tools which can be used not only to elucidate the ultimate mechanisms of drug action, but also to tell us more concerning the manner in which the eye puts itself together. In the case of avian embryos, techniques are already at hand for infusing water-soluble substances, either continuously or in a programmed fashion, into the extraembryonic vascular compartment.

E. There is need to study the developmental genetics of the eye. The chains of influence represented in the flow sheets of ocular development which are currently being drawn begin in several instances at the level of protein synthesis. An exciting, if formidable, task remains in linking what we understand of the morphogenetic chain of command with yet earlier events occurring at the level of gene activation and protein synthesis. What is desperately needed is an experimental vertebrate whose generation time is relatively short, whose embryos are available in large numbers and are readily accessible to experimental manipulation at all stages of development, and whose chromosomes are suitable for cytogenetic study.

X-rays and the monkey fetal retina

Roberts Rugh and Ludmilla Skaredoff

It is now well known that fetal neuroblasts are particularly radiosensitive. It is also known that such neuroblasts appear very early in development and in some animals may be found even after birth, particularly in the cerebellum. It is possible to x-ray the embryo or fetus of an experimental animal at any time and determine those organs or areas in which there are neuroblasts simply by noting the distribution of pyknotic nuclei in neural cells. Thus x-rays are useful in plotting the progress of nervous system development from early organogenesis through parturition.1-3

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In the human being it is known that neural development begins at about 18 days after conception and is particularly active for the following 3 to 4 weeks. For this reason radiologists have been cautioned to avoid diagnostic examination of the pelvis of female subjects of fertile age except during the first 9 or 10 days after the onset of menstruation, since an early pregnancy could go unnoticed. But any unnecessary radiation of a fetus during the first trimester is unwarranted and contraindicated because of the possibility of producing congenital anomalies of the central nervous system and the sense organs.

This study was made on three male monkeys, one a control and the other two x-rayed, in utero, at two different gestational ages and examined at various times during 23 months of postnatal life. Information on only three primates would seem to be inadequate until it is pointed out that such material is difficult to acquire, and, more important, the data conform in every way with prior studies on rodents.
Materials and methods

The materials consisted of three pregnant monkeys (*Macacus rhesus*) shipped directly from India, kept under surveillance for a week, palpated to determine the fact of pregnancy and probable age of the fetus, tested for tuberculosis, and then placed on a routine of food and care calculated to provide the best conditions for the completion of the pregnancy. Monkeys thus supplied are in various stages of pregnancy so that estimates of fetal age, based upon anal and abdominal palpation, can be only approximate. However, the age can be confirmed by the ultimate delivery date, since the monkey has a gestation period of approximately 150 to 160 days. In this experiment it was simply good fortune that provided three almost simultaneous pregnancies each involving a male monkey fetus.

The two experimental animals were anesthetized with subcutaneous injections of Nembutal (0.5 c.c. of veterinarians' Nembutal per kilogram of body weight) and when completely relaxed were placed under a single-tube x-ray source, limited by a lead cone 75 mm. in diameter. The x-rays were filtered through 0.28 mm. Cu and 0.050 mm. Al, the HVL was 0.6 mm. of Pb, and the factors were 184 KVP and 30 MA. The dose rate at the position of the fetus was 100 r per minute, but at a calculated depth of 3 cm, the air dose was attenuated to 77 r per minute. In order to equalize the exposure of the fetus, the gravid uterus was first x-rayed from the right and then from the left side; it received half of the aggregate exposure from each side. One monkey fetus (No. 40) received 300 r at 60 days' gestation, and the other (No. 38) received 200 r at 80 days; the control was No. 54.

The three monkeys for this study were born within a month of each other and during the next 23 months they were examined periodically for the effects of fetal x-radiation, including weight changes and skeletal growth (radiographs); at 8 months the control and one monkey (No. 40) which appeared to be the most adversely affected were tested with electroretinography. At the termination of the study (23 months), each monkey was killed; studies of organ weights and histopathology followed. This paper presents the effects on the eyes.

During their lives, the eyes of these monkeys were examined for the onset of cataractogenesis by a resident ophthalmologist and none was observed. The eyes appeared to be grossly normal except that those of No. 40, a male, which had been exposed to the higher dose at the earlier stage of gestation, appeared to be smaller than those of the control. The general behavior of the x-rayed monkeys was distinctly neurotic as compared with the control, but there was no behavioral evidence of severe loss in visual acuity.

At 8 months of age, and with the aid of those in the laboratory of Dr. Jerry Jacobson, electroretinograms were made of the control and of the most severely stunted of the x-rayed monkeys, No. 40. This monkey had been exposed as a fetus to 300 r at 60 days' gestation. The stimulus intensity for these retinograms was $2 \times 10^6$ FL. The interval of stimulation was once every 15 seconds, and the time constant of amplification was 1.0 second at 0.2 mv.

Observations and experimental data

The general histological, electroencephalographic, and electrocardiographic effects

![Fig. 1. Monkey brains at 23 months. No. 38, 200 r at 80 days' gestation. No. 40, 300 r at 60 days' gestation.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933618/)
of fetal x-radiation of these monkeys has
been reported in full by Rugh and co-
workers.5 It should be restated here, how-
ever, that the entire central nervous system
appeared to be affected in much the same
manner as was the retina of the irradiated
fetus. The brains at autopsy are shown in
Fig. 1; it can be seen that the control (in
the center) is the larger of the three
brains and that, while the two brains
from monkeys x-rayed in utero did not
react alike, they were both reduced in over-
all size. The brain to the left came from
monkey No. 38, which received the lower
exposure at the later gestational age, while
that to the right came from monkey No.
40, which received 300 r at 60 days' gesta-
tion. Thus, one can assume that probably
both the earlier gestational age and the
higher dose contributed to the greater
damaging effect on the brain from monkey
No. 40.

Electroretinography measures the func-
tional response of the retina to controlled
light stimulation, and must therefore be re-
lated to visual acuity, which is subjective
and cannot be measured in the monkey.
The ERG records (Fig. 2) show the con-
trol and the two eyes of the more severely
affected monkey, No. 40. It is obvious that
there was at 8 months considerable reduc-
tion in retinal function in this monkey.
The response was similar to that which one
would expect from the lowering of the
stimulation intensity, although the light
stimulation was identical for the control
No. 54 and the x-rayed monkey No. 40.

Volume measurements of the eyes of
these monkeys were not taken. However,
crude approximations of the relative sizes
are suggested by two-direction measure-
ments of the largest of the serial sections
from each of the monkeys. They are as fol-
lo ws:

Control monkey No. 54: 1.8 by 1.6 cm. =
2.88 cm.²
X-rayed monkey No. 38: 1.35 by 1.25 cm. =
1.69 cm.²
X-rayed monkey No. 40: 1.0 by 0.5 cm. =
0.5 cm.²

Fig. 2. Electroretinograms of monkey eyes at 8 months. A, No. 54, control. B, No. 40, left eye,
300 r at 60 days' gestation. C, No. 40, right eye, 300 r at 60 days' gestation.
Fig. 3. For legend see opposite page.
Likewise, measurements were taken of the thickest regions of the retinas:

Control monkey No. 54: 20 mm.
X-rayed monkey No. 38: 17 mm.
X-rayed monkey No. 40: 10 mm.

Thus these two sets of measurements do suggest graded damage, with monkey No. 40 showing the most severe effect in terms of volume and size reductions. But monkey No. 38, with an intermediate exposure at a later stage, also showed effects.

The histopathological analysis showed a perfectly normal and well-differentiated retina for the control. Monkey No. 38, which was x-rayed at 80 days to 200 r, did not show any persistent damage in its retina except for the reduction in the thickness of the nuclear layers. There were regions where it appeared that cells (or nuclei) had dropped out, leaving a small and clear space which might affect visual acuity even without correlating evidence in the ERG. Morphologically, it cannot be said that the retina of monkey No. 38 was entirely normal, but it was indeed reduced, and to determine visual acuity would require a subjective analysis which could not be made. In monkey No. 40, however, not only was there gross reduction in the size of the eye, the thickness of the retina, and the cellular constituents of the retina, but the ERG confirms that there were functional corollaries to the morphological effects (Fig. 3).

Illustrative evidence of permanent damage to the retina from fetal x-radiation is presented. It is seen that at 60 days (monkey No. 40) the posterior regions of the retina have accomplished their differentiation into neurons, but that the progression of this differentiation had reached only the midlateral levels. Here one can find abundant and extensive areas involving rosettes, even among the residual cells which are much reduced. More anteriorly (toward the...
ora serrata), the neural ectoderm does not seem to be similarly affected, and yet it is deficient in cellular material. Thus it appears that the neuroblasts at 60 days are most abundant midway between the optic disc and the ora serrata and that by 80 days an exposure of 200 r is too late to cause rosette formation, because neuroblast differentiation in the retina had been accomplished. Since at 80 days no rosettes were formed anywhere in the retina, it must be presumed that differentiation had progressed throughout the retina and that the differentiation in the more anterior portions of the retina occurred between the sixtieth and eighty-eighth days of gestation.

It is possible to demonstrate this in a composite drawing from another monkey which, as a fetus, was x-rayed at a time comparable to the 60 day gestation animal No. 40 (see Fig. 4). The combination of a drawing of the entire section of the eye (enucleated) with actual photographs of the various levels of the retina to show the cytological detail illustrates the fact that neurogenesis occurs in a wave moving from the region of the optic disc toward the ora serrata. In this combination illustration it is apparent that the posterior limit of the retina had achieved complete differentiation into neural retina, while the midlateral region was in the process of differentiation (with hypersensitive neuroblasts), and the more anterior region of the retina had not yet begun the process of differentiation. The greatest disorganization of the retina occurred in the midlateral region, although more anteriorly there was some evidence of spotty degeneration of neuroblasts. Only at the region of the optic disc was the retina fully constructed, with the nuclear layers in proper relation to each other and fully represented. On the basis of this sort of illustration one could say that the differentiated neural retina is radioresistant, the undifferentiated neurectoderm more anteriorly is sensitive only in scattered and spotty regions, and the lateral retina was almost completely disorganized because it was made up of neuroblasts in the process of differentiation.

The lowest x-ray exposure represented in this report is 195 r, and certainly this is far above the threshold level for any effect on the retina. Nevertheless this level of exposure, and the higher level of 300 r, demonstrated that neurogenesis in the x-rayed eye clearly illustrates the wide range of neural radiosensitivity. It has been estimated that 25 r may be the threshold of effect on neuroblasts, and the extent of damage from 195 r shown here would tend to support this probability even for the monkey's retina. The other aspect of this study is that radiation occurred some 26 months prior to this examination, some 23 months after birth, and yet there was no evidence of repair or recovery of the damaged retina, from either the functional or the cytomorphological studies. The rosettes do suggest attempts at repair, but the fact

Fig. 4. This plate is a composite of a drawing of the retina of the monkey fetal eye at about 60 days' gestation, showing the effects of x-radiation with 195 r at this time. Superimposed on the drawing are a series of six photographs from the specimen to illustrate the actual damage to the nuclear layers at different levels of the retina. It is demonstrated that the most posterior region is undamaged because it has already achieved differentiation, whereas the most anterior level, near the ora serrata, is also undamaged—but in this case because differentiation has not yet begun. These are neurectoderm cells and nuclei. In the intermediate zone, lateral retina, there is extensive and severe damage to the neuroblasts. Such a composite of diagram and photograph demonstrates that there is a wave of radiosensitivity paralleling the areas of active differentiation from the optic disc toward the ora serrata. Thus it is of great significance with regard to the stage of differentiation which will determine those regions of the retina which will be radiosensitive. (From Rugh and Skaredoff: Arch. Ophth. 74:382, 1965.)
that they too remain for 23 months suggests that they must be permanent. Therefore, radiation damage to the developing eye depends upon its progress through differentiation, and is probably permanent. This permanent damage is expressed in deficiencies in the nuclear layers and in the rosettes which must affect visual acuity.

Discussion

The earliest studies of the effect of ionizing radiations on the developing retina go back to 1937, 1938, and 1944, in which studies it was shown that the mitotic and premitotic cells were particularly radiosensitive. The word "recovery" was used, but even then it was not used to mean replacement but simply reorganization of the residual cells in an attempt to form a normal retina. There still is no evidence of recovery from radiation damage in the sense of proliferation and replacement of traumatized cells. Studies by Rugh and Wolff used lower and carefully controlled exposures to x-rays on both developing salamander larvae and the mouse fetus; they found that there was some ability to reconstitute the retina out of residual undamaged neurectoderm cells but that all irradiated retinas showed cytological deficits, particularly in the outer and inner nuclear layers. There appeared to be a rough correlation between the level of exposure to x-rays and the degree of irreparable damage to the retina of these developing vertebrates. These studies were later supported by Hicks on the fetal retina but not on the adult retina. Still later, Hicks and collaborators showed that ionizing radiations might be useful in studying neural differentiation. Others further confirmed the particular radiosensitivity of the developing mammalian retina. Neuroblasts are not easily identified or defined but, being highly radiosensitive, they can be located after exposure to ionizing radiations even at low levels.

In experimentally accessible retinas it is possible to demonstrate the tendency of excised neurectoderm to round up into rosette formation, whether the cells are in vitro or in vivo. This simply means that the radiologically damaged cells are eliminated by phagocytosis, leaving residual less damaged or entirely undamaged cells which attempt to deconstitute the retina. There is no evidence of any accelerated proliferation as a result of this radiation damage to certain cells, so that the retina is left with a deficit which expresses itself in these rosettes and areas of depleted nuclei. If an irradiated retina is examined as early as 24 hours after exposure of the embryo or fetus, numerous scavenging phagocytes can be identified as they devour these pyknotic cells and nuclei. When the radiation level is low, the resulting retina shows a lattice-type degeneration, with porous areas where destroyed cells have been eliminated but not replaced.

One of the major contributions of this current study is the fact that as long as 23 months after birth, or about 25 months after irradiation, the eyes show the persistence of the damaging effect of the x-rays in both the lattice-type degeneration and the rosette formation. The idea that neural tissue has powers of reconstitution, regeneration, replacement, or whatever it might be called, must be abandoned. Damage to neuroblasts in the embryo or fetus must invariably result in permanent deficits, in direct relation to the degree of exposure as well as to the time. Neither the neurectoderm, from which the neural retina develops, nor the fully differentiated retina are radiosensitive in the normal usage of that term. However, the neuroblasts which are the transitional stage between the primitive and embryonic neurectoderm and the formed neural retina are indeed so radiosensitive that it is believed that an exposure of as little as 25 r is lethal. This is a situation of tremendous importance in determining whether exposure of the human fetus at any time is without damaging effects, since neuroblasts are present throughout the body for the entire duration of fetal life and in fact for a short period after birth. Thus, it is now our
practice to consider 25 r as the outside limit of tolerance of radiation by the fetus during the second and third trimesters, but during the first trimester an exposure of 10 r should be considered borderline above which a therapeutic abortion should be advised. The human neural differentiation begins at about the eighteenth day after conception, and is very active during the next 3 or 4 weeks. It is during this early period of 6 to 7 weeks, during which the woman might not recognize her pregnancy, that even diagnostic levels of radiation could be severely teratogenic.

This study on monkeys does not reveal the threshold dose for cytological effects but it does reveal the progressive differentiation of the retina from the optic disc toward the ora serrata during the second of the 5 months of gestation. Retinal damage can be evaluated cytologically, but in this study such an evaluation was further supported by electroretinography. This method of determining the degree of retinal pathology in the monkey is not new—especially in focally irradiated eyes. But similar studies in man have been made after high-intensity flashes. Nagey and associates studied the gradual development of postnatal retinal function in the monkey by electroretinography. In our study we chose only the more severely damaged monkey and in this the disparity, when compared with the control, was almost in the extreme. The response was similar to what one would expect had the light stimulation been seriously reduced.

Summary and conclusions

1. Three male monkeys constituted the subject matter for this study, two of which were x-rayed in utero at two different gestational ages and at two levels of exposure. One was given 300 r at 60 days and the other 200 r at 80 days of gestation. All three were born within a month of each other and were studied at frequent intervals until they were put to death at 23 months of age.

2. This study dealt with effects of x-rays on the developing retina. In general, the response agreed with prior studies on all other vertebrates so that rodent findings are at least suggestive as to what one would find with primates.

3. It is obvious that there are tissue variations in response to ionizing radiations, and that the neuroblasts of the developing retina are particularly radiosensitive. It is further obvious from this study that the retina is not a unit, but that it exhibits a wave of differentiation from the optic disc toward the ora serrata. Thus the time at which the developing retina is exposed to the trauma of radiations will determine what part of the retina is damaged.

4. Structural damage to the differentiating retina (the neuroblasts) is irrevocable and irreparable; at autopsy at 23 months the eyes of the monkey x-rayed at 60 days showed the persistence of the damage in the form of severe nuclear deficits and rosette formation.

5. There is no evidence of proliferation of retinal cells or nuclei following x-radiation damage to the neuroblasts and their removal by phagocytes. Thus, when susceptible (differentiating) cells are traumatized by x-rays they are removed and the remaining cells (if any) attempt to reconstitute the retina. Obviously, this leaves a retina deficient in cellular requisites.

6. The visual function of the monkey eye is quantitatively reduced by fetal x-radiation at 60 days' gestation as indicated by electroretinographic recordings. One cannot evaluate the reduction in visual acuity on the basis of the cytological changes, nor on behavior alone. However, the ERG records probably are a more accurate measure of the visual decrement of each eye.

7. Neuroblasts appear to be particularly radiosensitive, and there is evidence that even cortical neuroblasts are similarly affected by x-rays. Neuroblasts are present in the human embryo and fetus from about day 18 not only through parturition but for several weeks thereafter; hence it is strongly recommended that in cases where the
human embryo or fetus is exposed to ionizing radiations in excess of 10 r during the first trimester there be a therapeutic abortion. When such an abortion is carried out it is a simple matter to section the eyes and to determine directly that neuroblasts were affected. The threshold dose for this effect has not been determined, but on the basis of rodent and other experiments it is believed to be somewhere between 10 and 25 r.

REFERENCES