincide generally with the appearance of differentiating

cell zones that are going to lead to these structures, but the modifying role of regeneration can be enormous.

Concluding Remarks

Ionizing radiations alter development (1) by mutation, (2) by disturbing developmental processes so as to copy mutations, and (3) by destroying primitive differentiating cells in the embryo. The latter effect is an extirpative one and the malformations that result are governed by the same principles of development that determine what happens in simpler vertebrates when parts of them are extirpated. Concerning (1) and (2) we still have very little quantitative data.

Most of what is known about the effects of radiation on neural development is based on work on mammals and simpler vertebrates. The general principals however are well enough established to make reasonable estimates about the mechanisms of the effects on man (Fig. 4).

- Fig. 1. Genetic anophthalmia in a mouse due to a pair of closely related recessive genes. The mutations may have originally resulted from radiation.
- Fig. 2. Timetable of Radiation Malformations produced in rats by 200 r.
- Fig. 3. Acquired anophthalmia in a rat (right) acquired during embryonic life (about 4 somites) as a result of direct radiation injury (200 r) to the earliest eye structures. This is not heritable.
- Fig. 4. Microcephaly in a child resulting from exposure to atomic radiation early in intra-uterine life. This is due to direct injury to the embryo and is not heritable. (By permission of the child's parent and the Atomic Bomb Casualty Commission).

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Fig. 1

MALFORMATIONS IN RATS CAUSED BY 200r

Fig. 2

FIG. 3

FIG. 4