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**Response of the Nervous System
to Ionizing Radiation**

Response of the Nervous System to Ionizing Radiation

Proceedings of an International Symposium
held at Northwestern University Medical School
Chicago, Illinois, September 7-9, 1960

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FOREWORD

The assembly of groups of people of divergent views for the purpose of educating each other is a goal which is not too often attained. However, these directions were the ones given to the Symposium and program chairmen by the committee that had decided it was time to look into effects of ionizing radiations on the nervous system.

This was a new approach, because many had said that the nervous system was insensitive to radiation, but the undercurrent arriving from many laboratories indicated that the statement was only partly true. If we wished to understand the radiation syndrome itself it would be necessary to consider the nervous system in our over-all outlook. Upon this basis it was decided that a beginning should be made by reviewing neonatal aspects, histopathological effects, ablation of specific central nervous system areas by particulate irradiation, evaluation of functional changes, and last but not least, the psychological effects of irradiation on animal performance.

To further these goals, investigators from many parts of the world joined with their colleagues in the United States to present the material contained in the following pages. We do not believe that all available information on the subject is contained herein, but we have strived honestly for a beginning in order that investigators will not only know what has been done and is being done, but also what the future may cause to have done.

This symposium was made possible by research grants to Northwestern University from the Institute of Neurology and Blindness, National Institutes of Health, Bethesda, Maryland, and the U. S. Atomic Energy Commission, Washington, D. C. Special thanks go to the Neurology Study Section of National Institutes of Health for the necessary services it rendered.

We hope that this modest beginning will inspire others to assist all to a better understanding of the various effects produced by irradiation of the nervous system.

THOMAS J. HALEY
Program Chairman
RAY S. SNIDER
Symposium Chairman

PREFACE

This is a meeting of two groups of minds, the basic neurologists and the basic radiobiologists. It is the first meeting of its kind and, if successful, we hope there will be subsequent meetings. It should not be necessary to point out that this select group has a double responsibility, that of pointing up not only what we know, but, equally important, what we don't know about radiation effects on the nervous system. This is a long neglected field, and we are all students with much to learn. On some of the points there is enough information for general agreement; on other points, general agreement is impossible. Perhaps the frustrated feelings will be so annoying that you will go back to your laboratories, design better experiments, and come to the next symposium with even better scientific papers.

The orientation of this meeting is the result of months of planning. There are five major topics of discussion. Each topic is being handled by a chairman, who is a specialist in the field, and is being introduced by a general survey speaker, who will cover much of the literature. The scientific papers are followed by discussion of the subject, which then is summarized by the individual chairman. The physical aspects of radiation and clinical studies will be discussed in a subsequent meeting.

Our present task is a noble one, *i.e.*, a mental cross-pollination of neurologists and radiobiologists interested in basic mechanisms. So without further comment, I welcome all of you and now ask you to capture your protons, your electrons, dendrons, axons, and neurons and orbit into new frontiers of learning.

RAY S. SNIDER
Symposium Chairman

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PART I

**Effects of Ionizing Radiation on the
Developing Nervous System**

INTRODUCTION TO PART I

The past 10 years and especially the last few have seen a great increase in interest in what ionizing radiation may do to the nervous system, both in respect to the malforming effects induced during early development and the functional and structural changes occurring in later stages. Formerly, it was said—and sometimes still is—that the embryo brain is very radiosensitive and the adult brain will stand almost anything. Unqualified, these phrases are almost meaningless today. We try to specify what *dose* of radiation produces a given effect, because one type of cell in the adult or embryonic nervous system may respond quite differently from another cell. It makes a difference whether one gives 200 r of conventional 250 kv x-rays to a fetal or neonatal rat in divided doses or in a single dose, or whether one gives a single dose of 700 r. Even the difference in effects between 200 r and 300 r on the fetal and neonatal cortex, cerebellum, and retina can sometimes be remarkable.

The term *radiosensitivity* now is used more carefully, because it has meant quite different things to different workers. We can no longer say, "this stage of embryonic life is the most radiosensitive" or "that enzyme system is the most radiosensitive" without qualification. To a geneticist, radiosensitivity means that a chromosome can be changed easily; to a pathologist, it has often meant that a tumor cell is easily killed; and to an endocrinologist, it may suggest that a cell's hormone production can be easily stopped. Many laboratories, including our own, are interested in discovering subtle radiation changes in neurons. For example, we are attempting to demonstrate changes in nucleic acids in adult rat cortical neurons by ultraviolet microscopy following exposure to 200 or 400 r of conventional 250-kv x-rays. I note this because it reflects the growing attitude that we ought to be looking for obscure and perhaps totally unexpected changes in the nervous system and other tissues following radiation. Certainly, among the most attractive areas for research are those which relate to the roles that DNA and RNA have in both the development and, later, the function of neurons.

A diverse array of approaches to problems of developmental radiobiology is represented in these papers on the effects of ionizing on the developing nervous system, including effects of successive small doses of radiation on neuron differentiation, chemical and histochemical changes in irradiated nerve cells of all ages, studies on nucleic acids of nerve cells

by the use of nucleic acid antimetabolites, and considerations of disturbances of function that radiation may cause. Development is not restricted to embryonic life, but continues through the life of the organism, and we will hear not only what radiation may do long before the embryo has a brain, but also what man-made radiation and radiation from outer space may be doing during adult life.

SAMUEL P. HICKS

Major Radiobiological Concepts and Effects of Ionizing Radiations on the Embryo and Fetus

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Introduction

As a radiobiologist, it is appropriate to initiate this symposium with a few general statements regarding the biological effects of ionizing radiations. We are concerned with ionizing radiations, not ultraviolet or infrared radiations. Ionizing radiations result in the excitation or loss of an electron from an atom, causing an unstable situation, whether it be in an atom of an inanimate or animate object. Such imbalance can be brought about by alpha or beta particles, by x-rays or gamma rays, or secondarily by neutrons. The ensuing physical imbalance results in a chemical change which, in turn, may effect a demonstrable biologic adjustment. While the ionization may be immediate or instantaneous, and is in most cases undetectable by the nervous system, the evidence of the biologic adjustment may take decades.

Radiations are so potent that the ionization of one molecule in 10,000,000 is sufficient to kill almost all organisms. It matters not whether the ionization is brought about by any of the different qualities of radiation, the basic biologic reactions are the same. However, the total dose absorbed and the dose rate are indeed important. There is a total absorbed dose which cannot be survived, and there is a dose rate so small that it can be tolerated. In all properly controlled radiobiologic experiments the ion density, the dose rate, and the total absorbed dose must be determined and recorded. In reporting radiobiologic experiments it should be made clear what specific organism, organ, tissue, or even cells are concerned, because tolerance is not uniform. This does not contradict the statement that the biologic reactions to ionizing radiations are basically the same. It does emphasize the fact that tolerance, restitution, and repair are properties which vary with the large variety of differentiated tissues. Organisms vary during their lifetime in their reaction to absorbed ionizing radiations, even as do cells during their process of dif-

ferentiation. The radiosensitivity of a single cell may vary 1,000 times from its undifferentiated to its differentiated state. The time has long since passed when data were adequately presented in terms of milligram hours of radium without consideration of the actual absorbed dose (rads), as well as the organ, tissue, or cell exposed.

There is a confusion of terms, such as *threshold*, *safe*, *permissible*, or *tolerable* doses of ionizing radiations. If by *threshold* one means an exposure below which nothing happens, it is very doubtful that such a level exists. A single ionization occurring at a critical point on a chromosome may not affect its bearer, but may have permanent and drastic effects on its progeny, which explains the extreme caution of the geneticists. At the level of the atom, any effect brought about by ionizations is probably all-or-none. It is another matter whether such an effect is *detectable* and, if detectable, whether it is *tolerable* to the biologic system. Certainly some changes in the central nervous system of the embryo or of the adult are both detectable and tolerable, if by tolerable we mean that the individual is able to survive.

At the cellular level the changes brought about by the absorption of ionizing radiations are irreversible, irrevocable, and irreparable, but they may still be tolerated by the cell. If the cell survives and is a germ cell, it may contribute a new mutant to its succeeding generations of cells, both detectable and tolerable, but not likely of any benefit. If the cell survives as a somatic cell, it may tolerate the damage and reproduce by mitosis for many years, ultimately to blossom out in two or three decades as a center of a malignancy. The ability to tolerate absorbed radiations is good for survival, but possibly not good for progenies.

The term *permissible dose* is used largely in Civil Defense directives and radiologic centers, and its dose level is generally somewhat lower than the *tolerable*. There is still another aspect of the tolerable dose, and this relates to the ability of the adult to repair or regenerate replacements for damaged tissue, usually scar tissue, or of the embryo to redirect its schedule and pattern of differentiation. The words have a wider meaning, therefore, and include reparative processes above the cellular level. A certain amount of radiation may be tolerated by an organism if the remaining tissue is sufficient for survival and can replace the void with protoplasmic mass. This ability is more evident in the embryo where undifferentiated cells are being directed and redirected after irradiation toward differentiation along the various routes which result in recognized tissues. Once this is completed, as in the adult, replacement of kind is not usual.

A corollary of this discussion is the matter of *cumulative effects*. Certainly one can see how a primitive germ cell, successively exposed to ionizing radiations, might well accumulate mutants at various points along its chromosomes. In the same way, a somatic cell may be bombarded successively by

ionizing radiations, and, as long as it survives, it too should accumulate effects until an intolerable composite of the damage would result in its death. In the somatic cell, exposure effects short of lethality may not be so graphically demonstrable as those produced by a mutated germ cell. Such a change in the somatic cell might never be detected. There is as yet no direct proof, but it may be conjectured that squamous cell carcinoma of the skin is more likely to follow repeated, accumulated, but tolerable exposures, than a single exposure. This presumption is based on the knowledge that squamous cell carcinoma, which can be produced by ionizing radiations, can result from tolerated exposures.

The human embryo or fetus, and to some extent the adult, have powers of sloughing off the undesirable or dead cells so that the only place that cumulative effects can be detected is in the progeny of surviving somatic and germ cells. This is why the low and tolerable exposures are so important. To kill is clear cut. To maim for the duration of life may be biologically tolerable, but psychologically and sociologically intolerable.

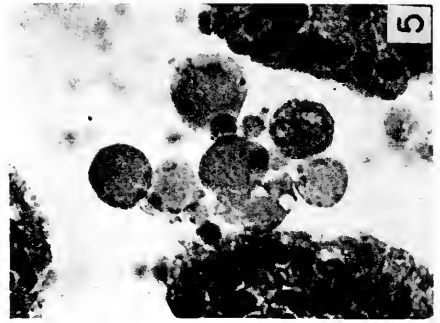
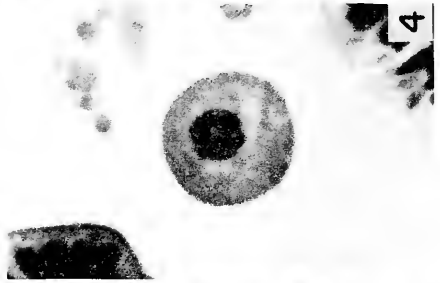
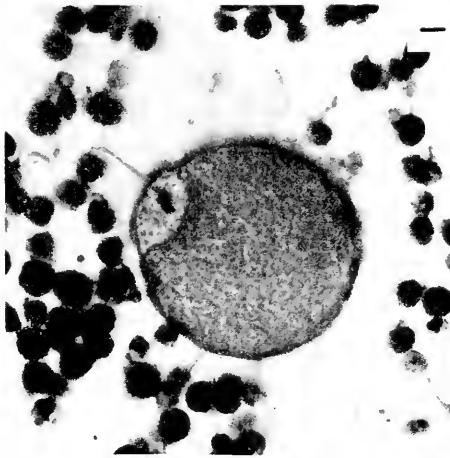
Finally, one must emphasize the difference between the somatic and the genetic effects. Since ionizing radiations can alter the central nervous system either through the germ cells or through direct irradiation, we are concerned with both genetic and somatic effects. Neither is apt to be immediate: both can be subtle and long delayed.

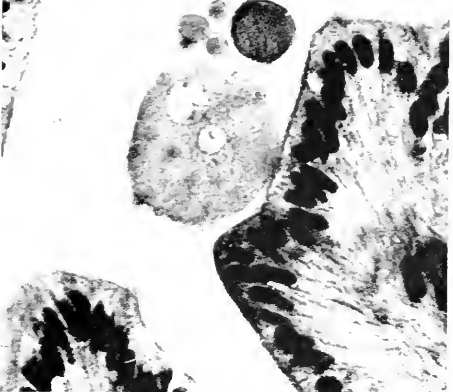
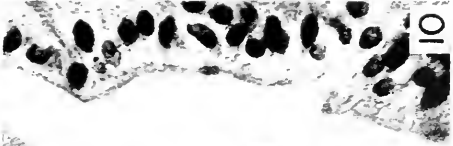
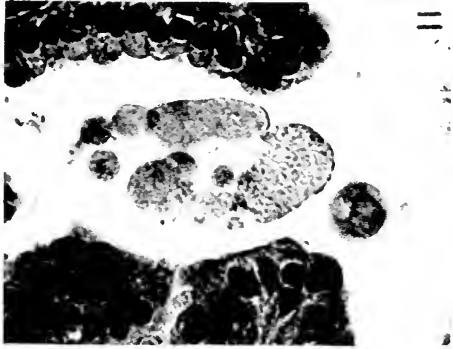
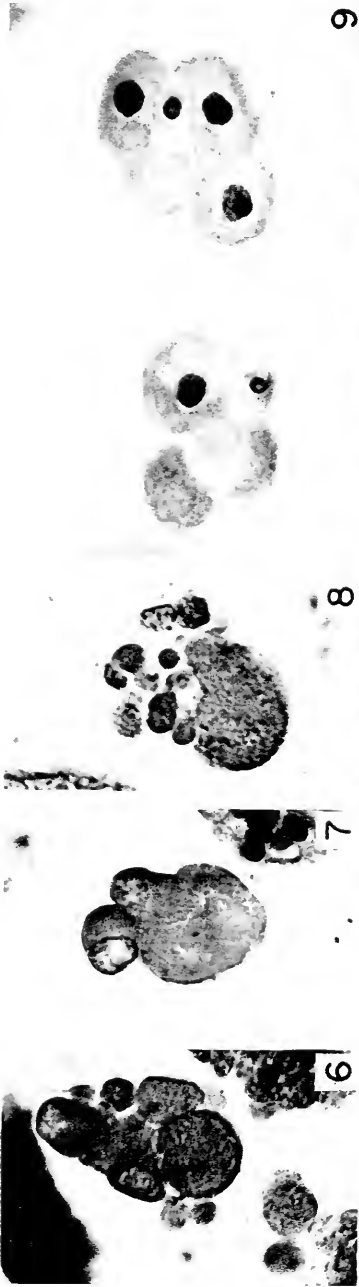
When the embryo or fetus is irradiated it must be realized that both the developing central nervous system and the developing gonads of the organism may have been exposed. Congenital effects resulting from direct irradiation of the developing organ primordia cannot have genetic corollaries except by coincidence, which is very unlikely. Congenital effects following direct irradiation cannot be inherited. However, concomitant with somatic exposure there may be germ cell exposure which may well result in different and possibly even more severe effects, but ones which cannot become apparent until they appear in a succeeding generation. Somatic exposures alone may alter the soma of one generation, but germ cell exposures may alter all succeeding progenies.

Effects on the Embryo or Fetus

In analyzing the embryonic effects one must have in mind four special situations:

1. The medium of the embryo is aquatic, and there is reason to believe that this enhances its radiosensitivity.
2. The embryo is a mosaic of actively differentiating centers with constantly changing but high mitotic indexes, both conditions enhancing radiosensitivity.





3. The embryo can be killed by irradiations which for any species are less than the lethal dose for the adult, and congenital effects may be produced by exposures of about 2% that of the LD/50/30 level (Figs. 1-27).

4. If the embryo survives the irradiation, it has powers of topographic repair which are not known to the adult. But it cannot step up cell production to replace cells lost from radiation necrosis, so that the net result is a deficient embryo or fetus—deficient in those cells or those tissues which are most damaged at the time of irradiation (Figs. 28-35).

Every one of its systems may be affected by exposure of the embryo or fetus to ionizing radiations, but the most obvious effects appear to be on the central nervous and the skeletal systems. Probably the most common and graphic effect, one most frequently reported from Hiroshima and Nagasaki, as well as in experimental radiobiology, is microcephaly. This is not an isolated condition, and all those so affected undoubtedly exhibit other anomalies. There is often stunting, microphthalmia, and loss or reduction of other parts indicating deficits (Figs. 21-35).

But anomalies designated as congenital may be caused by irradiation at times other than during differentiation. First, exposure of the sperm cell or its precursor may produce the anomaly in all succeeding generations due to chromosomal effects. Second, irradiation of the ovary may cause the anomaly to appear in successive generations. Third, the embryo is most likely to develop specific anomalies if the differentiating organ concerned is irradiated directly. Fourth, the embryo at any time from the moment of fertilization of the egg through the completion of organogenesis may be caused to develop the same type of anomaly. Once organogenesis is completed, congenital anomalies can no longer be caused by irradiation (Figs. 1-12 and 17-20).

Thus, congenital anomalies involving the central nervous system may be caused by irradiation of either germ cell, of the actively differentiating organism, or of any stage prior to this.

In our studies we have concentrated on the cerebral hernia or exencephalous condition where the midbrain protrudes through the cranial roof. This is a graphic and readily observable maldevelopment, and any fetus exhibiting this condition is presumed also to have other, possibly less graphic, but even more serious effects. Since this anomaly is readily observable, it has been a convenient marker of severe irradiation damage to the developing embryo (Fig. 21 and Table I).

Exencephalia has been produced by the irradiation of the mouse testis or ovary and has appeared in successive generations following a single exposure. True, its frequency is very low, but it is a severe and lethal anomaly which can be genetically produced. It has also been produced by exposing the mouse embryo at any time from fertilization through gestation day 8.5, and in the earlier stages with doses of as little as 15 r. Exposures of 5 r at certain

periods have increased the intrauterine mortality by 10%, so that in the early embryo we are probably dealing with the most radiosensitive stage in ontogeny. No exencephalies have appeared among thousands of unirradiated control embryos or in those irradiated after completion of organogenesis. It has been produced, however, by other traumatic conditions (Figs. 19, 20, 22, 24).

The term *low dose* should be defined here. Green (1959), the geneticist, says: "There is no totally safe dose of radiation," so that to him there is no

TABLE I

MALFORMATIONS AMONG NORMAL OFFSPRING OF $CF_1 \times CF_1$ MICE^a

<i>Offspring</i>	<i>Number</i>	<i>Per cent</i>
Pregnancies	61	—
Total embryos	630	—
Normal embryos	591	94
Dead embryos	2	0.3
Resorptions	37	5.7
Exencephalies	0	—

^a This table shows that among 630 unirradiated CF_1 mouse embryos, not a single exencephaly (brain hernia) developed. However, note that there were almost 6% resorptions and two dead embryos. This is an expected ratio and may be due to genetic causes. At no time in our experience, while examining thousands of mouse embryos, have we found the congenital anomaly of exencephaly among the control mice.

Figs. 1-12 are on pages 6 and 7.

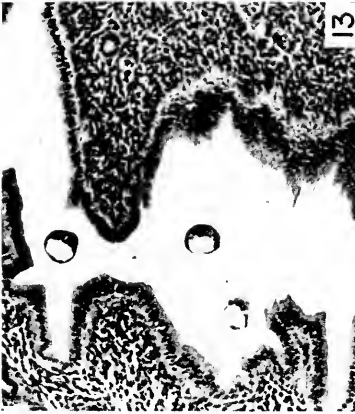
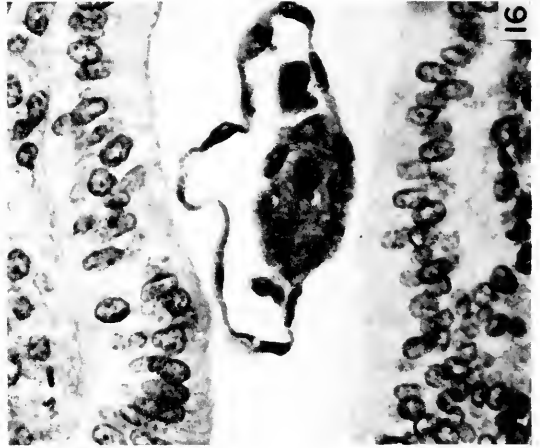
Figs. 1 and 2. These are normal mouse eggs seen during the first 24 hours after conception. FIG. 1 shows the egg at the moment of sperm entrance and FIG. 2 shows the two pro-nuclei and the first polar body. Highest percentage of resorption follows irradiation at this stage.

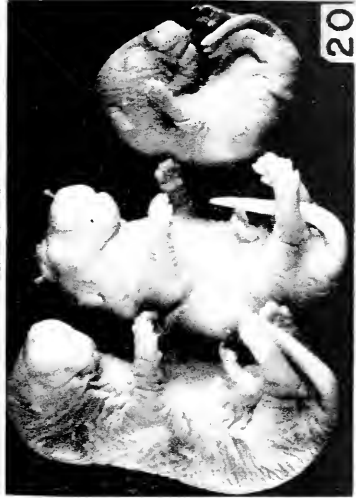
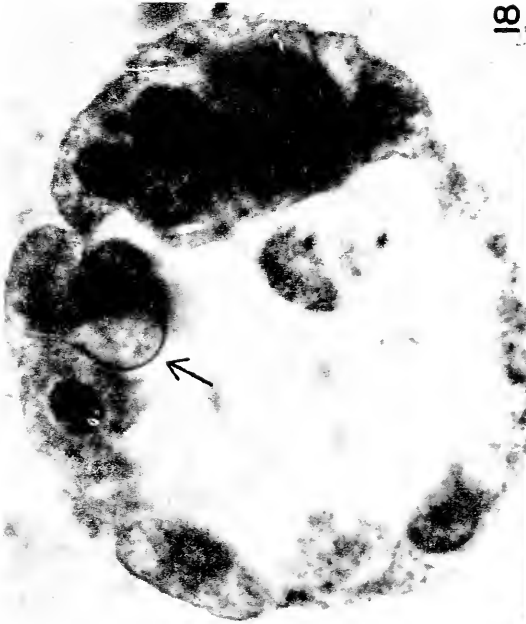
FIG. 3. This shows a group of normal mouse embryos at 1.5 days in the 2-cell stage. This is probably the most radiosensitive period with respect to the production of irradiation congenital anomalies.

FIG. 4. This is a mouse egg at 1.5 days which had received 50 r at 0.5 days and shows a hyperchromatic nucleus.

Figs. 5 to 8. These show various stages in the disintegration of the mouse embryos following exposure to 15 r at 1.5 days. Note that in some cases the pro-nuclei are being extruded from the cytoplasmic mass. It is unlikely that the residual cellular material could survive. However, the majority of eggs exposed at this time and to this level of irradiation would survive.

Figs. 9 to 12. These are all irradiated embryos exposed to 15 r at 1.5 days and examined at 2.5 days. Note pyknosis, hyperchromatism, and fragmentation of the embryos.





such thing as a low dose, every exposure is too high. In our times this may be impractical and unrealistic, though still a concept to be respected.

In Copenhagen, Dr. Hammer-Jacobson (1959) states: "Fetal doses of less than about 1 r are presumed to cause no noticeable injury. . . . Fetal doses between 1 r and about 10 r are assumed in some instances to cause injuries in the form of diseases, malformations, slow development, or reduced resistance, especially when the irradiation occurs between the 2nd and the 6th week. . . . If there are additional indications, therapeutic abortion should be assumed advisable. . . . Fetal doses above about 10 r are assumed to involve a rather great probability of fetal injury. In such cases induction of abortion should therefore be the general rule." (See Table II.)

In all of this, reference is made to the somatic effects, but certainly the geneticist would concur. A whole body exposure of as little as 12 r will cause some lymphopenia in the adult; and Brues (1959) refers to 25 r as a "low dose" for somatic effects, probably because there are always at least some hematologic changes. Thus, a dose of 5 r may not have measurable consequences for the somatic tissues of the adult, but would be seriously damaging to the embryo or to its progeny through effects on the gametes.

The embryo or fetus is not simply a miniature of the adult and must be regarded as a dynamic, tirelessly changing mosaic of differentiating areas all integrated into an over-all pattern under organismic influences which appear themselves to be immune to ionizing radiations. As long as there are undamaged building units for development which are adequate in number and basically intact, these influences will attempt to organize them into a topographically normal, balanced embryo. But the undamaged cells cannot replace those that were killed by irradiation. Any stimulus to excess cell production is cancerogenic, so that the embryo may be topographically well

Figs. 13-20 are on pages 10 and 11.

FIG. 13. This shows 3 normal mouse blastulae suspended within the uterus at 3.5 days.

FIG. 14. This shows the normal mouse embryos at the moment of implantation at 4.5 days.

FIGS. 15 and 16. Mouse embryos at 4.5 days (time of implantation), but following x-irradiation with 15 r at 1.5 days. They show pyknotic nuclei, discarded (necrotic) cells within the blastocoel, and failure at implantation. These embryos might survive to give rise to deficient fetuses.

FIG. 17. This embryo was likewise exposed to 15 r at 1.5 days and exhibits a giant cell with prominent chromosomes at 4.5 days. This is a common irradiation sequela.

FIG. 18. This is a mouse embryo treated as that of FIG. 17, showing a prominent cell with vacuolization. This is a frequent irradiation consequence.

FIGS. 19 and 20. These are members of litters dissected at 18.5 days showing stunting, anencephaly, and exencephaly, while other members of the litter appear superficially to be normal. When compared with controls they, too, are shown to be stunted.

TABLE II

ANOMALIES REPORTED FOLLOWING HUMAN FETAL X-IRRADIATION ^{a, b}

1. Microcephaly (most frequent)	16. Nystagmus
2. Hydrocephalus	17. Stillbirth increase
3. Poroncephaly	18. Decrease live birth weight
4. Mental deficiency	19. Neonatal and infant death increase
5. Mongolian	20. Ear abnormalities
6. Idiocy	21. Spina bifida
7. Head ossification defects	22. Cleft palate
8. Skull malformations	23. Deformed arms
9. Micromelia	24. Clubfeet
10. Microphthalmus	25. Hypophthalmism
11. Microcornea	26. Syndactyly
12. Coloboma	27. Hypermetropia
13. Strabismus	28. Amelogenesis
14. Cataract	29. Odontogenesis imperfecta
15. Chorioretinitis	30. Genital deformalities

^a This table lists thirty congenital anomalies found in humans following fetal x-irradiation. Note that the most frequent type of anomaly relates to the central nervous system. Most, if not all of these anomalies, have been produced in experimental animals by exposure during embryonic development.

^b It must be remembered that the levels of irradiation which are hazardous for the embryo or fetus are very much lower than those for the somatic tissues of the adult organism. It is, therefore, obvious that extreme caution should be exerted where either the reproductive organs or the developing embryo might be involved. We do not yet know the extent or the duration of radiation effects on the fetus or the germ cells.

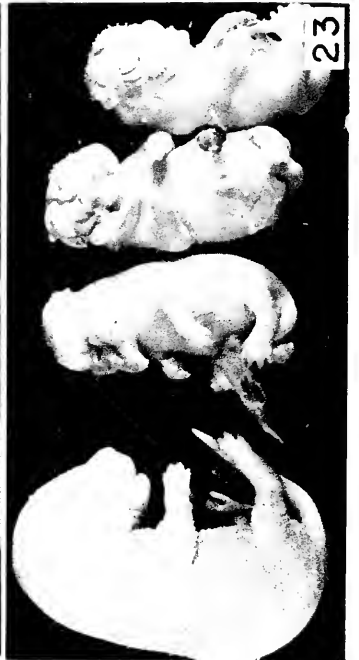
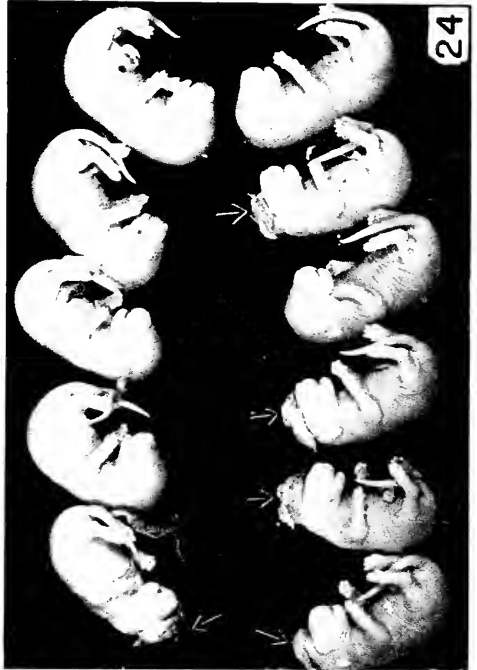
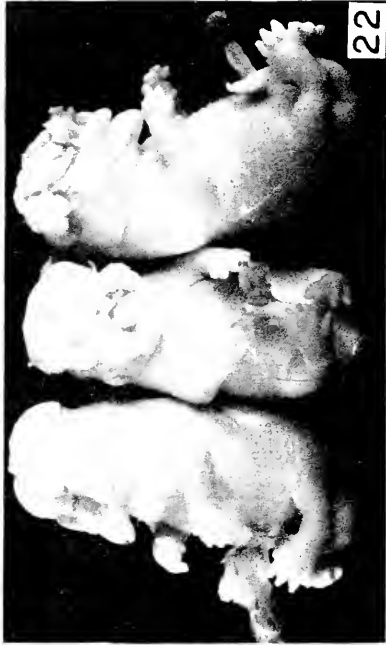
TABLE III

EFFECT OF LOW-DOSE X-RAYS ON THE EARLY
MOUSE EMBRYO ^{a, b}

	<i>Number of Embryos</i>	<i>Average litter</i>	<i>Normal (%)</i>	<i>Resorbed (%)</i>	<i>Dead (%)</i>	<i>Exencephaly (%)</i>
Controls	630	9.6	94.0	5.7	0.3	0.0
5 r at 1.5 day	80	10.0	85.0	15.0	0.0	0.0

^a This table presents data following 5 r exposure of the mouse embryo at 1½ days post conception. At this time, the mouse embryo is in the 2 cell stage. Eighty such embryos exposed to 5 r gave 15% resorptions which was almost a 10% increase over the expected 5.7% of the controls. No exencephaly appeared.

^b An increase of 9.3% in intrauterine deaths caused by 5 r exposure at 1-2 cell stage.



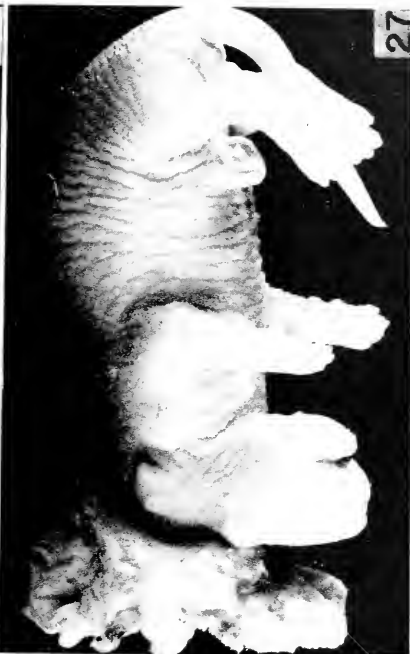


TABLE IV

X-IRRADIATION OF THE EARLY MOUSE EMBRYO ^a

<i>50 r x-rays at: Embryo age (days)</i>	<i>Total number implants</i>	<i>"Normal" (%)</i>	<i>Resorbed (%)</i>	<i>Exencephaly (%)</i>
0.5	52	58	42	0
1.5	90	95	5	0
2.5	95	73	24	3
3.5	76	88	9	3
4.5	53	92	8	0
5.5	37	77	17	6
6.5	77	92	8	0
7.5	25	96	0	4
8.5	51	96	0	4
9.5	12	90	10	0
	568	85.7	12.3	2.0

^a This table gives data from an extensive study of the effect of 50 r x-rays on the mouse embryo at various days from 0.5 to 9.5. In any somatic study 50 r would be considered a low level exposure, but from this study, when such an exposure kills 42% of the embryos at 0.5 days and large percentages at 2.5, 5.5, and 9.5 days, it is obvious that 50 r to the early developing mouse embryo is a high level of exposure. Of course, exencephalia was produced, the largest per cent being at 5.5 days.

FIGS. 21-27 are on pages 14 and 15.

FIG. 21. An enlarged view of exencephaly (brain hernia) in the mouse. This is a protrusion of the mesencephalon through the cranial roof.

FIG. 22. Three members constituting an entire litter, all showing severe exencephalic maldevelopment. This followed exposure of 50 r at 2.5 days.

FIG. 23. Note the same group of 3 congenital anomalies in a field including a normal control mouse fetus of the same age. This demonstrates that in addition to congenital anomalies there is often a stunting of the irradiated embryos.

FIG. 24. This shows an entire litter, as found in the bicornate uterus of the mouse at 18.5 days, following an exposure of 200 r at 8.5 days. Note that 5 of the 11 litter members exhibit exencephalia.

FIG. 25. This shows 4 members of a litter exposed to 50 r at 3.5 days. These are to be compared with a single control above. Note not only congenital anomalies but stunting of every member.

FIGS. 26 and 27. These mice were exposed to 50 r fractionated to 25 r each at two times during embryonic development, one exposure occurring before implantation and the second after implantation but before the completion of neurogenesis. Note the bizarre form of the extruded mesencephalon. The 2 litter members appear to be normal but are stunted.

balanced, but at the same time may exhibit gross deficiencies. The brain may appear to be grossly normal, but when compared with the control brain may be seen to be microcephalous. Once the neuron is differentiated, it is then almost completely radioresistant, but neurogenesis is not completed by the time of birth. Thus, irradiation effects on the central nervous system extend from the germ cell through the completion of neurogenesis of the next generation, at least. Put more succinctly, ionizing radiation should be respected by germ cells at all times and by all undifferentiated cells (Tables III and IV).

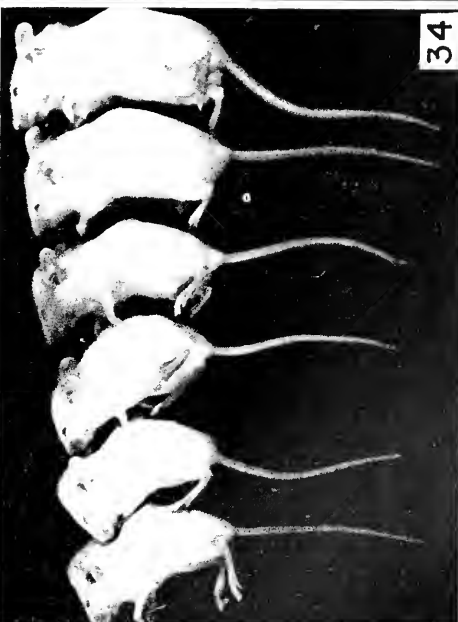
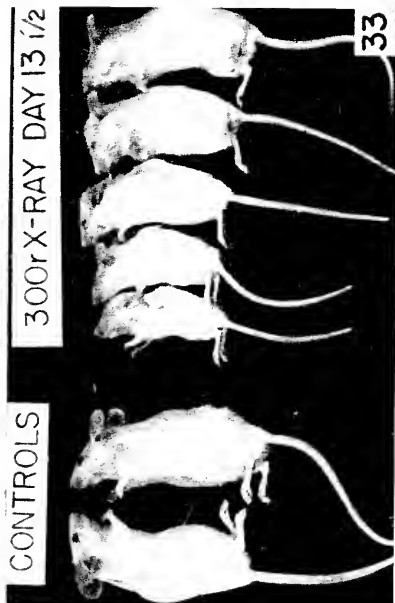
In treatment of central nervous system malignancies, doses of 10,000 r are sometimes accumulated. If killing a tumor by irradiation results in the saving of a life, it is certainly justified. If it results in the prolonging of a life with concomitant and permanent injury and possible germ cell exposure, the procedure may be questioned. When the individual is beyond the reproductive age, there is no place for this discussion. The emphasis here is on the germ cells which might be used, and on the embryo or fetus which should never be exposed if it can be avoided. Any exposure of the germ cells or early embryo is undesirable.

Gentry *et al.* (1959) have found a correlation between the areas in New York State of high congenital malformations and geographic concentrations of natural materials of relatively high levels of radioactivity, such as igneous or black shale rock. These may include C^{14} , K^{40} , Ra^{226} , Th^{232} , and U^{238} , and their decay products. The average exposure of individuals was estimated at 2.1 to 3.2 r per 30 years. The highest record for any single town was 66.7 congenital malformations per 1,000 births, and in an area particularly high in natural radioactivity. This was considerably above the average in the "unlikely areas" of 12.9 per 1,000 live births. There were some towns with no anomalies in low radiation areas. A reduction in birth weights also showed a correlation with increasing radioactivity. Detailed maps of radioactive concentrations fitted perfectly those of higher incidence of congenital malformations. Of all malformations, 15% involved the central nervous system, and of these 94.5% caused death. While some may doubt the conclusions of this study, it cannot be ignored.

A somewhat similar study has been made by Wesley (1960) in which he, as a statistician, finds that "96% of all deaths due to congenital malformations are caused by background radiation, and x-rays have caused a 6% increase in congenital malformations in the United States in the last 30 years." There was a low incidence of congenital anomalies in southeastern Asia and a high one in northern Ireland, correlated with background concentrations. There is no way of determining how many of the 5,000,000 mentally retarded United States citizens are products of irradiation injury.

Hicks *et al.* (1959) made the following statements, all of which emphasize





that fetal irradiation of the rat results in a neurologically deficient embryo:

"The cerebral hemispheres and diencephalon were a good deal smaller than normal. . . . The neocortex was seriously deficient, and about half as thick as normal at the vertex to about $\frac{2}{3}$ normal thickness laterally. . . . Small pallium. . . . The anterior commissure was a little less compact. . . . The midbrain was smaller in total cross area than normal due to somewhat flattened superior colliculi. . . . The cerebellum was altogether a little smaller than normal. . . . The lower medulla showed a slight reduction in total over-all size. . . . The lower brain stem and cerebellum were a little smaller than normal. . . . The cords were a little smaller in cross section than normal. . . . Most of the cells in the 13-day retina were killed by radiation."

Anomalies mentioned in this excellent study included:

"Radiation-killed cells in the periepidual primitive matrix threw the mitotic layer into rosettes which continued to proliferate brain, nonetheless. The result was an anomalous mass of ectopic cortex. . . . No corpus callosum. . . . Bizarre bundles of fibers. . . . Disorderly array of all sorts of cortical neurons. . . . The neurons were jumbled, scattered, and they were often upside down or pointed sideways."

Figs. 28-35 are on pages 18 and 19.

Figs. 28, 29, and 30. These represent members of litters from 3 successive generations following a single exposure of the ovary of the mother of those in Fig. 28 to 100 r. It was whole-body exposure, but we have reason to believe that the somatic effects of this exposure had nothing to do with these congenital anomalies. The fact that a single exposure of the ovary caused this brain anomaly to appear in three successive generations is genetically significant, even though its incidence was very low.

Fig. 31. These represent an entire litter from a grandfather who had received high-level exposure of his testis. The first generation appeared normal, were viable and fertile. This brain anomaly of exencephalia appeared in the next generation. One might expect it to appear in yet succeeding generations.

Fig. 32. The four embryos to the right show the variety of anomalies which appeared in the second generation following testis exposure. All were stunted, some died as fetuses (late in development). The single member to the left is a control of the same age.

Fig. 33. When mouse embryos are exposed after organogenesis to high but tolerable levels of irradiation, the effects are largely skeletal. Note particularly the variations in size within the single litter. One member of the litter is almost as large as the controls. The explanation of this is probably genetic.

Fig. 34. This is one litter, all of whom were exposed at the same time to the same irradiation, but which exhibit a wide range of difference in size.

Fig. 35. This shows photographs of Spalteholz's preparations of two mice at birth, the upper one being the control, the lower one x-irradiated at 13.5 days. Note that the irradiated embryo appears to be topographically normal but obviously is very much stunted. The developmental processes have been able to reorganize the undamaged cells to provide an apparently normally proportioned but stunted mouse.

All of these anomalies could be attributed to deficiencies during development caused by ionizing radiations.

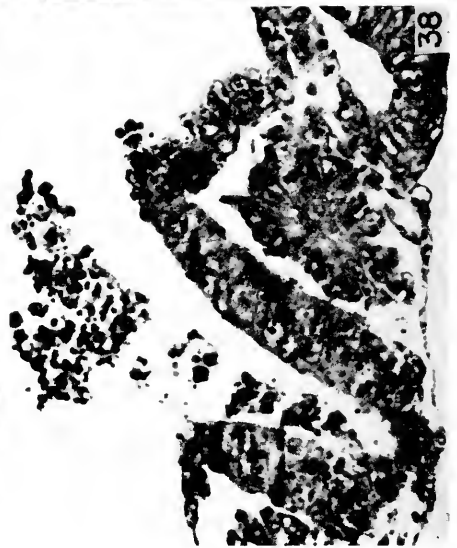
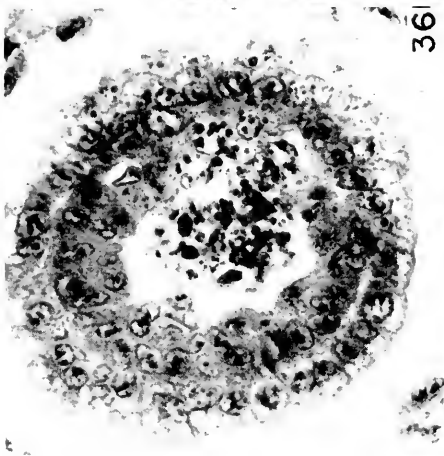
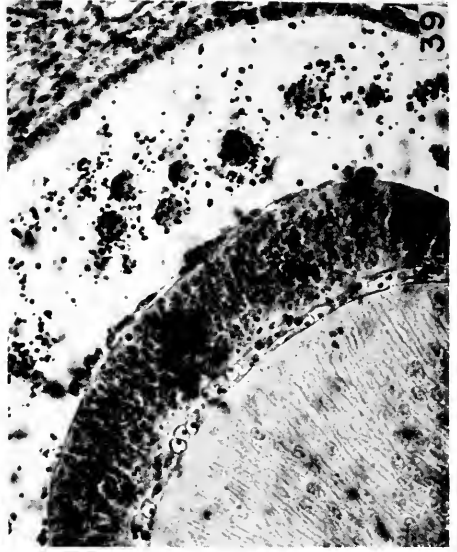
Their explanations of these central nervous system malformations included the following direct statements:

"There was a selective extirpation effect on certain primitive cells. . . . A patchy deficiency of cells. . . . Numerous dead cells spilled into the ventricles. . . . Virtually all of the primitive migratory cells in transit were killed. . . . The residual mitotic colony of lining cells was thrown into disorder because their support, the matrix of radiosensitive cells forming much of the wall, was gone."

The entire emphasis of this study seemed to be on the deficiencies following fetal irradiation.

The appearance of rosettes has often been described in both the neural retina and in the developing cortex following fetal irradiation. The presence of rosettes is proof that cells have been desegregated and that those still viable attempt neural organization. In the case of irradiation, the desegregation is due to the killing of radiosensitive cells, which are then removed, leaving loosely scattered, but viable cells. It has been shown recently (Moscona, 1960) that presumptive nerve, cartilage, and liver cells of mouse and chick embryos may be desegregated (disaggregated) by trypsinization and mixed together, only to reaggregate with respect to whether they were nervous, cartilage, or liver, and irrespective of whether they came from the mouse or the chick. In other words, presumptive nerve cells show an affinity for each other, regardless of their genetic source. When they come together without sustentacular materials, they tend to form rosettes which are an expression of disorganization. The rosettes are therefore not a peculiarity of post irradiation, nor of the mouse or rat, but rather of neural disorganization. A single rosette has been formed of neuroblasts from both the mouse and the chick embryos. These structures, usually temporary in the irradiated and developing embryo, simply represent a stage in the reorganization of viable nerve cells which are inadequate in number to accomplish structural normality (Figs. 36-43 and Table V).

Our current studies are utilizing low doses to determine the effect of ionizing radiations on the developing central nervous system as demonstrated by behavior, electroencephalographic records at various stages of maturation, and electron microscope and neuropathologic studies of the postnatal brain. It may develop that it will be the experimental psychologist who will spot the specific developmental stages most drastically affected by ionizing radiations. If our society is primarily concerned with the function of the central nervous system, we may be dealing with radiation changes which are beyond analysis by the conventional neuropathologic techniques or by the electron microscope. We expect to have information on this during the next year.



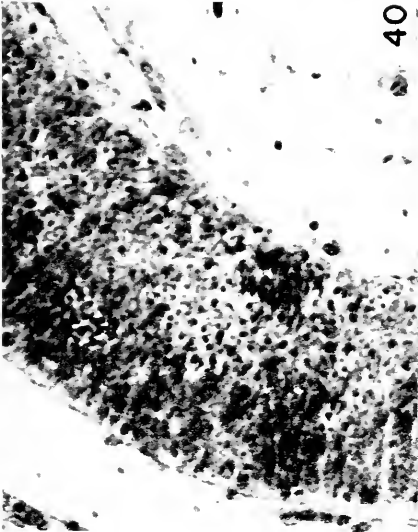


TABLE V

EFFECT OF FETAL X-IRRADIATION ON MOUSE EYES^a
(Measurements at 6 weeks of age)

	<i>Average diameter (in mm)</i> ^b	<i>Relative volume (%)</i>
Controls	3.555	100
150 r at 12.5 days gestation	2.970	69.6
250 r at 12.5 days gestation	2.670	50.6

^a X-irradiation of the developing mouse embryo seems to result in cellular deficiencies because the damaged cells are removed. When these irradiations occur early, before the development of a specific organ system, the deficiency resulting from the elimination of the necrotized cells results in a reduction of organ size. The data of Table IV show that with increasing irradiation at 12.5 days, there is a decrease in the relative volume of the diameters of the mouse eyes at 6 weeks of age. An exposure of 250 r reduced the volume to approximately 50%. There are no studies thus far relative to the visual acuity of these eyes.

^b Minimum of 8 diameters of fixed eyes taken for each average.

Summary

The early embryo is more radiosensitive than is the organism at any other time in its entire life cycle. The earlier the stage, the more sensitive, with regard to both survival and the development of anomalies.

At the cellular level, there is no such thing as "recovery" from irradiation damage, meaning a return to the preirradiated state. Since embryonic cells are precursors of all cells of the adult, irreparable damage to surviving cells results in such damage to all descendant cells of the adult organism. Ionizing radiations represent a very potent tool.

Figs. 36-43 are on pages 22 and 23.

FIG. 36. When mouse embryos at 6.5 days are exposed to x-rays, 24 hours thereafter they show the sloughing off of cells into the central cavity as seen here. The inner neurectoderm will be deficient to the extent of this cellular loss.

FIG. 37. This shows the neural groove at the level of the brain of 8.5-day embryos 24 hours after exposure to x-rays. Note the many pyknotic nuclei and the sloughed off cells into the neural groove.

FIG. 38. This is similar to FIG. 37 except it is at the level of somites.

FIG. 39. In this figure note the many phagocytes posterior to the developing retina, each of which contains a number of necrotic neurectoderm cells. This occurs about 24 hours after x-irradiation, but the retina will be deficient to the extent of this cellular loss.

FIG. 40. This is an enlarged view of the retina 4 hours after irradiation, showing many pyknotic nuclei.

FIG. 41. This is an enlarged view of single phagocyte containing 14 dead neurectoderm cells from the x-irradiated retina.

FIGS. 42 and 43. These are enlarged views of the retina of the control FIG. 42 and the irradiated FIG. 43 to show slight thinning of the various layers in the x-irradiated eye of the mouse.

The embryo, in contrast with the adult, has powers of reorganizing its residual and surviving cells so that topographic normality may be achieved. However, every such individual will be deficient, either in parts or in the stunting of the whole.

Deficiencies are seldom similar in litter mates, owing to the submicroscopic nature of ionizing radiations, the genetic variations in individuals, the varying abilities for restitution, and probably other factors.

Irradiation of the embryo is the only way to produce irradiation congenital anomalies, but such anomalies may be produced by other traumatic means. Following organogenesis, irradiation effects are similar to those one expects in the adult.

The embryo after a certain stage possesses gonad primordia or developing gonads, and these are subject to irradiation effects which may not be evident for generations.

Central nervous system anomalies may be produced by irradiation of the mature gamete of either sex, the fertilized egg, or any stage in development prior to completion of neurogenesis. Some formative cells are present even after the birth of the mammal. The range of radiosensitivity of gamete to formed organism is such that discussion of threshold is meaningless. We cannot now state the extent or the duration of irradiation damage to the developing central nervous system. There may well be subtle effects to be revealed by population studies over generations. Any exposure of the early embryo should be regarded as too much.

Finally, I would like to make four specific requests:

1. That we insist on better and more adequate controls in radiobiology.
2. That radiation dosimetry in all radiobiologic experiments be checked by a qualified radiophysicist and be fully reported.
3. That there be a pool of research information on neurologic effects, including critically reviewed information from the U.S.S.R. because of the language barrier.
4. That symposia of this sort be organized as frequently as the accelerating accumulation of data demands.

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Quantitative Histologic and Behavioral Studies on Effects of Fetal X-Irradiation in Developing Cerebral Cortex of White Rat *

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Introduction

Recent experimental studies on effects of fetal irradiation on nervous tissues have clearly shown (Hicks, 1954, and Hicks *et al.*, 1957) that primitive neuroblasts and spongioblasts are selectively damaged by ionizing radiation. It has also been demonstrated in this work that rather specific and predictable anomalies are produced in nervous tissues in postnatal life in the rat by radiation exposure on any given day in the gestation period between the 9th day and birth. At the same time, some behavioral studies (Levinson, 1952; Furchtgott and Echols, 1958a, b) have demonstrated serious behavioral deficits in rats irradiated as fetuses. While considerable attention has been given in the histopathologic studies to the regenerative ability and recovery of nervous tissues from such radiation exposure (Hicks, 1957) little effort has been devoted to analyzing the capacity of the cells surviving irradiation exposure for normal growth or the specific effects of the irradiation on cell growth. Further, the behavioral studies carried out thus far have not emphasized the possible relationships between cytologic deficits and behavioral deficits. It has been our purpose, therefore, in initiating the present series of investigations to analyze effects of fetal x-irradiation administered in fractionated and single doses on early postnatal growth of surviving cells in cerebral cortex and to determine what relationships may exist between alterations in normal growth patterns or cytologic deficits and behavioral abnormalities appearing later in life. The present report is concerned with our preliminary findings with fractionated doses administered during the latter half of the gestation period.

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Materials and Methods

Three groups of rats of the Sprague-Dawley strain were exposed *in utero* to fractionated doses of total body x-irradiation.

Two of the groups received 12.5 and 25 r per day at the rate of 60 r per minute on gestation days 10 through 17 giving total doses of 100 and 200 r, respectively. A third group (Brizzee *et al.*, 1961) received a total dose of 300 r given at 60 r per day on gestation days 10 through 14. These animals and a series of control animals treated in the same manner as the above groups, except for the exposure to radiation, were grouped according to age at 1, 5, 10, and 20 days with from 4 to 6 animals per group and the sexes equally divided. The tissues were fixed and stained as reported previously (Brizzee and Jacobs, 1959; Brizzee *et al.*, 1961) and subjected to quantitative histologic analysis. The parameters studied were neuron packing density, neuron nuclear, cytoplasmic, and soma volume, nucleocytoplasmic ratio, gray cell coefficient, glial packing density, and the glia/neuron index in area 2 (Krieg, 1946). In addition, total brain weight was determined and cortical thickness measured in areas 2, 4, 41, and 17 (Krieg, 1946). All of the volumetric, density, and thickness determinations were confined to the submolecular layers only. Methods employed in the quantitative histologic determinations have been described in earlier publications (Brizzee and Jacobs, 1959; Brizzee *et al.*, 1961).

In the behavioral studies, 9 pregnant rats were divided into three equal groups: a full-body group, a half-body group in which the lower half of the dam was shielded by lead, and a control group which received no irradiation. Irradiation took place each day from the 10th through the 17th days of gestation. Each irradiated animal received 40 r per day for a total of 320 r (60 r per min). For the half-body group, a shield made of blocks of lead $2 \times 4 \times 8$ in. was constructed so that only the thorax, neck, and head were exposed to radiation.

To assess the duration of the effects of x-irradiation on locomotor coordination, the three groups of rats were divided into three subgroups to be tested at different ages. Group one was tested at age 40 days, group two at age 90 days, and group three at age 140 days. The test of locomotor coordination required the rats to traverse a bridge made of two parallel rods.

At 115 days of age the rats in the 90- to 140-day groups were given 2 trials in a simple L-shaped water maze. The next day all the rats were run in a 14-unit multiple-T water maze patterned after the Stone design (Heron, 1930; Sharp, in press).

At age 50 days 6 rats from each group were sacrificed, and their cerebral cortexes studied in the same manner as in the first three groups.

In plotting the values of the various parameters in Figs. 1-6 the vertical

lines indicate the magnitude of the standard errors of the mean for the controls. Standard errors for the irradiated groups were not plotted in the interest of clarity in the figures.

Results

Neuron packing density in all groups (Fig. 1) was seen to decrease very rapidly between the 1- and 5-day stages, with a less notable decrease between the 5th and 10th days, and approached normal adult levels on the 20th day. The values for all irradiated groups and controls were in close agreement and showed no significant differences at any age level.

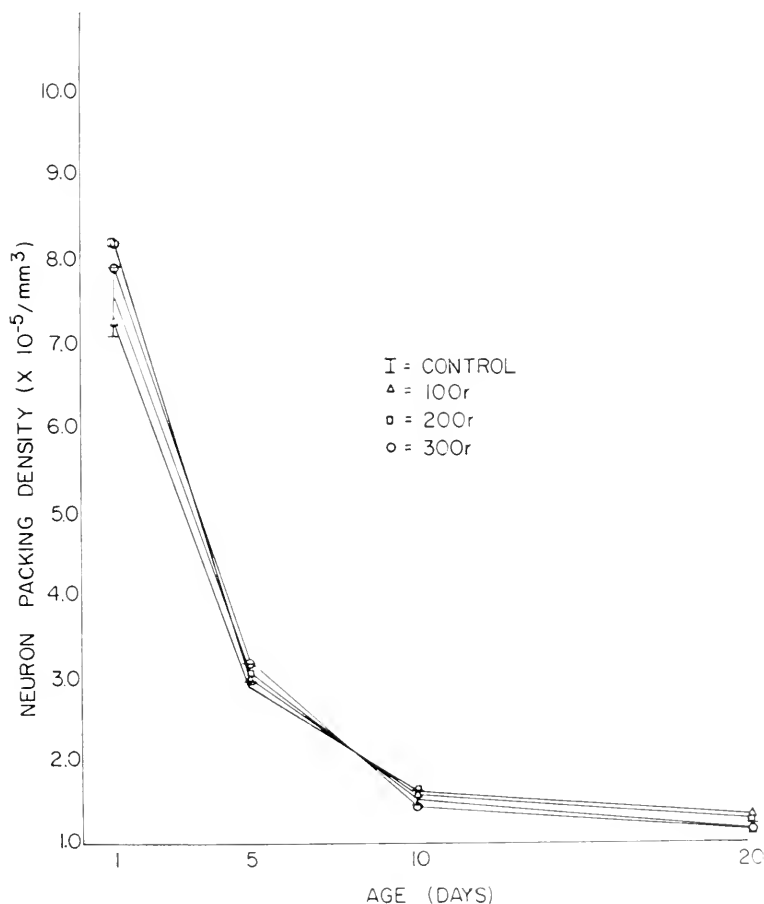


FIG. 1. Early postnatal changes in neuron packing density.

The mean values for neuroglial packing density in the control groups decreased from 45,000 cells/mm³ at 1 day to 27,000 at 20 days, but the differences between the various age levels in this series are not significant owing to a rather large variance in counts. The mean neuroglial packing density for all four age groups in the nonirradiated animals was 34,000 cells/mm³. The neuroglial density in the 100 and 200 r groups did not differ significantly from the controls, but in the 300 r group, the value for neuroglial density in 1-day-old animals was significantly higher (80,000 cells/mm³; $p < .05$) than in the nonirradiated groups. In later stages the differences were not significant.

The neuroglia neuron index (Fig. 2), almost entirely as a result of the

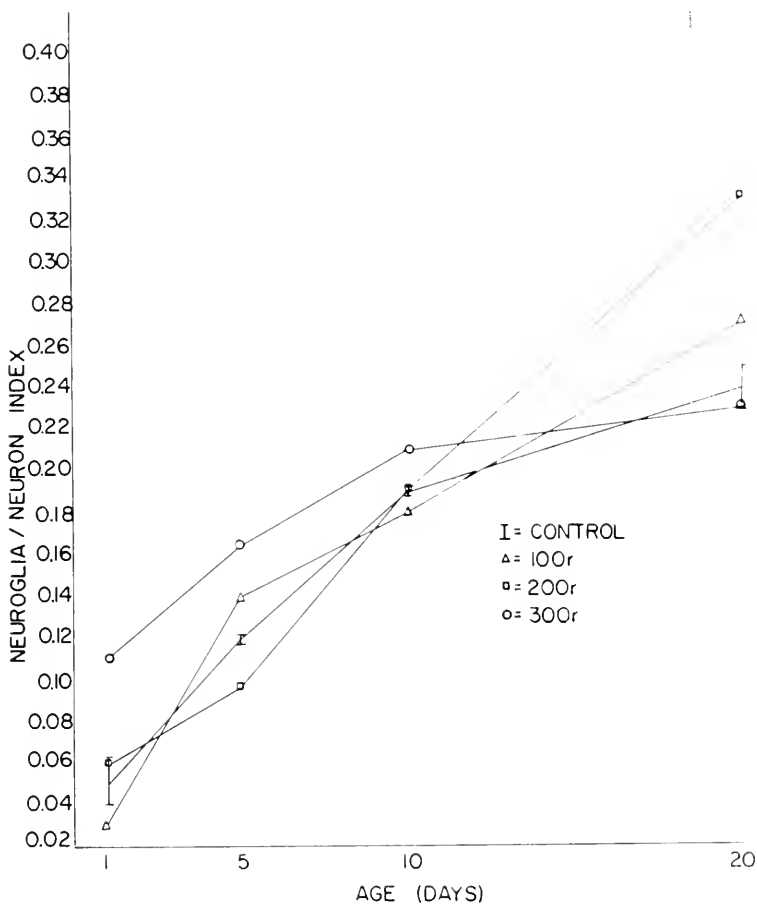


FIG. 2. Increase in neuroglia/neuron index from 1st to 20th postnatal day.

changes in the neuronal packing density, increased fairly rapidly in controls from the 1st to the 10th day and more slowly from the 10th to the 20th day. Differences in values for the neuroglia, neuron index between irradiated and control groups were not significant at the 5-, 10-, and 20-day stages. In the 300 r group, however, the neuroglia, neuron index in the 1-day-old rats is significantly higher ($p < .05$) than that in the non-irradiated rats of the same age. In 20-day animals the value for the index in the 200 r group is considerably higher than in the controls, but due to a large variance it is not statistically significant at the 0.05 level.

It is noteworthy that the levels for the neuroglia, neuron index at all ages studied are very low as compared with adult values in some other species as, for example, in man (1.78; Hawkins and Olszewski, 1957) or in the horse (1.24; Friede, 1954), although the index is comparable in our 20-day animals to the average values derived from Friede's data for the cerebral cortex in the mouse (.35) and the rabbit (.42).

Neuron nuclear, cytoplasmic, and soma (nucleus + perikaryon) volumes (Fig. 3) increased steadily from the earliest to the latest stage examined with the values in all groups in fairly close agreement. As in our preliminary studies describing the 300 r group (Brizzee *et al.*, 1961), however, the

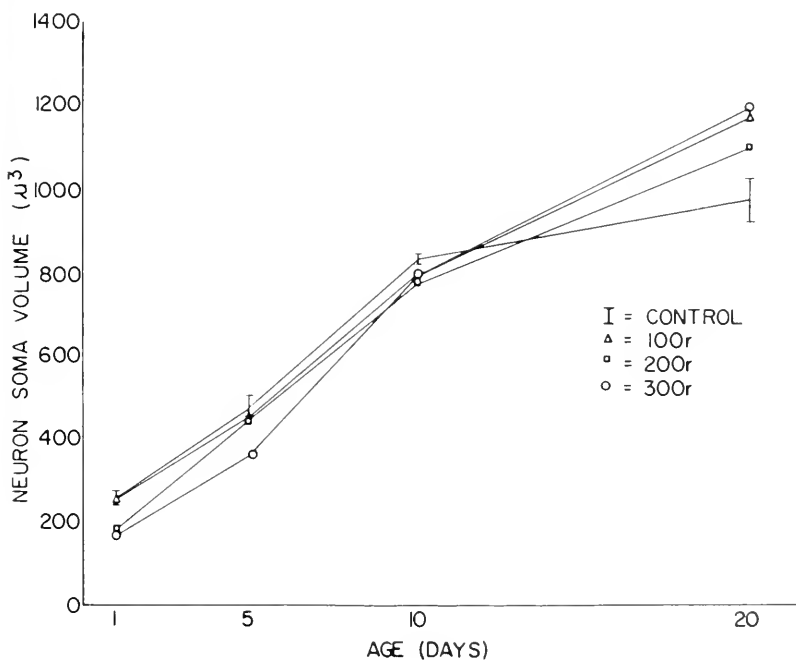


FIG. 3. Alterations in neuron soma volume in early postnatal stages.

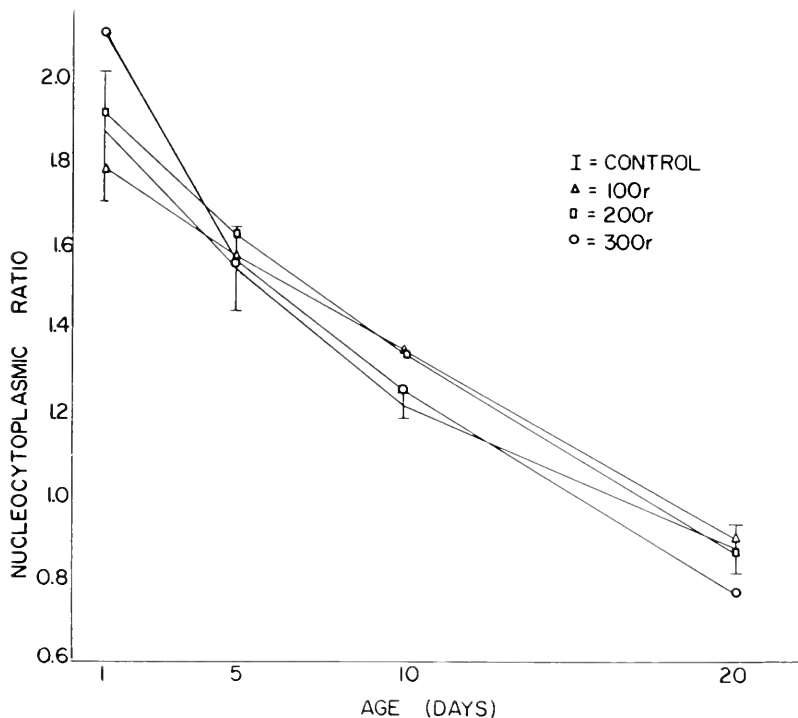


FIG. 4. Decrease in nucleocytoplasmic ratio between 1st and 20th postnatal days.

values for neuron soma volume (nucleus + perikaryon) in the 100 and 200 r series are lower than in the controls in the first three developmental stages and increase to values above the control level on the 20th day. The group receiving the highest dose of irradiation (300 r) diverged more markedly from control values than the animals given lower doses, but the differences are within the range of error of the methods employed and are not statistically significant. The nucleocytoplasmic ratio (Fig. 4), reflecting the changing relationships between nuclear and cytoplasmic (perikaryon) volume through the four stages of development, was seen to decrease steadily in all groups with no significant differences appearing among irradiated or control groups.

In contrast to the above findings, marked differences were observed in cortical thickness in area 2 (Fig. 5, Table I) between the controls and animals irradiated at 200 and 300 r ($p < .01$), and between the 200 and 300 r groups themselves ($p < .01$). No significant differences were noted between the 100 r groups and nonirradiated rats.

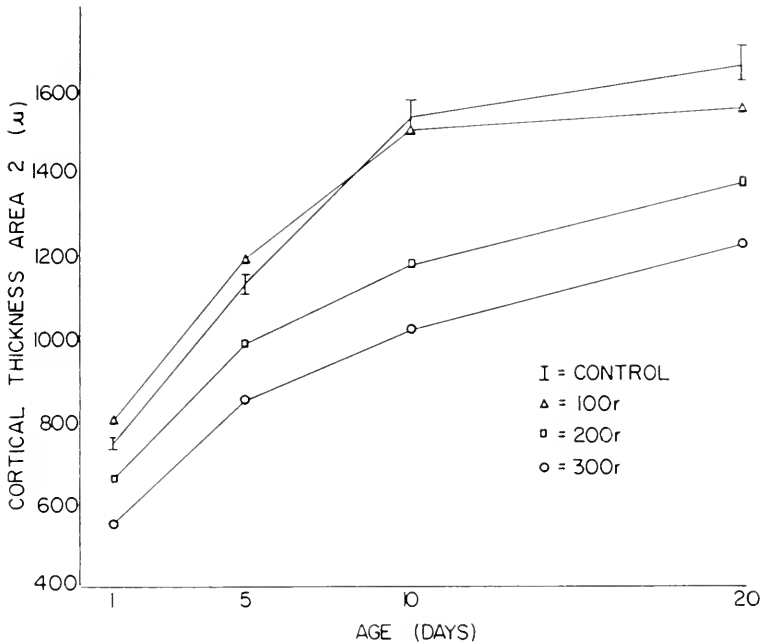


FIG. 5. Comparison of cortical thickness in area 2 in irradiated and control groups in early postnatal period.

It is particularly noteworthy that the curves illustrating the increase in cortical thickness for radiated and control groups tend to be parallel. While only the curves for area 2 are illustrated (Fig. 5), it was observed on plotting out the values for the other areas that the curves for each area are quite characteristic for that area, with the curves for all radiated and control series showing the same general trends. One notable exception to this rule was observed in area 4 in the 200 r series. Here the value for cortical thickness in the 200 r group drops below that for the 300 r animals. With this exception, the differences in cortical thickness in area 4, 41, and 17 are of about the same order as in area 2.

While cortical thickness thus appeared to exhibit an inverse relationship to radiation dose, no marked differences in relative thickness of layers were observed in area 2 between irradiated and nonirradiated rats even in the 300 r series. No detailed observations other than cortical thickness were made in areas 4, 41, and 17. No general cytologic differences between neurons in irradiated and control animals were observed in area 2, and no abnormalities of blood vessels were found. It is of course recognized that the latter would not be well demonstrated in Nissl preparations.

TABLE I
COMPARISON OF EFFECTS OF FETAL X-IRRADIATION AT VARIOUS
DOSES ON CORTICAL THICKNESS

Age (days) Area		Control	100 r	200 r	300 r
1	2	744 ± 14	800 ± 54	655 ± 11	546 ± 14
	4	690 ± 20	778 ± 57	560 ± 16	551 ± 85
	41	416 ± 26	467 ± 27	370 ± 8	314 ± 8
	17	445 ± 17	486 ± 12	396 ± 22	340 ± 9
5	2	1131 ± 16	1191 ± 15	982 ± 29	845 ± 48
	4	1086 ± 23	1227 ± 25	975 ± 66	778 ± 68
	41	700 ± 61	706 ± 9	545 ± 30	445 ± 29
	17	695 ± 17	675 ± 14	549 ± 14	458 ± 45
10	2	1529 ± 39	1500 ± 90	1177 ± 46	1015 ± 55
	4	1592 ± 28	1470 ± 48	908 ± 65	1021 ± 69
	41	861 ± 14	881 ± 35	858 ± 19	632 ± 80
	17	920 ± 29	896 ± 18	854 ± 10	624 ± 37
20	2	1654 ± 62	1551 ± 9	1370 ± 58	1221 ± 42
	4	1668 ± 40	1655 ± 37	1296 ± 92	1080 ± 60
	41	945 ± 23	936 ± 6	843 ± 40	673 ± 32
	17	1112 ± 25	977 ± 21	835 ± 46	670 ± 31

Differences in brain weight (Fig. 6) between the 200 and 300 r series and between these groups and the control animals were consistent and marked throughout the four developmental stages studied. Brain weight in the 100 r group, however, was higher in the 1- and 10-day animals and appreciably lower in the 10- and 20-day stages than in the controls. At the 10- and 20-day stages the differences in brain weight between all three radiated series and between each of these groups and the nonirradiated rats were significant at the .05 level or less.

Results of behavioral studies are summarized in Tables II and III and in Fig. 7. Table II shows the mean, variance, and number of rats for each of the nine groups in the experiment on locomotor ability. The higher the mean, the better the performance. From Table II it is clear that any initial differences between the groups are overcome by 140 days. An analysis of variance of these data, summarized in Table II, showed that the irradiation (totaling 320 r) did result in a decrement of locomotor coordination on the parallel bars for *young* rats.

In the water maze experiment, 73% of the control rats and 82% of the half-body rats reached the criterion of one perfect trial by the twenty-first trial, whereas only 26% of the full-body group did so ($X^2 = 15.5$, 2 *df*,

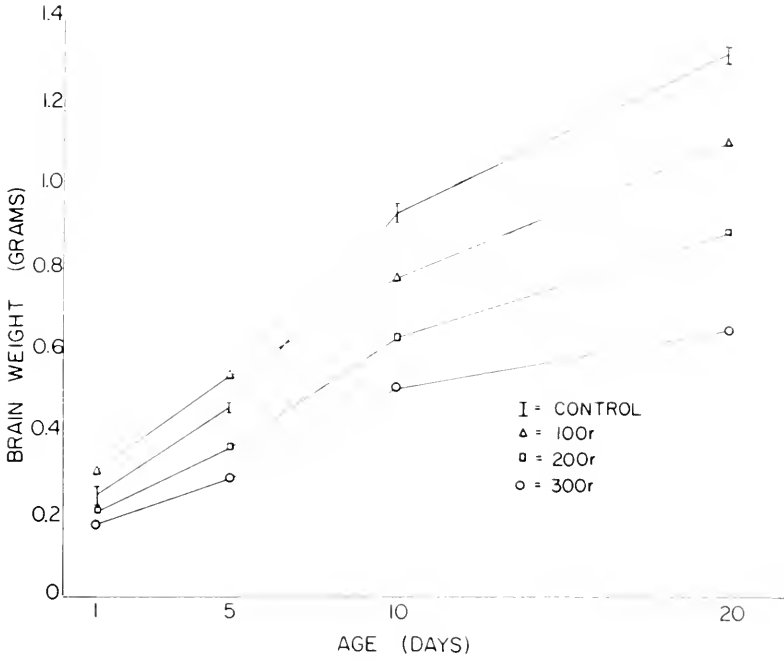


FIG. 6. Comparison of total brain weight in irradiated and control animals in first 20 postnatal days.

TABLE II

MAXIMUM 1/4-INCH GAPS SUCCESSFULLY NEGOTIATED
BY ALL GROUPS

Days tested	Conditions of Irradiation		
	Full-body	Half-body	Controls
40	$\bar{X} = 2.90$ $\sigma^2 = 1.69$ $N = 11$	$\bar{X} = 8.67$ $\sigma^2 = 7.02$ $N = 12$	$\bar{X} = 8.50$ $\sigma^2 = 3.50$ $N = 6$
90	$\bar{X} = 3.6$ $\sigma^2 = 2.04$ $N = 10$	$\bar{X} = 6.9$ $\sigma^2 = 9.49$ $N = 11$	$\bar{X} = 6.17$ $\sigma^2 = 3.76$ $N = 6$
140	$\bar{X} = 6.09$ $\sigma^2 = 3.10$ $N = 11$	$\bar{X} = 7.36$ $\sigma^2 = 12.85$ $N = 11$	$\bar{X} = 5.8$ $\sigma^2 = 6.20$ $N = 5$

TABLE III

SUMMARY TABLE FOR THE ANALYSIS OF VARIANCE OF THE
DATE FROM THE LOCOMOTOR EXPERIMENT

Source	df	MS	F	p
Condition of irradiation	2	107.61	18.75	.001
Age at test	2	8.20	1.42	—
Irradiation \times age at test	4	23.58	4.11	.01
Individuals	74	5.74	—	—

$p = .01$). Figure 7 represents the mean number of errors plotted against trials. One can see that the full-body group did not learn as rapidly as did the control and half-body groups and that the control and half-body groups were very similar. An analysis of variance of these data and the equivalence of the curves for the nonirradiated and half-body groups suggests that (a) the full-body group learned significantly more slowly than the other two groups, and (b) the effects of irradiating the mother with the uterus shielded did not cause a decrement in the future maze learning ability of their fetuses.

Quantitative histologic methods in 50-day animals from the groups subjected to the behavioral tests revealed essentially the same differences between full-body (320 r) groups and controls as were observed in the 20-day animals of the 300 r group described previously. That is, the only significant

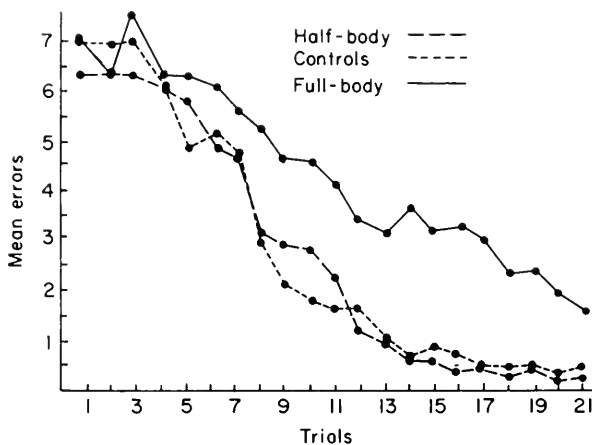


FIG. 7. The mean number of errors for each group across trials. The data points were computed on the assumption that once a rat reached criterion he would make no more errors on successive trials.

differences appeared in cortical thickness and brain weights and were generally of the same magnitude as reported for the 300 r series. The values for the half-body group did not differ significantly from controls in any of the parameters studied.

Discussion

Rugh (1959) has made the observation that loss of cells in the embryo or fetus due to irradiation may be obscured by the fact that the organism has remarkable powers of integrating the remaining undifferentiated, undamaged neurectoderm into a topographically normal but reduced whole organism. Our results appear to furnish considerable support for this view in reference to cerebral cortex.

While the curves illustrating increase in cortical thickness are plotted only for area 2 (Fig. 5), it is seen on plotting the values for the other areas that the pattern of increase in cortical thickness in all radiated and normal series is characteristic for each area. While cortical thickness varies inversely with radiation dose at the 200 and 300 r levels in all areas, the curves illustrating increase in cortical thickness at all dose levels and in control series tend to be parallel for a given cortical area. This strong tendency toward parallel growth within a specific area, together with the lack of any truly significant volumetric or density changes, indicates that the surviving cells must possess essentially the same developmental capabilities as the nonirradiated cells of the control animals.

The degree to which the orientation and pattern of branching of cell processes may differ in irradiated and control groups at the dose and rate levels used remains to be determined. The quantitative technique developed by Sholl (1953) and Eayrs (1955) should be admirably suited to the solution of this problem. However, it appears clear from our determinations that, at the dose levels employed, there are no significant differences in cell territory (computed from neuron packing density) in any of the irradiated or control groups. This indicates that, whether or not the cell processes may be altered morphologically, they must, on the average, occupy the same cortical volume in irradiated groups as in control animals.

Hicks (1959) has shown that rather specific, predictable nervous system anomalies may be produced with single exposures of x-irradiation from about 150 to 200 r at any given time in the gestation period after the 9th day. Our results suggest that with fractionated doses between the 10th and 17th gestational days it may be possible to produce animals with cerebral cortexes in which definite and predictable cellular deficits, rather than gross anomalies, exist in the absence of any volumetric, density, or general cytologic changes in the cells present. From our observations it appears that the

cellular deficiencies are generally distributed through all cortical layers and that neurons and neuroglia are equally affected. If this were not true the neuroglia/neuron index would reveal more consistent differences between control and irradiated animals than were observed. We believe that the high values for neuroglial density and the neuroglia/neuron index in the 300 r group in 1-day animals may reflect an increase in microglial elements as a manifestation of the terminal phases of the reparative processes which follow irradiation damage in the cerebral cortex as described by Hicks (1957).

The use of graded fractionated doses of x-irradiation during the latter half of the gestation period may offer a means for producing a series of animals with predictable, graded cellular cortical deficits. One might raise the objection, however, that fractionated doses administered on several successive days may produce cellular deficits in many or all parts of the neuraxis. This would obviously decrease the value of such preparations for the study of the effects of localized cortical cellular deficits on learning ability, locomotor function, or electrophysiologic phenomena. It seems probable that better localized cellular deficits without gross abnormalities might be produced by fractionated doses given within a more limited time than used in the present study at certain specific days in the gestational period as shown by Hicks in his timetables of radiation malformations. Such specimens should prove particularly useful in the field of experimental psychology, and investigations aimed at exploring this possibility are now in progress in our laboratory.

It is interesting to note that Hicks (1959) found that single doses of 150 to 200 r administered on the 16th day of gestation resulted in cortexes which were only one-half as thick as normal and bore little resemblance to the normal laminated 6-layered neocortex except in the lateral region. In our preparations, even at total fractionated doses of 300 and 320 r, while the cortical thickness in area 2 is reduced by approximately 30%, the laminated character of the cortex in rats 20 and 50 days of age is well preserved with surviving cells apparently cytologically normal, thus indicating that the surviving cells manage to overcome the effects of the daily low-dose radiation exposure to a great extent, even where the cumulative dose is rather high. In earlier stages, cell layers are less well developed in many irradiated animals, especially in areas 17 and 41.

Numberger and Gordon (1957) have recently called attention to the inadequacy of the more commonly used referents, such as wet weight and dry weight, for evaluating chemical and presumably metabolic properties of tissues and emphasized the desirability of employing more meaningful referents, such as cell density. We would like to emphasize the desirability of employing not only cell packing density, but additional referents, such as mean and total nuclear volume, cytoplasmic volume, glial packing density, glia/neuron index, dendritic territory, cell surface, and other such param-

ters as a frame of reference for properly evaluating the chemical properties and metabolic activity of nervous tissues. We emphasize this point because of the increasing interest in chemical and metabolic effects of irradiation. It is our belief that more significant results will be derived from such investigations if they are based on the cellular composition of the tissue, rather than on the more commonly used biochemical referents.

With reference to the behavioral studies, since the surviving cells in our irradiated preparations in area 2 appear cytologically normal, it appears that the explanation of the observed deficits in learning ability must rest on the assumption that the surviving cells possess physiologic deficits not amenable to measurement with quantitative histologic or ordinary cytologic methods, or that the deficit is entirely or in part the result of the numerical deficit in cells, or both. It is of considerable interest to note, however, that the locomotor deficit observed in the youngest animals tested is largely cleared up in the oldest group. Long-term studies are now in progress to determine if a similar phenomenon may occur with reference to learning ability in older animals following fetal irradiation.

Summary and Conclusions

Exposure to fractionated doses of total-body x-irradiation at 100, 200, and 300 r (60 r per minute) over a period of 5 to 7 days after the 10th day of gestation results in no significant difference in neuron packing density in area 2 in the first 20 days of postnatal life. Neuroglial packing density and the neuroglial neuron index are higher in the 300 r group in 1-day-old animals than in controls, but at later stages the differences are not significant. Mean values for neuron soma volume in 1-, 5-, and 10-day-old irradiated animals are consistently lower than in nonirradiated series, but are higher at the 20-day stage in irradiated than in the control animals. The differences are considered to be within the range of error of the methods employed. The nucleocytoplasmic ratio decreased steadily from the 1st to the 20th days, and no significant differences occurred between irradiated and control series.

Brain weight varied inversely with irradiation dose at the 10- and 20-day stages and in 200 and 300 r groups at the 1- and 5-day stages. Cortical thickness in the 100 r group was not significantly different from controls. In the 200 r and 300 r groups, however, cortical thickness varied inversely with dose level, and significant differences between the 200 and 300 r groups and between the irradiated and control groups occurred at the 20-day stages in all areas. It is concluded that the doses of fetal x-irradiation employed produced no significant differences in volumetric or density relationships or general cytoarchitecture between irradiated and control animals in area 2 but resulted in a significant decrease in total number of cells in this zone.

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Structural and Behavioral Alteration in the Rat Following Cumulative Exposure of the Central Nervous System to X-Irradiation *

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Introduction

This project is constructed to permit analysis of the cytologic, histochemical, and functional state of the rat's central nervous system. This analysis has been based on short- and long-term responses demonstrated by the central nervous system to cumulative levels of total-head x-irradiation. The following report is limited to the long-term responses.

Davidoff and others (1938) have described the microscopic effects of x-irradiation applied directly to the brain and spinal cord of monkeys. These workers described thickening of the blood vessel walls, swelling, gliosis, hypertrophy, and formation of gitter cells in the neuroglial elements. These changes were noted 322 days following exposure in animals receiving 1,856 r. The same authors expressed the opinion that there seemed to be two factors involved in governing histologic alterations: the dosage and the time interval between irradiation and autopsy.

Clemente and Holst (1954) subjected monkey heads to doses of x-irradiation ranging from 6,000 r to 15 r at 188 r per minute. They observed varying degrees of necrobiotic changes such as clumping, chromatolysis, degeneration, and neuronophagia of neurons with only slight changes below the 1,500 r level. These investigators observed gliosis 4 to 8 months after the date of exposure. Astrocytes were hyperchromatic and undergoing degeneration, while scattered oligodendroglia demonstrated swelling.

More recently Berg and Lindgren (1958) attempted to chart the extent of cerebral lesions produced by different divided and undivided doses and to determine the time-dose relationships for delayed reactions of brain tissue on application of a single dose and fractionated doses. These investigators reported various lesions in the rabbit brain: frank necrosis, partial destruc-

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tion of gray and white matter, as well as profound gliovascular alterations. The authors believe that their morphologic analysis supports the assumption that the vascular changes were primary and delayed; lesions in the brain were essentially the same type after fractionated as single dose exposure.

Materials and Methods

In this study approximately 120 male 9-month-old rats were used. Of these, 54 were placed in a group for long-term studies whose postirradiation sacrifice dates were selectively extended. These animals were grouped in six large cages, 9 rats per cage. During the first week of x-irradiation, a total of 36 animals were exposed; at random, 6 animals per cage received 1,000 r total-head irradiation. The remaining 18 animals, 3 in each cage, were maintained as controls. The rats received 1,000 r until the total accumulated dosage of the last remaining experimental group had reached 5,000 r.

Control animals and experimental animals scheduled for radiation were fed during a 45-minute period each day for 6 days. On the 7th day, each animal was placed in a Skinner box for 45 minutes. The animals' first experience in the box resulted in learning to press a bar for food reward. For the next two periods in the box, the animals were placed on short aperiodic reward schedules. The third time, all animals were placed on the aperiodic schedules and bar presses recorded during a 45-minute interval. After establishing a desirable level of individual performance, all of the various groups of animals underwent the scheduled exposure to x-irradiation and 5 weeks of testing.

The head of each experimental animal was exposed to x-irradiation from a 1,000 kvp x-ray unit filtered through 2 mm of aluminum and 56 cm of air. A Victoreen Chamber R-Meter was used at each operation to obtain equal and exact data of the total roentgens delivered in a prescribed interval of time. The rat's body was protected in a lead-lined box which was itself shielded by a wall of lead bricks 2-3 in. thick from which the animal's unshielded head protruded vertically above the body shield. Each unanesthetized animal was secured in the box by means of a modified burette clamp that formed a collar and limited the animal's movement. The lead-lined box containing the rat was rotated clockwise through 360° at 33 rpm by a turntable. Each rat's head was continuously exposed to x-irradiation from multiple angles until the animal accumulated at one exposure a level of 1,000 r, delivered at the rate of 237 r per minute and ½ r per minute to a partially exposed neck. Each animal remained approximately 4 minutes in the radiation chamber. As previously mentioned, this procedure was repeated every 7 days on the selected group of animals until the desired cumulative dosage was attained. The animal groups were then

sacrificed after receiving a total cumulative dosage of x-irradiation as follows: Group A, 1,820 r; Group B, 2,000 r; Group C, 3,000 r; Group D, 4,000 r; Group G, 5,000 r; and Group F, 5,000 r.

All animals, control and experimental, were anesthetized with sodium nembutal and surgically prepared for perfusion via the left ventricle of the heart. Perfusion pressure was maintained at approximately 90 mm of Hg. Initially, the major portion of the circulating blood volume was removed by washing the cardiovascular system with physiologic saline. A small incision in the right atrium provided a means of exit for the perfused fluids and blood. Subsequently, with the heart still pulsating, the fixatives were perfused. Three animals received saline-acacia formalin, while the fourth animal received acetic acid-alcohol formalin. The partially fixed brain was then rapidly removed and cut by coronal sections into three parts: a frontal, midcoronal, and occipital plus brain stem and cerebellum. Each of the sectioned brains was then immersed in its respective fixative, formalin and acetic acid-alcohol formalin along with the control for each fixative.

Following the usual histologic techniques, specimens fixed in acetic acid-alcohol formalin were processed for histochemical analysis utilizing the periodic acid-Schiff reaction (PAS) for glycogen. Specimens that were fixed in formalin-acacia were stained with toluidine blue for Nissl material and glia, azocarmine and Verhoeff's procedure for connective tissue and interstitial cell reaction, Weil's method for general identity of structures and axon pathways, Swank-Davenport modification of Marchi method for degeneration of myelin, and Bodian's method for nerve fibers and nerve endings.

Results

Following each exposure to x-irradiation the animals showed behavioral changes which were unremarkable and somewhat variable. The animals in general exhibited confusion, sluggishness, or malaise, as expressed by withdrawal to the back of the cage. Generally, during the 24 to 72 hours following exposure, most animals appeared to recover from a postirradiation malaise.

Group A animals (Table I and Fig. 12) survived 228 days after receiving a single exposure of 1,820 r and exhibited little or nothing in the way of physical changes. There occurred some slight weight loss during the first week which appeared to return to normal by the third week. One animal developed a head tumor, a benign involvement of the salivary glands, well encapsulated with a caseous center (sclerosing angioma).

Group B animals (Table I and Fig. 12) received two successive exposures of x-ray totaling 2,000 r. The animals in this group averaged approximately

a 12% decrease in body weight by the 228th day after initial exposure. These animals all demonstrated thinning or diffuse loss of hair about the head with complete epilation around the eyes. All had well-developed bilateral, posterior subcapsular cataracts. One animal developed a tumor of the head, a low grade carcinoma of the skin.

Group C animals (Table I and Fig. 12) received a total of 3,000 r during 3 weeks and were sacrificed 165 days after the initial exposure. The average decline in body weight over this time indicated approximately a 17% loss. These animals all demonstrated a rather severe diffuse loss of hair about the head with complete epilation around both eyes.

Group D animals (Table I and Fig. 12) accumulated a total dosage of 4,000 r during 4 weeks. Three of the animals were sacrificed after 158 days and demonstrated an approximate 20% weight loss. Two of the animals died after 44 days and demonstrated a loss of 31% of body weight. All animals showed diffuse loss of hair over the head with complete epilation around the eyes.

Group G animals (Table I) accumulated 5,000 r during 5 weeks. Two animals were sacrificed after 116 days and demonstrated approximately a 29% loss of body weight. All animals demonstrated severe, diffuse loss of hair over the head region with complete epilation around the eyes.

The animals indicated thus far, in experimental groups A, B, C, D, and G along with their controls, have been utilized in behavioral studies during the first 5 weeks following radiation. The weight loss demonstrated by these animals, including controls, during this testing procedure was in part due to forced food deprivation. The calculations for weight loss were based on per cent of difference between controls and experimentals which allows for the standard weight reduction due to a decrease in rations.

Certain animals died or were sacrificed as they became moribund, especially in the 4,000 and 5,000 r dose range. Apparently this high mortality rate in these groups after total head irradiation is more involved than apparent. One additional group of animals, group F, received the same treatment as did group G, with the exception that these animals were not utilized in behavior studies and did not undergo food deprivation. None of the animals in this group expired or appeared moribund before the date of sacrifice 228 days after initial exposure. All animals in this group had well-developed bilateral posterior subcapsular cataracts and the usual epilation.

All groups of animals with the exception of group F underwent a preliminary analysis of behavioral characteristics in operant conditioning equipment. Results from a preliminary analysis of animal behavior indicated that, from the first day of exposure to x-irradiation, all groups of animals through and including the 4,000 r group demonstrated some decrease in

TABLE I
 CUMULATIVE EFFECTS OF X-IRRADIATION
 PHYSICAL FINDINGS^a

Rat	Head dosage <i>r</i> ^b	Weights (gm)		PRT ^c (days)	Hair loss	Head		
		Con	Exp			Bleph- aritis	Cataract	Tumor
(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)
91 ^d	—	359	—	—	—	—	—	—
98	—	479	—	—	—	—	—	—
92	1820	—	580	228	—	—	+	—
93	1820	—	545	228	—	—	—	+ ^e
94	1820	—	440	228	—	—	—	—
95	1820	—	460	228	—	—	—	—
96	1820	—	405	228	—	—	—	—
97	1820	—	460	228	—	—	—	—
99	—	600	—	—	—	—	—	—
106	—	490	—	—	—	—	—	—
100	2000	—	470	228	+	—	+	—
101	2000	—	424	228	+	—	+	+ ^f
102 ^d	2000	—	292	36	+	+	+	—
103	2000	—	475	228	+	—	+	—
104	2000	—	400	228	+	—	+	—
105	2000	—	475	228	+	—	+	—
107	—	460	—	—	—	—	—	—
114	—	442	—	—	—	—	—	—
108	3000	—	400	165	+	+	—	—
109	3000	—	368	165	+	+	—	—
110	3000	—	387	165	+	+	—	—
111	3000	—	375	165	+	+	—	—
112	3000	—	406	165	+	+	—	—
113	3000	—	446	165	+	+	—	—
115	—	429	—	—	—	—	—	—
122	—	416	—	—	—	—	—	—
116	4000	—	299	158	+	+	—	—
117	4000	—	317	158	+	+	—	—
118 ^d	4000	—	173	115	+	+	—	—
119	4000	—	390	158	+	+	—	—
120 ^d	4000	—	303	44	+	+	—	—
121 ^d	4000	—	278	44	+	+	—	—
123	—	378	—	—	—	—	—	—
130	—	369	—	—	—	—	—	—
124 ^d	5000	—	318	44	+	+	—	—
125	5000	—	336	158	+	+	—	—
126 ^d	5000	—	292	44	+	+	—	—
127 ^d	5000	—	324	44	+	+	—	—
128	5000	—	286	116	+	+	—	—
129	5000	—	241	116	+	+	—	—
F46	—	—	—	—	—	—	—	—
F49	—	—	—	—	—	—	—	—
F48	5000	—	—	228	+	+	+	—
F47	5000	—	—	228	+	+	+	—
F50	5000	—	—	228	+	+	+	—
F51	5000	—	—	228	+	+	+	—
F52	5000	—	—	228	+	+	+	—
F54	5000	—	—	228	+	+	+	—

^a KEY: + presence; — absence;

— does not apply.

^d Moribund at sacrifice.

^b Roentgens delivered in air.

^e Benign encapsulated salivary gland (Sclerosing angioma).

^f Post-radiation time

^g Low grade carcinoma.

^h Animals not utilized for behavioral studies; all other animals utilized in behavioral studies.

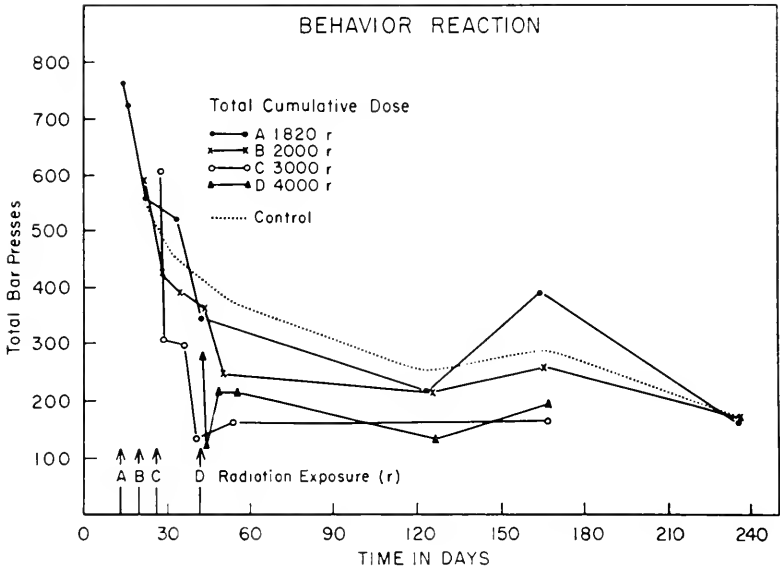


FIG. 1. Behavior reaction pattern demonstrated by control and experimental animals for food reward during 45 minute test periods. Slope of line to right indicates a gradual extinguishment of learned reaction. Bar presses are plotted against time in days.

bar pressing activity for food reward (Fig. 1). Those animals which received 1,000 r showed only minor deviation and after 228 days equaled the control animals. The 2,000 r animal group showed a more striking initial decrease but they, too, equaled the control animal performance by 228 days. Animals receiving the accumulated doses of 3,000 and 4,000 r both demonstrated a severe decrease in activity which reached the lowest performance rates 7 days after receiving their last exposures. During the remaining survival period the 3,000 and 4,000 groups demonstrated little tendency to increase from this low level activity. The obvious decline of all animal performance over the 5 weeks of testing, including controls, was attributed to gradual extinguishment of learned behavior.

Purkinje cells and granule cells of the cerebellum appeared to have undergone certain similar changes at the various levels of exposure and time intervals. These changes appear to have involved a mild to severe loss of Purkinje and granule cells. Hyperchromatic neurons and pyknosis were prevalent throughout most exposure levels. These alterations (Figs. 2 and 3) were scattered throughout the cerebellum.

Cerebral neurons demonstrated pyknosis and hyperchromatosis in scattered areas throughout all levels of radiation. Mild neurofibrillary or axonal

changes, characterized by beading and swelling, were noted in all groups (Fig. 4). Occasional areas of cell necrosis were observed in the 5,000 r level.

Hypothalamic neurons demonstrated occasional evidence of swelling, chromatolysis, pyknosis, and hyperchromatic staining qualities, but these changes were largely inconsistent. The presence of PAS-positive globules within the extraneuronal tissue was clearly evident over the entire dosage range of radiation, demonstrating no specific alteration.

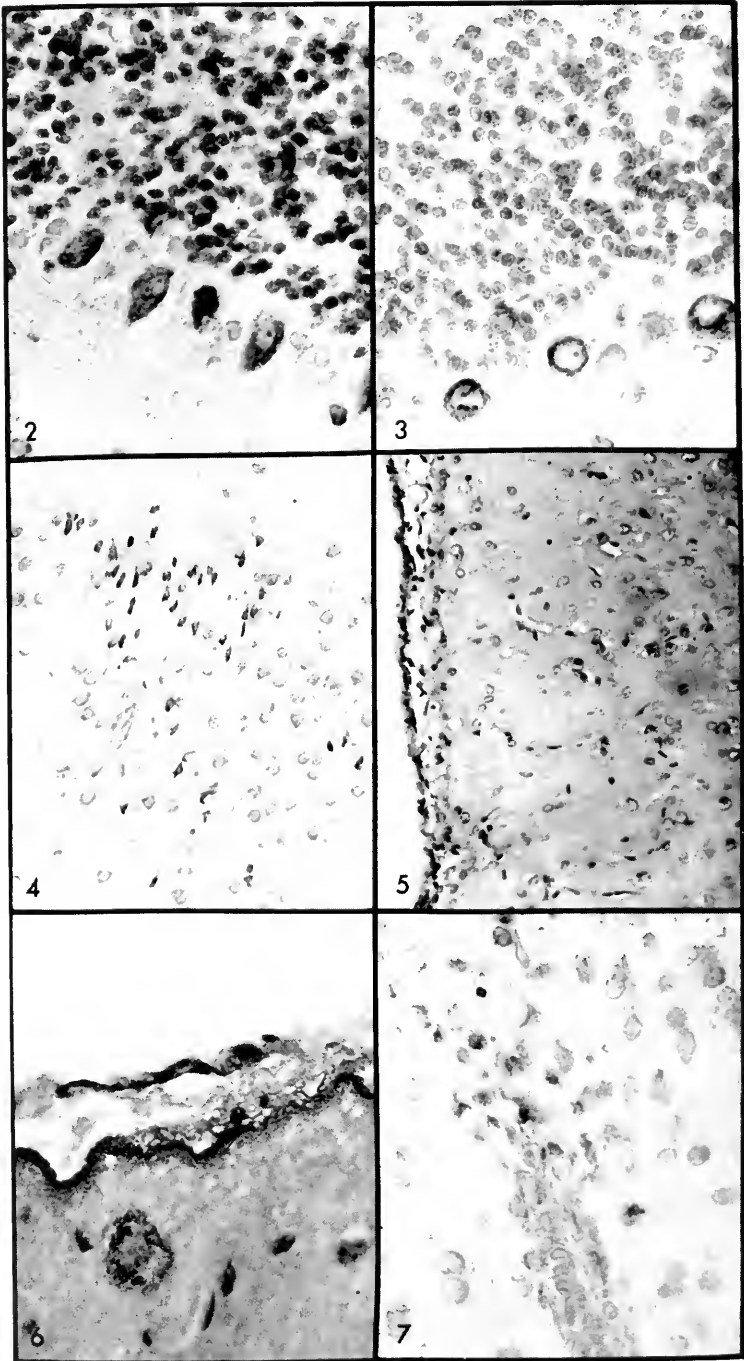
Brain stem neurons were not observed to have undergone major changes at any level of radiation. Those changes that were observed were mild to moderate chromatolysis, pyknosis, and hyperchromatism. In the 5,000 r level maintained 228 days, hyperchromatic neurons were in excess with no chromatolysis observed.

The neuroglial cells underwent slight but consistent degrees of scattered hyperplasia in the subpial cortex (Fig. 5). These changes were somewhat localized and similar at the 1,820, 2,000, and 3,000 r levels. Astrocytes seem to have been the most regularly reactive cells and in many instances could be termed gemistocytic. Glial cell hyperplasia and hypertrophy became quite noticeable in the cortex at the 4,000 and 5,000 r level, especially in the group surviving 228 days. Small areas of infarction (Fig. 6) were in the cortex and subcortical white matter in all groups of animals. There seems to have been a more intensive cellular and fibrillary gliosis about these areas in the 4,000 and 5,000 r animals.

The ependymal and subependymal cells were significantly reactive at all levels of radiation. There appeared to have been a thinning of cells in all groups. The pyknosis and hyperchromatic cells were scattered throughout the varied exposure levels in different ventricular areas as well as within the same areas. It was not uncommon to find nests of hyperchromatic subependymal cell foci (Fig. 7). PAS-positive accumulations of globules both within the basal position of the ependymal cell and subependymal areas were increasingly evident as the level of radiation was increased. Heavy deposits of such materials were noted in the 4,000 and 5,000 r groups.

Meninges, specifically the leptomeninges, and the pial-glial membranes underwent changes (Fig. 5), varying from mild thickening at 1,820 and 2,000 r to moderately severe in the 5,000 r range. Such changes were noted to have been scattered over the brain in focal areas. Throughout all of the varied dosage levels occasional cellular and fibrillary infiltrations were present in these foci. Astrocytes were gemistocytic in such areas along the pial-glial membrane.

Blood vessels underwent various alterations at the different dosage levels. Vessels most often involved were capillaries and small arteries. Mild to moderate hypertrophy and hyperplasia of endothelium and increase in adventitia were noted in animals receiving 1,820, 2,000 and 3,000 r (Fig. 8) and were



severe at 5,000 r. Some petechial hemorrhages were seen. The animals that underwent 4,000 r exposure showed a more severe reaction. Apparently there was a consistent increase in connective tissue, presumably perivascular, from mild to severe within the hypothalamus from 1,820 to 5,000 r (Figs. 9, 10, and 11).

Discussion

There were no remarkable or otherwise consistently observable neurologic deficits in animal activity during the 228 days following irradiation. If any such alteration could be detected by observation alone, one might describe malaise.

The animals' physical appearances were altered by cataracts and varying degrees of hair loss about the head. There was complete epilation of hair immediately surrounding the eyes in a circumscribing area approximately 3 mm wide. In most instances the hair over the remaining portion of the rat's body lost its usual healthy sheen and became ruffled. Some of the animals had inflamed margins of the eyes which might be described as blepharitis.

Analysis of weights indicated that beginning with group B at 2,000 r level there was a decline in animal body weight. This was in the approximate magnitude of 12% of control animal weight at the time of sacrifice 228 days after initial exposure. Additional cumulative exposure to x-ray revealed a similar weight loss in the animals at sacrifice. The per cent of difference between the remaining groups of control and experimental animal weights became increasingly greater with increasing dosage. The greatest factor of difference was at the 5,000 r level where a loss of 29% was noted. However, the percentage weight differences indicated were taken only from weight differences at the time of sacrifice for the group as an average. In

FIG. 2. Cerebellar granule cell pyknosis and hyperchromatic staining reaction 228 days following 5,000 r. Nissl; \times 320.

FIG. 3. Cerebellar granule cells demonstrating normal staining reaction. Nissl; \times 320.

FIG. 4. Cerebral cortex focal area of pyknotic and hyperchromatic stained neurons 228 days following 5,000 r. Nissl; \times 130.

FIG. 5. Cerebral cortex cellular gliosis and thickened pial-glial membrane with some cellular and fibrous infiltration of subarachnoid space 228 days following 5,000 r. Hematoxylin and eosin; \times 130.

FIG. 6. Cerebral cortical infarct. Small with slight encapsulation of necrotic area 228 days following 1,820 r. Verhoeff; \times 320.

FIG. 7. Ependymal cells and area of subependymal cell pyknosis and hyperchromatic staining reaction 228 days following 5,000 r. Nissl; \times 320.

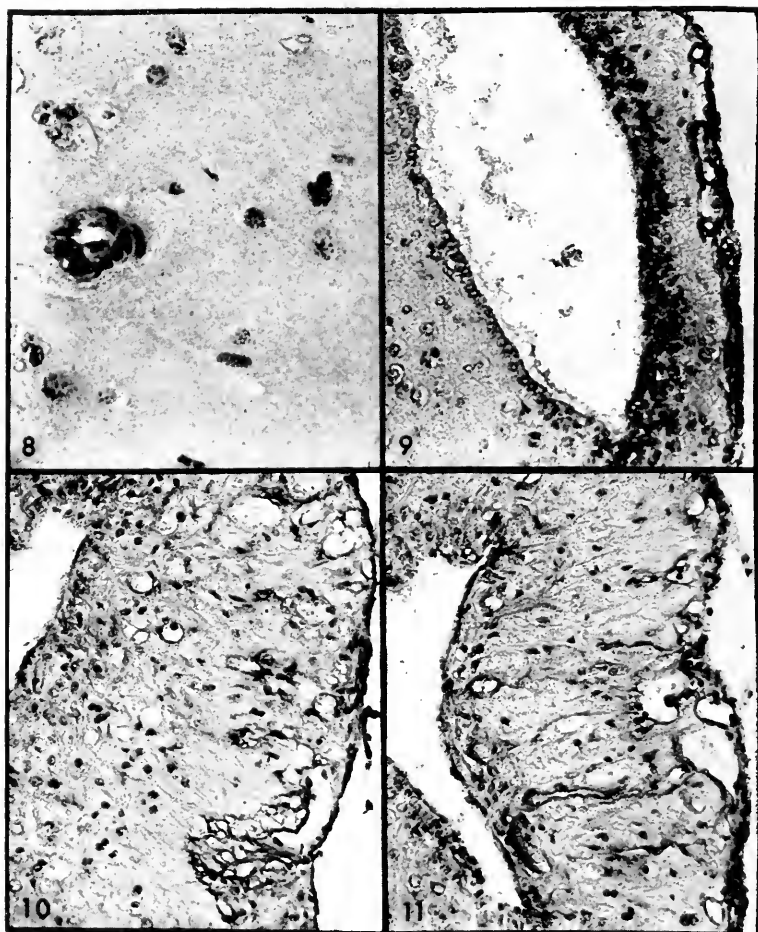


FIG. 8. Cerebral cortical arteriole demonstrating hypertrophy and hyperplasia of adventitia with large hyperchromatic nuclei 228 days following 1,320 r. Verhoeff; $\times 64$.

FIG. 9. Normal rat hypothalamus and third ventricle. Verhoeff; $\times 130$.

FIG. 10. Hypothalamus with increased vascularity and perivascular connective tissue. Increased numbers of nuclei 228 days following 2,000 r. Verhoeff; $\times 130$.

FIG. 11. Hypothalamus with increased vascularity and perivascular connective tissue. Numbers of nuclei present less than demonstrated in Fig. 9 228 days following 5,000 r. Verhoeff; $\times 130$.

nearly all cases there was a favorable increase in the weights approximately one week following the last exposure administered to that group. One notable exception was in group G after reaching the 5,000 r level. This group failed to show any tendency to increase weight until after 120 days.

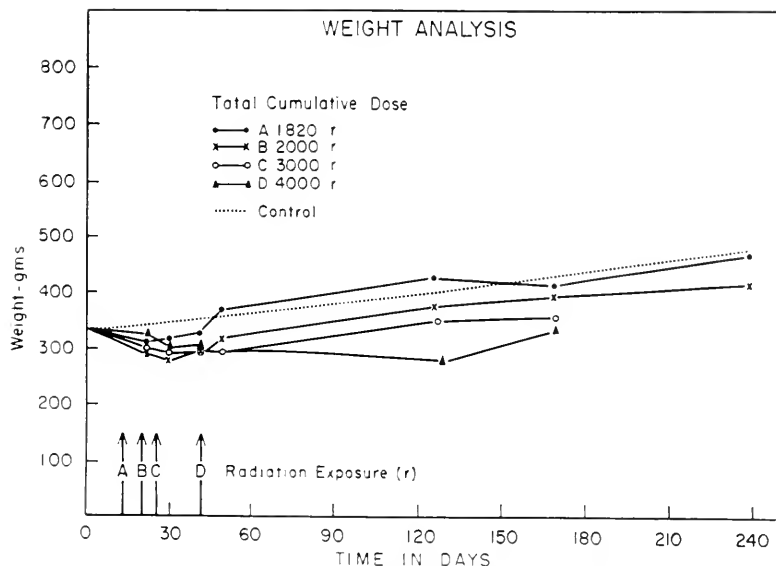


FIG. 12. Analysis of average animal weights in grams for control and experimental animals plotted against time in days.

In no instance were the experimental animals in groups B, C, and D capable of equaling their control litter mates' weight record during the 228-day interval before sacrifice. Animals in the control and experimental groups underwent 5 weeks of behavioral studies which required a substantial decrease in food intake during observation. During this period of food deprivation the control animal weights continued to rise (Fig. 12), emphasizing that the decrease in body weight and life span of the rat following x-irradiation of the central nervous system is further magnified by forced reduction in available nutritional requirements.

Cumulative exposure of the rat head to x-irradiation has been noted to cause certain physical and histologic alterations. In the total accumulated dosages 1,820, 2,000, 3,000, 4,000 and 5,000 r these alterations were strikingly similar in character and appeared to show a quantitative relationship to time and accumulated dose level.

The cell changes, although quite similar in appearance, did display certain specific sensitivity. For example, in the cerebellum both Purkinje and granule cell neurons underwent the severest reactions in rats receiving 5,000 r after 116 days. This may be contrasted to those lesser cellular changes that were moderate in rats receiving 5,000 r and surviving 228 days. It is possible that the intervening time between 116 and 228 days was sufficient to allow cell recovery. The cytologic changes were noticeably more severe in

TABLE II
 CUMULATIVE EFFECTS OF X-IRRADIATION

Physical data							Microscopic
<i>Head</i>			Neurons				
<i>Rats no.</i>	<i>dosage (r)^a</i>	<i>PRT (days)</i>	<i>Purkinje cerebellum</i>	<i>Granule cerebellum</i>	<i>Pyramidal cortex</i>	<i>Tuber hypothalamus</i>	<i>Large brain stem</i>
(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)
6 ^b	1820	228	Mild hyperchromatic	Mild scattered pyknotic, hyperchromatic	Scattered hyperchromatic, pyknotic, mild neurofibrillary changes	Mild to moderate, (PAS) positive globules, mild hyperchromatic pyknotic	—
5 ^b	2000	228	Mild hyperchromatic, pyknotic	Mild scattered pyknotic, hyperchromatic	Scattered pyknotic, mild neurofibrillary beading, hyperchromatic	Heavy, (PAS) positive globules, hyperchromatic, pyknotic	Mild chromatolysis, hyperchromatic, pyknotic
6 ^b	3000	165	Mild hyperchromatic, pyknotic	Moderate scattered pyknotic, hyperchromatic	Scattered pyknotic, mild neurofibrillary beading hyperchromatic	Moderate (PAS) positive globules, mild pyknotic, hyperchromatic	Mild chromatolysis, hyperchromatic, pyknotic
4 ^b	4000	158	Mild hyperchromatic, pyknotic	Moderate scattered pyknotic hyperchromatic	Scattered pyknotic, mild, neurofibrillary beading, hyperchromatic	Moderate (PAS) positive globules, moderate hyperchromatic, pyknotic	Moderate chromatolysis, hyperchromatic, pyknotic
2 ^b	5000	116	Severe hyperchromatic, pyknotic	Severe scattered pyknotic, hyperchromatic	Scattered mild neurofibrillary beading, cell necrosis	Heavy, (PAS) positive globules, hyperchromatic, pyknotic	Moderate chromatolysis, hyperchromatic, pyknotic
6 ^c	5000	228	Moderate to severe hyperchromatic, pyknotic	Mild scattered pyknotic hyperchromatic	Scattered pyknotic, mild neurofibrillary beading, cell necrosis	Heavy, (PAS) positive globules, hyperchromatic, pyknotic	Moderate to mild, hyperchromatic, pyknotic

^a Roentgens delivered in air.

^b Animals utilized in behavioral studies.

^c Animals not utilized in behavioral studies.

TABLE II (Continued)

CUMULATIVE EFFECTS OF X-IRRADIATION

Microscopic						
Glia			Non-neural			
<i>Oligo-</i>	<i>Astro-</i>	<i>Micro-</i>	<i>Ependyma</i> <i>sub-</i> <i>ependyma</i>	<i>Meninges</i>	<i>Blood</i> <i>vessels</i>	
(I)	(J)	(K)	(L)	(M)	(N)	
Scattered areas of cellular gliosis in sub-pial cortex gemestocytic astrocytes, occasional small infarct			Mild pyknotic, (PAS) positive globules	Mild scattered thickening, cellular infiltration	Moderate increase in perivascular connective tissue and blood vessels in hypothalamus, hypertrophy, hyperplasia of endothelium and adventitia	
Occasional small infarct scattered cellular gliosis in sub-pial cortex			Heavy (PAS) positive globules	Mild scattered thickening, cellular infiltration	Moderate increase in perivascular connective tissue and blood vessels in hypothalamus, hypertrophy, hyperplasia of endothelium and adventitia	
Scattered areas of cellular gliosis in sub-pial cortex			Hyperchromatic, pyknotic, (PAS) positive globules	Mild scattered thickening	Mild increase in perivascular connective tissue and blood vessels in hypothalamus, mild hypertrophy and hyperplasia of endothelium and adventitia	
Mild cellular and fibrillary gliosis around infarcts in white matter			Heavy (PAS) positive globules	Mild scattered thickening	Moderate increase in perivascular connective tissue and blood vessels in hypothalamus, mild hypertrophy and hyperplasia of endothelium and adventitia	
Mild cellular and fibrillary gliosis around infarcts in white matter			Heavy (PAS) positive globules	Moderate scattered thickening	Moderate increase in perivascular connective tissue and blood vessels in hypothalamus, moderate hypertrophy and hyperplasia of endothelium and adventitia	
Moderate cellular and fibrillary gliosis in sub-pial cortex, gemestocytic astrocytes, small infarcts in white matter			Heavy (PAS) positive globules	Moderate scattered thickening, cellular infiltration	Heavy increase in perivascular connective tissue and blood vessels in hypothalamus, severe hypertrophy and hyperplasia of endothelium and adventitia	

the granule cells than in Purkinje cells, appearing in both as nuclear pyknosis and hyperchromatic staining. There appeared to be an appreciable decrease in cellularity most severely affecting the 5,000 r level at 116 days.

Wilson (1960) irradiated monkeys with whole-body exposure to cobalt-60 (γ) from 400 to 40,000 r and stressed the changes that occurred in animals dying during the first 54 hours post-irradiation. Cerebellar granule cells underwent nuclear pyknosis especially within the innermost layers with a decreased cellularity explained on the basis of decreased nuclear area. Vogel (1959) administered massive doses of gamma radiation to the head of rabbits and dogs sacrificed at intervals up to 10 days. He reported that the altered granule cells of the cerebellum demonstrated pyknotic and hyperchromatic nuclei most notably within the first 24 hours after exposure. His opinion was that the recovery phase was completed by 72 hours after radiation.

The tissues studied in this project are decidedly those of chronic classification sacrificed up to 228 days after exposure. The results from our study indicate that x-irradiation of the rat head will elicit slight changes in cerebellar neurons after 228 days and that a dose as high as 5,000 r is capable of causing severe cell change during the 116 days. In all instances the changes described through and including 228 days after radiation were accompanied by decreased cellularity. It would only be speculation to propose cell loss. The only absolute measure of this would have to incorporate a quantitative analysis.

Wilson (1960) and Vogel's (1959) findings that the granule cells in the cerebellum undergo acute changes followed by recovery during a slightly later period after radiation, would seem to indicate the existence of secondary effects. This effect noted months after exposure may further demonstrate its recovery phase at a still later date.

Neurons in the cerebral cortex, hypothalamus, and brain stem seldom appeared to be necrotic and were probably of reversible nature. Such changes were described as shrinkage in both cytoplasm and karyoplasm or pyknosis. It is suspected that the decrease in cell or nuclear volume or both is paramount to the hyperchromatic staining quality, as well as to the appearance of decreased cellularity. Peculiar to these alterations was the obvious scattering of cell changes within identical structure, thus indicating certain differences in radiosensitivity within the particular structure under observation. In view of the perfusion-fixation methods utilized for this study, it is felt that these observations are reasonably free from artifact. These cell changes were present in varying amounts throughout all radiation levels, which seemingly indicates little or no direct qualitative relationship between dose and time within the parameters of this study. Only at the 5,000 r level were there evidences of significant neuronal necrosis. The absence of major

structural alterations among cortical neurons has been pointed out repeatedly by many investigators (Gerstner *et al.*, 1956; Haymaker *et al.*, 1954, 1958; Russell *et al.*, 1949) for a wide range of experimental variables.

Gliosis was evident in increasing quantity in direct relationship to the increasing level of cumulated dosage or the postirradiation time or both. The gliosis was characterized by increased cellularity and, for the most part, appeared within the molecular layer of the cortex, specifically the subpial area. The cell hyperplasia occurred predominantly in astrocytes, many of which appeared gemistocytic.

Oligodendroglia and microglia remained relatively nonresponsive or negative. Increased fibrillary responses were noted only as thickenings or encapsulating responses about small areas of infarct. Arnold and Bailey (1954) have placed particular emphasis on glial response in the monkey brain to high and low energy x-rays. They reported that glial responses were related to dose intensity and time. High energy, low doses, e.g., 3,000–5,000 r, initiated astrocytic responses as hypertrophy and hyperplasia months after exposure. Globus and others (1952) implanted radon seeds in the brain stem of dogs and after 106 days reported the appearance of astrogliosis. In an earlier study of glial response to 1,500 r x-irradiation in the guinea pig, Brownson (1960) found no significant alteration of either qualitative or quantitative relationships of perineuronal satellite glial cells in various ages of animals 33 days after exposure. Glia have been reported to demonstrate responses related to duration of time after irradiation, that is, initially inhibition, then recovery, and eventually intense gliosis (Arnold and Bailey, 1954).

Ependymal and subependymal cells lining the ventricular system appeared to demonstrate selective sensitivity. Rats, 7 days after exposure to cumulated doses between 1,000–5,000 r of total head x-ray, frequently demonstrated ependymal and subependymal cell swelling and pyknosis (Brownson, 1961). Hicks and Montgomery (1952) have noted subependymal necrosis in rats 6–12 hours following head exposure to 1,200 r. There were occasional scattered nuclear pyknosis of ependymal cells and nests of hyperchromatic subependymal cells in all levels of x-irradiation doses. Heavy accumulations of PAS-positive globules were present in all radiation groups. These dark globules were located within the basal portion of ependymal cells and subjacent extracellular areas and were not affected by previous treatment with salivary enzymes (diastase).

Changes in the walls of blood vessels were regular throughout the tissues studied. These alterations predominantly appeared as hypertrophy and hyperplasia of the adventitia, often with extra nuclei. Less obvious were changes in endothelium demonstrated as hypertrophy and hyperplasia. The total picture suggested that the dosage accumulated up to and including

5,000 r had little differential effect on blood vessels. However, the added factor of 228 days in either low level or high level dosage was critical, as evidenced by increased severity of reaction.

Lyman and his colleagues (1933), in an early study of the effects of roentgen rays on the central nervous system of adult dogs, reported that 6 months after irradiation various sized blood vessels of the brain had progressive changes. These changes were quite similar to those seen in our animals, with marked hyaline degeneration and obliterating sclerosis of arterioles.

In the hypothalamus of radiated animals there was especially heavy connective tissue, which might be thought of as an increased vascularity involving pial-glial extensions from the surrounding meninges. Furthermore, there appeared to be an increased number of nuclei, mostly neuroglial. Increases in cellularity in animals receiving 2,000 r at 228 days following exposure were less severe in animals which received 5,000 r after 228 days.

Conclusions

Rats were exposed to total head x-irradiation in doses of 1,000 r per week until accumulations by groups of six animals ranged from 1,820 to 5,000 r.

During the interval following each exposure to ionizing rays, animals in all dosage levels demonstrated changes in behavior, physical appearance, and weight. Quantitative food reduction had a deleterious effect on the general well-being and survival of the animals receiving the larger accumulated doses of x-irradiation.

Neurons in the cerebral cortex, brain stem, and cerebellum demonstrated slight to severe alterations throughout all dose levels. Generally, cell change became more frequent and in some instances more severe with increasing accumulation of total roentgens and time following exposure.

Neuroglial elements underwent variable minor alteration. Astrocytes were most frequently observed undergoing hyperplasia and hypertrophy.

Meningeal thickening with some evidence of cellular and fibrillary infiltration was observed throughout all dose levels.

Endothelial and adventitial changes were noted in small and medium size intramedullary vessels with perivascular accumulations of large nucleated cells. Changes in blood vessels were directly related to increasing dosage and time.

General observation indicated that changes in cells leading to necrosis were similar throughout the various dose levels and days after exposure. The increasing severity in reactions associated with time and dose were more quantitative than qualitative.

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Behavioral and Histologic Effects of Head Irradiation in Newborn Rats

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Introduction

The effect of ionizing radiation on the nervous system during various stages of its growth and development has elicited a broad spectrum of pathologic responses. Early experiments clearly demonstrated the relative vulnerability to radium and x-ray of the brain in young guinea pigs, rats, dogs, rabbits, and kittens compared to the adult (Danysz, 1903; Turner and George, 1910), resulting in stunted growth and abnormal neurologic findings. The teratogenic effects of ionizing radiation in the embryo has received the attention of many excellent studies clarifying the differential response during the various stages of development from the earliest embryonic stage to the end of gestation (Bagg, 1922; Job *et al.*, 1935; Warkany and Schraffenberger, 1947; Russell and Russell, 1954; Hicks, 1950, 1953a,b, 1954a,b). The nature of the cerebral anomalies depended less on the dose of radiation than on the time of gestation when administered. The degree of sensitivity has been related to the extreme sensitivity of the undifferentiated multi-potential cell in the very early embryonic stage and later in gestation to the early neural cell or neuroblast (Rugh and Grupp, 1959; Hicks, 1950, 1953a,b, 1954a,b).

Regardless of the ease with which the embryonic central nervous system can be damaged, an increasing gradient of radioresistance develops as gestation progresses. The degree of this sensitivity is demonstrated by the fact that 15 r can produce exencephalia in the very early embryo (Rugh and Grupp, 1959). In contrast, the minimal dose which has caused pathologic lesions in the adult monkeys has been 1,500 r when slight neuronal damage was seen, but some of the animals after 4 to 8 months had elapsed developed focal seizures. With larger doses there occurred blood brain barrier changes, astrocytic and neuronal damage with lesions, particularly affecting the hypothalamus and the medulla. Several neurologic signs of varying severity developed according to the radiation dose (Clemente and Holst, 1954;

Arnold *et al.*, 1954a,b,c; Davidoff *et al.*, 1938). Many adult rats receiving 1,000 r to the head alone appeared normal in every respect (Bennett, 1960). In young head-irradiated rats of unspecified age, it has been reported that subependymal cells of the lateral ventricle were destroyed with 200 r. However, the associated neurologic findings were not mentioned (Hicks and Montgomery, 1952).

The time sequence during which this radioresistance develops in the neonatal period associated with the maturation of the brain has not been as clearly demonstrated. In the fetal guinea pig, the neuronal differentiation is closely correlated with biochemic, functional, and other morphologic changes in the last trimester of gestation (Flexner, 1953). The monograph edited by Waelsch (1955) emphasizes that in the rat similar developments occur during the first 10 postnatal days. Ionizing radiation might well alter these highly integrated events suggesting the possibility of a changing pattern of response to irradiation with increasing age. Our study was undertaken to systematically observe the immediate and long term behavioral and histologic effects of single direct radiation graded doses to the brain of neonatal and young rats.

Method

A total of 112 rats, aged 8 hours to 15 days, received x-radiation to the head only. Single doses of radiation were given at four levels: 125, 300, 500, and 1,000 r. Observations were made on 48 control animals. From 48 to 72 hours after irradiation, 28 animals were autopsied. The remaining 84 animals were observed up to 14 months and sacrificed as described in previous reports (Yamazaki *et al.*, 1960; Clemente, *et al.*, 1960).

Observations

The most interesting data in our study indicated that the severity of the neurologic and pathologic findings were dependent on two factors: the intensity of the radiation dose and the stage of postnatal maturation of the brain at the time of radiation. The most severe brain reactions were found in rats radiated on the first 3 or 4 days postnatally. Animals irradiated on the 5th postnatal day definitely showed fewer neurologic signs than rats radiated on the first 4 days. In the 5-day rat, 500 r administered to the head produced neurologic signs in a little less than half of the irradiated rats, whereas all of the animals irradiated with 300 r and 500 r prior to this time developed some evidence of central nervous system involvement, and 1,000 r often proved lethal during the first few days of life. Animals radiated after the 5th postnatal day showed a marked decrease in the incidence and

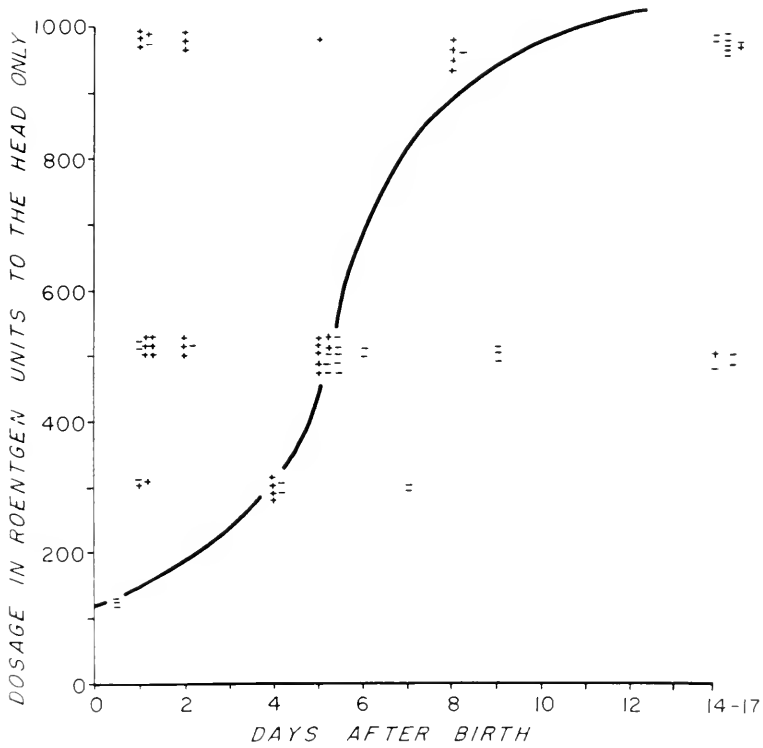


FIG. 1. A graph showing a dose-age curve with respect to the production of neurologic signs by head radiation in the neonatal rat. Most of the animals to the left of the curve had clinical signs involving the nervous system, while most of the animals to the right of the curve did not. (From Clemente *et al.*, 1960, p. 670.)

severity of neurologic signs, and radiation on the 15th day resulted in no visible neurologic findings, even though doses of 1,000 r were administered (Fig. 1).

Similar age-dose relationship was demonstrated in regard to mortality, retardation of body growth, head growth, and cataract formation. Tremor was the most frequently observed neurologic sign and appeared as early as the 2nd week in animals irradiated with 1,000 r during the first 5 days postnatally. Other neurologic findings were incoordination, paresis, a peculiar propulsive gait, and falling backward when animals attempted to stand on their hind legs. Seizures occurred in a few animals. Small heads were noted in 23 cases. Cataracts developed in 7 rats, 6 of whom had small heads, and the greatest incidence of cataract formation occurred in animals that had received 500 r on the 5th day. It is possible that a higher incidence

TABLE I

WEIGHT OF HEAD-IRRADIATED RATS AT 3 MONTHS OF AGE

	<i>No. of animals</i>	<i>Range (gm)</i>	<i>Average weight (gm)</i>
<i>Male</i>			
500 r			
Rx 1 - 5 days	10	170-280	234
Rx 6 - 15 days	5	255-290	269
1,000 r			
Rx 1 - 5 days	2	140-220	180
Rx 6 - 15 days	4	220-260	246
<i>Female</i>			
Controls	17	160-225	185
500 r			
Rx 1 - 5 days	12	125-185	165
Rx 6 - 15 days	12	170-220	198
1,000 r			
Rx 1 - 5 days	2	110-140	125
Rx 6 - 15 days	6	140-190	175

of eye pathology might have been found in animals radiated under 4 days of age had they been able to survive for longer periods.

Animals radiated during the first 5 days of life tended to weigh less than controls (Table I). Mortality was also greatest in the animals irradiated during this immediate neonatal period, and, with one exception, all deaths under 3 months of age occurred in this group. The average survival periods of the animals that died under 3 months of age were 0.9 months for the 1,000 r animals, and 2.3 and 2.4 months for the 300 and 500 r animals.

TABLE II

MORTALITY IN HEAD-IRRADIATED RATS

<i>Radiation dose</i>	<i>Age at the time of irradiation</i>			
	<i>(r)</i>	<i>Under 4 days</i>	<i>5-6 days</i>	<i>7-15 days</i>
125		0/3	--	--
300		3/9	--	0/2
500		5/13 ^a	5/17	0/3
1,000		9/11 ^b	1/2	2/14

^a One anesthetic death.

^b Two anesthetic deaths.

respectively. The difference in brain involvement between the animals irradiated during the first 5 days of life compared to the older animals is demonstrated by the following examples:

500 r: Rat No. 32 was irradiated on the 5th day of life. Tremors were first noted at 6 weeks. These became progressively more severe, and incoordination and weakness of the hind limbs were noted from the 3rd month. The eyes and head were small, and cataracts developed in one eye. Of 7 animals irradiated after the 8th day, only rat No. 18 developed any neurologic sign, a mild tremor first noted at the 10th month.

1000 r: Animals irradiated on the first days of life at this dose level demonstrate the marked vulnerability of the brain at this age. By the 2nd week of life they could easily be separated from their control litter mates by their small size, weighing an average of 18 gm in comparison to 39 gm for the controls. Marked tremor was already present, eyes were narrowed and small, the animals tended to drag their hind limbs and encountered difficulty in righting themselves. Other animals revealed a marked hyperactivity, darting about the cage in a purposeless, random manner.

The incidence and severity of the pathologic findings correlated closely with the neurologic findings in the irradiated animals.

The most consistent and outstanding finding was the ~~difference~~ decrease in size of the cerebellum in the irradiated animal in comparison to the controls. When the cranial vault is removed in a newborn rat, the cerebellum is in close approximation to the inferior colliculi of the brain stem. Within a week after birth, the hemispheric colliculi are overgrown by the developing cerebellum and cerebral cortex in the normal animal, whereas in the irradiated animals the colliculi are very prominent even 3 weeks after birth. The cerebellum remaining is underdeveloped. Petechial hemorrhages were noted on the surface of the cerebral cortex, although noticeably absent elsewhere. When the irradiated brain was cut, the tissue had a more gelatinous consistency than the normal brain.

In animals allowed to survive for longer periods of time, the generally retarded size of the brain was striking in addition to a distortion in the configuration of the cerebellum. The meninges often appeared thickened and fibrous, and the dura was firmly adherent to the brain surface, although it was possible to separate it from the cerebral cortex. The posterior part of the cortex often was depressed as if the cortex were thinner in this area.

In the animals sacrificed 48 to 72 hours after irradiation, widespread changes in the vasculature in the brain and brain stem were noted, and more specific lesions were identified in the choroid plexus and meninges. Vascular lesions occurred after dosages of at least 500 r in animals irradiated within the first 5 days postnatally and in animals administered 1,000 r between the 5th and 10th postnatal days. The most common vascular

phenomena observed 2 to 3 days after irradiation seemed to be simple swelling of the endothelial cell cytoplasm with a concomitant increase in nuclear basophilia (Fig. 2), petechial hemorrhages at the capillary level, and, in some instances, polymorphonuclear vasculitis. In the choroid

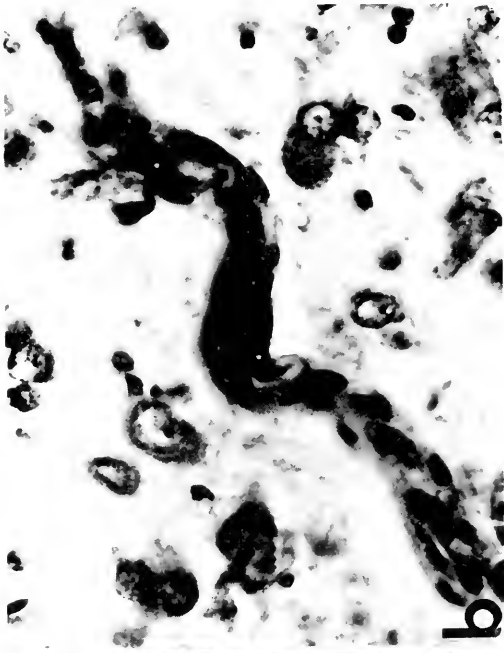
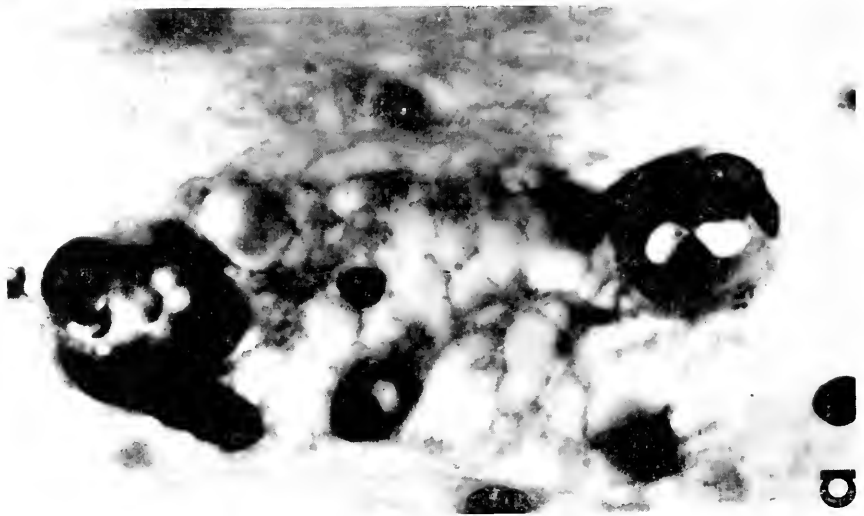


Fig. 2. Sections cut transversely (a) and longitudinally (b) through precapillaries in the medullary reticular formation of a 10-month-5-day-old rat that had been given 1000 r to the head on the 2nd day postnatally. Note the thickened vessel wall and increased basophilia. This animal had tremors, a wobbly gait, and other neurologic signs, and most of the small vessels of the brain appeared similar to those pictured here. Thionine stain. a, $\times 385$; b, $\times 190$. (From Clemente *et al.*, 1960, p. 670.)



plexus, in addition to the capillary changes which were at times limited to the plexus in the fourth ventricle, the parenchymal cells appeared swollen and cytoplasmic material more watery than normal, and the cells contained relatively little granular material. Acute inflammatory reactions noted in the meninges were observed in most animals given over 300 r within the first 8 postnatal days (Fig. 3). The meningitis, characterized by neutro-

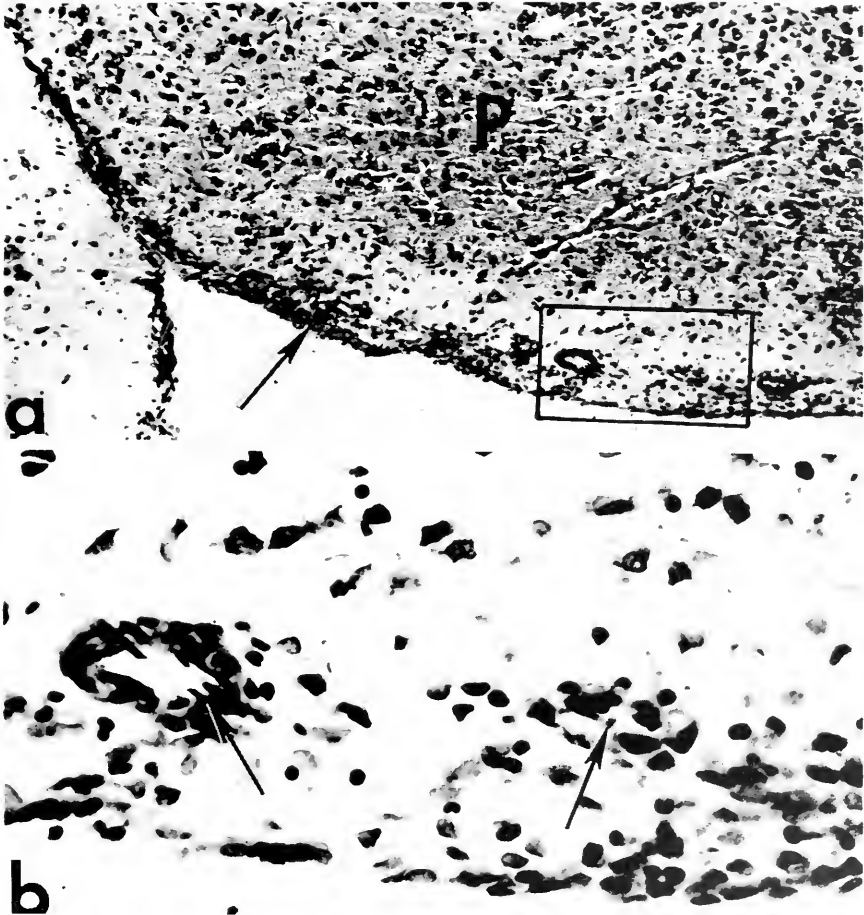


FIG. 3. (a) A photomicrograph showing, in transverse section, the ventral mid-pontile region in a 23-day-old rat that had been given 1000 r to the head on the first postnatal day. Notice the thickened meningeal coverings (arrow) and the thickened vascular channels of the pia mater. ($\times 32$) (b) An enlargement of the block outlined in a, showing more clearly the thickened vascular walls (arrows). The lumen of the vessel on the right was almost completely obstructed. Thionine stain ($\times 130$) (From Clemente *et al.*, 1960, p. 670.)

philic accumulation in the dura and around the vasculature of the pia mater, tended to be more focal with the lower radiation dose and more intense and generalized with the higher radiation dosages (1,000 r).

In the long term series (survival periods of 20 days to 14 months), vascular pathology consisted principally of an increase in the thickness of vessel walls with a consequent narrowing of the vascular lumen. This was especially evident in vessels that were seen within the meningeal layers on the surfaces of the brain, although at times deeper vessels also showed unmistakable signs of damage. The thickening in the blood vessel walls was most pronounced in the tunica media and in the tunica intima, so that the total vessel diameter did not increase, but the lumen decreased. Around such fibrosed vessels there often were signs of a minor chronic inflammatory process. In these instances, the inflammatory reaction, characterized by the presence of mononuclear cells, was possibly the residual of the more acute inflammatory reactions observed in the short term series.

Lesions observed over a longer time revealed hemorrhage and necrosis in areas where capillary damage was most evident in the brain of animals 48 to 72 hours after irradiation, suggesting that this initial lesion was an acute phase of the lesions observed at later periods. The areas that were most often involved included the cerebellum, basal ganglia (especially the globus pallidus and caudate nucleus), diencephalon, and medulla. In many rats, sections of the brain stem showed an excessive number of neuroglial cells, even though no distinct localized lesions could be seen. This hypergliosis unassociated with apparent neuronal damage was most intense in white matter and most frequent in the midbrain, pons, and medulla, but was also seen in the caudate nucleus and globus pallidus.

Evidence of early inflammatory lesions in the choroid plexus was visible as small contracted scars characterized by connective tissue proliferation. Marked enlargement of the lateral ventricles developed in animals receiving 1,000 r during the immediate neonatal period (Fig. 4). With 500 r the distension of the ventricles was not as noticeable. The third and fourth ventricles were never as severely involved. With lateral ventricular distension, there occurred a concomitant decrease in thickness of the cortical layers. The packing density of the cortical neurons looked like that of the 3-day-old rat, although some of the animals had lived from 3 weeks to 3 months. The cortical neurons appeared intact; however, the cortical mantle resembled that of a much younger animal.

The dura and pial coverings of the brain increased in thickness with time. Proliferation of connective tissue and a mild secondary mononuclear infiltration occurred, especially in animals irradiated with 1,000 r between the 5th and 14th day and surviving for 9 months to one year. With meningeal

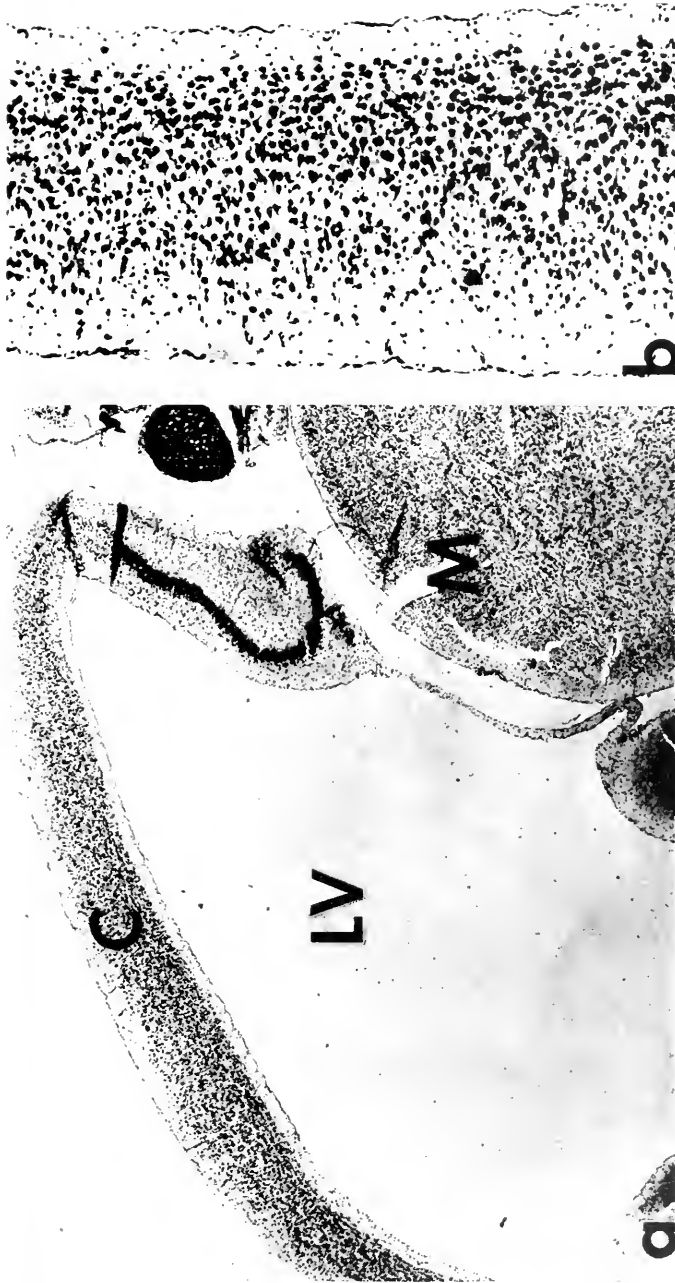


FIG. 4. (a) A transverse section through the midbrain (M), lateral ventricle (LV) and cerebral cortex (C) in a 20-day-old rat that had been given 1,000 r to the head on the second postnatal day. Notice the marked hydrocephalic brain. ($\times 20$) (b) A portion of the cerebral cortex in the animal shown in a, illustrating the closely packed cortical cells. The thickness of the cortex was approximately 50% that of a normal rat at this age. Thionine stain. ($\times 60$) (From Clemente *et al.*, 1960, p. 670.)

thickening, there invariably was some thickening in the walls of the medium sized and smaller arteries of the pial layers.

Discussion

The neurologic findings observed in the rats in this study are similar to the findings of other investigators in their studies on irradiated newborn and young guinea pigs, rabbits, kittens, and dogs (Danysz, 1903; Turner and George, 1910; Brunner, 1920; Nemenov, 1934; Demel 1926; Mogilnitzky and Podljaschuk, 1930). Tremors, clonic twitching, epileptoid seizures, paralysis of extremities, retardation of head and body size, and poor mental performance have been reported. Previously, however, a systematic sequential age-dose relationship during the neonatal period had not been demonstrated.

The effect of radiation on the developing human brain is not as well documented as are the animal studies. The occurrence of microencephaly, mental defectives, hydrocephaly, ossification defects of the cranial bones, eye defects, and skeletal abnormalities in children born after maternal pelvic irradiation has been reported (Murphy, 1929; Goldstein and Murphy, 1929). Intrauterine exposure of the fetus to atomic radiation in sufficient amount to cause acute radiation effects on the mother has resulted in children with significantly smaller head circumferences than in the control group (Plummer, 1952; Yamazaki *et al.*, 1954). Information concerning irradiation of the head alone in infants and children is scanty. Children irradiated for scalp lesions developed epilation in three weeks, and, after a year or so, hemiparesis and seizures developed in one patient (Lorey and Schaltenbrand, 1932) and a subdural hematoma was reported. In contrast to this, over 3,000 persons, many of whom were children, receiving epilating doses of radiation for treatment of tinea capitis had no evidence of injury to the brain (Mackee and Cipollaro, 1946). In another report, children over 3 years who received radiation to the head for a similar condition did not develop any abnormal neurologic findings (Macleod, 1909). Children under 3 years were not radiated to avoid any possibility of radiation injury. Periods of somnolence lasting from 4 to 14 days were noted in 30 out of 1,100 children epilating by radiation therapy for ringworm of the scalp (Druckmann, 1929). A similar type of reaction was noted in adult human volunteers who received approximately 150 r to the diencephalic area (Birkner and Trautmann, 1953). Disturbances of sleep-wake patterns and changes in gonadal function also were noted. In this regard, radiosensitivity of the hypothalamus and brain stem has been demonstrated in recent studies by Clemente and Holst (1954) and Arnold and his associates (1954a,b,c). A report on the survivors of the atomic bomb explosions who exhibited no sign of burns,

trauma, or generalized radiation illness, but who were assumed to have been radiated to the head alone, revealed that the most extensive involvement of the brain was in children (Uchimura and Shiraki, 1952).

The relatively abrupt change in the degree of radiosensitivity of the neonatal rat brain occurs at a time when unique morphologic, functional, and biochemical changes are taking place, and this seems to present an interesting temporal correlation with the findings reported in this study (Waelsch, 1955; Richter, 1957).

The general growth rate of the neonatal rat brain is reflected by the five-fold increase in weight by the end of the 2nd postnatal week and this represents almost 80% of the weight of the adult brain (Potter *et al.*, 1945; Folch-Pi, 1955). The cerebral cortex is gaining weight proportional to the growth of the brain as a whole, but the cerebellum is gaining nearly three times as much weight, and the brain stem is accumulating only one-half its birth weight during the same period (Sugino, 1917). However, the vascularity of the brain undergoes little change during the first 5 days, but between the 5th and 10th day a definite increase in vascular richness occurs. After the 10th day the density of the capillary bed increases rapidly, and a concomitant richness of the capillary bed increases in oxidase content and mitochondria simultaneously (Campbell, 1939). An example of the neuronal differentiation taking place during the neonatal period is presented by the change in the packing density of the neurons in the cerebral cortex. This density decreased rapidly between the 3rd and 4th day after birth and then more slowly, "no change taking place after the 17th day" (Haddara, 1956). This would indicate an increase in cytoplasmic constituents along with the development of a more elaborate cortical dendritic system.

It is during the first 2 week period postnatally that the electroencephalogram becomes more regular and assumes the characteristics seen in adult rats (Crain, 1952). The ability of the rat to withstand anoxia is greatest in the immediate postnatal period, and shortly after birth during the first 5 to 6 days there is a loss of tolerance to anoxia (Fazekas *et al.*, 1941).

Immature budding vessels may well be more differentially sensitive to noxious agents than fully developed ones. It has been shown that growing retinal vessels during the first postnatal week, but not the choroidal vessels, constrict when exposed to oxygen and may even be obliterated with prolonged exposure (Ashton and Cook, 1954). As the vessel reaches maturity, which takes place at about the 8th postnatal day in the rat, it gradually loses its ability to constrict when exposed to oxygen. In this regard, it is felt that the oxygen effect is an appropriate example since oxygen enhances ionizing reactions (Dowdy *et al.*, 1950). Moreover, the best protectors against x-rays, i.e., cysteamine and cysteine, sulfhydryl containing amino acids, are also the best protectors of mammals against oxygen poisoning (Bacq and Alexander, 1955). How-

ever, it remains to be demonstrated whether radiation actually affects maturing cerebral vessels in a like manner. The capillaries have been demonstrated to be the most radiosensitive of the blood vessels, and the degree of vascularity would seem to have a bearing on the pathologic picture. The degree of vascularity varies widely in the rat brain; for example, the globus pallidus has been demonstrated to be strikingly low in vascularity (Craigie, 1945). The globus pallidus was one of the areas where necrosis was most frequently involved.

These physiologic and morphologic changes in the rat brain during this initial postnatal 2 weeks when radioresistance is rapidly developing are also associated with dynamic biochemical transformation. Thus, oxygen uptake increases rapidly as does the lactic acid production (Greengard and McIlwain, 1955; Tyler and Van Harreveld, 1942). Marked increase in enzyme activities occur; there is a threefold increase in the respiratory enzymes succinic dehydrogenase and cytochrome oxidase during the initial 2 weeks and a fivefold increase of adenosine triphosphase activity, believed to be involved in making energy available to the cell to accomplish its differential growth (Potter, *et al.*, 1945; Flexner, 1953). Increased cholinesterase and pseudo-cholinesterase activity is similarly observed (Elkes and Todrick, 1955). The water content decreases significantly and levels of proteins and phosphatides are beginning to approach adult levels. However, the cerebroside which are importantly related to myelin formation accumulate only 11% of the adult weight by the 19th day (Folch-Pi, 1955). Deoxyribonucleic acid content which continues to accumulate during this period increases fourfold between the 2nd and 16th postnatal days, after which further increase is hardly noticeable (Mandel and Bieth, 1951). These examples amply demonstrate that the x-irradiation may well alter these manifold biochemical changes occurring in the rat brain shortly after birth.

Summary

Newborn rats ranging in age from 8 hours to 15 days received single doses of x-radiation to the head only. Doses of 125 r, 300 r, 500 r, and 1,000 r were administered. One group of animals was sacrificed at 48 to 72 hours after irradiation, and another group was autopsied at ages ranging from 14 days to 15 months.

The study demonstrated the development of a marked radioresistance in the brain by the 3rd postnatal week, compared to the easily damaged brain of the rat during the first postnatal week. The development of this relative radioresistance following the first week of life was abrupt. The radiosensitivity was manifested by an increased mortality, retarded growth, retarded brain size, production of cataracts, and abnormal neurologic signs in the

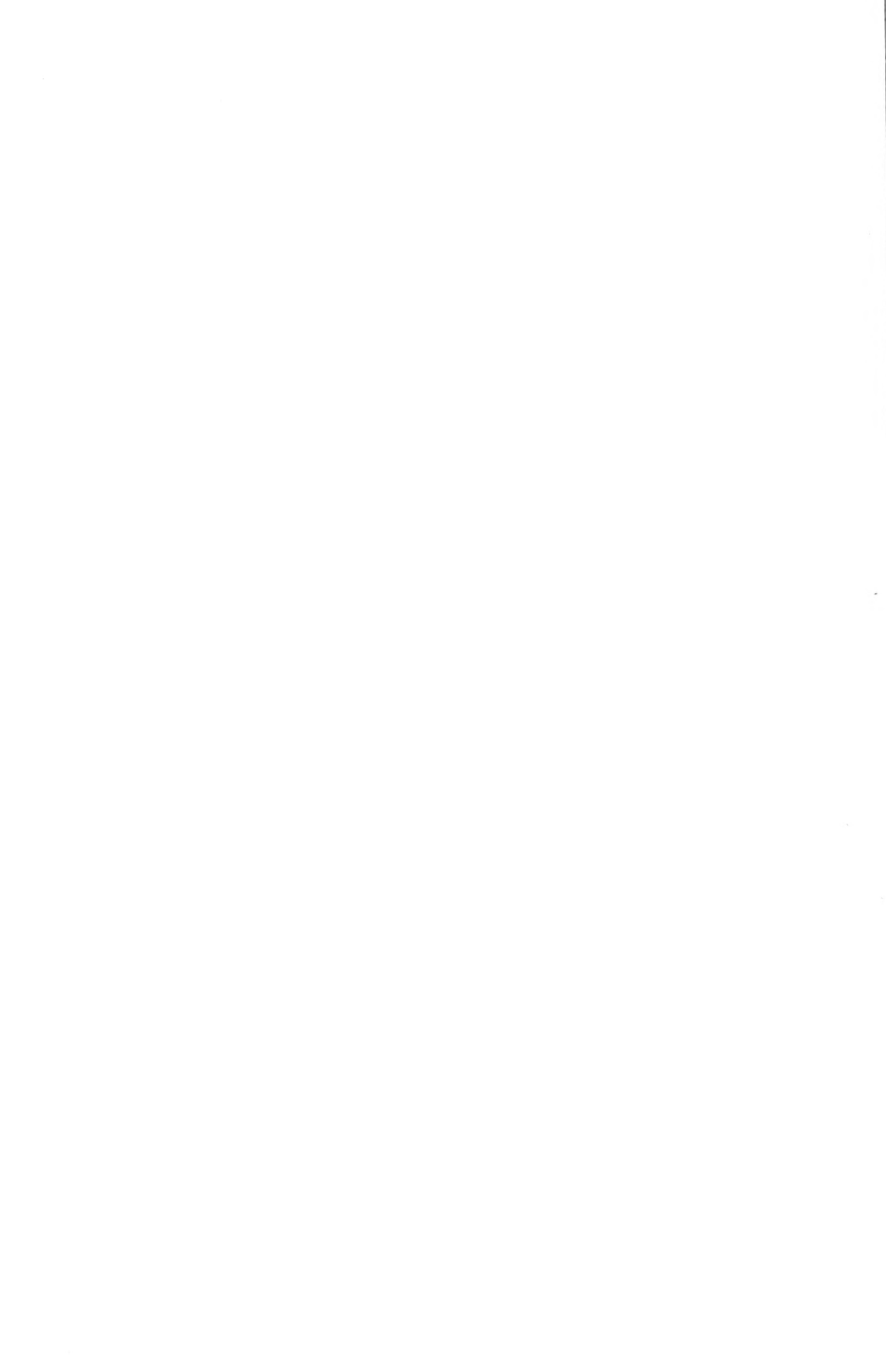
radiated animals. The neuropathologic findings correlated closely with the behavioral disturbances. Prominent among the early microscopic findings was damage to capillaries. Later histopathologic findings included perivasculitis, progressive thickening of the vessel walls and a narrowing of the vessel lumen, meningitis, inflammation of the choroid plexus, ventricular enlargement, delayed cerebral cortical and cerebellar development, focal necrosis, and gliosis.

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Cytoplasmic Inclusions Containing Deoxyribonucleic Acid in the Neural Tube of Chick Embryos Exposed to Ionizing Radiation*

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Introduction

The identification of deoxyribonucleic acid (DNA) with chromatin normally confines it to the nucleus of the cell. Nevertheless, reports of the occurrence of DNA in the cytoplasm are sufficiently numerous to suggest that its presence there may be a phenomenon of wide distribution, even though the origin and destiny of such DNA may remain obscure.

Perhaps not sufficiently appreciated is the large amount of cytoplasmic DNA which makes its appearance in young embryos a few hours following moderate irradiation. The striking picture presented by the numerous Feulgen positive bodies in the neural tube of early chick embryos that had received moderate doses of ionizing radiation prompted the present investigation. This enlarges on a preliminary report (Sauer, 1957) dealing with irradiated chick embryos. Since similar bodies have a normal occurrence (Glücksman, 1951; von Sallmann *et al.*, 1957), their interpretation as an injury response demands special caution. In the chick, the large number and wide distribution of these in irradiated embryos far exceed any normal appearance.

Materials and Methods

Chick embryos ranged from 2 to 4½ days in incubation age at the time of treatment, most being about 60 hours old.

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Embryos received either 200 or 500 r of x-rays through the unopened shell and were returned to the incubator. Control embryos were subjected to all treatment given the others, except the actual irradiation. Individual embryos were fixed at frequent intervals following irradiation, ranging from 1 to 76 hours. The following factors were employed in the irradiation: 250 kv, 30 ma, added filter 0.25 mm Cu plus 1.0 mm Al, 50 cm FOD, average intensity of 145 r/min.

Other embryos were labeled with tritium (Sauer and Walker, 1961). Some received only thymidine- H^3 , usually 50 mc per embryo, specific activity 1.6 c/mmole. Others had received 200 r x-rays immediately preceding treatment with thymidine- H^3 .

The embryos were fixed in Newcomer's (1953), Carnoy's, or Serra's fixative, or in 10 or 50% neutral formalin, embedded in paraffin and sectioned at 3 to 6 μ . Stains used included hematoxylin and eosin, toluidine blue O, May-Grünwald and Giemsa, Feulgen's, and methyl green-pyronin. The May-Grünwald and Giemsa stain following Newcomer fixation was preferred for general cytologic study. Kurnick's (1955a,b) procedure was followed for DNase and RNase. The enzymes were purchased from Worthington Biochemical Corporation, Freehold, New Jersey. For electron microscopy, pieces of embryos were fixed in Dalton's osmic-dichromate mixture, embedded in methacrylate, and sectioned with a Porter-Blum microtome.

Results

Numerous cells in the irradiated embryos have a striking appearance in that one or more bodies approximately 2-5 μ in diameter lie within their cytoplasm (Figs. 2-12). Such bodies occur in small numbers in normal chick embryos but greatly increase in number following irradiation. In 2- to 3-day-old chick embryos, they may begin to appear in the 2nd hour after exposure. In the early hours after treatment the cells containing the inclusions may be few or many, depending on the susceptibility of the particular embryo. The distribution of the affected cells at this time is wide but irregular: while some fields show many of the bodies, extensive regions may contain none or few. The bodies appear most constantly in the neural tube, especially in the brain. Their number increases with time, so that a 60 hour embryo that has received 200 r will 9 hours later contain large numbers of the inclusions in every system (Figs. 3-6). The body lies within the cytoplasm of an apparently normal cell. Its position adjacent to the nucleus is characteristic (Fig. 6) and it often flattens or indents the nucleus at the area of contact.

In the series receiving 200 r, the inclusions are very numerous in embryos fixed at 9 to 22 hours following irradiation. The number of inclusions then decreases rapidly. Only small numbers of the cytoplasmic bodies remain at

the end of 34 hours in any of the 200 r series, and by 57 hours recovery seems to be complete. Although a variable amount of degeneration occurs even at this lower dosage, manifested in the neural tube as scattered nuclear fragments or as areas with an indistinct luminal margin, there is no wholesale degeneration, and most nuclei remain essentially normal in appearance (Figs. 2-8). To what extent recovery consists of reversal of the process in the individual cell rather than replacement by unaffected cells can not be answered by this study.

Quite different results follow a dosage of 500 r. At 20 hours after exposure, numerous inclusions fill many cells, and there is considerable cell death. Debris is present in the ventricles of the brain and in the central canal of the neural tube. This series shows considerable reduction in the mitotic rate during the 20 to 30 hour period following treatment, while no such secondary delayed period of mitotic arrest follows the 200 r dosage. The series receiving 500 r shows complete recovery at 76 hours.

From the small series of older embryos (4 to 5 days) receiving the same amount of radiation as the two groups just described, it was determined that age is not a factor in the mere appearance of inclusions, although their distribution and time of maximum development differ in the two stages.

Table I summarizes the staining reactions of most of the cytoplasmic inclusions. Each body commonly displays a deep staining region, or center, (Figs. 2 and 10) which is strongly basophilic and appears to contain a high concentration of DNA. Both with Feulgen's stain and methyl green-pyronin, the centers give a strongly positive reaction for DNA; the negative reaction when DNase precedes the staining confirms the DNA content of the centers.

Alfert (1955; Vendrely *et al.*, 1958) showed that reaction to methyl green is not a reliable indicator of the degree of polymerization of DNA, as held by Kurnick (1955a,b). The intimate union of DNA and protein in normal chromatin keeps many groups unavailable for dye binding. Although the DNA of pyknotic nuclei does not decrease until the fragmentation stage, the intense green which pyknotic nuclei display can not reflect the amount of DNA present, but only means that autolytic changes accompanying pyknosis have unmasked stainable groups.

The staining reactions carried out (Table I) support the concept that these bodies also contain ribonucleic acid (RNA). With Kurnick's (1955a, b) modification of the methyl green-pyronin stain, for which he claims specificity for each of the types of nucleic acid, the body stains with a green center surrounded by a red periphery. The color resembles the deep green of the metaphases rather than the lighter green of other nuclei. The red color of the periphery might mean either RNA or depolymerized DNA. The negative Feulgen stain and the absence of red color when the stain is applied

TABLE I

STAINING REACTIONS OF THE BODIES WHICH APPEAR IN
THE CYTOPLASM AFTER X-IRRADIATION

<i>Method</i>	<i>Centers</i>		<i>Periphery</i>		
	<i>Color</i>	<i>Significance</i>	<i>Color</i>	<i>Significance</i>	
Hematoxylin-eosin	Purple	Basophilic	Red	Acidophilic	
Toluidine blue 0	Deep blue	Presumably nucleic acid	Pale blue	—	
Feulgen's	Deep red	DNA	Green	Not DNA	
Omitting	No red		Green		Green
hydrolysis					
After DNase	Deep red		—		
After RNase	Deep red				
Methyl green- pyronin	Deep green	DNA (polymerized?)	Red	RNA (not de- polymerized DNA, for Feulgen- negative)	
After DNase	No green on slide		Red		
After RNase	Deep green		No red		
May-Grünwald- Giemsa	Black	DNA and RNA?	Black or gray	—	
After DNase	Black	—	Gray	—	
After RNase	Mostly red, but a few remain black	—	—	—	

after digestion with RNase indicate that it is RNA. The deep purplish black staining of the center or often of the entire body with the May-Grünwald and Giemsa stain would be consistent with this conclusion. Jacobson and Webb (1952) found that chromatin changes in its reaction to this stain during the sequence of mitosis from red in the interphase to black during metaphase. These authors apparently demonstrate that black indicates presence of RNA in addition to DNA, but the specificity of this has been questioned (Swift, 1953; Theorell, 1955). In our hands black of the metaphase was more resistant to each type of nuclease than were the other cellular elements and that of the inclusions was even more resistant than were the metaphases. Following DNase, the bodies stained as black as before; following RNase, digestion was incomplete, some resistant bodies always remaining.

The response of neural tube cells of embryos exposed for as long as 12 hours to tritiated thymidine resembled the response to x-ray (Sauer and Walker, 1961). However, there was no cessation of mitosis in the tritium-

treated embryos, while mitosis was inhibited for 1½ and 3 hours following the 200 and 500 r exposures respectively. Tritium, once added to the egg, remains available over a prolonged period, so that a high degree of incorporation results (Sauer and Walker, 1959, 1961). Assuming that the mitotic stages are the ones most sensitive to radiation, x-ray affects only those in mitosis at the time of treatment. In the tritium-labeled embryos, however, within the course of a few hours practically every neural tube cell would undergo mitosis and thus be exposed to radiation at its sensitive stage. Those embryos exposed to the combined action of tritiated thymidine and 200 r did not appear to show much greater injury than those that had received thymidine alone.

With only light microscopy, there remained the possibility of error as to the actual cytoplasmic location of the bodies. The electron microscope proved invaluable in demonstration of electron dense bodies of complex form not found in normal material (Fig. 12).

Discussion

Feulgen-staining granules located outside the nucleus have been described in many species under a wide variety of conditions. They occur normally, especially in embryonic development (Glückmann, 1951; Chang, 1940), but also in the adult in certain locations (Corner, 1932); pathologically following cell death (Barthels and Voit, 1931) and in association with viruses (Leuchtenberger *et al.*, 1956); and experimentally in tissue cultures of normal vertebrate embryos (Maximow, 1925) and in irradiated material (Alberti and Politzer, 1924; von Sallmann *et al.*, 1957). Wherever encountered, they resemble in their deep staining the chromatin of mitotic stages and are surrounded by a portion of cytoplasm more deep staining than the remainder.

Extranuclear chromatin bodies assume prominence in developmental stages in both plants and animals in connection with death of superfluous cells, as in regression of transient structures or following excessive cell production. The latter is probably an almost universal growth phenomenon.

In the intensely studied field of insect development, Feulgen-positive bodies regularly occur both within the cytoplasm and extracellularly. Wigglesworth (1942) in an extensive review established two facts: a) whole nuclei break down, and b) the granules are often intracellular. They are most numerous during active mitosis, when excess cells would be formed. Incorporation of the remnants of a dead cell by a neighboring cell seemed a distinct possibility in epidermis with its intercellular connections; also, in rapid cell division, of one of the daughter nuclei died before the cytoplasm had divided, the dead cell would remain as a cytoplasmic inclusion. In one

known situation, nuclear division regularly occurs without cytoplasmic division, and one of the daughter nuclei degenerates to become a DNA-containing cytoplasmic inclusion. Since origin from degenerating nuclei in this case is undisputed, Wigglesworth inferred such origin for all cases. Linder's (1956; Linder and Anderson, 1956) more recent observations support this conclusion.

Glücksmann (1951) in his classic review listed numerous descriptions of prominent, dark-staining bodies usually interpreted as degenerating cells in normal embryos. The Feulgen stain, whenever carried out, indicated nuclear material. Glücksmann concluded that the bodies in all cases represented nuclear degeneration which typically began with pyknosis, further changes occurring either in the isolated remnant or inside a neighboring cell that had resorbed it. Chang (1940) pointed out the large number and wide distribution of the bodies in mouse embryos. He held that the bodies are within the cytoplasm, being phagocytized fragments of dead cells. According to Hamburger and Levi-Montalcini (1949), the entire body resembles a macrophage in its reaction to vital stains.

Nucleic acid normally moves from nucleus to cytoplasm by submicroscopic particles, but the same function may occasionally be accomplished by transport of large bodies. Here probably belong the examples of cytoplasmic DNA granules in certain plants (Sparrow and Hammond, 1947; Chayen and Norris, 1953). A number of species of nematodes and insects undergo a chromatin diminution process in connection with the segregation of the germ cells from somatic cells, whereby the somatic cells regularly cast out into the cytoplasm what may be a large part of the chromosomes (Wilson, 1934; Painter, 1959).

Extranuclear DNA in nonexperimental pathologic states is usually interpreted as degenerating nuclear remnants (Barthels and Voit, 1931). Other possibilities, especially in malignant cells, are nuclear buds which become enclosed in the cytoplasm, explained as an adjustment of the nuclear-cytoplasmic surface ratio, and a direct extrusion of chromatin into the cytoplasm, leaving a hypochromatic nucleus (Ludford, 1942). Von Sallmann *et al.* (1955) in studying radiation-induced changes in the lens of laboratory animals, where extranuclear Feulgen-positive bodies apparently are the predominant pathologic finding, pointed out the striking analogy with age-induced changes. They considered the bodies to be extruded from the nucleus. Loewenthal (1957) interpreted the Feulgen-positive bodies found in large numbers in chick embryos homozygous for the "creeper" mutation as degenerating nuclei. Cytoplasmic inclusions containing DNA characterize a number of virus diseases. Leuchtenberger *et al.* (1956) recently applied electron microscopy and quantitative measurements of the DNA to the bodies constantly present in rectal polypoid tumors and concluded that they were viral.

Maximow (1925) recognized that the peculiar granules occurring in tissue cultures of young rabbit embryos were inclusions within the cytoplasm of otherwise normal appearing cells, and that, except for their enormous increase in number, they were identical with the inclusions of normal embryos. They were especially abundant in the neural tube and mesenchyme and were usually more numerous in the central part of an explant. He stated that they always first appeared as small granules in close proximity to the nucleus, and he assumed that the large granules resulted from the growth of small ones.

Temperature changes (Chèvremont *et al.*, 1958) and chemical agents (Dustin, 1947; McLeish, 1954; Chèvremont *et al.*, 1958) may evoke DNA-containing cytoplasmic bodies. Explanations have varied. Dustin (1947) saw evidence that dividing cells respond to colchicine and a series of other mitotic poisons by nuclear pyknosis, followed by fragmentation and engulfing of the debris by the cytoplasm of surrounding cells. "Micronuclei" may result from chromosome breakage and be the source of DNA-containing bodies in the cytoplasm. Chromosome breakage may occur from intracellular metabolic disturbances resulting from changes in temperature or oxygen tension (Koller, 1954) and following exposure to chemicals (McLeish, 1954; Frederic *et al.*, 1959). Chèvremont *et al.*, (1958; Baeckeland *et al.*, 1957) found that fibroblasts cultivated in the presence of DNase contained numerous Feulgen-positive granules in their cytoplasm. This DNA, which may amount to 90% of the normal diploid nuclear value, was newly synthesized, as demonstrated by labeling with tritiated thymidine (Chèvremont *et al.*, 1959). Since other nonphysiologic agents, including chilling to 20°C, gave similar results (Chèvremont *et al.*, 1958), and in view of the evidence that cytoplasmic DNA may mean only that dead nuclear fragments have been phagocytized, the authors' interpretation that the bodies are altered mitochondria must be accepted with caution.

Extranuclear DNA following irradiation may be the result of cell death by nuclear pyknosis, "micronuclei" resulting from chromosome breakage, or a manifestation of other mechanisms.

According to Spear and Glücksmann (1938) and Glücksmann (1951), cell death from radiation injury is by pyknosis, and the Feulgen-positive bodies represent pyknotic degeneration, which they subdivided into three stages: chromatopycnosis, consisting of the separation of the chromatic from the nonchromatic material and the precipitation and coalescence of the chromatin into granules; hyperchromatosis of the nuclear membrane, in which the chromatin, having united into a single body, lies against the nuclear membrane as a deeply staining rim or partial rim; and chromatolysis, with loss of the Feulgen reaction. The entire process may take place in about an hour. Since onset of prophase in itself effects separation of chromatic from nonchromatic elements, cells degenerating in mitosis omit the first stage.

This behavior furnishes a means of distinguishing, on the basis of the Feulgen-positive granules, between death of mitotic and of interphase stages. The granules become cytoplasmic when resorbed by a neighboring cell.

In Fig. 1, based on the neural tube of the irradiated tadpole during the prolonged prophase extending from 11 to 24 hours, the low metaphase count means that heavy casualties occur on completing prophase. These casualties

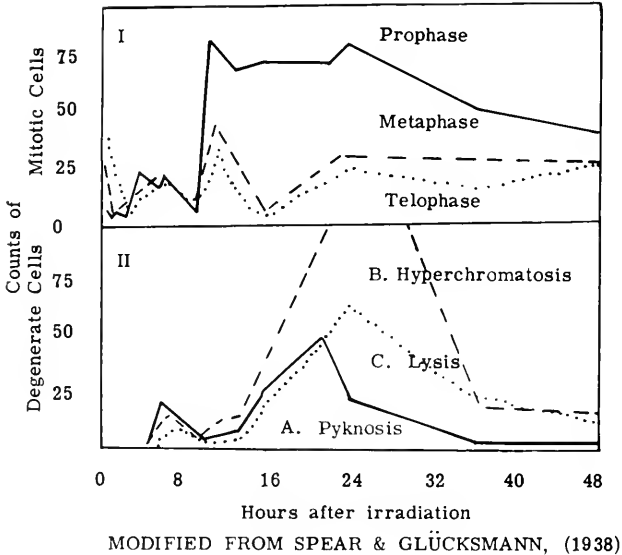


FIG. 1. Chart showing the number of mitotic and degenerate cells in the brain and retina of young tadpoles for 48 hours after exposure to gamma radiation (268 r). I. Mitosis. Key: prophase, — — —; metaphase, — — —; anaphase and telophase, Although prophases are normally fewer than metaphases, from 11 to 24 hours after radiation the number of prophases greatly exceeds the number of metaphases, indicating a prolongation of the prophase period. The subsequent decrease in the number of prophases without change in the number of metaphases indicates degeneration of many of the prophases. II. Degeneration: Three stages, as applied to the nucleus. *Stage A*. Chromatopycnosis, — — —. The chromatic material separates from the non-chromatic, with the chromatin material appearing as scattered granules. *Stage B*. Hyperchromatosis of the nuclear membrane, — — —. The chromatin granules have united into a single, deeply staining mass sitting as a cap on the nuclear membrane. *Stage C*. Chromatolysis, The chromatin breaks into fragments, and loses its Feulgen staining properties.

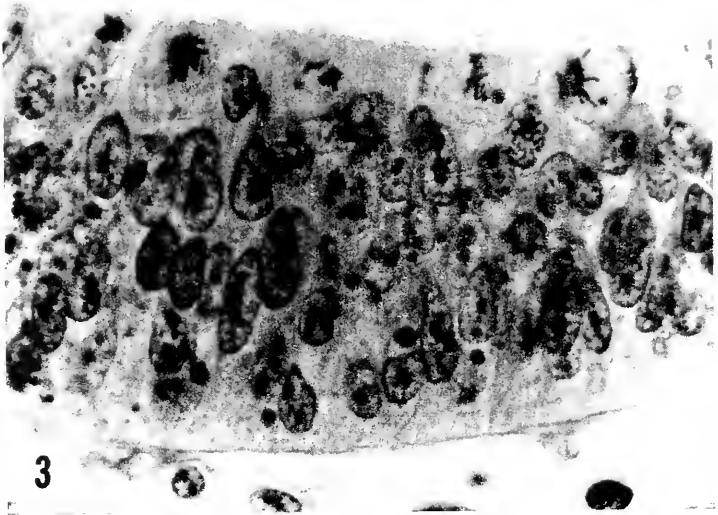
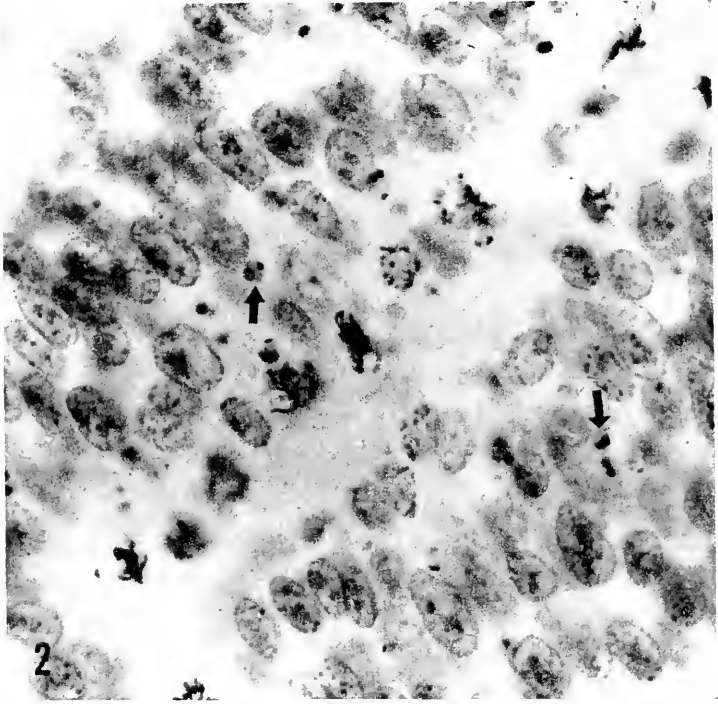
Since the mechanism of prophase effects separation of chromatic from non-chromatic material, a cell which reaches prophase before degenerating omits the first stage and passes directly into hyperchromatosis. The high increase in hyperchromatosis, beginning shortly after the peak of the prophase curve, indicates that the greatest number of casualties occurs after the cell reaches prophase. (Modified from Spear and Glücksmann).

cause a rapid rise in the degenerate cell count, beginning at about 13 hours, and since the dying cells are mitotic stages, this rise is in the hyperchromatic type of granule. The smaller rise in the pyknotic stage shows that some cells also break down before beginning division.

So-called micronuclei are a well known and classic effect of radiation. Although first seen by Koernicke (1905) in irradiated roots, it remained for Alberti and Politzer (1924) to show in the corneal epithelium of salamander larvae what becomes of a piece broken from a chromosome if it does not rejoin. Lacking a centromere, it does not move to one of the poles in anaphase, but lags on the spindle and is usually not included in either daughter nucleus at telophase. Remaining in the cytoplasm, it becomes spherical and lies beside the chief nucleus. They named these *Teilkerne* or partial nuclei. Several isolated chromosome fragments may unite into a single larger micronucleus (Ohnuki and Makino, 1960).

The great majority of so-called micronuclei are nonliving, spherical, deep staining bodies about 2μ in diameter. Those few acentric fragments that contain both an adequate amount of heterochromatin and a nucleolar organizer may continue to live, however, playing an active part in cell metabolism and dividing synchronously with the main nucleus for several subsequent life cycles (LaCour, 1953; McLeish, 1954). The completeness of the chromosome set is supposedly necessary for the normal functioning of the cell; consequently, the daughter cell with the deficient chromatin, presumably the one in whose cytoplasm the micronucleus became enclosed, has been assumed to be short-lived. However, Ohnuki and Makino (1960) proved survival throughout at least one mitotic cycle, and Hornsey (1956, 1960) showed that their maximum number appeared at the end of the first mitotic cycle (prolonged by irradiation) and that their subsequent decrease was exponential, depending only on dilution by further cell division.

Chromosome breaks may occur from exposure of a cell to irradiation in any stage of its life cycle. However, interphases in which the chromosomes are widely dispersed show a special resistance to chromosome breakage as compared to cells irradiated in the premitotic and mitotic stages when the chromosomes are tightly condensed. Muller (1954) reviews the factors involved. Lagging chromosome fragments are the chief change resulting from irradiation in interphase, being few in early stages and becoming more abundant as interphase progresses. Chromosomes irradiated in late prophase to early telophase, even though effectively broken, give no evidence of being broken at the time, for when in the condensed condition they are held as if by some enveloping material and can not fall apart into fragments. However, when the chromosomes recondense at the next mitosis, after an intervening interphase has elapsed, more structural changes appear than would have followed irradiation in the interphase. Consequently, micronuclei are



rare until cells have undergone at least one mitosis subsequent to irradiation. Micronuclei are more prominent in some material than in others (La Cour, 1953). Both the number of fragments per cell and the number of cells with fragments increase with the dose (Koller, 1947). At their height, micronuclei may occur in 10% or more of cells (Gray and Scholes, 1951; von Sallmann, *et al.*, 1957; Friedkin, 1959).

Most ideas in the literature as to the nature of the bodies in the cytoplasm are based on interpretations of a static picture rather than on direct observation. Of indisputable origin are micronuclei. Recordings with time-lapse photography have been made of the formation of micronuclei in irradiated cells and of their movement into the cytoplasm (Bajer, 1958; Bloom *et al.*, 1955; Ohnuki and Makino, 1960). The origin of a chromatin body in the cytoplasm through degeneration of a sister nucleus in a cell which did not complete division is well founded in a restricted field (Wigglesworth, 1942) and has also been observed in irradiated tissue cultures (Stroud and Brues, 1954). Direct extrusion of nuclear material into the cytoplasm has often been postulated as the method of formation of these bodies, but apparently has not often been observed in irradiated material, nor has the gradual growth of a cytoplasmic body *de novo* in the cytoplasm, nor actual phagocytosis of degenerated nuclei.

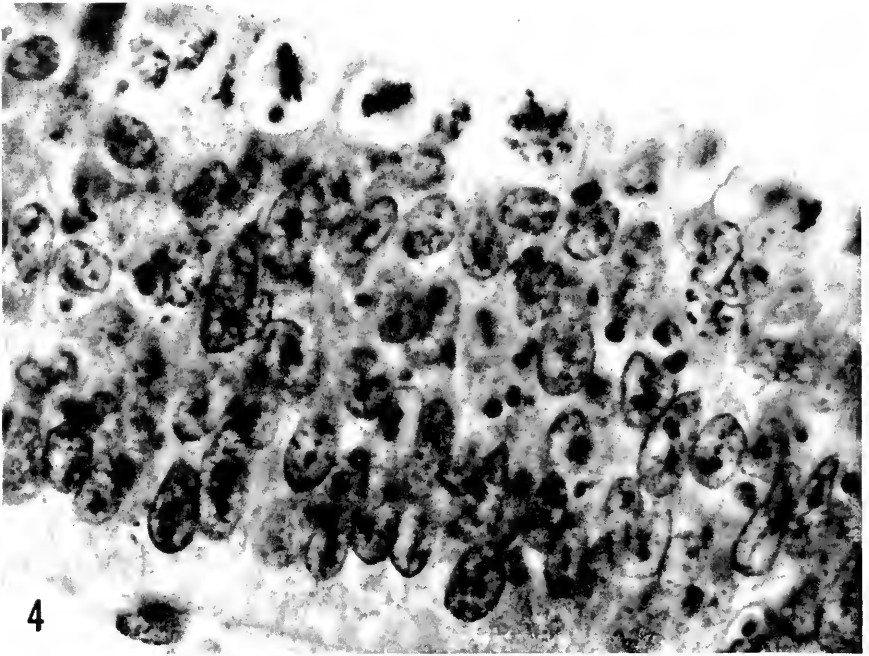
Our material confirms the observations of others (Butler, 1936; Schneller, 1951) working with irradiated chick embryos. Attention has been centered on the large amount of extranuclear Feulgen-positive material present, especially in the neural tube. Apparently the presence of DNA in the cytoplasm is not a universal reaction to irradiation. Mitchell (1942) found the cytoplasm of irradiated tumor cells consistently negative to the Feulgen reaction.

Our material justifies the conclusion that many of the bodies lie within the cytoplasm (Figs. 2-11). This could often be demonstrated with the light microscope with Zeiss stereoscopic eye caps to give an exaggerated view of depth. The electron microscope pictures are indisputable (Fig. 12) Wanko *et al.*, 1959.

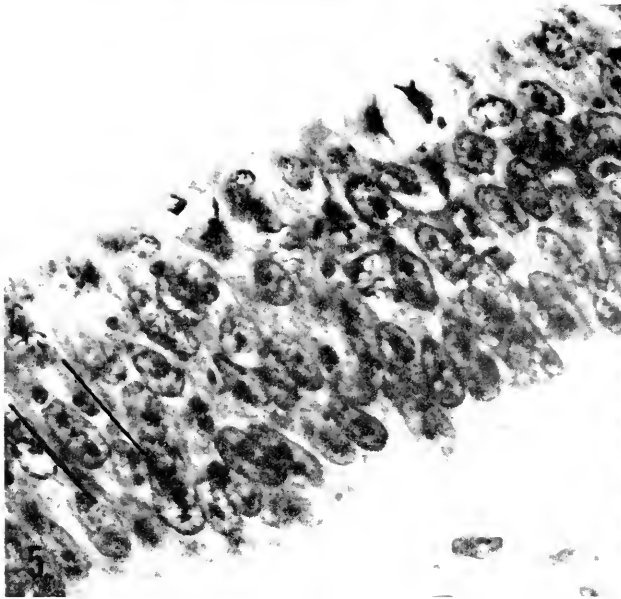
Spear and Glücksmann's (1938) and Glücksmann's (1951) distinction between two types of chromatin bodies, depending on whether death of the cell occurred in interphase or in mitosis, aids in identification of certain

FIG. 2. Feulgen stain of the neural tube of a 3-day chick embryo which had received 200 r of x-rays 7 hours previously. Many of the cytoplasmic bodies are of compound nature (see arrows) containing one or several Feulgen-positive centers, accompanied by Feulgen-negative material. Compare FIG. 10. Newcomer fixative. $\times 1200$.

FIG. 3. Brain of a $2\frac{1}{2}$ - to 3-day chick embryo which had received 200 r of x-rays 9 hours previously. The lumen is at the top of the figure. This field shows only minor change compared to much of the embryo. Carnoy fixative: May-Grünwald and Giemsa stain. $\times 1000$.



4



6

FIGS. 4-6. From the same brain as FIG. 3 (2½- to 3-day chick embryo, 200 r, 9 hours). Carnoy fixative; May-Grünwald and Giemsa stain.

FIG. 4. The lumen is at the top of the figure. $\times 1100$.

FIG. 5. The lumen is at the top of the figure. $\times 800$.

FIG. 6. Enlargement of cell indicated in FIG. 5. $\times 3000$.

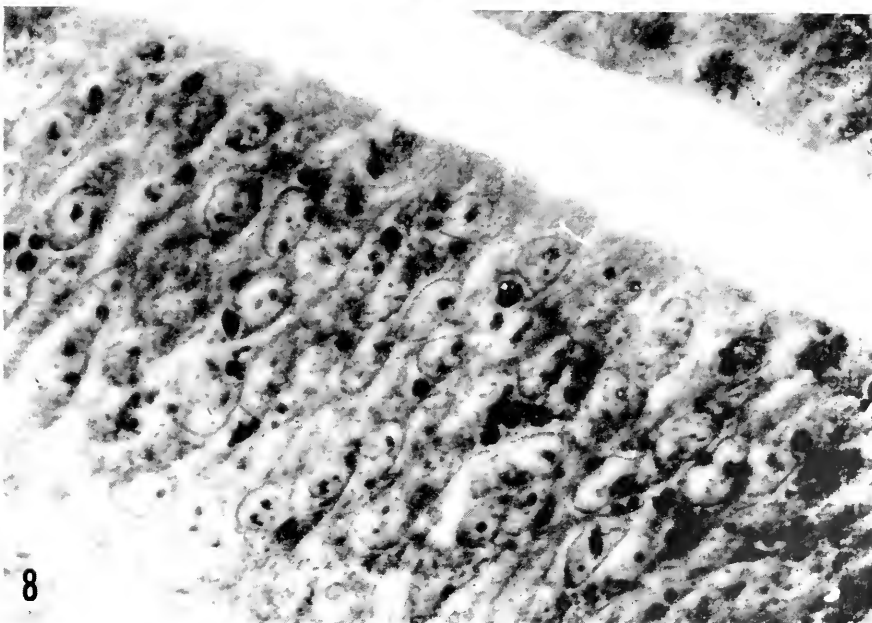
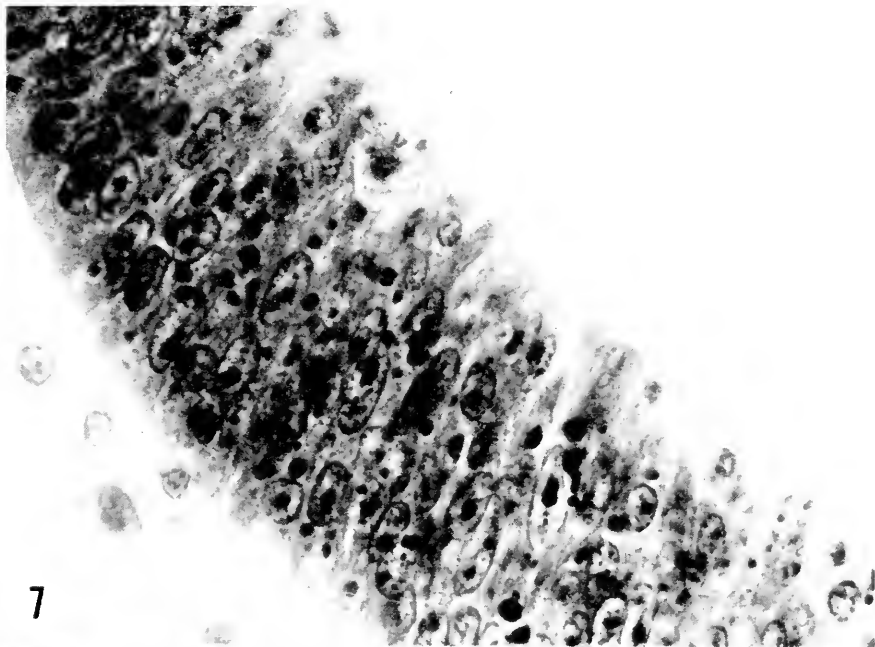


FIG. 7. Brain of a $2\frac{1}{2}$ -day chick embryo which had received 200 r of x-rays $11\frac{1}{2}$ hours preceding fixation. Lumen is at the right. Newcomer fixative; May-Grünwald and Giemsa stain. $\times 900$.

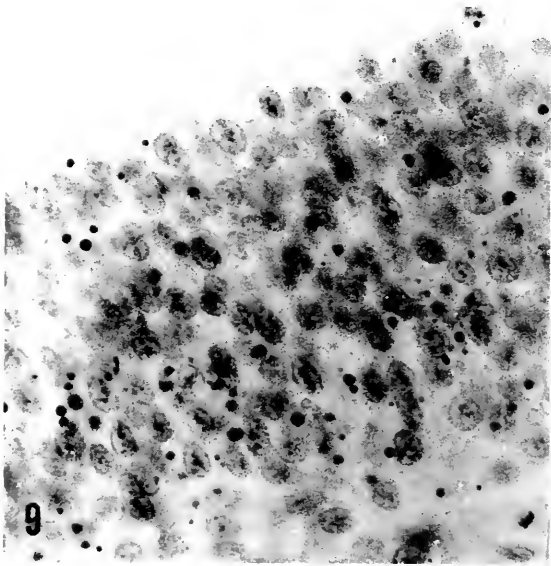
FIG. 8. Neural tube of a $2\frac{1}{2}$ - to 3-day chick embryo which had received 200 r of x-rays $14\frac{1}{2}$ hours previously. Newcomer fixative; May-Grünwald and Giemsa stain. $\times 1000$.

bodies. Those which are dish-shaped, lens-shaped, broken ring-shaped, and possibly also those consisting of several granules, are features of the neuroepithelial layer during the period of highest mitotic activity (8 to 15 hours after 200 r) when numerous cells break down in division. This period is approximately coextensive with the first mitotic period following irradiation. Since these bodies occur at all depths of the neuroepithelial layer, while dead cells are chiefly observed adjacent to the lumen, the bodies must have been moved into the depths of the wall following cell death. Although some are cast into the central canal, it may be speculated that others are resorbed at the lumen into the cytoplasm of adjacent nuclei which are beginning their postmitotic migration peripherally (Sauer, 1935; Sauer and Chittenden, 1959; Sauer and Walker, 1959; Sidman *et al.*, 1959; Watterson *et al.*, 1956). Evidence for the actual process of phagocytosis is completely lacking in sectioned material; however, to one who has marveled at the antics of cells in tissue cultures, as demonstrated with time-lapse photography, rapid absorption of degenerated cell remnants seems plausible. The many chromatin bodies surrounded by only intact cells can be explained readily in this way.

In addition to the irregular bodies which seem to be degenerated mitotic cells, after irradiation there are also larger, Feulgen-positive bodies more nearly approaching a cell nucleus in size. After 500 r these are the first bodies to appear in the neuroepithelial layer. They begin about the time of resumption of mitosis and become numerous 4 to 5 hours following irradiation (Fig. 9). The picture is clean-cut, in contrast to the same region seen overlain with small fragments at 10-14 hours following 200 r. Similar large, spherical bodies occur in enormous numbers in the mantle layer of our older irradiated chick embryos and of irradiated Chinese hamster embryos of comparable age. Their large sizes suggests whole pyknotic nuclei. It is assumed that they are differentiating cells which have died a pyknotic type of death in interphase. Since they begin to appear even in the period of mitotic arrest, they are unrelated to mitosis. (Hicks, 1958 and Hicks *et al.*, 1959) has studied this radiosensitive form extensively. Some in the neuroepithelial layer are definitely cytoplasmic. In the mantle layer, few intact cells remain.

Those Feulgen-positive bodies definitely enclosed in mitotic stages at the lumen are considered to be micronuclei. In some material they are rather numerous. As the nuclei of these mitotic cells migrate into the depths of the wall in their postmitotic period, their micronuclei will be drawn with them. Consequently, some of the mitotic bodies deep in the wall must also be micronuclei.

How many of the numerous, spherical bodies, about 2 μ in diameter and apparently cytoplasmic, are actually micronuclei is unknown. That some may represent a direct extrusion from the nucleus is a possibility. This might



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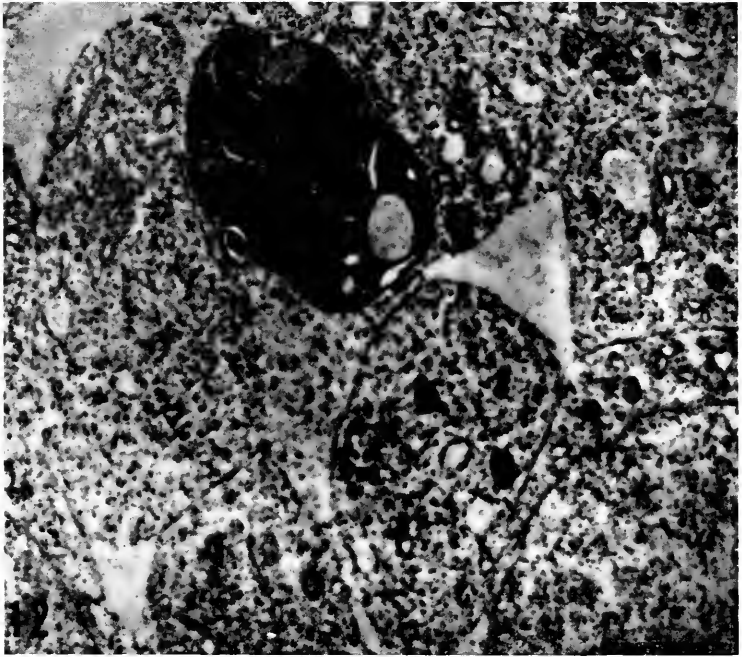


FIG. 12. Electron microscope picture of a tangential section of the neural tube. $\times 15,000$. Line indicates 1 micron.

not represent essential DNA but an excess formed in a period of deranged metabolism or the reversal of a synthesizing cell to its presynthetic stage. A related idea is an accumulation in the cytoplasm of nuclear material which diffuses freely but is normally present in only small amount. RNA increases in the cytoplasm following irradiation (Mitchell, 1942). Consequently, more DNA from the nucleus is transforming into RNA of the cytoplasm. Some of the cytoplasmic bodies might result from a derangement in this process.

This has been a fascinating study, but it has raised more questions than it has answered. What is the potentiality of early neural tube cells for resorp-

Figs. 9-11 are on page 89.

FIG. 9. Feulgen stain of the brain of a $4\frac{1}{2}$ -day chick embryo irradiated with 500 r 3 hours previously. The lumen is at the top of the figure. Newcomer fixative. $\times 750$.

FIG. 10. Feulgen stain of the brain of a 3- to $3\frac{1}{2}$ -day chick embryo which had been irradiated with 500 r $3\frac{1}{2}$ hours previously. The lumen is at the top of the figure. Most of the bodies contain both Feulgen-positive and negative regions. Carnoy fixative. $\times 1450$.

FIG. 11. Neural tube of a $2\frac{1}{2}$ -day chick embryo irradiated 8 hours previously with 500 r. The lumen is at the left. Newcomer fixative; May-Grünwald and Giemsa stain. $\times 1400$.

tion of contiguous solid material? What use is made of all of this cytoplasmic DNA? Surely a ready made supply of its own brand of DNA is a material too valuable to rapidly dividing cells to be wasted. What is the role of DNase, or its absence, in connection with cytoplasmic DNA? Finally, there remains the unsolved problem of the great radiosensitivity of the differentiating neuroblast. What change has suddenly come over this cell, probably still with mitotic potentiality, to increase its sensitivity and, with neurofibril appearance, to leave it again to make it among the most resistant of cells?

Summary

The material consisted of 2- to 4-day chick embryos subjected to 200 to 500 r of x-irradiation or labeled with thymidine- H^3 of high specific activity.

Moderate doses of radiation led to a structural change in the cytoplasm in numerous cells of the early neural tube. A striking feature a few hours following exposure to ionizing radiation was the presence within the cytoplasm of one or more dense, basophilic bodies approximately 2-5 μ in diameter. These typically consisted of one or several Feulgen-positive centers, surrounded by an RNA-containing rim. The centers were digested by DNase. They represent a relatively large amount of extranuclear DNA. Electron microscopy demonstrated their great density and confirmed their cytoplasmic location. At the lower dosage of x-ray, the process was completely reversible, without an intervening period of degeneration. The bodies were not confined to the neural tube, although they attained great prominence there, but were widely distributed throughout the embryo.

It is concluded that the bodies are of several types. It is possible to distinguish between those resulting from degeneration of mitotic stages and those of interphases on the basis of their morphology. Micronuclei are another type, sometimes present in considerable number.

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Biochemical Effects of Irradiation in the Brain of the Neonatal Rat*

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Introduction

From several perspectives the brain of the neonatal rat may be regarded as embryonic. The cortex and the cerebellum are relatively small compared to the brain stem (the more basic and primitive structure), and even in the brain stem the nerve tracts are incompletely myelinated (Folch-Pi, 1955). The cell nuclei are larger and more hydrated than adult nuclei, and the neurons have not yet assumed their characteristic elongate and dendrite configurations. During the first 2 weeks, extensive cell division takes place in the cerebellum and cortex. In the brain stem cells increase greatly in total volume, but there is less cell division.

Hicks (1952) has shown that neuroblasts in the brains of newborn rats may be exposed to more than an hour of total anoxia without breakdown, a finding that indicates a well developed glycolytic mechanism and relates these structures metabolically to other embryonic tissues (Gal *et al.*, 1952).

Aside from purely practical applications of studies on irradiation damage to brain tissue in young animals, these embryonic properties of the brains of neonatal rats make them useful tools in the assay of radiation effects on cell mechanisms in general (and on nervous tissue in particular). The neonatal brain presents a continuously changing aspect over a period of weeks. During this time radiation-produced lesions may manifest themselves in various ways. Conversely, certain mechanisms of differentiation may be further elucidated by the changes induced by radiations given soon after birth.

The present work is a preliminary survey of the effects of sublethal doses of x-irradiation to the heads of newborn rats. Several aspects of brain morphology, biochemistry, and metabolism have been followed up to 40 days after birth.

*These studies were partially supported by the Atomic Energy Commission.

Materials and Methods

Litters of Wistar strain rats were divided into two groups. The individuals of one group received 750 r of x-irradiation to the head only (the pituitary was irradiated as well as the brain) at 2 days of age and were kept for varying lengths of time before sacrifice. Individuals of the other group served as litter-mate controls.

At sacrifice (by decapitation), the brains of all rats were separated into three precisely resolvable parts: brain stem, cerebellum, and cortex. Each sample was frozen and stored in a freezer. On thawing, the total wet weights of the organs were determined, as were total water contents, total solids, total nitrogens, total lipids, and total phospholipids. Selected samples were analyzed for wet volumes of nuclei, mitochondria, and microsomal fractions; total lipids were extracted and their fatty acid profiles obtained by gas chromatography.

Water content was determined by disintegrating the thawed tissue in a piston-type disintegrator, adding acetone to a weighed sample of the wet mash, evaporating thrice under a stream of nitrogen at room temperature (Sperry, 1955), and then drying in an Abderhalden apparatus over boiling water for 3 hours in the presence of PO_5 . Water loss was measured gravimetrically. Total lipid was extracted from the dried solids by addition of 10 ml of a 2:1 chloroform-methanol mixture. The extract was purified by Sperry's modification of the procedure of Folch-Pi *et al.* (Sperry, 1955), the total lipid being measured gravimetrically. Total phospholipids were determined gravimetrically by extracting the total lipids with 15.0 ml of acetone to which one drop of saturated MgCl_2 had been added. Nitrogen analyses on the solid residue remaining after lipid extraction was done by the micro-Kjeldahl technique.

Subcellular components were isolated by differential centrifugation, and measurements of wet volumes of the fractions were carried out as detailed in a previous publication (Schjeide, *et al.*, 1960).

Gas chromatography of the fatty acids derived from nuclei and mitochondria¹ was performed on the Model 10 Barber-Coleman apparatus, using a 50-in. column packed with commercially obtained ethylene glycol succinate at 170°C. The detector was of the argon-ionization type. A 3 μl aliquot of 2.5% in petroleum ether solution was injected into the column for each analysis.

¹ The methyl esters of these fatty acids were prepared by solubilizing the total lipid in 1 ml of dried benzene, placing this in a 15-ml glass-stoppered centrifuge tube to which was added 2 ml of 4% anhydrous methanolic HCl, refluxing 4 hours over methanol, extracting the pentane-soluble lipids in 10 ml of pentane, washing thrice with distilled H_2O , shaking with magnesium sulfate (to remove residual H_2O), and finally absorbing away the nonesterified lipids on powdered alumina.

Results

As the brains of young or irradiated rats were removed from their brain cases, they appeared less firm and more hydrated than the brains of old or nonirradiated rats.

Some of these tissues were analyzed immediately after removal from the animal. The brains of the youngest rats (7 days) contained an average of 88.0% water. No modifying effect of x-irradiation was noted. By the time the rats were 40 days old, the cerebellums and cerebral cortexes contained 80.0% water and the brain stems only 76.0%. X-irradiation elevated the water content to 80.0% in the brain stems. Only slight hydration was noted in irradiated cerebellum and cortex.

Figures 1, 2, and 3 illustrate the effects of radiation on the *total* dry weights of brain stem, cortex, and cerebellum.

During the first 2 weeks after 750 r of x-irradiation, enlargement of the brain stem followed a normal course, but then the growth rate became somewhat depressed in all groups of animals (Fig. 1). The dry weights of irradiated cerebral cortex were comparable to those of control rats until 16 to 20

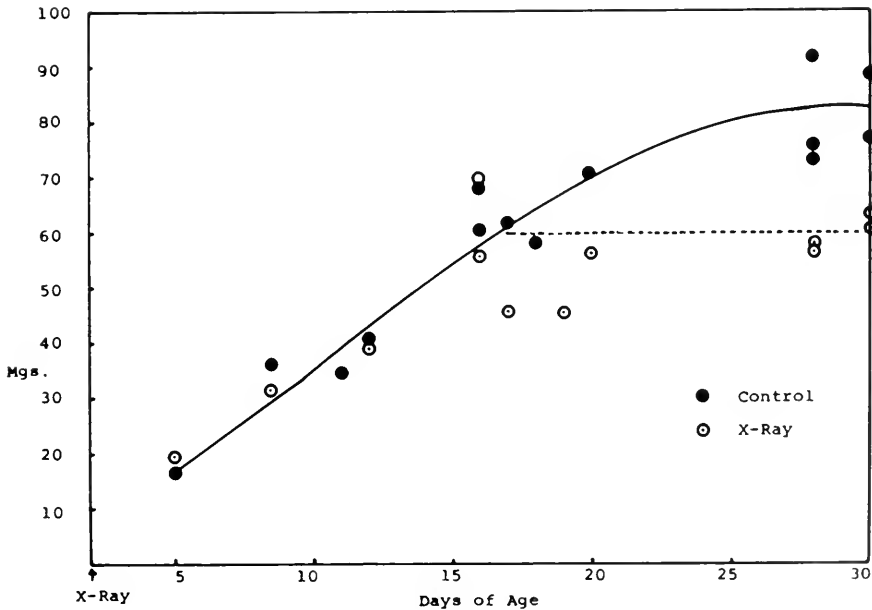


FIG. 1. Dry weights of brain stems from control and x-irradiated neonatal rats.

days after exposure. At this time they fell off markedly (Fig. 2). Cerebellums were profoundly affected by radiations, retardation in growth being evident

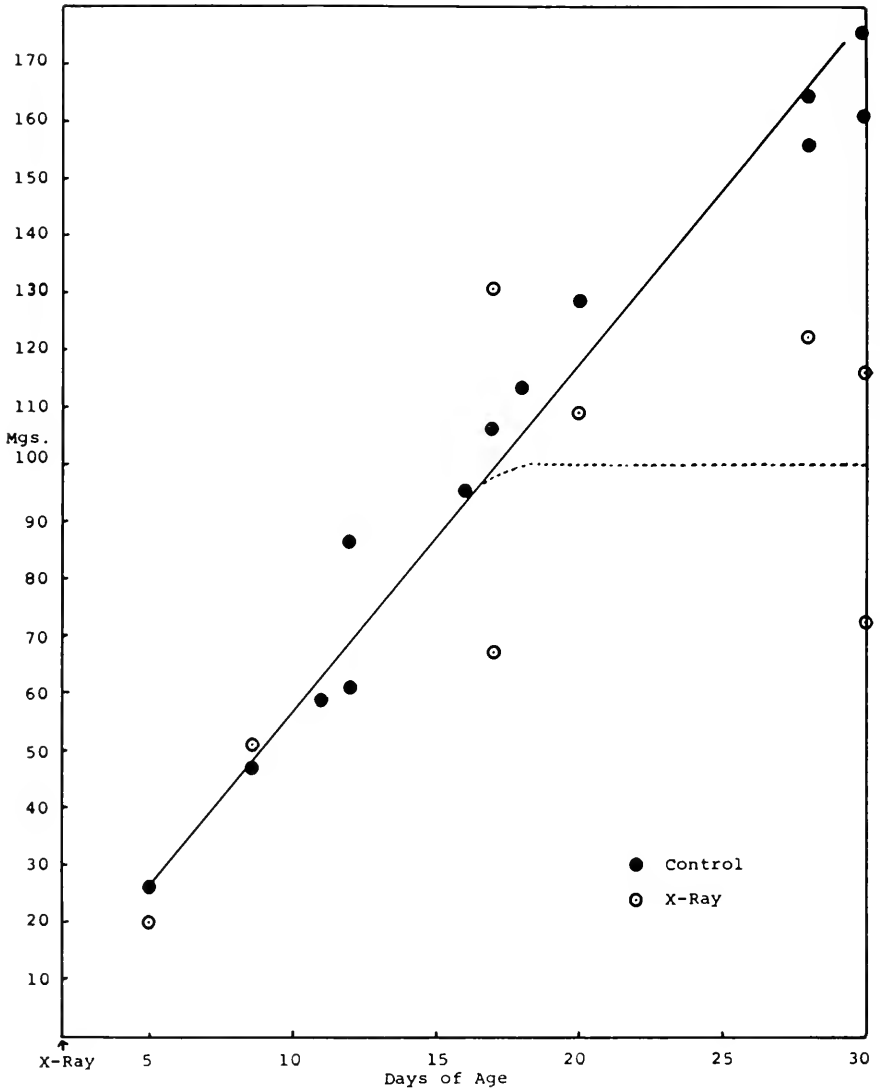


FIG. 2. Dry weights of cortex from control and x-irradiated neonatal rats.

during the first week after x-irradiation (Fig. 3).

Figures 4 and 5 show that the amounts of total lipid *per unit dry weight* of cortex and cerebellum increased at the same rates with increasing age despite irradiation. Obviously, both total lipid and phospholipid were reduced per whole brain in those animals that displayed decreases in brain

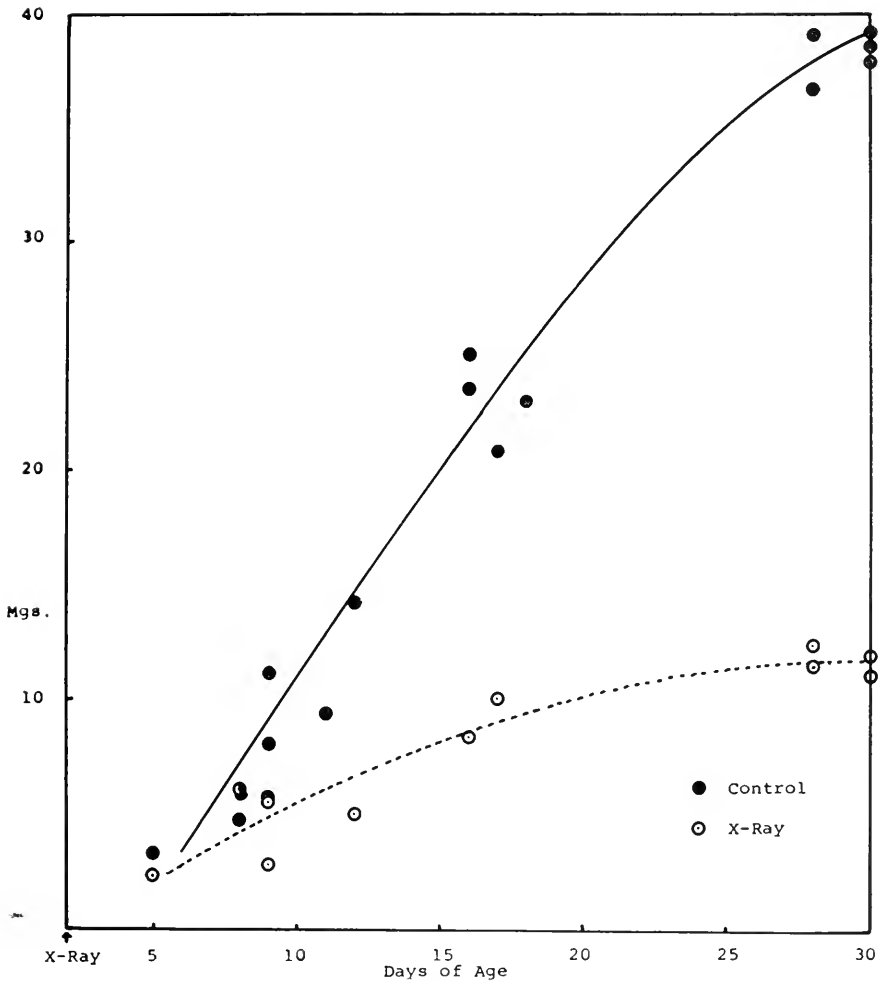


FIG. 3. Dry weights of cerebellums from control and x-irradiated neonatal rats.

dry weights following radiation exposure. Preliminary analyses for total phospholipid revealed no consistent differences either among the different brain components or between control and irradiated animals (phospholipid averaged approximately 80% of the total brain lipid in both cases). However, the unit lipid content of irradiated *brain stem* was *decreased* significantly at 16–20 days of age (Fig. 6). This inhibition of lipid synthesis was closely proportional to a retardation of normally occurring dehydration of the whole brain stem tissue.

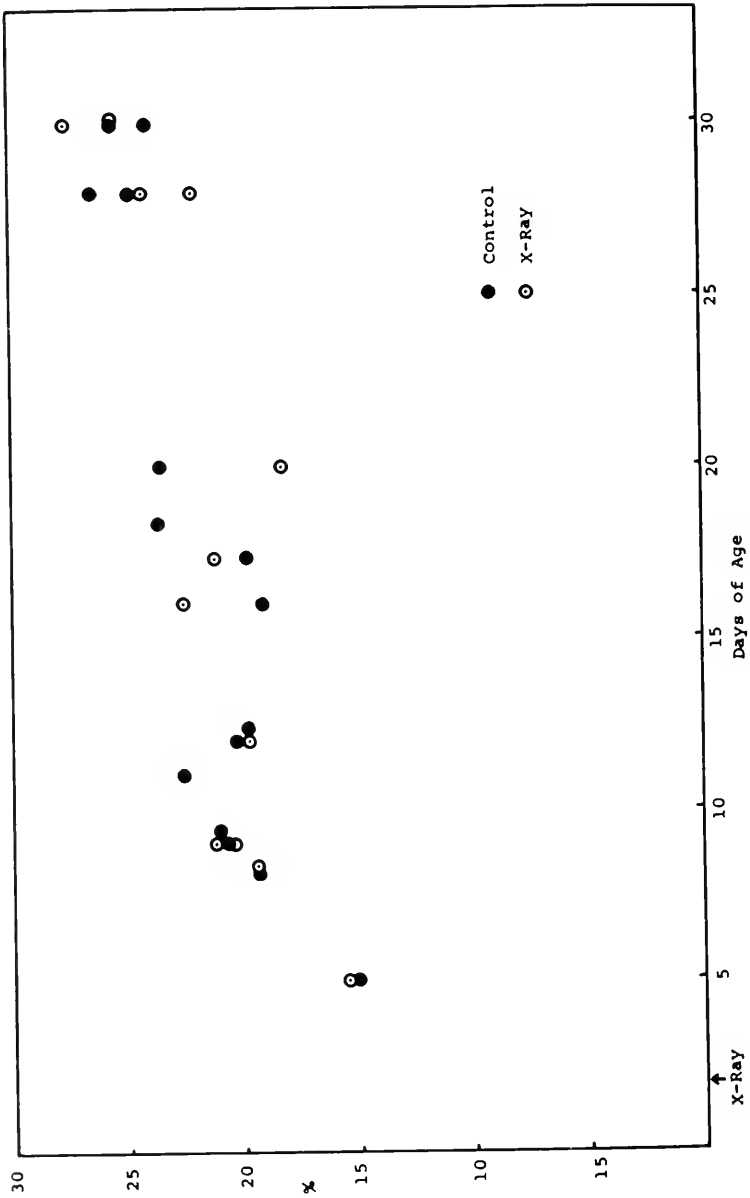


Fig. 4. Percentages of lipids in dry cortices of control and x-irradiated neonatal rats.

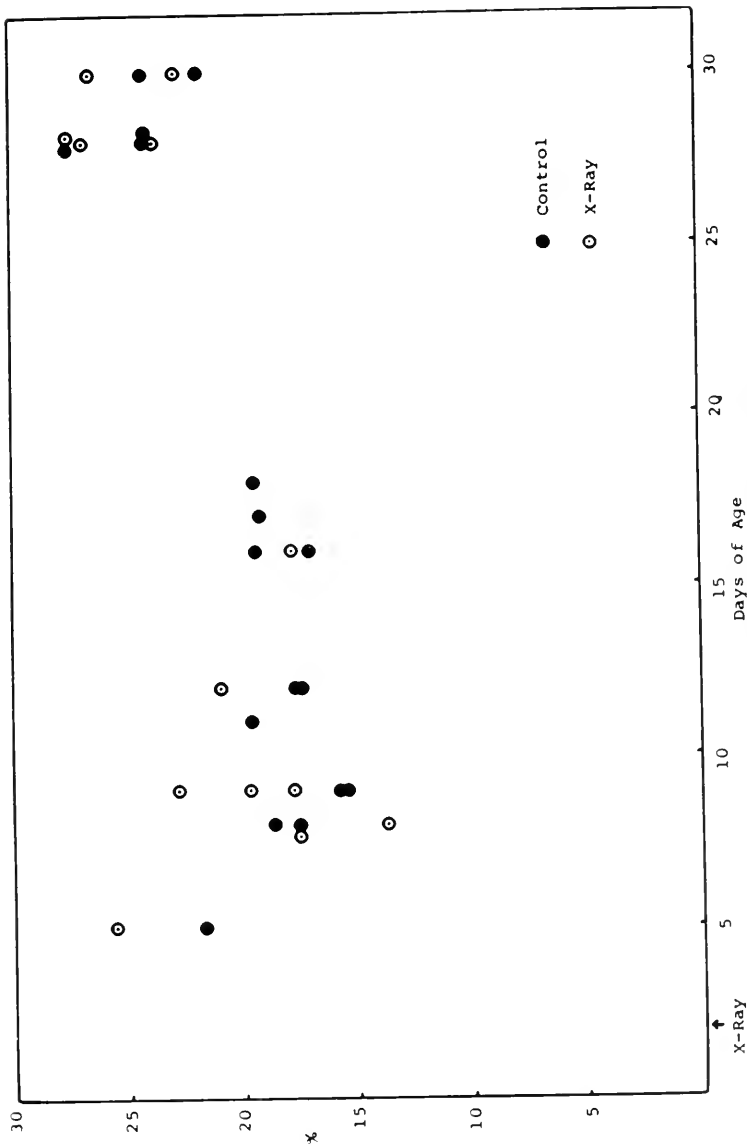


Fig. 5. Percentages of lipids in dry cerebellums of control and x-irradiated neonatal rats.

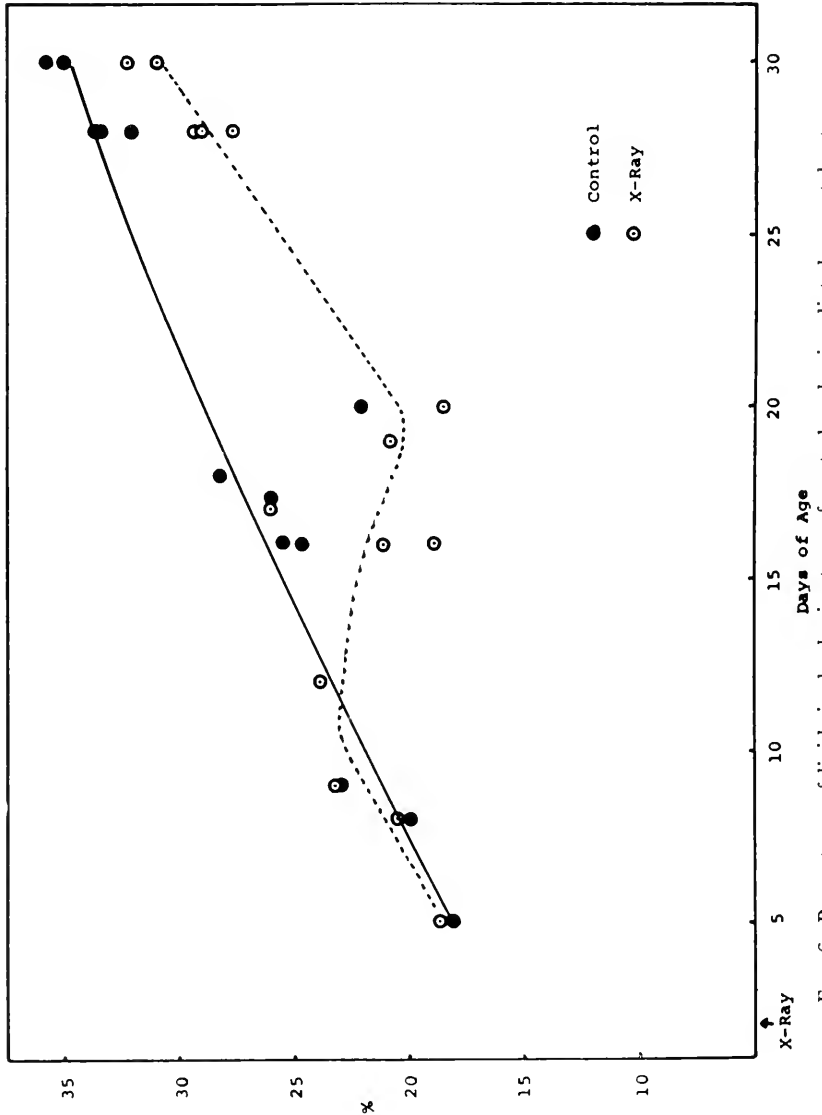


Fig. 6. Percentages of lipids in dry brain stems of control and x-irradiated neonatal rats.

Nitrogen values for lipid-extracted brain solids were obtained for rats aged 11-18 days. In nineteen groups, no significant differences could be demonstrated either as a function of age or of x-irradiation. There was an average of 83% protein and amino acid in the lipid-extracted solid material. The potentially most interesting stages with respect to variations in intra-

cellular nitrogen (and DNA), namely rats less than 1 week of age, have not yet been studied.

Proportions of centrifugally isolated nuclei, mitochondria, and microsomes for cortices and brain stems of various age groups (with and without prior x-irradiation) are presented in Table I. Mitochondria in the cortex appeared to increase per unit cellular volume with increasing age (and development). The only consistent changes following irradiation appeared to be decreases in wet volumes of mitochondria in some of the younger (8- to 9-day) and older (28- to 30 day rats). However, since electron microscope examination of brain mitochondrial fractions obtained by the technique of Schneider and Hogeboom (1950) reveal the presence of materials other than mitochondria, the present results can only be regarded as tentative.

Gas chromatograms of fatty acids from nuclei and mitochondria in

TABLE I

WET VOLUMES OF INTRACELLULAR COMPONENTS OF CORTEX AND
BRAIN STEM, WITH AND WITHOUT IRRADIATION

Number of animals	Age (days)	Organ	Treatment (in r)	Volumes (Ml 4 ml) ^a		
				Nuclei	Mitochondria	Microsomes
6	8	cortex	control	0.20	1.24	0.92
9	8	cortex	750	0.21	1.04	0.80
5	8	cortex	control	0.48	2.34	1.60
5	8	cortex	750	0.30	1.80	2.00
5	8	brain stem	control	1.20	4.80	.56
5	8	brain stem	750	0.40	2.24	2.64
11	9	cortex	control	0.39	1.07	1.00
12	9	cortex	750	0.34	1.00	.80
5	16	cortex	control	0.76	2.80	1.96
4	16	cortex	750	0.80	2.96	1.80
4	20	cortex	control	0.30	2.42	1.60
5	20	cortex	750	0.42	2.40	1.90
4	20	brain stem	control	0.75	2.66	1.50
3	20	brain stem	750	0.40	2.60	0.68
6	28	cortex	control	0.78	4.80	1.91
4	28	cortex	750	1.36	3.73	1.60
4	30	cortex	control	0.32	4.05	2.02
3	30	cortex	750	0.27	2.68	3.46
	30	cortex	control	0.30	1.80	
	30	cortex	750	0.36	1.44	

^aMl per ml4 of starting tissue. In some cases the sum of the wet volumes of the intracellular components exceeds that of the starting tissue. This phenomenon is due to uncontrolled hydration of the solids and inefficient packing in the volumetric tubes.

cerebral cortices of rats aged 8–9 days to 30 days appear in Fig. 7. The most prominent fatty acids in these cross sections include palmitic, stearic, oleic, arachadonic, and linoleic, in that order. Experience with fatty acids from the developing livers of chicken embryos has shown that, as the cell matures, the percentage of palmitic acid in the nucleus decreases and the percentage of oleic acid increases markedly (Schjeide, 1960). X-irradiation retards the adjustment of these fatty acid ratios, and it may that this is a reflection of inhibition of differentiation in the nucleus. Re-enforcing this finding is the observation that the fatty acid profiles of mitochondria from irradiated chicken embryos contain an *increased* percentage of oleic acid.

Although the nuclear fatty acids of neonatal rat brains failed to show changes as dramatic as those observed in the embryonic livers, there was generally a decrease in the ratio of palmitic acid to stearic acid and oleic acid in nuclei as a function of increasing age (Fig. 8). In cortices of all stages the decrease of this ratio was retarded by a dose of 750 r (Fig. 8). The decrease in ratio was also retarded in nuclei of irradiated brain stems (Fig. 8). Significantly, control fatty acid ratios of the nuclei in brain stems (the more mature portion of the brain at this early age) were those assumed to be more characteristic of adult-type nuclei (Fig. 8).

Linoleic acid in brain lipids decreased with age, reflecting the development of the "blood brain barrier." Irradiation did not appear to have any consistent effect on the percentage of linoleic acid in the brain lipids.

Discussion

Although the foregoing represents a purely introductory survey of biochemical effects of radiation on maturing brain, three points of interest stand out at this early stage.

First, per unit dry weight of cortex and cerebellum, the total lipid, total phospholipid, and total nitrogen appear relatively unchanged by exposure of the heads of rats to 750 r of x-irradiation. Thus, in these respects, the irradiated neonatal cortex and cerebellum can be considered essentially as miniatures of their control counterparts, differing primarily in having fewer total cells per organ and having these cells poorly arranged in the greater structural context. A similar situation appears to exist in the deformed skeletons of rat embryos irradiated between 12 and 16 days of gestation (Russell, 1954). As far as is known, calcification mechanisms of the structurally-poor skeletons are unimpaired by the radiations that initiated the deformity; i.e., the enzymes involved in calcification appear to be present in sufficient quantity in irradiated bone.

Contrasting with the lack of biochemical effects of radiations hereto observed on cerebellum and cerebral cortex is the inhibition of lipid synthesis

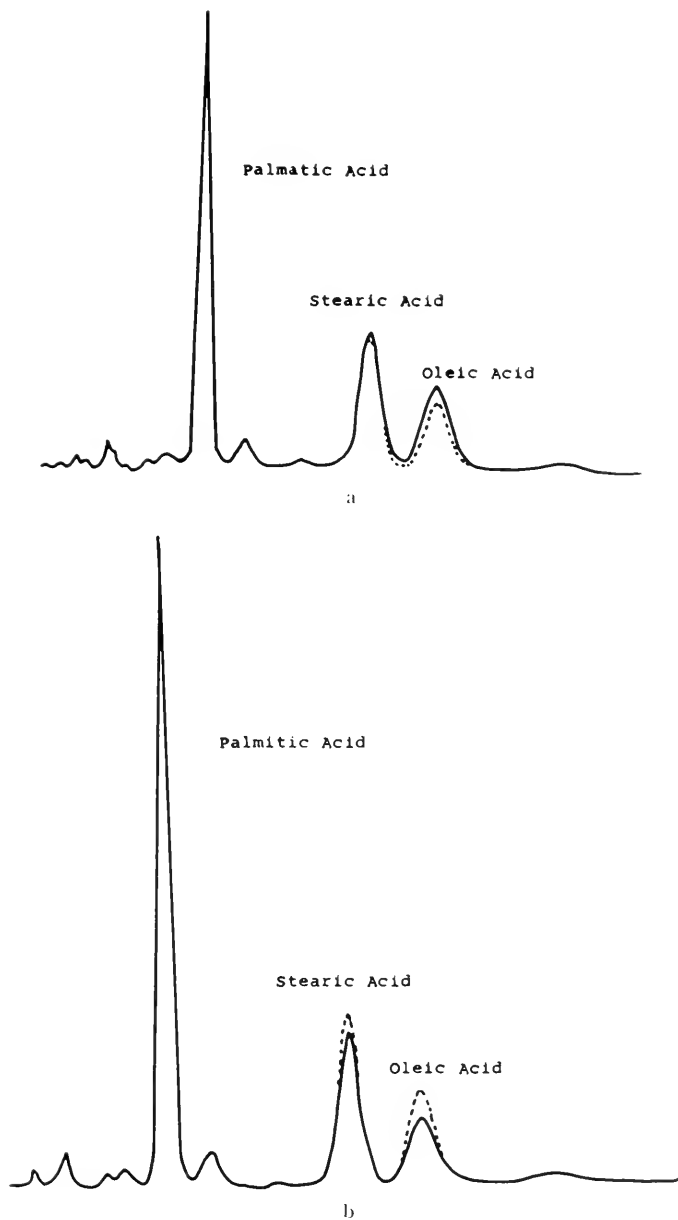


FIG. 7(a) Fatty acid profiles of nuclei from cells of 8-day cortices. Solid line=control pattern. Dotted line=pattern from x-irradiated cortices.

FIG. 7(b). Fatty acid profiles of mitochondria from cells of 8-day cortices. Solid line=control pattern. Dotted line=pattern from x-irradiated cortex.

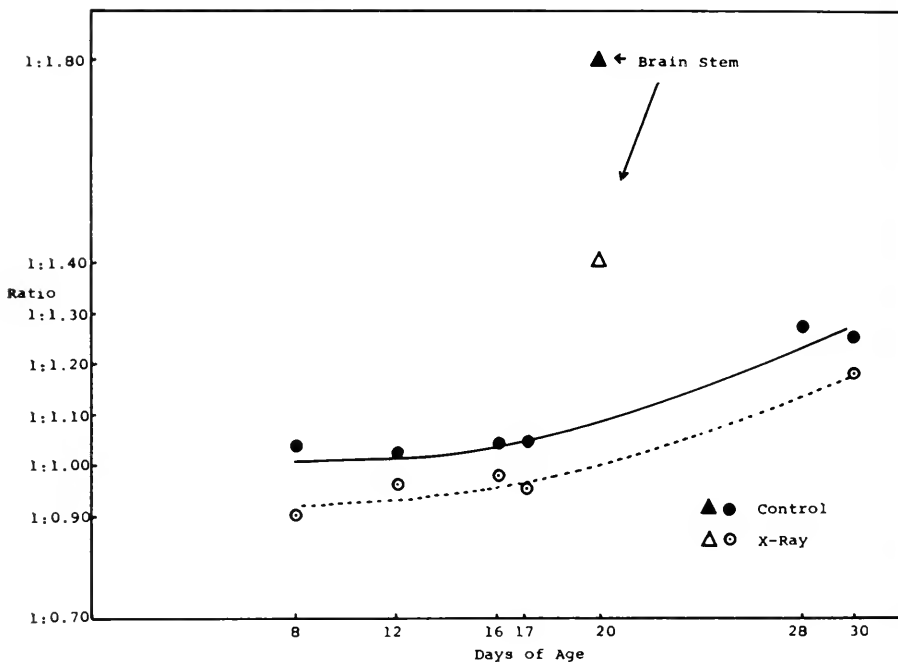


FIG. 8. Ratios of palmitic acid to combined total of stearic and oleic acids in nuclei of control and x-irradiated neonatal rat cortices and brain stems.

in the brain stem. Because of the important relationship that has been (indirectly) established between the neural cells and closely adjacent oligodendroglia cells with respect to the production of myelin (lipid), it would appear to be important to determine histologically the ratio of oligodendroglia to neural cells in irradiated brain stem, for in this organ complex, not only does the inhibition of lipid synthesis correlate closely with the retardation of dehydration, but the inhibition is first noted (ca 16 days) at a point in development when a large increase in myelination processes takes place. These results point up the fact that the various sections of the brain are by no means homogeneous tissues and offer the possibility that in brain stems the oligodendroglia are vulnerable to radiations at a time when the strictly neural elements may be relatively less so. (Such vulnerability could be expressed either as outright destruction of the cells or alteration of their synthetic abilities.) Histological studies currently being carried out by one of the authors of this paper (C. D. Clemente) should help in the elucidation of this issue.

A second point of interest concerns the radiation-induced decreases in mitochondrial populations as observed in the cortices of some younger and

older rats. Such an effect is seen in cells of the embryonic chicken liver (Schjeide, 1960) and is thought to be indirect because of the time required (3 days) for the fall in population. It remains to be determined whether the decrease in mitochondria observed in the neonatal rat cortex is consistent and due to direct effects, to damage to a controlling mechanism in the nucleus, or to elicitation of toxic blood-borne factors.

A third interesting result which may be a harbinger of better things to come with respect to elucidation of radiation effects on cell organelles, is the inhibition of change in fatty acid ratios of nuclei in all irradiated rats.

The changes in nuclear fatty acid ratios that occur normally as a function of age have tentatively been interpreted as reflecting an advance in the maturity of these organelles. Thus, one of the interpretations for the inhibition of these shifts in ratios following irradiation is that maturation (or differentiation) of the nucleus has been retarded due to injury by oxidizing radicals. However, in a heterogenous tissue, such as brain, changes in organelle fatty acid ratios may merely reflect changes in the proportions of resident cells. The importance of good histology as an adjunct to biochemical studies in these tissues is thus emphasized.

In most animals receiving irradiation to the head only, there was a decrease in total body weight and the weights of such organs as liver, spleen, kidney and heart. Although the changes in weights of the various brain components did not appear to correlate closely with the weight changes of the above organs, the influence of irradiation on the pituitary (and hence a probable change in output of certain hormones) is a possible factor influencing the observed results. However, a personal communication from Dr. Van Dyke, of the University of California at Berkeley, indicated that growth hormone administered to irradiated neonatal rats has no effect in delaying the onset—or modifying the intensity—of neurological aberrations.

Summary

All three major divisions of the brain (brain stem, cortex, and cerebellum) were inhibited in growth following irradiation (750 r) to the head at 2 days of age. Growth of brain stem was not retarded until about the 16th day, and due to a relatively slow rate of growth in the control animals, the difference in dry weight of this part of the brain at 4 weeks postirradiation was not great. In cortex, the inhibition of growth was also first discernible at about 16 days, but due to a relatively fast rate of increase in the control animals, the differential between control and irradiated cortices at 4 weeks was very significant. Cerebellum was most profoundly affected by x-irradiation, the decrease in size being quite apparent early in the 2nd week following exposure.

Although in this preliminary survey no differences could be detected between irradiated and control cortices (and cerebellums) in terms of relative lipid, relative phospholipid, and relative nitrogen, irradiated brain stem appeared to contain relatively less total lipid beginning at about 14 days following exposure. The inhibition of lipid synthesis in the brain stem (failure of myelination) was accompanied by a parallel retardation of the dehydration normally occurring as a function of age.

Some tissues from irradiated brain revealed decreases in wet volumes of mitochondria. In no case was there a significant increase of mitochondria in irradiated tissue.

The ratio of palmitic acid to stearic acid and to oleic acid in the nuclei decreased as a function of age. The development of this ratio was retarded in the irradiated tissues examined.

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Some Effects of Nucleic Acid Antimetabolites on the Central Nervous System of the Cat*

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Among the biologic effects imputed to ionizing radiation is a disturbance in nucleic acid metabolism. The deleterious effect of ionizing radiation on DNA, particularly in proliferating tissues, is well known (Seed, 1960). Its influence on RNA metabolism has received less attention. That it is not without effect on the latter, however, is suggested by several recent studies (Krogh and Bergeder, 1957; Schummelfeder, 1957). It may be germane to this symposium to describe some of the effects of certain nucleic acid antimetabolites on the mammalian central nervous system.

These studies had their inception in observations made earlier with the aid of tagged precursors which showed that neurons, oligodendroglia, and certain other cells are site of active RNA and protein turnover (Koenig, 1958a,b). A slow labeling of DNA also occurs among these cells, neurons excepted, which probably indicates cell division. We have attempted to interfere with these metabolic activities through the use of nucleic acid antimetabolites. Intrathecal administration was used to circumvent the blood-brain barrier and to attain adequate local concentration of antimetabolites in the nervous system without damaging hematopoietic and other susceptible tissues. Of many purine and pyrimidine analogs tested, several fluorinated pyrimidines were found to produce interesting neurologic disorders (Koenig, 1958c). The neurotoxic antimetabolites were 5-fluoroorotic acid (FO), the analog of orotic acid (the natural precursor of pyrimidines) and the ribosides, 5-fluorouridine (FUR) and 5-fluorocytidine (FCR) (Fig. 1). The pyrimidine bases, 5-fluorouracil (FU) and 5-fluorocytosine (FC), were without overt effect, even in large doses. The clinical, pathologic, and biochemical effects of these analogs, particularly FO, on the feline neuraxis have been under investigation for several years. Although their biochemical effects have not been

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Fig. 1. Structural formulas of orotic acid and 5-fluoroorotic acid.

worked out completely, sufficient data have been collected to warrant the conclusion that the neurologic effects of these compounds are attributable, directly or indirectly, to disturbances in pyrimidine nucleotide or nucleic acid metabolism or both.

Intracisternal Administration

Intracisternal administration of 5–15 mg of sodium salt of FO (or 2–5 mg FUR) in cats produces a progressive rhombencephalopathy and, in some animals, a cervical myelopathy (Koenig, 1958c). Signs of neural dysfunction appear on the 4th to 6th day after injection. The first indication of disease is a mild clumsiness of gait, which worsens in time. The gait becomes broad-based, unsteady and dysmetric. Decomposition of movement, oscillation of trunk and limbs, and reeling gait complete the picture of cerebellar ataxia. This usually becomes so disabling that the animal is incapable of locomotion or alimentation by the 2nd or 3rd week after injection of the antimetabolites. Many animals have signs of neuronal irritation, including fasciculations of facial musculature, myoclonic jerks of forelimbs, and various tonic and runnings seizures. Animals die of inanition, seizures, or bulbar failure by the 3rd week. Outstanding pathologic disturbance is a depletion of Nissl substance in Purkinje neurons of the cerebellum and in neurons of the brain stem and cervical spinal cord.

Intraspinal Injection

Injection of FO into the lumbar subarachnoid space (10–15 mg divided into two doses and injected 3–4 hours apart) in cats produces a progressive myelopathy, which becomes evident on the 2nd or 3rd day (Koenig, 1960). FUR and FCR produce a similar disorder in doses of 2–4 mg. Signs of neuronal irritation appear first. These consist of muscle fasciculations, hyperesthesia, and sometimes myoclonic jerks in the hindquarters (Fig. 2) and are associated with clumsiness, mild weakness, enhanced stretch reflexes, and

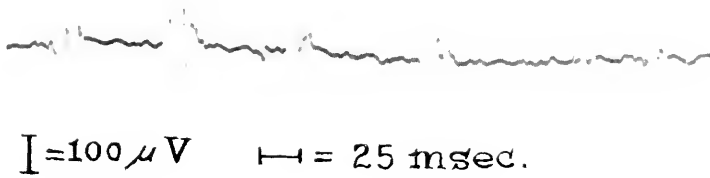
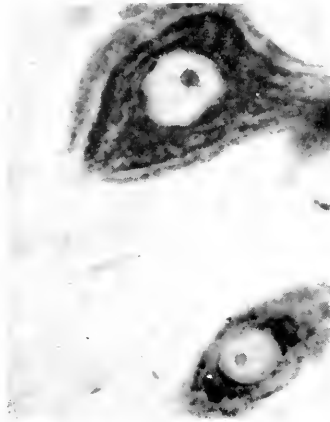


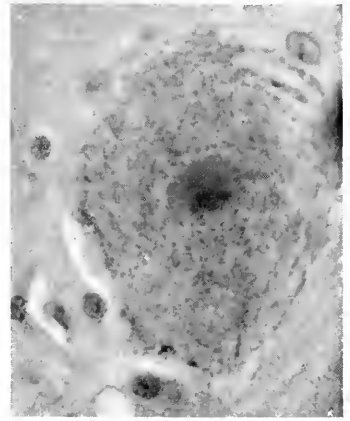
FIG. 2. Fasciculations recorded electromyographically in the hamstrings 3 days after FO.

hypertonia of flexor muscles in the hindlimbs. In some animals this may not progress beyond a hyperreflexic paraparesis. In more severely affected animals, the signs of neuronal irritation diminish and paraplegia with loss of muscle tone and stretch reflexes ensues. Sensation becomes obtunded, and the sphincters are paralyzed. These signs indicate a loss of neuronal function. Denervation atrophy with fibrillary potentials may appear in the 2nd week, presumably because some motoneurons are destroyed. Animals with a mild myelopathy, i.e., a hyperreflexic paraparesis, after a period of stability or improvement enter a second stage of illness during the 3rd to 4th week. Their condition worsens, and within a day or two they exhibit an extensor paraplegia with dulling of sensibility in the hindquarters and acute urinary retention. Spastic weakness of the forelimbs sometimes occurs later. In most severely afflicted animals, flaccid paraplegia appears in 4 to 5 days. The forelimbs are affected early, and death occurs from respiratory failure by 5 to 7 days. In general, the larger the dose of the analog, the more severe is the myelopathy.

The histopathology of the myelopathy produced by FO has been carefully investigated (Koenig, 1960). The changes initially are confined to neurons. Inflammatory or vascular lesions do not occur. The nucleoli of nerve cells become small and less basophilic. Within 3 or 4 days a fragmentation and loss of peripherally situated Nissl substance is seen in spinal motoneurons and interneurons (Fig. 3). Depletion of Nissl substance progresses to involve much of the perikaryon by 7 to 10 days (Fig. 4). Signs of recovery then appear in viable neurons. These consist of a striking hypertrophy and an increase in basophilia of nucleoli (Fig. 5) followed by increasing amounts of Nissl substance. Regeneration is well advanced by 35 to 50 days, and some neurons are even chromophilic. White matter is structurally intact for 2 to 3 weeks, but thereafter it almost always exhibits some spongy or myxocystic degeneration with varying quantities of neural fat, either free or in macrophages (Fig. 6). A thinning of oligodendroglia is seen in white matter at this



3



4

FIG. 3. Peripheral chromatolysis with reduction in size and basophilia of nucleoli in neurons of L-7 three days after FO. Thionin, $\times 700$.

FIG. 4. Generalized chromatolysis of lumbar motoneuron seven days after FO. Gallocyanin, $\times 800$.

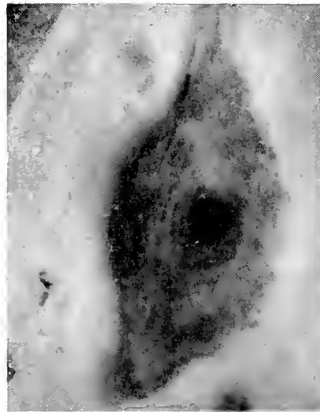


FIG. 5. Recovering lumbar motoneuron 21 days after FO. Note hypertrophy and increased basophilia of nucleolus. Gallocyanin, $\times 700$.

stage. The white matter lesion occurs at a time when neurons are recovering and probably results from oligodendroglial disease.

Signs of neuronal irritability are correlated with a minimal to moderate depletion of Nissl substance. Loss of neuronal function is associated with severe loss of Nissl substance. In most severely affected animals, a necrosis of

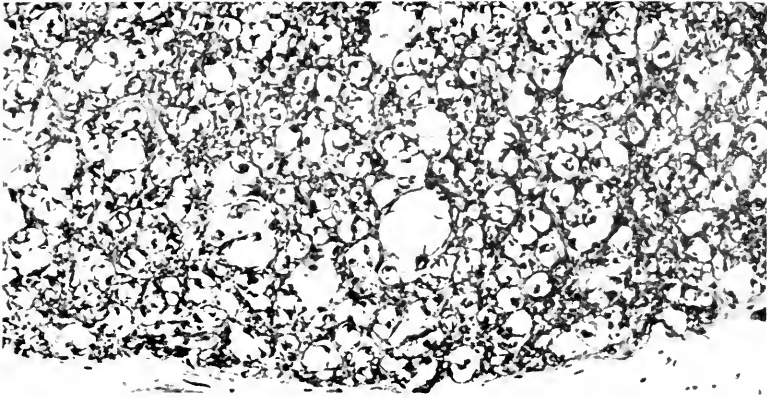


FIG. 6. White matter. L-1, 50 days after FO. Note status spongiosus. Weil stain, $\times 195$.

gray matter appears early, but this is not common unless 20 mg or more of FO are used. The delayed, subacute deterioration in spinal cord function is associated with spongy degeneration of white matter.

Intracerebral Administration

The injection of 4–6 mg FO (2–3 mg FUR) into the sensorimotor cortex of the cat (3–5 mm under the pial surface through a 26 gauge needle) produces a focal cortical encephalopathy (Kurth *et al.*, 1960). A moderate hemiparesis, proprioceptive deficit, and impaired contact placing reaction appear in the contralateral limbs 3 or 4 days after injection. Focal motor seizures are sometimes seen. The disorder progresses for several days and then becomes stationary or improves slightly. Neural dysfunction persists for several months. A focal electroencephalographic defect is demonstrable in the vicinity of the injection. Normal rhythms are replaced in part by slow waves, sharp waves, and high voltage spikes (Figs. 7 and 8). Focal seizure activity appears spontaneously or may be provoked by joint movement or skin pinching of the contralateral limbs. Biochemical studies have revealed a high incorporation of FO-2-C¹⁴ into RNA near the injection site. Indeed, there is a close correlation between the presence of electroencephalographic abnormalities and a high uptake of FO into RNA of affected cortex.

The introduction of FO or FUR into the temporal lobe of cats may produce alterations in personality and epileptiform seizures (Koenig *et al.*, 1960b). Some animals become withdrawn, unfriendly, hostile, and even aggressive, with periods of confusion, stupor, and other disturbances of behavior indicative of temporal lobe seizures. Sometimes the contralateral pupil

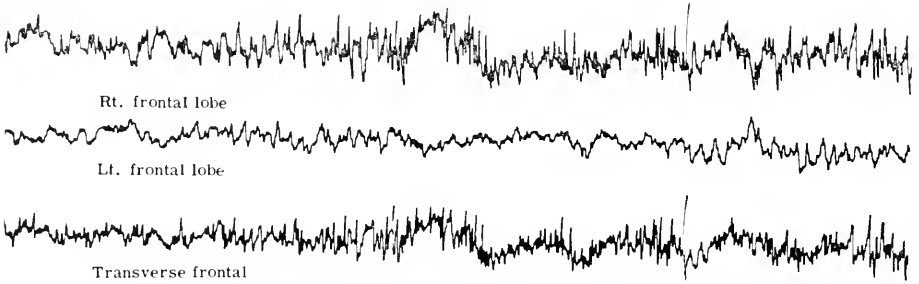


FIG. 7. EEG 16 days after injection of 6 mg FO into right frontal lobe. Note numerous spikes. Pentobarbital anesthesia.

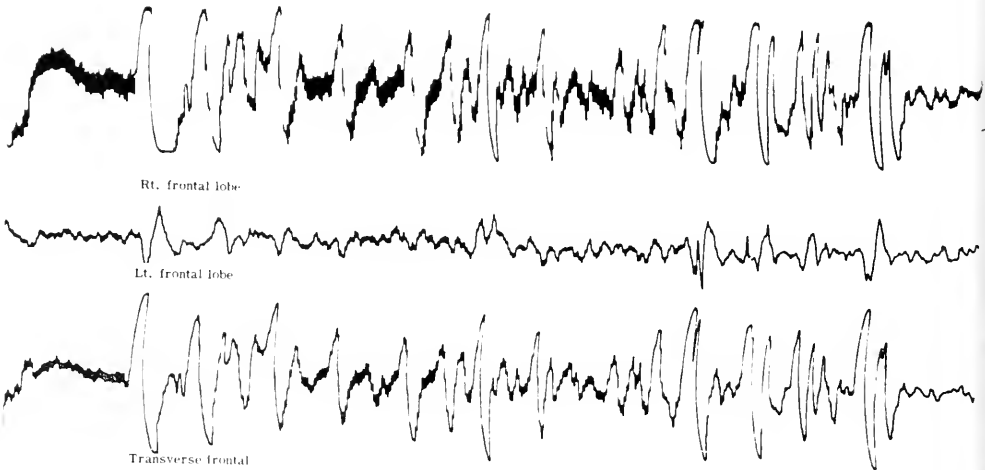


FIG. 8. EEG 15 days after injection of 6 mg FO into right frontal lobe. Note paroxysm of high voltage activity. Pentobarbital anesthesia.

becomes dilated, and the nictitating membrane retracts. Focal electroencephalographic defects appear 2 to 4 days after injection, with slow waves, sharp waves, and spikes (Figs. 9 and 10). Some of these abnormal rhythms are propagated to the opposite temporal lobe. Spontaneous focal and generalized electrical seizures occur, even though animals are under pentobarbital anesthesia. Cytopathologic changes occur in cortical neurons which are similar in character to those observed when FO is administered elsewhere in the central nervous system. However, the changes are mild and often unrecognizable when neuronal dysfunction is present.

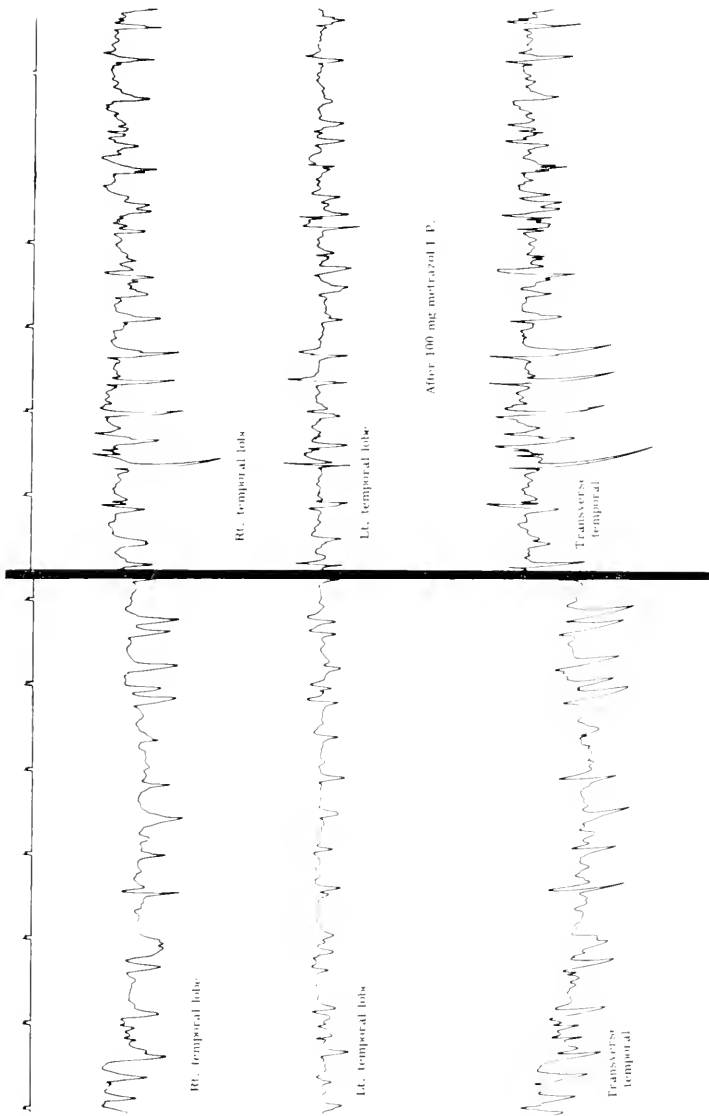


FIG. 9. EEG 15 days after 12 mg FUR into right temporal lobe. Note random spikes before metrazol and the increase in frequency and voltage of spikes after metrazol activation. Many spikes are propagated to left temporal lobe. Pentobarbital anesthesia.

Metabolism of FO

The metabolism of tagged FO (FO-2-C¹⁴) in the spinal cord of cats has been investigated by autoradiographic and biochemical methods (Koenig and Young, 1960; Koenig *et al.*, 1960a). Column and paper chromatography have been used for nucleotide analysis. FO is metabolized similarly to orotic

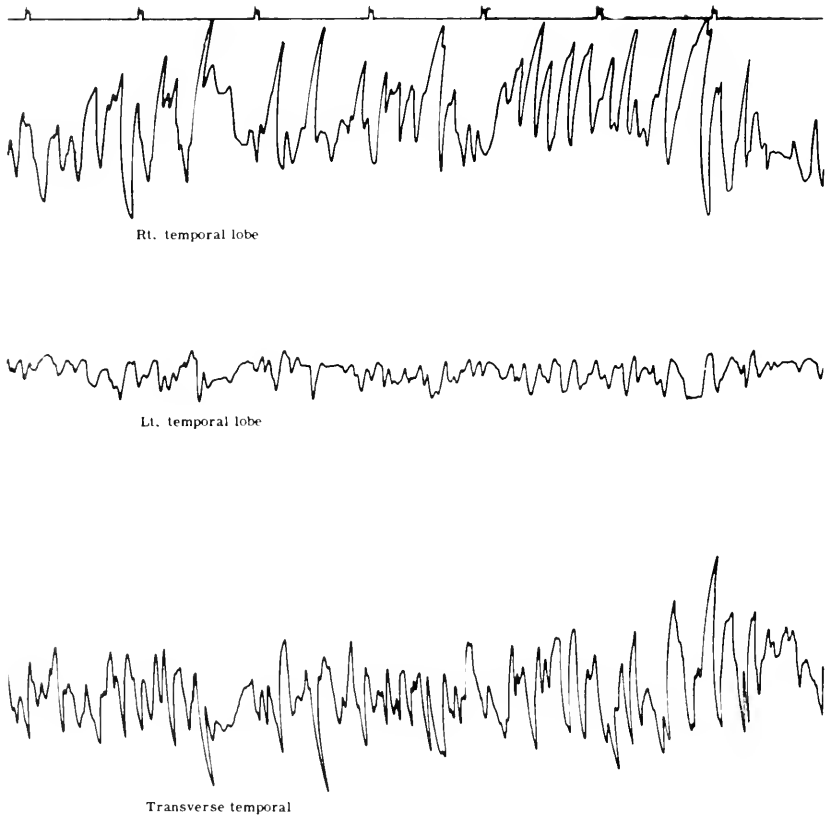


Fig. 10. EEG 6 days after 12 mg FUR into right temporal lobe. Note seizure activity. Pentobarbital anesthesia.

acid, the natural precursor of pyrimidines in the nervous system. These studies have disclosed that FO is efficiently converted into the following acid-soluble nucleotides in nervous tissue: fluorouridine monophosphate, diphosphate, and triphosphate; fluorouridine diphosphate-glucose and diphosphate-acetylglucosamine, and fluorocytidine monophosphate (Fig. 11). FO is incorporated into RNA as fluorouridine monophosphate, but is not incorporated into DNA. Unlike FO, 5-fluorouracil (FU), is not converted efficiently into acid-soluble and RNA nucleotides in the feline neuraxis. Uracil itself also is poorly metabolized by this tissue.

These observations suggest that nucleoside phosphorylase, the enzyme which converts pyrimidine bases to their ribosides, is present in scanty amounts in the central nervous system of the cat. Poor anabolic conversion

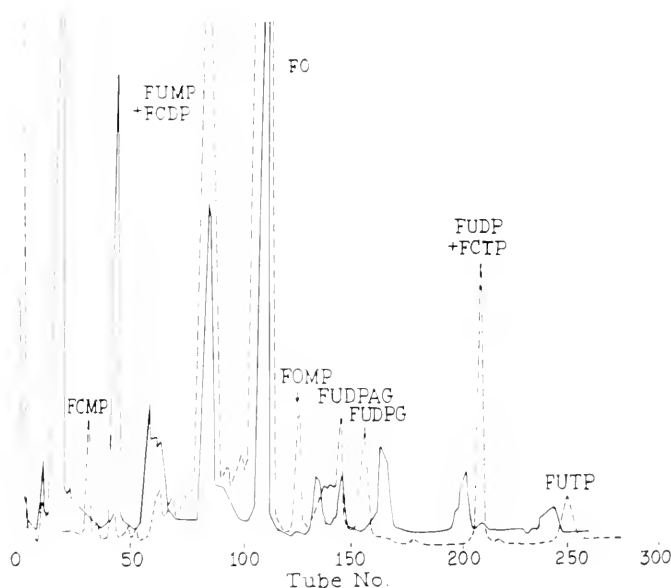


FIG. 11. Chromatogram of acid-soluble fraction of spinal cord obtained by extended gradient elution with formic acid-ammonium formate from Dowex 1 column. Tissue removed 4 hours after the intraspinal injection of FO-2- C^{14} .

Key: —D 260 μm ; - - - Radioactivity of C^{14} .

of FU and FC probably accounts for failure of these analogs to produce neurologic disturbances. Similar anabolic conversions of these fluorinated pyrimidines have been described in other organs (Harbers *et al.*, 1959).

The morphologic distribution of incorporated FO has been examined by high resolution autoradiography (Koenig and Young, 1960). FO is taken up into RNA of neurons and oligodendroglia and leptomeningeal, ependymal, and Schwann cells. Initially FO is incorporated into nuclear RNA. Some labeling of cytoplasmic RNA is discerned in nerve cells after a day or so; however, FO persists in nuclear RNA for a number of weeks.

Metabolic Effects of FO

The histopathologic changes produced by FO suggest a depletion of neuronal RNA. The results of biochemical analysis corroborate this inference (Koenig *et al.*, 1960a). The concentration of RNA in gray and white matter of spinal cord diminishes by 30–50% after 1 week. A greater depression in RNA concentration occurs in animals with areflexic paraplegia than in those with hyperactive reflexes. The incorporation of labeled orotic acid and

adenine into RNA *in vivo* is depressed by FO (Fig. 12). FO evidently interferes with the biosynthesis of RNA in neural tissue. The mechanism by which FO brings about a depletion of RNA, however, has not been ascertained at the time of this writing.

The well-known participation of RNA in protein synthesis has led us to investigate the uptake of labeled amino acids into protein. Initially, FO does not depress the incorporation of tagged methionine and lysine into neural protein. A depression of 50–85% is observed after 1 week, however. Significantly, the greatest depression in uptake is observed in cases of severe neuropathy, i.e., when areflexic paraplegia is present. Thus, a loss of neuronal function is associated with a greater depletion of RNA and a severe defect in protein biosynthesis in affected nerve cells (Fig. 13). The formation of spurious RNA molecules also could contribute to the defect in protein synthesis.

The role of pyrimidine nucleotides as cofactors in lipid and polysaccharide biosynthesis and in interconversion of sugars has been recognized recently (Henderson and LePage, 1958). The formation of spurious fluoropyrimidine nucleotides suggests that disturbances in lipid and carbohydrate metabolism may be partly responsible for the neurologic disorders that are produced by the fluorinated pyrimidines. This possibility is being investigated. The biochemical basis for the neuronal hyperirritability also remains to be elucidated.

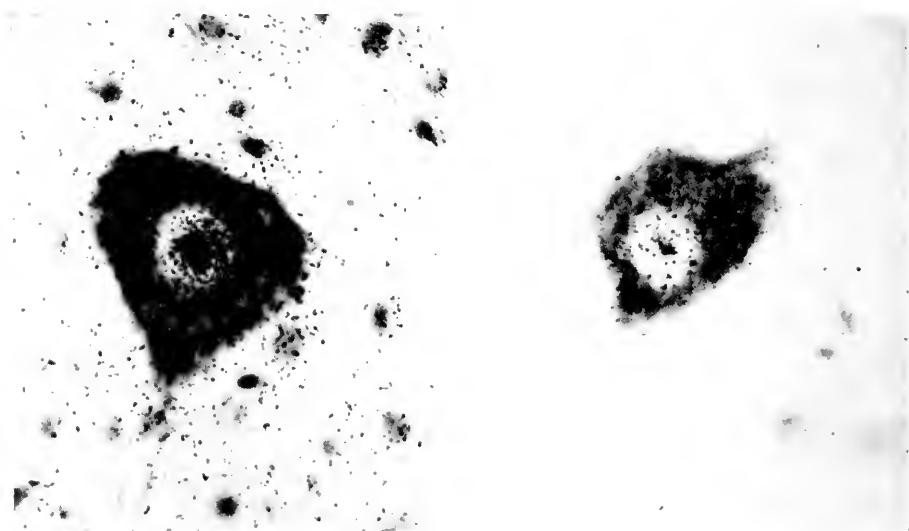


FIG. 12. Autoradiographs showing depressed uptake of orotic-6-C¹⁴ into RNA of lumbar motoneuron 2 days after FO. Control on left, experimental on right. $\times 700$.

Summary

Intrathecal administration of the fluorinated pyrimidines, FO, FUR, and FCR, results in interesting neurologic disorders, the nature of which depends on the injection site. Myelopathy, rhombencephalopathy, and cortical encephalopathy are produced by the intralumbar, intracisternal, and intracerebral routes, respectively. An asymptomatic "incubation" period precedes the appearance of neural dysfunction. Signs of neuronal hyperirritability appear

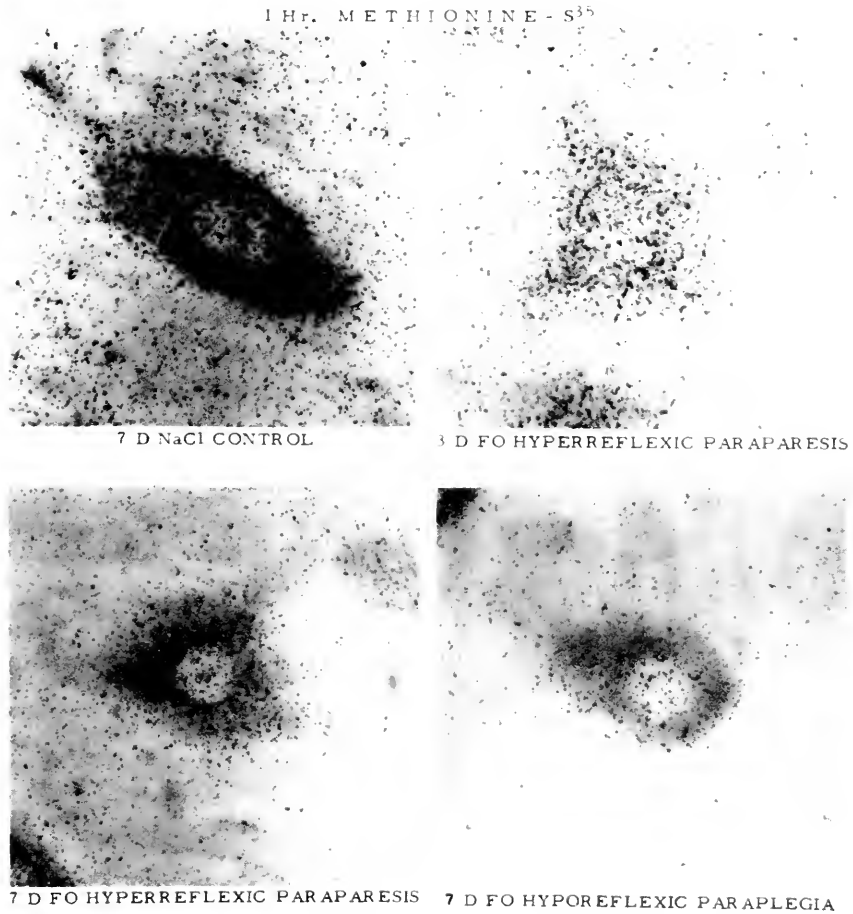


FIG. 13. Autoradiographs of L-7 segment showing uptake of methionine-S³⁵ into neuronal protein. Note reduction in blackening over experimental neurons three and seven days after FO, most marked in animal with hyporeflexic paraplegia. X 700.

first. In more severe intoxications, loss of neuronal function follows the stage of neuronal irritation. Alterations in neuronal structure accompany the neurologic disorder. RNA-containing structures, i.e., nucleoli and Nissl bodies, are conspicuously affected. The neuronopathy may be reversible or may result in necrobiosis, depending on the severity of intoxication. Spongy degeneration of white matter occurs later, probably caused by oligodendroglial disease.

FO undergoes conversion to acid-soluble nucleotides and is incorporated into RNA in the feline neuraxis. Indeed, anabolic conversion of the fluorinated pyrimidines seems to be a requirement for the production of neural dysfunction. A depletion of RNA and a depression in protein biosynthesis appear later and are most marked in neurons that become inexcitable. Disturbances in other metabolic spheres may exist, but have not been demonstrated. Many points of similarity, both physiologic and pathologic, can be discerned between the disorders described and some virus infections and degenerative diseases of the nervous system. It seems possible, therefore, that derangements in pyrimidine nucleotide or nucleic acid metabolism may be present in some of the latter disorders. The fluorinated pyrimidines are useful tools for the production of focal neuronal disease. Their use may provide experimental models for some of the degenerative neuronal diseases that afflict man.

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Geographic Distribution of Multiple Sclerosis in Relation to Geomagnetic Latitude and Cosmic Rays*

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Introduction

Despite the fact that multiple sclerosis has been known as a clinical entity for over a century, its etiology is still an enigma (Schumacher, 1960). Moreover, there is no specific treatment for the disease, which in its later stages often results in severe crippling. The disability results from interference with the processes of electrical conduction along nerve fibers in the brain and spinal cord as the myelin sheath of the fibers degenerates in localized regions; the term "demyelinating disease" is accordingly used.

One of the interesting aspects of the disease is its geographic distribution. It has been a clinical impression for some years (Steiner, 1938) that multiple sclerosis does not have a uniform distribution throughout the world, and several epidemiologic surveys have been undertaken to clarify this distribution (McAlpine *et al.*, 1955; Hyllested, 1956; Kurland *et al.*, 1957). The disease appears to be appreciably more common in northern than in southern latitudes in North America and in Europe, but uncommon in the Orient, South America, Africa, and the tropics and subtropics.

There have been several possible explanations advanced for this geographic distribution, some of which I have reviewed elsewhere (Barlow, 1960), but none has appeared to be consistent with all of the available data for the distribution. More recently, Acheson *et al.* (1960) have found that the geographic distribution of multiple sclerosis among veterans in the United States correlates strongly in an inverse manner with the average solar radiation of place of birth, and in particular with the December solar radiation, the implication being that this agent may in some way act as a preventive or protective agent against multiple sclerosis. When the distribution by residence at onset of symptoms was examined, the correlation appeared best

* This work was supported by the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

with geographic latitude. Since, according to these authors, the isolines for winter solar radiation follow the lines of geographic latitude fairly closely, it is not clear that the relationship observed between December solar radiation and distribution of multiple sclerosis by birthplace would prevail in a similar manner for other areas of the world, for geographic latitude itself does not appear to be a good correlate when data for the disease from different areas of the world are examined (Barlow, 1960). It may well be, however, that the geographic distribution of the disease is determined by several factors, and the importance of each of these may vary in different areas.

The possibility that geologic factors, perhaps in trace elements, may have a role in the distribution of multiple sclerosis recently has been reiterated by Warren (1959).

Latitude Distribution of Multiple Sclerosis

In the United States in recent years, Dr. Leonard T. Kurland of the Epidemiology Branch of the National Institute of Neurological Diseases and Blindness has been particularly concerned with surveys on multiple sclerosis, and it was as a result of his epidemiologic summary at the First International Congress of Neurological Sciences in Brussels in 1957 that the present approach had its origin. At that congress, Kurland presented data for North America which indicated that the frequency of the disease is strongly dependent on latitude, and he suggested that any satisfactory explanation of the etiology must take the geographic factor into account (Kurland *et al.*, 1957), a view also held by other observers (McAlpine *et al.*, 1955).

The variation of the disease with latitude particularly interested me, and I undertook to determine if there were any similarity between this latitude effect and the intensity of cosmic rays, whose distribution is well known to be dependent in part on latitude (Barlow, 1959).

The variation of cosmic ray flux is determined by the earth's magnetic field and therefore is related to geomagnetic latitude rather than to geographic latitude. A map indicating geomagnetic latitude in relation to geographic latitude is reproduced in Fig. 1. The lines of constant geomagnetic latitude are skewed with respect to those of constant geographic latitude, derived from the fact that the earth's magnetic axis is inclined at an angle of about 10° with respect to its axis of rotation. For the eastern United States, the geomagnetic latitude for a given location is about 10° greater than its geographic latitude; for western Europe, the two are approximately the same, and for eastern Asia, the geomagnetic latitude for a particular location is about 10° less than its geographic latitude. It is apparent then that the two latitudes may differ from one another by an amount up to plus or minus 10° , a total range of about 20° .

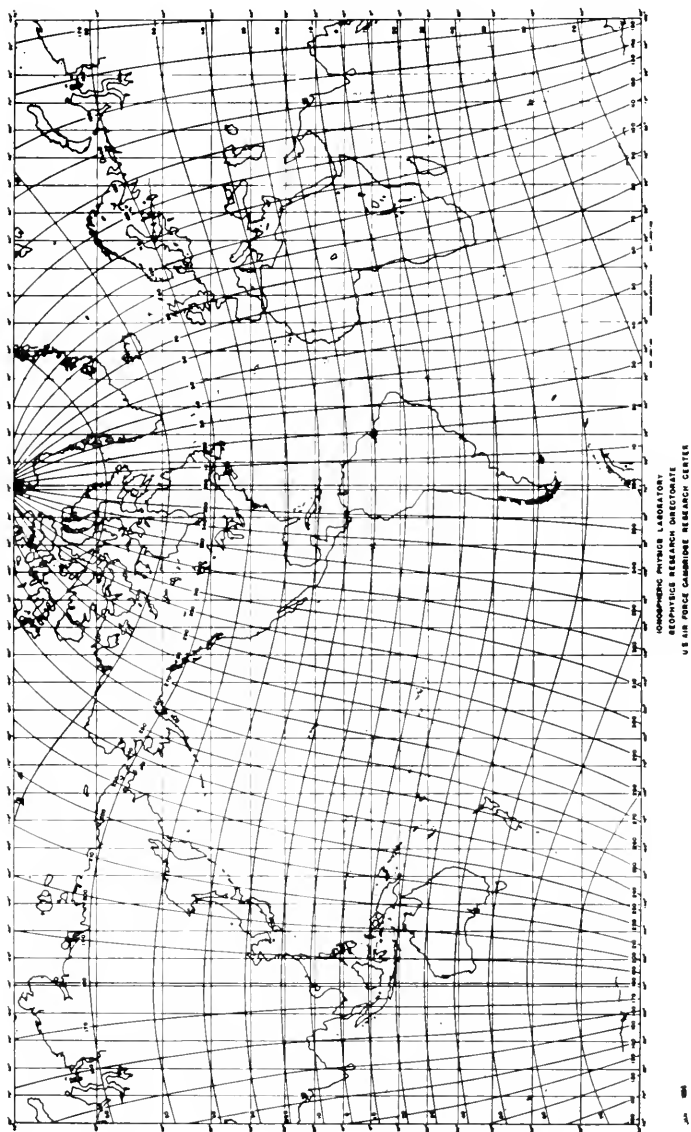


FIG. 1. Map of the world in geographic and geomagnetic coordinates. (From Chernosky and Maple, 1952.)

Prevalence Surveys

To study the latitude effect for multiple sclerosis, several independent sets of statistics for the United States and other areas in the world were examined (Barlow, 1960). These included mortality data, prevalence data, (i.e. data

concerning the total number of cases in a population at a particular time), incidence data (i.e. the number of new cases appearing in a population per year), and statistics concerning hospital admissions for the disease.

Since multiple sclerosis is a chronic disease (the average case has a duration of approximately 20 years after onset of symptoms), it appears that the most reliable indication of its distribution may be obtained from prevalence data. Table I shows results from a series of surveys in the United States and

TABLE I
MULTIPLE SCLEROSIS PREVALENCE RATIOS FOR THE WHITE POPULATION
IN SELECTED COMMUNITIES IN THE UNITED STATES AND CANADA^a

City	Geographic latitude ($^{\circ}$ N)	Geomagnetic latitude ($^{\circ}$ N)	Prevalence per 100,000 population ^b	Prevalence relative to Winnipeg
Winnipeg	50	60	42 (40)	1.0 (0.95)
Boston	42	53	41	0.97
Denver	40	48	38	0.90
San Francisco	37	43	30	0.71
Charleston, S. C.	32	43	18 (12)	0.43 (0.29)
New Orleans	30	40	13 (6)	0.31 (0.14)

^a Data from Kurland *et al.*, 1957.

^b Values in parentheses are corrected values on "clinical review" of the reported cases.

Canada. In the present study, emphasis is laid on relative rather than absolute frequencies of occurrence of the disease in different areas, and the table indicates the prevalence in each city relative to that for Winnipeg. Relative

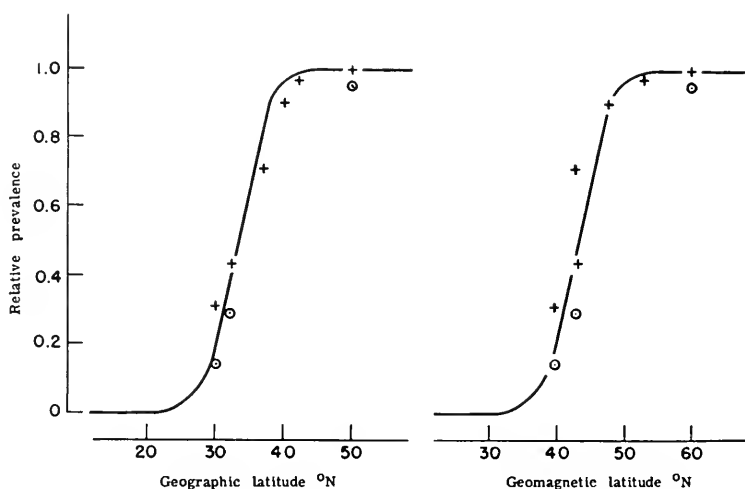


FIG. 2. Relative prevalence of multiple sclerosis in selected communities in the United States and Canada (see Table I).

prevalence is plotted against geographic and geomagnetic latitude in Fig. 2, and the points suggest a sigmoid curve. A normal sigmoid curve (i.e., the integral curve for a normal distribution) has been included in each plot, and it is evident that it is not possible to conclude from these data drawn from a limited range of longitude whether geomagnetic latitude is a better parameter than geographic latitude. The general trend shown by the points between approximately 40° and 50° has been confirmed from several other types of data for the United States (Barlow, 1960; Acheson, 1959), although the presence of a "knee" at about 50° geomagnetic latitude is not clear from these latter data. Evidence that a rapid rise in the frequency of the disease between geomagnetic latitudes of approximately 40° and 50° appears for other areas of the world in both the northern and southern hemispheres has previously been presented (Barlow, 1960).

For further examination of the latitude distribution, prevalence data from recently conducted surveys presented at the Geomedical Conference in Copenhagen in June, 1959 (Hyllested, 1960) are listed in Table II. It also includes the data from Table I and results of other prevalence surveys. As in Table I, the mean prevalence ratio for locations of 50° or greater geomagnetic latitude was determined, and prevalences relative to this standard (48 cases per 100,000) are shown. These data are plotted against geographic and geomagnetic latitude in Fig. 3. Since a wide range of longitudes is represented in these plots, the sigmoid curve for the *geomagnetic* plot of Fig. 2 is reproduced in both the geographic and the geomagnetic plot of Fig. 3, and it will be reproduced in subsequent plots for comparative purposes.

It is apparent from inspection of the two plots that at any given latitude the scatter of the points is greater for the geographic plot than for the geomagnetic plot, at least for latitudes of less than 50° . The scatter of the points above 50° is such that a "knee" or leveling off is not as clearly suggested as in Fig. 2. There is some indication, however, that the rapid increase of prevalence between 40° and 50° geomagnetic latitude does not continue upward in the same manner beyond 50° .

Several independent surveys are represented in Fig. 3; therefore it is not possible to state how much of the scatter of points above 50° geomagnetic latitude is due to differences in survey procedures and how much is due to real differences in prevalence among the population groups. It is probably unlikely that the scatter is entirely due to differences in survey procedures. Even if uniform survey procedures were used, such a scatter of points could conceivably occur if the prevalence ratios among different population groups formed a normal distribution about a mean value, and the scatter might further be accentuated by differences in the size of the population groups. Particularly if the population is very small, chance variations in the observed prevalence ratios may be pronounced for occasional communities (Deacon *et al.*, 1959).

TABLE II
PREVALENCE OF MULTIPLE SCLEROSIS AT VARIOUS LATITUDES

<i>City or country</i>	<i>Geographic latitude</i>	<i>Geomagnetic latitude</i>	<i>Prevalence per 100,000 pop.</i>	<i>Relative prevalence^a</i>	<i>Investigator</i>
Surveys described at the Geomedical Conference ^b					
Faeroe Islands	62	65	19	0.40	Fog (1960)
West Norway	60	61	17 ^c	0.36	Presthus (1960)
East Norway	60	60	37 ^c	0.77	Oftedal (1960)
Göteborg, Sweden	58	57	62	1.3	Borman (1960)
Denmark	56	56	64	1.3	Hyllested (1960)
Durham & North- umberland Coun- ties, England	55	57	54	1.1	Miller (1960)
Gronigen, Holland	53	54	56	1.2	Dassel (1960)
Spessaert, Germany	50	51	75	1.6	Bammer and Schaltenbrand (1960)
Switzerland	47	47	50	1.0	Georgi and Hall (1960)
Missoula, Montana ^d	47	55	55	1.3	Siedler <i>et al.</i> (1958)
Halifax, Nova Scotia ^e	46	56	32	0.70	Alter <i>et al.</i> (1960)
Sapporo, Japan	43	33	1.6	0.04	Okinaka <i>et al.</i> (1960)
Turkey	39	35	2.5	0.05	Mutlu (1960)
Charleston, South Carolina ^d	33	44	14	0.30	Alter <i>et al.</i> (1960)
Kumamoto, Japan	33	21	1.8	0.04	Okinaka <i>et al.</i> (1960)
Egypt	27	24	0.2	0.004	Georgi and Hall (1960)
Yemen	15	11	"Unknown"	—	Georgi and Hall (1960)
East Africa	-10	-6	"Very few"	—	Georgi and Hall (1960)
Surveys cited by Kurland <i>et al.</i> (1957)					
Northern Scotland	58	61	55	1.1	
Northern Ireland	55	58	41	0.86	
Rochester, Minnesota ^d	44	53	64	1.3	
Kingston, Ontario ^e	44	55	53	1.1	
Winnipeg, Manitoba ^e	50	60	40	0.83	
Boston, Massachusetts ^d	42	53	41	0.86	
Denver, Colorado ^d	40	48	38	0.80	
San Francisco ^d	37	43	30	0.63	
New Orleans ^d	30	40	6	0.13	
Other surveys					
Budapest	47	46	20	0.42	Halasy (1957)

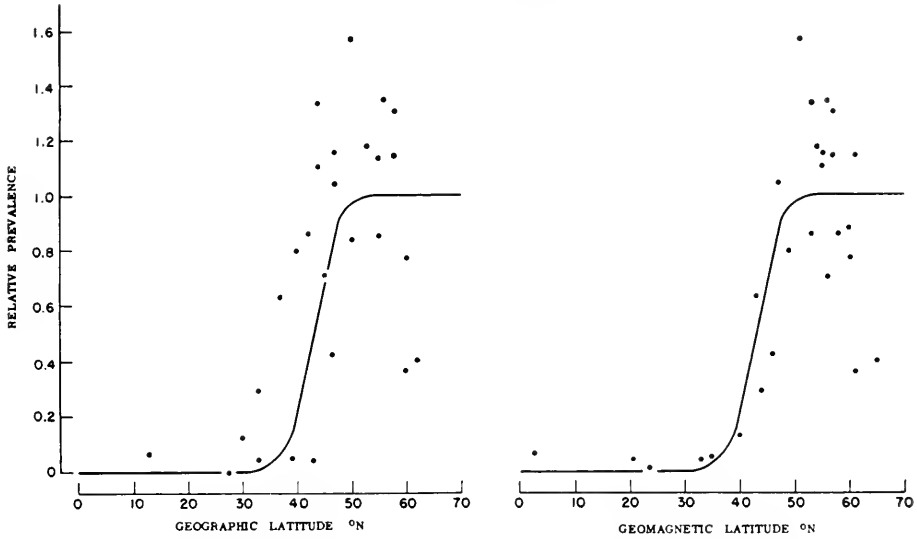


FIG. 3. Relative prevalence of multiple sclerosis for the localities listed in Table II. The sigmoid curve included in this and in subsequent figures is reproduced from that for the geomagnetic plot in Fig. 2.

Two surveys included in Table II are of special interest, for they were conducted at different latitudes in areas with almost the maximum possible range of difference between geographic and geomagnetic latitudes, i.e., in North America and Japan. Moreover, each of the two surveys included the

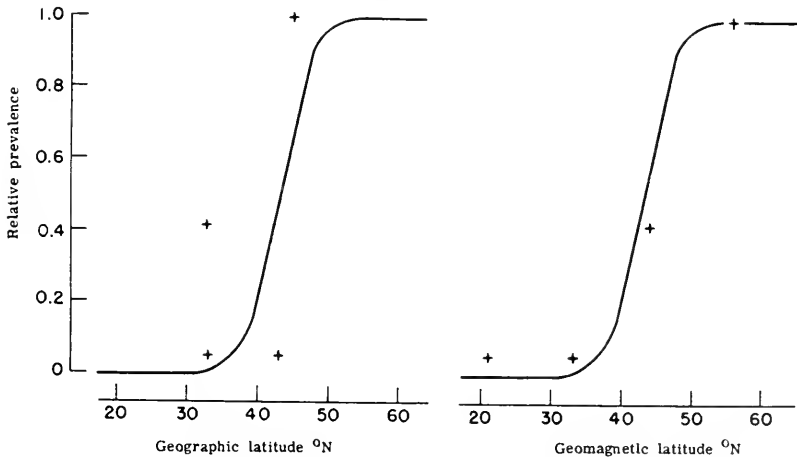


FIG. 4. Relative prevalence of multiple sclerosis for four communities in North America and Japan (see Table III).

FOOTNOTES TO TABLE II ON FACING PAGE 128

^a 1.0 corresponds to the mean prevalence for 50 or greater geomagnetic latitude (48 per 100,000).

^b Hyllested, K. (ed.) 1960. Report on the Geomedical Conference in Copenhagen, 1959. Studies in Multiple Sclerosis III. *Acta Psychiat. Neurol. Scand.* 35, Suppl. 147, 158 pp.

^c Provisional.

^d U.S.A.

^e Canada.

TABLE III
 RELATIVE PREVALENCE OF MULTIPLE SCLEROSIS IN NORTH AMERICA AND JAPAN

City	Geographic latitude	Geomagnetic latitude	Population (1955)	Number of cases	Prevalence per 100,000	Relative prevalence
Halifax, Nova Scotia ^a	45	56	198,000	64	32.3	1.0
Charleston, South Carolina ^a	33	44	188,000	26	13.5	0.42
Sapporo ^b	43	33	426,000	7	1.6	0.050
Kumamoto ^b	33	21	332,000	6	1.8	0.056

^a Data from Alter *et al.* (1960).

^b Data from Okinaka *et al.* (1960).

same team of observers for the two latitudes studied and similar diagnostic criteria (Alter *et al.*, 1960; Okinaka *et al.*, 1960). Additional details for these two surveys are shown in Table III, with prevalences relative to that for Halifax. Plots for this table are reproduced in Fig. 4, and it is evident that for these two surveys the geomagnetic coordinate provides a much better parameter than does the geographic coordinate, a conclusion not appreciably altered by assumption of a reference prevalence of 33.6 per 100,000 population instead of the 48 per 100,000 of Fig. 3 or of 42 per 100,000 of Fig. 2. Moreover, it is apparent that the sigmoid curve in the previous geomagnetic plots is also a good fit for this plot in Fig. 4.

Multiple Sclerosis in the Soviet Union

Because of its considerable geographic extent, data on the occurrence of multiple sclerosis in the Soviet Union would be of considerable interest. Grashchenkov and his associates (1960) have recently investigated the morbidity due to this disease in different regions of the Soviet Union by comparison of the percentages of cases of multiple sclerosis in relation to the total number of patients of services of neurology during 1948–1957 (Table IV). Such data on the relative frequency of a disease are not directly comparable with mortality statistics or prevalence data for other areas of the

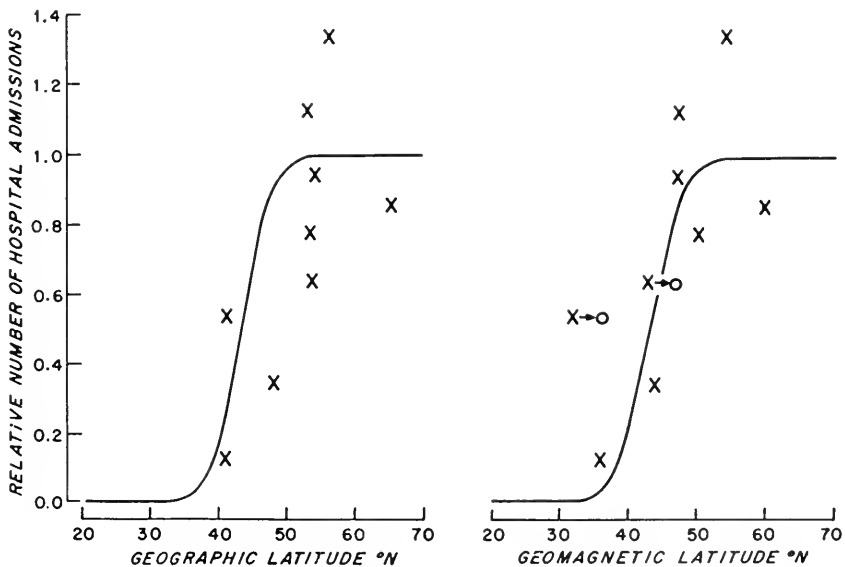


FIG. 5. Relative number of hospital admissions for multiple sclerosis in selected communities in the Soviet Union (see Table IV).

TABLE IV
 RELATIVE HOSPITAL ADMISSION RATES FOR MULTIPLE SCLEROSIS
 IN SELECTED REGIONS OF THE SOVIET UNION ^a

<i>Regions and cities represented</i>	<i>Geographic latitude</i>	<i>Geomagnetic latitude</i>	<i>Mean geographic latitude</i>	<i>Mean geomagnetic latitude</i>	<i>Percentage of cases with M.S.</i>	<i>Relative number of cases ^c</i>
Central European USSR			54	50	2.96	0.78
Kursk	52	48				
Voronezh	51	48				
Ryazan	54	50				
Kalinin	57	53				
Western European USSR			56	54	5.1	1.35
Riga	57	55				
Kaunas	55	53				
Minsk	54	53				
Southwest USSR			48	44	1.3	0.35
Odessa	47	43				
Chernovitsi	48	46				
Stalino	48	44				
North			65	59	3.24	0.86
Archangeł	65	59				

Table IV (Continued)

Urals			54	47	3.6	0.95
Sverdlovsk	57	49				
Ufa	54	47				
Orenburg	51	44				
Siberia			54	43	3.6	0.64
Novosibirsk	55	44				
Irkutsk	52	41				
Volga			53	47	4.28	1.13
Kuzychev	53	48				
Stalingrad	49	44				
Kazan	56	50				
Central Asia			41	32	2.03	0.54
Tashkent	41	32				
Alma-Ata	43	34				
Stalinabad	39	30				
Transcaucasia			41	36	0.49	0.13
Baku	40	34				
Tiflis	41	36				
Sochi and Sukhumi	43	38				

^a Data from Grashchenkov *et al.* (1960).

^b Relative to the total number of patients of services of neurology.

^c 1.0 corresponds to the mean of percentages for locations of 50° or greater geomagnetic latitude (i.e., 3.8%).

world, but their trend with latitude can be compared, and hence the plots shown in Fig. 5 were constructed from the last column of Table IV.

Although there is little to choose between the geographic and the geomagnetic plots with respect to the better fit by the sigmoid curves that have been included, superimposition on the plots of Figs. 2, 3, and 4 is possible only if the geomagnetic parameter is chosen.

From their data, Grashchenkov and his collaborators (1960) separately examined the effect of maritime climate on the frequency of multiple sclerosis by comparison of the statistics obtained for several maritime cities in the

TABLE V
RELATIVE HOSPITAL ADMISSION RATES FOR MULTIPLE SCLEROSIS
IN SELECTED MARITIME CITIES IN THE SOVIET UNION ^a

City	Geographic latitude	Geomagnetic latitude	Percentage of cases with M.S.	Relative number of cases ^b
Archangel	65	59	3.2	1.06
Riga	57	55	2.8	0.90
Odessa	47	43	1.6	0.53
Sochi & Sukhumi	43	38	0.58	0.19
Baku	40	34	0.45	0.15

^a Data from Grashchenkov *et al.* (1960).

^b 1.0 corresponds to the mean of the percentages for the two cities of greater than 50° geomagnetic latitude (i.e., 3.0%).

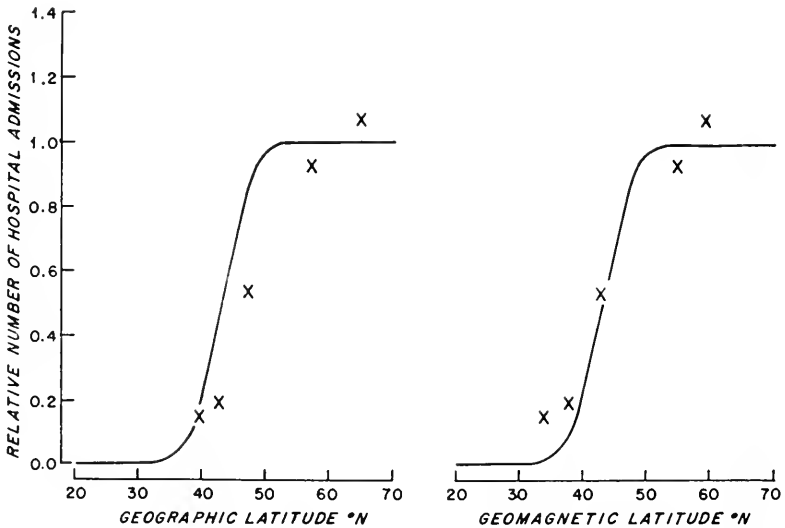


FIG. 6. Relative number of hospital admissions for multiple sclerosis in selected maritime cities of the Soviet Union (see Table V).

Soviet Union. These data (Table V) also clearly indicate a greater frequency in the northern regions. Again, geomagnetic latitude is the better parameter if the results plotted in Fig. 6, are superimposed on those in Figs. 2-4.

Predicted Geographic Distribution of Multiple Sclerosis

To permit comparison between the data represented in the preceding figures and those from future surveys or from other sources, geomagnetic latitudes corresponding to relative prevalences of 0.1 and 0.9 were determined from the sigmoid curve in Fig. 2 and are approximately 38° and 48° , respectively. These geomagnetic coordinates are indicated as isoprevalence lines by solid lines in Fig. 7. For inhabited areas of geomagnetic latitude less than 38° (i.e., the area between the two 0.1 lines), relatively low prevalence ratios of the order of 4-6 per 100,000 population are predicted. North and south of the 0.9 lines in the northern and southern hemispheres, respectively, relatively high prevalence ratios of the order of 40-60 are predicted. A rapid increase of prevalence with increasing geomagnetic latitude is predicted to lie between the 0.1 and 0.9 lines in both hemispheres.

The predictions from such a map appear to be in reasonably good agreement with much of the available data concerning the distribution of multiple sclerosis (Barlow, 1960). The general distribution being relatively common in northern Europe and in northern North America, but relatively uncommon in the Orient, South America, Africa, and the tropics appears to be in accord with the map.

Comparison of Multiple Sclerosis with Hodgkin's Disease

The geographic distribution of multiple sclerosis has been contrasted with that for Hodgkin's disease on several occasions (Ulett, 1948; Kurland, 1952; McAlpine *et al.*, 1955), since Hodgkin's disease, at least in the United States and Canada, appears to show little geographic variation. It is of interest to compare statistics for the two diseases from the present standpoint. For this purpose, mortality statistics for the two diseases in several localities throughout the world, compiled by McAlpine *et al.* (1955), are compared in Table VI and are plotted in Fig. 8. The statistics have limitations and may be subject to considerable sampling error since only one year is represented for each locality. Nonetheless, it is apparent that the latitude trend of relative mortality for multiple sclerosis is somewhat similar to that suggested by the prevalence data in Fig. 3. Moreover, there is possibly less scatter of the points about the sigmoid curve (reproduced from the geomagnetic plot of Fig. 2) for the geomagnetic plot than for the geographic plot. For Hodgkin's

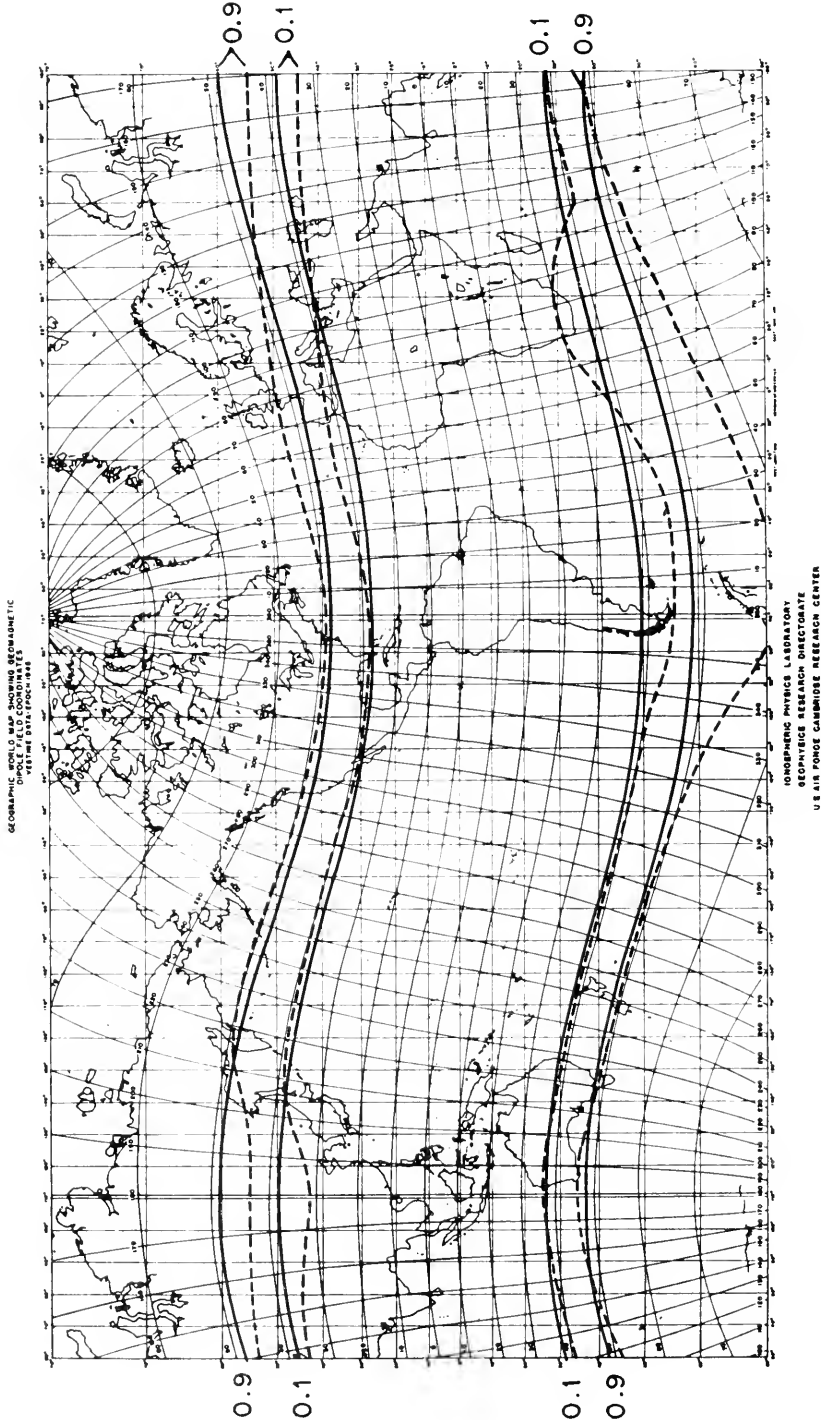


Fig. 7. Expected geographic (relative isoprevalence lines) for multiple sclerosis. Key: ———, according to lines of constant geomagnetic latitude; - - - - - , according to lines of constant cut-off rigidities for primary cosmic ray protons. For explanation see text.

TABLE VI
RELATIVE MORTALITY FROM MULTIPLE SCLEROSIS AND FROM HODGKIN'S DISEASE
FOR SELECTED COUNTRIES AND SELECTED YEARS^a

Country or city	Geographic latitude ^b	Geomagnetic latitude ^b	Year	Multiple Sclerosis		Hodgkin's Disease	
				Mortality rate ^c	Relative mortality ^a	Mortality rate ^c	Relative mortality ^a
Capetown	34S	34S	1950	0.5	0.27	2.5	1.7
Egypt	28	25	1947	0.02	0.01	0.1	0.07
Canada	46	56	1948	1.7	0.92	1.3	0.9
Newfoundland	48	60	1949	0.3	0.16	0.6	0.41
U.S.A.	37	47	1949	1.0	0.54	1.7	1.20
Rio de Janeiro	22S	12S	1933	0.2	0.11	0.5	0.35
Trinidad	10	20	1949	0.3	0.16	0.8	0.55
Venezuela	8	20	1946	0.7	0.38	1.4	0.97
Bombay	19	10	1940	0.0	0.0	0.3	0.21
Ceylon	8	2S	1949	0.02	0.11	0.03	0.02
Malaya	5	6S	1938	0.3	0.16	0.2	0.14
Singapore	1	11S	1948	0.1	0.54	0.2	0.14
Australia	30S	40S	1949	0.6	0.33	1.2	0.83
New Zealand	41S	46S	1946	0.9	0.49	1.1	0.76
Bulgaria	42	41	1937	0.1	0.05	0.1	0.07
Czechoslovakia	49	50	1933	1.2	0.55	0.7	0.48
England and Wales	53	55	1950	1.8	0.98	1.5	1.03
Hungary	48	47	1941	0.9	0.50	0.5	0.35
Italy	46	46	1942	0.5	0.27	1.4	0.47
Netherlands	53	54	1940	2.0	1.1	1.7	1.2
Northern Ireland	55	57	1951	3.1	1.7	1.6	1.1
Scotland	57	60	1949	2.7	1.5	1.8	1.2
Vienna	48	49	1948	2.8	1.5	2.4	1.8

^a Data from McAlpine *et al.* (1955).

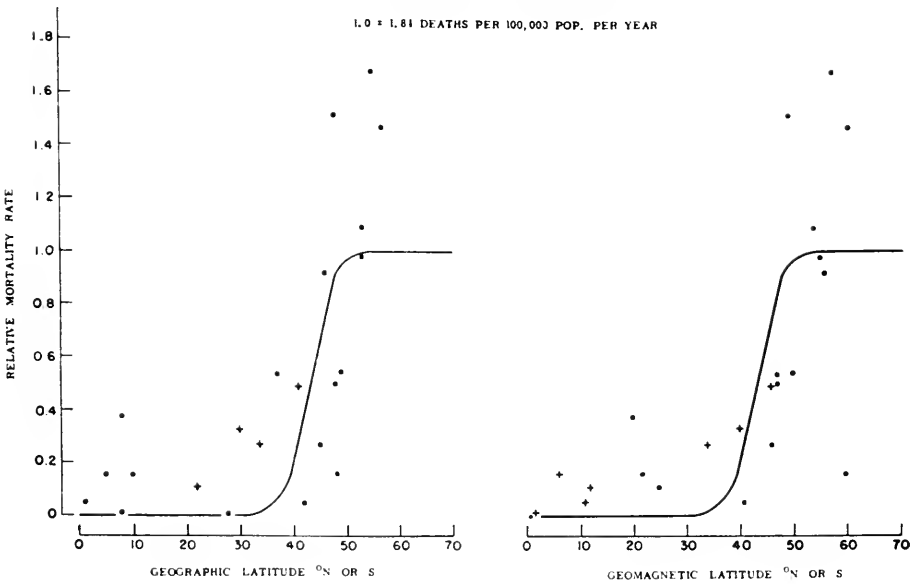
^b For countries of considerable extent in latitude, an average figure is taken.

^c Deaths per 100,000 population per year.

^d 1.0 corresponds to the mean of mortality rates for locations of 50° or greater geomagnetic latitude: for multiple sclerosis, 1.84; for Hodgkin's disease, 1.45.

MULTIPLE SCLEROSIS

1.0 = 1.81 DEATHS PER 100,000 POP. PER YEAR



HODGKIN'S DISEASE

1.0 = 1.45 DEATHS PER 100,000 POP. PER YEAR

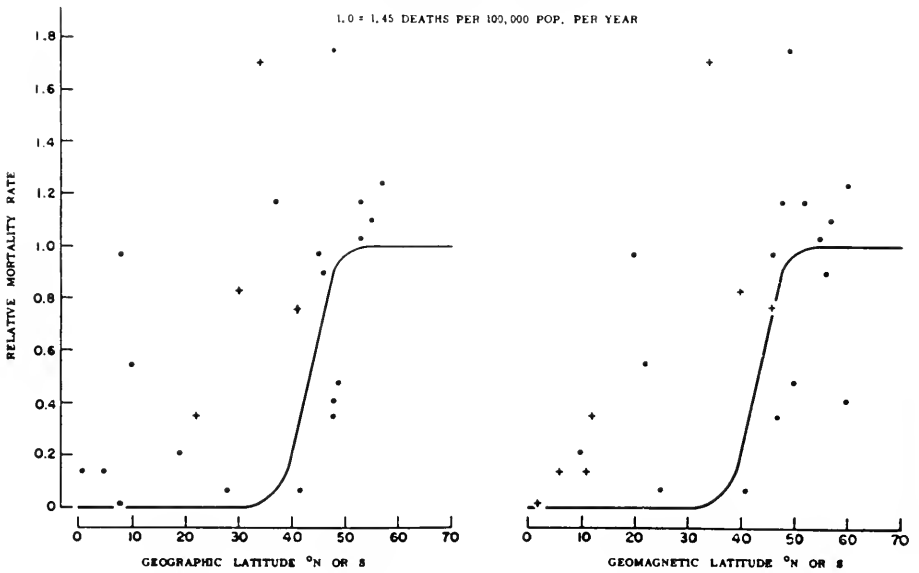


FIG. 8. Latitude distribution of multiple sclerosis and Hodgkin's disease by relative mortality rates (see Table VI).

disease, however, the latitude trend is considerably more poorly defined, although there is some suggestion of an increasing mortality with increasing latitude. Further, there is little to choose between the geographic and the geomagnetic plots for Hodgkin's disease.

Latitude Effect for Cosmic Rays

The statistics for multiple sclerosis that have been examined here, as well as those considered previously (Barlow, 1960), appear to suggest that the geographic distribution of this disease is better correlated with geomagnetic latitude than with geographic latitude. Since the phenomenon of cosmic radiation is the only one known to be related to geomagnetic latitude,¹ it is appropriate to examine the latitude effect for various cosmic ray parameters for comparison with the latitude effect for multiple sclerosis. Variation with altitude must also be considered. For multiple sclerosis the available data do not indicate any clear variation with altitude (Barlow, 1960), whereas for cosmic rays the altitude effect is generally large compared to the latitude effect. Such is the case for the ionization produced by cosmic rays as determined by an ionization chamber (Fig. 9). At sea level, the latitude effect between 0° and 50° is only 14%, a much smaller effect than is apparent in Figs. 2-6. Between 40° and 50° , it is even smaller. The altitude effect of 50° , however, is such that there is about 70% more ionization at an altitude of 2,000 meters (6,500 feet) than at sea level.

The latitude effect for multiple sclerosis thus is not in accord with that for the ionization produced by cosmic rays, except some of the data for multiple sclerosis are suggestive of a "knee" at about 50° , and a "knee" at this latitude is apparent in Fig. 9. Neither the meson component nor the nucleonic component (protons and neutrons) of cosmic rays near sea level had a latitude effect as pronounced as multiple sclerosis apparently has.

More pronounced latitude effects are found at the top of the atmosphere (i.e. at about 80 kilometers or 50 miles), at heights where the atmosphere has not yet exerted its filtering and diffusing effects on the incoming cosmic ray flux. Thus, the total number of particles incident vertically at high latitudes is some ten times greater than the number incident at the geomagnetic equator (Fig. 10). Such a curve represents the flux for primary particles of all energies, and if specific energy ranges are examined, even more pronounced latitude effects are apparent, as indicated in the theoretically computed curves reproduced in Fig. 11. The sigmoid curve from Figs. 2-6 has been included in Fig. 11, and a close parallel is seen between this curve and that for primary cosmic ray protons of 4.5 Bev energy. This order of energy

¹ The aurora borealis and australis appear to be indirectly related to cosmic radiation and the earth's magnetic field through the intermediary of the van Allen radiation belts (van Allen, 1959).

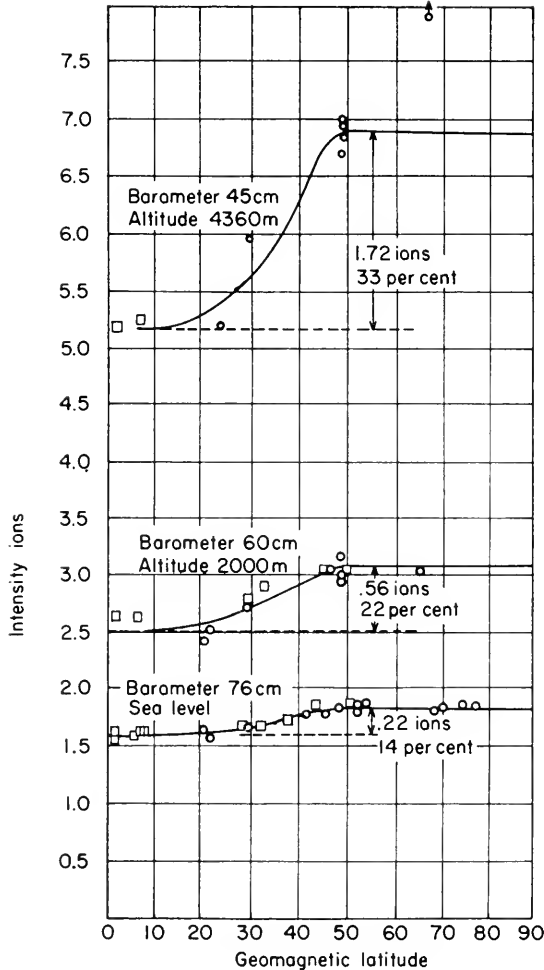


FIG. 9. The latitude effect for the ionization produced by cosmic rays at different altitudes. The altitudes 2,000 and 4,360 meters correspond to 6,500 and 14,000 feet, respectively. (From Janossy, 1950, and adapted from Compton, 1933.)

is somewhat more than twice the energy that corresponds to the rest mass of the proton or neutron and hence is in the range of the threshold energy for production of nucleon-antinucleon pairs (Segrè, 1958). It is also in the same energy range as that required for a primary proton to penetrate the atmosphere and come to the end of its path in a thickness of some 10–60 cm of water equivalent (Aron *et al.*, 1949), i.e., to come to rest in the brain or spinal cord of a human in the open air. The greatest biologic effect of

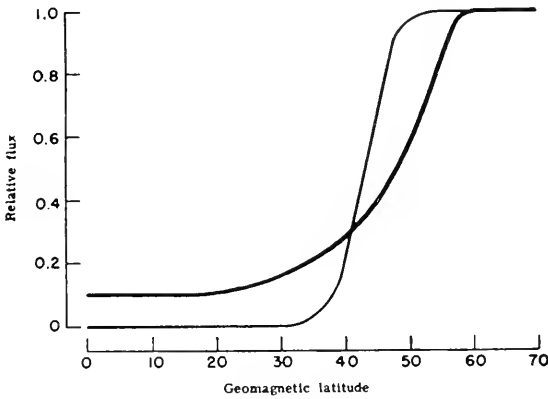


FIG. 10. Vertical flux of cosmic ray particles near the top of the atmosphere. (After Curtis, 1956, and from Puppi and Dallaporta, 1952.)

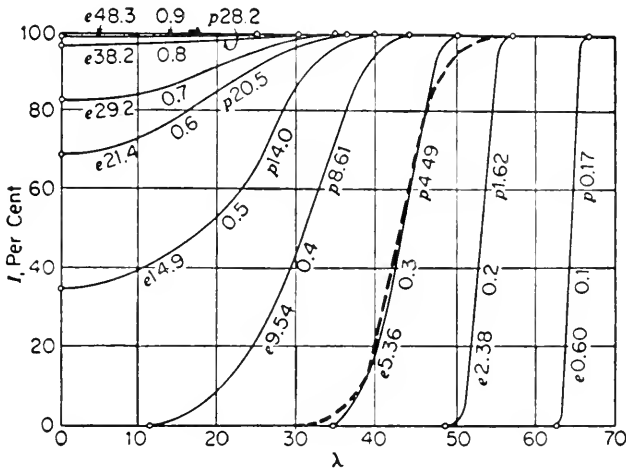


FIG. 11. Dependence of primary cosmic ray intensity on magnetic latitude (λ). The ordinate represents the relative intensity for each of the various energies (p = energy of protons in billions of electron-volts; other parameters shown on the curves are not relevant to the present discussion), expressed as the per cent of the maximum possible intensity for that particular energy. (From Richtmyer *et al.*, 1955, and adapted from Lemaître and Vallarta, 1933.)

protons appears just before the end of their trajectory (Malis *et al.*, 1957). However, the number of protons of a similar terminal energy at the surface of the earth which have been produced by the complex interaction of primary cosmic rays with the atmosphere is far greater than the number of

primary protons in this category (Wilson and Wouthuysen, 1958), and the latitude effect for such secondary protons is much less than that for primary protons (their altitude effect is also appreciable). For these reasons, the striking similarity between the two curves in Fig. 11 must be considered fortuitous.

Corrections to the Geomagnetic Effect

Since a rather narrow range of geomagnetic latitudes appears to correspond to the zone of rapid increase in prevalence of multiple sclerosis, further clarification of the details of the exact relationship between the latitude effect for cosmic rays and the earth's magnetic field possibly may be of importance for the present study (Katz *et al.*, 1953). Thus, corrections for local variations in the earth's magnetic field may be necessary to provide a better fit for observed cosmic ray phenomena than geomagnetic latitude *per se*. The dashed lines in Fig. 7 indicate isoprevalence lines constructed on this basis and determined from data for cosmic rays published by Quenby and Webber (1959). There is little difference in the location of the two sets of isoprevalence lines in inhabited areas of the world except in central Asia, and the circles in Fig. 5 indicate the corrections that would occur on this basis for the two locations in this area represented in the figure.

Discussion

Cosmic radiation has been implicated as a factor in human disease in other studies (Morris and Nickerson, 1948; Wesley, 1960), but the marked latitude effect for multiple sclerosis appears to provide a greater possibility for distinguishing between geographic and geomagnetic latitude as a parameter, a test which originally firmly established the relationship between cosmic rays and the earth's magnetic field (Compton, 1933).

The present data and that examined previously (Barlow, 1960) appear to suggest that cosmic radiation in some way may be related to the occurrence of multiple sclerosis.

Since at a given location multiple sclerosis apparently is distributed randomly among the susceptible population (Kurland *et al.*, 1955) and since plaques of demyelination of this disease are largely randomly distributed in the white matter (Adams and Kubik, 1952; McAlpine *et al.*, 1955), it would be an attractive possibility to attempt to relate a randomly occurring cosmic ray event to the trigger mechanism that Lumsden (1951) suggests may occur in the initiation of plaques of demyelination. Several considerations militate against such a direct relationship, if there is any relationship at all between cosmic radiation and multiple sclerosis. Among these consid-

erations are the generally greater level of radiation from terrestrial sources of naturally occurring radioactivity² as compared with that from cosmic radiation (Libby, 1955; Neher, 1957; Solon *et al.*, 1960) and the lack of an altitude effect for multiple sclerosis.

Since direct effects of cosmic rays at the earth's surface cannot be implicated, it is of interest to examine the latitude distribution of atomic nuclei made radioactive by cosmic ray events. These radionuclides include T (the hydrogen isotope, tritium), Be⁷, Be¹⁰, C¹⁴, Na²², P³², P³³, S³⁵, and Cl³⁹ (Suess, 1958). The *production rate* for cosmic ray-induced radionuclides is said to be greater by a factor of four at the poles than at the geomagnetic equator, following the latitude effect for the neutron component of cosmic rays (Suess, 1958; Kaufman and Libby, 1954; Simpson, 1951). Their *concentration* at the surface of the earth, however, is dependent on several additional factors, including half-life, diffusion in the atmosphere and the oceans, and meteorologic factors. Thus, the half-life of C¹⁴ (5,570 years) is so long that diffusion processes tend to minimize any variation of its concentration with latitude. Diffusion effects should be relatively small for elements with short half-lives, P³² for example (14.5 days).

In any event, it would not appear possible that the latitude distribution of any cosmic ray-induced radionuclide would be as pronounced as that for multiple sclerosis. A latitude effect for the distribution of fall-out from heavy cosmic ray primary nuclei might be considered additionally (Wesley, 1960).

Consideration of the problem of the latitude distribution of radioactive nuclei associated with cosmic rays is even further complicated by the fact that some of these nuclei are produced in the explosion of atomic and hydrogen bombs. Thus, the amount of T that has been produced by hydrogen bombs is comparable to or larger than the total inventory of natural T on the surface of the earth, and artificially produced C¹⁴ had by 1957 increased the C¹⁴ content of atmospheric CO₂ by about 10% (Suess, 1958). Although there is a pronounced distribution with latitude for fall-out debris, at least as indicated by Sr⁹⁰ in the northern hemisphere (Fig. 12), the variation is with *geographic* latitude and not with geomagnetic latitude. It should of course be remembered that multiple sclerosis was well-known as a clinical entity long before the era of bomb testing.

Linear vs. Nonlinear Dose-Response Relationships

The above considerations concerning comparisons of the geomagnetic latitude distribution of multiple sclerosis with those for various cosmic ray

² Gentry *et al.* (1959) have recently found that areas with increased rates of congenital malformations in New York State appear to be associated with increased levels of background terrestrial radiation.

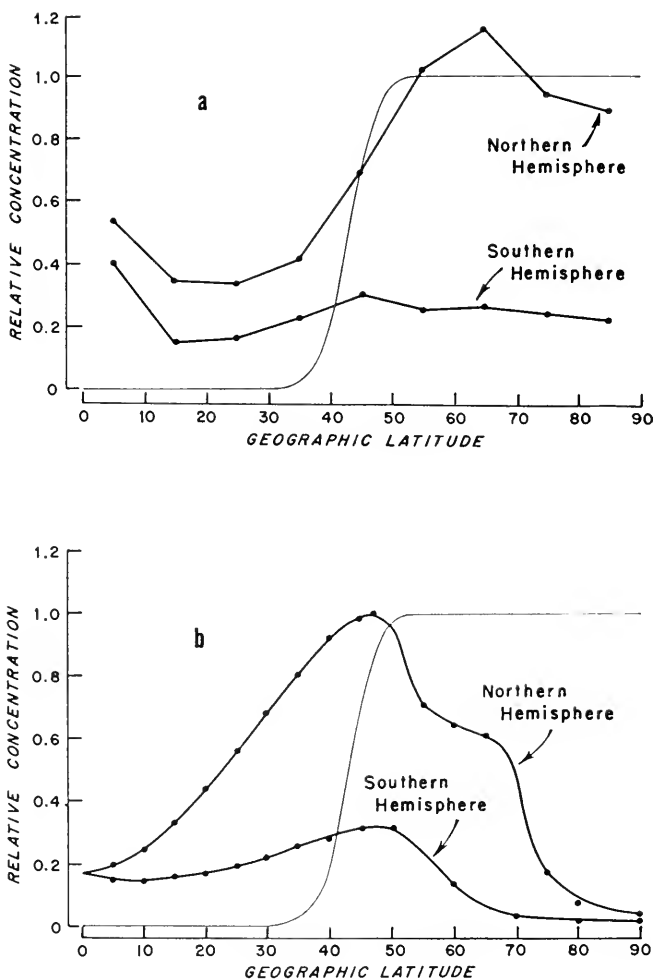


FIG. 12. Relative concentration of Strontium-90 versus geographical latitude.
 a. In the stratosphere, averaged over the period November 1957–November 1958. 1.0 = 12 mc per square mile. (Data from Feely, 1960).
 b. On the ground in November, 1958. 1.0 = 4.1 mc per square mile. (Redrawn from Wexler, 1960.)

parameters are based on the assumption of a one-to-one (*i.e.*, linear) correspondence between the exposure and the number of cases of the disease. Should a nonlinear relationship obtain, it theoretically would be possible for a more pronounced latitude distribution for the disease to result from a cosmic ray phenomenon of a given latitude distribution. (The altitude effect

for such a phenomenon would still have to be small compared with its latitude effect.) Such a nonlinear relationship between inciting agent and disease can obtain, for example, if the susceptibility of individuals in the population is distributed in a normal (Gaussian) manner. A normal distribution for susceptibility and the concomitant nonlinear relationship between per cent incidence and dose rate at low doses appears to obtain for the occurrence of tumors in mice following exposure to ultraviolet light, and it has been suggested that a similar relationship may occur for the appearance of cancer in man following exposure to ionizing radiation (Blum, 1959a,b).

Duration of Exposure to Inciting Agent

Whatever is the cause of multiple sclerosis, a most interesting question arises in connection with the incubation period for the disease. Acheson *et al.* (1960) have pointed out that if the inciting or protective agents are prolonged in their effect, then the important factor in the history of individual cases will be that of place of residence over an extended period; alternatively, only the place of residence early in life might be the important factor. These workers suggest that a long incubation period may be implicated by their own findings as well as by the observation of Dean (1949) that multiple sclerosis is almost unknown in persons of European stock born and raised in the Union of South Africa, whereas it is more frequently described in persons born in Europe who have emigrated to South Africa. An analogous observation in Israel by Rozansky (1952) is being verified by Alter (1960).

The present approach does not help in elucidation of this question, for it is conceivable that radiation could have either a single-shot effect, perhaps analogous in animals to the graying of hair produced by heavy cosmic ray nuclei at very high altitudes (Chase and Post, 1956), or alternatively it might have a cumulative effect, as does the carcinogenic property of ultraviolet radiation.

Possible Experimental Approaches

Despite the difficulties of establishing more than a correlative (and therefore possibly fortuitous) relationship between cosmic radiation and multiple sclerosis, it is perhaps in order to consider briefly some possible experimental approaches to the problem, particularly if additional carefully collected epidemiologic data substantiate the results so far obtained. These experimental approaches differ somewhat according to whether it is the place of residence in adult life, or in early life, that is established as being the important factor in the geography of the disease.

The early plaques of demyelination in multiple sclerosis appear preponderantly around small veins (Adams and Kubik, 1952), and a similar localization appears for the demyelinating lesions of experimental allergic encephalomyelitis in some animals (McAlpine *et al.*, 1955; Waksman, 1960). Further, induction of the lesions of the latter disease in predetermined locations in the brain by use of physical agents has been reported by Clark and Bogdanove (1955). It might be of interest to determine whether lesions of experimental allergic encephalomyelitis could also be induced in predetermined locations by low doses of radiation from well focused beams of high energy particles (for example, of appropriately filtered high energy protons). Perivenous staining with trypan blue has been reported in the demyelinating plaques of multiple sclerosis (Broman, 1949) and in lesions of experimental allergic encephalomyelitis in animals (Barlow, 1956; Waksman, 1960); a similar focal staining might be looked for following focused irradiation at low doses, since it is a known finding with much larger doses (Clemente and Holst, 1954). Should positive results be found from either of these experiments, a possible protective effort might be explored for some of the agents (e.g., cysteine) which are known to lessen the biological effects of ionizing radiation.

If place of residence early in life is the important factor, then possible relationships (direct or indirect) might be explored between radiation and immunochemical processes in early life which might underlie demyelinating processes in adult life.

In connection with the experiments outlined, it should be noted that if cosmic radiation is at all related to multiple sclerosis, the mechanism of its action is likely to be different from the biologic effects of cosmic rays at high altitudes (Yagoda, 1957; Schaefer, 1958).

Finally, the possibility should be kept in mind that some other factors (for example, certain types of infections, perhaps not known) might act as intermediaries between cosmic rays and multiple sclerosis.

Summary and Conclusions

Multiple sclerosis appears to exhibit a fairly well marked geographic distribution, being appreciably more frequent in northern than in southern latitudes in certain areas of the world. It is generally agreed that this geographic distribution must be taken into account in any satisfactory theory of etiology of the disease. Possible correlates of the geographic distribution which previously have been advanced have not appeared entirely satisfactory; certainly geographic latitude itself appears to be a poor correlate. Several independent sets of statistics on the distribution of the disease were examined from the standpoint of geomagnetic latitude, a parameter that is related to

the earth's magnetic axis in the same way that geographic latitude is related to the earth's axis of rotation. That the distribution of multiple sclerosis might be examined in this way was suggested by the fact that a variation with geomagnetic latitude is well known for the intensity of cosmic rays. The frequencies of the disease in widely separated areas were found to vary in a systematic manner with geomagnetic latitude; therefore, the possibility arises that cosmic rays in some way might be a factor in the occurrence of the disease. The latitude distribution for the disease bears some resemblance to that for a particular component of the primary cosmic radiation at the top of the atmosphere; no similarly good correlate is apparent, however, among the various cosmic ray components at the surface of the earth, hence the similarity may be entirely fortuitous. Some possible intermediary factors examined appear to offer no great promise. A world map, on which is indicated the expected geographic distribution of multiple sclerosis on the basis of geomagnetic latitude, is presented for comparison with results of future surveys on the disease so that the present results may be evaluated further.

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GENERAL DISCUSSION

PAUL HENSHAW (*U. S. Atomic Energy Commission, Washington, D. C.*): I am indeed interested in the information Dr. Rugh presented with his spectacular slides of early embryos. Seeing evidence of deteriorating embryos at an early stage is to be expected in view of what is known about the quality of germ cells and the uterine bed in some situations. Certainly, degeneration is inevitable as a consequence of some of these conditions. Dr. Rugh has called attention to the kinds of abnormalities that occur following different levels of exposure to germ cells and to early embryos, and it was particularly interesting that abnormalities show in organisms exposed to doses of 5–15 r. This is an extremely low level of irradiation, and I am sure it will be quoted repeatedly. I feel, therefore, that we should ask questions about the confidence he has in the findings. Dr. Rugh has shown abnormalities which indeed do show in samples of organisms that have been exposed to low doses of radiation, but such abnormalities will show as a consequence of other agents as well. I would be pleased if Dr. Rugh would cite the specific evidence he has that permits him to say the low level changes are due to radiation. The second point pertains to the abnormality that showed in the third generation. Were these actually due to radiation change involved in a germ cell? We know something about how cerebral hernias will result from damage to neural fold primordia, the failure to close the neural crest, which permits the brain to turn inside out. This is a developmental abnormality not connected with germ cell damage. If the same can come from irradiation of germplasm, this is exceedingly interesting. I would like Dr. Rugh to indicate whether he feels there is evidence that cerebral hernias may result from mutations in germ cells. Third, Dr. Rugh has emphasized that low levels of radiation produce developmental abnormalities and went so far as to call attention to the possibility that a large portion of the naturally occurring developmental abnormalities may be due to background radiation. If I were a physician, I think I would go along with Dr. Rugh's warning and be cautious about any exposure of embryos or germ cells, but I am a laboratory man. I would like Dr. Rugh to give his strongest evidence that environmental radiation is having a significant effect. Does he feel that background radiation can or does account for a substantial proportion of the abnormalities, having knowledge as we do that many things can produce the kind of abnormalities described?

ROBERTS RUGH (*Columbia University*): In regard to your first question concerning whether we are dealing with low level effects of radiation or possibly other traumatic effects: We are currently studying the embryos found in 98 pairs of uteri of mice exposed to 15 r at 1½ days, because we felt this sort of statement had to be quantitative and proven. We will have statistical data from over 1,000 embryos which received 15 r at 1½ days with an appropriate number of controls. It is true that we get anomalies without irradiation, that is, without superimposed

irradiation from laboratory sources in addition to background irradiation; and it is also true, as you saw from the first slide, that we get about 7% death and resorption of embryos in the controls. This may be due to cosmic radiation or natural background radiation, or even may be due to genetic causes. We do not know. We have been dealing with exencephaly as an anomaly that was definitely produced by irradiation. We have never seen this anomaly except following irradiation, and we have produced this at the low level of 15 r in the early embryo. The second question dealt with genetic effects. I did not state that the three generations of exencephaly found following irradiation of the ovary or the testes were due to 15 r. It was due to a much larger dose as for instance, 100 r to the ovary of the great-grandmother. The important point was that the effect was carried through three generations. Obviously, it would be necessary to determine the statistical frequency. However, that it occurs at all following irradiation of the ovary is of concern to every potential mother. The point to emphasize is that this anomaly was produced by irradiation of the germ cells and was found in three successive generations. It therefore had a genetic origin. Snell showed about 1935 that x-irradiating the testes and having the male mate with a normal female produced in the second generation something like 35% of such anomalies. We have carried it from both the sperm and the egg through several generations, and we were simply emphasizing that this anomaly appears to be similar, whether it is derived from an effect on the chromosome or an effect directly on neurogenesis. How it is produced genetically we do not know, but having had considerable experience in experimental embryology with amphibia and chicks, it seems to me it is probable that the damage was to chromosomes which at the time of gastrulation caused such disruption in organogenesis that any gross change could follow. This happens to be one that is simply produced due to failure of closure of the cranial roof and loss of neuroblasts and probably osteoblasts during development. The third question related to the dangers of cosmic radiation. Like taxes, we are all faced with cosmic and natural radiation, and there is nothing much we can do about it. This may actually be good for evaluation! I think, however, in line with the last paper and those of Drs. Gentry, Wesley, and others, it may be proven statistically that there is some correlation between the amount of background radiation and the incidence of congenital anomalies. If our thesis is correct, at this early stage of development the embryo is so extremely radiosensitive that 5 r causes a 10% increase of resorptions and 15 r causes exencephaly in the embryos which develop later.

PERCIVAL BAILEY (*University of Illinois*): This afternoon I am handicapped by lack of intimate knowledge of the embryonic cerebral cortex and by the quantity of the material presented. You cannot really judge histologic material by a few projections. The material presented here seemed inadequate for any fine cytologic study. I suppose I should be happy that it is so, because that leaves an opportunity for somebody else to make a good cytologic study of the effects of radiation on the cells of the cerebral cortex, with more adequate cytologic technic.

ORVILLE BAILEY (*University of Illinois*): Dr Brownson, were you radiating the whole animal or the head only? And did you conclude that fractional doses of radiation produced less or more effect than the same total dose at one time? How long did the animals live?

ROBERT H. BROWNSON (*Medical College of Virginia*): To answer the first question, this was total head x-ray. Second, this was a cumulative exposure, and we have not compared the total radiation effect accumulated at one time with it. Concerning the effects of radiation, the alteration was cumulative in the direction of change which was more quantitative than qualitative. The effects we saw did not seem to show more severity in themselves individually, but more intensity through the actual quantity of such change. The total picture which we saw at the end, beginning with 2,000 r as a minimum dose, demonstrated individual changes at 228 days similar to those changes that we could see using the 5,000 r level following a shorter postirradiation. The problem was to have these animals survive. Many did not survive 228 days, probably due to being stressed with an additional nutritional deficiency. In testing these animals psychologically, we had to deprive them of some food, and this influenced the death rate which accelerated with the increasing cumulative radiation. The group which exhibited the greatest change was the 5,000 r cumulative group, which were not subject to any type of psychologic testing and went through a relatively normal span of 228 days. Much of the problem in correlating the changes of one of the animals with 18-20 r with 5,000 r was that these animals had gone through 228 days normally while the 5,000 r animals did not survive. Most of the changes were quantitative and in general increased in direct proportion to cumulative dosage and, to a degree, to time after exposure.

ORVILLE BAILEY: In the terms I ordinarily use it seems as the intensity of the radiation goes down, the amount of damage per total dose also falls. One can build up almost grotesque amounts of x-ray dosage without damage if given slowly enough over a long period of time. Most of the lantern slides which Dr. Brownson showed were in the acute phase of the reaction which is difficult to evaluate. The focal neuronal changes that were described seemed like small foci of "dark neurons," the change which Dr. Gammermeyer has studied. They are artifact or at least, reflect some terminal state of activity in that particular cell. Most of the changes in the Purkinje cells, as Dr. Vogel and associates have demonstrated, are quite frequently found in control monkeys.

E. C. ALVORD (*University of Washington School of Medicine*): I would like to stress one minor theme that was developed by Dr. Rugh and has recurred in rather low notes through most of these papers. This is the concept that the body as a whole is made up of a mosaic of many structures, each of which has vastly different sensitivities to radiation. This concept of a mosaic also applies within a part of the body, namely the nervous system itself. There are a number of syndromes that have been delineated, particularly by Maisin of Belgium, on the basis of survival times following various doses of x-rays to various parts of the body. He speaks of a "delayed head syndrome," which occurs in rats after 1,000 r to the head, the rats dying about 5 months later, and of an "oropharyngeal syndrome," which suddenly appears at 1,500 r and cuts the survival time down to 10 days. I would like to ask Dr. Brownson to define the exact site of the irradiation to the heads of his rats. I doubt that this included the whole head, since Maisin and others have found it difficult for rats to live beyond 10 days following irradiation of the whole head with 1,500 r or more. This "oropharyngeal syndrome" has been found to be due to the inclusion of the oral pharynx, tongue, and lower

jaw of the animal, damage to these particularly sensitive areas causing death by means that are not clearly defined. It would be particularly interesting if repeated doses of 1,000 r can avoid this syndrome and allow as much as 5,000 r to be given, but I would suggest that Dr. Brownson has irradiated only the forebrain so that he sees relatively little of the change in the cerebellum because of a slight rostral advancement of the posterior margin of his x-irradiation. My own experience is only with the adult animal. I am sure that in the adult guinea pig one has to include the cerebellum and has to go well below the cerebellum to produce neurologic signs and death of the guinea pig in a short time. This leads me to Dr. Schjeide's paper, in which, unfortunately, the wet weights are not available. I would predict that, when the wet weights become available, the most striking chemical change will be in the degree of hydration with marked edema of the cerebellum. Dr. Sauer, have you with tritiated thymidine been able to apply this at certain times after the irradiation, with the idea of establishing whether these DNA bodies are dead or still metabolically active?

WOLFGANG ZEMAN (*Indiana University Medical School*): I think we should strive for a more accurate definition of cumulative or fractionated doses. In 1949, I tried to arrive at an understanding as to the radiobiologic effectiveness of fractionated doses as compared to a single dose. My data at that time were rather scanty, but in the meantime Lindgren (Stockholm) arrived at a simple formula for converting fractionated doses into single exposures. He determined the x-ray dose which was necessary to produce radiation-induced brain damage in 50% of rabbits. He used various single and cumulative dosages and found that in plotting the morbidity dose (50%) in r logarithmically against the total amount of days over which this dose was given, also logarithmically, a straight line results which has a slope of about 0.26 for the adult rabbit brain. In other words, a dose of say 2,000 r given in one day, compares to a dose of 2,000 r times $10^{0.26}$ given over 10 days. For the human brain the slope has been shown to be about 0.34, and it stands to reason that each different species does have a specific slope. I would predict that within one species, the slope might be dependent on the developmental stage. I wonder whether Dr. Brownson would be good enough to convert his data into terms which would make for an easy comparison to the radiobiologic effectiveness of cumulative doses with single dose exposures.

L. J. PEACOCK (*University of Georgia*): I would like to ask Dr. Brownson whether the decline in response rate in his rats was due to an error in their timing behavior or to a decrease in motivation. That is, was there a decline in the overall food intake of these animals, or was it a matter of their not being able to properly time the intervals and schedule?

ROBERT H. BROWNSON: Dr. Alvord, the radiation instrumentation was conducted by our physicists in the biophysics department. Each animal was prepared by placing it in a cage in which the head was elevated out of the cage and held by a clamp with the remainder of the animal shielded. A Victoreen Chamber R-Meter was used to monitor the dosage. Each animal received 1,000 r delivered at the rate of 237 r per minute. The animal was given 1,000 r per week, so that it accumulated as scheduled per week the desired total roentgen dosage. Our experience with guinea pigs has indicated that they are more liable to radiation

death than rats. Our rats did well with 5,000 r cumulative total head exposure at the end of 228 days, provided they were not stressed as they were when they were placed in the Skinner box and tested for positive food reward. To reply to Dr. Peacock's question: to our way of thinking this change in the animal's behavior as related to its performance in the Skinner box was one that seemed to be motivation. We tested our animals between and after each dosage and following the end of the first 5 weeks on food intake. The food intake of the x-irradiated animals versus the controls was not so different as to make us think that this was the whole picture. The controls when placed on a deprivation diet maintained their normal weight, which tended to climb slowly. We believe there is a definite food problem involved, but there may have been one of motivation also. We are now utilizing shock avoidance and positive food reward together in anticipation that this will help clarify the matter.

JOHN L. FALK (*Harvard School of Public Health, Boston, Massachusetts*): I was happy to see that Dr. Brownson was using long-term testing. Short-term tests involving food intake or various food-motivated performances might cause radiation sickness. Did Dr. Brownson use a variable interval schedule? We have been making readings in medial nuclei of the hypothalamus and getting increases in bar-pressing rates on variable interval schedules. Since there did seem to be some hypothalamic involvement in Dr. Brownson's animals, I wonder if perhaps there was more involvement in the lateral nuclei, possibly indicating classical aphagia?

ROBERT H. BROWNSON: I think the only answer we will have to this question is dependent on the results obtained with shock avoidance. We anticipate improved testing methods in our future plans. The schedule was aperiodic, and the tapes were run for 45-minute intervals. The periods ran 4-224 seconds apart with an average of 62 seconds between reinforcements.

CORNELIUS A. TOBIAS (*University of California, Berkeley, California*): I had the pleasure of discussing with Dr. Barlow his interesting statistical findings and trying to encourage him and other people who are taking a similar approach to the explanation of some diseases similar to this as to etiology. However, there are several aspects that need further study and improvement. It seems to me that if multiple sclerosis is due to radiation, it is most likely that it should be due to early effects in the embryologic or even germ cell stage. It appears that neither the studies by Dr. Hicks nor Dr. Rugh show that multiple sclerosis is a frequent occurrence in animals developing from irradiated embryos or germ cells. Secondly, findings such as these should be correlated with other findings which were also discussed in Dr. Rugh's paper. For example, those by Gentry, who finds that in certain areas of the United States with high natural radioactivity background congenital malformations occur more frequently than elsewhere. If the Barlow and Gentry studies do not correlate, then it would seem that it is not the low ionizing component of cosmic rays that would cause the effect in multiple sclerosis, but some other component of cosmic radiation that occurs perhaps only rarely. There are some cosmic ray phenomena for which further work may be needed before correlations can be established. For example, the so-called Auger showers which are energetic showers arrive at ground level from primary cosmic rays which can give well measurable doses to a single individual. On the average one such

shower passes through the body of an individual once a year. Another possibility is that a small percentage of the primaries that come in would come down to ground level and would produce highly ionizing secondaries. If highly ionizing particles are more effective than x-rays, one could perhaps understand the lack of results in x-irradiated animals. One would still expect to find an altitude effect; for example, multiple sclerosis incidence in Denver should be higher than in New York. Secondly, one would expect that in some way the incidence of multiple sclerosis would correlate with the 11-year cycle. The primary cosmic radiation events near the North Pole change radically in this 11-year sunspot variation period, and perhaps babies born in periods of low sunspot activity might exhibit a higher statistical incidence of multiple sclerosis. If heavy particles should cause multiple sclerosis, this hypothesis could be tested by exposing embryos to radiations produced in accelerators, such as the heavy alpha particles and other nuclei.

JOHN S. BARLOW (*Massachusetts General Hospital*): The points made by Dr. Tobias are well taken; I might make a few additional comments. Multiple sclerosis per se is known only in man. There are naturally occurring demyelinating diseases in animals, but as far as I know these exhibit no latitude effect. At a given latitude, there are regional variations in the occurrence of the diseases, and it is an interesting suggestion to determine whether these are correlated with variations in background terrestrial radiation. The Auger showers may well be of biologic importance, but I think that the energy of the original cosmic ray particles giving rise to such showers is so great that no latitude variation for the showers would be expected. Those few primaries that do reach the earth's surface certainly would have a high relative biologic effectiveness, but they apparently are far overshadowed in numbers by similar secondary particles for which the latitude effect is not very pronounced between 40° and 50° geomagnetic latitude and for which an appreciable altitude effect is present. The question of variations with the 11-year solar cycle merits further investigation. For several of the recent surveys of multiple sclerosis, data for a 10-year period were collected, and no systematic fluctuation with time is apparent from these data. They have not, however, been examined, as far as I know, from the standpoint of year of birth of the patients, as suggested by Dr. Tobias. Dr. John A. Simpson, of the University of Chicago, has informed me that the solar variation at 50° geomagnetic north is about 25% and decreases to some 6% at the geomagnetic equator; an effect of this size might well be obscured in statistics for the disease collected for localities of latitudes of 50° or less. Studies in localities further north would be of particular interest from the standpoint of the solar cycle.

SUMMATION BY CHAIRMAN*

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The investigators have given summaries, and the discussants have added to this invaluable by emphasizing important points and questioning others. I will comment on matters that were not discussed and add information about the mechanisms of retinal, cortical, and cerebellar malformations produced in fetal and neonatal rats by 150–300 r of 250 kv conventional x-rays, a dose range widely used because of its selective effect and tolerability. The malformations are of prime importance to many of the experimental and behavioral studies to be reported later.

In presenting his experiments Dr. Rugh has raised the important question of whether low doses (a few r) may impair the development of the embryo long before it begins to differentiate a nervous system. It is well-known that such early embryos have extraordinary powers of regulation and restitution, and a substantial number of cells may be lost; yet apparently normal individuals result. Dividing early embryos into two parts may result in two apparently normal individuals. What we don't know is whether such embryos are really normal, although they have been reported to be so. The work Dr. Rugh and others are doing aims at exploring one aspect of this. At the morphologic level, the problem remains one of establishing a statistically sound relationship between the presence of necrotic cells in early embryos and an effect of low doses of radiation. There is no problem with higher doses—cells are killed. "Spontaneous" cell death has a way of turning up in a variety of circumstances during development, sometimes as a necessary normal process. As the dose of conventional x-rays is increased above 20 r at almost any stage of embryonic life, the number of dead cells increase, yet it is difficult to show that development has been impaired. In some later stages, for example when the neural folds are forming, the rat embryo can recover to a remarkable degree from excessive cell loss after 100 r or even 200 r, and it does so in a manner such as that described long ago in amphibians by Harrison and by Detweiler. Contrary to what Dr. Rugh said, injured parts do catch up successfully. In the face of this remarkable capacity for embryos to regulate structure after cell loss, we may have to look at other parameters when the resulting animal looks normal, but does not measure up in some aspects of function or behavior. Mutations and chromosome aberrations in the early embryonic somatic cells, alterations in other organ systems that indirectly affect the brain, and the indirect effects that irradiation of the mother has on her fetuses are some things to be taken into account.

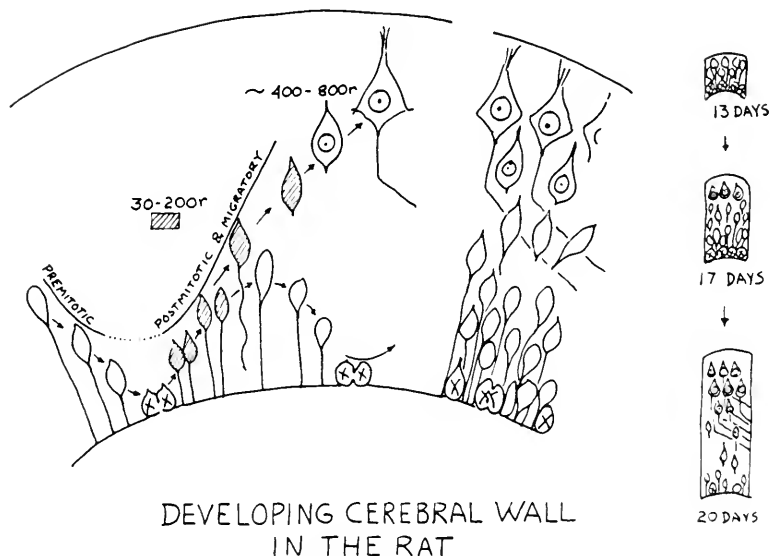
Dr. Brizzec's approach to the study of the effects of radiation on cortical growth is a new and promising one because some of the divided doses he gives probably kill few cells. Sixty r will kill some of the primitive cells. The single doses around

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200 r employed by us and others extirpate certain classes of primitive proliferative and migratory cells and at a given stage produce specific deficits which are translated by ensuing morphogenetic sequences into adult patterns of cortical and other malformations. Dr. Brizzee's effects seem to be different and involve sublethal alterations of the differentiating neurons and glia. He reports that the overall cytoarchitectural pattern in the cortex is well organized in rats treated, say, with 25 r successively on fetal days 10 to 17, yet there are significant cytologic deviations from normal. These include alterations of the neuron, of the size of the nucleus in relation to cytoplasm, and in how closely packed the cells are. Some neurons were much too large. Closer packing of cells may have reflected subnormal proliferation of dendrites or other deficiencies in fiber development. What controls the size of a cortical neuron? How much influence do afferent fibers have on determining cortical cell types, and what alterations in DNA and RNA might lead to these cytologic abnormalities? Dr. Brizzee's further studies on the morphogenetic sequences of events may tell.

Dr. Sauer, extending the studies of the late Prof. F. S. Sauer on the nature of the proliferative neuroepithelium, has come up with a new concept of partially destructive injury to the nucleus of the radiosensitive primitive neural cells. We had always thought that the quickly destructive effect of 200 r on the postmitotic migratory and other sensitive cells was an all-or-none phenomenon. Histologic studies of a series of embryos removed by Cesarean section at hourly or 2 hour intervals up to 9 hours after exposure still confirms this for the most part, but there is probably no discrepancy in our findings. In the chick, 200 r kills relatively fewer cells than in the rat, and Dr. Sauer clearly showed that after this lesser injury sublethal effects occurred. What happens to these partially incapacitated cells? Do they grow up to be abnormal neurons like those in Dr. Brizzee's rats? Some of his doses in rats may have produced effects corresponding to those following 200 r in the chick.

The malforming effect of radiation on the developing mammalian central nervous system and retina depends on factors that include the stage of development, the individual growth characteristics of the species, and the doses of radiation. The dose largely determines what cells are killed and, therefore, what kind of malformative sequences of growth will be set in motion. Considerable data are now at hand on the morphology, the mechanisms, and the reproducibility of the malformations induced in albino rats by 200 r of conventional 250 kv x-rays when given at any stage from the 9th day of embryonic life to more than a week after birth. The acute extirpative effect of this exposure seems to be chiefly limited to the post-mitotic and primitive migratory neural cells, but obviously some chromosomal damage with delayed cell death must also occur. Figure 1 indicates in schematic form how different doses kill cells in the young brain. The cerebral vesicle of a 17 day fetal rat is represented and shows that the neuroepithelial zone is a thick pseudostratified layer of tadpole-shaped cells which replicate their chromosomes in the outer part of this zone and slide in to mitose in the lining. This was demonstrated by F. C. Sauer in 1935 and confirmed by Watterson *et al.* (1956), M. E. Sauer and Chittenden (1959), Sidman *et al.* (1959; Sidman, 1961), and by Hicks *et al.* (1961a, b). Postmitotic cells are shaded, and they are the



DEVELOPING CEREBRAL WALL IN THE RAT

FIG. 1. Schematic representation of the neuroepithelium. (Adapted from Hicks and D'Amato, 1960a.)

principal radiosensitive ones, we believe. By radiosensitive we mean killed and visibly necrotic in 2 or 8 hours. The threshold of this effect is about 20-30 r. Above 200 r the selectivity is gradually lost, and more and more mature cells are killed. Also, the time after radiation that they die may lengthen from hours to a day or more. A good many young cortical neurons are killed by doses of several hundred r, but most of them escape for a while. Even after 800 r some members of the proliferative cell colony remain, and for the few days that an embryo so exposed may live, these residual cells actually go on proliferating brain or other neural structures as best they can. The little figures at the right in Fig. 1 emphasize the changing ratios of mature to immature cells as the fore-brain develops. At 13 days most of the primitive cells are involved in the proliferative cycle, and a cell no sooner divides into two postmitotic cells than the daughter cells enter the premitotic stage of the cycle again. This frenzied growth subsides by term, although a series of bursts of mitotic activity occurs in the fore-brain proliferative colonies between 13 days and birth, complicating the assessment of just which cells are radiosensitive.

A detailed account of the brain malformations, especially in the cortex, and the mechanisms of their formation can be found in Hicks (1958), Hicks *et al.* (1954, 1959), and Hicks and D'Amato (1960a). The patterns of cortex of a rat irradiated with 200 r on day 13 is so completely different from one irradiated on day 20, or day 16, that from the neurologic standpoint, lumping such animals together in behavioral experiments as "prenatally irradiated" would be absolutely meaningless. Considerable data on the patterns of malformation of the retinas

and the mechanisms involved in their formation, as well as similar information about the cerebellum, are now available (Hicks and D'Amato, 1960a, b); Hicks *et al.*, 1959). There is no simple formula for the eye and retinal malformations, and radiation on any day from the 9th fetal to the 8th postnatal day presents its own problem, as Fig. 2 shows. Proliferation in the retina of the albino rat ceases about 8 days after birth. Like the forebrain, the eye passes through a series of stages in which radiation does a variety of things. Restitution seems to be complete or almost complete, after the severe destruction that 200 r causes on day 13, and rosettes do not form. Rosettes are the cell balls that resemble distorted neural tubes and form from residual neuroepithelial cells after destruction of surrounding cells by radiation or other agents. Such distorted neuroepithelium continues to proliferate brain, cord, or retina, as the case may be. On day 20, for complex reasons, the retina is not very radiosensitive in the sense used here, yet on day 19 or 21, mostly because there are greater numbers of sensitive migratory cells present, permanent changes occur. Of special interest for behavioral experiments is the great severity of the malformations that characterize the retinas of rats irradiated on the first few days after birth. From the morphogenetic standpoint, some immature bipolar cells are already present at birth, but when radiation destroys much of the adjacent layer of primitive migratory cells, these infant bipolar cells are suddenly stimulated to grow and sprout fibers, forming a precocious plexiform zone. When the residual neuroepithelium begins to catch up, it spawns another layer of bipolar cells and itself forms rosettes as it differentiates into the rod cell layer. (Fig. 2).

Malformations of the cerebellum are just as complex in their mechanisms as those of the cortex, thalamus, spinal cord, or retina. Figure 3 shows drawings of

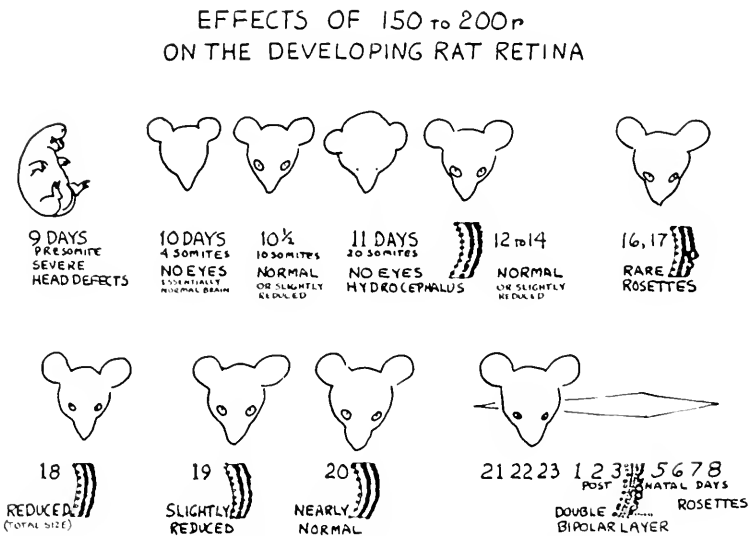


Fig. 2. The spectrum of eye malformations produced by 200 r.

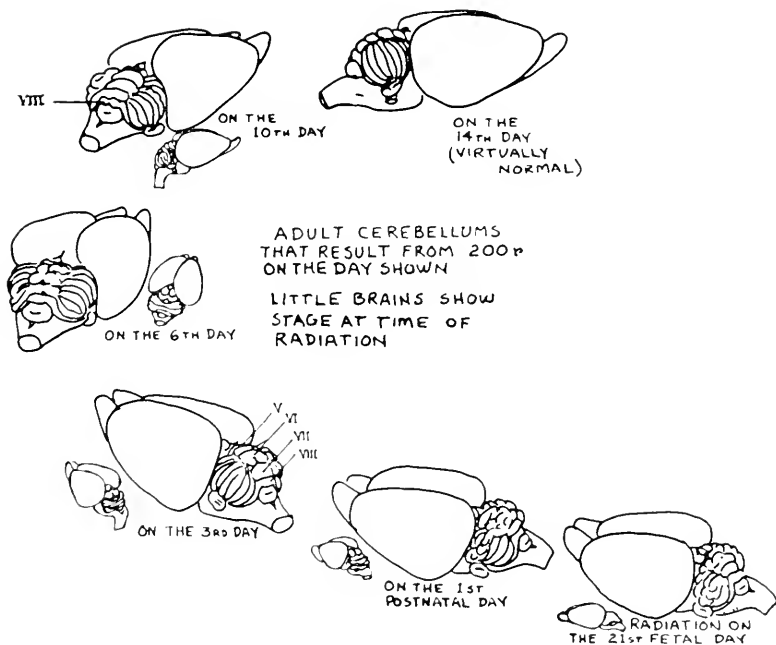


FIG. 3. The spectrum of cerebellar malformations produced by 200 r from late fetal to neonatal life. (Adapted from Hicks and D'Amato, 1960b.)

actual brains representing the gross patterns of cerebellar malformation in the albino rat that result from 200 r on the days indicated. In the gray rat, as in the mouse, the cerebellum is a little more advanced in its schedule of development than in the albino rat. The little brains, drawn to scale, show the fetal or neonatal brain as it was when radiation was given. Malformations induced at earlier stages are described by Hicks *et al.* (1959). In certain respects there is a front to back sequence of maturation of folia and a lesser center to lateral sequence, which is reflected in a corresponding sequence of deformations. To press this generality further would be misleading, because different regions, and even parts of folia, wax and wane in their growth patterns. While the folial pattern is maturing, the cytologic characteristics of the cerebellum are also being unfolded, and the cyto-architectural malformations do *not* at all parallel the folial malformations. The malformations induced in late fetal life are characterized by a normal cyto-architecture, while those representing the end of the 1st week are characterized by an ectopic, extra granule cell layer, outside rather than deep to the Purkinje layer. Damage to the neuroepithelium, which in the developing cerebellum comes to lie on its surface instead of lining a ventricle, results at this stage in a precocious coarse growth of the Purkinje dendrites. This, we think, blocks the further migration of the primitive cells which would complete the granule layer. The late comers simply stop in the molecular layer. Irradiation on the first days after

birth presents still other cytoarchitectural anomalies, including irregular ectopias of both granule and Purkinje cells.

In summary, a certain amount of radiation delivered at given stages of early development results in unique patterns of response. The resultant morphologic patterns are distinctive for each stage, and it follows that the corresponding behavior patterns must also be distinctive.

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PART II

**Histopathological Changes Resulting
from the Irradiation of the
Nervous System**



Basic Problems in the Histopathology of Radiation of the Central Nervous System

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This survey of the histopathologic changes resulting from radiation of the central nervous system will be confined to those detected by light microscopy in postnatal experimental animals and largely to the effects of gamma and roentgen radiation. Its purpose is to state some of the problems in tissue reaction following radiation as they are met by the neuropathologist and to illustrate rather than offer solutions for these questions.

Materials and Methods

A series of experiments were carried out over many years in the Neurosurgical Research Laboratory of the Children's Medical Center, Boston, under the direction of Dr. Franc D. Ingraham, in association with Drs. E. A. Bering, Jr., R. L. McLaurin and others. Many results of these studies have been published (Bailey *et al.*, 1957, 1958; Bering *et al.*, 1955; McLaurin *et al.*, 1955), but the histologic findings, especially in the spinal cord, have not been described in detail.

The experiments were of three types. In the first, tantalum¹³² wires covered with polyethylene were inserted into the cerebral cortex of 40 monkeys (*Macaca mulatta* and *Ateles geoffroy*), 1.5–2.0 mm posterior to the motor strip of the right cerebral hemisphere. The wires were removed after 2.5–4,770 r had been delivered. Monkeys were allowed to survive from 2 hours to 33 months after completion of radiation. The polyethylene encasement was regarded as sufficient to prevent any tissue effects of beta radiation from the activated tantalum wire.

In the second series of experiments, a piece of tantalum wire activated in the atomic pile at Oak Ridge was encased in polyethylene and placed on the dorsal surface of the spinal cord—or, in a few animals, beneath the skin overlying the spine and fixed in place to the paravertebral fascia). Experiments were carried out in 18 *Macaca mulatta* monkeys with dosages varying from

208 r to 55,000 r, measured at the center of the cord. Survival times were from 1 day to 36 months.

The third series was concerned with the effects of roentgen radiation in 17 *Macaca mulatta* monkeys receiving 138 r per minute to the lower dorsal region while under Pentothal or Nembutal anesthesia. Dosages varied from 4,000 to 54,500 r, and survival times from 5 days to 18 weeks. In one additional monkey, both Ta¹³² and roentgen radiation were used.

Time Factor in Tissue Responses

The influence of time on the appearance, extent, and character of the histologic lesions produced by roentgen radiation has been recognized for over 50 years, the early studies being based on the skin of those engaged in therapeutic use of this agent (Wolbach, 1909). Using dogs Nemenov (1934a, b) studied physiologically in regard to conditioned reflexes. Lyman *et al.* (1933) have demonstrated the increase in the intensity of histologic lesions in the brain caused by roentgen radiation as the interval between the end of radiation and sacrifice is increased. This paper also contains a thorough review of the literature to 1933, as does that of Warren (1943).

The effects of radiation in experimental animals has been further considered by Scholz (1934, 1935). In fully grown animals, the immediate reaction is not detectable microscopically, but if the animals survive from 4½ weeks to 1 year after radiation, histologic lesions are striking. In a study of delayed lesions of brain and spinal cord in the dog, Davidoff *et al.* (1938) pointed out that the rapidity with which clinical evidence of spinal cord injury appeared is proportional to the dosage and that disabilities in the monkeys tend to be progressive. The importance of time as a crucial factor in the appearance and interpretation of cerebral changes induced by roentgen radiation was carefully studied by Russell *et al.* (1949). They have shown that with dosages of 2,850 r in rabbits no histologic changes were found before 82 days, but were present after that in all but one animal (of 7). Behavioral changes and abnormal neurologic signs did not appear until about 100 days after radiation, yet three rabbits killed before these changes appeared (82, 85, and 90 days) showed well defined lesions. The brains of rabbits killed earlier than 82 days after radiation showed no changes detectable by light microscopy. They also found that reducing the dose of radiation lengthened the latent interval.

These and other substantial contributions have clearly established that the intensity of the histologic changes induced by roentgen radiation increase with time interval between radiation and sacrifice or death of the experimental animal. There are indications that the character of the lesions also alters with time.

Such progression of lesions apparently does not take place in response to beta radiation, at least not to the same degree as with roentgen radiation (Campbell and Novick, 1949; Edwards and Bagg, 1923).

More recently, interest in the neuropathology of radiation has tended to be focused on the acute phase of the reaction (Haymaker *et al.*, 1958; Vogel *et al.*, 1958). By the use of relatively large doses of gamma radiation, it has been possible to characterize the acute lesion as seen by conventional methods of light microscopy as one of acute inflammatory changes and degenerative alterations in the cerebellum (Vogel *et al.*, 1958). These changes are the direct effect of radiation on the brain, since they do not appear when the whole body of the animal is radiated and the head shielded. While the acute changes are definite, they are mild in comparison with those which develop as time after radiation is increased.

In personal studies, the experimental procedure precludes critical evaluation of the acute phases of radiation reaction. Tantalum¹⁸² delivers radiation at a rate such that several days are required to accumulate the dosages necessary. When Ta¹⁸² wire, shielded with polyethylene to prevent beta radiation from complicating the picture, is inserted into the cerebrum of



Fig. 1. Coronal section of monkey brain, showing representative lesion induced by insertion of Ta¹⁸² wire. Dosage, 600 r in 5¾ days. Left hemiplegia developed after 3 months. Sacrifice 1 year after radiation. Lesion measured 7x4 mm.

monkeys posterior to the motor strip, an area of necrosis is produced (Fig. 1). Observation of such animals gives some indication of progression in the lesions. Hemiparesis which developed in 6 of 21 monkeys did not appear until 3 weeks to 20 months after radiation, the animals previously being neurologically normal (Bailey *et al.*, 1958).

Electroencephalograms a few days after radiation showed decreased voltage and some slow waves on the radiated side, while on the opposite side there was a predominant frequency faster than the one before radiation. This condition remained and was most prominent 6 to 8 weeks after radiation. The EEG pattern then reverted almost to normal. At longer intervals, slow waves again appeared on the radiated side, and many records demonstrated voltage asymmetry with decreased voltage on that side. Two years after radiation, 2 animals developed runs of spikes and fast activity localized to the region of radiation. One of these developed generalized clonic and tonic seizures 30 months after completion of radiation (Bailey *et al.*, 1958). There was thus some EEG evidence of progression. These results are in fair agreement with those of Ross *et al.* (1954), though the conditions of radiation are so different that direct comparison is difficult.

The histologic changes in these animals became more striking as the interval between radiation and sacrifice was lengthened. There was more evidence of irregular streaks of injury extending out from the necrotic region in which the Ta¹⁸² wire had originally been placed (Fig. 1).

Studies of radiation effects in the spinal cord of experimental animals have not been numerous (Cairns and Fulton, 1930; Davidoff *et al.*, 1938; McLaurin *et al.*, 1955; Pendergrass *et al.*, 1922; Peyton, 1934; Sicard and Bauer, 1907). However, the spinal cord has proved a very favorable area for the study of radiation effects.

In personal studies, clinical observations of monkeys gave some evidence of the time factor as an important consideration. With Ta¹⁸² radiation of 18 monkeys, 2 developed transitory paraplegia with complete recovery, 6 permanent paraplegia, and 1 early paraplegia, then recovery followed by permanent paraplegia after 6 weeks. When roentgen radiation was used, 6 of 17 animals developed paraplegia. In some monkeys dying with complete paraplegia within a few days after either form of radiation, there were no histologic changes or only scattered vacuolation of myelin in white fiber tracts. In view of the striking alterations described in monkeys surviving for long periods after radiation, there is good evidence that the histologic changes become progressively more obvious, at least to light microscopy, as the interval between completion of radiation and sacrifice is lengthened.

The time factor thus becomes a dominant consideration in defining dosage effective in causing histologic change and especially in determining the ultimate effect on a living organism subjected to ionizing radiation.

High Energy versus Low Energy Radiation

In previous experiments, the tolerance of the spinal cord of *Macaca mulatta* for gamma radiation was approximately 135 r per hour and about 125 kv r for roentgen radiation (McLaurin *et al.*, 1955). Radiation from activated Ta has a much higher energy than the roentgen radiation used in our studies. These results suggest that, under the experimental conditions used, low energy radiation is slightly more effective in producing paraplegia than high energy radiation. This agrees with the findings of Arnold *et al.* (1954a). However, other investigators (Hicks *et al.*, 1956) have found that central nervous system tissues are more sensitive to high energy radiation.

It seems difficult to reconcile these divergent results. Among the studies, there are data from different animals, and sources of radiation also vary somewhat. Even so, when both types of radiation have been carried out in the same laboratory under conditions as nearly controlled as possible, contradictions in results remain. The situation is no clearer in regard to the sensitivity of tissues outside the central nervous system. The question remains a significant one for further investigation.

Effect of Intensity of Radiation

The intensity of radiation has emerged as an important factor in the tissue response in the nervous system (Hicks *et al.*, 1956; McLaurin *et al.*, 1955). Intensity as a factor in other organs has produced more equivocal results (Brunschwig and Perry, 1936; Pack and Quimby, 1932).

The effect of the rate at which a given dose of radiation is administered is strikingly demonstrated by 2 monkeys in personal material. Each received the equivalent of 55,000 r of gamma radiation to the spinal cord, at the rate of 4,000 r per day in one and at 1,870 r per day in the other. The first animal developed a flaccid paralysis in the 2nd week after radiation, which progressed steadily in severity until death at 2 months. The second monkey showed no neurologic deficit until its death from an independent cause 4 months after completion of radiation.

In the series in general, it was found that 7,500 r as a single dose were required to produce paraplegia, but two doses of 5,000 r were necessary (McLaurin *et al.*, 1955). These results are somewhat different from those of Davidoff *et al.* (1938), who found 5,000 r sufficient to cause paraplegia. However, they used only 1 animal at that dosage.

Differences in Tissue Reaction with Age

There is evidence that changes in young animals are different from those in adults of the same species. In young animals, smaller doses of radiation are required to produce behavioral and histologic changes in the brain than in

fully grown ones, and the period between radiation and overt tissue degeneration is shortened (Clemente *et al.*, 1960; Mogilnitzky and Podljaschuk, 1928, 1929; Scholz, 1934, 1935; Yamazaki *et al.*, 1960; Zimmern and Chavany, 1931).

Clemente *et al.* (1960) found that as little as 125 r of roentgen radiation to the head may result in microcephaly and cataracts if given to rats 8 hours old, while 300 r produces abnormal neurologic signs and histologic changes in most rats at 1 day and 4 days of age, but not at 7 days. They feel that resistance of the brain to radiation becomes significantly increased toward the end of the 2nd week of postnatal life. Their histologic studies indicate a high degree of correlation between abnormal neurologic signs and histologic lesions in these immature mice. While some changes, predominantly vascular, are found in rats sacrificed at 48-72 hours, they state, "It seemed as though processes were under way which would result in larger necrotic sites, especially in rats administered 1,000 r, had these animals been allowed to live for longer periods. This assumption seems especially valid since other animals radiated at the same postnatal time and sacrificed 1 to 14 months later showed larger lesions in the brain." (Clemente *et al.*, 1960).

This work is in some way reminiscent of Hicks's (1953, 1954) results in antenatal development. The occurrence of cerebellar changes and microcephaly is in accord with his timetable. The eye defects are of a different type from those Hicks produced by radiation early in pregnancy.

For this reason, the results of Clemente *et al.* (1960; Yamazaki *et al.*, 1960) in some ways may correspond to late prenatal radiation in certain other species of animals in which brain development is more advanced at birth. However, they are of particular interest because they are quantitative studies in brains with no, or restricted, regenerative capacity. They are also in accord with results in puppies reported by others (Lyman *et al.*, 1933; Scholz, 1934, 1935).

It seems reasonable to conclude that the nervous system of young animals is considerably more sensitive to radiation than that of adult animals of the same species, that the time interval between radiation and overt histologic evidence of degenerative changes is less in young animals, and that abnormal neurologic signs, behavioral changes, and histologic lesions are regularly produced with lower dosages of radiation in young animals than in old ones. The particular alterations that accompany maturation in the neuron so that it changes its response to ionizing radiation in these ways remains an important problem.

Vascular Responses to Radiation

One characteristic of the reaction to radiation in all tissues is the development of degenerative and occlusive changes in blood vessels. The central

nervous system is no exception. There is general agreement that alterations in vessel walls are found in all phases of radiation reaction in the brain and spinal cord. The sequences involved and the importance of these changes in the total picture of radiation injury are still not entirely established.

Clemente *et al.* (1960) consider the earliest and most constant vascular reaction to be a swelling of the cytoplasm of endothelial cells and an increase in the intensity of basic staining in the nucleus. They feel that this process may be reversible or arrested or may progress to capillary rupture and inflammatory cell infiltration. Polymorphonuclear leucocytes appear soon after radiation, as early as 6 hours (Clemente and Holst, 1954), and tend to disappear after about five days (Clemente and Holst, 1954; Haymaker *et al.*, 1958). Rachmanow (1926) has demonstrated accumulation of trypan blue in the endothelial cells at this stage. These early reactions are most conspicuous in capillaries, where they may be associated with detectable necrosis of the wall. Such lesions of capillaries would account for the frequent occurrence of minute hemorrhages in the acute phase of radiation reaction (Alquier and Faure-Beaulieu, 1909; Clemente and Holst, 1954; Haymaker *et al.*, 1958). In animals surviving longer after radiation, hemosiderin deposits mark the location of previous small hemorrhages (Fig. 1). Larger hemorrhages rarely have been described (Rachmanow, 1926; Scholz, 1935) and usually in animals surviving 3 or 4 weeks after radiation. Capillary changes may be related to the increased permeability of the blood-brain barrier (Clemente and Holst, 1954; Mogilnitzki and Podljaschuk, 1930) and perhaps less directly to brain swelling noted by several authors (Gerstner *et al.*, 1954; Ross *et al.*, 1953).

Damage to larger vessels becomes more obvious at longer intervals after radiation. Severe necrosis with disruption of vessel walls occurs in the brain, spinal cord and meninges. This is accompanied by cellular infiltration which presumably is at first polymorphonuclear, but at the stage usually seen is predominantly lymphocytic with a component of macrophages. In well marked examples, few vestiges of the structure of the vascular wall remain (Fig. 2). The line of the endothelium and its basement membrane are separated from an adventitia which is heavily infiltrated with inflammatory cells, but is not necrotic (McLaurin *et al.*, 1955). With time, there is repair and reshaping of the vascular wall, the media retaining its form, but being composed mostly or entirely of fibrous tissue (Fig. 3). Vascular occlusion is often completed by an organized thrombus filling the lumen. This process as a general phenomenon of vascular repair has certain analogies with the repair of vessels in hypersensitivity reactions (Hawn and Janeway, 1947, especially their Fig. 12). Such similarities should not be interpreted as indicating a related pathogenesis, but as reparative phenomena in necrosis of similar distribution.

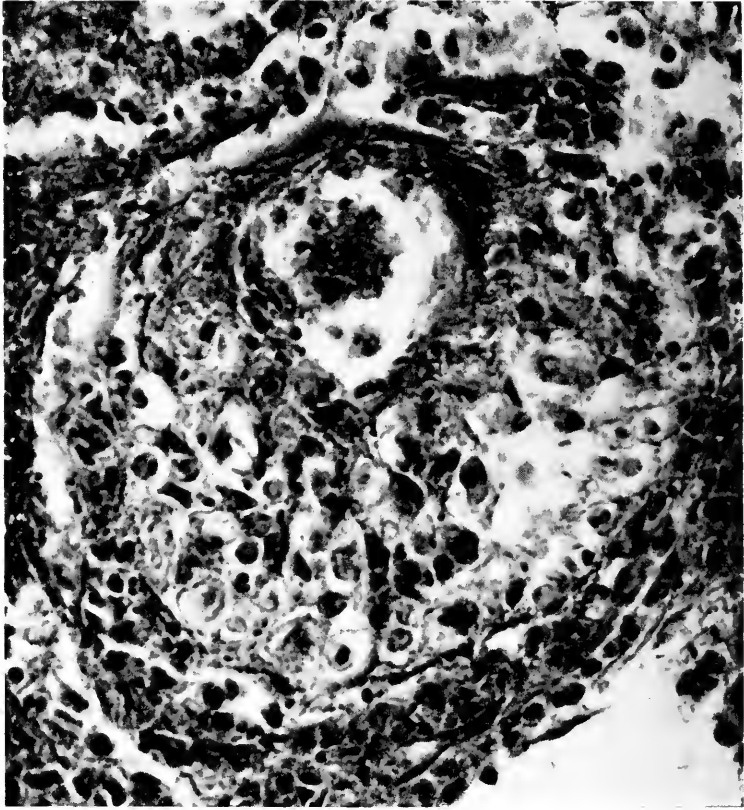


FIG. 2. Vascular lesion in small leptomenigeal vessel near an area of myelomalacia. There is heavy inflammatory cellular infiltration of the necrotic wall, but the endothelial layer appears intact. Gallocyenin-van Gieson $\times 250$. Dosage 5,606 r Ta^{132} in 3 months. Paraplegia at end of radiation; death 1 week later. Autopsy: myelomalacia 10th thoracic to 2nd lumbar segments.

There is at least one other type of vascular occlusion in the central nervous system of chronic animals. This occurs when there has been further necrosis of the collagenous tissue produced in repair followed by secondary collagenous response, eventually producing enlarged, bizarre vascular channels with tiny lumina (Fig. 4) or none at all.

These striking changes in vessel walls of chronic animals are not nearly so widespread in the area of radiation as in the vasculitis of smaller vessels in the acute phase. There is considerable evidence that the late vascular changes are segmental. Hence more vessels may have an occluded segment at some point than would be inferred from a single microscopic section or even from several sections of one tissue block.

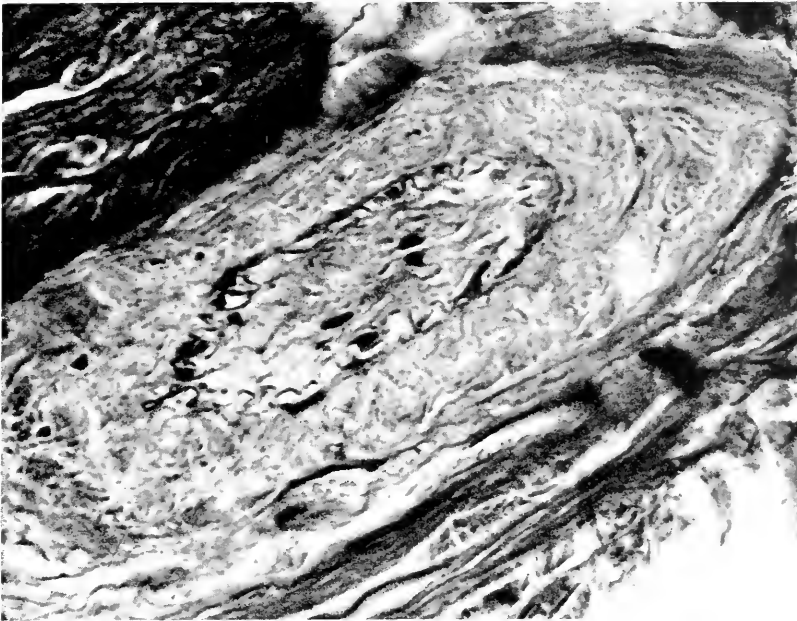


FIG. 3. Vascular lesion in small leptomeningeal vessel near an area of myelomalacia. End result of radiation change with fibrosis of wall and complete obliteration of the lumen. Hematoxylin-eosin $\times 250$. Dosage 4,440 r Ta^{132} in 29 hours. No neurologic deficit. Sacrifice 4 months after radiation. Autopsy: partial myelomalacia at 12th thoracic and 1st lumbar segments.

One of the most perplexing problems in the histopathology of radiation reactions is the relation of such lesions to other changes induced by this agent. As long ago as 1921, Bagg stated that x-ray injury to the brain is secondary to vascular change. Since that time, workers have been divided as to whether the changes in the parenchyma are ischemic and infarctive or whether they are direct effects of ionizing radiation without mediation through vascular mechanisms. The opinion of the author, based on personal material, is in agreement with Arnold's *et al.* (1954b) that the effects on the nervous system are direct effects. The occlusion of vessels could account for areas of complete infarction in the region of their distribution. Such effects, however, are only a minor part of the response of the central nervous system to ionizing radiation.

Effects of Radiation on the Neuron

Largely through the work of Hicks (1953, 1954), it has become generally recognized that the developing neuron is highly susceptible to ionizing radia-

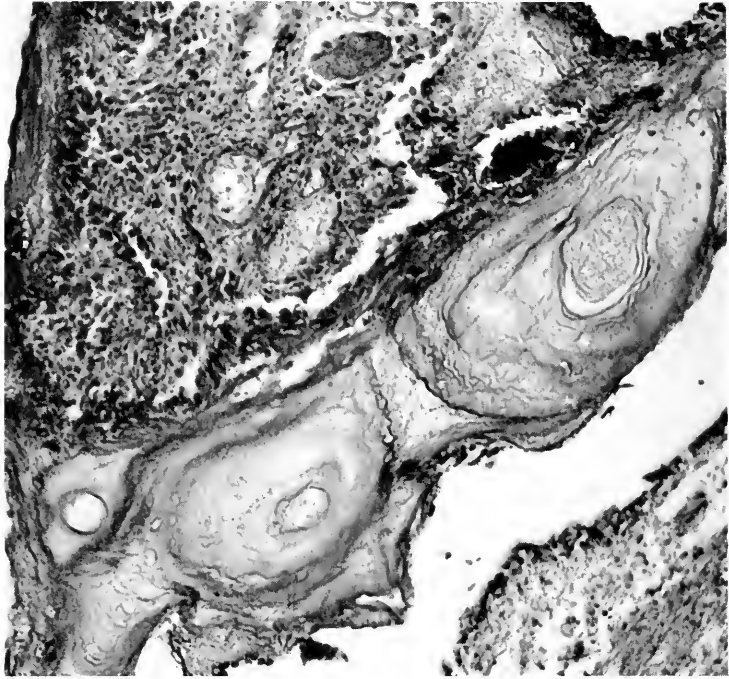


FIG. 4. Marked vascular lesions after intracerebral implantation of Ta^{182} ; Weil's method $\times 30$, Dosage 553 r in 13 days; sacrifice 16 days after completion of radiation. No neurologic deficit. Lesion measured 8 mm in diameter.

tion. Resistance to such injury increases in older animals. There is less general agreement as to whether the adult neurons are directly injured or whether the nerve degeneration is secondary to vascular lesions.

In the acute phase of the radiation reaction to gamma and roentgen rays, the changes visible by light microscopy in the nerve cells are not striking. Alvord and Brace (1957) found that there is pyknosis of granule cells in the cerebellum, which is probably reversible and coincides with a period of clinical neurologic dysfunction. This is maximal at 8 hours after 7,500 r of whole body radiation. Similar changes are produced if only the hindbrain and cerebellar regions are radiated, but no alterations occur when this area is shielded. The same type of pyknosis in the cerebellar granule cells has been produced by Vogel *et al.* (1958) using cobalt⁶⁰ (10,000 r), and they also feel that this effect is transient and reversible. The results of Hicks *et al.* (1956) are in agreement. In none of these studies is there evidence of vascular

changes in close association with the cerebellar lesions; the alterations in the granule cells are regarded as direct effects of radiation.

Campbell *et al.* (1946), on the other hand, have found early changes in Purkinje cells. Haymaker *et al.* (1958) have noted somewhat similar changes in Purkinje cells, but also have found these types of alteration in a control monkey. Nerve cell bodies in other regions of the nervous system are little affected in the acute phase of the radiation reaction. Brownson (1960), in determining whether any effects on these structures can be detected by changes in the perineuronal satellite cells, found no statistically significant alteration in neuron-neuroglia relationship of the cerebral cortex after 1,600 r.

Though less extensively studied, beta radiation apparently exerts an effect directly on the nerve cells (Campbell and Novick, 1949).

Delayed necrosis of the paraventricular and supraoptic nuclei of the hypothalamus has been demonstrated by Arnold *et al.* (1954b). This is a specific effect and is produced by doses of 3,000 r or less, no radioselectivity being noted with larger doses. Clemente and Holst (1954) also have encountered consistent involvement of the hypothalamus.

There is a high degree of radioselectivity for the white matter (Arnold *et al.*, 1954b). The neuronal necrosis becomes progressively more evident as the interval between radiation and sacrifice of the animal is lengthened. This delayed reaction is one of the most striking differences in histology between the acute and late phases of radiation change.

An extensive literature is in almost complete agreement that the brainstem is the most sensitive region (Arnold *et al.*, 1954a, b, c; Colwell and Gladstone, 1937; Demel, 1926; Ellinger, 1942; Ellinger and Davison, 1942; Mogilnitzky and Podljaschuk, 1928). Hicks and Montgomery (1952) also describe special sensitivity in parts of the "olfactory brain." At least in the dosages generally used, the injury to the brainstem involves both white and gray matter. In fact, Colwell and Gladstone (1937) emphasize nerve cell changes in these regions and in the central gray matter of the cerebrum.

Personal material, using the monkey spinal cord, is in accord with the literature cited in regard to late effects on the neuron. The nerve cell bodies in both anterior and posterior horns show no detectable changes after extradural application of Ta^{152} or roentgen radiation, except when in an area of total necrosis.

Nerve fiber necrosis in the posterior portion of the spinal cord is irregular, but widely distributed, and extends little beyond the immediate zone of radiation, either with Ta^{152} or roentgen radiation. In the monkeys sacrificed in less than 2 weeks after completion of radiation, the only change found was occasional myelin degeneration in isolated segments. In later stages, the extent of myelin degeneration is somewhat greater than that of demonstrable

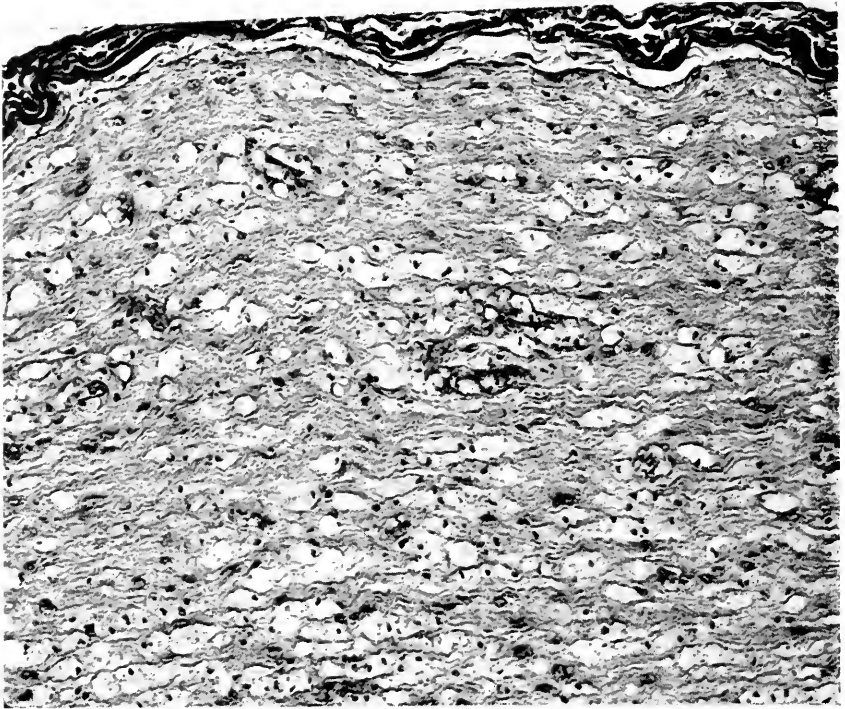


FIG. 5. Longitudinal section through posterior columns of spinal cord to show myelin sheath degeneration. Gallocyanin-van Gieson $\times 250$. Dosage 6,220 r Ta^{182} in $43\frac{1}{2}$ hours. Slight weakness of legs and loss of sphincter control 3 days after radiation; gradual recovery over 10 weeks; monkey then normal 1 month; gradual onset of paraparesis, persisting until sacrifice 9 months after radiation. Compare with Fig. 6.

neuron disintegration, a finding in agreement with Reynolds (1946), who compares this effect with that obtained with multilayer films of lecithin radiated on a water surface. Doses as low as 600 r destroy the normal molecular arrangement.

In embedded sections, this myelin degeneration appears as scattered vacuoles (Fig. 5), corresponding in distribution with droplets stained with oil red O (Fig. 6). The areas which fail to take such stains for the demonstration of myelin as Weil's method are considerably larger than shown by the other two techniques. Reasons for this difference are not clear.

The nerve fibers themselves are interrupted by irregular zones of necrosis affecting individual fibers (Fig. 7). Adjacent nerve fiber segments are swollen or partially fragmented. While the distribution of such injured nerve fibers corresponds closely to the area of radiation, it is possible with smaller



Fig. 6. Longitudinal section through posterior columns of spinal cord stained with oil red O, $\times 250$, to show the similarity in distribution of droplets stained by this method and vacuoles seen in Gallocyenin-van Gieson (Fig. 5). Same monkey as Fig. 5.

doses or at the edge of the zone of reaction to find patches of degenerated fibers (Fig. 8).

The distribution, time of demonstration by histologic methods, and the relation to vascular changes all support the view that the effects of gamma and roentgen radiation are direct effects on the parenchyma of the central nervous system and are not mediated through vascular insufficiency. The long latent period before these changes become apparent to the light microscopist is a time when the more quickly demonstrable vascular changes dominate the histologic picture. But this does not imply that the vascular lesions are causative. It suggests that changes at a submicroscopic, possibly molecular, level have been initiated at the time of radiation, changes which are compatible with preservation of morphologic structure for weeks or months. An endpoint must be reached not only before the light microscope can detect the changes, but also before the cellular sequences of repair are initiated.

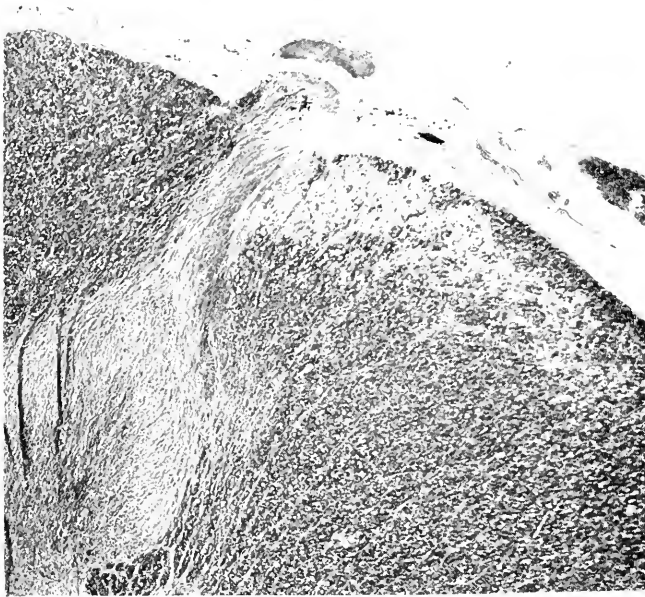


FIG. 7 (legend on facing page).



FIG. 8 (legend on facing page).

There is evidence (Bailey *et al.*, 1958; Gerstner *et al.*, 1955; McLaurin *et al.*, 1955; Yamazaki *et al.*, 1960) that in this latent period there may be functional changes related to the radiation injury before histologic change is established. The demonstration and definition of this latent interval is the role of the light microscopist; the electron microscopist and chemist must elucidate the processes during that time. Such enzyme studies as that of Cammermeyer and Haymaker (1958) are promising.

Effects of Radiation on Glia

Arnold and P. Bailey (1954) have pointed out that the response to x-radiation involves damage to all types of adult glial cells, depending on the total dose of radiation, the intensity of dose administration, the uniformity of dose distribution, and the time intervals between radiation and sacrifice of the animals.

The oligodendroglia are early and severely affected, with swelling of the cytoplasm (Arnold and Bailey, 1954) followed by pyknosis and disintegration of the cells (Hicks and Montgomery, 1952; Hicks *et al.*, 1956). In view of the generally accepted role of oligodendroglia in the maintenance of the myelin sheath, this finding is of considerable interest because of the relatively early disintegration of myelin in radiated areas (Hicks *et al.*, 1956) and the severity of radiation damage at the period of active myelination (Clemente *et al.*, 1960).

There is little, if any, formation of compound granular corpuscles in areas of necrosis (Arnold and Bailey, 1954), indicating inhibition or destruction of microglia.

A similar but more striking effect is exerted on the astrocytes. They at first swell, then fragment, and by 4 to 6 weeks after radiation the area is devoid of astrocytes (Arnold and Bailey, 1954). Only after many months is there resumption of the expected astrocytic proliferation in repair of necrotic areas.

FIG. 7. Patchy loss of myelin in dorsal spinocerebellar fasciculus, suggesting direct injury not mediated through blood vessel changes. Weill's method $\times 30$. Monkey received 12,048 r Ta^{182} in 67 hours. X-ray 10,000 r in 3 days given 5 months after completion of Ta^{182} radiation. Complete paraplegia 48 hours after Ta^{182} radiation; partial recovery in 2 months; paraplegia again complete after x-radiation. Sacrifice 6 weeks after completion of x-radiation. See also Fig. 11.

FIG. 8. Degeneration and fragmentation of nerve fibers in posterior columns of spinal cord. Hortega's silver carbonate $\times 350$. Dosage 10,000 r x-radiation in 2 days. Complete paraplegia 36 hours after completion of radiation. Sacrifice 5 days after radiation.

Campbell and Novick (1949) have found that astrocytes are most susceptible to beta radiation, oligodendroglia and microglia being highly resistant.

Haymaker *et al.* (1958), using barium¹⁴⁰-lanthanum¹⁴⁰ as the source of gamma radiation obtained results which differed from those of Arnold and P. Bailey (1954) with roentgen radiation. They conclude that alterations in glial cells are relatively inconspicuous. It is difficult to reconcile these two observations.

Personal studies of material using Ta¹⁸² as a source of gamma radiation give results entirely in agreement with those obtained by Arnold and P. Bailey (1954). While the experimental procedure was not suitable for the study of early changes, the specimens studied at relatively short intervals after radiation showed disintegration of oligodendroglia coextensive with myelin loss and almost complete, if not total, inhibition of compound granular corpuscle formation. The disintegration of astrocytes extended throughout the area of maximum radiation effect and for a short distance beyond where neurons were demonstrably affected. In later stages, the inhibition of the expected astrocytic proliferation was a striking feature of the radiation response, but at the longest intervals (up to 3 years) after radiation, brisk astrocytosis was again resumed. It is difficult to be precise in regard to the time when astrocytes begin to respond in the expected way as a part of the sequences of repair. Arnold and P. Bailey (1954) placed it at "many months." Our material can make this interval no more exact.

Behavior of Collagen in Radiated Areas and its Significance in Reparative Sequences

Little attention has been paid to the effects of ionizing radiation on the collagen of the central nervous system parenchyma. Confined as it is under normal conditions to the region of blood vessels, it is relatively inconspicuous. However, it is important in the normal nervous system and participates in reparative sequences.

In personal material, the growth of collagen is the dominant feature of the initial tissue response in brain and spinal cord areas where radiation has produced total necrosis. This type of lesion has been studied to best advantage in the spinal cord where no operative procedure had been carried out within the parenchyma. Necroses studied a few weeks or months after radiation fail to show any evidence of cellular repair (Fig. 9), a finding in agreement with Arnold and P. Bailey (1954). Some of these necroses extend completely across the spinal cord (Fig. 9) and occasionally a part of the necrotic material protrudes as small elevations into the subarachnoid space in small zones of partial destruction of the pia (Fig. 10). No instances of complete necrosis of the pia or escape of necrotic material into the subarach-

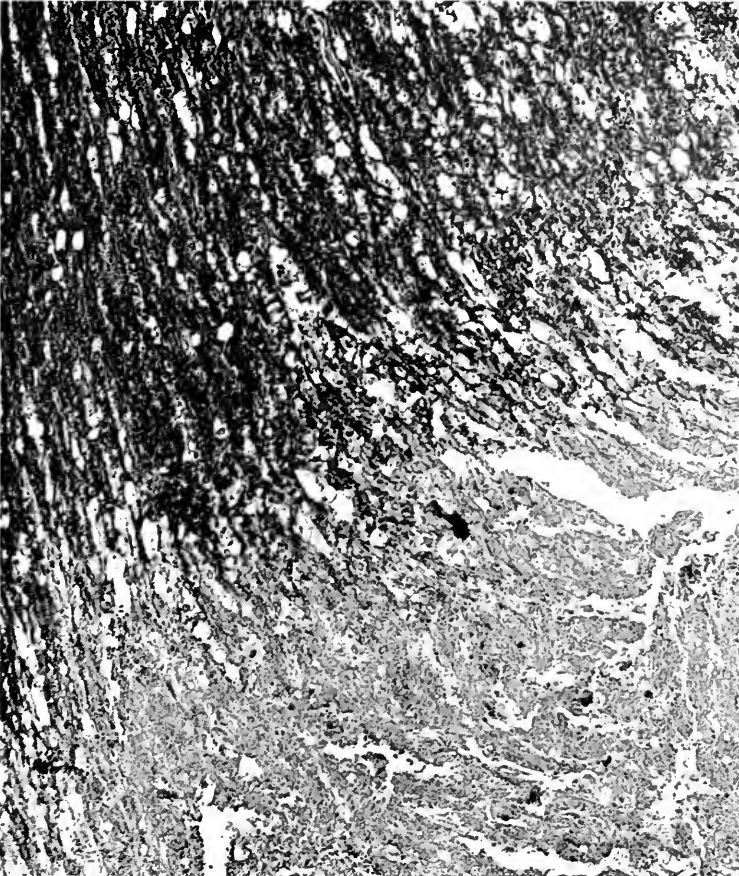


FIG. 9. Longitudinal section of spinal cord to show the junction of a zone of total radiation necrosis with surviving spinal cord tissue. Weil's method $\times 40$. Dosage 54,500 r x-radiation given in 23 days. On the day after completion of radiation, the monkey developed paraparesis which progressed to complete paraplegia in a few hours. Sacrifice 1 week after radiation. Autopsy showed a constriction in the spinal cord at the 7th and 8th thoracic segments. See also Fig. 10.

noid space have been encountered. Other necroses are smaller and involve localized regions of white matter.

When repair begins to take place after some months, the collagen proliferates long before the inhibition of astrocytes has ceased. Compound granular corpuscles make their first appearance at about the time that production of collagen is resumed.

The result of these alterations in the growth capacities of the cells usually involved in repair of central nervous system lesions is a localized, walled-off

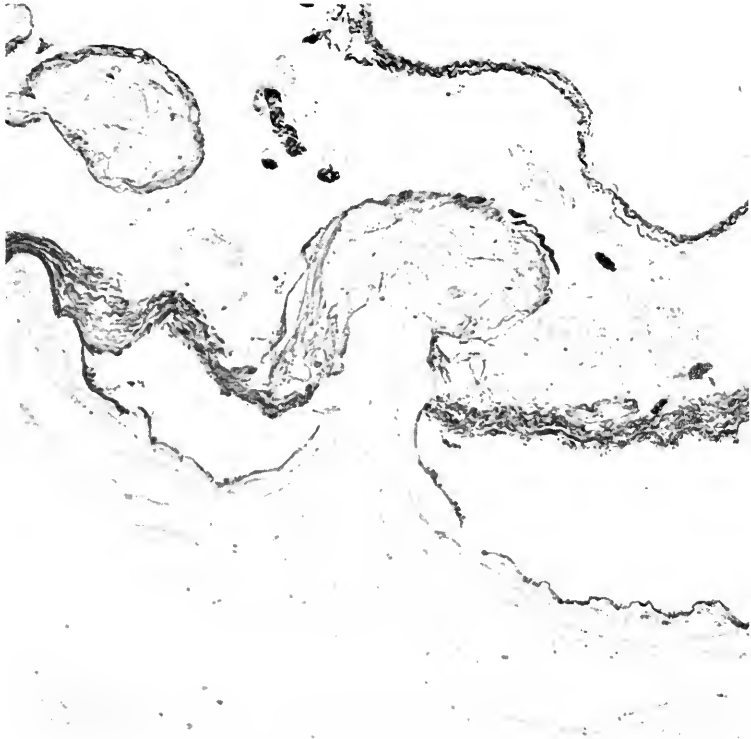


FIG. 10. Longitudinal section of spinal cord, showing herniation of necrotic tissue in an area of damage to the pia. Galloyanin-van Gieson $\times 150$. Same monkey as Fig. 9.

area. The layer next to the surviving spinal cord tissue is composed of collagenous tissue with its fibers oriented about the central necrotic material, now fragmented and amorphous (Fig. 11). Compound granular corpuscles are found in the meshes of the collagenous tissue and in the necrotic central region, but astrocytes are absent. The lesion at this stage is somewhat more reminiscent of repair of necrosis in tissue outside the central nervous system than within it.

In monkeys sacrificed at still longer intervals (usually 8 months and longer), proliferation of astrocytes is conspicuous outside the zone described, but the collagenous scar remains unpenetrated by astrocytes in its central dense zone.

Further opportunities to study the behavior of collagen are afforded by the monkeys in which Ta^{182} activated wire has been inserted into the corona radiata. It is difficult to evaluate collagen behavior in or near the point of

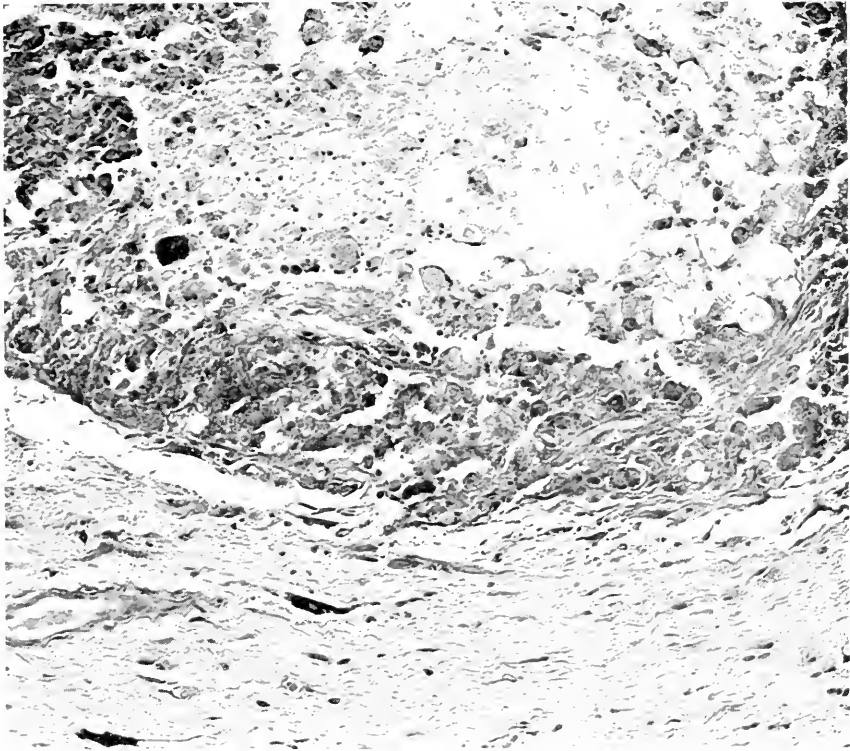


Fig. 11. Area of necrosis in the spinal cord with connective tissue proliferation and compound granular corpuscle formation, but without astrocytosis. Gallocyanin-van Gieson $\times 225$. Same monkey as Fig. 7.

insertion because of the possible participation of meningeal collagenous tissue. However, in the depths of the lesions at a few weeks or months after radiation, the same proliferation of collagenous tissue without admixture of astrocytes is present (Fig. 12). Occasionally, it can be seen that collagenous tissue is growing into regions of fibrin deposition (Fig. 12), but usually such a relationship cannot be established. When astrocytic proliferation is resumed, these areas of collagenous scar remain for the most part intact.

An attempt has been made to find out whether there is secondary degeneration of the collagen and regrowth of new collagen, as has been described in radiation reactions in the skin (Wolbach, 1909). Occasionally, hyalinized collagen fibers into which secondary proliferation of collagenous tissue has taken place, can be seen, but this is uncommon. Some vessel walls (Fig. 4) have appearances suggestive of repeated collagenous repair.

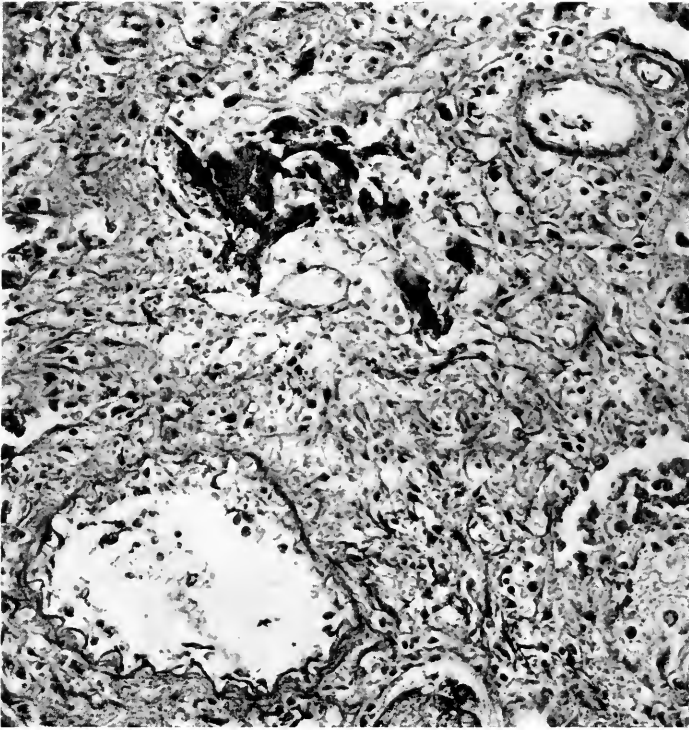


FIG. 12. Collagen production in the depths of an area of radiation necrosis at a stage before proliferation of astrocytes has begun. Gallocyanin-van Gieson $\times 250$. Dosage 600 r Ta^{132} in 8 days to right corona radiata. Mild hemiparesis developed. Sacrifice 20 days after completion of radiation. Area of necrosis 7 mm in diameter.

Radiation Reactions in Other Structures

In the acute phase of the radiation reaction in meninges, inflammatory changes with polymorphonuclear leucocytic infiltration are conspicuous with doses of 300 r or larger. The process is localized with smaller doses, but tends to spread more widely as the dose is increased. At longer intervals after radiation, the cellular infiltrate becomes mononuclear with some connective tissue proliferation. Vascular lesions occur within the meninges and undergo the same sequences as those within the brain parenchyma (Clemente *et al.*, 1960; Haymaker *et al.*, 1958; personal material).

In the choroid plexus inflammatory changes similar to those in the meninges are present in the acute phase, occasionally accompanied by small hemorrhages. The end result of these changes is a small fibrous scar. All portions of

the choroid plexus are about equally affected (Clemente *et al.*, 1960; Haymaker *et al.*, 1958).

Dilatation of the ventricular system has been seen after radiation (Clemente *et al.*, 1960; Demel, 1926), but this is variable from animal to animal. It is greatest in young animals.

In the pituitary, both anterior and posterior lobes are severely damaged in head or whole-body radiation (Haymaker *et al.*, 1958; Mogilnitzky and Podljaschuk, 1928; Vogel *et al.*, 1958).

Comment

In spite of numerous disagreements in the results of various workers, a general pattern of degeneration and repair is beginning to be defined in the tissue reactions of the central nervous system to gamma and roentgen radiation. It is quite possible that further advances can be made by particular study of points where contradictions in results now exist.

The series of studies reviewed have been carried out on widely divergent species of animals, ranging from goldfish to monkey. It is not certain how many of the contradictory results are dependent on species variation or to what characteristics of individual species these differences are related. The demonstration of certain constant features in a wide range of experimental animals suggests that species differences may be more related to details than to the general pattern of response. The high degree of sensitivity of the brainstem in animals far apart in the phylogenetic scale is an instance in point.

In nearly all experiments reviewed and in personal material, there are even more puzzling variations from animal to animal when radiation source, experimental conditions, and other technical aspects have been kept as constant as possible. Special studies of the animals which are particularly sensitive or resistant in a given set of experimental conditions may suggest factors not well recognized.

Some such factors may involve the biologic state of the animal at the time of radiation. Rugh (1958) has stated, "Probably the most effective physical factors which influence irradiation sensitivity at any biological level are: (a) state of hydration, (b) degree of oxygenation, and (c) amount of activity or movement." The abolition of early cerebellar effects by barbiturate anesthesia, as demonstrated by Alvord and Brace (1957), may be related to such factors. The apparently simple question of what is the minimum dose of radiation which produces damage to the central nervous system is actually one of great complexity.

Summary

A review of the literature related to the effects of gamma and roentgen radiation of the central nervous system is presented and compared with personal material, with a few comments on the effects of beta radiation.

The reaction of the central nervous system hours or a few days after radiation is an acute inflammatory one, dominated by vasculitis, meningitis, and choroid plexitis. Regressive changes, apparently mostly reversible, are present in the granule cells of the cerebellum.

Young animals are more susceptible to radiation damage than adults, but histologic effects can be produced at any age with sufficient dosage.

Different and more extensive degenerative processes become evident as the time interval between radiation and sacrifice is lengthened. This interval can be reduced by increasing the dose of radiation, increasing the intensity of radiation, and possibly by other factors.

In late stage of radiation reaction, there is extensive damage to neurons, with selectivity for the white matter and particular sensitivity of the brain stem and hypothalamus. Degenerative changes in blood vessels, sometimes with complete occlusion, can be demonstrated at this stage.

The effects on neurons are considered by most, but not all, workers to be direct effects and not mediated through vascular insufficiency.

All types of glia show degenerative changes after radiation, and there is a prolonged inhibition of glial response in repair. Proliferation of collagenous tissue is important in the first stages of repair. Compound granular corpuscle formation is resumed before astrocytic proliferation begins. Some months after radiation, astrocytosis becomes exuberant.

Despite many contradictions in results and interpretation, a basic pattern of degeneration and repair in response to gamma and roentgen radiation is becoming apparent by light microscopy. The most striking single feature is the progressive increase in evidence of damage as time after radiation is lengthened. It is impossible to predict end results from any characteristics of the acute response. Investigations by all techniques will be required to explain the processes going on in the interval, and studies of this type may well have biologic implications beyond the field of radiation pathology.

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Sequence of X-Radiation Damage in Mouse Cerebellum

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Since the time of Brunner and Schwartz (1918; Brunner, 1920, 1921), who in 1918 were the first to observe that the cerebellar granule cells of young dogs and cats could be easily injured by x-radiation, little attention was directed to the radiovulnerability of this part of the brain until the past few years. Our work in this field was concerned with the effect of x-rays on the cerebellum of mice (Schümmelfeder 1957, 1959a, b; Schümmelfeder *et al.*, 1957; Krogh and Bergeder, 1957). We used single x-ray doses and studied the irradiated tissue by morphologic, histochemical, and fluorescence techniques. The x-ray dosage ranged from 250 to 60,000 r. Fields of the cerebellum 3×3 mm and 0.5×2 mm were irradiated by a half-wave x-ray unit (50 kv, 20 ma, focal distance 6 cm, 0.12 mm Al filter) at 3,000 r per min to the surface of the cerebellum. The irradiated animals were sacrificed at intervals up to 6 months.

Observations

Morphologically demonstrable radiation effects were seen in the range of 2,000 to 60,000 r. The latent period between irradiation and the first morphologic changes decreased correspondingly. At 2,000 r, damage was first noted at the end of 4 months, while at 60,000 r, changes were observed in 30 minutes. Irradiation doses less than 2,000 r induced no morphologic changes in the cerebellum during the observation time of 6 months.

A few typical experiments will indicate the nature, severity, and sequence of damage after exposure of the cerebellum of mice to different x-ray doses.

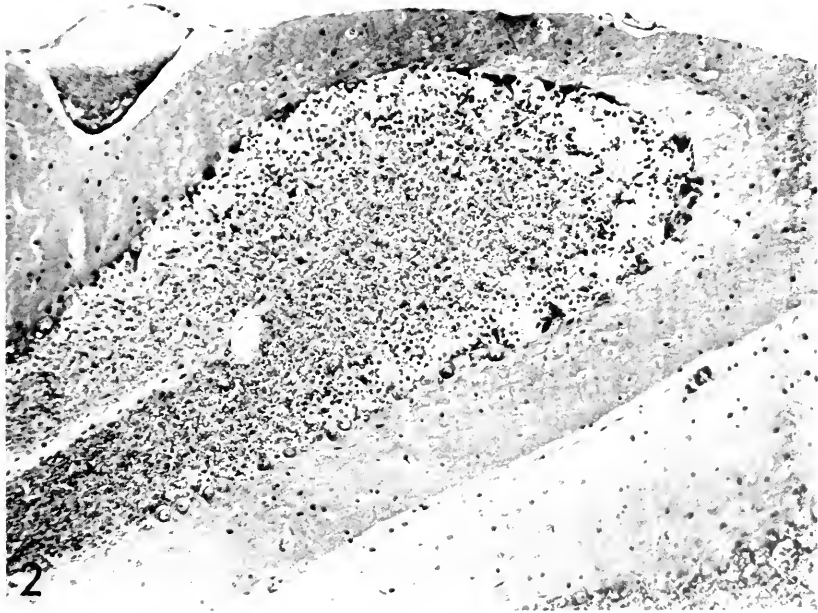
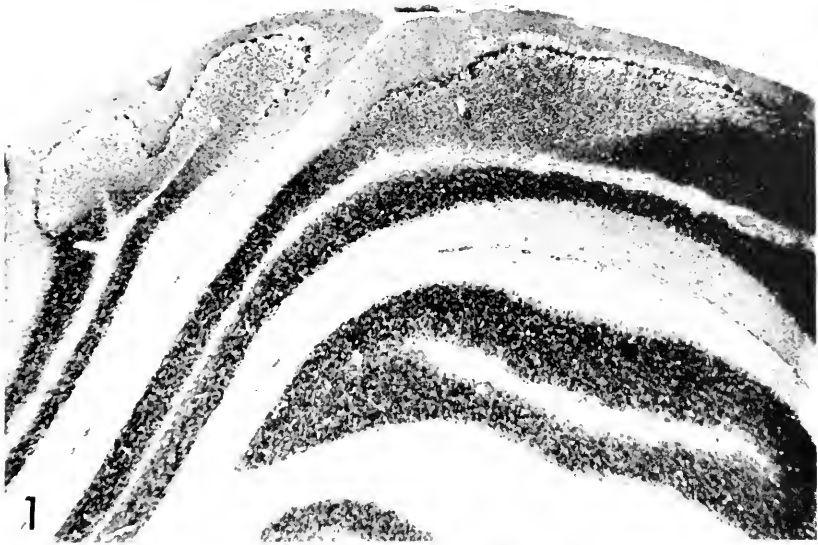
TOTAL NECROSIS OF CEREBELLAR TISSUE FOLLOWING EXPOSURE TO X-RAY DOSES RANGING FROM 60,000 TO 10,000 R

In the range from 60,000 to 10,000 r, x-irradiation induced total necrosis of cerebellar tissue within a time depending on the dosage.

In 30 minutes following exposure to 60,000 r, swelling of nuclei and cytoplasm occurred in granule cells, Purkinje cells, and basket cells in deeper parts of the molecular layer. The interstitium of the molecular layer exhibited slight vacuolation.

In 1 hour after 60,000 r, there was greater swelling of the various nerve cells, and the granular layer was loosened. Vacuolation of the molecular layer was increased. The chromatin in the swollen nuclei of the granule cells was condensed in the form of irregularly shaped coarse bodies located on the nuclear membrane. The swollen nerve cells within the molecular layer had a clear space around their nuclei. These spaces contained small, thread-like, ragged or flaky, cytoplasmic residues, often attached to the nuclei (acute cell swelling). The degree of nerve cell swelling was slight in upper parts of the molecular layer and increased progressively down to the Purkinje cells. Within the molecular layer, the nuclei of glial cells were slightly swollen or occasionally pyknotic. Vacuolation (*status spongiosus*) of the molecular layer was evident; the vacuoles were at first round, then oval, and increased in size downward from the cerebellar surface to the Purkinje cell layer. Occasionally, the vacuoles were arranged in vertical columns. The vacuoles seemed to contain a protein-free aqueous solution, because even with special staining methods and histochemical reactions no other material could be demonstrated. Henceforth, the Purkinje cells, in particular their nuclei, showed hydropic swelling. Within the swollen cytoplasm the Nissl bodies had usually disappeared. In other cells, the Nissl substance was dispersed as dust-like particles over the entire cytoplasm. The Bergmann glial cells occasionally had swollen nuclei, but they seldom displayed any nuclear pyknosis.

At 2 hours after 60,000 r, the regressive changes were still more advanced (Figs. 1 and 2). Within the lower part of the molecular layer, the vacuolation had progressed to tissue sponginess. Within the uppermost parts of the granular layer, the tissue looseness had also increased. Some nuclei of the irradiated granule cells were no longer swollen, but were shrunken and pyknotic. Correlated with the decrease of the x-ray dose with distance traversed, there was an upper zone composed mostly of pyknotic nuclei, then a transitional zone with pyknotic as well as swollen nuclei, and a lower zone containing solely swollen nuclei. Apparently as a consequence of the pressure from the swelling of the molecular and granular layers, many Purkinje cells within the center of the damaged area were deformed. These cells were oval, and their longitudinal axes were parallel to the granular layer. In the lateral part of the irradiated field, several Purkinje cells exhibited acute swelling. Near the border of the damaged area, which was evident because of the changes in the granular layer, the Purkinje cells showed slight or no morphologic alterations.



FIGS. 1 and 2. Radiation damage 2 hours after exposure to 60,000 r. Plane of the section is parallel to the direction of the x-ray beam. Vacuolation of molecular layer, shrinkage of Purkinje cells, and pyknosis of the nuclei of granule cells occur in the most severely damaged area of the granular layer, and nuclear swelling in the lower part. FIG. 1: $\times 40$, Hematoxylin-eosin. FIG. 2: $\times 170$, v. Gieson-stain.

At 4 to 5 hours after x-irradiation with 60,000 r, nearly all the granule cells in the irradiated part of the granular layer were shrunken and their nuclei pyknotic (Fig. 3). These cells were surrounded by a clear space. In the molecular layer, the nuclei of the swollen nerve cells were now pyknotic, while the degree of vacuolation in this part of the irradiated cerebellar cortex was the same as before. The morphologic changes in the molecular and granular layers were strictly localized, limited to the irradiated field. The border between the irradiated and the unchanged cerebellar tissue was sharp, as though drawn with a ruler. During this time interval, the white matter of the cerebellum was slightly, if at all, modified by the irradiation. There was neither detectable edema in the white matter nor any substantial change in the axis cylinders or in glial cells.

Within the first hours after irradiation, no evidence of a conspicuous

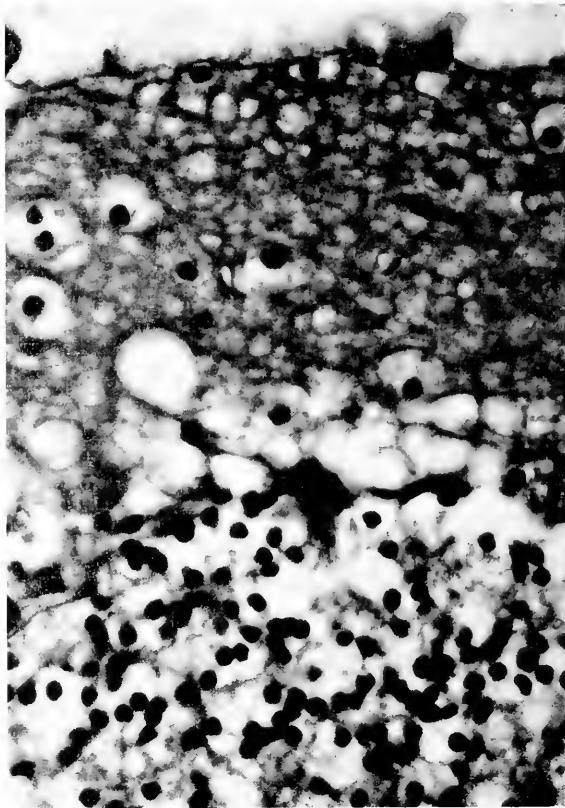


FIG. 3. At 4 hours after irradiation with 60,000 r, showing severe vacuolation of the molecular layer, shrinkage of Purkinje cells, and pyknosis of granule cell nuclei. $\times 780$. Hematoxylin-eosin.

change in the blood vessels was observed. Occasionally a slight dilatation of the capillaries was seen as an expression of hyperemia.

In 5 to 6 hours after x-irradiation of a 3×3 mm field with 60,000 r the mice died. Further evolution of radiation damage could be followed only after use of a dose of 60,000 r through a smaller aperture (0.5×2 mm) or by using lower x-ray doses (40,000 to 20,000 r).

At 12 to 14 hours after 60,000 r, using a 0.5×2 mm field, the pyknotic nuclei of the granular layer underwent disintegration, mostly as karyorrhexis. The damaged area was still limited to the irradiated field, as a section cut transversely to the direction of the x-ray beam illustrates (Fig. 4). The Purkinje cells also exhibited signs of disintegration. Some of these cells had greatly swollen cytoplasm and nuclei and showed lytic changes (Fig. 5). Within other Purkinje cells, the nuclear chromatin was initially condensed, simulating pyknosis, and then the cytoplasm and nucleus underwent lysis.

At 20 to 30 hours after irradiation with 60,000 to 40,000 r, necrosis was completely established in the superficial part of the cerebellar folia, i.e., nearest the radiation source (Fig. 6). The necrosis was strictly limited to



FIG. 4. Radiation damage 12 hours after exposure to 60,000 r (0.5×2 mm field). Plane of section is transverse to direction of the x-ray beam. Pyknosis and disintegration of nuclei in the granular layer. $\times 40$. Hematoxylin-eosin.

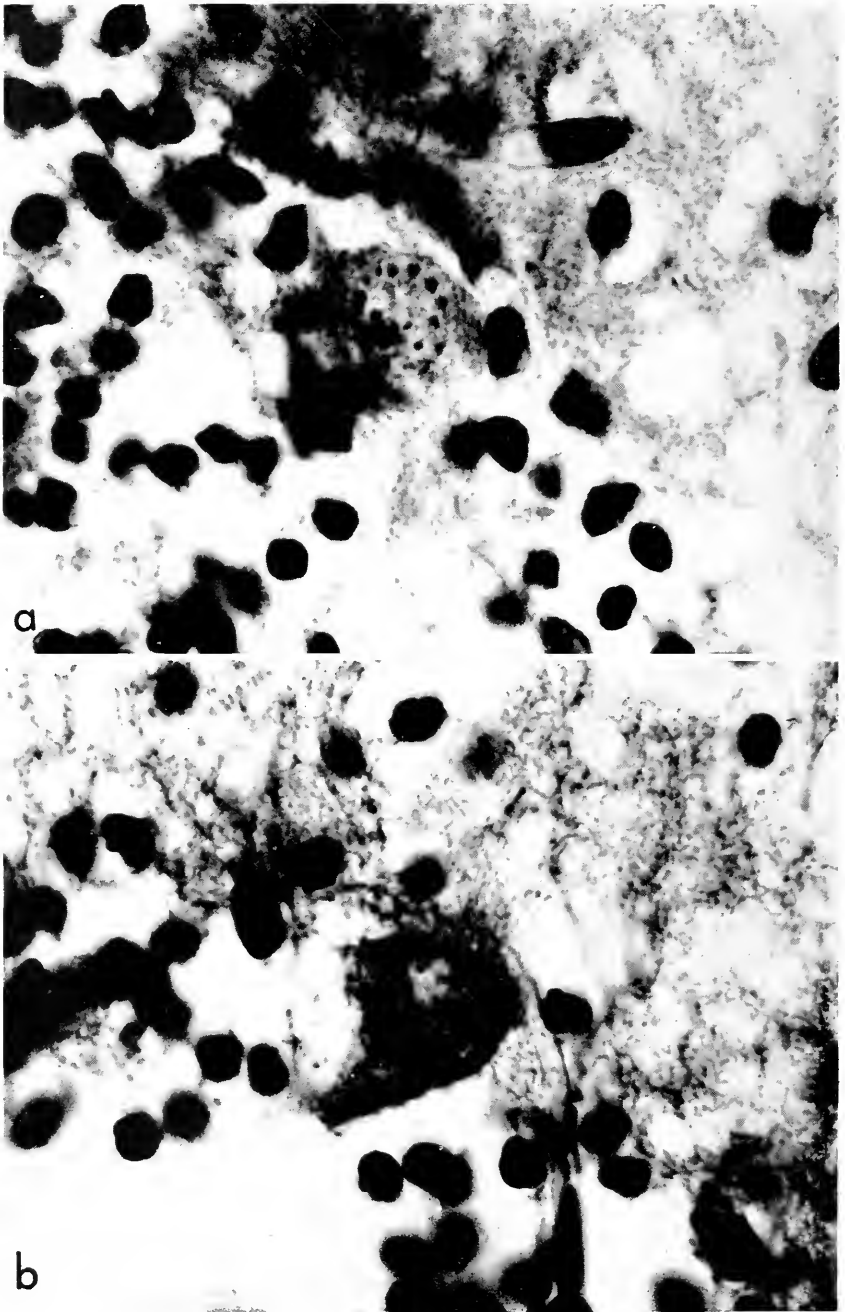
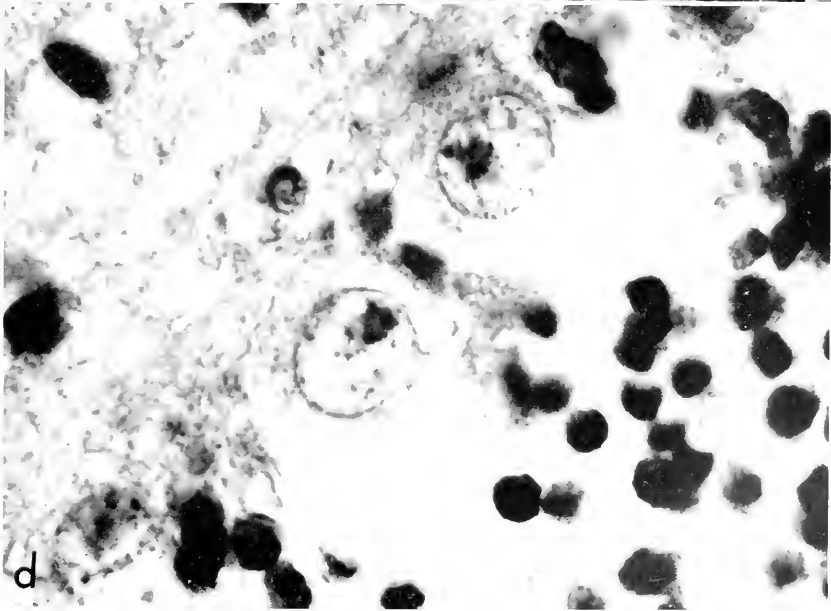
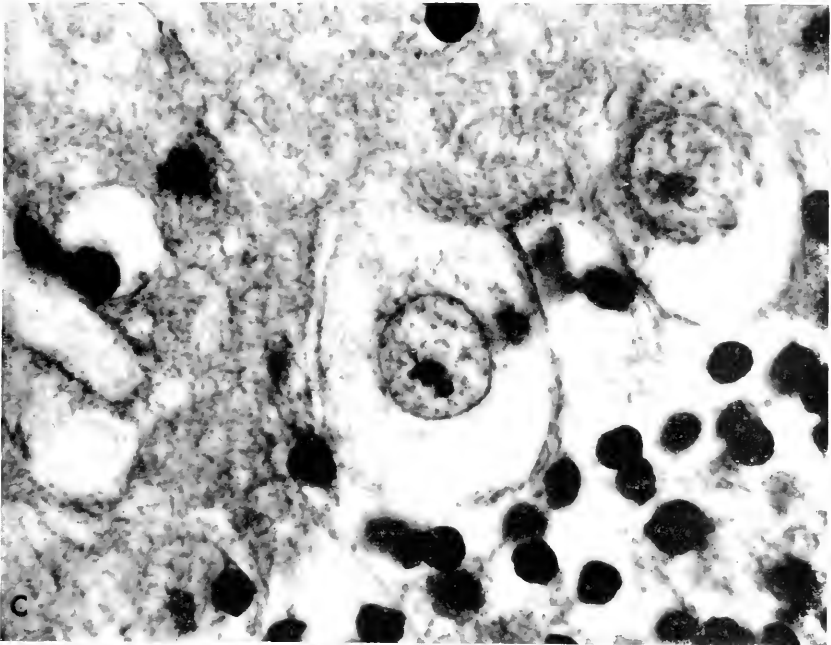


FIG. 5. Different types of nerve cell change after irradiation: (a) condensation of the nuclear chromatin, (b) swelling and partial vacuolation of the cytoplasm, (c) severe swelling of the cytoplasm, (d) severe nuclear and cytoplasmic swelling, incipient lysis of the cytoplasm. $\times 1440$. Gallocyanin-chromalum stain.



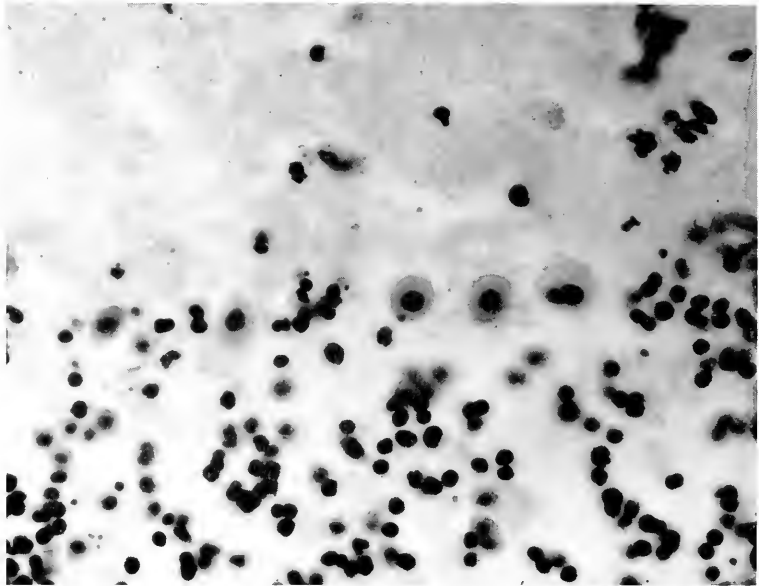


FIG. 6. At 20 hours after irradiation with 40,000 r, showing the homogenization type of nerve cell necrosis. $\times 700$. Hematoxylin-eosin.

the irradiated field and was sharply demarcated from the nonirradiated part of the cerebellum. The molecular layer showed granular and clumped areas of disintegration. Only some of the nerve and glial cells were preserved. Most of them contained pyknotic nuclei and showed all stages of disintegration or lysis. Within the necrotic granular layer enlarged by edema, the preserved nuclei were pyknotic, and between them nuclear debris was frequently found. Hemorrhages and extravasations of plasma proteins were remarkable neither within the necrotic granular layer nor within the white matter. The nuclei of the glial cells in the white matter of the more superficial part of the cerebellar folia (nearest the radiation source) were pyknotic, and in deeper parts of the cerebellum they were swollen. Some blood vessels in the necrotic area were preserved, but dilated. Frequently, they were surrounded by a hollow space, the ground membrane had often undergone hyaline thickening (hyalinosis), and there was swelling of endothelial and adventitial cells.

Within the irradiated field the Purkinje cells had, in part, disappeared. Most of the preserved Purkinje cells showed homogenization. They contained pyknotic nuclei and exhibited a strong cytoplasmic eosinophilia. Many were in all stages of disintegration, including cell shadows.

At 90 hours after irradiation, necrosis was completely established in all

parts of the irradiated cerebellar tissue (Fig. 7). Within the necrotic area there were scavenger cells and gitter cells as well as proliferating and naked glial cells. The nuclei of most altered granule cells were disintegrated and lysed. Only segregated groups of pyknotic nuclei together with nuclear debris were observed within the irradiated field. In contrast to the animals of shorter survival, edema was seen within the Bergmann layer and in adjacent parts of the molecular and granular layers. Frequently, the edematous process extended a short distance into the nonirradiated part of the Bergmann layer and was associated with hydropic swelling of adjacent Purkinje cells. Homogenization of Purkinje cells was no longer found, as cells which had suffered this change seemed to have been removed.

When the survival period was extended by reducing the x-ray doses to 20,000 to 10,000 r, resorption and repair occurred in the irradiated cerebellar tissue in the same manner as in necrosis of brain tissue resulting from other causes. The final stage consisted of cystic liquefaction of the necrotic brain tissue. By 20 days after irradiation with 16,000 r much of the necrotic cerebellar tissue had been removed. The resulting cyst-like areas contained remnants of necrotic debris and were traversed by partly preserved blood vessels. The processes of resorption and repair were evident at the margin

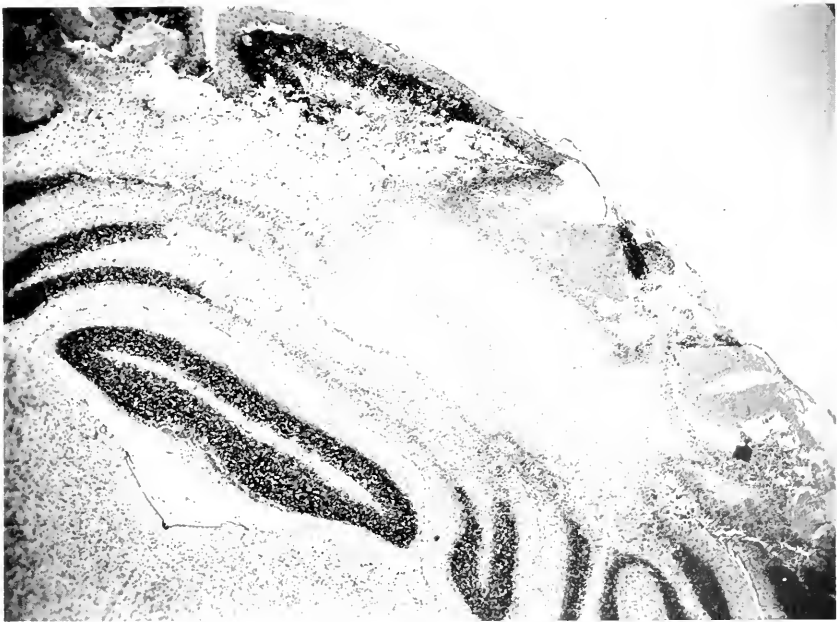


FIG. 7. Total tissue necrosis 90 hours after irradiation with 70,000 r (0.5×2 mm field). $\times 35$. Hematoxylin-eosin.

of the damaged brain tissue in the form of numerous gitter cells. Within the preserved tissue only slight glial reaction had occurred.

PARTIAL NECROSIS OF CEREBELLAR TISSUE FOLLOWING EXPOSURE TO X-RAY DOSES FROM 10,000 TO 4,000 R

At radiation doses from 10,000 r down to 4,000 r, there was necrosis only of the granular layer and a loss of single nerve cells in the other parts of the cerebellar cortex. The latent period between irradiation and the development of morphologic changes was longer than with higher doses.

Thus, with a dose of 5,000 to 4,000 r, the first morphologic changes appeared at approximately 12 hours. At this time only a few scattered pyknotic nuclei of granular cells were observed. The other parts of the irradiated cerebellar tissue were not modified.

At 5 days the more superficial part of the granular layer had undergone partial dissolution. Only adjacent to the Purkinje cell layer was there a zone of preserved pyknotic granule cell nuclei, and it was narrow. Occupying regions in which granule cells had undergone dissolution were numerous gitter cells and isolated pigment-bearing scavenger cells. Purkinje cells were destroyed only in the most severely damaged regions of the irradiated field. In their place, proliferated glia were seen. Other Purkinje cells were being phagocytized. The cerebellar white matter was loosened strikingly, and in some foci the white matter was completely destroyed. Some of the blood vessels, especially the capillaries, were greatly dilated and were surrounded by a mantle of mononuclear cells including a few neutrophilic leucocytes. Endothelial cells of occasional blood vessels were swollen.

At 10 days after irradiation the destroyed Purkinje cells were replaced by glial shrubberies (*Gliastrauchwerk*) extending from the Bergmann layer into the molecular layer. The uppermost part of the molecular layer showed decided shrinkage, as did other parts of the cortex (Fig. 8).

At 20 days after exposure to 5,000 r, extensive perivascular hemorrhages were often found in damaged cerebellar tissue. Shrinkage, pronounced glial proliferation, and cicatrization (gliosis) occurred in the irradiated tissue. The glial fibers within the damaged molecular layer, particularly those of the Bergmann cells, were thickened remarkably. These coarse fibers could easily be demonstrated by Lendrum's (1947) method.

Persisting Purkinje cells and nerve cells of the molecular layer often showed regressive changes, e.g., swelling of cytoplasm and nucleus or chromatolysis. Even at this time interval, some of the granule cells in the irradiated area were pyknotic. Such pyknosis had probably developed in the course of the radiation damage. The periphery of the most severely damaged area was marked off by pronounced vascularisation. The vessels

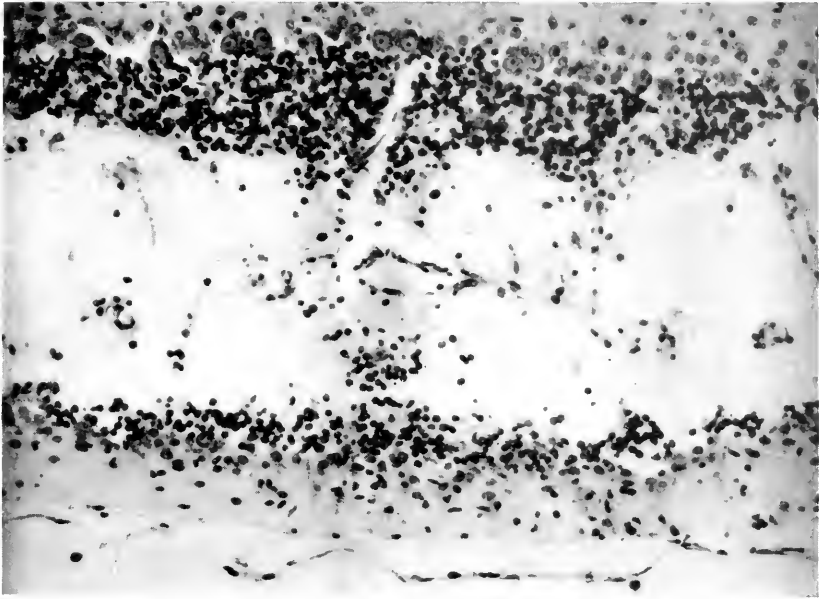


FIG. 8. Radiation damage 10 days after irradiation with 5,000 r. Dissolution of central parts of the granular layer and destruction of Purkinje cells within the uppermost layer followed by glial proliferation extending into the shrunken molecular layer. $\times 215$. Galloxyanin-chromalum.

were irregularly dilated, and their walls showed remarkable hyalinosis. Circumscribed aneurysmal distention of smaller blood vessels, previously described by Scholz (1934a, b, 1937) in brain tissue damaged by irradiation, was noted at the 20-day stage.

At 40 days after irradiation, the glial reaction in the irradiated cerebellar tissue had progressed further. The entire damaged area was shrunken. Some granule cells were preserved, but it was difficult at times to distinguish them from proliferated glial cells. As at shorter time intervals, hemosiderin-containing scavenger cells were often seen. Hemosiderin indicated previous hemorrhage.

In contrast to higher x-ray doses, 5,000 to 4,000 r produced only partial necrosis of the cerebellar tissue. The molecular layer and the white matter of the cerebellar folia were little affected. All the well known processes of resorption and repair took place in the irradiated cerebellar tissue in the same manner as in partial cerebellar necrosis due to other cause. Glial scarring was the final outcome.

SINGLE CELL NECROSIS WITHIN THE CEREBELLUM AFTER EXPOSURE TO
X-RAY DOSES BELOW 4,000 R

X-ray doses below 4,000 r resulted in loss only of single cells or small cell groups, not in necrosis of larger areas of the irradiated cerebellar tissue. The latent period between irradiation and the appearance of damage was increased. Following an x-ray dose of 3,000 r, morphologic changes within the irradiated cerebellar tissue were not observed in less than 45 days. At this time, focal loss of granule cells and destruction of some Purkinje cells were noted in the most superficial cerebellar folia, i.e., those nearest the radiation source. The better preserved Purkinje cells often showed regressive changes, which were of differing types. Cell shadows were occasionally observed. Together with fresh hemorrhages within the damaged brain tissue were residues of older ones in the form of hemosiderin in scavenger cells. The blood vessels were dilated, and their walls often showed hyalinosis. Perivascular infiltrates of mononuclear cells were found over the entire irradiated field.

At 4 months after irradiation with 3,000 r the various components of the irradiated cerebellar folia were relatively well preserved. The granular layer showed patchy looseness due to disseminated loss of granule cells. This layer contained proliferated glial elements and was traversed by capillaries. Hyaline thickening was found in the wall of blood vessels in the leptomeninges and brain tissue. The lumens of many vessels were dilated. Some Purkinje cells were destroyed and replaced by glial nodules from which glial shrubberies extended into the molecular layer. The preserved Purkinje cells seemed unaltered.

EARLY CHANGES IN CELLULAR NUCLEOPROTEINS OF IRRADIATED
CEREBELLUM

All these observations indicate that local irradiation of the cerebellum of mice with sufficiently high doses of x-rays results in marked alterations of cerebellar tissue which are limited to the irradiated field. One of the most remarkable changes is the pyknosis of nuclei of granule cells. Another important observation is that during the course of the radiation damage different types of regressive changes are observed in the Purkinje cells.

We have applied the fluorescence technique, using the basic fluorochrome acridine orange, to study alterations in nerve cells. This histochemical method is especially suitable for investigation of cytoplasmic and nuclear nucleic acids (Schümmelfeder *et al.*, 1958). Using buffered acridine orange solutions, pH 4.0 to 7.0, all ribonucleic acid (RNA)-containing material, e.g., the cytoplasm of Purkinje cells, fluoresces bright orange or red. In

contrast, the highly polymerized deoxyribonucleic acid (DNA) of the nuclei shows an intense yellow or yellow-green fluorescence. Other elements of the brain tissue present a weak green fluorescence. The difference in the staining reaction of RNA and DNA is due to the high degree of polymerization of the nuclear DNA in comparison with the cytoplasmic RNA. After depolymerization, e.g., by placing the tissue slices in boiling water or hydrochloric or perchloric acid, the DNA stained with acridine orange shows a red fluorescence. The depolymerized DNA behaves, therefore, like RNA, which is always more weakly polymerized. The acridine orange method is more sensitive than the methyl green, which can, however, bring out wide differences in the degree of polymerization in DNA. The occurrence of depolymerization in DNA thus can be proved by using the acridine orange fluorescence technique.

We tried to determine with this method whether alterations occur in the structural organization of the DNA in the pyknotic nuclei of irradiated granule cells. Many other cells besides those of the cerebellar granular layer show clumping or other changes in the nuclear chromatin. In an histochemical study of nuclear changes in the superficial epithelium of the tongue of mice that had received radioactive chromic phosphate, Burstone (1953) observed enlargement and hyperchromasia of the nuclei. In contrast to nonirradiated controls, treatment with deoxyribonuclease (DNase) resulted in decreased staining capacity of these nuclei to the Feulgen reaction and to the methyl-green-pyronin method. Burstone (1953) believed that this decreased resistance of the irradiated nuclei is due to a somewhat decreased aggregation of the nuclear DNA. Based on such observations and on radiation experiments on DNA solutions which show a splitting of DNA molecules (Scholes and Weiss, 1952) some still believe that the clumping of chromatin after irradiation is a result of depolymerization. If such depolymerization of DNA should occur in the pyknotic nuclei of irradiated cerebellar granule cells as a primary effect or a secondary reaction to ionizing radiation, then it should be demonstrable with acridine orange by the nuclei exhibiting red fluorescence. We have never found this. Disregarding the point that pyknosis causes a higher density which provokes a somewhat more intense fluorescence, no difference in the fluorescent color of these pyknotic nuclei compared to normal nuclei has been observed.

The fact that the DNA of these pyknotic nuclei is not significantly depolymerized can also be shown by using methyl green. This dye stains only highly polymerized DNA (Kurnik, 1950, 1952; Kurnik and Forster, 1950; Kurnik and Mirsky, 1950; Pollister and Leuchtenberger, 1949; Vercauteren, 1950). The pyknotic nuclei should not be stainable with methyl green if there had been depolymerization of nuclear DNA. But, as compared with nonirradiated cells, no substantial difference in the staining capacity

of the pyknotic nuclei was observed. This result corresponds to that of Sparrow *et al.* (1952) on *Trillium* nuclei.

Analogous to Burstone's (1953) observations, Kaufmann *et al.* (1955) have shown in experiments on meristematic cells of onion roots that DNA in irradiated cells is more easily dissolved by DNase than in nonirradiated controls. In contrast, similar experiments on grasshopper embryos showed a higher resistance of the irradiated nuclei, i.e., by their DNA, to enzymatic hydrolysis. These different results stimulated us to seek information on whether the development of pyknosis of granule cell nuclei following irradiation alters their response to depolymerizing and hydrolyzing agents.

The results of these experiments showed that pyknotic nuclei of granule cells are more resistant than nonpyknotic nuclei to treatment with depolymerizing agents, e.g. to boiling water or to hydrochloric or perchloric acid. Provided the reaction conditions are favorable, only in the nonirradiated nuclei did exposure to these agents result in depolymerization. These nuclei showed red fluorescence after staining with buffered solutions of acridine orange, pH 5.0 to 7.0, whereas the pyknotic nuclei still fluoresced bright yellow owing to retained high polymerization of DNA. The difference in color and intensity of the fluorescence was so conspicuous that each individual pyknotic nucleus could easily be observed.

Methyl green, which stains only highly polymerized DNA, has yielded the same results in companion sections. The pyknotic nuclei were still stainable with methyl green, whereas unaltered granule cell nuclei could not be stained after pretreatment with depolymerizing agents.

Further experiments showed that enzymatic breakdown of DNA, using DNase, occurs more slowly in pyknotic than in intact nuclei. This result is similar to that observed by Kaufmann *et al.* (1955) in irradiated grasshopper embryos.

Hydrolysis with hydrochloric or perchloric acid removed DNA from the nuclei of unaltered granule cells, whereas DNA of the pyknotic nuclei in irradiated granule cells was only slightly depolymerized. Using favorable conditions of hydrolyzation, it is easy to demonstrate selectively the pyknotic nuclei after staining with acridine orange, whereas nonpyknotic nuclei, which are not altered by the irradiation and which are deprived of the DNA by the foregoing hydrolysis, remain unstained.

In our experimental study we were unable to determine whether increased resistance of the irradiated pyknotic nuclei to DNase and to depolymerizing and hydrolysing chemical agents is an immediate and specific effect due to the action of ionizing radiation on the DNA of the nuclei. The observations of Kaufmann *et al.* (1955) have indicated that the structural organization of the nucleoproteins in irradiated nuclei is changed. On the other hand Yakar (1952) has demonstrated in plant cells that the speed of enzymatic

hydrolysis of chromatin decreases if pyknosis is induced by chemical agents. It is conceivable that the increase in resistance of the pyknotic nuclei which we found is attributable to greater density of the nuclear mass in that the increased density reduces depolymerization and hydrolysis.

We have emphasized that when RNA-containing material, e.g., the cytoplasm of the Purkinje cells, is stained with acridine orange, pH 4.0 to 7.0, it takes on a bright orange or red fluorescence. Since different types of regressive changes can be observed in Purkinje cells during the course of radiation damage, we have used the acridine orange method to study the behavior of their cytoplasmic nucleic acids. Because of regressive changes in these cells and because of the nuclear pyknosis in granule cells, the irradiated area of the cerebellar tissue can easily be demonstrated by this method. Since regressive changes in the Purkinje cells usually occur more strikingly in the center of the irradiated area than along its margins, the red fluorescence exhibited by the more peripheral cells gradually decreases in intensity toward the center of the irradiated zone. The cytoplasm of unaltered Purkinje cells fluoresces bright orange-red. Acute shrunken nerve cells show the same fluorescence because their cytoplasm contains abundant RNA. Immediately following irradiation, the swollen and vacuolated Purkinje cells give off a slightly decreased orange fluorescence, but after sufficient time has passed the cytoplasm exhibits only yellow or yellow-green fluorescence because the RNA content of their altered cytoplasm is decreased. Purkinje cells showing the homogenization type of necrosis have a green fluorescence because they have lost all cytoplasmic RNA. Since color and intensity of fluorescence in these Purkinje cells is similar to that of the neuroglia of the molecular layer, it is somewhat difficult to recognize necrotic and homogenated Purkinje cells. It bears emphasis that the nuclear pyknosis in Purkinje cells as well as in granule cells is not associated with depolymerization of DNA, since, when stained with acridine orange, the pyknotic nuclei still fluoresce yellow-green.

Discussion

There have been apparently conflicting reports in the literature as to the primacy of irradiation damage in the central nervous system, whether in vessels or in nerve cells. The cerebellum seems especially suitable for investigation of this problem. Some other workers already have directed attention to the radiovulnerability of this part of the brain. In macaque monkeys, Haymaker *et al.* (1958) have studied the effect on the central nervous system of whole-body BA^{130} - LA^{130} (gamma) radiation. Evidence of nerve cell damage in the cerebrum was scanty, but granule cells of the cerebellum were pyknotic within a dose range of 5,000 to 30,000 r. The pyknosis occurred earlier and

was more severe at higher dose levels. In other experiments on the macaque, in which 10,000 r Co^{60} (gamma) radiation to the head alone or to the whole body was used, Vogel *et al.* (1958) also observed pyknosis of granule cells in the cerebellum. Both Haymaker *et al.* (1958) and Vogel *et al.* (1958), whose animals survived no longer than one week, observed that the pyknosis of granule cells was reversible by about the 3rd day. In a subsequent study of the effects of cobalt⁶⁰ (gamma) radiation on the cerebellum of macaque monkeys at the same dose levels, Wilson (1960) confirmed the observation that under these conditions the granule cell pyknosis is transitory and reversible, but he found that pyknosis was somewhat briefer than reported by the other authors. Similar results have been obtained in rabbits after exposed to a Co^{60} source (Vogel, 1959) and in guinea pigs after x-irradiation (Alvord and Brace, 1957). Vogel (1959) noted that after a dose of 15,000 r gamma radiation from a Co^{60} source, granule cell pyknosis was evident in 15 hours and that by the 10th day practically all the granule cells of exposed folia had disappeared. According to Hicks (1953; Hicks and Montgomery, 1952; Hicks and Wright, 1954; Hicks *et al.*, 1956) the same holds for rats and mice. In these animals they found that nerve cells or cerebellar tissue could readily be damaged by x-rays, depending on the dose.

Our observations coincide with those of Hicks *et al.* (1956) on the mouse. We have shown that sufficiently intense x-irradiation of the cerebellar tissue results in primary tissue changes. Incidence, pattern, and course of these changes are clearly related to the x-ray dose. Under the conditions of our experiments, nuclear pyknosis in irradiated granule cells of adult mice is a sign of irreversible cellular change leading to cellular necrosis. In this respect our observations on mice do not coincide with those of Haymaker *et al.* (1958), Vogel *et al.* (1958), and Wilson (1960) on macaque monkeys. We are unable to explain this difference, but points to be taken into consideration are species and age of the animals, nature of irradiation, radiation energy and rate of dosage.

There is still little knowledge of the earliest histopathologic and histochemical changes occurring in the cerebellum following irradiation or in the sequence in which the changes develop. Our histochemical investigations show that during early radiation damage of cerebellar tissue following high x-irradiation dose, changes occur in the nucleic-acid-containing components of granule and Purkinje cells. Particularly in Purkinje cells, alterations occur in the cytoplasmic RNA that are secondary effects due to regressive cellular alterations, since swelling of the nerve cells was observed initially and decrease in cytoplasmic nucleic acid content later on. The change in the structural organization of the nuclear DNA in the pyknotic granular cells is possibly also a secondary effect due to the increased density of the pyknotic nuclei. But it could also represent a primary change in the physicochemical

quality of the DNA caused by the action of ionizing radiation. Further investigations are necessary to clarify this problem. As brought out by the acridine orange fluorescence technique, the increased resistance of pyknotic nuclei to depolymerizing and hydrolysing agents allow clear identification of damaged irradiated pyknotic cell nuclei.

Summary

Development and course of radiation lesions in the cerebellum in mice were studied after exposure to single doses of x-rays, 250 to 60,000 r, applied to one cerebellar hemisphere through apertures 3×3 mm or 0.5×2 mm in diameter. Degree and severity of radiation damage in the cerebellum was correlated in terms of time-intensity relationships with the x-ray dose.

After a dose of 60,000 r, morphologic changes of different cerebellar structures (e.g., nerve cells of the molecular layer and Purkinje and granule cells) were visible as early as 30 minutes following exposure and were fully developed at 60 minutes. Within 90 hours, complete necrosis of the irradiated cerebellar area with concomitant resorptive and reparative changes was observed. Cystic liquefaction eventually occurred.

After less intense x-ray doses, 60,000 to 10,000 r, similar radiation lesions were observed, but the latent period of their inception was longer. Exposure to doses below 10,000 r down to 4,000 r resulted in necrosis of the granular layer and in destruction of single nerve cells in other parts of the cerebellar cortex. The latent time was more prolonged. Radiation damage finally resulted in the formation of a glial scar.

At x-ray doses below 4,000 r, only single nerve cells or cell groups were damaged, and the latent period was correspondingly lengthened. In such lesions, proliferation of glial elements and capillaries ultimately occurred, and hyalinosi developed in the walls of distended blood vessels. Since no evidence of vascular alterations was observed before development of nerve cell lesions, the visible radiation damage was interpreted as due to a direct effect of the ionizing radiation on the cellular elements of cerebellar tissue.

The acridine orange fluorescence technique was used to determine whether alterations occur in the structural organization of the DNA in pyknotic nuclei of irradiated granule cells and in the RNA content of Purkinje cells. Our results indicated that after irradiation DNA of pyknotic nuclei in the granular layer is not depolymerized to a noticeable degree. These pyknotic nuclei were more resistant than normal to treatment with depolymerizing and hydrolysing chemical agents and DNase. Without further experimentation it is difficult to say whether this increased resistance of irradiated, pyknotic nuclei is an immediate and specific effect due to the action of ionizing radiation on the DNA or whether it is a nonspecific effect due to in-

creased density of the nuclear mass. Within the region damaged by irradiation, Purkinje cells in a state of regression showed remarkable decrease or loss of cytoplasmic RNA which was obviously secondary to cellular regression.

Acknowledgment

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Morphological Effect of Repeated Low Dosage and Single High Dosage Application of X-Irradiation to the Central Nervous System *

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This preliminary presentation deals with the effects of x-rays on the tissue of the central nervous system and the ensuing pathogenesis. Although we shall refer repeatedly to x-ray dosage, its relation to the time of manifestation of tissue changes, and the severity of these changes, it is not our intention to determine an exact time-dose relationship as this has been done by Berg and Lindgren (1958). We have tried to find a connection between the delayed x-ray lesions seen after moderate single or fractionated doses and the acute tissue necroses occurring within hours after a single application of massive doses of high intensity up to 80,000 r. It is now almost universally accepted that the delayed lesions originate from changes of the vessels. A breakdown of the hematoencephalic barrier has been considered significant since Mogilnitzky and Podljaschuk (1930) described the passage of trypan blue into repeatedly irradiated central nervous tissue. When we made our first investigations and experiments with dogs in 1932-1935 (Lyman *et al.*, 1933; Scholz, 1935), the histologic picture was dominated to such a degree by plasmatic transudations with and without erythrodiapedesis into the central nervous tissue, it was difficult to admit any direct influence of the x-rays on nervous tissue constituents. While it was true that the x-ray doses applied through different portals to the skull ranged from 4,400 r to about 8,000 r, the intensity of irradiation was so low that to apply 12 skin erythema doses corresponding to about 6,600 r, a radiation time of 6 hours was required. Since that time, x-ray technique and the accurate measurement of dosage has improved so considerably that it seemed worthwhile to re-examine our former results with new experimental material and methods.

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Accordingly, since 1956, we have studied the neuropathology of x-ray lesions produced by irradiation of the spinal cord of rabbits.¹ A 3 x 6 cm field on the back, corresponding to the upper thoracic segments of the cord, was irradiated. The technical conditions were: 180–200 kv, 18 mA, 0.95–1.12. Cu half value layer, filter 0.5 Cu, 60 r per min, focus—skin distance 50 cm, and pendulum angle 70° on both sides. The average dose was 250 r daily; total doses were 3,000 to 11,000 r given over 12 to 40 days. All animals acquired paralysis of the hind legs and loss of sphincter function within 4 to 33 weeks after the beginning of irradiation. This investigation was published in *Psychiatria et Neurologia Japonica*, 1959, and only the important features will be mentioned here. The white matter of the spinal cord was much more affected than the gray substance and showed more or less circumscribed areas of disintegration following the radial distribution of the spinal vessels entering from the vasocorona along the whole periphery of the cord. Regressive changes of the small vessels with slight perivascular astrocytic reaction within the focal lesions suggest the transudation of a fluid histologically not demonstrable, causing swelling and disintegration of the myelin fibers. Only in a few cases did the gray substance participate in the changes. Here, plasmatic extravasation, partly with erythrodiapedesis, could be observed, followed by an astrocytic reaction and some regressive changes in some nerve cells. Although a reaction of the neuroglia was observed, it remained rather scanty, especially in the white matter. Regardless of the fact that some lesions were 3 to 4 weeks old, sudanophilic material was not observed. In both white and gray matter, plasmatic disintegration of the walls of larger vessels was encountered occasionally, but plasmatic exudation was seen only in the gray substance. Thus the morphologic feature pathogenetically pointed to the primacy of processes of transudation, exudation, and erythrodiapedesis, that is, to a breakdown of the barrier function of the spinal vessels. This concept was further supported by intravital injection of trypan blue (Fig. 1). There are blue stained foci in the white matter



FIG. 1. Delayed lesion of the spinal cord of a rabbit after fractionated x-irradiation, intravitaly stained with trypan blue. Beneath some foci of disintegration in the white matter, the whole gray substance although demonstrating no disintegration, has taken the blue color.

¹ We received the material from the radiologist Dr. Breit, who was interested in questions of tolerance dose, dose fractioning, and concentric application by a pendulum x-ray machine.

caused by destruction of tissue. The remarkable fact is the sharply limited blue staining of the whole gray matter, which demonstrates no disintegration at all.

These investigations have been continued in 21 additional adult rabbits. Equivalent changes in the spinal cord could be produced using the same technique with fractionated doses totaling 3,000 r. No exact relation between the amount of applied r and the length of the interval can be stated, but on the average the intervals became longer with diminution of the total dose. The minimum single dose sufficient to produce severe changes in the cord after 5 months was 2,000 r. Some figures demonstrating the pathologic findings in this material show again the important role which the breakdown of the blood-brain barrier plays in the pathogenesis of changes in the nervous tissue. Thus we see in Fig. 2 that the numerous focal changes in the white matter follow the distribution of the small arteries entering the cord from the vasocorona. In this case, a single dose of 3,500 r was followed by paralysis 15 weeks later. Two small vessels in the white matter (Fig. 3)



FIG. 2. Delayed lesion of the spinal cord of a rabbit, 3 $\frac{3}{4}$ months after a local single x-ray dose of 3,500 r. Numerous areas of disintegration in the white matter follow the radial direction of the entering vessels of the vasocorona. Myelin stain (Schröder).

show a swelling and disintegration of their walls caused by infiltration with a plasmatic material which is stained yellow in van Gieson preparations.

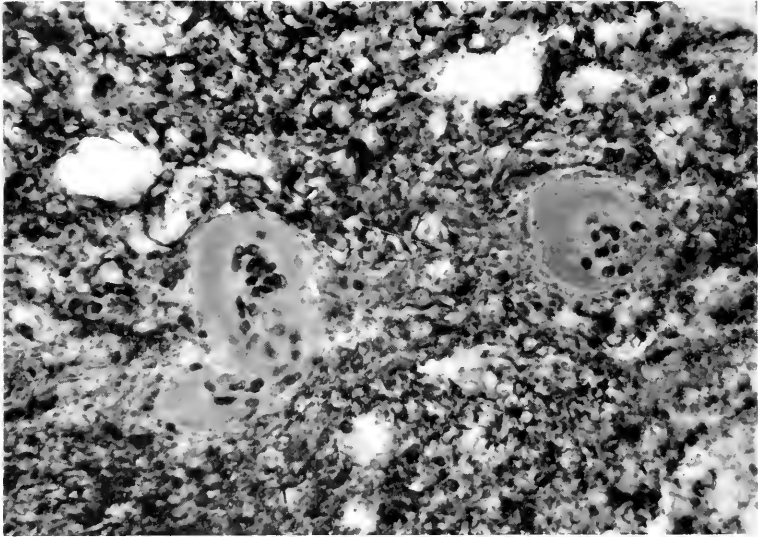


FIG. 3. Plasmatic swelling and disorganization of the walls of two small vessels in the white matter, still without effect on the neighboring tissue, 5 months after a single dose of 2,000 r. van Gieson.

No change of the neighboring myelin fibers can be demonstrated. These changes occurred 5 months after the application of a single x-ray dose of 2,000 r. Within an area of spongy dissolution of the white matter in the same case, plasmatic material with red blood corpuscles spreads out from such vessels (Fig. 4). These conditions are demonstrated more distinctly with the Mallory method in Fig. 5. The fibrinoid disorganization of the vessel walls is here followed by erythrodiapedesis, hemorrhages, and fibrin-containing fluid in the gray matter. In all places where the plasmatic fluid spreads into the tissue, oxygen diffusion is inhibited and the cellular elements become necrotic.

On the whole, the findings in this second series confirm and complete the results of our first investigation on x-ray changes in the spinal cord of adult rabbits. The pathogenic mechanism seems the same as in the brains of dogs, observed more than 20 years ago. We have not seen any proof for a primary effect of ionizing radiation on the neuronal constituents of the tissue. Whenever a damage of neuronal constituents could be observed, it was accompanied and often preceded by changes in the barrier function. The focal lesions of the white matter are distributed irregularly within the field of x-irradiation of the cord. Some sections are filled with areas of demyelination, whereas at other levels not a single one can be seen. This may account



FIG. 4. Plasmatic infiltration and erythrodiapedesis into the tissue in an area of disintegration of the white matter from the same case as FIG. 3. van Gieson.

for the fact that in routine investigations of single cases, no morphologic changes may be observed, although the animals are paralyzed.

In the second series, transformation of the tissue debris into sudanophilic material could not be observed, although in many cases the clinical symptoms indicated that morphologic changes were 3 to 4 weeks old.

In addition to these experiments on the spinal cord of rabbits, the brains of approximately 100 Syrian hamsters were irradiated to examine the effect of a high and intensive single x-ray dose using a special Siemens x-ray machine with a beryllium tube. The animals were fixed on a small table (Fig. 6), and the whole body was covered with a half tube of lead, except for the head which was held in position by two metal clips. A small brass cylinder, 1 cm in diameter, was used for local application of x-rays and



FIG. 5. The same case as FIG. 3. Fibrinoid (plasmatic) disorganization of greatly enlarged angioectatic vessels with erythrodiapedesis and transudation of fibrin-containing fluid into the tissue of the posterior horn producing a necrotizing effect. Masson stain.

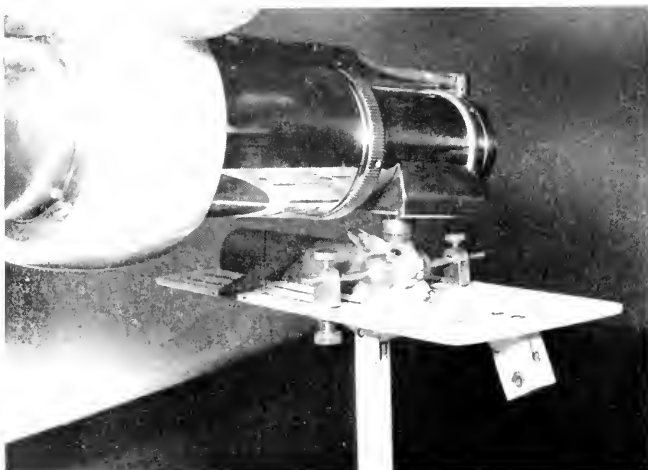


FIG. 6. (see text).

positioned on the mid-dorsal skull. Technical conditions included: 40 kv, 25 ma, filter 0.3 mm Al, and focus-skin distance 5.5 cm. To determine more exactly the actual intracerebral radiation, the x-ray dosage was measured by a Siemens dosimeter at a level to include skin, bone, and 1 mm of brain substance, which means that within a distance of 2.5 mm about 50% of the surface dose was measured. Further values were obtained by using 1 mm plates of a phantom material, Cellon, which has the same absorption value as brain tissue. This procedure indicated a diminution of the dose in different regions (Fig. 7). Effective x-radiation values of 1,000 to 80,000 r were administered to the cerebral cortex with application times of 28 sec to 37 min 34 sec, respectively. Doses of 20,000 r and more were badly tolerated by the animals; generally they died spontaneously 2 or 3 days later. Young animals seemed to be more sensitive than older ones, and on the average showed more severe morphologic changes. With the application of 30,000 r, sharply limited zones of total necroses, sometimes containing numerous small hemorrhages, could be produced and were fully developed after 67 to 68

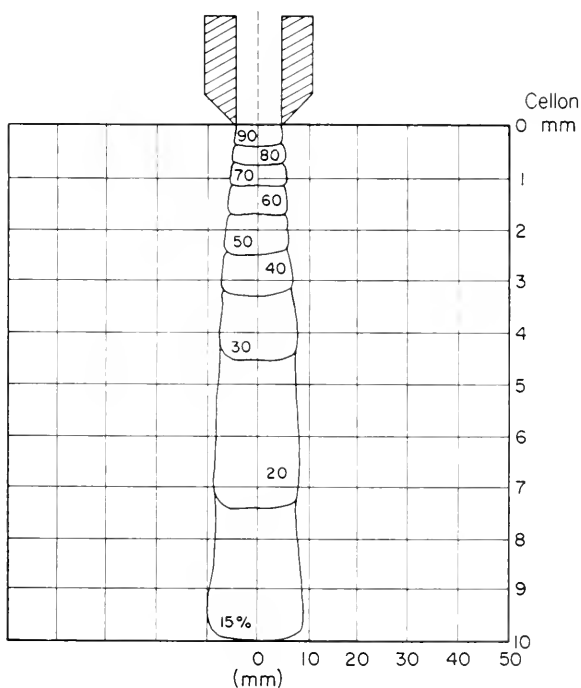


FIG. 7. Equivalent doses for special tube used with soft x-ray radiation. The diagram demonstrates the diminution of the x-ray dose at different levels in the tissue. The figures represent the effective dose in percentages of the surface dose. The abscissa demonstrates the diffusion of the x-ray beam within the tissue.

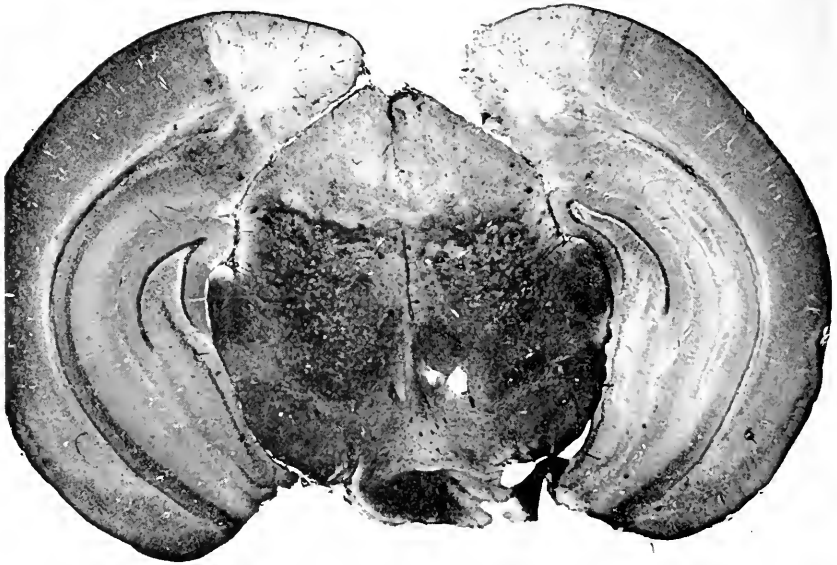


FIG. 8. Sharply limited total radionecrosis of semicircular shape in the brain of a Syrian hamster, following the application of 30,000 r after 67 hours of survival. Numerous diapedetic hemorrhages, some of them far from the necrotic zone in the thalamus and midbrain. H. and E.

hours (Fig. 8). It was not possible to identify the different types of cells in the necrotic zone, which included the dorsal part of the thalamus. The perikaryon had disappeared and all nuclei, including numerous polymorphonuclear leucocytes, were in a state of pyknosis or rhexis (Fig. 9). A fairly large number of small hemorrhages could be observed at some distance from the necrotic zone. No progressive interstitial reaction of the glial or mesenchymal tissue was seen.

It does not seem possible to determine the pathogenesis of these necroses, which have been designated as anemic by Russel *et al.* (1949) because of their pale appearance. We did not find occlusions of pial vessels or larger arteries, and certainly the necrosis does not involve a particular region of arterial irrigation. Rather, it is restricted to just the irradiated field with a semicircular penetration into the depths of the cerebral tissue. To exclude an ischemic condition as the cause of the necrosis, India ink was injected into the left ventricle of the heart of living anesthetized animals. The freely circulating blood carried the indicator substance throughout the capillary bed of the area that had received 20,000 r 50 hours before (Fig. 10). In some places, where erythrodiapedesis had occurred, the India ink pene-

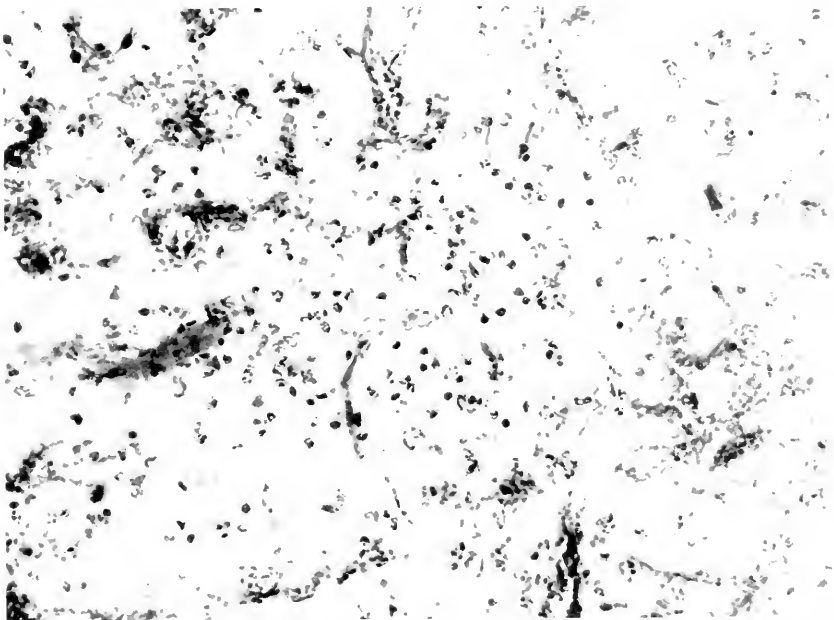


FIG. 9. Multiple hemorrhages, pyknosis, and rhexis of tissue cells and of emigrated polymorphonuclear leucocytes in a necrotic region of the same type as in FIG. 8 and produced by the same x-ray dose, H. and E.



FIG. 10. Nearly complete representation of the capillary bed of the cerebral cortex by India ink, 50 hours after local irradiation of a Syrian hamster with 20,000 r.

trated into the tissue. This region was completely stained blue with trypan blue injected subcutaneously shortly after the irradiation.

To clarify these observations, the experimental technique was modified in two different ways: (1) we diminished the x-ray dose to 1,000 r, and (2) we shortened the survival time to 1 hour, using x-ray doses large enough to produce complete tissue necroses. With the first of these methods, the size of the necrotic area decreased, so that with 5,000 r only a small tangential zone of the cortex including the first and second layer was affected. These lesions developed within about 8 days. The nerve cells and glia cells lost their cytoplasm, and their nuclei were shrunken. A few polymorphonuclear leucocytes were scattered throughout the necrotic tissue. This small necrotic zone was surrounded by a broad region of spongy tissue containing numerous tiny hemorrhages. Within 6 days, an application of 10,000 r produced a larger zone of necrosis extending through the whole cortex (Fig. 11). In these cases the first reactive processes had begun, and several fat granular cells and progressive glial cells were found at the borders of the necrotic tissue, especially near the pia. However, within the center of the necrosis nothing seemed viable. Here again, the zone of hemorrhages was considerably more extensive than the necrotic area. The necrotic zone,

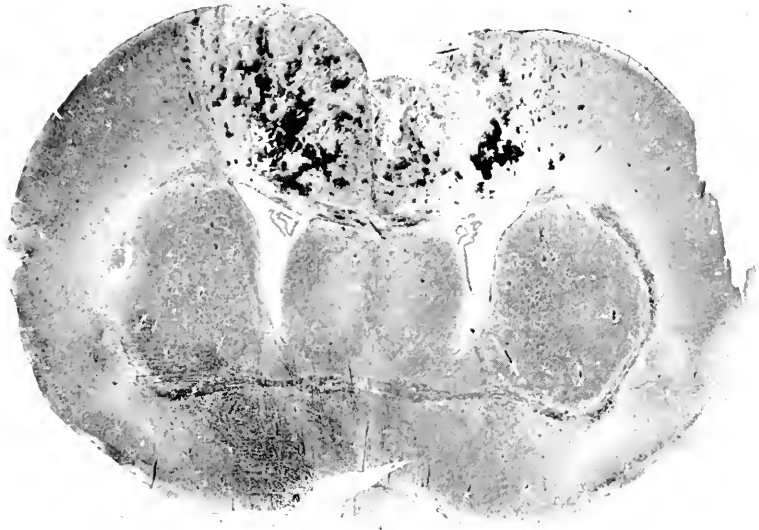


FIG. 11. Large, sharply bordered areas of acute radionecrosis involving the whole cortex and corpus callosum and containing numerous, partly confluent, diapedetic hemorrhages; 10,000 r with 6 days survival. Azan stain.



FIG. 12. Intravital trypan blue staining of the necrotic region of the same brain as in FIG. 11.

even when small, was surrounded by a broad shell of spongy tissue where the cellular elements showed only minor changes. To demonstrate the barrier function, a series of animals received a subcutaneous injection of 1 cc of 1% trypan blue solution from 1 to 6 hours after irradiation. As is well known, the brain remains unstained under normal conditions. With disintegration of tissue and destruction of vessels, the dye may enter the tissue. Figure 11 demonstrates such a necrotic zone which had developed within 6 days after x-irradiation with 10,000 r and which involves the whole thickness of the cerebral cortex. From the numerous hemorrhages in the necrotic zone, trypan blue spread diffusely all through the destroyed tissue (Fig. 12). These cases do not serve to illustrate a disrupted barrier function unless the blue staining surpasses the necrosis and extends to the surrounding spongy area where only minor cellular changes are seen. This could be observed in some cases, but it is difficult to demonstrate.

By shortening the survival time of the animals, we attempted to observe the earliest stages of tissue necrosis. A standard x-ray dose of 20,000 r was applied within 10 min 36 sec. This dose proved sufficient to produce clear necrosis within 24 hours. Surprisingly, we could produce lesions of similar size with a considerable diminution of the x-ray dose. After 24 to 26 hours, a large semicircular zone of obvious necrosis covering the irradiated field could be seen extending from the dorsal surface of the cerebral cortex to the corpus callosum. With shorter survival times and in older animals, the depth of the necrotic area flattened (Fig. 13). Here again, a sponginess of



FIG. 13. Earlier stage of radionecrosis in the brain of a Syrian hamster, 24 hours after the local application of 20,000 r. The tangentially situated zone of clear necrosis has a spongy character and is demarcated by a strip of even more pronounced sponginess. Azan stain.

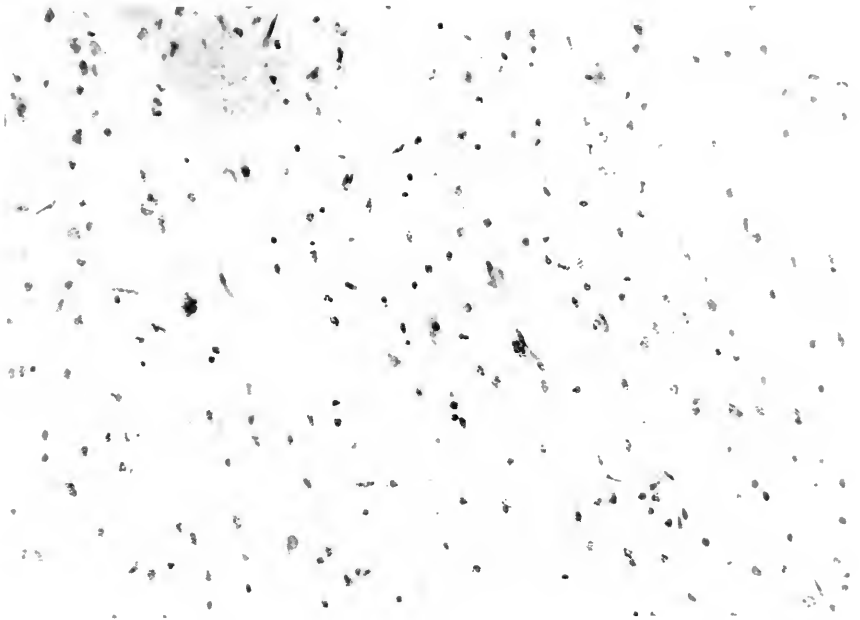


FIG. 14. The same case as in FIG. 13. In the necrotic zone, the cellular constituents can scarcely be differentiated, with only a few exceptions. The plasma has become unstainable; the nuclei are pyknotic. Some emigrated polymorphonuclear leucocytes are also seen. Gallocyanin.

the bordering zone with granular breakdown of the astrocytic processes could be observed in Cajal preparations. In the necrotic zone, it was difficult to identify the different types of cells (Fig. 14). Only a few nerve cells could still be recognized by their acidophilic cytoplasm. In all other cells, the perikaryon became unstainable. Almost all cells, including glial elements, demonstrated pyknotic nuclei. Some polymorphonuclear leucocytes could also be recognized. With further shortening of survival time to 7 hours, only two small zones of spongy loosening of the tissue were seen, situated almost symmetrically in both hemispheres (Fig. 15). They were rather sharply

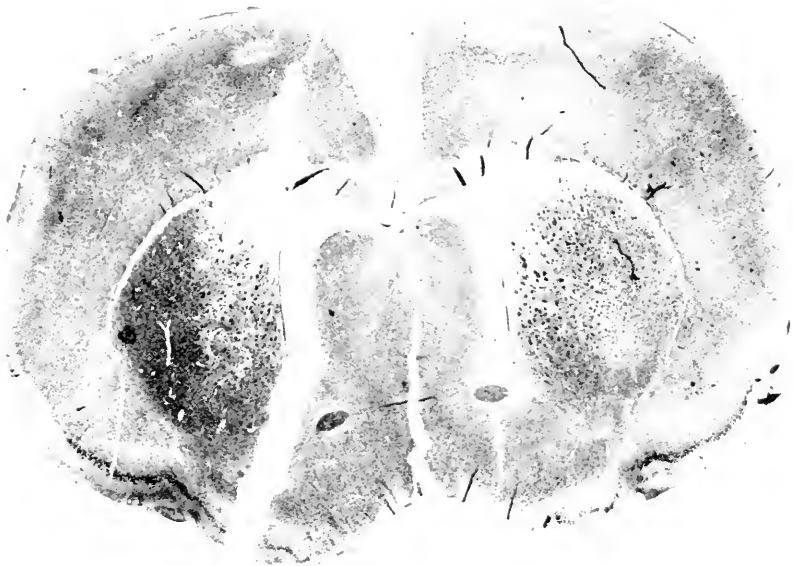


FIG. 15. Two sharply limited small foci of spongy loosening, symmetrically and rather superficially located in the cortex. This is an early stage, 7 hours after local application of 20,000 r. H. and E.

limited by intact nervous tissue. The cellular elements in the spongy zone exhibited only minor changes, such as shrinkage with a clear nuclear structure (Fig. 16). Sometimes, however, groups of nerve cells showed signs of dissolution, including vacuolization of the cell cytoplasm. After local irradiation with 45,000 r and a survival time of 6 hours, small areas of sponginess of tissue of the same type and size reaching from the surface to the 3rd cortical layer could be observed (Fig. 17). The nerve cells exhibited only minor changes, such as diminished affinity for galocyanin, and leucocytes were absent. With low power, this deviation can be more clearly seen as a

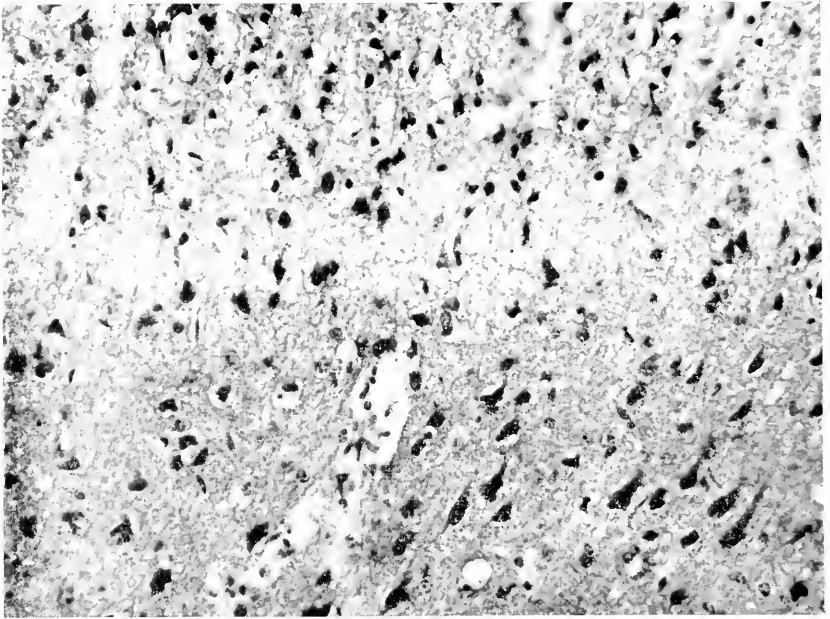


FIG. 16. The same preparation as in Fig. 15. Bordering zone of the spongy area, with tissue cells showing only minor changes. H. and E.

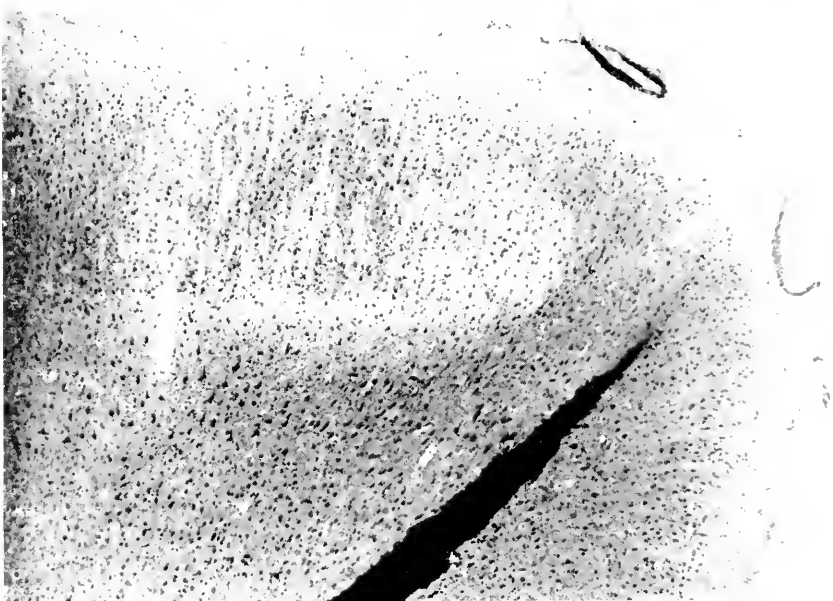


FIG. 17. Symmetrically and radially arranged sponginess of tissue in both hemispheres following local x-irradiation of 45,000 r after 6 hours. H. and E. method. In this zone, the cells appear less stained.

slight pallor in the cortex (Fig. 18). High power demonstrates disappearance of Nissl bodies in the large pyramidal cells so that the perikaryon is generally pale and diffusely stained, whereas the nuclei of the smaller nerve cells contain rather coarse chromatin particles (Fig. 19). It is extremely difficult to judge the whole tissue situation from deviations in appearance of the cortical nerve cells alone. The significance of such deviations seems to require a special cell study as performed by Krogh and Bergeder (1957) using the gallocyanin stain of Einarson and by Schümmelfeder (1957) in his

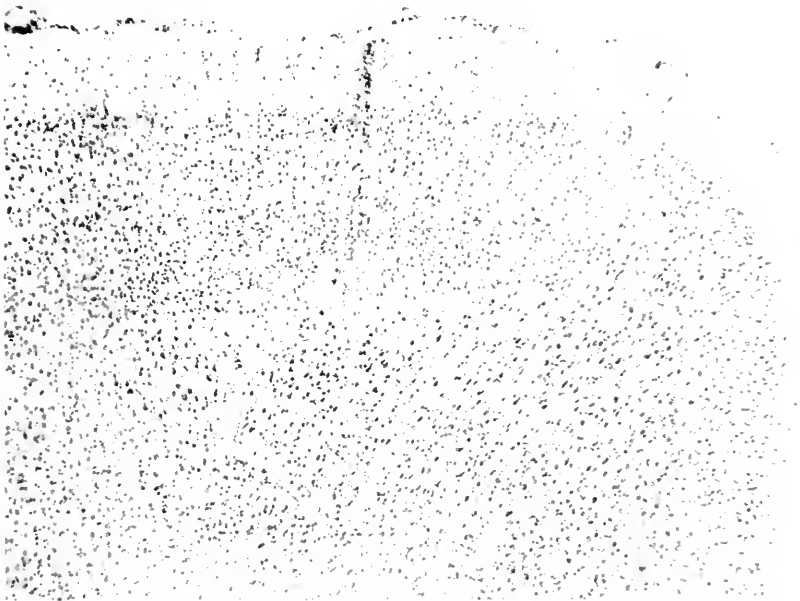


FIG. 18. Equivalent of the spongy region in Fig. 17, stained with the gallocyanin method. In this zone, the cells appear less stained.

histochemical investigations with fluorescent acridin-orange on the nerve cells of the cerebellar cortex. With regard to the different types of nerve cell changes after irradiation of 5,000 to 20,000 r, they made similar observations on cortical nerve cells, and they considered this to be an expression of cell necrosis. Certainly, it requires a high level of experience to decide from the appearance of the cell alone that cell shrinking with dark staining of the perikaryon and a pyknotic nucleus is not reversible and must inevitably lead to necrosis. In cresyl violet and gallocyanin preparations, we have seen such cells distributed over wide regions including the cortex of control animals which had never been irradiated. Their significance is not

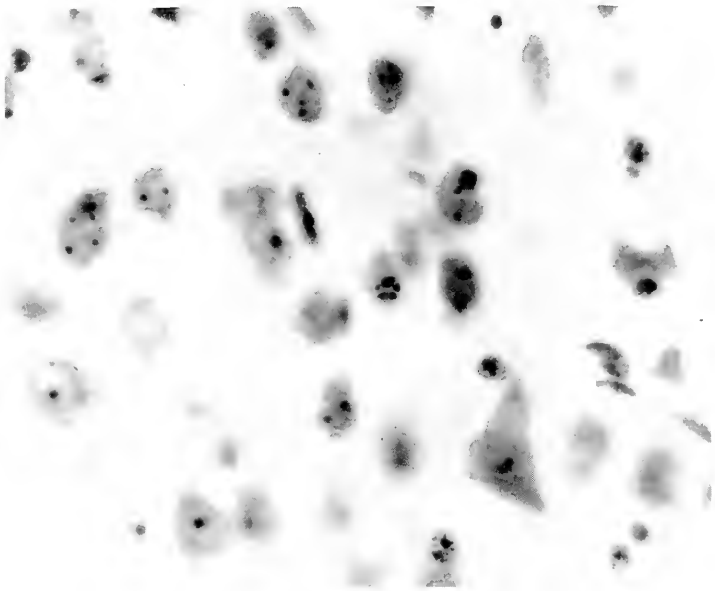


FIG. 19. Detail of FIG. 18. The large nerve cells are of normal shape; their perikaryon is lightly stained, and the nucleus is not strikingly altered. The nuclei of the smaller nerve cells contain rather coarse chromatin particles.

known, but the experiments of Scharrer (1933) and of Cammermeyer (1960) suggest that they can be produced artificially by simple mechanical pressure on the cortex. A high degree of swelling and vacuolization of the cytoplasm with loss of ribonucleic acids and the occurrence of coarse chromatin particles within a nonpyknotic nucleus seem to constitute evidence of necrosis. But again, the nerve cell nuclei of rodents may normally have rather coarse chromatin particles. The third variety of nerve cell alteration referred to by these authors seems to resemble the so-called ischemic type of nerve cell change. Certainly, as soon as a breakdown of the nucleus is established, death of the cell must be accepted if postmortal processes can be excluded. We have observed the cytoplasm of such cells to be acidophilic, staining with eosin and even with azocarmine and acid fuchsin, but we have had no opportunity to make comparisons with the results obtained by Schümmelfeder (1957) with acridine-orange.

Krogh and Bergeder (1957) did not state whether the pyknosis of the granular cells is due to a direct influence of irradiation, or if edema of the granular layer may play an intermediary role. Schümmelfeder (1957) favors a direct influence of ionizing rays. However, it is not easily understood why the same cause in the same quantity elicits a shrinking one time and a high

grade swelling of the nerve cells another. In approaching this question, it seems necessary to consider the condition of the whole tissue concerned and not solely the nerve cells, since they are only a part of the tissue, and a necrosis of nerve cells alone does not cause tissue disintegration as seen, for example, in anoxic selective neuronal necrosis. Only if the oligoglia and astrocytic glia are also destroyed, does the continuity of the tissue break down. As we have seen in Cajal preparations, astroglia processes show a granular decay rather quickly, long before regressive changes in the vessel walls are demonstrable. Schümmelfeder (1957) also mentions disappearance of ribonucleic acid in glia cells. This would indicate a much more severe lesion of the nervous tissue; however, definite tissue disintegration needs a certain time to become manifest with clear structural changes. In initial stages, when necrosis is not yet clearly apparent, the most reliable histologic phenomenon seems to be the immigration of single leucocytes into the tissue and the beginning of erythrodiapedesis. We have tried to study the earliest stages of cortical necrosis in regard to the function of the hematoencephalic barrier. We did not succeed in staining intravitaly with trypan blue during initial stages of spongy tissue transformation (Figs. 15-19), when seemingly only minor alterations such as moderate swelling and dissolution of the Nissl substance without significant changes of the nuclei were seen. A positive result was attainable, however, before distinct symptoms of tissue disintegration became evident. Thus, the blue staining with trypan blue was complete as soon as single diapedetic hemorrhages of small size in the zone of irradiation could be observed or single polymorphonuclear leucocytes had immigrated into the nervous tissue. This is demonstrated by a section stained with the azocarmine Mallory method of Heidenhain (Fig. 20) and, for comparison, by a macrophoto of the blue stained field on the surface of the brain (Fig. 21).

Discussion

We must emphasize that the delayed radionecroses of the spinal cord are not significantly different from those in the brain. We observe the same phenomena of altered vascular permeability which in the white matter results chiefly in transudation and in the gray matter chiefly in plasmatic infiltration, often with erythrodiapedesis. It now seems established beyond doubt that these processes are primary to all other destruction of nervous tissue in delayed radionecrosis, although in many cases structural changes of the vessels are not evident. The same pathogenic mechanism in delayed radionecrosis is valid in the human brain, as has been demonstrated by Fischer and Holfelder (1933), Markiewicz (1935), Scholz and Hsü (1938), Zeman (1949, 1955) and others. In a necrotic zone, even after many years

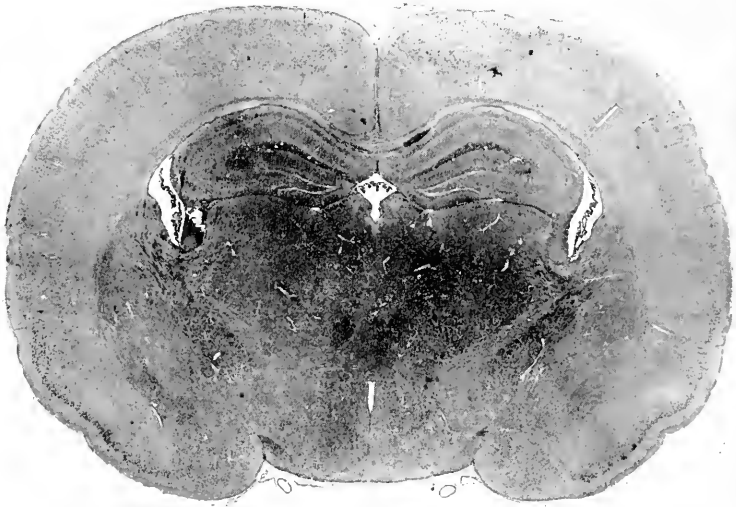


FIG. 20. Early and incomplete radionecrosis, 20 hours after local x-irradiation with 20,000 r. Only small single hemorrhages and slight diminution of stainability of the cortical tissue at both sides of the median fissure are visible.



FIG. 21. Intravital staining of the irradiated zone of the brain from which FIG. 20 is taken.

the plasmatic material can be found as an amyloid-like substance, resistant to absorption and behaving like a foreign body. This substance has not been demonstrated in large amounts in the central nervous system of experimental animals. Whereas most investigators, including in recent years Berg and Lindgren (1958), and Zeman (1955), acknowledge this pathogenic mechanism of the delayed radiolesions, it is much more difficult to explain the pathogenesis of the acute radionecrosis occurring within hours following the single application of extremely large doses of ionizing radiation of high intensity. We did not see convincing morphologic changes either with 80,000 r after $1\frac{3}{4}$ hours or with 45,000 r after $1\frac{1}{2}$ hours; however, necroses were fully developed with 30,000 r after 67 hours. They cover exactly the field of irradiation, are apparently not related to regions of arterial irrigation, and do not depend on vascular occlusions. As to the restriction of the lesions to the field of irradiation and the morphologic appearance of the nerve cell changes, our results are largely in accordance with the observations of Krogh and Bergeder (1957) and of Schümmelfeder (1957), though there was some difference in the application and measurement of the x-irradiation. It is true that fully developed acute radionecroses closely resemble anemic infarctions, but we think that such a designation, used by Russel *et al.* (1949), is not justified, since during the development of the necrosis, the free passage through the capillary bed has been made evident by India ink. In these cases, we find hemorrhages not only in the region of necrosis but also at some distance from the necrotic zone, invading the unaltered nervous tissue which was exposed to a lower intensity of irradiation and indicating a disturbance in the permeability of the blood vessels. Usually, the centers of the necroses are surrounded by a broad shell of spongy tissue which raises the question of whether we deal simply with a demarcation zone as seen around every more or less complete necrosis. However, the initial stages of such necroses produced by an irradiation of 20,000 and 45,000 r after 7 and 6 hours, respectively, consist only of such spongy loosened tissue, suggesting a local edema and demonstrating no distinct signs of cellular necrosis. Efforts to stain these earliest lesions intravitaly with trypan blue were not successful, probably because the disorder of the blood-brain barrier is still incomplete in this stage. But already in the next stage, when only minor changes of the cellular constituents of the tissue manifest themselves and only a few vessels show beginning diapedesis, we find a distinct blue coloration, pointing to the important role of permeability disorders in the pathogenesis of the acute radionecroses also. As soon as distinct necrosis is present, the trypan blue staining spreads far beyond the necrotic center throughout the whole spongy zone. It may therefore be reasonable to consider the sponginess of the tissue as well as the multiple hemorrhages within and out of the necrotic center as being due to a disturbance of permeability of the blood-brain barrier.

primarily caused by irradiation. Krogh and Bergeder (1957), who produced cerebellar lesions by a high x-ray dosage, did not decide whether the breakdown of the granular layer is secondary to edema or is a primary lesion. In experiments with Co^{60} , Vogel *et al.* (1958) produced a reversible pyknosis of the same granular cells. Since we know that the granular layer of the cerebellar cortex is rather sensitive to edema, we must consider the possibility that it is the radiation-induced edema that produces the granular cell changes. However, this does not mean that all tissue changes should be considered secondary to the edema-like loosening of the tissue. In the cerebral cortex, nerve cells remain resistant to a simple edema for a long time. Moreover, we failed to find in acute radionecroses the dangerous plasmatic infiltration of tissue that can inhibit oxygen diffusion. As all cellular elements demonstrate an early and rapid structural breakdown, the assumption may be justified that x-irradiation of high dosage and intensity may cause a coordinated breakdown of the hematoencephalic barrier and a primary destruction of all other tissue constituents as well. It seems that in delayed lesions, the latent period becomes progressively shorter with an increase of x-ray dose and intensity, and the hematoencephalic barrier, in common with other tissue constituents, is at last simultaneously affected by the increased ionizing radiation.

Conclusions

X-irradiation of the spinal cords of 36 rabbits produced results similar to the previously reported delayed x-ray lesions in the brains of dogs. The fractionated application of doses up to 11,000 r within 40 days and single doses of 2,000 r were followed by focal zones of disintegration, mainly situated in the white matter, but also affecting the gray substance. These changes occurred after latent periods of from 4 to 33 weeks. The fibrinoid disorganization of the vessel walls, erythrodiapedesis, and infiltration of plasmatic material into the central nervous tissue seem to be the primary lesions and demonstrate a breakdown of the hematoencephalic barrier. All other changes of the tissue, including demyelination and breakdown of cellular constituents, were preceded by permeability disturbance and may be considered secondary. In the brains of approximately 100 Syrian hamsters, acute radionecroses were produced by single applications of large x-ray doses of high intensity. In animals receiving 5,000 r, necroses were fully developed within 8 days; within 6 days after 10,000 r, and within 3 days after 45,000 r. Several animals receiving 20,000 r showed distinct disintegration of tissue in the field of irradiation after 24 hours, and initial stages in these cases could be detected after only 7 hours. The sharply limited semicircularly shaped lesions covered exactly the field of irradiation and decreased in depth with the diminution

of the x-ray dose and abbreviation of the survival time. In fully developed necroses, all cellular elements were broken down, and erythrodiapedesis from more or less numerous vessels occurred not only within, but also out of the necrotic zone. Initial stages demonstrated a spongy transformation of the nervous tissue with only minor changes of the cellular constituents, such as plasma and nuclear shrinkage. Here, erythrodiapedesis was still lacking. A disorder of the hematoencephalic barrier in this initially spongy territory could not be demonstrated by intravital trypan blue staining, while a blue coloration of a large area including the surrounding region of spongy loosening became visible before a distinct disintegration of tissue, indicated by signs of cell necrosis, were demonstrable. Solitary small diapedetic hemorrhages and the immigration of single leucocytes seem to indicate a degree of permeability disorder sufficient to allow an intravital staining with trypan blue. This behavior and the appearance of more or less small hemorrhages at the borders of, and sometimes far from, the necrotic foci point to the significance of an early disorder of the blood-brain barrier. Since nerve cells have been shown to be rather resistant within zones of edematous loosening of tissue, and astrocytes even may become progressive, the whole process of necrosis cannot be considered as solely secondary to the permeability disorder. From the morphologic facts, it seems justified to admit that in acute radionecrosis from large x-ray doses of high intensity a direct effect on the nervous and astrocytic tissue constituents may occur simultaneously with vascular damage. Processes of transformation, resorption, and organization develop slowly and are established regularly only at the borders of the necrotic zones.

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A Demyelinating or Malacic Myelopathy and Myodegeneration— Delayed Effect of Localized X-irradiation In Experimental Rats and Monkeys

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Introduction

For many years the central nervous system was considered highly resistant to radiation damage—and statements to this effect occasionally still appear. It is manifest that there must be some qualification by reference to the part of the system exposed, the conditions and dosimetry of irradiation, and the species and age of the animals used. The earlier experimental irradiation work on the normal nervous system was reviewed by Warren (1943) and Hicks (1952). We are concerned here with our initial experimental irradiation studies on the spinal cord of rats and with some observations on experimental monkeys.

In man the hazard attached to x-irradiation of the brain or spinal cord, whether by deliberate design for radiotherapy or unavoidably when extraneural sites must be exposed, is well established. Late or delayed irradiation effects on the nervous system are different from acute massive radionecrosis which follows extremely high doses. The problems associated with the two types of damage are multiple and complex, and the literature was reviewed by Zeman (1955) and by Zollinger (1960). Many original papers on human cases have been perused, e.g., Lyman *et al.* (1933), Stevenson and Eckhart (1945), Pennybacker and Russell (1948), Greenfield and Stark (1948), Boden (1948), Friedman (1954), Itabashi *et al.* (1957), and Dynes and Smedal (1960). It is not necessary to deal with these contributions individually, but we can reiterate the pathologic problems to be faced. The number of reported cases in the literature is an index neither to the incidence of delayed irradiation lesions in the spinal cord, nor to the importance of the hazard, as is evident from discussions with neurologists, neuropathol-

ogists and radiotherapeutists. As Itabashi *et al.* (1957) pointed out, when a neoplasm has been the target for irradiation, neurologic signs which might appear later have usually been attributed either to metastasis or extension of the primary lesion, especially when there has been some apparent improvement. Further, many human cases may not be followed up after x-irradiation of the spine: in others, autopsies may not be possible when death occurs years later, or it may not be possible to examine the spinal cord at autopsy. Some papers on delayed irradiation myelopathy are based largely on clinical data, and the lesions have not been comprehensively studied. Dynes and Smedal's series included 10 therapy cases, and Friedman (1954) estimated that the incidence of delayed neurologic damage was 10% in 100 patients with testicular carcinoma whose spinal cord had received 5,000 rads or more. In all cases, subsequent to irradiation, there is a latent period ranging from many months to many years, during which there are no neurologic signs or symptoms due to irradiation. The clinical onset can be abrupt or insidious, with a variable neurologic syndrome leading to paraplegia and inevitably to death, although some patients have lived for years with paraplegia (Dynes and Smedal, 1960).

Lesions in the spinal cord in such cases have been variously reported as radionecrosis, postirradiation myelitis, or myelopathy; but whatever the designation, the damage can be devastating and appears no different from that described by us (cf. Itabashi *et al.*, 1957, one of the few papers describing spinal cord damage in man). In the delayed postirradiation process, it is not the most superficial layers of nervous tissue which are most radiosensitive, but the white matter in both brain and spinal cord. Neuroglial response has varied according to different reports, but it can be absent or negligible, and in some late lesions neuroglia must have been destroyed at a rate equal to the damage to white matter or rendered incapable of response. In both the human brain and cord, observers have commented on the difficulty of separating the damage done specifically by the irradiation on tissue already traumatized by another cause, such as a tumor.

The fundamental question, still not answered, is whether the damage is a direct effect of the radiation or an indirect one caused by a primary change in vascular walls which leads to interference of the normal blood supply, possibly then emanating from chronic hypoxia or ischemia. If it were a direct effect, then the question arises as to what happens to the neural tissue in the latent period before the eventual neurologic signs and symptoms. Years ago, Scholz (Scholz *et al.*, 1959) and Zeman (1955) observed in such late lesions the deposition in and around vessels of an "amyloid or paramyloid material," the nature of which has never been unequivocally established by histochemical methods. The thickening of vessel walls and subsequent constriction of lumina were thought to cause

hypoxia or dynamic alterations in the vascular walls resulting in increased capillary permeability and seeping through of plasma. This was considered sufficient to account for the lesions in areas of neural substance supplied by the affected vessels. These themes have been discussed repeatedly (see Zeman, 1955, and the most recent paper by Scholz *et al.*, 1959).

It is doubtful if experimental work has helped greatly, whether we are dealing with brain or spinal cord (Malamud *et al.*, 1954; Pennybacker and Russell, 1948; Warren, 1943; Davidoff *et al.*, 1938; Clemente and Holst, 1954; McLaurin *et al.*, 1955; Scholz *et al.*, 1959). Nor are the reasons hard to find. The acute necrosis produced by massive doses of x-rays does not help to explain the pathogenesis of the late delayed damage. After x-irradiation, experimentalists have noted the utterly unpredictable variations (a) of the reaction in different animals of the same species and age, some animals remaining unscathed under the same experimental conditions and dosimetry which causes marked late lesions in others, (b) in the latent period before neurologic signs develop, and (c) in the occurrence of the vascular changes, because thickening or deposition of "amyloid material" has not always been observed to be associated with the neural lesion.

An experimental approach with any animal species brings out that it is different than working with established transmissible neurotropic infections which can be reasonably controlled—the dose of causal agent related to a regular incubation period and a specific pathologic effect. The varying latent periods (Table I) set a formidable barrier in designing an experiment on a quantitative basis. Many animals may not develop neurologic signs or lesions under the same conditions of experiment, and to determine this with certainty, it might be necessary to wait much longer than 1 year. Little progress toward the solution of the problem may be expected until a lesion can be produced consistently in *small animals*, under controlled conditions related to dosimetry and exact localization of exposure. Monkeys and dogs can hardly be used in large numbers because of the expense involved, although delayed cerebral and spinal cord lesions have been produced in both species, and more meticulous clinical observations are possible with the larger animals. If this were possible, it might open the way for study of the pathogenesis of the myelopathy by examination of a large series of animals from a few days postirradiation up to a year or more. Of equal importance would be more accurate determination of the minimal pathologic dose to effect the spinal cord damage. Our observations were made with these facts in mind, and their importance may lie in the consistency of production of spinal lesions in rats, together with the continuing study of the pathogenesis.

The work was initiated by observations on rats exposed to upper body irradiation in which some clinical signs suggested the animals might be suffering from myelitic pain, evidenced by irritability, sensitivity to touch,

and changes in behavior. Some animals developed paralysis of the hind legs many months after irradiation. After identification of the demyelinating myelopathy in a few rats, a series of experiments were planned in which, by adequate shielding, only the vertebral column from about sixth cervical to second thoracic segment was irradiated. The first experiments were concerned with definition of the pathologic process and its topographic neuro-anatomic distribution.

Techniques

White female rats were housed two in a cage and given food and water *ad libitum* throughout the experiment. They were 3 to 6 months of age at irradiation. Before and after irradiation, they were weighed and examined daily.

Rats were anesthetized with intraperitoneal injections of sodium pentobarbital, 45 mg per kg of body weight. In the groups exposed to upper body irradiation, the animals were marked with dye at the xiphoid process and placed in 2-in. diameter lucite tubes. A ¼-in.-thick cylindrical lead shield was placed around the lower half of the tube at the dye mark, so that the upper body and head of the animal were exposed to the x-ray beam. In rats receiving thoracic exposure, a second lead shield was placed over the head end of the lucite tube at the manubrium. Animals indicated as spine exposed were completely surrounded by ¼ in. lead shields containing a small hole in a position so that only the spine from sixth cervical to second thoracic segment was exposed (Table I).

The irradiations were made with a General Electric 250 kV Maxitron x-ray machine. The radiation factors were: 250 kvp, 30 ma, 0.5 mm Cu filter, Aluminum parabolic filter for field uniformity, target to skin distance from 6.5 to 9 in., dose rate of 200 to 350 rad per minute measured in a tissue equivalent phantom, half value layer of 2.15 mm of copper. All animals (rats and monkeys) received 3500 rads.

Clinical Findings

After irradiation, a latent period varying from 5 to 9½ months occurred in rats. The first sign of untoward involvement of the spinal cord was incontinence of urine, which persisted to some degree in some rats for weeks before any motor weakness or incoordination of the hind legs developed. Thereafter, the rats became obviously unsteady on their hind feet and were ataxic, and the tail lost its tonicity. This syndrome progressed until the animal's hind quarters were completely immobilized, without the tail or limbs becoming completely flaccid. An affected animal could move around,

TABLE I

EXPERIMENTAL RATS

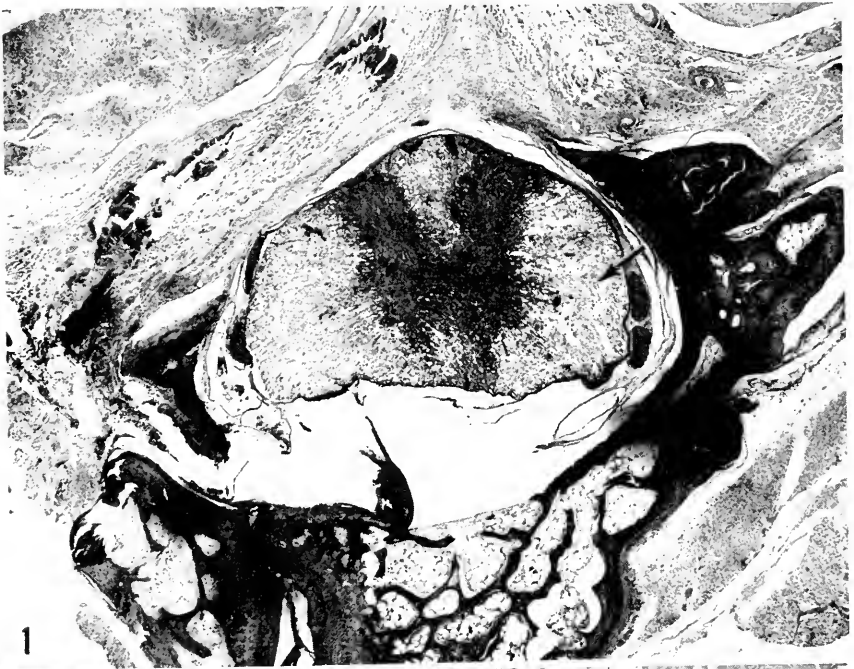
<i>Rat number</i>	<i>Irradiation (3500 rads)</i>	<i>Latent period (months)</i>	<i>Duration of clinical signs until sacrifice (days)</i>
1 (42/59)	thorax	5	3
2 (70/59)	thorax	6½	24
3 (96/59)	spine	7½	2
4 (9/60)	spine	5	4
5 (11/60)	spine	5	28
6 (17/60)	thorax	7	2
7 (18/60)	thorax	5½	2
8 (21/60)	spine	7	No clinical signs, but lesions in spinal cord
9 (27/60)	spine	6½	8
10 (67/60)	thorax	9	28
11 (81/60)	spine	9½	No clinical signs

pulling itself by its forelimbs with the hind limbs dragging helplessly behind. Complete loss of sphincter control of bladder and rectum resulted in the hind quarters becoming permanently wet and soiled. The "paralyzed" rats were allowed to survive for a few days up to 28 days before being sacrificed for neuropathologic study (Table I).

Pathologic Findings

As a routine survey for lesions and their extent and distribution, the spinal cord was fixed *in situ* in the vertebral column with its surrounding skeletal muscles. Subsequent to fixation and decalcification, the column was sliced transversely throughout its entire length in 1-2 mm pieces. The caudal surface of each slice was sectioned and stained with hematoxylin and eosin. Usually this amounted to 28 or more blocks (including usually 3 of the brain) being cut from each rat. The distribution of the lesions was plotted for each rat as in Fig. 5. We do not include any detailed reference to examination of the spinal cord of the many rats irradiated and killed at intervals from 1 day to 1 month, or to extensive histologic and histochemical work on embedded and frozen sections. The spinal cords from nonirradiated control rats were also studied.

As might be expected from the variability of the latent period and the time allowed between onset of signs and sacrifice, there was some variability in extent of damage to the spinal cord.



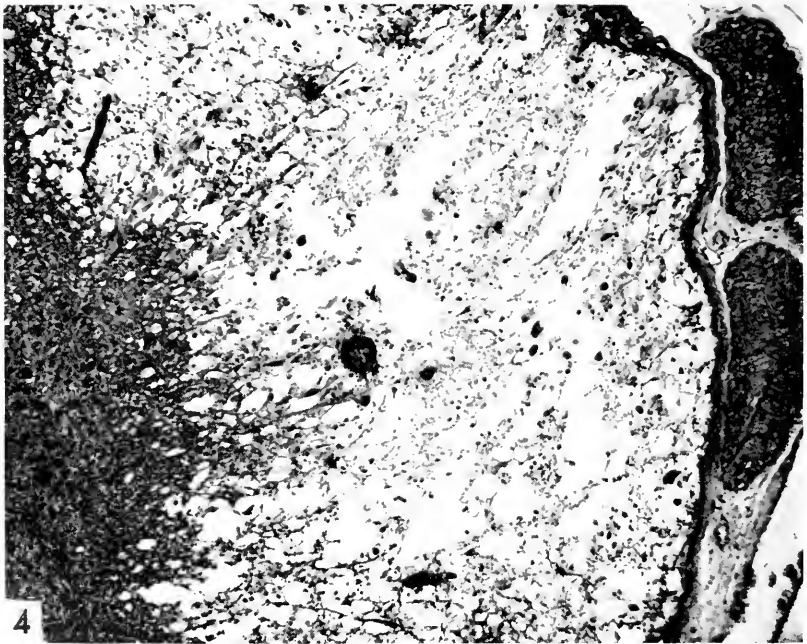
At the level in the cord where the lesion was seen in its fullest development (Figs. 1, 2, and 3), the process had developed into acute malacia terminating in severe liquefaction of the white matter. To some extent, the ventrolateral white columns were more damaged than the dorsal columns, for although dorsal and ventral areas were affected in some rats—e.g. Fig. 1—the dorsal columns were never selectively changed. In paraffin sections, the affected areas were spongy or reduced to holes and cystic spaces, bridging across which were scattered skeins of glial and reticular fibrils and minute vessels. As Figs. 1–3 show, the process constantly appeared more severe under the leptomeninx, gradually decreasing in intensity inwards. In most rats the gray matter was intact and never showed acute softening as in the white substance. In the spongy and cystic areas, there were no gitter cells, or so few as not to be noticeable, nor was there any astrocytic or fibrillary response—glial or reticular (Fig. 4). Parts of fragmented axis cylinders were scattered throughout the malacic focus, sometimes within what was presumed to be ballooned and liquefied myelin sheaths. There was no meningeal reaction, no hemorrhage, and the spinal nerves and ganglia in the same area were undamaged.

The lesion described corresponded in its regional distribution to the irradiated area of the body. For example, in animals in which the thorax had been irradiated, only the thoracic cord was affected. In others, where the spine was selectively irradiated from about sixth cervical to second thoracic segment, only that area showed the acute damage. Above and below the focus, secondary (Wallerian) degeneration was clearly evident, depicted in the paraffin sections by numerous "holes"—i.e., liquefied myelin sheaths and axis cylinders.

As an example of distribution of the lesions in a thorax-irradiated rat, Fig. 5 is a diagram of slices of the spinal cord cut at different levels. As sections are studied starting at the first cervical and proceeding in series to the sacral level, normal spinal cord and vertebral marrow is found until the irradiated area is reached. The lesion then may start on one side, then the other, and continues until it merges into an irregular funicular focus of

FIG. 1. Spinal cord, rat, spine irradiated. Latent period 6½ months, sacrificed after 8 days duration of neuromyolysis. Severe myelomalacia of all parts of white matter—dorsal and ventrolateral columns. Fatty marrow. Lesions in muscle (upper left) not too clear at this magnification (See Figs. 7A and 7B). Hematoxylin-eosin. About $\times 16$. (Area marked by arrow shown in Figure 4).

FIG. 2. Another case, rat (17/60), thorax irradiated. Latent period 7 months, duration of signs 2 days. See Fig. 5 for distribution of lesions. Malacia confined at this level to lateral column of white matter on one side. Fatty marrow. Hematoxylin-eosin. $\times 16$.



malacia. This is related to the well known irradiation damage which affects the marrow of the surrounding vertebrae.

Two rats that showed no clinical signs were killed at 7 and 9½ months after irradiation, and small lesions (Fig. 6) were found in the white matter at an early stage of development.

Severe myodegeneration and necrosis of the vertebral skeletal muscles in the same area as the spinal cord damage was almost a constant concomitant finding (Figs. 7A and 7B). Such changes have not been reported in experimental studies by others or in human cases of postirradiation myelopathy.

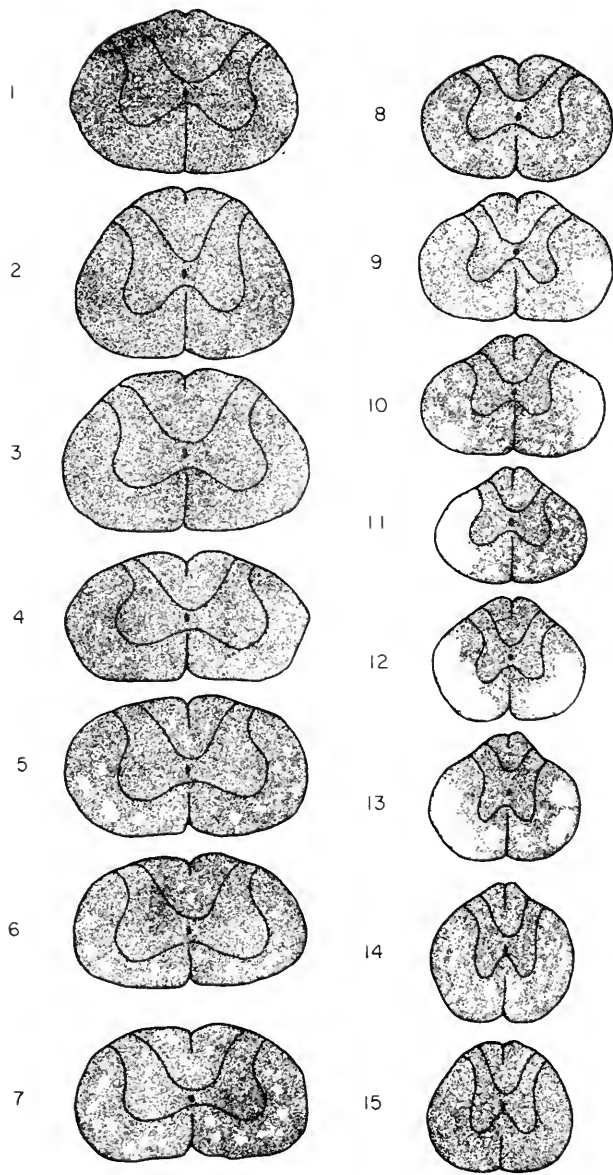
Experimental work on x-irradiated monkeys

Concurrently with the rat studies, comparable experiments were carried out on adult monkeys, and they will be reported upon separately (neuropathologic studies made in collaboration with Webb Haymaker, M.D., Armed Forces Institute of Pathology, Washington, D.C.). These can be briefly summarized. Five monkeys were irradiated by the same method and exposures (3500 rads) were restricted to the same area of the vertebral column as in the rats, i.e. to include the area from the sixth cervical to second thoracic segment of the spinal cord.

One monkey died from pneumonia 3 months 3 days after irradiation without showing neurologic signs, and no lesions were found in the irradiated part of the spinal cord. One monkey developed neurologic signs 5 months 13 days after the irradiation: motor weakness of the lower limbs started and progressed until there was complete paralysis without the legs or tail being flaccid. There was also loss of sphincter control. In spite of this, the monkey remained very agile and climbed around its cage and tree by use of the arms alone. The animal was sacrificed for study of the nervous system after a clinical course of 4 weeks. A malacic myelopathy similar to that in experimental rats was found in the irradiated part of the spinal cord. Two of the monkeys developed neurologic signs between 6 and 6½ months postirradiation. Another monkey developed signs 8 months 13 days after irradiation, but the paralytic course was thereafter very acute and the animal was killed when moribund, 4 days after the onset of clinical signs.

FIG. 3. Another rat. Latent period 5½ months, duration of clinical signs 2 days before sacrifice. At this level, the lesion is more pronounced on one side of ventrolateral column than the other. Hematoxylin-eosin $\times 16$.

FIG. 4. High magnification from area in Fig. 1 marked by arrow. Pia mater and nerve roots on right; gray matter, left. Almost complete tissue dissolution of white matter with no neuroglial response. Hematoxylin-eosin. $\times 100$.



5

FIG. 5. Same case as Fig. 2, showing distribution of lesions in the cord. Malacia represented by white areas. Secondary degeneration above and below shown by smaller scattered white blobs, from No. 5 to about No. 9. Lesion starts about No. 9 and develops to its maximum intensity about No. 12, fading out about section No. 14. Numbers 1-8 roughly represent the cervical cord and its segmentation; the remainder, thoracic cord.



FIG. 6. Spinal cord of rat spine irradiated. Killed after 7 months with no neurologic signs. About C 8-T 1. Early stage of small malacic focus about C 8-T 1 on the left. Fatty marrow. Hematoxylin-eosin. $\times 16$.

Discussion

A severe myelopathy, localized to the area of irradiation, can be produced with some consistency in rats, at a dose level within the therapeutic range used in man, and in which neuroparalytic accidents have occurred after deliberate or accidental exposure of the spine. In rats, there is also the parallel of an unpredictable latent period, sometimes many months before the onset of progressive neurologic signs. The extent and localization of the myelomalacia in the rats is thus responsible for the syndrome starting as motor weakness of the hind legs and progressing to ataxia and paralysis. The clinical picture is thus what is commonly called "posterior paralysis" in animals, which by itself means little. Neurologic examination of small laboratory animals is restricted to observations on a few cardinal objective signs, and may be no indication of what is ultimately found after neuropathologic studies. For example, in the course of this experimental work and by virtue of extensive histologic work, we found pituitary chromophobe adenoma in 3 rats, one spinal ependymoma, one sarcoma, and more recently

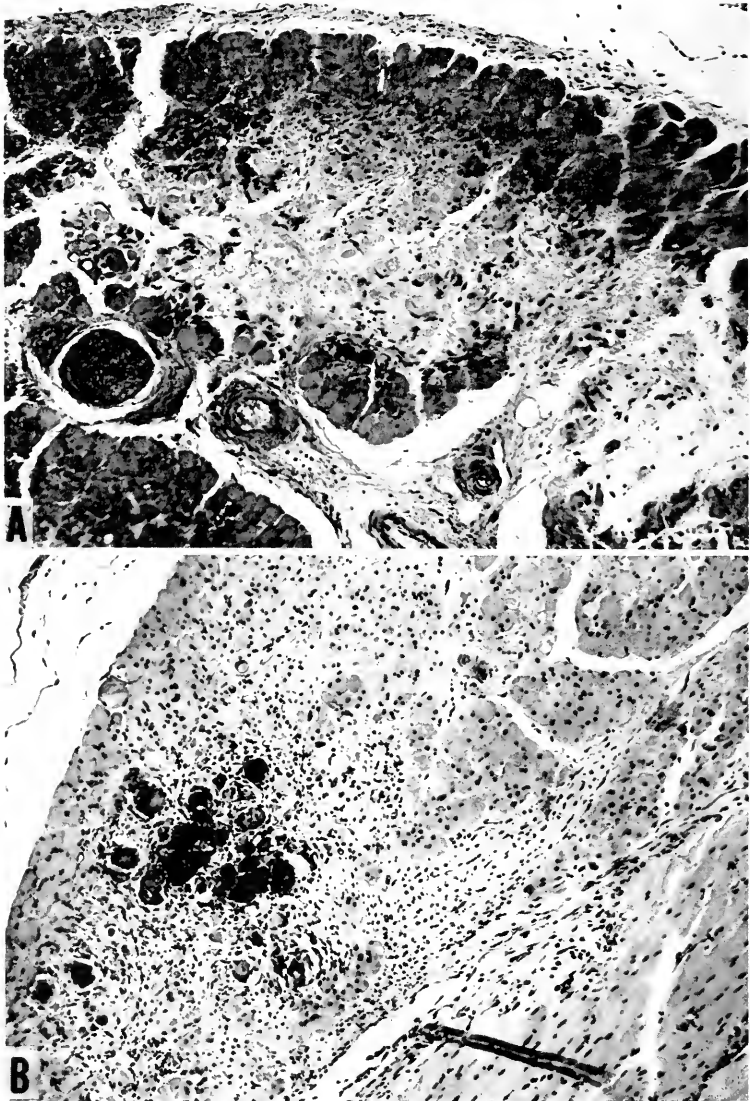


FIG. 7. A. Vertebral skeletal muscle from same rat as Fig. 2, showing necrosis, waxy degeneration, loss of muscle nuclei in center, and some proliferation at periphery of lesion. B. Another case with waxy degeneration, calcification and early fibrosis. Hematoxylin-eosin, $\times 80$.

a spinal oligodendroglioma (Innes and Borner, 1961). Apart from some head tilting and circling in the rats with the cranial tumors, all showed virtually a comparable clinical picture.

The malacic lesion occurring after a long latent period and due to

x-irradiation of the spinal cord in experimental rats is different from the ravaging necrobiosis wreaked on all areas of the spinal cord (gray and white matter) when the animals are exposed to extremely high doses, with survival from a few days to a week. From the few neuropathologic studies on human cases (Itabashi *et al.*, 1957), delayed irradiation myelopathy in rats appears to be a similar process, with the exception of the absence in rats of unmistakable changes in the wall of vessels. In the spinal cord, white matter seems more sensitive than gray. Irradiations could possibly have some direct effect on myelin sheaths and axis cylinders, aside from any changes caused in the endothelium or walls of vessels. This might be suggested by the work of Leboucq (1934), on the inhibitory effect of irradiation on the developing myelin of baby rats.

There might be some concern about the definition of the process, whether it should be designated a demyelinating or malacic one. It is demyelinating in the sense of predilection for attack on the white matter, and no doubt it could start as such. However, it seems that once the lesion starts it is a rapidly progressive one and then it cannot be regarded as anything but a very severe liquefactive process. A large variety of histochemical methods on sections through such a lesion failed to identify with precision any specific degradation products of myelin breakdown. The almost negligible glial reaction is of importance, and in the white matter the oligodendroglia seem to disappear as fast as the myelin sheaths.

Regarding the changes in the walls of vessels, which have been depicted as hyaline, amyloid, or para-amyloid degeneration in human postirradiation myelopathy (Zeman, 1955; Scholz *et al.*, 1959; Pennybacker and Russell, 1948) but which were not found by O'Connell and Brunschwig, (1937) it is important to note there was no evidence in the rat lesions of the deposition of any unusual degenerative substance in or around vessels. Whether dynamic alterations in capillary permeability are responsible for an irrevocable destruction of white matter is another problem.

A spontaneous demyelinating disease of the spinal cord (and not the brain) in two rats was reported by Pappenheimer (1952). From his descriptions and illustrations, the condition cannot be distinguished clinically or pathologically from experimental postirradiation myelopathy. We know that comparable types of disease processes can be produced in the nervous system by divergent types of causal agents; but that spontaneous demyelinating myelopathy can also occur in rats, should be recognized. Pappenheimer was unable to transmit the disease to other rats or mice; the cause was never ascertained, nor has any similar disorder been reported by others. Despite this, we cannot relegate into the limbo of forgotten things the remote feasibility that Pappenheimer's murine disease was caused by a virus and that irradiation might not light up some latent neurotropic infection.

There remains to be considered the degeneration and necrosis of the

vertebral muscles in the direct vicinity of the spinal cord damage, i.e., in the same irradiated area. The specificity of this myodegeneration in that it was caused by the irradiation is undoubted for the lesion is certainly not artifact or traumatic due to handling the rats. The changes are no different in kind or severity from those seen in types of myodegeneration of man, domestic, or laboratory animals, and which can be produced by a multiplicity of causes, perhaps most characteristically in natural and experimental alpha-tocopherol deficiency (Hadlow, 1961). That some of the lesions were old chronic ones was evident by the frequency of calcareous depositions, and again no changes in the walls of arteries supplying affected muscles were seen. Skeletal muscle is regarded as radio-resistant, but there are few observations on muscle in concurrent studies of any more deep-seated process which follows irradiation of the nervous system.

The study is being continued using both rats and monkeys, with the particular aim of seeking clues to determination of the early stage of damage to the nervous system and thus to a better understanding of pathogenesis. The clinico-pathologic studies on monkeys along with serial EEG recordings is but part of this study. In rats, groups of animals have also been irradiated in the lumbar enlargements of the spinal cord. Finally, as such experimental work has clear medical radiotherapeutic implications, groups of animals are now being irradiated with the same dose (3500 rads), but in divided doses following patterns used in radiotherapeutic treatment of human beings.

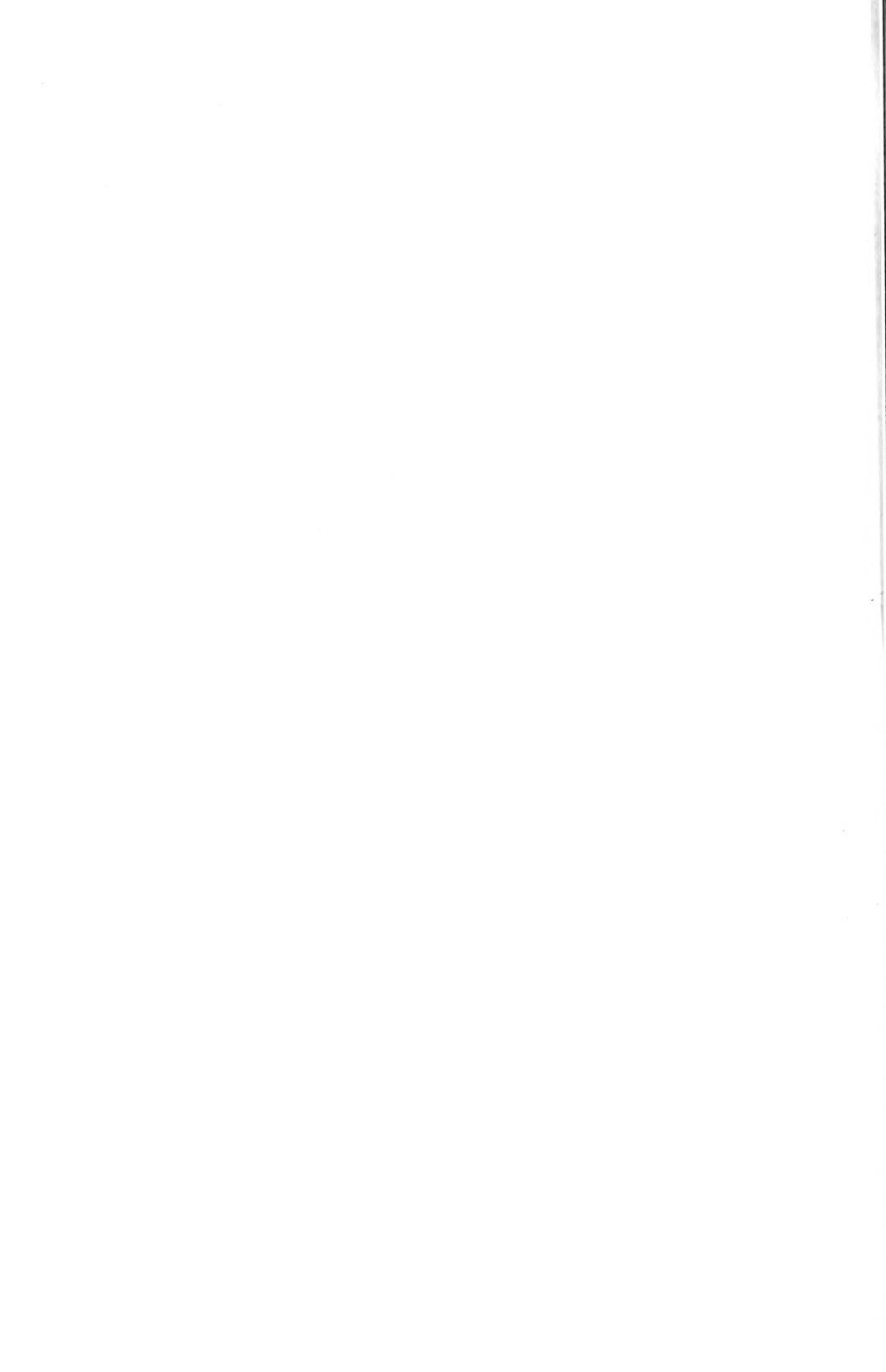
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Effects of High-Dose Gamma Radiation on the Brain and on Individual Neurons*

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Massive doses of ionizing radiation regularly and promptly bring about characteristic morphologic alterations in certain neural tissues and in the mesenchymal structures in and around the brain (Arnold *et al.*, 1954; Haymaker *et al.*, 1958; Vogel *et al.*, 1958; Wilson, 1960). Most notable among these changes are contraction and pyknosis of the nuclei of the granule cells of the cerebellum and leucocytic infiltration into the walls of the cerebral blood vessels, in the leptomeninges, and choroid plexuses. These were conspicuous in monkeys exposed to large doses of gamma radiation from Ba^{140} - La^{140} and Co^{60} sources, as have been described elsewhere (Haymaker *et al.*, 1958; Vogel *et al.*, 1958).

There is much evidence that the pyknotic change in the cerebellar granule cells in cats (Brünner, 1920), monkeys (Vogel *et al.*, 1958), rabbits (Gerstner *et al.*, 1956), guinea pigs (Alvord and Brace, 1957), mice, and rats (Hicks and Wright, 1954) is transitory, and ancillary studies indicate that similar transient structural changes occur in these cells grown in tissue culture and exposed to ionizing radiation. Nevertheless, recent observations have made it clear that in dogs this cellular response, although initially characterized by nuclear contraction, is often followed promptly by karyorrhexis with cellular death (Vogel, 1959). When examined with the electron microscope, the pyknotic and karyorrhetic cells regularly show distinctive alterations in intracellular fine structure (Vogel, 1959). These provide in-

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formation about the pathogenesis of transitory and lethal cellular responses of neurons to ionizing radiation.

Methods

Most procedures employed in these studies have been described in detail in individual publications.

Total body radiation was administered to 67 young, 2- to 4-year-old, male *Macacus rhesus* monkeys, in graded doses from 1,000 to 30,000 r from a Ba¹⁴⁰-La¹⁴⁰ source, at 1,000 r per minute. Detailed morphologic examinations were made of all animals promptly after death (Haymaker *et al.*, 1958).

Gamma radiation, in total doses of 10,000 r, was administered at 1,000 r per minute from a Co⁶⁰ source to 48 young male *Macacus rhesus* monkeys, divided into 3 groups of 16 animals each. One group received radiation to the head with the body shielded; another, to the body with the head shielded; and a third, to the entire animal. At periodic intervals up to 96 hours later, pairs of animals from each group were killed (Vogel *et al.*, 1958).

Explants of cerebellar tissue from mice, 3 days old, were grown on slides with prepared media (Gillete and Findley, 1958) in Carrel flasks and Maximow chambers. Gamma radiation was administered in doses of 10,000 r from a Co⁶⁰ Ticker machine at 160.5 r per minute at a distance of 30 cm. Irradiated and nonirradiated cultures were examined periodically by the phase microscope, and tissues were removed from the cultures, stained by hematoxylin and eosin, and examined by light microscopy.

Young healthy mongrel dogs and adult white albino rabbits were exposed to 15,000 r of gamma radiation from a Co⁶⁰ Ticker machine over the superior cerebellar region with a field 5 cm in anterior-posterior dimension and 7 cm across. The rate was 160 r per minute with a source to skin distance of 30 cm and a half value layer of 11 mm of lead. Tissues were taken from the cerebellum of anesthetized animals, fixed immediately in 1% osmium tetroxide solution, and prepared by standard methods for electron microscopy (Vogel, 1959).

Observations

MORPHOLOGIC EFFECTS OF GAMMA RADIATION ON THE BRAIN AS VIEWED WITH THE LIGHT MICROSCOPE

Exposure of animals to massive doses of ionizing radiation is followed promptly by conspicuous morphologic alterations in the brain and mesenchyma, notably by a pyknosis of the cerebellar granule cells and inflamma-

tion of the cerebral blood vessels, leptomeninges, and choroid plexuses. These lesions were not evident in monkeys exposed to 1,000 r. They were present in some animals, but equivocally or in minimal intensities, after exposure to 2,500 r. They were found with increasing frequency and intensity after doses of 5,000 and 10,000 r. They showed slight increases with greater dosages up to 30,000 r. The severity of the lesions differed appreciably from animal to animal, even when exposure and survival conditions were essentially identical.

The cytologic changes were well established within 2 hours after radiation. They were dynamic, for their intensity increased rapidly within the next 8 to 24 hours and then regressed precipitously, the lesions being minimal or absent 96 hours after exposure.

The pathologic alterations were of the same character and intensity whether the head and body or only the head was irradiated. They did not occur when the radiation was applied to the body with the head shielded, the findings provided evidence that these cytologic responses were induced by the ionizing rays acting directly upon the intracranial tissues, neither being initiated nor enhanced by exposure of other regions of the body.

GRANULE CELL CHANGE

The morphologic appearance of the affected cells was notably similar in monkeys and rabbits, being regularly characterized by a reduction in the diameter of the nucleus to as much as one-half the normal, with marked condensation of the intranuclear chromatic material. Narrow margins of basophilic cytoplasm were visible about some of the contracted nuclei, and these often stained deeply with pyronin. Up to 50% of the granule cells of a single animal were severely altered; most others remained normal; few showed intermediate degrees of change. Usually the affected cells were haphazardly distributed throughout all portions of the internal granular layer and cerebellum. In some animals, there was preferential localization, the vermis and deeper portions of the granular layer being most often so involved. Golgi and Purkinje cells were regularly spared. The pyknotic cells were more widely separated from one another than nonpyknotic ones. The appearance resembled that caused by extracellular edema, although regularly unaccompanied by coagulated fluid. The widened intercellular spaces were infiltrated by only a few leucocytes and macrophages and rarely contained hemorrhages. Neuronophagia was absent. Perivascular cuffing by leucocytes was minimal in the cerebellar cortex, particularly so in the granular layer.

The initial cytologic changes in the granule cells of dogs was also characterized by contraction of the nucleus, but this was accompanied by nuclear

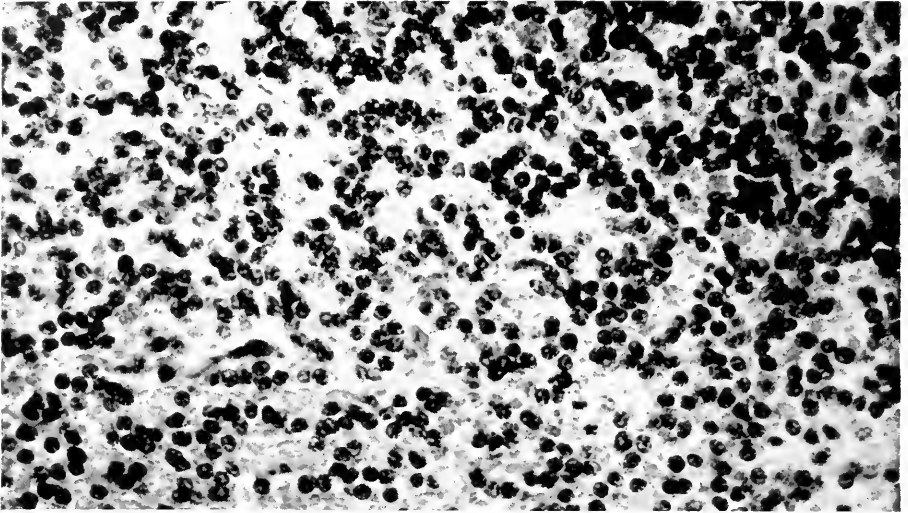


FIG. 1a. Granular layer of the cerebellum of a normal dog. The granule cells have uniform, rounded nuclei with a nucleolus and fine chromatin material, but no visible cytoplasm. Hematoxylin and eosin stain. $\times 400$.

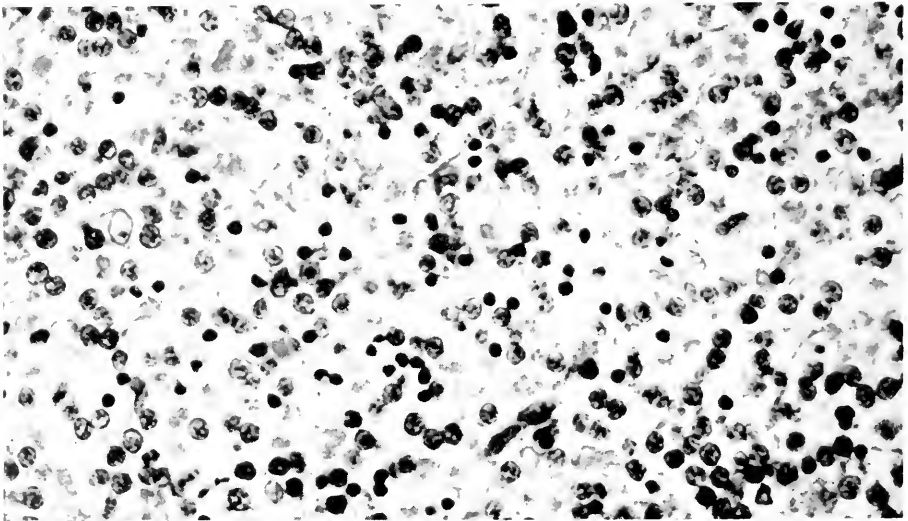


FIG. 1b. Granular layer of a dog 15 hours after exposure of the head to 15,000 r of gamma radiation from a Co^{60} source. Many nuclei are shrunken and hyperchromatic. Some show karyorrhexis. Hematoxylin and eosin stain. $\times 400$.

fragmentation in some (Figs. 1a, b). Macrophages, with phagocytized cellular debris, were present. Notable losses of granule cells became evident in animals killed 8 days after exposure, and a mild astrocytic proliferation was present at this time. Golgi and Purkinje cells remained intact (Fig. 2).

VASCULITIS

An exudate of leucocytes appeared promptly in all layers of the cerebral blood vessels. Veins and arteries were involved about equally. Vessels of all sizes were affected. The vessels in the cerebral nuclear masses were generally more intensely involved than those in the cerebral cortex, while those in the white matter shared in the process, but to lesser degree. The vessels of the brain stem, cerebellum, and spinal cord were similarly affected, also in lesser intensity. The leucocytes rarely penetrated into the surrounding neural substance, but usually concentrated in the adventitia and perivascular spaces. Hemorrhage was rare. Vessels stained specifically for collagen and elastic tissue regularly showed no notable alterations in these components. With

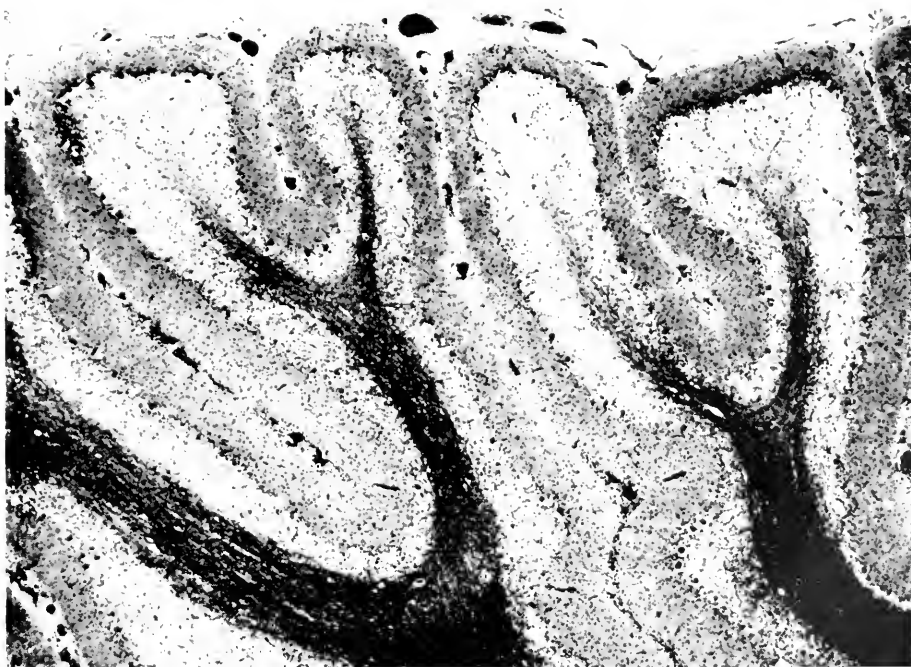


FIG. 2. The rarified granular layer of a dog 10 days after exposure to 15,000 r of gamma radiation contains Golgi cells and an increased number of astrocytes, but is largely devoid of granule cells. Hematoxylin and eosin stain. $\times 55$.

the passage of 72 to 96 hours, the inflammatory cells lysed and the exudate lessened, but with residual perithelial edema and scant numbers of macrophages and lymphocytes still present at the latter times.

MENINGITIS

Initially the exudate was perivascular and spotty. It persisted as such in some animals, but disseminated over the gyri and spread into the sulci in most. The cellular composition varied with duration after exposure, but also differed somewhat in animals with identical postirradiation states. Earlier polymorphonuclear leucoctyes predominated in great numbers; later, with decreases in the cellular concentrations, lymphocytes and macrophages were relatively more abundant. Generally, macrophages persisted and with their contents of cellular debris constituted the inflammatory residue in animals killed 96 hours after exposure.

CHOROID PLEXITIS

The choroid plexuses in the lateral, 3rd, and 4th ventricles were equally involved. The intensity and cellular composition of the inflammatory exudate generally paralleled that in the meninges and in the cerebral blood vessels. Edema of the fibrous tissue stroma usually antedated the exudation of cells into these regions. The choroidal epithelium was generally spared, but ulceration followed on frons with unusually heavy exudates. The epithelium was reconstituted, and the inflammation had regressed in most animals by 96 hours after exposure.

MORPHOLOGIC EFFECTS OF GAMMA RADIATION ON GRANULE CELLS IN TISSUE CULTURE

The cells in explants of cerebellum from new born mice proliferated rapidly and migrated in sheets centrifugally onto the glass. Many of these cells had rounded nuclei with one or several small nucleoli, finely particulate chromatin material, a well defined nuclear membrane, and scant or undetectable cytoplasm. Slender, short processes radiated from the perikaryon of some. These cells with distinctive cytologic features as seen with the phase microscope and in sections stained with hematoxylin and eosin were considered granule cells (Fig. 3). Also abundant in cultures 5 to 10 days old were cells with more elongated nuclei, coarse chromatin material, and bipolar or diffusely radiating cytoplasmic strands. These resembled fibroblasts derived from other tissue sources and were identified as such with phase and light microscopy. Some with similar appearances were viewed as

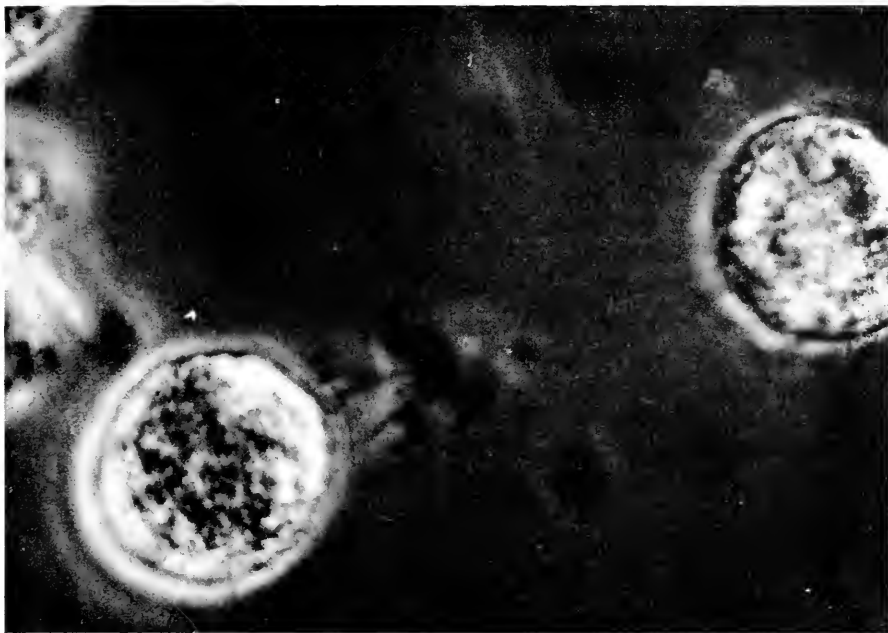


FIG. 3. Granule cells of the cerebellum of a 3-day-old mouse grown for 10 days in tissue culture. The cells have rounded nuclei with extremely scant perikaryon. Phase Microscopy. $\times 1200$.

astrocytes. Fewer cells had a larger nucleus, usually with a prominent nucleolus, and abundant perikaryon that formed a single dominant process and occasionally lesser ones. These cells were identified as neurons derived from the cerebellar cortex from regions other than the granular layer.

Preliminary studies have made it clear that only cells with the cytologic characteristics attributed to the granule cells showed notable structural changes in the immediate postirradiation period. These changes closely resembled those noted previously in histologic preparations of the irradiated cerebellar cortex, being characterized by a contraction of the nuclei to approximately $\frac{2}{3}$ normal size. The perikaryon that was normally scant about the granule cells in tissue culture became conspicuously wider and the over-all dimensions of many cells increased (Fig. 4). As noted in sections stained by hematoxylin and eosin, the nuclei were contracted and the chromatin material compressed and hyperchromatic. The perikaryon was more abundant than normal, stained intensely with eosin, and it was often foamy and vacuolated. Altered cells were most abundant 24 hours after exposure. Periodic examinations of the cultures with the phase microscope



FIG. 4. Granule cells, as in FIG. 3, 24 hours after exposure to 10,000 r of gamma radiation from a Co^{60} source. The nuclei are markedly contracted and the nuclear membranes are serrated. The cytoplasmic spaces are enlarged. The over-all size of these cells is somewhat less than normal; in others, it was greater. Phase Microscopy. $\times 1200$.

and in stained sections made it clear that these cytologic changes were transitory. The cells in individual clumps survived and became normal in appearance. Cells in nonirradiated cultures did not undergo these cytologic changes.

MORPHOLOGIC EFFECTS OF RADIATION ON GRANULE CELLS AS VIEWED WITH ELECTRON MICROSCOPE

The fine structure of nonirradiated granule cells of rabbits was indistinguishable from that of the cells in dogs, and in each species they were strikingly uniform. The cells possessed a large, spherical nucleus with uniformly distributed, finely granular, abundant intranuclear granules. The dual nuclear membranes were uninterrupted and lay parallel except for a rare out-folding of the external one. The cytoplasm was regularly scant, but clearly visible about the entire nucleus with expansions at the axon hillock.

Mitochondria were small and sparse, rarely more than 6 in a single cross section of a cell. Their cristae were delicate and inconspicuous. The endoplasmic reticulum was also scant, but was most abundant at the axon hillock. A Golgi apparatus frequently occupied this region. Many granule cells lay side by side with cytoplasmic membranes in apposition. Others were encased in part or totally by dendritic processes (Fig. 5).

The earliest recognizable cytologic changes, abundantly evident in the tissues of rabbits examined 24 hours after exposure, were characterized by a contraction of the nucleus and clumping of the intranuclear granules, with increased serration of the nuclear membranes and broadening of the cytoplasmic space with dispersion of the cytoplasmic constituents. With

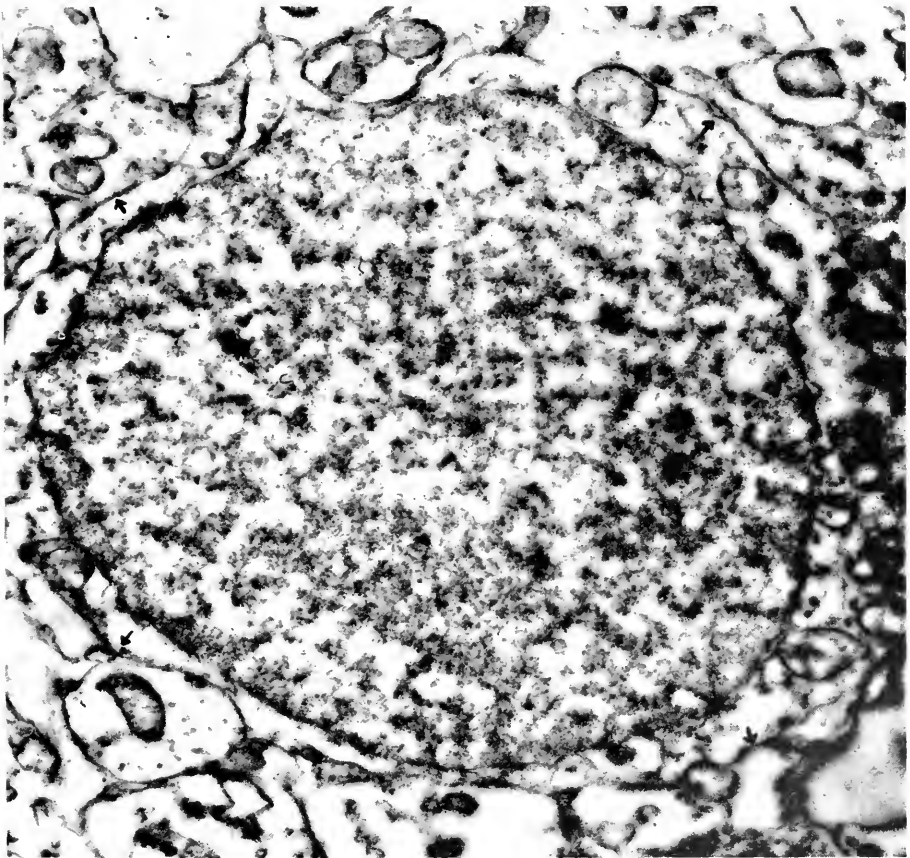


FIG. 5. A normal granule cell of a rabbit is encased in dendrites and has scant cytoplasm (↑) with few mitochondria and sparse endoplasmic reticulum. Approximately $\times 18,000$.

further contraction of the nuclei, the intranuclear material was greatly compacted, but still remained particulate and discrete, the granules being separated from one another by an electron-lucent margin of uniform width. Although the nuclear membranes became redundant with extreme contraction of the nuclear mass, in the rabbit they did not fragment, but folded and coiled. The expansion of the cytoplasm was conspicuous, in most cells exceeding the volumetric decrease in the nucleus. The over-all size of the cells increased. The endoplasmic reticulum was widely dispersed in the expanded cytoplasm, but did not show consistent structural change (Fig. 6).

The recovery phase, as judged by light microscopy, was completed by 72 hours after radiation. Tissues taken at this time and examined with the

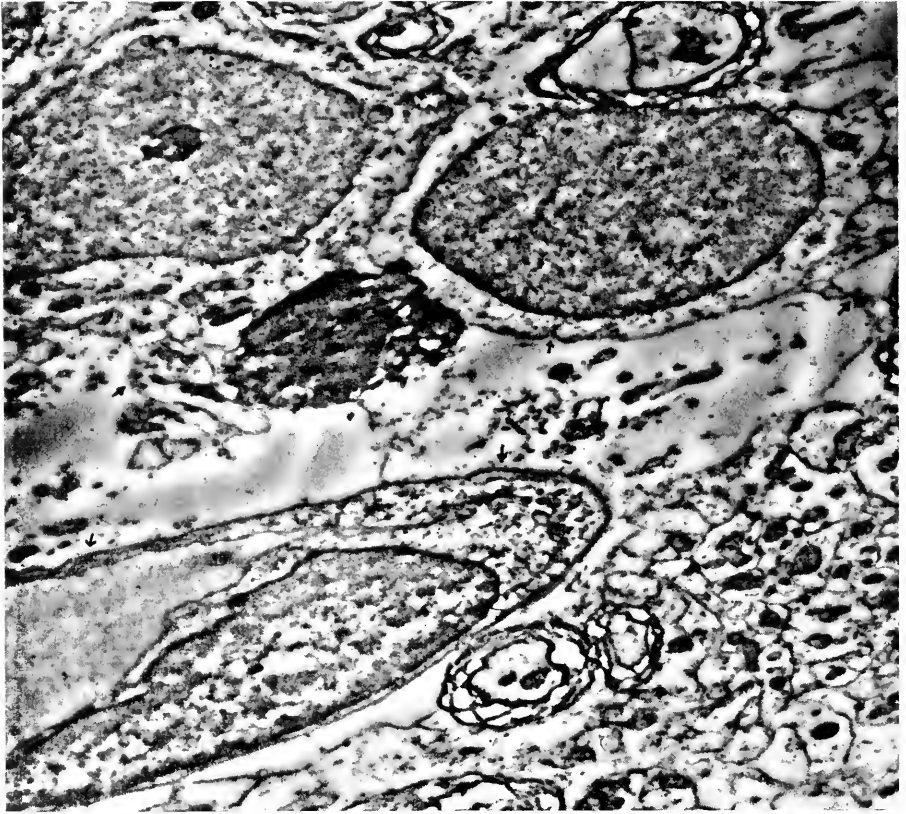


FIG. 6. Electron micrograph of a pyknotic granule cell of a rabbit 24 hours after exposure to 15,000 r of gamma radiation. The nucleus of one cell is extremely contracted; the intranuclear granules are condensed and the cytoplasmic space (↑) is greatly expanded, with dispersion of the cytoplasmic constituents. Two unaltered granule cells and a capillary surround the pyknotic one. Approximately $\times 10,000$.

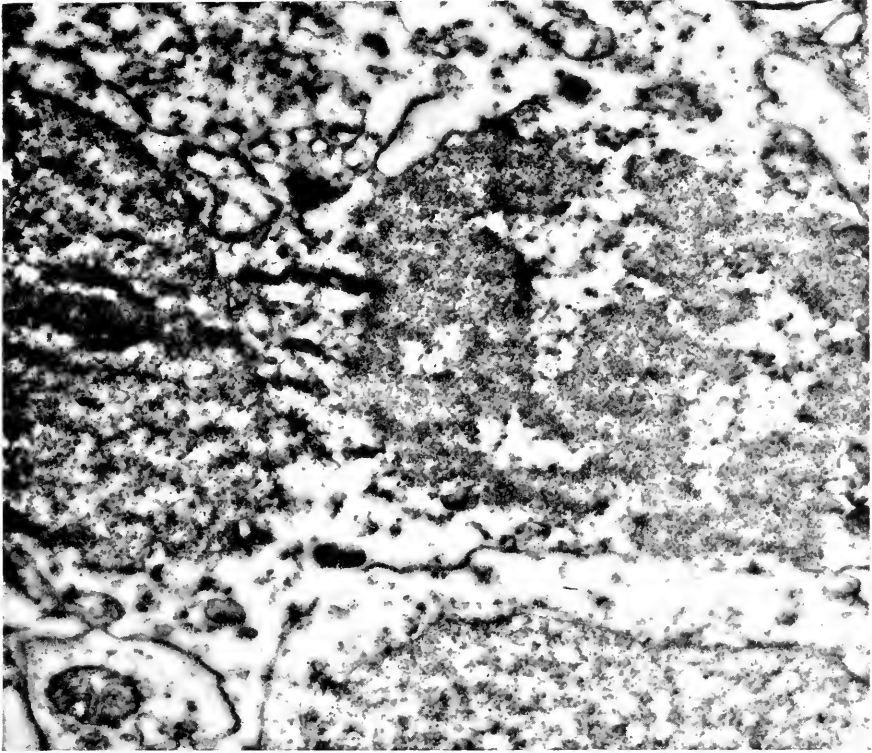


FIG. 7. Electron micrograph of karyorrhetic granule cell of a dog 24 hours after exposure to 15,000 r of gamma radiation. There is fragmentation of the nuclear membrane and extrusion of the cytoplasmic constituents. Approximately $\times 15,000$.

electron microscope showed most of the granule cells to have fine structure that was essentially normal. Minor stigmata were present in some, indentations of the nuclear membranes being the most conspicuous.

In general, the altered neurons in radiated dogs were not notably different from those in rabbits. The initial cytologic changes were, as in rabbits, shrinkage of the nuclei, clumping of the nuclear granules, folding and redundancy of the nuclear membranes, and expansion of the cytoplasm. In addition, and apparently as a further progression of these changes, there was fragmentation and disintegration of the nuclear membranes of some of the contracted nuclei (Fig. 7).

COMMENT

The findings make it clear that exposure of the granule cells in the intact animal or in tissue culture to ionizing radiation initiates rapid volumetric

changes in the intracellular compartments. It seems most likely that these changes are manifestations of a rapid transfer of electron-lucent "nuclear sap" from the karyoplasm into the perikaryon. Such volumetric changes might result from alterations in the permeability of the nuclear membranes, from hypotonicity in the nuclear chamber, which causes an egress of fluid, from hypertonicity in the cytoplasmic compartment, which attracts fluid, or from several of these factors acting simultaneously. The expansion of the cytoplasmic space, which in many cells notably exceeded the volumetric decrease in the nucleus, and the increase in the over-all size of the cells make it seem likely that extracellular fluid is also imbibed by the perikaryon. Considered together, the findings suggest that radiation induces a marked hypertonicity of the cytoplasm that, in turn, initiates a series of cytologic changes that are transitory in the granule cell neurons of rabbits and other animals, but often lead to cellular death in those of the dog.

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Electron Microscope Observations on the X-Irradiated Central Nervous System of the Syrian Hamster*

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Relatively few reports on brain damage following massive dosages of x-radiation have been published in the light microscope literature (Hicks and Montgomery, 1952; Hicks *et al.*, 1956), and only Vogel (1959) has utilized the high resolving power of the electron microscope in his study of x-ray induced pyknosis of the granular cells of the cerebellum. Our work was undertaken to demonstrate the morphologic alterations produced by x-radiation and to ascertain if there exist in the ultrastructural range cytologic alterations specific for this type of energy. The brains from which specimens were removed for electron microscopy were also utilized in the extensive light microscope investigations of Scholz which are reported elsewhere in this symposium. Only ultrastructural observations will be considered. Because of the sampling limitations inherent in electron microscope technique, time-dosage relationships will not be discussed.

Material and Methods

A circumscribed region of the mediodorsal skull of unanesthetized Syrian hamsters was subjected to a single exposure of x-radiation. A Monophos ray machine was used with a target distance of 5.5 cm. The technical conditions were as follows: 40 KV, 25 mA, 0.3 Aluminum with a half value depth in tissue of 1 mm. Dosages ranged from 7,500 to 45,000 r, and animals were sacrificed after periods ranging from 4 hours to 30 days. Specimens taken from the living animal were fixed in buffered osmium tetroxide,

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treated in the usual fashion, and embedded in methacrylate. Ultrathin sections were examined in a Siemens UM 100 electron microscope.¹

Results and Discussion

This preliminary report is concerned only with dosages sufficiently high to produce necrosis.

Within a few hours after exposure to the radiation the first significant change that could be seen with the electron microscope consisted of swelling

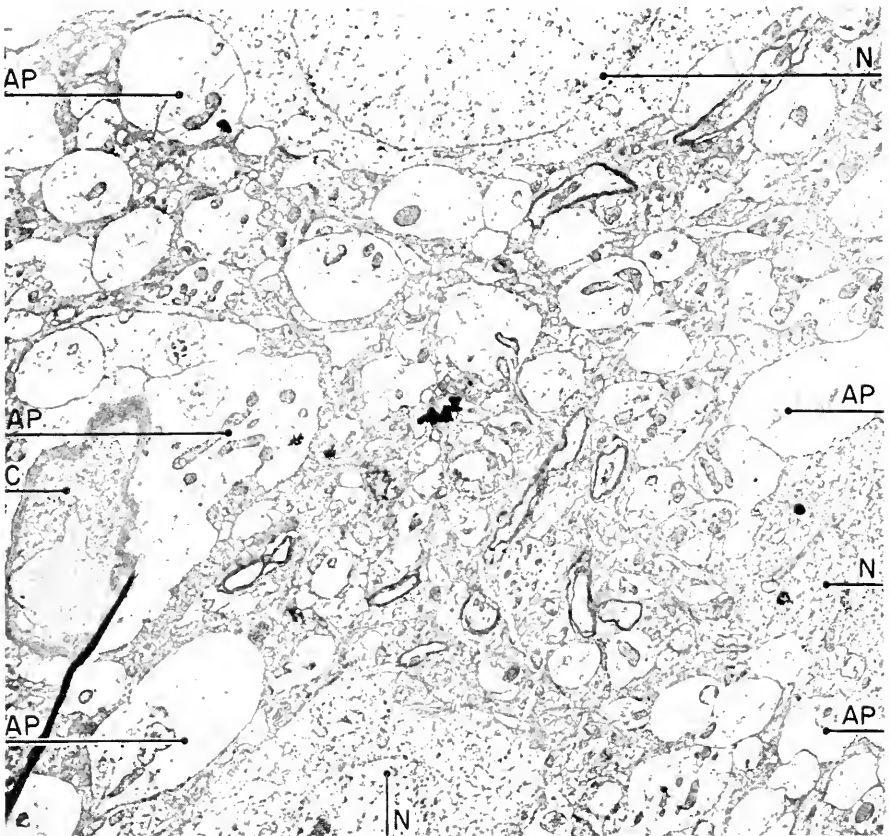


FIG. 1. Cerebral cortex, 15 hr after exposure to 15,000 r. Swelling of astrocytic processes (AP) in the neuropil and around a capillary (C). N, nerve cells. $\times 4,250$.

¹The authors gratefully acknowledge the technical assistance of Miss Luh and Mr. Fellner and the cooperation of Professor Dr. Rollwagen in allowing the use of the electron microscope facilities of the University of Munich.

of the astrocytes' cytoplasm (Fig. 1). All astrocytic processes are considerably enlarged and even more pale and "watery" than usual. Around a small capillary there is disruption of astrocytic membranes, probably due to a combination of artifact and edema. Under different experimental conditions De Robertis *et al.* (1958) have produced similar changes and emphasized the role of the astrocytes in water and ion metabolism of the brain tissue. It seems probable that this astrocytic swelling represents the electron microscope equivalent of the sponginess and loosening of the cortical neuropil that is usually considered reflective of edema. This morphologic manifestation of radiation-induced edema is restricted to astrocytic cytoplasm, and other cells within the central nervous system seem to be unaffected. Furthermore, the narrow intercellular gap which represents the only anatomic extracellular space in the brain (Hager, 1959; Horstmann and Meves, 1959) is not increased in this situation. The neurons appear normal at this stage. The appearance of mitochondria is somewhat difficult to evaluate, since similar changes in these organelles can occur as the result of incomplete or delayed fixation.

Figure 2 demonstrates erythrodiapedesis around a small vein. The red blood cells lie outside the perivascular space and within the intercellular gap. This is more easily seen in Fig. 3, where the erythrocyte is situated between completely intact cell processes, most of which are astrocytic. Contemporarily with the appearance of astrocytic swelling and erythrodiapedetic bleeding, there occurs slight, but probably definite, swelling of the capillary endo-

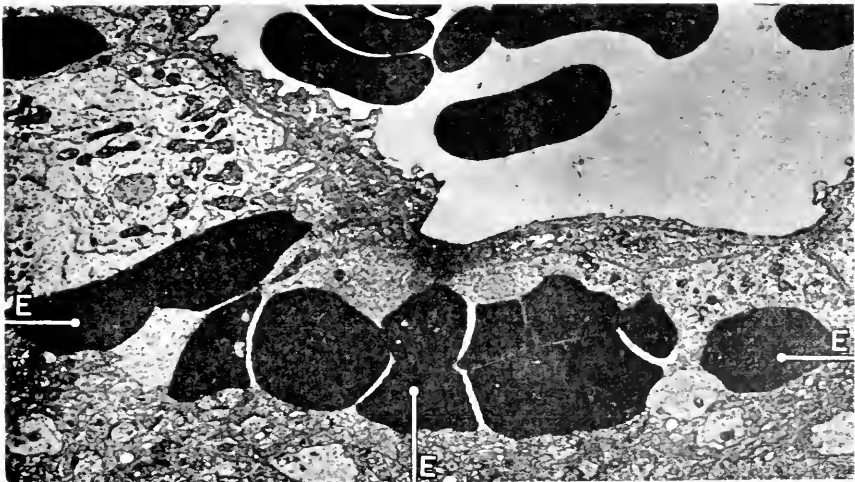


FIG. 2. Cerebral cortex. 69 hr after exposure to 45,000 r. Erythrodiapedesis around a small vein outside of the perivascular space. E, erythrocytes lying between astrocytic processes. V, vessel wall; N, nerve cell. $\times 4,800$.

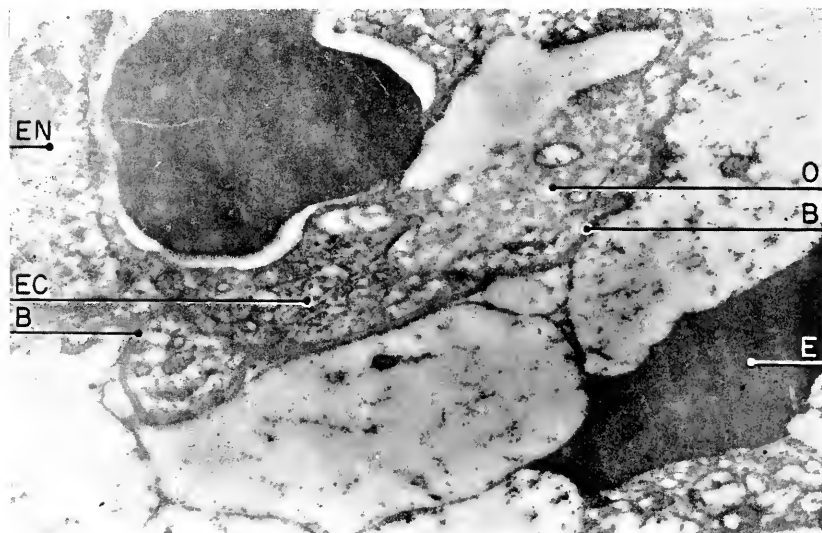


FIG. 3. Cerebral cortex, 48 hr after exposure to 45,000 r. A diapedetic erythrocyte (E) lies between completely intact cell processes in the neighborhood of a capillary. EC, swollen endothelial cytoplasm; EN, endothelial nucleus; O, outpouching in the capillary lumen; B, basement membrane of capillary. $\times 10,800$.

thelium. Occasional outpouchings and irregularities in the capillary lumen can also be seen. Cytoplasmic organelles and vascular basement membranes show no significant alterations.

Similar changes in the blood vessels and remarkable edematous astrocytes are also noted in irradiated cerebellar tissue (Fig. 4). Here, however, nerve cells are also involved, though there is a striking difference in the appearance of granular cells and Purkinje cells. The latter are relatively unchanged, while the granular cells are shrunken and hyperchromatic with clumping of the nuclear material. A higher magnification of such changes is seen in Fig. 5, where normal and abnormal granular cell nuclei can be contrasted. These pyknotic changes in granular cells following radiation have been recognized by several investigators (Alvord and Brace, 1957; Br nner, 1920; Haymaker *et al.*, 1954, 1958; Sch mmelfeder, 1957; Vogel *et al.*, 1958), and similar electron micrographs have been published by Vogel (1959).

In Fig. 6 the extensive plasma exudation following radiation is apparent. The perivascular space bounded by the glial and vascular basement membranes (Nelson *et al.*, 1961) is filled with structureless material, which has a density similar to that of plasma and contains inflammatory cells. Neither leucocytes nor erythrocytes have so far been seen penetrating the glial basement membrane that constitutes the external margin of the perivascular space,

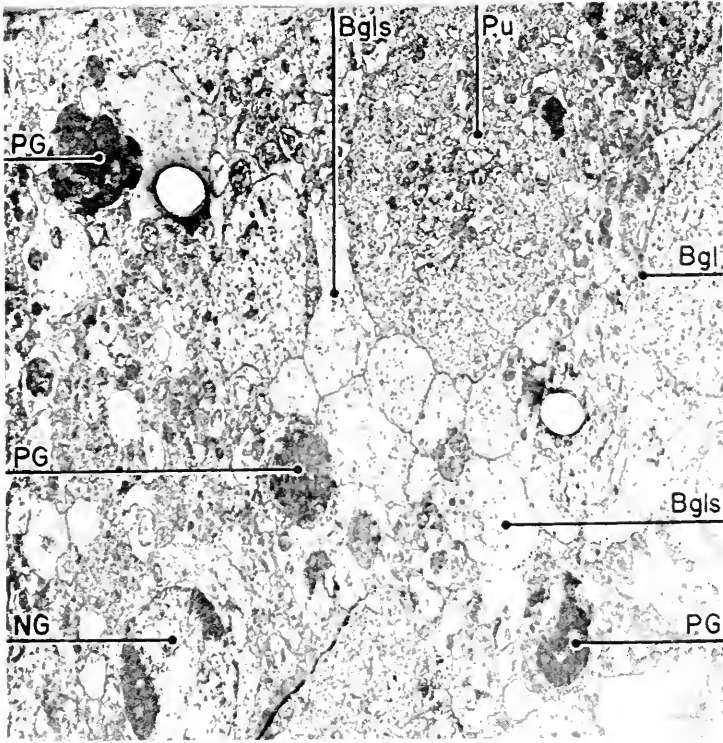


FIG. 4. Cerebellar cortex, Purkinje cell layer and superficial granular layer, 22 hr after exposure to 40,200 r. Scattered pyknosis of granular cells (PG). NG, normal granular cell; Pu, perikaryon of a Purkinje cell; Bgl, Bergmann glial cell; Bgls, Swelling of Bergmann gliocytic processes. $\times 4,800$.

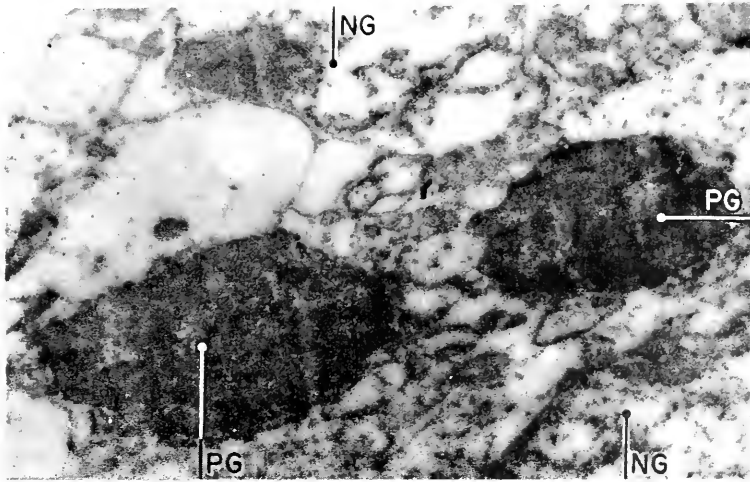


FIG. 5. Cerebellar cortex, granular layer, 46 hr after exposure to 30,000 r. Nuclei of granular cells show severe shrinkage and clumping of the nucleoplasm (PG). NG, normal granular cell nucleus. $\times 10,800$.

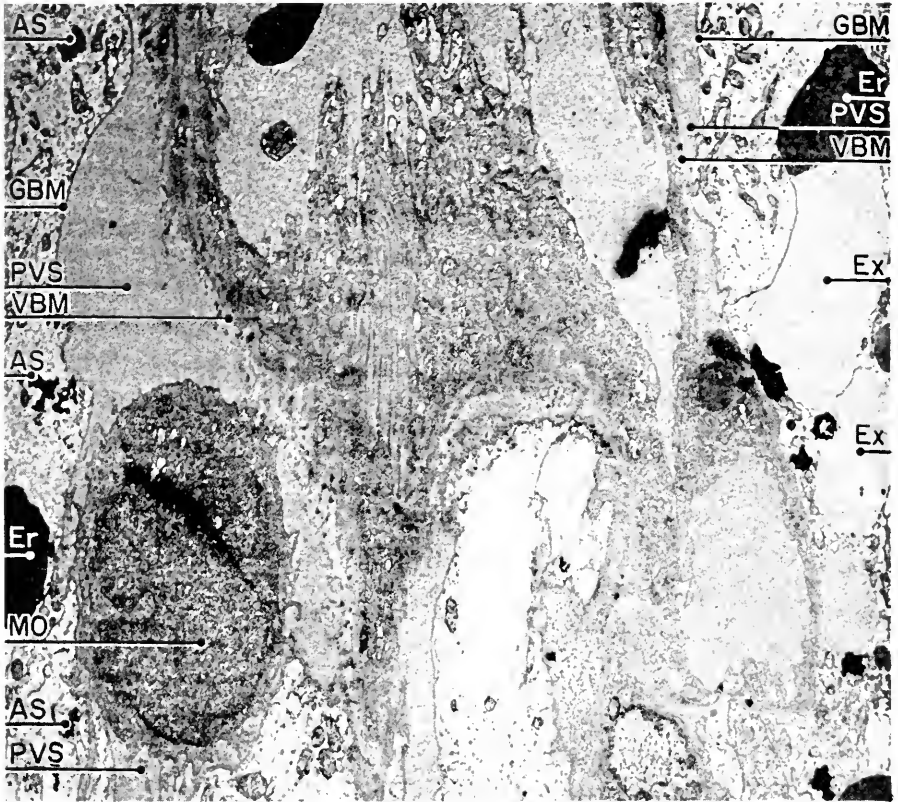


FIG.6. Cerebral cortex, 45 hr after exposure to 15,000 r. Tangential section through a small vein. The perivascular space (PVS), bordered by glial (GBM) and vascular (VBM) basement membranes, is filled with exudate and also contains a monocyte (Mo). Ex, exudate within partially necrotic tissue; Er, diapedetic erythrocytes; As, astrocytic processes containing densely osmiophilic inclusions. $\times 4,500$.

though polymorphonuclear leucocytes have been literally caught in the act of passing through the vascular basement membrane. This process has been discussed elsewhere (Nelson *et al.*, 1960), but we are still ignorant of the factors that prevent or permit such cellular movement. Plasma exudate is also seen outside the confines of the perivascular space, but it is difficult to determine whether this is in the intercellular space, as are the erythrocytes, or if it is partly intercellular in these incompletely necrotic tissue. A rather common finding is the occurrence of densely osmiophilic inclusions in astrocytic cytoplasm. Figure 7 shows exudate associated with more definite early necrosis, with dissociation of the normal, intricately arranged components of the neuropil and a disruption of some of their membranes. Nerve cells, such

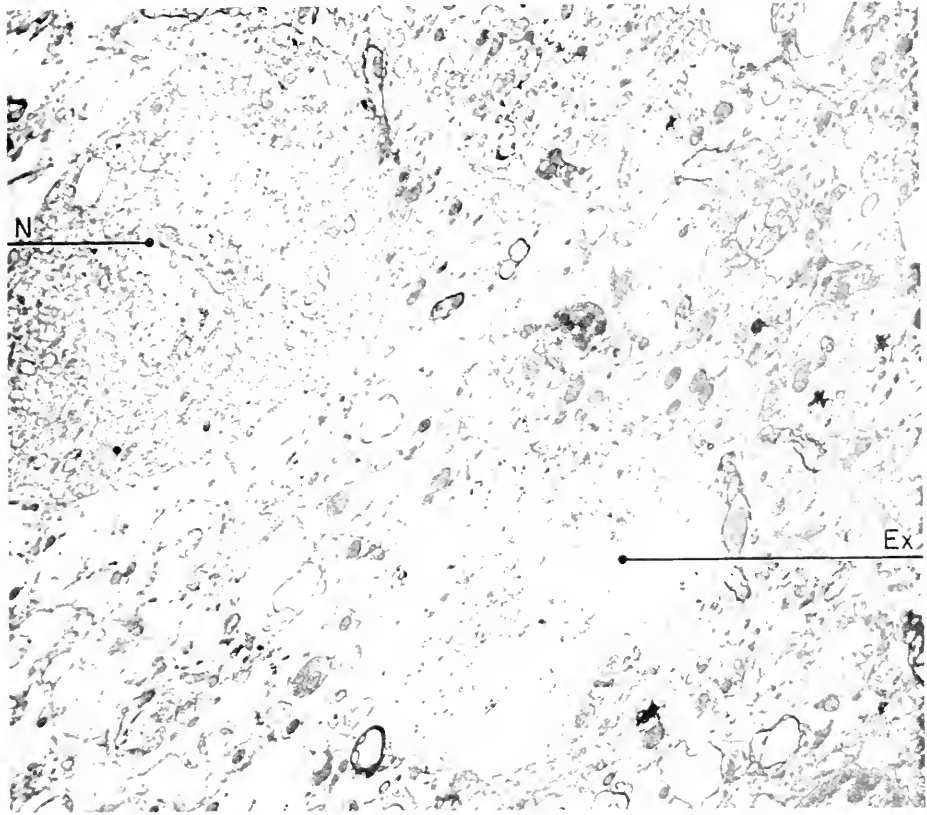


FIG. 7. Cerebral cortex, 73 hr after exposure to 45,000 r. Beginning necrosis with dissociation of the components of neuropil and disruption of some of their membranes. Nerve cell (N) shows widening of the endoplasmic reticulum and swelling of mitochondria. Ex. exudate. $\times 4,000$.

as the one shown here, contain a nucleus which morphologically is still essentially normal, while the cytoplasm shows changes of questionable significance, including widening of endoplasmic reticulum and mitochondrial swelling. There is good preservation of RNA granules.

In Fig. 8 one can see a still more severe stage of tissue destruction. Here, a small artery is in a state of relatively good preservation, despite extensive necrosis of the surrounding tissue. The outer margin of the perivascular space is no longer intact. Perivascular macrophages and leucocytes are seen in contact with the necrotic neuropil. The dark round bodies are probably ingested fragments of erythrocytes.

Figure 9 illustrates total dissociation of tissue components so that only cell

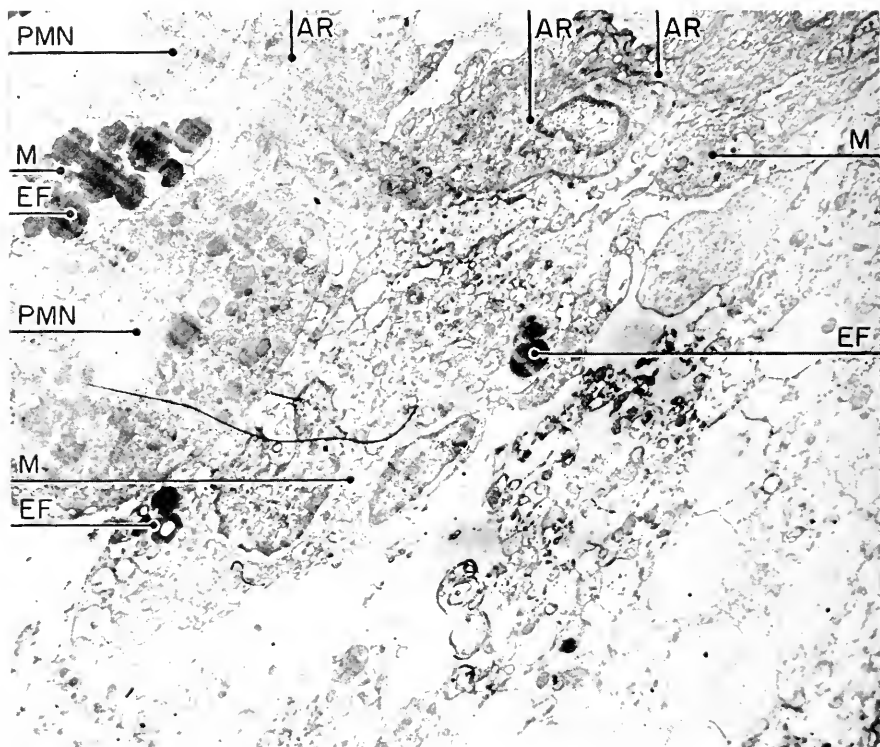


FIG. 8. Cerebral cortex, 73 hr survival after exposure to 45,000 r, showing a relatively well preserved small artery (Ar) surrounded by macrophages (M) and polymorphonuclear leucocyte (PMN) in an area of extensive necrosis. EF, ingested erythrocyte fragments. $\times 4,000$.

membranes and occasional mitochondria are still recognizable. Erythrocytes and macrophages are seen swimming free in the severely damaged tissue.

Figure 10 is a high magnification of a similar region to show the variety of intracytoplasmic inclusions in macrophages. The significance and origin of similar products of decomposition in cerebral necrosis following mechanical trauma has already been reported. (Hager, 1960). In the same figure, the persistent preservation of membranous components of the neuropil is evident. Figure 11 shows a nerve cell also in a severe stage of destruction. Nuclear and cytoplasmic membranes are interrupted. The internal arrangement of the mitochondria is broken down, and the normal fine structural organization of the cytoplasm is greatly altered. The nucleoplasm is vacuolated, no longer fills the nuclear envelopes, and is obviously abnormal, though the appearance differs greatly from the shrunken pyknotic nuclei of the granular cells of the cerebellum. Capillaries from areas of total necrosis,



FIG. 9. Cerebral cortex, 172 hr after exposure to 7,500 r. Fully developed necrosis with total dissociation of most tissue components, though cell membranes are still recognizable. Macrophages (M) and erythrocytes (Er) are seen free in the necrotic tissue. $\times 4,000$.

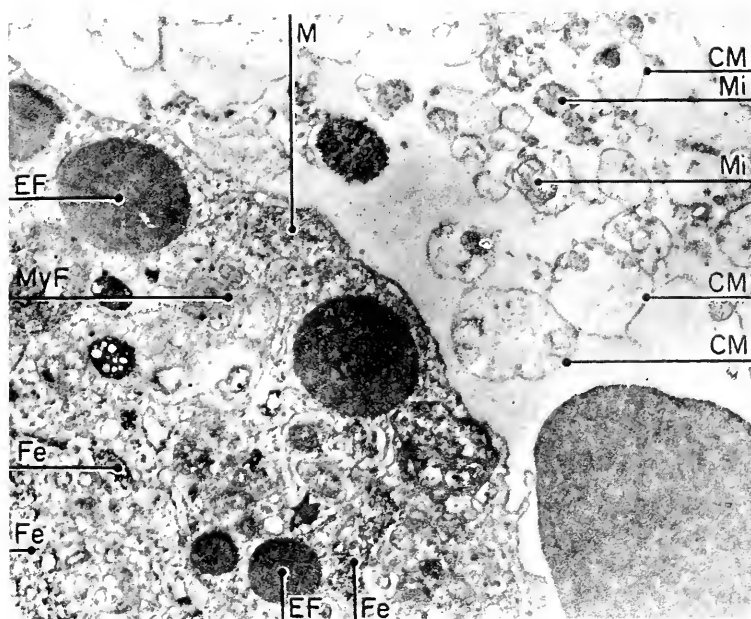


FIG. 10. Cerebral cortex, 172 hr after exposure to 7,500 r. Various cytoplasmic inclusions in a macrophage (M). EF, erythrocyte fragments; MyF, myelin figures; Fe, hematogenic pigment, probably iron-containing; CM, cell membranes of dissociated components of the neurophil; Mi, mitochondria. $\times 10,800$.

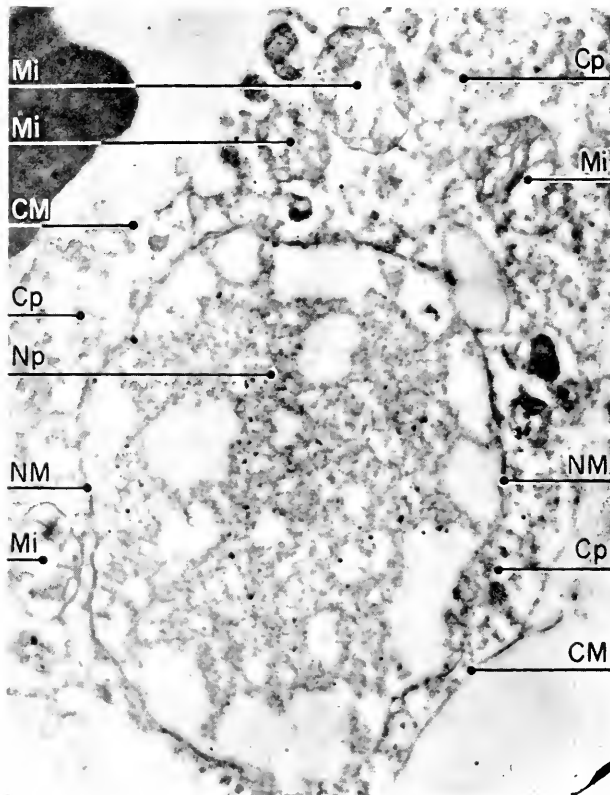


FIG. 11. Cerebral cortex, 172 hr after exposure to 7,500 r. Interruption of nuclear (NM) and cytoplasmic (CM) membranes of a nerve cell with breakdown of internal arrangement of mitochondria (Mi). Severe changes in appearance and distribution of nucleoplasm (Np) and cytoplasm (Cp). $\times 21,600$.

as in Fig. 12, are also strikingly altered. One can recognize enormous swelling of the endothelial cytoplasm, vacuolation, and various types of inclusion bodies, so that these cells appear to have a phagocytic function. The basement membrane remains relatively well preserved. In Fig. 13, at the border zone of a necrotic area, two large reactive astrocytes are seen. The cytoplasm of these cells is clearly of the same type as the pale, watery cytoplasm that previously has been considered characteristic for astrocytes (Farquhar and Hartmann, 1957; Schultz *et al.*, 1957). The presence of intracytoplasmic fibrils in these cells, which can be seen in Fig. 14 at higher magnification, would seem to be a significant point in the positive identification of this cell type as an astrocyte and not as an oligodendrocyte as has been maintained by some investigators (Brünner, 1920; Luse, 1958).

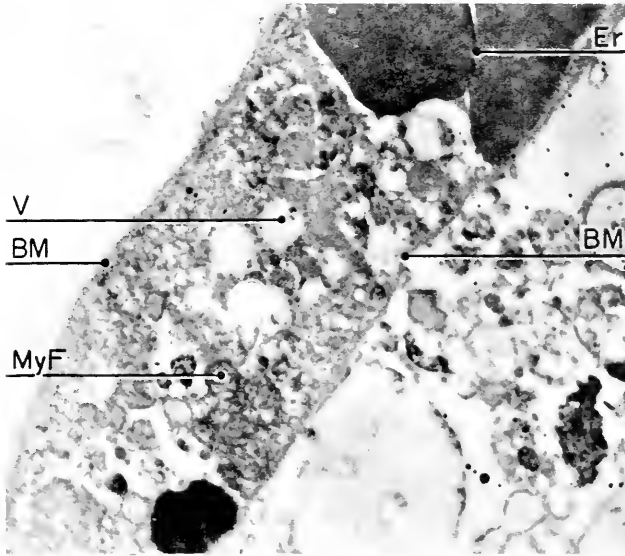


FIG. 12. Cerebral cortex, 168 hr after exposure to 7,500 r showing alterations in a capillary in an area of total necrosis. Basement membrane (BM) is still preserved, while endothelial cytoplasm is swollen and contains vacuoles (V), myelin figures (MyF), and various other types of inclusions. Er, erythrocytes. $\times 10,800$.

Conclusions

The early alterations consisting of astrocytic swelling, plasma exudation, and erythrodiapedesis are all to be considered secondary to increased vascular permeability. It would seem that an acute effect of ionizing radiation is to interfere with basic cellular mechanisms of the cell and, while probably affecting all elements of the central nervous system, to have its most dramatic effect on the blood vessels which manifest this functional derangement by increased permeability, thus allowing fluid and erythrocytes to pass. Changes in the granular cells of the cerebellum similar to those following x-irradiation have been described in a number of conditions where there was definite or presumptive evidence of edema (Leigh and Meyer, 1949; Nötzel, 1955; Olsen, 1959a, b; Schmidt, 1958; Upners, 1939). It is, therefore, entirely possible that the striking change of granular cells is not a direct radiation effect, but is a nonspecific reaction of this type of cell to the presence of edema. A study on ultrastructural alterations in the brain following extensive cerebral trauma has been made in our laboratory, parallel with investigations on the effect of x-ray-induced damage (Hager, 1960). These different, but complementary, studies can profitably be compared when seeking cytologic

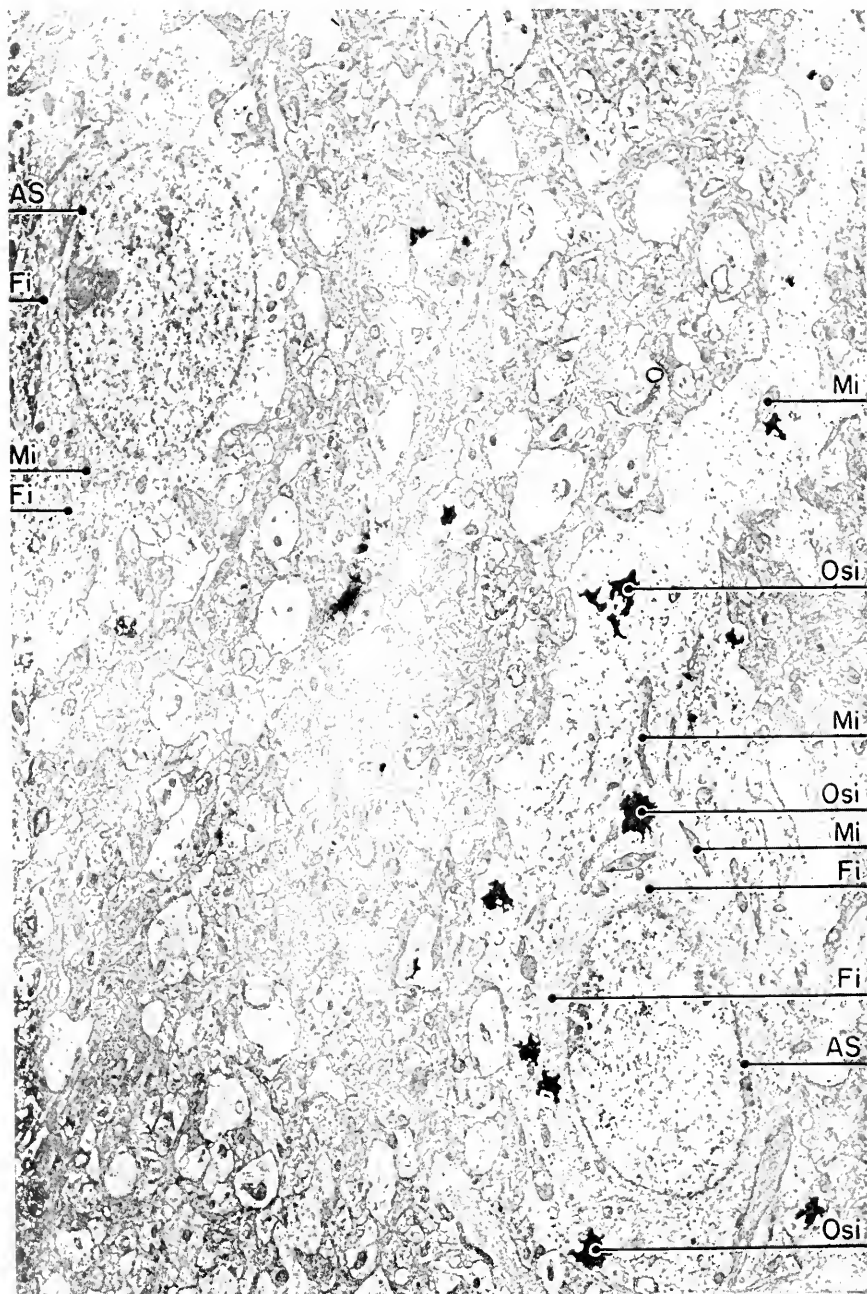


FIG. 13. Cerebral cortex, 218 hr after exposure to 7,500 r. Large reactive astrocytes (As) in the border zone of a necrotic area. Intracytoplasmic fibrils (Fi) can be seen in the perinuclear regions. Osi, osmiophilic inclusions; Mi, mitochondria. $\times 4,000$.

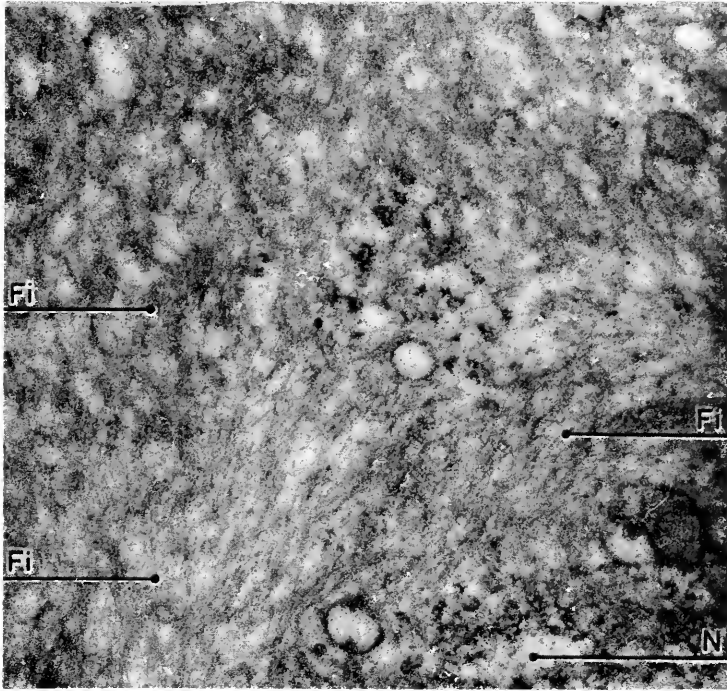


FIG. 14. Cerebral cortex, 218 hr after exposure to 7,500 r showing perinuclear region of a reactive astrocyte. The cytoplasm contains many fine fibrils (Fi). N, nucleus. $\times 21,600$.

alterations that might be specific for ionizing radiation. With our present knowledge, it is not possible to be dogmatic about the significance of observations which, though the result of examining many hundreds of microphotographs, still are based on alterations in only a relatively small number of cells from limited areas. Nonetheless, it appears that in areas of necrosis following x-radiation there is a depression of phagocytic elements in number and activity. Thus, from areas of total traumatic necrosis, the macrophages are filled with vacuoles and inclusions, while in regions of correspondingly complete x-ray induced necrosis, the phagocytes are fewer and their phagocytic function less prominent.

It is possible that ionizing radiation affects the ability of macrophages to proliferate in and around a necrotic area, as well as altering the enzymes involved in the breakdown of phagocytosed material.

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Bioelectric Effects of High Energy Irradiation on Nerve*

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Introduction

There is little in the literature concerning the action of high energy radiation on the bioelectric properties of nerve. Most papers deal with the exposure of nerve to x-rays (Audiat, 1932; Audiat and Piffault, 1934; Audiat, *et al.*, 1934; Bachofer, 1957; Bachofer and Gautreaux, 1959, 1960a, b; Gerstner, 1956; Gerstner *et al.*, 1955; Janzen and Warren, 1942) or beta rays (Gasteiger, 1951, 1952, 1959; Redfield *et al.*, 1922). The goal of this investigation was to determine the dose of high energy radiation that would inhibit the excitatory process of frog's sciatic nerve. Synchrocyclotron-produced 910 Mev alpha particles, and 455 Mev deuterons were employed as irradiation beams.

The effects of high energy alpha particles and deuterons have medical implications because of the increasing application of cyclotron beams to stereotaxic radiosurgery in the central nervous system (Tobias *et al.*, 1952, 1958; Born *et al.*, 1959). In space exploration and in long time exposure projects, such as lunar colonization, the biologic effects of high energy particles might be a limiting factor. Evaluation of this hazard has been speculative. A practical way to study this problem is to engage existing cyclotron facilities for biologic research.

Methods

Frogs (*Rana pipiens*) were housed under low temperature conditions (10°C) for about a week prior to experimentation. They were sacrificed by decapitation followed by spinal cord pithing. Both sciatic nerves were excised from over 200 frogs and placed in Ringer's solution (Mitchell, 1948). One

* This study is based on work performed under contracts with the U.S. Atomic Energy Commission.

nerve of each pair was irradiated, while its companion functioned as a control.

To determine neural activity, a nerve was placed on Ag-AgCl electrodes in a moist chamber (Fig. 1) through which circulated a mixture of 95% oxygen and 5% carbon dioxide saturated with water vapor after passage through 3 gas-washing cylinders. Monophasic, rectangular stimuli 0.1 millisecond in duration were delivered from a Grass stimulator (Model S-5) through an isolation unit to the nerve at 60 pulses per second. Recording electrodes detecting the propagated neural impulse ran to a push-pull, A. C. pre-amplifier (Grass Model P-5), which then fed the signal into a Textronic oscilloscope (Model 532) with a high-gain, differential input amplifier (Textronic type 53/54 D). In conduction velocity studies a fast rise, dual-trace input stage amplifier (Textronic type 53/54 C) was employed. The displayed action potentials were photographed by a Fairchild polaroid oscilloscope camera (Model F-286).

The Lawrence Radiation Laboratory's 184-in. frequency modulated cyclotron was available as a source of 910 Mev alpha particles and 455 Mev deuterons (Tobias *et al.*, 1952, 1958). By appropriate magnetic focusing techniques, these high energy nuclei were made to travel in parallel, approximately monoenergetic beams. An ionizing chamber placed in front of the bombarded nerve was used to monitor the delivered dose (Birge *et al.*, 1956). A summary of the specifications of the 184-in. synchrocyclotron is presented in Table I.

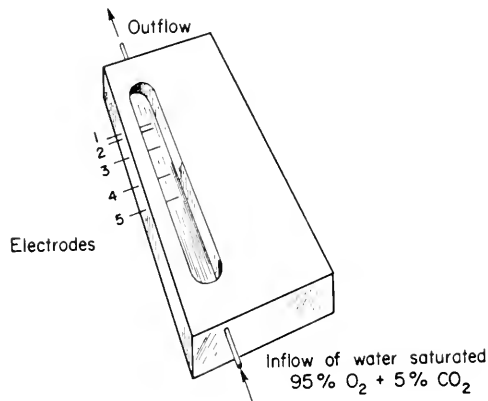


FIG. 1. Lucite chamber for keeping a nerve moist during the study of propagated potentials. Electrodes (1) and (2) are stimulating electrodes, while recordings can be made from electrodes (3) and (5) or (4) and (5). The distances from the first electrode are: 3 mm, 15 mm, 30 mm, and 40 mm.

TABLE I

SUMMARY OF SPECIFICATIONS OF THE 184-INCH SYNCHROCYCLOTRON

<i>Beam particles</i>	<i>Deuterons</i>	<i>Alpha particles</i>
Beam energy—maximum (Mev)	460	915
Beam intensity—average current (μ a)	0.75	0.10
Beam intensity—peak current (μ a)	120	16
Time required for acceleration (msec)	4.5	4.5
Number of revolutions during acceleration	110,000	110,000
Distance traveled during acceleration (miles)	550	550
Velocity at maximum energy (v/c)	0.59	0.59
Mass increase at maximum energy (% of rest mass)	24	24
Range of particles (in. of Al)	12	7
Range of particles (gm/cm ² of tissue)	44	22

Under most experimental conditions, the dose rate received by nerves was 2 krad per min (1 krad = 10^5 ergs absorbed per gm or 1.07×10^3 rep absorbed in tissue). High energy nuclei were generated by the 184-in. synchrocyclotron in pulses of 500 microsec duration with 64 pulses per sec. In special experiments the effect of varying the dose rate of the cyclotron's beam from 0.5 to 8.0 krad per minute was tested to determine if this was a significant factor in altering neural activity. The linear energy transfer (also referred to as stopping power and rate of energy loss) of alpha particles was 15 Mev-cm² per gm (Born *et al.*, 1959), i.e., approximately the same linear energy transfer of secondary electrons from a 250 kev x-ray machine.

Results

BIOELECTRIC STUDIES

In exploratory experiments nerves mounted in a moist chamber were placed in the horizontal path of high energy particles generated by the 184-in. synchrocyclotron. Irradiation of the nerve was beyond the stimulating electrodes (maximum beam diameter was 44 mm). Every 10 krad, the cyclotron's beam was interrupted, and the action potential of the nerve being irradiated was recorded photographically until the electrical activity was abolished. Large doses of alpha particles were required to block excitation. It is now known that there is a serious difficulty with this type of procedure because a greater dose than minimal was received by the nerve to eliminate its electrophysiologic response.

To determine the effect of high energy particles on neural activity, it was deemed prudent to follow the time course of the survival of bioelectric activ-

ity after exposure to some specified dose of irradiation. For this purpose the following method was adopted. After obtaining oscillograms of the preirradiated neural activity of both isolated sciatic nerves of a frog, one nerve of the pair was bombarded in the cyclotron's beam while contained in a plastic vial filled with Ringer's solution. Following irradiation, the neural activities of the exposed and control nerve were again monitored after transferral to a moist chamber (Fig. 1). This routine was repeated at 2 hour intervals for a minimum of 24 hours. Control nerves maintained in Ringer-filled vials were treated in an identical manner.

In Fig. 2 are shown three rows of oscillograms of the action potentials of the right (upper photographs) and left (lower photographs) sciatic nerves of a frog. Preirradiation action potentials were recorded, and the right sciatic nerve was subjected to 72 krad of 910 Mev alpha particles. The left sciatic nerve functioned as a control. Immediately after alpha particle irradiation (oscillograms above "0 hr" in Fig 2), a transformation in the action potential complex of the exposed nerve was apparent. Oscillograms recorded at 2, 4, 6, 8, 10, and 12 hours after irradiation trace the deleterious effects caused by alpha particles. At 14 hours postirradiation, there was complete cessation of the bioelectric activity of the alpha-bombarded nerve, while the action potential of the control nerve was still present.

The spike potential changes for the irradiated and control nerve illustrated in Fig. 2 are summarized in Fig. 3. On the ordinate of Fig. 3 (and also on the ordinates of Figs. 4, 5, and 6) is plotted the percentage of the initial spike potential, i.e., the ratio of the amplitude of the spike potential at some t -hours after irradiation over the preirradiated spike potential amplitude multiplied by 100.

In Fig. 4 is presented a sample of the data obtained for alterations in the neural activity resulting from alpha particle irradiation. It is clear that larger doses of alpha particles (greater than 300 krad) eliminate neural excitability rapidly. With lower doses of 910 Mev alpha particle irradiation, the survival of neural activity is progressively extended. It would appear from Fig. 4 that at 6 hours postirradiation there is considerable enhancement of the neural output. That all this enhancement is a direct consequence of irradiation seems doubtful, because when the irradiated nerve of a pair demonstrated an enhanced neural output, so did its nonirradiated control (Fig. 5). However, bombarded nerves with enhanced activity were usually 5 to 10% higher in their neural output than their controls. The nonirradiated nerves manifested the enhancement phenomena most strongly during the winter season.

The time course for the abolition of neural activity was also studied as a function of deuteron dose. A sample of the findings for the degeneration of the spike potential due to different doses of deuterons is presented in Fig. 6. Deuteron experiments, which were carried out in the spring and summer seasons, showed only a small enhancement of neural output.

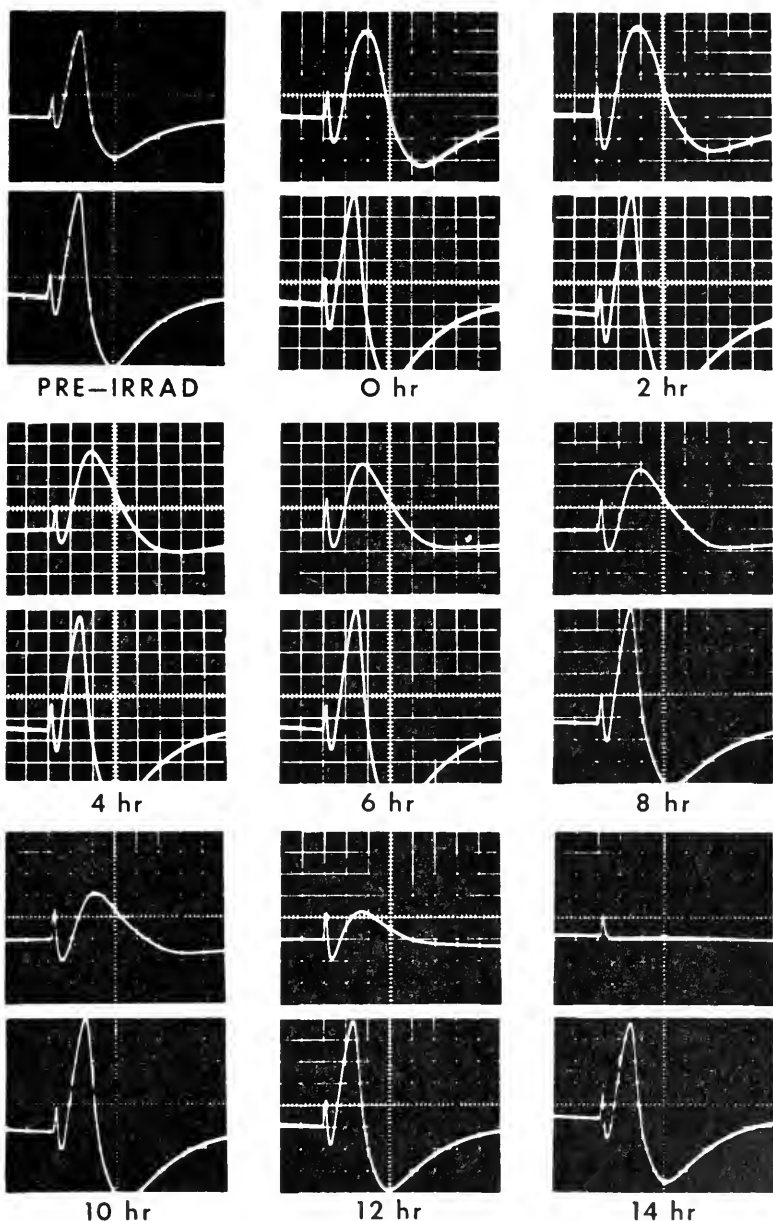


FIG. 2. A composition of action potentials from the right and left sciatic nerves of a frog before and after irradiation. The nerve producing the action potentials in the upper section of each row was exposed to 72 krad of 910 Mev alpha particles. The nerve producing the action potentials in the lower section of each row served as a control. Conduction block occurred in the bombarded nerve 14 hours following irradiation. The oscillograms have 8 units on the vertical axis and 10 units on the horizontal axis. On the ordinate, 1 unit is equivalent to 2.5 mv; on the abscissa, 1 unit is equivalent to 1 msec.

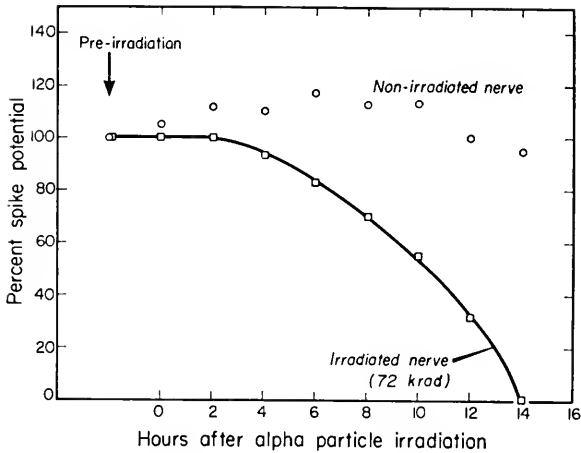


FIG. 3. The percent of the initial spike potential is plotted against postirradiation time for a nerve exposed to 72 krad of alpha particles and for its pair control. The oscillograms in Fig. 2 provided the data for the construction of Fig. 3.

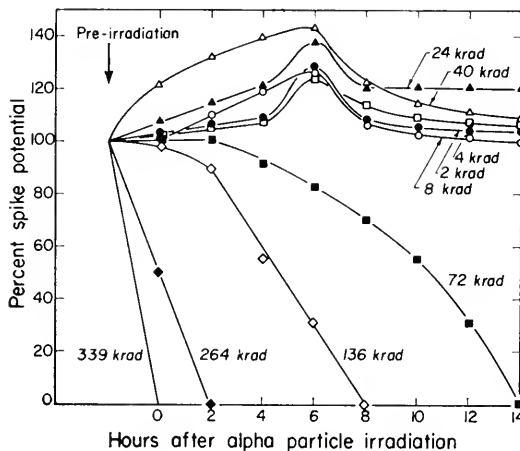


FIG. 4. Percent of initial spike potential plotted against the time after irradiation by 910 Mev alpha particles for doses between 2 and 339 krad.

The relative inhibitory effects of alpha particle and deuteron irradiation on excitability are exemplified in Fig. 7. The time for the complete extinction of spike amplitude is a logarithmic function of the absorbed dose within certain limits. Below 30 krad for alpha particles and 60 krad for deuterons, no demonstrable suppression of the spike potential of sciatic nerve due to irradiation can be reported. Irradiated nerves after more than 24 hours showed

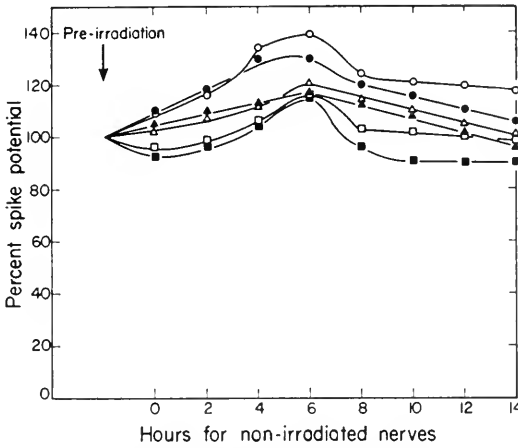


FIG. 5. Nonirradiated control nerves exhibit a variation in the amplitude of the spike potential when plotted as percentage of the initial spike potential.

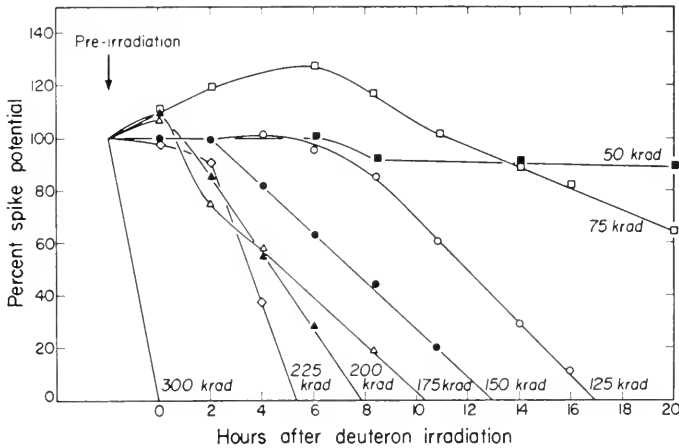


FIG. 6. The time course for the inhibition of the spike potential of nerves bombarded with 455 Mev deuterons in the dose range 50 to 300 krad is pictured. Alterations in the magnitude of the action potentials are given in terms of relative spike activity, i.e., percentage of the initial spike potential.

deterioration of spike potential activity, but the degree of impairment was mimicked by the nonirradiated controls. The slope of the alpha particle dose-survival line is double that of the deuteron line (Fig. 7). This alpha particle-deuteron slope ratio is taken as evidence that alpha particles have twice the relative biologic effectiveness of deuterons.

Conduction velocities of propagated impulses have been computed from

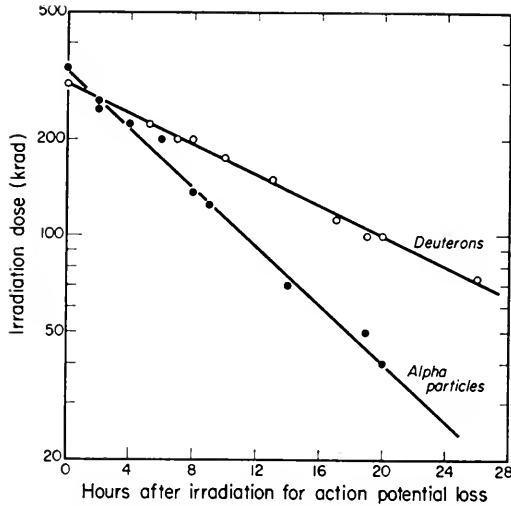


FIG. 7. The logarithm of the dose of irradiation (910 Mev alpha particles and 455 Mev deuterons) is plotted against the survival time of neural excitability. High energy particles given in doses near 300 krad promptly inhibit the action potential of frog sciatic nerve. Above 100 krad each point on the diagram is the mean of 2 experiments; below 100 krad each point is the average of 3 experiments.

the time delay between 2 spike peaks on oscillograms and the distance between recording electrodes. Alterations from irradiation in conduction velocity, latent period, and stimulus strength do not appear strongly related to suppression of the spike amplitude, because when the propagated impulse was 90% abolished, conduction velocity was retarded by only 25 to 30% of its original value, and the stimulus strength and latency period were changed by approximately 25 and 20%. From recent studies employing 2 Grass stimulators (Model S-4), it was found that the refractory period increases (after a small transient decrease) before conduction velocity reduction, action potential depression, latency period prolongation, and stimulus strength alterations and is, thus, the earliest index of radiation damage that the author has noted.

DOSE RATE STUDIES

The influence of modifying the dose rate at which alpha particles were administered to isolated sciatic nerve was investigated. The cyclotron's beam was adjusted to deliver high energy particles at the rate of 0.5, 1.0, 2.0, 4.0, and 8.0 krad per minute in 8 experiments. The survival of excitability was found to be independent of the intensity at which irradiation was absorbed

and dependent on the quantity of dose absorbed. The inhibition of neural activity resulting from irradiation was not reversible.

RADIOACTIVE STUDIES

The influence of alpha particle irradiation on sodium ion permeability of sciatic nerve can be presented here only as a brief, preliminary report. Nerve sheaths were left intact in order to prevent volume changes.

Long life Na^{22} was used as a radioactive tracer in Ringer's solution ($1 \mu\text{c}$ per ml of Na^{22}). The proximal ends of isolated nerves were ligated with 3 mil tantalum wire to allow manipulation of the nerves. To determine the time course for the penetration of radioactive sodium, the nerve was immersed in "hot" Ringer's solution, and the activity accumulated during this soaking period was estimated by removing the nerve from the Na^{22} Ringer's solution to a 4 ml vial of nonlabeled Ringer's. A scintillation spectrometer registered the radioactivity of the sample, and the nerve was restored to the Na^{22} Ringer's solution for additional radioactive tracer uptake. The Na^{22} which diffused from the nerve during the counting time could subsequently be estimated by recounting the vial of contaminated Ringer's solution.

Results revealed that nerves given less than 150 krad of alpha particle irradiation did not differ significantly from their nonirradiated controls in the kinetics of sodium penetration. In the dose range 150-200 krad, the rate of Na^{22} uptake for irradiated nerves was increased to only a small extent over controls (Fig. 8).

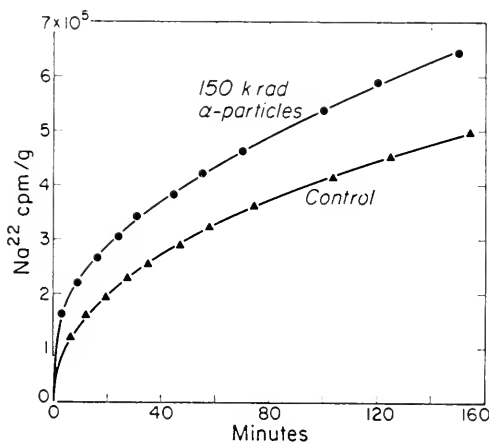


FIG. 8. The time course for the entry of radioactive tracer sodium into sciatic nerve exposed to a 150 krad dose of 910 Mev alpha particles and its pair control. The experimental temperature was $21^\circ\text{C} \pm 1.5^\circ\text{C}$.

The technique for studying the emergence of Na^{22} from isolated sciatic nerve was similar to that described by Shanes (1954). Nerves were immersed in Na^{22} Ringer's solution for approximately 12 hours at 10°C , brought to room temperature (21°C), and irradiated in the beam of the 184-in. synchrocyclotron. The emergence of Na^{22} from the "loaded" nerves into frequently replaced vials of inactive Ringer's was measured with a satisfactory degree of accuracy (counting error less than 1%) by a scintillation spectrometer.

Figure 9 illustrates that after an exposure to 200 krad of alpha particle irradiation, there was a small decrease in the rate of movement of sodium ions from the irradiated nerve when compared to its control. From 8 experiments in which nerves were administered doses below 150 krad of alpha particles, there was no evidence of an alteration in the rates of loss of Na^{22} as a consequence of irradiation.

From these limited radioactive studies, it can be inferred that with alpha particle irradiation in excess of 150 krad there is probably a rise in the sodium ion content of sciatic nerve due to an increase in the rate of sodium ion penetration coupled with a decrease in the rate of sodium ion loss:

In these experiments the studies on the rate of Na^{22} loss began 5 minutes after irradiation was completed, while Na^{22} uptake studies started 1 hour postirradiation.

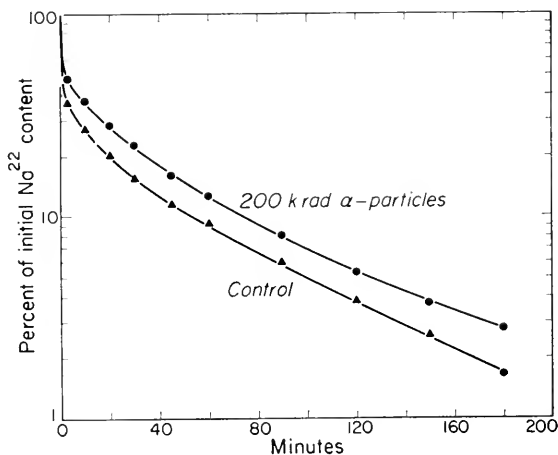


FIG. 9. Decline of the Na^{22} content (percent initial) of sheathed sciatic nerves by diffusion into Ringer's solution. Each of the experimental points on the 200 krad alpha particle line is the average data from 4 experiments, as are the points on the control line.

Discussion

The present findings indicate that irradiation of frog nerve with 30 krad or less of high energy alpha particles or deuterons was below the minimal dose required to evoke an early impairment of neural activity. Such a result is in general agreement with other observations found in the literature. Schmitz and Schaefer (1933) reported no functional damage to frog sciatic nerve when exposed to 10 kr or x-rays. For rat sciatic nerve, no apparent effect on neural conduction after exposure to 10 kr of x-rays has been observed (Janzen and Warren, 1942). Similarly, Rothenberg (1950) administered 50 kr to squid's stellar axon and reported that when the preparation was electrically stimulated, good action potentials were present. From the data offered in this paper, it is reasonable to report that 30 krad of 910 Mev alpha particles represents a threshold dose for the destruction of bioelectrical activity of the amphibian nerve. An explanation on the molecular level which would account for what determines the functional resistivity of nerve to ionizing radiation is not found in the literature.

For a prompt inhibition of bioelectric activity of frog sciatic nerve, about 300 krad of alpha particles or deuterons was required. Using a frog muscle-nerve preparation, Audiat (1932) and Audiat *et al.*, (1934) observed that administering 300 kr of x-rays caused a loss of neural excitability. Gerstner (1955; Gerstner *et al.*, 1956) stated that the sciatic nerve of bullfrog (*Rana catesbeiana*) suffered a conduction block when exposed to about 300 kr of high intensity x-radiation. The neural alterations of mammalian nerve during x-irradiation have been investigated by Bachofer (1957) and Bachofer and Gartereaux (1960a, b), and they established that approximately 500 kr will extinguish the amplitude of the spike potential of the ventral caudal nerve of the rat. Extirpation of axonal activity of the median and lateral single giant nerve fibers of the earthworm (*Lumbricus terrestris*) was shown to occur after 246 and 306 kr of x-rays (Bachofer and Gautereaux, 1959). The neural mechanisms affected by these massive doses of irradiation have not been established.

The sodium influx into squid giant axon immediately after x-irradiation has been reported by Rothenberg (1950) using Na^{24} . After 125 kr, sodium influx was increased markedly. On exposure to 50 kr, the rise in sodium permeability was smaller, but significant. The Na^{22} experiments on frog nerves (described in this report) after alpha particle irradiation are in harmony with the view that irradiation increases sodium ion permeability. However, the alpha particle dose must be near 150 krad to express a sodium permeability increase.

Experiments have revealed that the relative biologic effectiveness (RBE) of alpha particles is twice that of deuterons in inhibiting neural activity. It

is known (Zirkle, 1954) that the linear energy transfer (i.e., the stopping power or rate of energy loss) along a particle's track varies as the square of its charge. The linear energy transfer on an alpha particle is 4 times that of a deuteron of the same velocity. Since biologic effects in general vary with the linear energy transfer, it would be expected that the RBE of alpha particles with respect to deuterons would approach 4 as a limit.

MEMBRANE MODEL

In the following section a membrane model for nerve is outlined with the hope that such a model may suggest how the function of nerve is affected by radiation energy.

Direct evidence of neural membrane structure, in terms of lipid and protein components, must await a detailed study of lipids and lipid-protein systems. Whatever may be the ultimate interpretation of the molecular organization of the axon membrane, it is probably safe to say from electron microscope studies that the unit membrane includes two protein monolayers allied with a double layer of lipid molecules (Schmitt, 1959).

If it is assumed that the protein molecules of the neural membrane are helical in nature and form an oriented structural layer, certain insights into membrane properties are revealed. When 3 protein molecules of macromolecular diameter are closely packed, a 4th element is created—an interstice or fault which for convenience will be referred to as a "channel." When 3 protein macromolecules 28.2 Å in radius are most efficiently packed, an intermolecular channel about 4 Å in radius is obtained (Fig. 10).

It is known that models of membranes based on the concept of a continuous lipid layer are untenable because experiments reveal that biologic membranes are crossed by molecules of water and numerous compounds insoluble in fat. This is a property of a membrane with channels rather than a solution process in a lipid film. Comparison of rates of water entrance into cells under the influence of osmotic pressure gradients and simple diffusion gradients gives a rough indication of what may be the "equivalent channel size" (Koeffed-Johnson and Ussing, 1953; Prescott and Zeuthen, 1953). Values of channels range from 5 Å in red blood cells to 16 Å in squid axons (Nevis, 1957; Solomon *et al.*, 1957). When frog nerve is placed in a medium labeled with deuterium, tritium or O¹⁸, the half time for equilibration is only 1 minute (Tobias and Nelson, 1959). Hence, the existence of a channel pathway through the ultrastructure of cell membranes to water and small ions seems likely.

It has been suggested by Mullins (1956) that the number of water molecules associated with each ion in traversing the neural membrane is limited to the same minimum, say one. In physiologic solutions sodium ions are

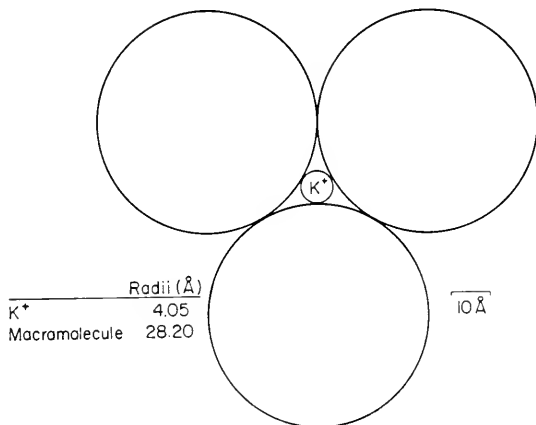


FIG. 10. Protein macromolecules are schematically represented as circles oriented hexagonally. At the junction of 3 macromolecules, an interstice is formed which in the three-dimensional model would be a channel. Drawn to scale it can be seen that potassium with its primary layer of hydration fits the channel created by the macromolecules.

considered to be larger than potassium ions, because sodium ions orient more layers of hydration due to the intense electric field created by the charge on the ion (Ling, 1952, 1957). Potassium ions with a lower energy of hydration than sodium have effectively fewer oriented shells of hydration and hence a higher mobility in an aqueous medium. Kortum and Bochriss (1951) point out that for cations, water molecules on the first layer (primary hydration) are held so tightly that the primary hydration shell moves as a unit with the ion. However, water molecules beyond the primary hydration layer are loosely oriented and exchange readily with surrounding water molecules. Hence, it is an acceptable hypothesis that ions with one layer of hydration migrate through neural membranes. From Fig. 11 it is seen that the radius of primary hydrated potassium is 4.05 \AA which is larger than primary hydrated sodium (3.67 \AA in radius; Fig. 12). The crystal lattice radii are taken from Pauling (1945), and the width of concentric water shells of hydration is that of the diameter of a water molecule, 2.72 \AA (Buswell and Rodebush, 1956).

The intermolecular forces between protein elements of the membrane are no doubt subjected to lateral straining pressures produced by thermal motion (kinetic and vibrational) and cytoplasmic streaming. As a consequence, a channel is never a fixed size, but statistically distributed, probably in a Gaussian fashion. The mode of the channel size distribution of the resting neural membrane is assigned to the ion empirically known to have the high-

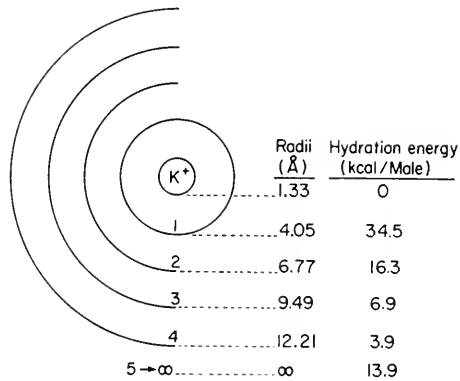


FIG. 11. Representation of the potassium ion with a crystalline radius of 1.33 \AA and the 1st, 2nd, 3rd, and 4th hydration shells. The diameter of a water layer is taken as 2.72 \AA . Hydration energies for a given hydration shell are computed on the basis that hydration energy exponentially decreases with the distance from the charge on the ion.

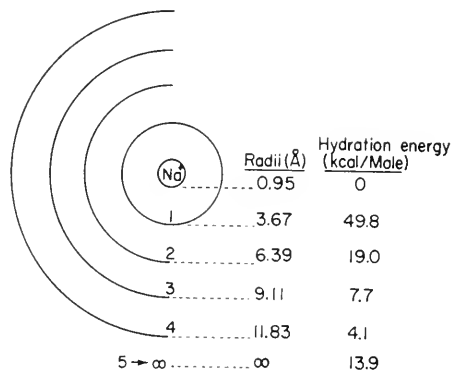


FIG. 12. Representation of the sodium ion with a crystalline radius of 0.95 \AA and the 1st, 2nd, 3rd, and 4th hydration shells.

est relative membrane permeability, potassium (see Fig. 13, resting state). The spread of the Gaussian distribution curve for the resting state is adjusted so the area representing potassium ion channel is 25 times greater than the area representing sodium ion channels, which is in harmony with Hodgkin and Katz's (1949) evidence that the relative permeability of potassium to

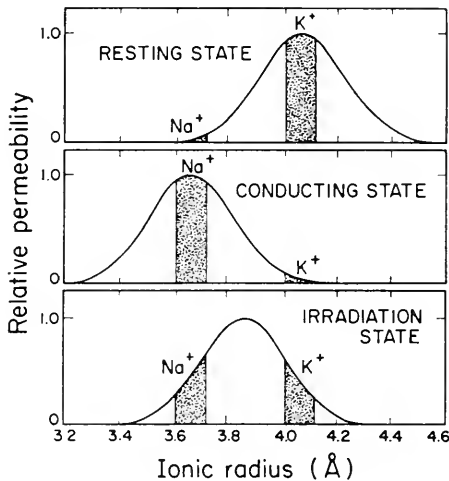


FIG. 13. The relative permeability of an ion (ordinate) is considered a physiological term interchangeable with density of channel size. The ordinate on these diagrams could just as conveniently read "number of available channels per unit area of membrane." It is a reasonable assumption that channel size (abscissa) is distributed according to a Gaussian curve. For neural membranes it is assumed the mode of the distribution curve is (a) in the resting state that of a potassium ion, (b) in the conducting state that of a sodium ion, and (c) in the irradiation state somewhere between (a) and (b).

sodium ions across the axonal membrane is 25 to 1. During excitation these relations are reversed, i.e., the permeability of potassium to sodium is 1 to 25. Hence, the mode of the distribution curve during activity is assigned to sodium (Fig. 13, conduction state).

The concept that the neural membrane behaves as though it were a molecular sieve is not practical. Such a proposal does not offer an explanation of how the cell discriminates between potassium and sodium ions as indicated by permeability studies. A molecular sieve model for the membrane permits a small ion to pass through any channel of greater size than itself. On this basis, sodium ions 3.67 Å in radius should have free permit through channels that sterically just pass potassium ions 4.05 Å in radius. Hence, a molecular sieve model fails to explain selective ion permeability. This difficulty is removed if solvation (interaction with membrane components) is "quantized." That is, an ion on entering a channel has all of its hydration shells beyond the primary hydration level solvated by the wall of the

channel (Mullins, 1956). It is maintained that only whole shells of hydration for ions are replacable by the membrane's wall in the process of solvation. In this sense, a 3.67 Å sodium ion would not "fit" into a 4.05 Å potassium ion channel because the quantum solvation provided by the wall of the potassium ion channel does not match a sodium ion. This could be true only if some level of hydration (secondary, tertiary) for sodium did uniquely match some hydrated size of potassium. By similar reasoning, potassium ions do not fit sodium size channels. A comparison to Bohr's theory in which electrons exist in integral energy levels is only an analogy, but it may help one's thinking. For the membrane's channel wall, it is held that instead of an infinite number of solvation levels, there are only a restricted number with properties represented by functions of n , where n is an integer.

If the quantized view of membrane solvation is correct, and ions penetrate the membrane with only the primary layer of hydration, then it will cost the cell more in solvation energy to transport sodium (44.7 kcal/mole) than potassium (40.5 kcal/mole). On the evolutionary scale it would appear that the cheaper ion was selected to balance the intracellular negative charges.

The initial suggestion of a helical protein structure does not violate our knowledge about the architecture of proteins. In terms of our membrane model, the helical nature of protein provides a key to the interpretation of the excitation phenomena of axons. Protein membrane molecules are conceived to be in a contracted or coiled state, while the nerve is in the resting state. A threshold stimulus permits the constrained, helical macromolecule to become relaxed or uncocked, thus diminishing the radius of the macromolecule. Intermolecular attractive forces maintaining membrane structural order cause a decrease in the mode of the channel size distribution when coiled macromolecules uncock. If, on stimulation, the membrane macromolecules alter their radii from 28.2 Å to 26.2 Å, the new mode of the channel size distribution will be 3.67 Å, the size of primary hydrated sodium ion (see Fig. 13, conducting state). It is naive to imagine that the helical protein molecules have characteristics of a mechanical spring. A coiled spring can be stretched a good deal before a decrease in radius is effected. The reduction of the radial dimension of the membrane's helical molecules is perhaps due to the action of London forces.

A pleasing consequence of this membrane model is the number of neural characteristics it can interpret. The all-or-none law for axons on the molecular level can be viewed as the states which the membrane helical macromolecules can occupy; either a stimulus is sufficient to uncock the macromolecule, or it is not. If the stimulus is sufficient, the channel size mode shifts from potassium to sodium and ions follow their electrochemical gradient generating a bioelectric impulse. Molecularly translated, the refrac-

tory period of a nerve is the time element required to restore the helical macromolecule to the constrained state.

Hodgkin and Katz (1949) have presented evidence showing that at rest the ionic permeability of potassium is 25 times that of sodium. During excitation these ionic permeabilities are quickly reversed, so that sodium is 25 times as permeable as potassium. The burden of accounting for this sudden ionic shift has undone many an ingenious membrane hypothesis. The cocked-uncocked performance of helical molecules in the present membrane model supplies an adequate explanation not only for the permeability events triggered by excitation, but also for the time constants for the limbs of the action potential.

The character of the cocked protein molecule is such as to allow for a rapid change to the relaxed structural state, thus accounting for the fast time constant of the ascending limb of the action potential. To "reconstrain" the relaxed helical macromolecules suggests a need for an energy input. Since the ratio of heat produced during activity over that produced in recovery shows that the latter requires most of the energy, we have another observation that does not violate the model, but agrees with it.

Developing a membrane model has made the task of interpreting how radiation energy influences neural functioning, relatively easy. Only an average energy of some tens of electron volts can be accepted by a molecule. Successive energy transfers occurring along the path of high energy particles (kinetic energies in the thousand or million electron volt range) supersede the acceptable energy level, and a defective molecule is the consequence. For conducting axons, it is construed that the most radiation-labile molecules are the membrane protein macromolecules. When the structure of these molecules is damaged, excitability is affected.

Radioactive tracer studies of resting nerve (Rothenburg, 1950; also see *Radioactive Studies* in this paper) reveal that radiation causes an increase in sodium ion permeability, i.e., a shift in the mode channel size from potassium toward sodium (see Fig. 13, resting state versus irradiation state). Bioelectric studies on single myelinated nerve fibers after irradiation (Gaffey, 1960) indicate that there is an increase in potassium ion permeability (revealed by a decrease in the slope of the falling limb of the action potential) and a decrease in sodium ion permeability (connoted by a decrease in the slope of the rising limb of the action potential). The deterioration of selective ion permeability in the conducting nerve is viewed as a translation in the mode channel size from sodium toward potassium (Fig. 13, conducting state versus irradiation state). These early signs of neural impairment would be expected as a consequence of the partial loss of the ability of the helical molecules of the membrane to fully coil-uncoil. The terminal degener-

ative steps due to radiation take place rapidly and could be interpreted as a full loss of the membranes' helical molecules capacity to change states, perhaps as a result of a loosening of their structure. This would cause a broadening and flattening of the channel size distribution curve, which in essence eliminates selective ion permeability, thus producing a rapid loss of excitability.

In conclusion, it can be argued that certain doses of radiation are threshold for neural injury as a result of the membrane's macromolecules being irreversibly impaired in their ability to change states.

Summary

Isolated sciatic nerves from *Rana pipiens* were exposed to cyclotron accelerated beams of 455 Mev deuterons and 910 Mev alpha particles. The degree of electro-physiologic damage was found to depend on the dose of irradiation and the elapsed time from irradiation.

With massive doses of alpha particle or deuterons (greater than 300 krad) the action potential of the frog's sciatic nerve was promptly suppressed.

Within the range 30 to 300 krad for alpha particles and 60 to 300 krad for deuterons, the survival time of the action potential was a logarithmic function of the absorbed dose.

Alpha particles were found to have twice the relative biologic effectiveness of deuterons in blocking excitation.

An increase in the refractory period was manifested before conduction velocity reduction, action potential depression, latency period prolongation, and stimulus strength alteration in the high energy irradiated nerve.

Alpha particle irradiated nerves were shown not to increase in sodium ion permeability for doses less than 150 krad. Between 150-200 krad the rate of Na^{22} penetration was slightly increased, while the rate of loss of Na^{22} from the nerve was decreased.

Variations in the exposure rate from 0.5 to 8.0 krad per min failed to induce a dose-rate effect for alpha particles.

The inhibition of neural activity resulting from alpha particle and deuteron irradiation was not reversible.

A model for the neural membrane was outlined, and the action of radiation was interpreted on the basis of this model.

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Morphologic and Pathophysiologic Signs of the Response of the Nervous System to Ionizing Radiation

(Review of main works published in the USSR)

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Introduction

The decisive role in the complex interrelationship between animal organisms (including human) and their environment belongs to the nervous system. All environmental agents, whatever their nature, address themselves in the first instance to the nervous system.

The degree to which an animal or human organism reacts to various harmful stimuli is determined by the properties of functional mobility and working unity of the vegetative nervous system with the endocrine system of the organism and by the interdependence between these systems and the peripheral and central nervous system.

During the last few decades a new environmental factor has appeared—ionizing radiation. The degree of concentration of this radiation in the environment is inevitably increasing owing to the further development of atomic weapons, the growth of powerful installations, electric power plants, ships, and submarines which process radioactive substances, and the increasing quantity of scientific research using radioactive substances. The result is that an ever increasing number of people are being subjected to the action of ionizing radiation.

To protect man from the harmful action of ionizing radiation we need thorough investigation and accurate knowledge of the mechanism by which ionizing radiation acts on the animal and human organisms. Once this is known, a series of preventive and therapeutic means can be suggested which would eliminate or reduce the force of the harmful action of ionizing radiation on the organism as a whole or on particular systems and organs. It is generally admitted that ionizing radiation attacks the human organism first

* Much of the work entailed in compiling this review and the attached bibliography has been done by the staff of the laboratory of Dr. B. M. Hecht and Dr. A. M. Wein.

and chiefly through the nervous system, in particular the vegetative nervous system, and the blood and its principal morphologic elements.

Owing to the special conditions under which physiology has been developing in the U.S.S.R. and the exceptional attention paid to the leading role of the central nervous system in the physiologic functions and in the development of disease pathogenesis in man, a great deal of research in the U.S.S.R. has been devoted to the morphologic and structural changes which represent the nervous system's response to ionizing radiation.

This review does not cover all the published literature but merely gives an idea of some of the main publications on this subject.

Morphologic Changes in the Nervous System Under the Effect of Ionizing Radiation

Our ideas about the resistance of the nervous system to radiation are based mainly on the negative morphologic picture observed after irradiation. A definite discrepancy between the morphologic and the functional shifts has come to light, providing evidence as to how the nervous system reacts to radiation. A number of factors lie at the root of these apparent contradictions. For one thing the methods in use today for studying the morphology of the nervous system are fairly crude and not always adequate for the purpose of estimating slight shifts occurring in the tissues. Progress in this field, therefore, depends on the use of more precise methods of investigation, particularly histochemical methods. Further, in cases of extremely serious lesions, the death of the animal occurs quickly, and the morphologic changes do not have a chance to develop over a long period. Finally, in recent years fairly definite morphologic changes which account for shifts of a neurodynamic character have been demonstrated.

In the opinion of many Soviet investigators, the term radiosensitivity is not a happy expression. It would be more correct to speak of *radioreactivity*, which would be described in terms of a change in some functional reaction on the part of the nervous system, and of *susceptibility to radiation damage*, which would be described in terms of morphologic symptoms.

When animals are subjected to large doses of radiation which produce the pattern of radiation disease, the following phenomena are seen in the central nervous system: swelling of the cells, argentophilia, displacement of the chromophil substance at the height of the disease, and hemorrhagic diathesis, often in the thickness of the meninges. Degenerative necrotic changes, such as vacuolation of the ganglionic cells, increased precipitation of silver, distension of the cells, and changes in the processes (Kraevsky, 1957), are observed primarily in the zones of the higher vegetative subcortical centers. Some authors have not detected any reaction on the part of the microglia (Kraevsky, 1957), while others found dystrophic changes (Aleksandrovskaia, 1959).

It has been demonstrated that there is a relationship between the size of the radiation dose and the character of the morphologic changes (Aleksandrovskaya, 1959). With a dose of 650 r, cerebral anemia occurs macroscopically, with hyperemia in some parts of the brain. Histologically, dystrophic changes can be detected in the cortex, subcortical ganglia, and hypothalamus and vacuolation accompanies partial destruction of the nerve elements in the corpora quadrigemina, nuclei of cranial nerves, and structures of the reticular formation. Swelling or edema becomes established, and the nerve fibers break up. Wrinkling of the astrocytes takes place. Aleksandrovskaya (1959) characterizes the above picture as toxicohypoxic encephalopathy with dystrophic changes of the nerve cells and productively dystrophic reactions on the part of the glia.

All these changes were deeper and more diffuse after irradiation with a dose of 1,300 r. Subarachnoid and perivascular hemorrhages were observed. Portugalov (1957) found the most substantial changes in the diencephalon and mesencephalon. Several authors have pointed to morphologic changes (necrobiosis) of particular cell groups in the cortex, accompanied by chromatolytic and karyolytic phenomena [Kurkovsky, 1958 (see in this review the following authors: Kyandaryan; Papoyan; Beglaryan, Zagotskaya, Arutyanyan, 1957)]. Bibikova (1959) obtained acute or chronic demyelination after irradiating dogs with 5,000–30,000 r.

Smirnov (1960) observed changes in the distribution and quantity of tigroid substance (Nissl bodies) in the neurons of the reflex arc, an index to the functional condition of a neuron.

Manina (1959) demonstrated the destruction of neuroblasts and severe disturbance of the architectonics in different parts of the cell as a result of irradiation with radioactive phosphorus (P^{32}).

Definite morphologic shifts occur under the effect not only of large doses of radiation but also of single or repeated small doses (Aleksandrovskaya, 1957). These shifts take the form of proliferation responses by microglia and ectodermal glia. Profound morphologic changes are observed in the progeny of rats irradiated with a dose of 150–200 r during the antenatal period.

Shabadash (1957) has demonstrated that irradiation with a dose of 25–100 r causes a shift of -0.3 to -0.4 in the pH of the nucleoproteins. The response is detected first in the mitochondria, then in the tigroid substance. These changes are caused by depolymerization processes and reduction in the quantity of nitrous bases. A typical feature is that the huge number of mitochondria in the diencephalic region and the reticular formation suffer from ribonucleoprotein deficiency as a result of irradiation; this indicates local respiratory disorders. There is undoubtedly a good prospect that this cytochemical change will be useful in revealing the mechanism by which ionizing radiation affects the organism.

These findings are evidence that morphologic changes occur in the brain

after radiation. Not all sections, however, are equally damaged (the marked changes in the higher vegetative centers of the hypothalamus are noteworthy), nor are all the structures which form part of a given section equally involved. Portugalov (1957) attempted to account for this on the grounds that those microstructures which were in an active condition at the moment of irradiation suffered most damage.

It has also been demonstrated that there are two stages in the development of morphologic changes in the brain which occur during the first days of the disease and at its climax. In the first period, the changes in the nervous system are regarded as the result of direct action by radiation on the nerve elements. As to the second period (late necroses), some authors are inclined to regard these changes as the result of a hemodynamic disorder, while others consider them the result of a metabolic disorder and a manifestation of toxic elements. The latter hypothesis seems to be better founded, since sometimes there are no hemodynamic disorders, while it has been demonstrated that definite substances of lipoid origin, hemolysins, are present. They are formed when tissues are subject to direct irradiation (Tarusov, 1957; Benevolensky, 1957).

The radiosensitivity of nerve elements in young animals and embryos is not disputed, and there is a considerable amount of literature to attest to it (Zavarzin *et al.*, 1936; Emdin and Shefer, 1935; Olenev and Pushnitsyna, 1952; Manina, 1959; Aleksandrovskaia, 1959).

It is possible to determine the morphologic changes which occur in the peripheral nervous system during irradiation of the organism. Oleinikova (1936) observed degenerative changes in the nerve trunks only 30–60 minutes after irradiation. These changes reached a peak at the height of the radiation disease. The nerve fibers broke up into segments; some were in a state of granular decay, and partial demyelination phenomena were observed. Changes were also noted in the cells of the Auerbach and Meissner plexuses. In 1953, Lebedev showed that Auerbach's plexus was involved and that Meissner's plexus had great stability. He noted, however, reactive and destructive changes in the sensory cerebrospinal and craniocerebral (trigeminal and vagal) ganglia and in the sympathetic ganglia.

Anisimova and Aleksandrova (1959) consider the afferent nerve channels and their terminal receptor apparatus to be the most sensitive. Kraevsky (1957) also notes that of all the parts of the peripheral nervous system it is the peripheral parts of the sympathetic nervous system which are most susceptible. Gracheva (1959) is the only author who has not detected convincing morphologic changes in the nerve cells of the vegetative and sensory ganglia. Damage to the peripheral nervous system, accordingly, consists primarily in disorders of the constituent parts of the vegetative nervous system.

Pathologic Signs of the Action of Ionizing Radiation

One cannot discuss the effect of ionizing radiation on the functioning of the nervous system without mentioning the early research on this subject in the Pavlov-Nemenov laboratories in the late 1920's. This was the period when the view that the nervous system is highly radioresistant was widely propagated in the literature—an opinion held to this day by a great many foreign authors, but based on the morphologic research of that time.

Nemenov (1932, 1950), however, by using the conditioned reflex method was able to demonstrate that this view was incorrect and to provide confirmation that the nervous system is radiosensitive. Today we have accumulated extensive information on disturbances of the higher nervous activity by exposing various experimental animals and human subjects to radiation and studying its effect on their conditioned reflex activity. As this material has been dealt with in a number of reviews and monographs, I shall confine myself to outlining the results obtained from work done in the last few years.

These researches have shown that various types of ionizing radiation lead to phase changes in the neurodynamics of the cortex. During the initial periods the cortical activity is stimulated, but later, depression sets in. The duration of each period depends both on the condition of the nervous system and on the dose and characteristics of the radiation.

In recent years the work of Kotlyarevsky and co-workers (1957), Livshits (1955, 1956a, b), Lomonos (1953), and Piontkovsky and co-workers (1957) has provided confirmation that phase changes occur in the higher nervous activity after whole body exposure of experimental animals to small or medium doses of radiation. Lomonos (1959) noted phase changes in the conditioned reflex activity after the body of a dog had been exposed first to a single and then to repeated doses of radiation with its head shielded. In several cases, prolonged disturbances of the conditioned reflex activity have been observed without any clinical signs of radiation sickness.

Klimova (1957) observed persistent changes in the higher nervous activity without clinical symptoms of radiation sickness following internal irradiation of animals (dogs) fed $1 \mu\text{c}$ each of radioactive strontium for 6 months. She found that the conditioned reflex activity was at first heightened, then depressed, and that phase conditions appeared in the cerebral cortex. Four to 6 months after irradiation, similar disturbances were observed in the unconditioned reflexes. Restoration of the nervous activity took place unevenly. The motor defense reflex was the first to return to normal at the end of the 1st month, followed by the motor food reflex, and 2–2½ months later by conditioned reflex activity. Various defects were still observed in the dogs' higher nervous activity even 6 months after the experiment.

With large doses of ionizing radiation, the initial phase of cortical activa-

tion may not occur. Minaev (1957), for example, observed no phase during which the excitability of the brain increased when he exposed the head of a dog to a dose of 7,000 r.

Of great practical interest is the research which has been done on the influence of small doses of ionizing radiation on the conditioned reflex activity of the brain. More and more evidence is accumulating to indicate that small doses of 5 to 10 to 20 r lead to changes in the higher nervous activity, mainly to disturbance of internal inhibition, disinhibition of the capacity to differentiate, and disturbance of the inhibiting conditioned reflexes. Gorsheleva (1958), Airapetyants (1958), Malyukova (1958), and Rokotova and Gorbunova (1958) noted a change in the conditioned reflex activity after the administration of an even smaller dose (2 r).

Even such small doses of radiation are capable of having a cumulative effect. Malyukova (1958) and Meizerov (1958) have shown that systematic exposure to x-rays in doses of 3–15 r led to serious disturbances of the higher nervous activity when the total dose reached 130–190 r. Tests performed on human subjects by working with radiation doses approaching the permissible limit and using the speech-motor method have also revealed definite disturbances of the higher nervous activity: delay in forming the conditioned reflex connections, prolongation of the latent period of the conditioned reflex, and a number of other signs indicating loss of mobility and equilibrium in the nervous processes (Morozov, Drogichina *et al.*, 1957).

Voevodina (as quoted by Cherkasov, 1960) has shown that even after rats have been exposed to x-radiation in a 10 r dose, the conditioned reflex activity is disturbed. After the rats had been exposed to a triple dose of 10 r, the conditioned reflexes could not be elicited for 42 days. Prolongation of the latent period and disturbance of the capacity to differentiate were still observed for the next 6 months. Even many months after the brain had been exposed to this dose, functional weakening of the nervous system caused by the injection of morphine again led to disturbance of the conditioned reflex activity.

Work done in Kupalov's laboratory established the fact that after animals were subjected to small doses of radiation, aminazine (equivalent to chlorpromazine) in a dose of 0.5 mg per kg caused acute excitation in the animal, with serious disturbance of the conditioned reflex activity; whereas before irradiation, similar or even larger doses of aminazine caused only some depression of the cerebral activity. This led Kupalov to the conclusion that the nervous system goes on acquiring other properties for a long time after irradiation and possibly even permanently.

Electrophysiologic methods have yielded further evidence confirming what has been discovered about the radiosensitivity of the nervous system by the conditioned reflex method.

In a series of papers published during the last few years, Livanov and

co-workers (1959) and several other authors have described results obtained by using ordinary EEG and corticogram traces or from investigations performed with implanted electrodes. EEG's show that in rabbits and dogs exposed to whole body irradiation in large doses (about 1,000 r), the bioelectric activity and the irritability of the brain tissue are at first heightened and subsequently lowered. Hypnotic phases are also observed in various parts of the brain.

Grigoryev (1958) found that variations in the EEG's of human subjects, taken during therapeutic exposure to x-rays, could be detected 18 sec after the beginning of the treatment. This effect occurred irrespective of whether the head, abdomen, or whole body was exposed to the x-rays. After the first exposures, however, the excitability of the nervous system was observed to increase: subliminal (3-irradiation) stimuli gave distinct physiologic effects, and the stimulation thresholds fell from between 6 and 12 sec to between 2 and 4 sec.

Further irradiation led to a drop in the responsiveness of the cortex. The number of paradoxical and ultraparadoxical responses to a strong light stimulus increased. Stimulation by caffeine, instead of ordinary activation of the electrical potentials, lowered the bioelectric activity; this indicates a lowering of the working capacity of the nerve cells. Toward the end of treatment, depression of the bioelectric activity usually set in, and the alpha rhythm became faster and more widely diffused throughout the cortex. The results of the first exposures were not stable and soon disappeared. Subsequent exposures produced radiation effects which lasted much longer. Comparing his findings with the dynamics of the excitation and inhibition processes discovered by other methods, Grigoryev (1958) says that after irradiation, the stimulatory process intensifies first, then diffuse inhibition occurs.

On the theoretical level, Grigoryev's observation that vegetative rhythms appear in the cortex after irradiation, duplicating in frequency the cardiac and respiratory rhythms, is of distinct interest. In his opinion and in that of several other authors, the emergence of this rhythm indicates that the cerebral cortex is being more intensively influenced by excited subcortical formations.

Geinisman and Zhirmunskaya (1953) and Grigoryev (1958) stress the fact that variations in the EEG are less pronounced after each consecutive x-ray sitting and regard this as a manifestation of the central nervous system's adaptation to repeated exposures by developing compensatory mechanisms. Nevertheless, along with the adaptation phenomena there cannot fail to be an accumulation of the variations occurring in the central nervous system under the effect of ionizing radiation.

Grigoryev (1956) and Tsy-pin (1956) found that when rabbits were exposed to whole body gamma radiation, even doses of 0.05–1.3 r caused distinct changes in the electric activity of the brain. In many animals the

cortical responses were at first enhanced when tested by photic stimulation, but when the dose was increased to 7.8 r, cortical responsiveness and excitability diminished to the point at which there was no response at all to light stimulus.

It follows from the research described above that even small doses of radiation energy (0.05 to 2, 5, or 10 r) are sufficient to cause detectable trace functional changes in the nervous system. The accumulation effect indicates that physiologic changes are at the root of this phenomenon. It can be assumed that even the natural level of radiation leads to similar changes, although this will overlap with repair processes in the nerve tissues.

The changes in the nervous activity described, occur under the effect of whole body radiation and under local irradiation of the head (Meshchersky, 1958; Fanardzhyan *et al.*, 1960).

The dynamics of the EEG's display certain characteristic features which make it possible to differentiate the reflex from the local responses of the brain to irradiation.

Grigoryev (1958) notes that a lowering of the electrical potentials is observed following irradiation of the head. In whole body irradiation, the EEG response depends on the initial background, and the variations in the EEG are more generalized and as a rule localized in the opposite hemisphere to the one which is irradiated.

With local irradiation of the temporal regions, variation in the electrical activity is observed within the irradiated zones of the brain or, at any rate, predominantly in these. We can therefore assume that the variations observed in the EEG's are both local and reflex in etiology.

The considerable shifts observed in the interrelationship of the vegetative and endocrine systems as a result of radiation lesions are naturally leading investigators to pay attention to the influence of ionizing radiation on the functions of the hypothalamic and diencephalic parts of the brain and on the activities of the different links in the vegetative nervous system.

Work done in the laboratory of Livanov and Efremova (1957) has shown that 1 to 3 days after single whole body exposure (1,000 r), there is an abrupt change in the bioelectric activity of a rabbit hypothalamus. The variations in potential become more intensive, and there are recurrent spasmodic discharges in sharp waves. The response of the cerebral cortex to hypothalamic stimulation undergoes sharp variations and at times is even distorted. There is a lowering of the thresholds of the stimulations required to elicit the characteristic behavior responses (sniffing, licking the lips). The rise in bioelectric activity is subsequently replaced by a lowering.

Phase disturbance of the hypothalamic activity after mass irradiation of experimental animals has also been noted by Smirnova (1958) and Kondrateva (1957).

There is an increasing amount of evidence that other links in the vegeta-

tive nervous system are also involved in radiation diseases.

Klimovskaya (1958) noted increased lability of the preganglionic fiber of the cervical sympathetic nerve after irradiation.

Nakhil'nitskaya (Lebedinsky's laboratory, 1959) demonstrated that the lability of the postganglionic sympathetic nerve is reduced after irradiation. This enabled Lebedinsky (1959) to suggest that "discoordination" of the different vegetative apparatus may play a considerable part in the genesis of responses to radiation.

Disturbances or distortions of the vegetative responses from certain reflexogenic zones may constitute one of the major factors in the genesis of responses to radiation.

Popova noted in 1954 that the reflex reaction of the blood process and respiration in response to stimulation of the gastric and rectal interoceptors of cats was first heightened and then lowered after the animals' heads had been exposed to 1,500 r. In 1954, Yaroshevsky noted a disturbance of the reflex leucocytosis under similar conditions.

In 1957, Chernichenko, experimenting on rabbits, showed that exposure to 600 r leads to a phase variation in the vegetative reflexes of the urinary bladder interoceptors. Komarov established in 1957 that the thresholds of the stimulation required for reflex heightening of the blood pressure were raised as a result of irradiation.

A number of other authors have noted postirradiation variations in the pressor responses to adrenaline, a distortion of the response to Corazol (Metrazol) and lobeline, and a lowering of sensitivity in the chemoreceptors in response to a rise in the CO₂ level in the blood.

An observation of great interest was made by Kondrateva in 1957 in connection with the change in the character of the vasomotor and respiratory responses to direct stimulation of the hypothalamus after radiation, from which we may assume that the regulation of a number of physiologic processes is distorted in the course of a radiation disease, precisely because of a disturbance of the reflex irritability of the hypothalamus.

Another factor closely connected with functional disturbances of the central vegetative apparatus, and possibly even depending on its function, is the severe variations in the activity of the endocrine organs. In this connection, great interest attaches to the functional disturbances of the adrenal cortex noted by several authors, such as Tonkikh in 1958.

Preliminary investigations performed in our laboratory, indicate that the cortical layer of the adrenal glands is involved in the response to radiation.

As the scope of investigation widens in regard to both objects and methods, our knowledge of the influence of radiation on various parts of the nervous system is steadily expanding. Today we have convincing evidence of the influence of ionizing radiation on the activity of the trunk apparatus of the nervous system and the cerebellum.

Mushegyan and Abovyan showed in 1950 that radiation of the optic thalamus in frogs leads to characteristic inhibition of the spinal reflexes.

In 1957 Yanson established that, after rabbits had been exposed to 1,000 r of x-radiation, a phase change began to occur in the condition of the labyrinthine and cervical tonic reflexes. This change intensified during the first 2 days but was unstable from the 3rd day on. During the first 3 days, a development of the plastic tonus in the extremities was observed and maintained for 7–10 days.

Biryukov (1957) assumes that ionizing radiation acts on the reticular formations of the brain stem, basing this view on the similarity between certain physiologic effects produced by the action of aminazine and those produced by ionizing radiation in birds.

Livanov and co-workers (1959) regarded the diffuse character of the EEG variations observed when the organism is exposed to ionizing radiation, as grounds for assuming that the reticular system of the brain stem must be included among the structures which condition the responses of the nervous system to radiation. The EEG was similar to that of a desynchronization response. Livshits, exposing the cerebellar region to directed x-rays, observed sympathetic phenomena similar to the responses elicited by direct stimulation of that organ.

The influence of ionizing radiation on the function of spinal mechanisms is widely known from the literature, but new discoveries have been made in the last few years. Fedorova (1958) and Kudritsky (1957) have shown that even small doses of radiation (10 r) lead to a change in the time taken by the flexor reflexes of the posterior extremities of rabbits. When the rabbits were exposed to daily radiation doses approaching the permissible limits (0.1–0.5 r) for 14 days, the total activity (1.4–7.0 r) produced a lowering of the flexor excitability. After 37 exposures, the flexor response threshold ceased to alter, but its response was distorted when urethan was injected.

Kudritsky (1957) showed that a change in the time taken by the flexor reflexes of the knee occurs mainly as a result of variations in the activity of the central apparatus and, to a lesser extent, of variations in the terminal apparatus of the reflex arc.

Godin and Gorshkov (1957) note that in rats fed with small doses of radioactive sodium ($0.5 \mu\text{c}$) the time of the defense reflex is slightly reduced, and the spread in the variations of this reflex is increased. When $250 \mu\text{c}$ were administered, the reflex time was first reduced and then began to lengthen. When the dose exceeded $300 \mu\text{c}$, the unconditioned reflex activity was immediately inhibited—an indication that the time taken to effect the reflex had increased.

In peripheral nerve, the functional variations following irradiation are seen to follow a phasial course. In 1934, Makarov noted parabiotic phe-

nomena in frogs after prolonged exposure of an isolated nerve to beta rays. Vasilev (1957) observed the development of parabiosis in a nerve conductor after its exposure to alpha radiation. Bakin, Dolgachev, and Lomonos (1952) pointed out that not all the constituent parts of a peripheral nerve possess equal radiosensitivity. The excitability of the sensory parts is first observed to increase; later, sensitivity diminishes, and only after this has occurred do we find disturbance of the motor functions.

Disturbances of the receptors and the consequent prolonged pathologic transmission of impulses from the periphery can play no small part in the nervous system's response to irradiation.

Geinisman and Zhirmunskaya (1958) observed intensified transmission of impulses from the carotid sinus zone and the skin of a frog after these structures had been exposed to radiation.

Tsy-pin (1956) noted a sharp intensification of the impulses reaching the nervous system from the eyes immediately after exposure to radiation.

Zaretskaya (1956) noted phase variations in the impulses arriving from the chemoreceptors of the lymphatic ganglia of cats after exposure to radiation.

Delitsina (1957) showed that after a rabbit's extremities had been exposed to 500 r there was at first a sharp intensification of impulses from the irradiated part, followed by subsequent weakening.

Chernichenko (1957), by exposing the urinary bladder and one of the intestinal ansae to local irradiation, established that this caused a disturbance in the character of impulses arriving from the receptors of the affected organ.

The fact that tolerance of radiation damage varies greatly from one subject to another has led several research workers to pay attention to individual characteristics. It has been found that resistance to the effect of radiation depends largely on the type of higher nervous activity. Animals with well balanced higher nervous activity withstand ionizing radiation best; disturbances of the nervous activity due to the effect of radiation develop later in these animals, even after exposure to large doses.

Lomonos (1959) notes that in dogs with a strong type of higher nervous activity, the conditioned reflex activity and the capacity to differentiate are maintained even on exposure to lethal doses of radiation.

Khruleva (1958) showed that a temporary lowering, followed by a prolonged heightening of the conditioned reflex activity, occurred in dogs with poorly balanced higher nervous activity when the animals were exposed to comparatively small doses of gamma radiation (50 r). In animals with a weak type of nervous system, such irradiation caused serious disturbances of the conditioned reflex activity, passing into prolonged neurosis.

Similar results were obtained by Klimova (1957), Kurtsin (1957),

Fadeeva *et al.* (1957), Kotlyarevsky *et al.* (1957), and other authors using animals exposed to various doses and types of radiation.

The nature of the nervous system's response to the effect of radiation depends also on its condition at the moment of irradiation.

Grigorev (1956), by administering various drugs to a patient before x-ray therapy, showed that when the stimulatory process was intensified after caffeine, the EEG response became much more intensive. When Bromural was administered, variation in the electrical activity was barely noticeable, and the EEG response to radiation was even less pronounced after administration of quinine.

Pomerantseva (1957) did not observe any radiation responses in animals exposed to radiation during sleep. Death caused by radiation after the animal had been awakened occurred a good deal later than in the control animals.

Kazaryan and Saakyan (1960) describe how the severity of radiation damage is considerably reduced in animals in hypothermia.

Soviet research workers have also studied the condition of the hematoencephalic barrier in animals exposed to radiation.

Stern and co-workers have shown that as a result of exposure to ionizing radiation the resistance of the hematoencephalic barrier changes in two stages. After irradiation, the transition of various indicators from the blood to the brain, which can be noted only 45 minutes after irradiation, becomes more intensive (Stern, 1957, 1960; Gromakovskaya and Rappoport, 1957, 1960; Goncharenko, 1960). Zaiko (1960) found that the peak increase in the permeability of the barrier occurred the 4th day after exposure with radioactive phosphorus (P^{32}). In the second phase the resistance of the barrier increases.

Stern's co-workers noted that by administering neurotropic preparations (atropine, Novocaine, morphia), it was possible to alter at will the permeability of the barrier structures. This result at the same time confirms the fact that nerve factors play a part in the mechanism by which the barrier is disturbed.

Arlashchenko (1955), using fluorescein as an indicator in investigating the condition of the hematoophthalmic barrier, showed that there is a sharp increase in permeability directly after exposure to radiation.

It has been found that the degree of disturbance to the histo-hematic barriers is directly dependent on the dose of radiation (Arlashchenko, 1955; Goncharenko, 1960).

There is every reason to assume that disturbance of the function of the histo-hematic barriers (in particular the hematoencephalic barrier) is one of the important basic factors in causing damage to nerve tissue exposed to ionizing radiation by making it easier for a number of alien and toxic sub-

stances formed in the organism during irradiation to pass into the central nervous system.

Clinical Symptoms Appearing as a Result of Ionizing Radiation

Much work has been done in recent years on the clinical changes occurring in the nervous system in humans exposed to various types of ionizing radiation.

Patients suffering from chronic radiation sickness complain first of tiredness, torpidity, apathy, poor sleep, irritability, loss of memory, vertigo, nausea, and a tendency to weep; in other words, complaints of an asthenic nature are predominant in the first stage.

The symptoms revealed by objective research are predominantly those of vegetative dysfunction: lability of cardiac activity, increased activity of the vasomotor nerves, pronounced red dermographia, abundant sweating, intensification of the pilomotor reflex, acrocyanosis, and variation in the pulse rate and arterial pressure.¹ Along with these symptoms of vegetative dysfunction certain symptoms of damage to the somatic nervous system have also been identified: lowering of the reflex from the mucosa, Chvostek's sign, pseudobulbar signs, hypesthesia in the distal parts of the hands and feet, and lowering of the sensitivity to vibration.

Trophic disturbances are also pronounced: brittleness and exfoliation of the nails, trichorrhea, hemophilia of the gums, and xeroderma. In more serious cases these symptoms are accompanied by pains in the extremities, distinct symptoms of sensory strain, disorder, nystagmus, and extrapyramidal disorders.

Allowing for slight shades of difference, these symptoms indicate, in the main, the nervous system's response to all types of radiation (gamma rays, x-rays, radioisotopes, radioactive luminous compounds). The picture of damage to the nervous system is, accordingly, dependent on the dose rather than on the properties of the type of radiation.²

The basic factor determining the neurologic picture is vegetative disturbances. The forms of damage mentioned are regarded by some authors as caused basically by functional disorders of the higher vegetative centers situated in the hypothalamic region (Kurshakov, 1954; Kozlova *et al.*, 1957; Kozakevich, 1957; Shamova, 1958; Morozov *et al.*, 1957). This hypothesis is supported by the fact that pronounced vegetative trophic and endocrine phenomena are combined in the pathologic picture, as Kozakevich pointed

¹ Disturbances of the oculocardiac reflex, attacks simulating Menière's syndrome, and stenocardiac manifestations. Skvirskaya (1956) draws attention to distinct manifestations of a regional spasm.

² Certain changes in the nervous system, however, can be detected even when the organism is exposed to small doses (Guskova, 1960).

out. Moiseev (1957) detected serious morphologic changes in the hypophysis of guinea pigs after irradiation.

Shamova (1958) demonstrated the distortion of several vegetative reflexes such as Scherbach's heat reflex, and variation in the sensitivity to ultraviolet radiation, which, with the clinical picture supports the hypothesis that the hypothalamus plays a leading part in onset of the disease.

These findings are in full agreement with our own morphologic research, which also indicates that exposure to radiation causes pronounced damage to the hypothalamic region. Various authors suggest different systems of classification for the clinical symptoms that have been discovered.

The early phase is described as moderately pronounced cerebral asthenia which manifests itself as an asthenovegetative syndrome. More pronounced changes are described as an astheno-organic syndrome (encephalopathy).

There are also a good many dubious descriptions. Khazanov and Korenevskaya (1958), for example, have described a case of neuritis of the femoral nerve as being due to the effect of radiation.

The number of works devoted to acute radiation sickness is considerably smaller. Pigalev (1954) divides the disease into three phases: acute, sub-acute, and chronic. In cases of severe damage death occurs before the end of the acute phase.

Kurshakov (1954) subdivides the acute form into four periods: first period, a few hours after exposure; second or latent period, lasting a few days to 2 weeks; third period, grave symptoms; fourth period, recovery.

It follows that ionizing radiation affects the activity of all links in the nervous system (the receptor apparatus, peripheral nerves, the peripheral parts of the vegetative nervous system, spinal cord, different apparatus of the brain stem and hypothalamic-diencephalic region), and it has a direct effect on the cerebral cortex.

It is difficult to determine which of these many interrelated parts is most affected, since the disturbance of any one leads to deviations from the normal condition in all parts of the nervous system.

In my view, the most important damage done by ionizing radiation is to the receptor apparatus, the higher vegetative and endocrine centers of the superior parts of the brain stem and the cerebral cortex.

One of the main effects of ionizing radiation on the receptor structures (resulting either from the direct action of radiation or from the action of the radicals formed in tissues during irradiation) is that the normal transmission of the nervous system's information is disturbed and several reflex responses are distorted.

As the well known research by Vvedensky and Ukhtomsky has shown, the excessive inflow of impulses favors the development of parabolic states and pathologic dominants at different levels of the nervous system.

Functional and organic changes occurring in the hypothalamic and di-

encephalic region and in the vegetative structures situated in this region also have great significance.

In the conditions created as a result of exposure to radiation, the corrective activity of this part of the nervous system in relation to the various endocrine and vegetative structures becomes particularly important. Disturbance of this correlation or of the correct interrelationships between the diencephalic structures and the cerebral cortex will obviously bring about a whole series of vegetative and endocrine disturbances, such as are observed at all stages of radiation sickness.

Functional disturbance of the higher parts of the brain depends on the direct influence of radiation on the nerve cells and on disturbance of the activity of the subcortex and receptor structures.

Functional disturbance of the cerebral cortex has an effect on the character of the higher nervous activity and leads to the loss of one of the main functions of the cortex, namely, correct adaptation of the individual's reactions to environmental changes.

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GENERAL DISCUSSION

PERCIVAL BAILEY (*University of Illinois*): I was happy to hear that the effect on the nerve cells can be primary and not secondary to the disturbance of the circulation. That I have always believed from long years of examining brains after irradiation for brain tumors and from results of work in my laboratory by Dr. Arnold and his associates with the effect of x-rays of high energy produced by the betatron.

WEBB HAYMAKER (*Armed Forces Institute of Pathology*): Dr. Bailey, I believe you mentioned that radiation "can" injure the nerve cell. I suppose you used "can" intentionally, rather than "may"?

PERCIVAL BAILEY: Naturally. Nerve cells can be injured also by disturbance of the circulation, and they are injured at times by disturbance of the circulation in brains that have been radiated. I believe there is also a primary effect on the nerve cells which is unrelated to the circulation.

CARMINE D. CLEMENTE (*University of California Medical Center*): The problem of direct or indirect effects of radiation on the various elements in the nervous system is something that has plagued this field for a long time. I don't think we can quite let Dr. Bailey get away with what he has said without replying with some remarks. When we think about the radiosensitivity of the various elements in the brain we must talk about dosage. If one considers radiating an entire brain with high doses such as 5,000 r, 10,000 r, 50,000 r, or 100,000 r, that is one experiment, but if one considers the effects of brain radiation in the same tissue areas of maybe doses of 1,000 or 2,000 r, that is a completely different experiment. Furthermore, I believe that when a small part of the brain is radiated in comparison to the entire brain it becomes a third experiment, and the dose-brain volume variable must be considered. When effects of high dosage radiation are described in the realms of 10,000 rad and 15,000 rad, it certainly is conceivable that neurons are directly affected. On the other hand, not only are the neurons affected, but the neuroglia and the blood vessels are equally affected. With these high doses, it seems to me that there is an analogy of taking a piece of brain and putting it in a frying pan, and thereby getting a lot of pathology in the tissue, and then saying "look at what's happened to the neuron." Let us think, however, of discussing the differential radiosensitivity of endothelial cells, glia and neurons, and discuss low dose effects. Under these circumstances an endothelial cell in the brain is a much more radiosensitive element than the neuron.

ARTHUR ARNOLD (*Chicago, Illinois*): Dr. Clemente brought out the important point of total dose, namely dealing with 1,000 r or 10,000 r. Another fact or equally important is dose rate. One can take a dose of 3,000 r and create an effect at 50 r per minute, and increase that rate to 200 r per minute and have an entirely different effect, not so much a qualitative effect, but a quantitative one. For example, I can take a monkey and deliver 10,000 r from a betatron through the frontal lobes in a single small beam, and it will lobotomize the animal,

providing I have given a dose rate of 200 r or more. If I reduce the dose rate to 50 r then the effect is one-half or one-third as much. I think the factor of dose is important in responsiveness in the nervous system, and the total over-all effect depends on the dose as well as the dose rate. Dr. Innes brought out a feature that I thought was probably the highlight of the whole program, the problem of control. He showed in the rat that some of the animals, after a period of time, can develop neurolytic lesions in the field of demyelination which probably are due to a virus. I have seen the same thing in rabbits and dogs, and would emphasize that, if you have to do a great deal of radiation studies, stay away from these animals. I have followed monkeys for as long as 8 or 9 years and have never seen such demyelinating disorders, which points to the monkey as the ideal animal for radiation studies on the nervous system. There seems to be a difference in opinion as to whether we are dealing with direct or indirect effects and as to whether there is a total over-all effect from a massive dose; but I firmly believe there is a differential particularly when you are dealing with the glial cells and, through numerous studies on tumors, we know that there can be a differential response in a tumor cell. One would say that this is a different problem than the over-all nervous system; but there appears to be, in the adult nervous system in the well developed animal, differences in responses between the types of glial cells as compared to, for example, the vessel structure. I have noticed that glial cells responded earlier than the vessels, and yet we have also noted that in time some of the late delayed necrotic effects are obviously vascular, but the myelin changes may be related to all of those.

WEBB HAYMAKER: Professor Grashchenkov alluded to these delayed lesions, and he made a distinction between the vasomotor theory and another theory. Would you mind elaborating a little on that matter of the delayed radionecrosis?

N. J. GRASHICHENKOV (*Moscow, U.S.S.R.*): It is not my personal investigation, but from my view, you have serious destruction of certain cells of the nervous system, like those of the subthalamus and hypothalamus. Some lesions were connected with other signs of destruction such as the spinal effects, caused by damage to cells of the spinal cord. Mainly, however, the damage is located in the thalamic tissue.

WEBB HAYMAKER: One point that Professor Schummelfeder brought out with respect to these late lesions was that in the literature it was suggested that they may represent a sensitive reaction in the vessels. I wonder if Dr. Leon Roizin would comment on, from what he has seen this morning of Professor Scholz's presentation, whether he sees anything in these vessels in relation to his studies on sensitivity reaction.

LEON ROIZIN (*New York State Psychiatric Institute*): Orville Bailey has emphasized the time factor in relation to delayed reactions, particularly as they were affecting blood vessels. He said the character of the lesions increased with time. Professor Scholz also emphasized the fact of certain delayed reactions. We have studied for several years in connection with experimental vascular encephalomyelitis by using as the main antigen total brain or brain fractions. The technique consists of brain or brain fraction suspensions emulsified in antigens and injected subcutaneously or intramuscularly. After the injection there is a delay of from 3 weeks

to several months. During this so-called latent period of sensitization, the animals show a mosaic of neurologic symptoms. Some who are sacrificed or die during the acute phases showed edema and vascular changes of the necrobiotic or inflammatory coverings around the veins. Of particular interest to me was the reaction which Professor Scholz showed in the H and E preparations and with the elastic stain. He emphasized the fact of so-called plasmodic or fibroid material which has involved the vascular material as well as necrosis. We have observed similar changes during the early phases of the allergic reaction in experimental encephalomyelitis. It occurs to me that possibly the x-ray effects on the tissue produce a necrobiotic phenomena; and this phenomena, which requires a certain period of sensitization, initiates a chain of reactions with particular involvement of the blood vessels. I would like to suggest the possibility that delayed reactions could be produced by a necrobiotic process, induced by irradiation.

J. R. INNES (*Brookhaven National Laboratory*): I am pleased to hear Dr. Arnold mention that he thought the monkey was the ideal animal for radiation; however, although they don't suffer the legion of disorders which affect the nervous system of man, Van Bogaert, Schair, and others, show that they do suffer a remarkable variety of neurologic diseases, some of them unique. Curiously, some show lesions like the ones I showed and Dr. Scholz showed in his rabbits, which are specific in monkeys, except the gorilla and chimpanzee. In any work involved with monkeys where they are kept a long time, you must be alert to neuroparalysis, palsy, blindness, and leucoencephalomyelosis, which although uncommon is a well recognized condition. In this disease, brain as well as spinal lesions appear scattered, spotty, and more denominational. Concerning the specific effect of irradiation on the myelin system, I think this effect on the neuron is specifically on white matter and far more than on the axis cylinder. In the cord it seems to affect the ventrolateral more than the dorsal. Let us remember that there is such a thing as a conducting cylinder, the myelin system.

PERCIVAL BAILEY: It is true that monkeys have diseases of the brain, like other animals. But on our animals we never saw anything of the kind. Furthermore, it is not possible that any such disturbance could have occurred in them, because we used a beam produced by the betatron which makes a constant lesion without any scatter, and in one hemisphere using the opposite hemisphere for the same animal as a control.

WEBB HAYMAKER: We would like to hear from Dr. Alvord because of his sensitivity studies with the granular layer of the cerebellum under different physiologic conditions.

E. C. ALVORD (*Seattle, Washington*): The answers we get usually depend on the methods we use. I would like to develop the idea that there is specificity within the nervous system in its susceptibility to the effects of radiation and to bring up for comment the irreversibility or reversibility of some of these changes and the site of swelling or edema. When a large part of one cerebral hemisphere has been removed and a guinea pig is given 7,500 r in the head, the normal guinea pig will die in 24 hours. If you provide internal decompression by surgery, it can live 4 to 6 days. Those sacrificed 4 hours after irradiation look reasonably normal. The others show swelling of the entire brain, with cerebellar consular herniation.

By comparing the wet and dry weights of the cerebral hemispheres, brain stem, and cerebellum, we see that the accumulation of water is localized to the cerebellum and that the cerebral hemispheres, operative or inoperative side, are such that the brain cell shows practically no swelling. With this prolonged time it becomes obvious that in the cerebellum the granular cells go much further than we had thought in the normal guinea pig. After 5 days, there is karyorrhexis, as well as pyknosis, but there is no evidence of reversibility. I would ask Dr. Vogel if his remarkable, almost inconceivable, recovery of the monkey at 72 hours might not be a reflection of the individual variation in sensitivity of monkeys to irradiation near the threshold level, probably around 10,000 r and that the monkey that showed no change at 72 hours really didn't show any 48 hours earlier.

F. STEPHEN VOGEL (*Cornell Medical Center*): The graph that was shown with the spectrum of changes was from a group that were all exposed to 10,000 r. It was felt that this dose was capable of regularly producing pyknotic changes in the granular cells. Someone could postulate that any animal sacrificed at one time or another had either shown this change or would show it at the time of sacrifice. The pairs of animals were killed so that we had not one animal at 72 hours, but four animals. I think similar findings have been seen in the rabbit in our laboratory and elsewhere, coupled with the fact that there was no evidence of inflammation or karyorrhexis later nor evidence of pyknosis or decrease in number of cells. I think one is justified in suspecting that these four animals showed the same type of change as the much larger group of about 20 animals that were killed earlier. With the observation that in tissue culture this change appears to be transitory, we feel that this is a difference in sensitivity. I think one would have to increase the dose above 30,000 r, perhaps, before one reached the stage where you could destroy the granular cells of the monkey.

L. M. H. LARRAMENDI (*University of Illinois*): In using the sciatic nerve of the bullfrog, I assume the action potentials that Dr. Gaffey showed were of the A fibers. I would like to know if he has tried the same experiment to the B and C fibers. Anatomically they are different. And I think Dr. Grashchenkov mentioned that the autonomic nervous system is more sensitive, and the B and C fibers are related to the autonomic system. Concerning previous comments by several persons about the granular layer of the cerebellum, they were referring continuously to the granules of the granular layer of the cerebellum. The implication I think was that all the granular cells were within the granular layer of the cerebellum and that they are neurons. This is not the case.

C. T. GAFFEY (*Donner Laboratory, University of California, Berkeley, California*): Concerning the species of frog, the *Rana catesbeiana* is the bullfrog, and the *Rana pipiens* is the grasshopper frog. A question was raised concerning the sensitivity of A, B, and C fibers. It is my understanding that the *Rana catesbeiana* does not have B fibers from a histologic point of view. Gerstner has studied sensitivity to x-irradiation within the A fibers (*American Journal of Physiology*, 1956). I believe the A-gamma fibers have essentially the same sensitivity, but the B fibers are more resistant. Most of the changes are rather minor between these fiber types in the A group. He also thought the C fibers were too thin and delicate.

JAMES LOTT (*North Texas State College*): Since Dr. Gaffey's results seem to not coincide with Dr. Bachofer's and mine, working with peripheral nerves in the rat, I would like to ask him about some of his techniques. I would like to know how often he stimulated the nerve during the control period, how long after he removed the nerve did he begin his recordings, how often he stimulated the nerve during irradiation, when he was taking his recordings, and whether he has ever seen an increase in the action potential due to alpha or deuteron irradiation.

C. T. GAFFEY: The stimulation period of the frog was short. About half a minute to a minute. Once every 2 hours the nerve was monitored. So, essentially there would be no change in the nerve due to the stimulation effect. It is known that stimulations at low frequency, compared to high frequency, will change the magnitude of the potential of the compound A fibers. These stimuli that were given were maximal for a response. Enhancement of neural output is demonstrated, but there is some confusion in the season of the year in which the enhancement is shown. In winter frogs, enhancement is strongest. In summer and fall frogs, enhancement is minor. It would appear that the potassium gradients across the fibers are seasonal and reflect the degree of enhancement shown in any particular experiment.

ORVILLE T. BAILEY (*University of Illinois*): The pathology of radiation in the central nervous system leads to a series of questions which are very difficult to separate and on which we have conflicting opinions which may be related to extraneous factors. In regard to the long latent period, I am perhaps somewhat influenced by experiences with radiation changes in skin. Experience with these makes this long latent period more understandable. One patient seen over many years with repeated biopsies was an eminent scientist who, as late as 35 years after he stopped using x-ray, was not only developing new carcinomas but was developing patches of typical x-ray dermatitis in previously normal skin. The participation of allergic responses in the pathology of radiation is an open question. Similar end results do not necessarily mean similar pathogenesis. Because the reconstituted vessels in repair have the same appearance when these processes are finished that they have an allergic arteritis does not necessarily mean that all the stages or that the pathogenesis is the same. Finally, one of the strongest arguments for differential effects of radiation on the various types of cells in the central nervous system is the alteration in the sequences of histologic repair.

CHAIRMAN'S SUMMATION

WEBB HAYMAKER: We have heard a good deal about the cerebellum. One of the interesting observations pointed out by Dr. Vogel was with reference to the cerebellar cells in his tissue culture. Granular cells underwent significant changes in response to radiation, but the inner cells of the cultures apparently were unaffected. This must mean that granule cells are more radiovulnerable than other cerebellar cells, as we already know. The point of Dr. Vogel's observations is that nuclear pyknosis and early enlargement of the cytoplasm, pointed out also by Dr. Schummelfeder, took place without the benefit of circulatory disturbance. In his presentation on the cerebellum of the mouse, Dr. Schummelfeder considered the effect of circulatory disturbances on granule cell changes, and at all doses between 60,000 r to 2,000 r granule cells were always altered first. Then after a latent period, the vessels underwent morphologic changes. To him, and apparently to the two Drs. Bailey, quite definitely the ultimate pathologic picture when the latent period has passed is apparently the outcome of the combination of direct effects and the effects of vascular origin. The paper by Drs. Hager, Hirschberger, and Breit aroused particular interest because it dealt with observations under electron microscopy. They too, found that the granule cells of the cerebellum were particularly radiovulnerable, but they dealt also with the broader aspects of the problem of pathogenesis. This area of pathogenesis is a kind of twilight zone without a horizon, where observation still blends too much with opinion. Dr. Hager and associates, using high doses of 40 kv x-rays, provided the following timetable according to electron microscopy as seen in the brain of the Syrian hamster: First, there was increased vascular permeability as manifested by swelling of cells which they called astrocytes, by plasma exudation, and by erythrodiapedesis. Their observations showed that at these dose levels the first stage seen in the electron microscope was vascular disturbance. Dr. Hager and his associates feel that the loosening of tissue and the fine sponginess seen after radiation is merely a reflection of the accumulation of fluid in the astrocyte, or what has been called the astrocyte, and that gross sponginess is attributed to astrocytic and other membranes either *in vivo* or during tissue processing or both. These cells called astrocytes become watery. At the same time that the astrocytic change occurs, they said, the capillary endothelium shows suggestive swelling, and about this time a structureless material considered to be plasma exudate is seen in the perivascular space. Only later are nerve cell changes seen, and still later changes are seen in the large blood vessels. Dr. O. T. Bailey emphasized the relative radiovulnerability of the astrocyte. Professor Scholz and his associates demonstrated with high dose x-rays that astrocytic processes break down at an early stage, at a time when only minor changes are visible in nerve cells. I think we are now at the stage of knowledge at which we can generalize and say that when radiation upsets the transport in the metabolic mechanism of the astrocyte, nerve cells are bound to suffer as a consequence. Dr. Innes and Dr. Carsten showed us the later stages of

what happens after radiation. One of the interesting points was the variable latent period, namely, anywhere from 3 to 9 months after the animals were exposed to 3,500 r, before lesions became apparent. This matter of the latent period, as it concerns individual variation, is a common problem, and it always requires a large series of animals before one can reach dependable conclusions. This brings us to the concept of the delayed radionecrosis described by Professor Scholz. He demonstrated swelling and disintegration of vessel walls before there was any visible change in the surrounding spinal cord substance. The question arises as to whether these vascular changes and exudative phenomena, the existence of which there can be no question as seen in Professor Scholz' lantern slides, may be the outcome of some peculiar sensitivity reaction. It is interesting that Dr. Roizin is of the opinion that what he saw today might possibly be construed as a sensitivity reaction and that the change was primarily in the vessels rather than in the nerve substance around the vessels. Dr. Vogel pointed out, as did Dr. Schummelfeder and others, that radiovulnerability of the cells in the central nervous system varies with the species. This is a general principle of which we must all be aware, but which some of us now and then tend to overlook.

PART III

Particle Irradiation of the Central Nervous System

The Use of Accelerated Heavy Particles for Production of Radiolesions and Stimulation in the Central Nervous System

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The central nervous system has a more intricate organization than any of the other organ systems of the human body: its functions seem to depend on the spatial configuration of its delicate structural elements, and on spatial and temporal interrelationships of its neuronal discharges.

Through past centuries up to the present, a tremendous amount of knowledge has accumulated with respect to gross, microscopic, and submicroscopic anatomical structures of the central nervous system; and relatively recently it has become possible by the use of surgical instruments to make lesions in various parts of the brain, then follow the abnormal pattern of physiological and electrical activity thus created by a variety of techniques. In surgical interference with brain structure, whether this be done for the purpose of physiological study or with therapeutic aim, serious limitations are encountered: the entire path of the surgeons needle or knife produces injury from surface to depth with interruption of neuronal pathways and blood vessels. The result can be hazard for hemorrhage, subsequent necrosis, and later formation of scar tissue along the pathway of surgical injury. The presence of scarring by itself can lead to disturbances of electrical function and to new injury with the production of more extensive lesions. While there are constantly new improvements in operative technique and while much new knowledge is gained by the current methods, one should maintain an interest in novel approaches to the problems of neurophysiology. The role of accelerated heavy particles will be outlined here in historical background. This is done to illustrate that man's curiosity in relatively remote and disconnected, "useless" aspects of nature can sometimes lead to new tools, new methods, and new knowledge in very practical and useful realms.

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Acceleration of Atomic Nuclei

The original reason for artificially accelerating nuclei of light atoms was the realization that such particles might penetrate the atomic nucleus. About the same time that Ernest Lawrence built the first cyclotron (see Lawrence and Livingston, 1932) for acceleration of deuterons, protons, and alpha particles, it was realized by Zirkle (1932) that atomic nuclei, particularly alpha particles, were more efficient in killing cells than x- or gamma rays, and that the major effect of alpha particles was on cell nuclei. Acceleration of heavier nuclei (e.g., lithium, mercury) was attempted in the early 1930's (Sloan, 1935), but did not become practical until about 1940 (Alvarez, 1940; Tobias and Segrè, 1946), when the nuclei of carbon were accelerated in the Berkeley 60 in. cyclotron to about 120 Mev kinetic energy. In about the same year, the Swedish scientist Edlen (1941, 1942) discovered that multiply charged atoms of various elements, including carbon, calcium, and iron, were constantly present in the solar corona. These elements are either a result of emissions of solar plasma into surrounding space, or come about by interaction of the interplanetary dust with solar radiations. In 1948, Freier *et al.* discovered in observations taken on photographic emulsions in high altitude balloons, that primary cosmic rays reaching the earth at the top of the atmosphere were protons, alphas, and heavier nuclei, many of them with billions of electron-volts kinetic energy. It was demonstrated that at least part of the heavy nucleons arriving at the earth originate in the sun or solar corona.

For 30 or 40 years physicians concerned with tumor therapy realized the desirability of concentrating radiations in limited regions in the body. High energy x-rays, gamma rays, and neutrons were tried in succession. In 1946, Robert Wilson, then at the University of California, realized that high energy protons, due to their particular ionizing properties, might be useful for irradiation of deep seated tumors in the body. In 1948, with completion of the first large cyclotron, the Berkeley group undertook systematic investigation of the usefulness of protons and deuterons in biological research. Tobias *et al.* (1952) demonstrated that high energy particles (190 Mev deuterons) are useful for production of localized radiolesions in the body, and that the Bragg ionization peak for tumors in mice does indeed possess some advantages. A technique was developed for hypophyseal radiation of the rat (Tobias *et al.*, 1954; Van Dyke *et al.*, 1959) and monkey (Simpson *et al.*, 1959), which led to a well-defined, chronic hypophysectomized state in the animals. In 1955, encouraged by the mounting evidence for hormone dependence of many human cancers and by the initial successes of surgical hypophysectomy (Luft and Olivecrona, 1953), and following initial studies of hypophyseal radiation of dogs with advanced mammary carcinoma (un-

published), investigation of human hypophyseal proton radiation was begun. Today more than 100 patients have had pituitary radiation (Tobias *et al.*, 1958; Born *et al.*, 1960). Many cases of advanced metastatic mammary carcinoma and a few of prostate carcinoma, advanced diabetes mellitus, acromegaly, malignant exophthalmos, leukemia, and Cushing's disease were treated, and it appears that the hypophyseal radiation is useful in producing some regressions and in allowing studies of hormonal aspects of the above mentioned diseases. The radiation is so well concentrated in the pituitary that only occasional mild secondary neurological effects are encountered.

Several years ago it was also realized that accelerated particles made the production of lesions in the brain possible, so that the Berkeley group has initiated some studies on the hypothalamic radiation syndrome (Anderson *et al.*, 1957). It was found that for production of radiation lethality the hypothalamic region is more sensitive than either the cerebral cortex or the pituitary. The hypothalamic lethal syndrome is a symptom complex integrated from failures of a number of homeostatic regulatory functions and often results in abdominal or intestinal bleeding as a cause of death. It was also shown that lesions in the region of the median eminence lead to diabetes insipidus, glycosuria, thyroid abnormality, and delayed sexual development. Roberts, Thorell, and associates (1957) demonstrated that a lesion in the posterior hypothalamus, not identical with the "appetite center," leads to decreased rate of growth and degeneration of eosinophilic cells in the pituitary. An interesting use of the Bragg ionization peak of alpha particles was made by von Sallmann *et al.* (1955) who selectively irradiated the lens of the rabbit eye and determined the relative effectiveness of alpha particles and deuterons on lens epithelium for producing cataracts.

In 1955 and 1956, the author and his colleague, Victor Burns,¹ had the opportunity to collaborate with a team at the University of Uppsala, at a time when their proton synchrocyclotron was adapted to tumor studies and neurologic irradiations (Larsson *et al.*, 1956). Larsson and his associates developed a method for production of cutting "knife edge" lesions with a narrow beam of 185 Mev protons which were passed through a narrow slit made of absorbing material. Knife edge lesions of the spinal cord were studied. By the use of a 1.5 mm wide beam on a rabbit spinal cord, it was possible to produce a sharply limited lesion with only minimal hemorrhages (Larsson *et al.*, 1958; 1959). Rexed *et al.* (1959) produced cutting lesions in the upper anterior part of the rabbit brain with doses of 20,000 rad and found destruction of myelin sheaths, axons, and nerve cells. The lesions in the first 3 months were confined to the region irradiated. Working with pigeons, Fabricius and Larsson (1959) are in the process of studying the localization

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of control of instinctive behavior by production of lesions that decerebrate the animals. In 1959, Leksell, who has pioneered in the applications of radiations to brain surgery, and the Uppsala team initiated proton neurosurgical applications by producing bilateral cutting lesions in the frontothalamic bundles in a patient with schizophrenia (Leksell, 1961). Sourander and associates (Andersson *et al.*, 1961) have supplied very interesting neuro-histopathological studies.

While studies with deep lesions were in progress with high energy nucleons, another group of approaches took place with particles of a few million volts per nucleon. Pollard (1953) and associates have used protons for basic studies of enzyme molecules and phage particles. Zirkle and Bloom (1953) applied a microbeam of protons to partial cell irradiation. Soon after the nature of cosmic ray primaries were known (Freier *et al.*, 1948) speculations arose that heavy primary cosmic rays could exert great radiobiological effects, particularly in the brain, where it was believed no recovery from radiation effects would take place (Schafer, 1950; Tobias, 1952). The first exposure of living cells to carbon particles was done in 1952 at the cyclotron (Sayeg *et al.*, 1959), and an increased biological effectiveness of heavy ions on yeast cells was demonstrated. In 1957, both at Berkeley and at Yale University new accelerators became available which can deliver particles up to argon nuclei with about 10 Mev energy per nucleon, and detailed information is becoming available on the manner of action of these radiations (Brustad, 1960; Hutchinson, 1960). The chairman of this session and his associates (Malis *et al.*, 1957) showed that a monoenergetic beam of protons can produce in animals "laminar" lesions in the brain only about 80 microns thick, running quite closely parallel to the exposed brain surface. Malis *et al.* (1961) are presenting new data here. Haymaker and associates have traced the nature of pathological development of such radiolesions with alpha particles (Janssen *et al.*, 1961). Van Dyke *et al.* (1961) have pointed to the great variation of radiosensitivity of the brain among different species and demonstrated the vulnerability of the blood-brain barrier by showing that fluorescent dyes can penetrate the irradiated region early in the course of lesion development. It was also shown that very high doses in rat cerebellum with low energy beams can lead to sharp surface lesions with scarring virtually absent.

Today, accelerated particles can be used for the production of a variety of well-defined radiation lesions. Work is in progress not only on the involved steps that lead to the development of radiation-induced necrosis, but also the accurately placed lesions are being utilized to obtain answers for many pressing problems. The role of histologically defined neuronal zones in the gray matter, anatomical studies of retrograde degeneration, the problem of regeneration, and that of the origin and nature of the electrical function of

the brain (Tiltsjar-Lentulis and Tobias, 1959) are only examples of studies currently active.

One of the major tools of the neurological research worker is electrical stimulation. We have shown during the last few months that radiation, too, can be used for stimulation of nerve activity. For some years it has been clear that specialized structures, such as the retina, can be stimulated with penetrating radiations. Hug (1960) only a year ago demonstrated that tentacles of the snail can "feel" the presence of x-rays. Conard (1956) has studied the effect of radiations on contractions of parts of intestine *in vitro*. In our laboratory (Tobias *et al.*, 1961) pulsed beams of alpha particles were used on the cornea to elicit the so-called "corneal blinking reflex." It is clear that deep stimulation of structures of the central nervous system by heavy ion beams is a distinct possibility. Since the diameter of such beams can be made very small, and pulsing of the beams can be accomplished in various ways, a distinct possibility exists that in the future one might be able to scan limited regions of the brain surface with a deflected beam of penetrating particles, delivering a "message" to certain cells in the brain by a pre-arranged spatial and temporal code, without the trauma of surgical intervention.

Physical Techniques of Producing Lesions

The physical characteristics of accelerated nucleons have been described in detail (Tobias *et al.*, 1952; Larsson *et al.*, 1956; Birge *et al.*, 1956; Brustad, 1961). Here we wish to give only an outline of those properties of radiobiologic importance. Usually an attempt is made to produce an almost parallel beam of particles in an accelerator. This task cannot be accomplished perfectly because in the course of acceleration in a cyclotron, radial and axial oscillations occur; in a linear accelerator there are also "defocusing" effects. In a linear accelerator it is possible, with the aid of electromagnetic focusing, to have almost all particles emerge from the machine in a single spot of perhaps 1 mm in diameter and within 0.1% monoenergetic. In the cyclotron, the particles emerge from a nonhomogenous magnetic field, and they are not parallel. In this instance one may focus them to obtain a slightly convergent or divergent beam, and one usually applies a slit system to select particles traveling nearly parallel. This way beams of about 0.1 degree angular divergence can be obtained, but a great part of the beam which does not satisfy the criteria of parallelity is not actually used.

The monoenergetic particles penetrate nearly the same distance in tissue. Figure 1 gives the range energy relationship in water for protons, alpha particles, and for heavier ions as a function of the kinetic energy per nucleon. Also, on this figure are indicated the actual energy and range values

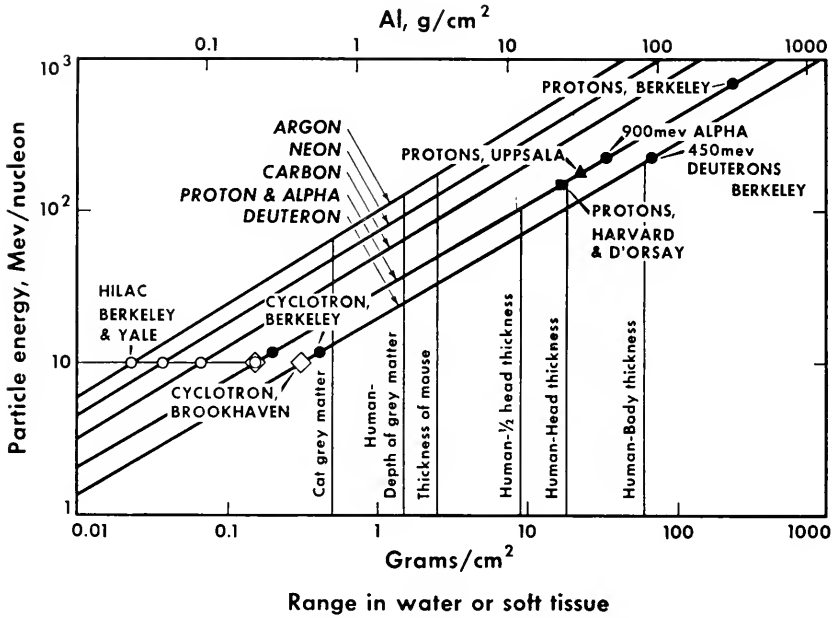


FIG. 1. Range energy relationship for protons, alpha particles, carbon, neon, and argon nuclei. Black dots indicate ranges of the cyclotron beams available in Berkeley. Black triangle signifies the protons of the Uppsala cyclotron. Black square is the proton beam of the Harvard and French Atomic Energy Commission cyclotrons. Open circles indicate beams of the Yale and Berkeley heavy ion accelerators.

which have been utilized at various laboratories in biological and medical studies. Since biological experimentation has usually been secondary to the use of machines in physics research, we may note that certain interesting and desirable energy ranges have not as yet been explored with biological specimens. For example, one does not have ions heavier than alpha particles with kinetic energy greater than 10 Mev per nucleon. Unlike x-rays or electrons, nuclear particles of equal energy travel almost to the same depth in an absorber. Some particles are, however, lost because of inelastic collisions. Others show slight differences in their respective ranges owing to straggling and multiple elastic scattering. The root mean square fluctuation $\sqrt{\Delta R^2}$ of the range R increases as the particle energy and penetration increases.

$$\sqrt{\Delta R^2} \text{ (observed)} = \Delta R^2 \text{ (initial energy spread)} + \Delta R^2 \text{ (straggling)} + \Delta R^2 \text{ (scattering)}$$

The exact relationships are complicated. However, the fractional straggling $\sqrt{\Delta R^2}$ for particles of the same velocity is independent of charge and varies with the square root of the mass of the particle.

$$\frac{\sqrt{\Delta R^2}}{R_1} \cdot \frac{R_2}{\sqrt{\Delta R^2}} = \sqrt{\frac{M_2}{M_1}}$$

Thus, a proton has more than 40 times less straggling than an electron;

a heavy particle, e.g., oxygen has 4 times less straggling than a proton. For a given range, experimentally minimum range variation is achieved by making ΔE (initial energy spread) as small as possible. Thus ideally, one would wish to accelerate particles with the proper energy for each range used in order to have optimum ionization properties.

As a parallel beam of particles enters an absorber, due to multiple elastic scattering there will be a radial spread in the beam. Having crossed a distance X in an absorber, the mean square radial distance \bar{r}^2 from theoretical straight line trajectory is

$$\bar{r}^2 = \frac{1}{3} \bar{\theta}^2 X^2$$

where $\bar{\theta}^2$ is the mean square of the angular spread (in radians)

$$\sqrt{\bar{\theta}^2} \approx \frac{z\sqrt{Z}}{T} X \rho c$$

where T is the kinetic energy, Z the atomic number of the absorber, ρ is its density, z is the atomic number of the fast charged particle, and c a constant that depends on the allowable elastic scattering angles θ . For the same velocity a lighter particle has a longer range and undergoes more scattering than a heavier one. Also, the root mean square angle of scattering is proportional to the rate of energy loss; thus at high energy, where the rate of energy loss is low, there is less scattering than at low energy; the radial spread of the beam will be less if an absorber of high atomic number is used. After a beam has emerged from an absorber, to the air, the spread of the scattered beam is much more noticeable than in a solid absorber, since in air there are greatly reduced numbers of collisions. The energy transfer to the absorbing medium along the path of an ionizing particle has been calculated by Bohr (see Evans, 1955). Over a wide range of energies the rate of energy loss is almost inversely proportional to the kinetic energy T of the particles ($T^{-1.1}$); thus low energy particles ionize much more heavily than high energy ones. The relationship breaks down near the end of the range where the particles pick up electrons as they stop, and also at very high energies, near 1 Bev per nucleon, where relativistic effects appear. Comparing protons to heavier accelerated particles of charge z , we find that at the same velocity the heavier particles transfer more energy to the medium, in proportion to z^2 . The rate of energy loss of various individual particles is plotted in Fig. 2 as a function of their kinetic energy per nucleon. Unlike x-rays, which show great dependence of energy transfer on the atomic number of the absorber, heavy accelerated particles interact mostly with atomic electrons; thus the stopping per electron is nearly the same for all elements. For accurate dose calculations it is necessary to know the stopping power of the tissue, relative to nitrogen or other gas, where the ionization is measured. While more experimental work needs to be done in this field, some stopping powers are accurately known (Tobias *et al.*, 1952).

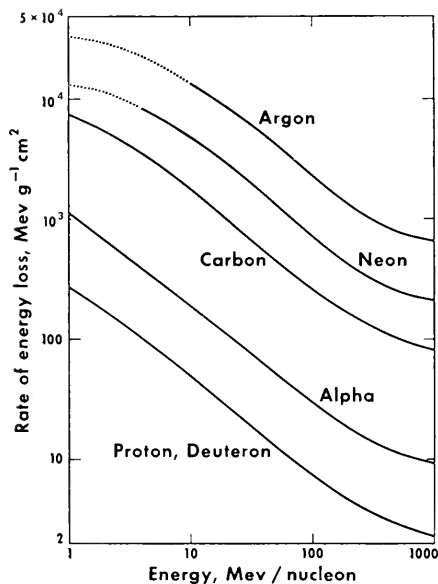


FIG. 2. Rate of energy transfer from various particles to water as function of the energy per nucleon. Rate of energy loss in soft tissue is within 2% of this value.

An actual beam of nearly monoenergetic particles ionizes along the so-called Bragg ionization curve, usually arrived at experimentally. This relationship takes into account all phenomena of scattering and straggling. Figure 3 shows the Bragg ionization curve as measured for 40 Mev alpha particles for the purpose of the work reported at this conference with Haymaker *et al.* Such a beam can cause laminar neurological lesions. In order to understand the way such lesions originate, Fig. 3 also contains a hypothetical dose-effect relationship of the multiple hit type. It is easy to understand that pathologically visible lesions occur only in regions where the dose, as represented by the Bragg ionization curve, exceeds a certain threshold value.

Experimentally the ratio $(\sqrt{\Delta^2 R})/R$ is between 1/40 to 1/60 in the energy range of 10 Mev/nucleon to 200 Mev/nucleon. The lateral spread of the beam is such that it is possible to maintain a lateral variation of $(\sqrt{r^2})/R$ somewhat less than the magnitude of the range variation $(\sqrt{\Delta^2 R})/R$. For an alpha particle beam of 10 Mev/nucleon the range is about 1300 μ , in tissue; the width of the Bragg peak is about 100 μ and the lateral spread of the beam in 1300 μ distance is about 10 μ ; for 190 Mev deuterons the

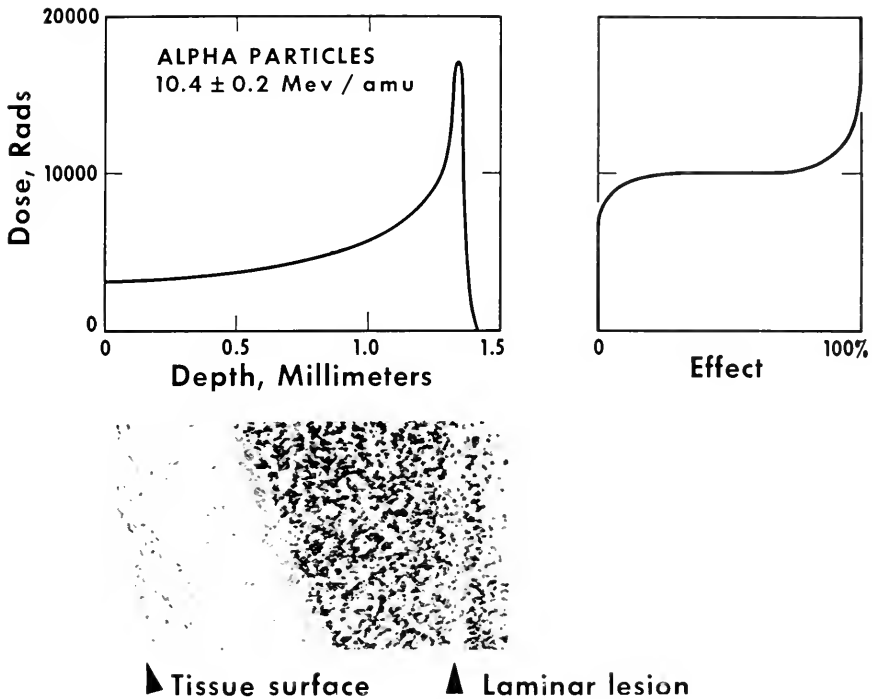


FIG. 3. Depth dose distribution for 40 Mev alpha particles. On upper right is a hypothetical dose-effect relationship. Assuming that effects greater than 50% are visible under the microscope, the lower curve gives profile of the "laminar lesion."

range in tissue is about 14.5 cm, the width of the Bragg peak is about 4 mm, and the mean lateral spread in the entire range about 3 mm. Figures such as these give an indication of depth and minimal practical lesion size of the particles in question. A great deal of experimental work remains as yet to be done to explore the limitations of the technique. It is practical to use knife edge lesions of 1 mm in diameter to a depth of about 4 cm in the brain: as the beam penetrates deeper, however, the lesion becomes less and less definite.

The cross-sectional area of the beam may be shaped by metal apertures of thickness greater than the range of the beam. Usually, one monitors the beam with ion chambers as it emerges from the accelerator and calculates the ionization as function of depth in tissue from the known stopping power and composition. Experiments with phantoms are possible.

An illustration of the relative lack of scattering is shown in Fig. 4. Here a beam of 190 Mev deuterons, $1 \times 2 \text{ mm}$ in cross section, was passed through



FIG. 4. Photograph of head of rat which received 20,000 rad of deuterons in a small beam 1×2 mm in diameter passing laterally through the head. White spot in hair indicates passage of beam. Entry and exit spots are indistinguishable. From Tobias *et al.* (1954).

the head of a rat. The hair of the animal turned white where the beam crossed the skin. It is impossible to tell which is the entrance or exit spot on the skin. There are many problems one encounters in actual practical radiologic procedures. Accurate evaluation of the isodose curves themselves present a great problem, since the dose varies very quickly with depth. The usual technique used is the exposure of a spectroscopical quality of photographic material to the beam in an accurately constructed phantom with identical apertures and rotations as in the actual biologic exposure. One may then evaluate the dose distribution from photographic densitometry. Any such procedure presupposes knowledge of the dose-effect relationship in photographic film (Tochilin *et al.*, 1955; Welch, 1955). Some corrections are necessary for various factors, e.g., oblique incidence of the beam, spread caused by motion of particles in air, etc. Other useful methods are solid state dosimeters, dosimetry by radioactivation, or the use of a chemical oxidation-reduction system.

At the Berkeley 184 in. cyclotron, a device is available for exact positioning, by means of x-ray diagnostic techniques, of any desired part of the head. With the use of a three-dimensional Descartes coordinate system, it is possible to deliver a radiation lesion of arbitrary size to the desired point. It is then possible, by merely changing one single coordinate, to deliver another lesion in the exact bilateral location. Usually the head of the animal that

is to be exposed is rotated around the point to be irradiated, while a cylindrical beam constantly radiates the center of rotation. Use of the Bragg ionization peak for such irradiation is also possible, but more elaborate preparations are necessary, since the depth of the range needs to be controlled at all times.

RADIOBIOLOGIC CONSIDERATIONS

The nature of the radiation effect on the adult central nervous system poses great and as yet unsolved problems. The tissue has heterogenous architecture: its structure is built for optimum maintenance of its most essential elements, the neurons, and this function is accomplished by an intricate architecture of the varied cellular components. Proliferating elements of neural tissue are the astrocytes and glial cells. Neurons in the adult do not seem to have mitotic activity, and their functions appear to be mainly in transmission of action potentials and, in some instances, perhaps neurosecretion. Our cellular radiobiologic knowledge comes mainly from studies of rapidly proliferating cells in the course of the cell division process, and we know that the maximum killing effect expresses itself mainly during and following the cell division process.

Since neurons do not divide, it is no wonder that they are thought to be relatively radioresistant. However, we do know that the developing embryo nervous system is extremely radiosensitive (see R. Rugh, 1961), and studies in progress also point to radiosensitivity of the adult brain; usually, however, a considerable time elapses before the damage is developed to the point where it is pathologically observable. The observed change is usually necrosis, that is, disappearance of all cellular elements. Current radiation studies point to the need of understanding the detailed biochemical and cellular processes of necrosis and its initiation.

There is no definite dose-effect relationship as yet established for nerve tissue. We do know that therapeutic x-ray irradiation of a large part of the human brain may result in late degenerative changes, including demyelination, scarring, and necrosis (Druger *et al.*, 1954), Lindgren (1958) has collected human material and demonstrated that protracted irradiation schedules are less effective in causing necrosis than a single dose and that the dose-effect relationship for protracted dose schedules is similar to that demonstrated by Strandquist (1944) for skin. Arnold *et al.* (1961) studied radiation effects on brain of primates and found demyelination, the first observable chronic deleterious effect, one that may be caused by a dose of a few hundred rads. Lindgren's (1958) data on rabbits indicates somewhat higher dose thresholds. At Berkeley many irradiations have been carried out on animals over the last few years, usually with the proton or deuteron beam,

in well-localized regions of the cortex, thalamus, brain stem, spinal cord, or cerebellum. Generally two relationships were observed: first, the higher the single dose, the sooner the onset of degenerative changes. Secondly, the larger the irradiated volume, the sooner the degenerative changes appear. Combining these two observations, we found it possible to compute the "integral dose," that is, dose times volume irradiated. As seen in Fig. 5, the integral dose and the time of death due to necrosis correlate reasonably well. Recently, Zeman *et al.* (1959) produced radiation lesions in rats with very small beams of alpha particles. It was found that many tens of thousands of rads are necessary to cause an effect as seen from Fig. 5.

The effects of protracted dose schedules and of the size of the irradiated volume make it appear that some other factors enter in brain radiosensitivity besides direct interaction of radiation with neurons.

One suspects the state of the capillary bed as being essential for the integrity of neurons, particularly knowing that proper oxygen supply or nutrition is essential for the maintenance of the latter. Capillary walls are known to regenerate more effectively following protracted dose schedules than a single large dose. Certainly vasodilation following radiation and increased permeability of the blood-brain barrier have been observed in the early post-irradiation period (see communications by Van Dyke *et al.*, Haymaker *et al.*, this conference.)

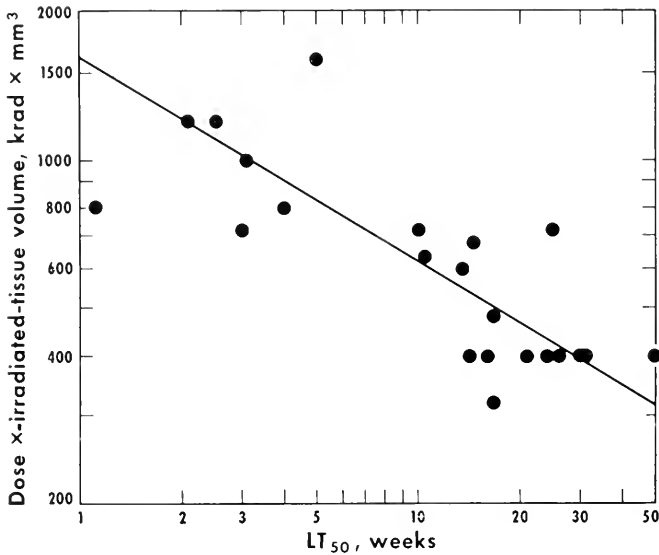


FIG. 5. Relationship of integral dose to time of death due to necrosis and hemorrhage in a number of experiments.

In the course of localized irradiation of the brain, special attention is given to the possibility that if the body and nucleus of neuronal cells are in the radiation field, the effect might be more profound than if only nerve trunks are irradiated. Unfortunately, so far there seems to be no definite evidence for such effect; in fact, demyelination of fibers seems to occur chronologically earlier than some other effects. However, it is possible that following proper doses, fibers recover or even regrow from initial damage, while it is anticipated that neurons might not recover from nuclear damage.

Early radiation-induced changes include alteration of the blood-brain barrier. If the lesion is massive, increased intracranial pressure might be one of the early gross signs of damage. It is possible that electric disturbances, resembling epilepsy, which sometimes follow massive head irradiation are in part due to the increased intracranial pressure. Tilsjar-Lentulis and Tobias (1959) in our laboratory observed cortical potentials following radiation in rats and found that the epileptic fits were absent when the brain was decompressed due to opening a large bone flap. In fact, using the cyclotron radiation technique it was found that irradiation of one cerebral hemisphere with doses in excess of 100,000 rads leads to immediate cessation of electrical activity in that hemisphere. The other irradiated hemisphere still had some activity, but the alpha rhythm was about three times slower than normal. Changes in excitability and transmission as a result of local irradiation in various parts of the brain are the object of current studies.

STIMULATION BY MEANS OF IONIZING PARTICLES

The usefulness of nuclear beams in neurology would become even greater if one were able to use radiation to stimulate nerve discharge, perhaps in well-defined locations inside the brain. Several classes of observed psychologic effects of radiation (e.g., see Miller *et al.*, this conference) indicate that neuronal function is altered in some way following radiation, and we do know that the retina can be directly stimulated with low doses of x-rays (Lipetz, 1955). Hug (1960) has recently demonstrated interesting responses in snails. Conard (1957) followed radiation effects in muscles of rabbit intestine following radiation exposure. In preliminary studies at Berkeley, it was realized that instantaneous stimulation of action currents in nerve might require high instantaneous radiation intensity for a brief period of time in a similar fashion as high electric current densities are required for brief periods of time to stimulate action currents.

The Heavy Ion Linear Accelerator was chosen because it could deliver pulses of one millisecond duration, up to 10^9 rad min in intensity. For the initial work a reflex was chosen which can be very easily demonstrated: the corneal blinking reflex. As seen in Fig. 6, the cornea of unanesthetized rabbits

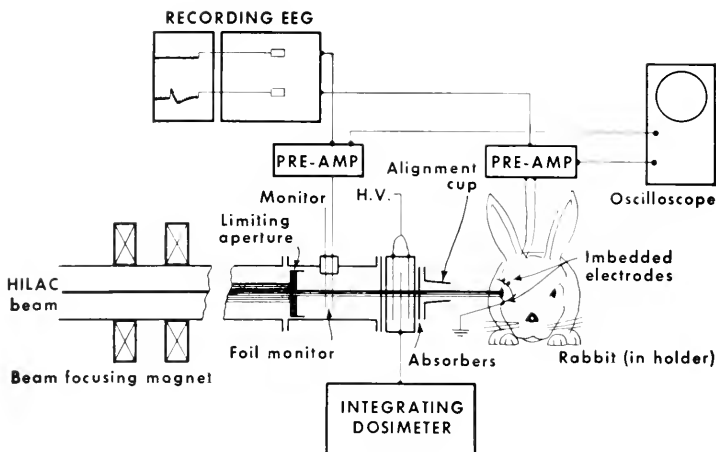


FIG. 6. Method of exposure of rabbit cornea to pulsed heavy ion beams. One or more radiation pulses of about 2 millisecon duration are allowed to fall on the cornea. The beam pulse as well as contraction of the auricular muscle are simultaneously recorded, and the blinking directly observed by closed circuit television.

was exposed to one or more pulses of accelerated alpha particles, over an area 5 mm in diameter. The response of the auricular muscle was followed by recording the potential between the electrodes inserted into it. (Tobias *et al.*, 1961). It was found that single or multiple pulses of beam did not cause blinking if less than about 20,000 rad was delivered within 1 sec to the substantia propria. Pulses larger than the threshold of about 30,000 rad per pulse did cause reflex muscle action with a delay of about 0.2–0.3 sec. By adjusting the range of the alpha particles it was also found that maximum stimulation occurred when the Bragg ionization peak is at depth 100–200 μ under the corneal surface; local anesthesia by a few drops of tetracaine abolished the effect. Such blinking reflex could occur if light, secondary to radiation, falls on the retina. That this was not a major part of the effect, was demonstrated by showing that the response is still present when the optic nerve is cut. The energy required for stimulation is sufficient to raise the local temperature (at the Bragg ionization peak) by about 0.1°C; it is known that absorption of infrared rays of about the same energy content can also initiate the blinking reflex (Dawson, 1961). Figure 7 reproduces some typical electrical records obtained in the course of radiation stimulation. The dose in a single beam pulse is sufficient to cause permanent pathologic changes; these are presently being studied by S. Kimura.

The corneal fibers that respond to such stimulation are believed to be pain fibers: it is possible that other type nerve endings or synapse will exhibit different and perhaps greater radiation sensitivity. At the present time one

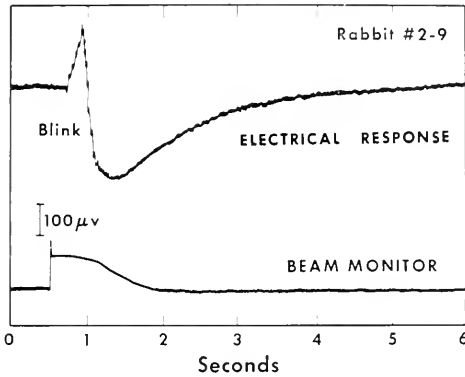


Fig. 7. Typical record of stimulation of blinking reflex.

would not wish to use such large dose stimulation in humans. In animals this may be a different problem. We know that needles used in stimulation inflict permanent trauma on brain tissue. It may well be that radiation-induced trauma will actually be less.

Looking into the future, one might visualize some of the advantages of heavy ion pulses for stimulation and study of the cerebral cortex. One should probably try to stimulate with heavier ions than alpha particles: perhaps even a single accelerated heavy nucleus of some ion such as iron might be capable of stimulating, owing to its great linear energy transfer. It seems quite possible to produce microbeams of heavy ions, perhaps $10\ \mu$ in diameter, then direct pulses of such beams to arbitrarily selected spots in the brain, and to vary the depth of penetration at will. One must admit that due to scattering and straggling the resolution becomes poorer as the beam reaches to greater depth in the brain. Thinking about future possibilities for radiation stimulation of the brain, I wish to present a preliminary scheme of the Heavy Ion Scanning Stimulator (HISS), shown in Fig. 8, which is in construction at Berkeley. Here magnetic fields provide a pre-coded scan pattern, as the beam is pulsed at some predetermined sequence. The response of the brain to such space and time coded radiation "messages" to the surface of the cerebral cortex might prove to be illuminating to our knowledge of cerebral cortical function and behaviour.

Summary

The chronological development of techniques for use of accelerated ions in neurology are described. Methods for deep localization of lesions, production of laminar lesions, and knife-edge lesions are described. The stimulation of reflex action by alpha particles is demonstrated.

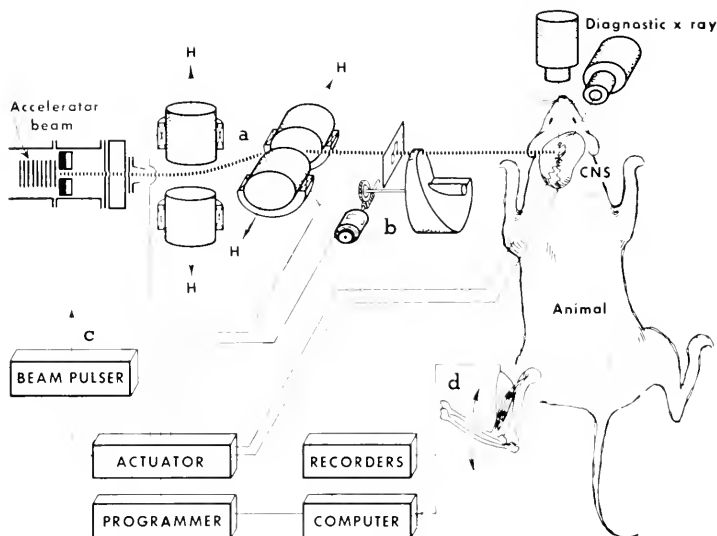


FIG. 8. Schematic concept of future uses of heavy ion pulses for conveying "coded" messages to the brain. The coding may occur by (a) magnetic deflection of beam in predetermined sequence, e.g., scanning; (b) by passage of beam through absorbers of predetermined profile to position the Bragg ionization peak where the stimulation occurs; (c) by coding time sequence and intensity of pulses of beam; (d) by using "feedback" information from periphery to change coded messages.

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Effect of Local Irradiation of the Central Nervous System with High Energy Protons

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High energy beams of light atomic nuclei have physical properties which make them suitable when small volumes of tissue are to be uniformly irradiated. Tobias *et al.*, (1952), Anderson *et al.*, (1957), made lesions in the hypothalamus of the rat using 190 Mev deuterons. Malis *et al.*, (1957), making use of the thin ionization peak at the end of the beam, produced laminar lesions at a depth of 0.8 mm in the cerebral cortex of the cat with 10 Mev protons. Larsson *et al.*, (1958) briefly described several types of localized lesions in the central nervous system within 2 months after irradiation by a 185 Mev proton beam. The histopathology of lesions produced by this technique in the spinal cord and brain of the rabbit was described in detail by Larsson *et al.*, (1959) and Rexed *et al.*, (1960). Zeman *et al.*, (1959), when investigating the effects of primary cosmic rays on nervous tissue, irradiated mouse brains with 22.5 Mev deuterons and studied the relationship between the size of the impact area and the threshold dose for a radiogenic lesion. Larsson (1960) described blood vessel changes following local irradiation of the rat's brain with high energy protons.

The present report is a summary of the neuropathologic findings in rabbits and goats after local irradiation with a beam of 185 Mev protons from the 230 cm synchrocyclotron at the Gustaf Werner Institute, Uppsala. Lesions up to about 1 year are considered. The experiments were performed to get fundamental data concerning local effects of heavy particle beams on the central nervous system, having in mind their possible application to stereotaxic neurosurgery.

Material and Methods

Irradiations were performed with a beam of 185 Mev protons from the synchrocyclotron as described by Larsson *et al.*, (1959). By focusing magnets

and sweeping coils an almost parallel beam with a uniform cross section of variable size was obtained. The desired beam profile was determined by a final aperture. The mean dose-rate was 1,000–2,000 rad per min. The doses were measured with an ionization chamber and by activation dosimetry. The results obtained by the two methods were in fair agreement. Single proton beams were used for irradiation of the rabbit's spinal cord and brain. Cross fire irradiation was applied in the experiments on goats.

In 55 rabbits and 5 goats, 3 types of lesions were produced: transection of the spinal cord, transverse lesion of the cerebral hemispheres, and restricted lesion in the depth of the brain.

The spinal cord provides a suitable region to evaluate the effect of radiation on nervous tissues because of its regular arrangement of gray and white matter, and because disturbances of function are easy to observe. Proton beams, 1.5, and 10 mm broad, were directed across the spinal cord under deep intravenous nembutal anesthesia. The position of the animal was checked by roentgenograms before and after irradiation. After irradiation the animals were examined regularly, and neurologic symptoms noted. In most cases, paresis of varying degree developed. The animals were sacrificed by exsanguination under chloroform anesthesia at different periods following appearance of paresis.

In a second series of experiments performed in rabbits, the 1.5 mm proton beam was directed across the upper anterior part of the brain. A dose of 20 krad was used. The skull was fixed to a frame to avoid movement. During irradiation the animals were observed on a television screen. The animals were allowed to survive from 2 to 56 weeks. During this time no functional disability occurred which could be ascribed to the lesion.

In view of the possible application of irradiation with high energy protons to neurosurgery, experiments on goats were performed. The goat has a rigid skull which can be conveniently fixed in the same stereotaxic apparatus as that constructed for man. The goat's brain is rather large, so that the volume of necrotized tissue can be made relatively small compared to the size of the whole brain. This fact reduces the risk of side effects, e.g., generalized edema of the brain. One goat was irradiated with a single beam of protons (dose = 20 krad). In 3 goats a disc-shaped lesion with a diameter of about 1 cm was produced in the internal capsule by the stereotaxic method using cross fire irradiation. A beam 2 mm x 7 mm or 2 mm x 10 mm and 20–22 fields were used (the dose at the desired site was in 2 cases 20 krad and in 1 case 38 krad). The head of the goat was fixed to the stereotaxic instrument by drills as described by Leksell (1951, 1957). When cross fire irradiation was applied, a point in the right internal capsule was selected as center of rotation. The lesions were planned so as to appear transverse to the direction of the fibers of the internal capsule. Before the operation, the goats

were given chloralhydrate by a stomach tube, and later on, nembutal anesthesia was inducted intravenously. The goats were killed 1 to 4 months after irradiation by decapitation under nembutal anesthesia.

Spinal cords and brains were fixed in 5% neutral formal-saline. From the irradiated part and adjacent regions of the cord, longitudinal and serial sections were cut in a frontal plane. The rabbit brains were cut sagittally, and the slices were embedded in paraffin or cut in the frozen state. The goat brains were cut horizontally in slices about 5 mm thick and examined macroscopically. The target region was embedded in celloidin and cut by serial sectioning. The principal staining methods used were hematoxylin and van Gieson's mixture, phosphotungstic acid hematoxylin, Heidenhain's modified Mallory stain for collagen, thionin, Grothionin and Palmgren's silver impregnation for axons, Loyez and Heidenhain's myelin sheath stains, and Ranke's Victoria blue for astrocytes. In a few cases, supplementary stains for demonstration of iron and fat were used.

Results

TRANSECTION OF THE SPINAL CORD

Eight rabbits were exposed to a single dose of 20 krad with a 1.5 mm beam. The first neurologic symptoms, consisting of lively tendon reflexes in the hind legs and slight knee and ankle clonus, appeared about 8 days after irradiation; later, flaccid paralysis developed. In the meninges and cord, a band of grayish discoloration 2 mm wide marked the radiated portion of the cord. No hemorrhages or inflammatory signs were visible to the naked eye. Microscopic examination revealed a narrow and sharply delimited necrotic zone corresponding to the path of the beam (Fig. 1). The lesion was broader in the white substance and had about the same breadth as the proton beam. The damaged gray matter was only about half the breadth of the beam (no correction for shrinkage due to fixation was made). In the path of the beam, nerve cells, axons, myelin sheaths, and glial cells were necrotized. No evidence of selective radiation effect on the various components of the neural tissue was seen. Minimal hemorrhages surrounding capillaries and venules were observed at the margin and sometimes in the middle of the irradiated zone. Large hemorrhages were never seen. Nuclear fragments, slight proliferation of astrocytes with bulky cytoplasm, and a few macrophages were noted in a small marginal zone between the irradiated and nonirradiated tissue (Fig. 2). Occasional nerve cells adjacent to the irradiated zone were degenerated, others being rather well preserved. In the white substance, the marginal zone was characterized by degenerating axons and myelin sheaths. Iron pigment occurred in a few glial cells at the edge of the necrosis. Fat



FIG. 1. Longitudinal section of rabbit's spinal cord; myelin preparation showing localized damage 12 days after irradiation with a 1.5 mm beam of high energy protons. Dose: 20 krad. Heidenhain. $\times 9$. The photographs are published by courtesy of *Acta Radiologica*.

stain was always negative. The occurrence of a small number of well preserved axons and myelin sheaths in the subpial region of the irradiated part of the cord was remarkable and constant.

To study the relationship between the breadth of the beam and the type of radiation damage, a group of animals was irradiated with a beam 10 mm broad, the dose being the same as in the first series. Three to 4 days after irradiation, rapidly progressing paresis developed in the hind legs. These animals were killed 1 to 6 days after irradiation. Macroscopically, hyperemia of the meninges and spinal cord corresponding to the path of the beam was seen 1 to 3 days after irradiation, and numerous petechial bleedings 3 to 6 days after irradiation. The earliest histologic changes found, at 1 and 2 days after irradiation, consisted of dilated capillaries and veins containing large

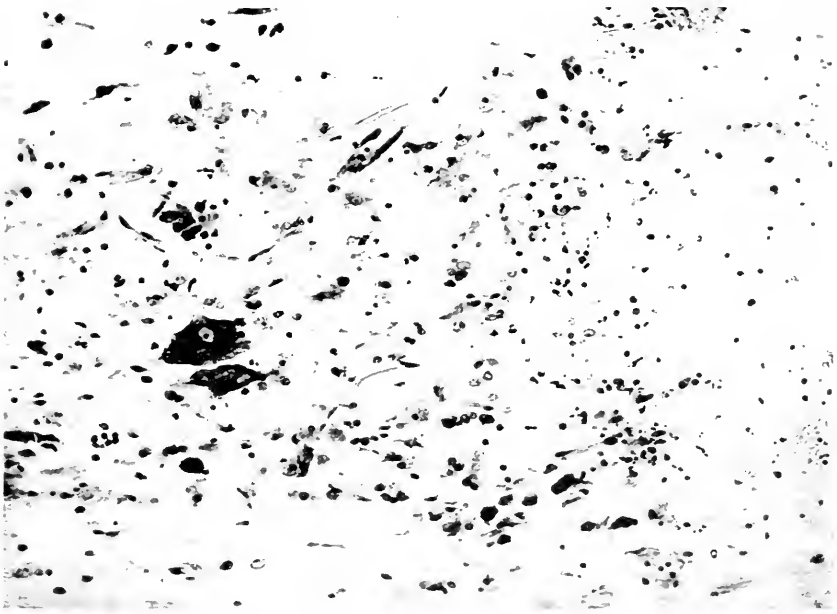


FIG. 2. The sharp demarcation of the irradiated zone in the gray matter of the spinal cord is seen to the right. Bordering the irradiated zone is a band of nuclear fragments and adjacent to them numerous astrocytes with swollen cytoplasm and a few macrophages; further away rather well preserved nerve cells are observed. Proton beam 1.5 mm broad. Dose: 20 krad. Thionin. $\times 150$. The photographs are published by courtesy of *Acta Radiologica*.

aggregations of erythrocytes. The nerve cells, axons, myelin sheaths, and glial cells appeared to be intact. After 3 days the gray matter within a zone about 10 mm wide showed exudation of plasma as well as multiple, and sometimes rather large, hemorrhages. The nerve cells were degenerating. The white matter was relatively spared and showed only slight perivascular disintegration of the nervous tissue. Slight inflammatory cellular proliferation was noted in the meninges and cord corresponding to the irradiated region. At 4 to 6 days after irradiation, widespread degeneration and necrosis of the white matter and abundant hemorrhages in the gray columns were seen (Fig. 3).

The approximate relationship between dose and time of appearance of signs was studied in a series of animals irradiated with 5 to 40 krad. Animals irradiated with 12 krad or less did not, over a year, show any clinical signs or histologic changes in the cord. After a dose of 40 krad, paralysis of the hind legs developed in 2 days. The histologic picture 5 days after irradiation revealed necrosis considerably more extensive than that seen after a dose of 20 krad. In addition, numerous small hemorrhages appeared.

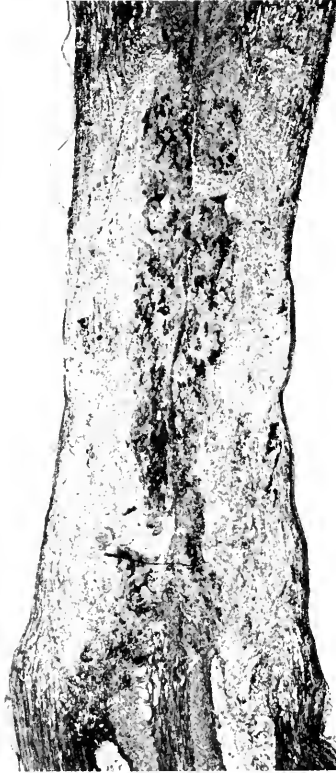


FIG. 3. Myelin preparation showing extensive necrosis and multiple perivascular hemorrhages 4 days after irradiation with a 10 mm high energy proton beam. Dose: 20 krad. Heidenhain. $\times 9$. The photographs are published by courtesy of *Acta Radiologica*.

TRANSVERSE LESION OF THE CEREBRAL HEMISPHERES

Twenty rabbits were irradiated with a 1.5 mm broad beam directed transversely through the upper part of the frontal lobes. The dose was 20 krad. The rabbits remained functionally unaffected during the time they were allowed to survive, up to about 1 year. Macroscopic changes consisted of a red or green groove on the anterosuperior surface of the brain, and in some instances, on the medial surfaces of the hemispheres as well. No large hemorrhage was seen, and the leptomeningeal vessels in the groove were not thrombosed. Sagittal sections of the brain showed a well demarcated lesion in the cortex, white matter, and caudate nucleus, which up to 3 months after irradiation was approximately restricted to the path of the beam. After 3 months the lesion was slightly broader than the beam (Fig. 4).



FIG. 4. Sagittal section of rabbit's brain 13 weeks after irradiation with a 1.5 mm beam of high energy protons. A discrete lesion corresponding to the path of the beam is seen. The cortical laminae are interrupted and the myelin destroyed. Increased vascularity is noted in the irradiated region. Dose 20 krad. Loyez stain for myelin. $\times 45$. The photographs are published by courtesy of *Acta Radiologica*.

Two weeks after irradiation, collections of fluid and small perivascular hemorrhages were observed in the irradiated zone. Within the lesion many thick-walled capillaries lined by large endothelial cells were present. The nerve cells, axons, and myelin sheaths in the path of the beam were mostly destroyed. In the damaged tissue, proliferation of astrocytes had occurred, and collections of macrophages were seen, particularly around the vessels. Four weeks after irradiation, cells with large nuclei, 2 or 3 times the normal size, were seen among the proliferating astrocytes. Numerous large atypical glial cells appeared within 3 months after irradiation (Fig. 5). Some of

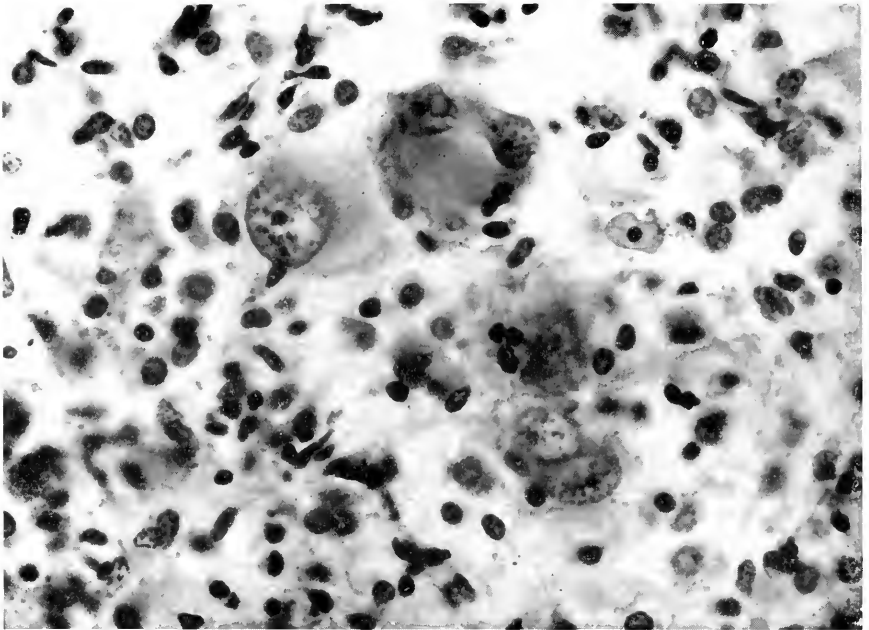


Fig. 5. Atypical glial cells appeared in the lesion at 4 weeks after irradiation and were more frequent at 10 weeks. Thionin. $\times 640$. The photographs are published by courtesy of *Acta Radiologica*.

these were binucleated or multinucleated; many had a huge nucleus of bizarre shape. Inclusion bodies were frequently seen in the nuclei of these cells. Some of the giant cells resembled nerve cells in that they had a large nucleus with a distinct nuclear membrane and a central nucleolus; the nucleus was often eccentric, and there was a large amount of glassy cytoplasm. The various types of atypical cells were most frequent at the margins of the lesion. They were never seen in other parts of the brain.

Cavities of varying size and shape were macroscopically seen in about half of the irradiated rabbits. They involved mainly the white matter, but in some cases the cortex was also affected (Fig. 6). Cavitation was first observed 10 weeks after irradiation. In some animals, surviving up to a year, no cavities appeared. The cavities were not encapsulated, but were lined by glial cells and giant cells and contained clear fluid and macrophages.

Teleangiectasis of the irradiated region was a striking feature. It was present in all animals surviving more than 23 weeks. The cortex and white matter exhibited numerous blood-filled, thin-walled capillaries of wide caliber lined by a single layer of endothelial cells. Occasionally some of these capillaries were thrombosed, and sometimes small hemorrhages and collections of hemosiderin-filled phagocytes were found around them (Fig. 7). Large hemorrhages were never encountered.

A thin zone of the cortex immediately underlying the pia matter showed less damage than the rest of the irradiated cortex.

It was an unexpected finding that the ependyma and choroid plexus al-

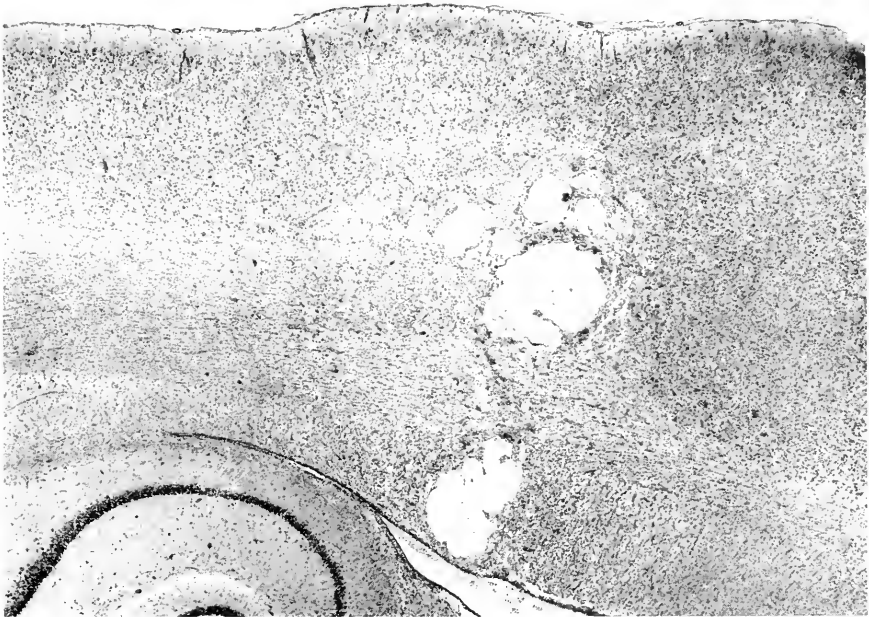


FIG. 6. Sixteen weeks after irradiation. The damaged region shows loss of substance and the formation of small cavities in the deeper parts of the cortex and white matter. Ependyma undamaged. The lesion is slightly broader than the beam. H. van G. $\times 20$. The photographs are published by courtesy of *Acta Radiologica*.

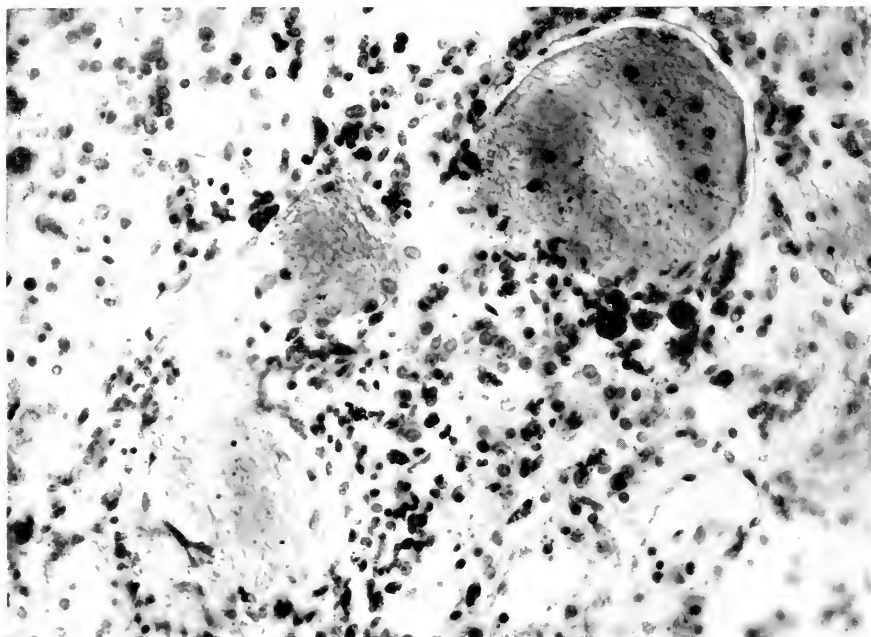


Fig. 7. Twenty-three weeks after irradiation. Newly formed wide vessels lined with flattened endothelium and surrounded by macrophages, some of them containing hemosiderin, now appear in the lesion. H. van G. $\times 200$. The photographs are published by courtesy of *Acta Radiologica*.

ways were unaffected, though the proton beam often passed through parts of these structures.

RESTRICTED LESION IN THE DEPTH OF THE BRAIN

To be able to compare the radiogenic brain lesions in rabbit and goat with each other, 1 goat was irradiated with a single beam directed through the frontal lobe of the right cerebral hemisphere (dose = 20 krad). The brain was examined 1 month after irradiation. The lesion in this goat bore a strong resemblance to the lesions in the brains of rabbits irradiated under similar conditions.

Lesions were produced in the internal capsule of 3 goats by the stereotaxic method using cross fire irradiation. A dose of 20 krad was given to 2 of these goats. They remained in good condition and did not show any symptoms of cerebral injury. Four and 7 weeks after irradiation, they presented restricted lesions in the selected areas (Fig. 8). The damaged region was roughly lenti-form, with a maximum thickness in the anteroposterior direction of about 3 mm. The longest diameter of the lesion was in the dorsoventral direction



FIG. 8. Horizontal section of the right hemisphere of a goat's brain showing a sharply delimited lesion in the internal capsule 7 weeks after cross-fire irradiation with 20 krad. Gros-thionin. $\times 5$. The photographs are published by courtesy of *Acta Radiologica*.

and measured 13–16 mm. The histopathologic picture of the brain lesions of these 2 goats corresponded closely to each other. Within the white matter the lesion was sharply limited, and its central part was completely necrotic. The capillaries were destroyed, and around them were seen shadows of erythrocytes and plasma exudate. There were no large hemorrhages inside the lesion or around it. A narrow zone surrounding the definite lesion showed degenerating axons and myelin sheaths. Slight cellular proliferation was noted. In the lesion examined 7 weeks after irradiation, a few macrophages were seen around distended vessels at the margin of the damaged region. Outside the lesion there were no signs of pathologic processes in the brain.

A dose of about 40 krad given by cross fire irradiation to 1 goat produced a different lesion. The volume of necrotic tissue was large. The tissue around the necrotic area in some places contained wide, thin-walled, newly formed vessels and was densely infiltrated with lymphocytes and macrophages. The affected region surrounding the necrosis was at least the size of the necrotic area itself. Only minimal hemorrhages were noted in the outer zone. The irradiated hemisphere was swollen, but did not show any other pathological changes outside the lesion.

Discussion

The proton beam as an agent for production of lesions in the central nervous system has the advantage that the particles of the beam proceed along practically straight tracks and undergo little scattering. The ionizing effect is thereby restricted, and it should be theoretically possible, therefore, to cause a sharply defined lesion.

The lesions in nervous tissue caused by high energy protons was that of an acute necrosis and similar to that described by Arnold and co-workers (1954) after irradiation of the brain of the monkey with high energy roentgen radiation. In our experiments on the spinal cord, as well as in those by Arnold and co-workers (1954) on the brain of the monkey and by Larsson (1960) on the brain of the rat, it appeared that the latent period for radiation necrosis depends on the dose of radiation. A dose of 20 krad with a 1.5 mm-broad proton beam gave a well defined lesion after 9 days. With larger and smaller doses, the latent period was shortened or prolonged, respectively. A radical increase of the dose above 20 krad, considered optimum dose, induced in the spinal cord, as well as in the brain, a more widespread tissue reaction. This observation stresses the importance of selecting the dose carefully when planning any surgical procedure.

The breadth of the beam proved to be a factor of great importance. In the experiments on the spinal cord a 10 mm beam not only produced necrosis of about the same breadth as the beam, but also produced a lesion considerably more quickly and of a histologically more severe type than a thinner 1.5 mm beam. With the thin beam, only slight vascular damage and minimal hemorrhages were seen. The same dose and a broad beam caused, even after 1 day, signs of vascular damage, and after 3 days, plasma exudation and numerous hemorrhages, especially in the gray substance. It is assumed that the peculiar type of vascular arrangement in the spinal cord and the poor development of collaterals in its thoracic part favored the appearance of large hemorrhages when a considerably large portion of the cord was irradiated.

In experiments on the tolerance of mouse brain tissue to high energy deuterons, Zeman and co-workers (1959) found that the dose required to produce a threshold lesion in mouse brain increased from 30 krad with a beam 1 mm broad to 1.1×10^6 rad with a beam 0.025 mm in diameter. According to these authors, a possible explanation would be that the microbeams cause a predominantly direct radiation injury, whereas the broad beams produce additional indirect effects, e.g., vascular disturbances. Arnold and co-workers using 10 or 25-mm-broad beams of high energy roentgen rays did not find evidence that the lesions produced by broad beams were secondary to vascular occlusion. In our experiments the narrow-

est beam used was 1.5 mm broad, and lesions produced by this beam were not studied before 9 days after irradiation. When the 10 mm-broad beam was used and the histology of the spinal cord studied 1 to 6 days after irradiation, vascular changes were found at the time when only slight destruction of myelin sheaths had appeared. This observation stresses the possible importance of vascular changes among the causal factors of delayed radionecrosis in the nervous system. Furthermore, the presence of a thin peripheral zone of the cord with undamaged nerve fibers may speak in favor of vascular factors, since it is well known that the peripheral region of the cord is relatively resistant to impaired circulation. The studies of Larsson (1960) on blood vessel changes following local irradiation of the rat's brain with high energy protons indicate that disturbances in capillary circulation may precede increased permeability of the blood-brain barrier system for trypan blue. The latter type of changes was accompanied or succeeded by destruction of nervous tissue.

In the rabbit's brain irradiated with high energy protons, capillary proliferation and sometimes teleangiectasis appeared within the lesion. It might be supposed that such changes of vessels would predispose to hemorrhage, but in fact a large hemorrhage was never seen either in the late or early stages after irradiation. Teleangiectasis was also observed by Berg and Lindgren (1958) in the rabbit's brain after roentgen irradiation.

The various types of giant cells found at the margins of the radiolesion in rabbit's brain were probably of glial origin and resembled the cells found in gliomata. These cells were always confined to the margins of the lesion and were never found to have spread to other parts of the brain. The number of the giant cells did not increase during the experiment of one year. Exceptionally large astrocytes were described in rabbits after roentgen irradiation of the brain by Russell *et al.* (1949), and giant, atypical glial cells have been observed in primates by Arnold and Bailey (1954) and Berg and Lindgren (1958).

The experiments reported show that, with a narrow beam of high energy protons, sharply delimited acute lesions at a desired site in any region in the central nervous system can be produced. Furthermore, it is obvious that by careful selection of doses and dose distributions, well circumscribed intracerebral lesions of appropriate size and shape can be caused. However, this statement is valid only for the earlier stages of the radiolesion, as only relatively short survival periods are considered. To what extent high energy protons may be used in stereotaxic surgery of the human brain depends on the late changes. The long-term effects of high energy proton irradiation are at present being studied in a series of experiments on goats, in which deep lesions are to be followed up over several years.

Summary

For the past several years experiments were carried out to determine the local effect on the central nervous system of high energy protons. The irradiations were performed on rabbits and goats with a 185 Mev proton beam from the 230 cm synchrocyclotron at Uppsala. The experiments have shown that it is possible with high energy protons to produce small well circumscribed cerebral lesions at a desired site in the brain without damaging the surrounding tissue. The importance of performing long-term experiments to determine the late effects of high energy protons on the brain is stressed.

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Production of Laminae Lesions in the Cerebral Cortex by Deuteron Irradiation*

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Our interest in radiation has been aroused by the possibility of utilizing the cyclotron beam for investigation of connections and functions of the cerebral cortex.

The mammalian cerebral cortex consists of an outer layer, almost devoid of nerve cells, and of several inner layers, more or less densely packed with radially oriented nerve cells. This makes it appear laminated. The functional significance of this lamination is obscure. We thought it of interest to produce lesions restricted to one or several layers in order to study them electrophysiologically and histologically.

Other workers employing heavy, ionizing particles usually used a high energy beam. With such a beam, one can irradiate with only that portion of the beam path along which the loss of energy is almost uniform. The terminal sector of the beam path (with great differences in energy loss) is arranged to lie outside the irradiated organism. With this technique, it is possible to produce substantially uniform destruction along the beam path if the dose is high enough. Destruction deep within the brain can be achieved by multiple irradiations. The beam path for each irradiation traverses different sectors of the brain, and the intensity of each is below threshold for destruction of the tissue. All beam paths, however, are made to converge on the target area, which thus receives the desired radiation dose (Anderson *et al.*, 1957a,b; Larsson *et al.*, 1959; Tobias *et al.*, 1954).

In contrast to these techniques introduced by Tobias and his co-workers (1954), we have used a beam of only moderate energy which is stopped entirely within the cerebral cortex. With such a beam, it is fairly simple to

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produce a restricted lesion deep in the cortex with a single irradiation, since one can take advantage of a sharp increase in the number of ionizations produced for a short distance near the end of the beam path, just before all ionizations drop abruptly to zero as the particles are stopped by the tissue (Malis *et al.*, 1957, 1958).

Figure 1 illustrates a typical laminar lesion 65 days after irradiation with 20 Mev deuterons. The laminar lesion is a sharply demarcated strip completely free of nerve cells. There is a noticeable loss of nerve cells in cortical layer IV just above the laminar lesion; the still more superficial cortical layers (II and III) and the cortical zonal lamina (layer I) appear substantially intact.

A laminar lesion covers an area commensurate with the aperture used. Its dorsoventral dimensions are always limited, though the width of the lesion is a function of the radiation dose. With smallest effective doses, the width of

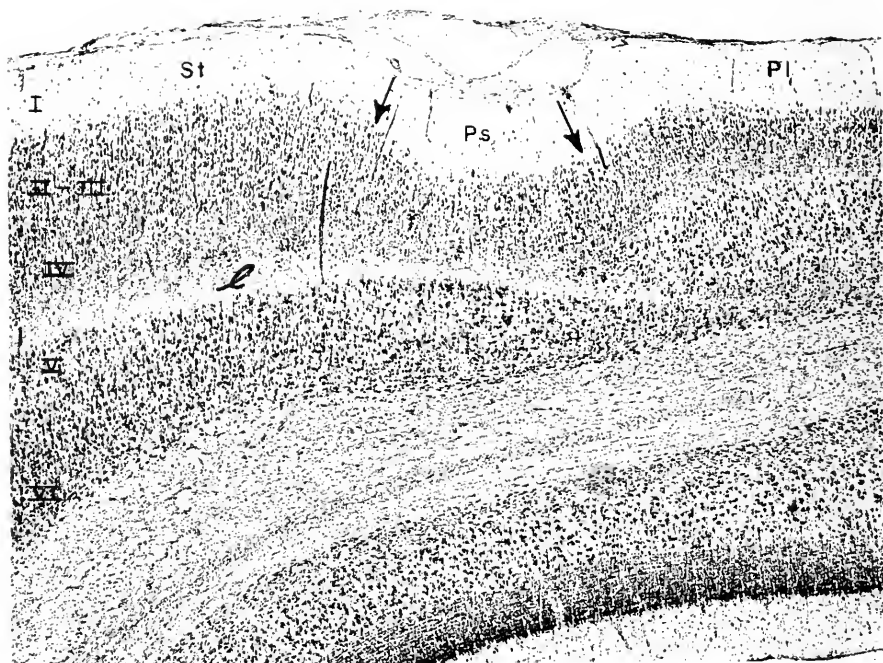


FIG. 1. Laminar lesion (l) in the striate (St) and peristriate (Ps) areas of a rabbit. The extreme edge of the lesion just extends into the posterior limbic region (Pl). Due to the scatter of the beam, the lesion is narrower in the peristriate than in the striate field. 65 days after irradiation. Peak dose: 33,000 rads; average dose: 12,000 rads; surface dose: 7,000 rads; number of deuterons per cm^2 : 8.8×10^9 I=1st cortical layer, II-IV=cellular cortical layers. $\times 30$.

the lesion may be as narrow as 60μ ; with higher doses which produce typical laminar destructions this width is close to 180μ ; still higher doses may produce lesions over 200μ wide. (All the figures refer to stained preparations.)

One striking observation in regard to the laminar lesions is that their glial content is moderate in animals surviving 6 weeks or longer. In Fig. 1 the glial content of the laminar lesions appears approximately the same as that of the zonal lamina. This is typical, even though a laminar lesion in early stages displays a vascular and glial reaction, which may be intense if the radiation dose is nearly maximal for laminar destruction.

We have conclusive evidence that older laminar lesions regularly display dendritic processes and a wealth of axons which form irregular and often denser patterns than do normal cortical sectors (Figs. 2-4). In Figs. 2-4, showing a laminar lesion in the upper part of layer VI 204 days after irradiation, it is apparent that the laminar lesion is filled with nerve fibers that form a dense, abnormal striation, which rather faithfully follows the undulations of the laminar lesion. From a study of a large amount of material (pre-

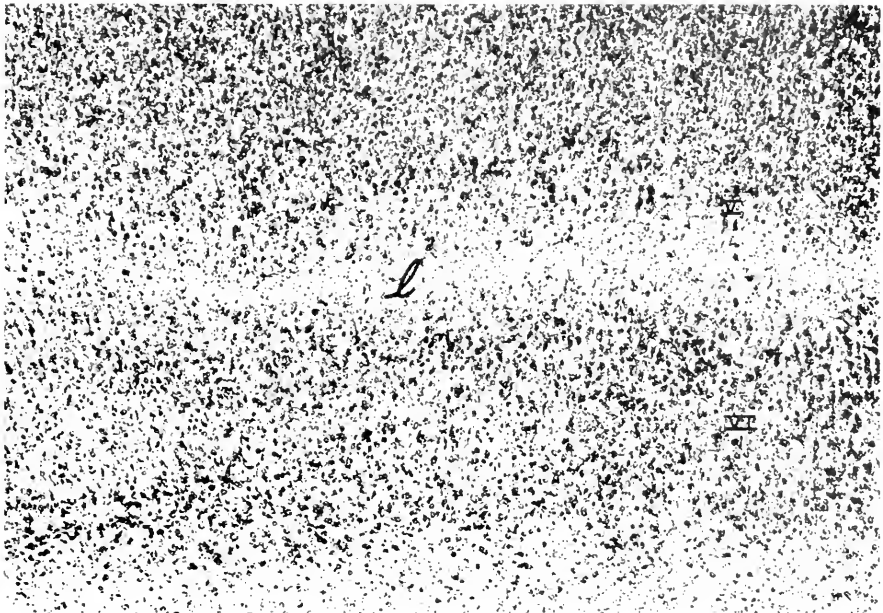


FIG. 2. Laminar lesion (l) in the postcentral region of another rabbit 204 days after irradiation. Almost normal cortical sector at the extreme left of the figure. Peak dose: 27,000 rads; average dose: 9,000 rads; surface dose: 6,000 rads; number of deuterons per cm^2 : 7.4×10^9 . Formalin fixation, frozen section, Nissl stain, $\times 50$.

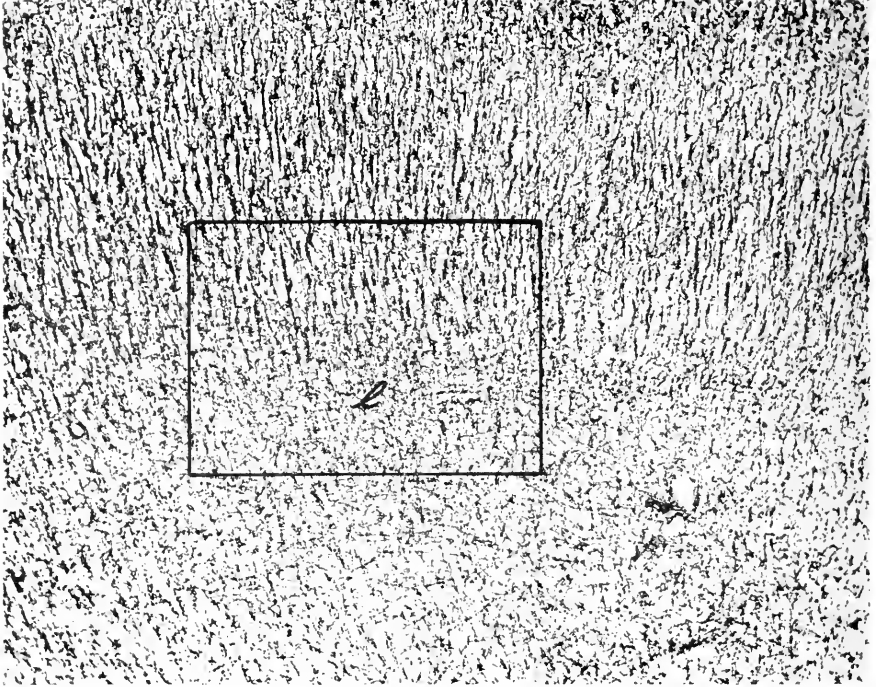


FIG. 3. Section adjacent to that shown in figure 2. Schultze's silver stain for fibers. Lamina lesion (1) is filled with nerve fibers which form a dense striation and interrupt the orderly radial arrangement of the fiber bundles. Notice the near normal appearance of the remaining portion of the cortical field. $\times 60$.

pared with a variety of histologic techniques† from brains of animals surviving from several days to 18 months after irradiation, we concluded that many nerve fibers in the laminar lesion must be new sprouts.

We have reported on the technique as well as on the histologic appearance of the laminar lesions and the problem of fiber growth in considerable detail elsewhere (Malis *et al.*, 1960; Rose *et al.*, 1960). However, considering the interest in the problem of the radiation dose, we will repeat a few points concerning the technique as well as some problems arising in regard to the determination of the dose.

All irradiations were carried out with the beam from the 60 in. cyclotron at Brookhaven National Laboratory, which delivers 20 Mev deuterons, 10 Mev protons, or 40 Mev alpha particles. Irradiations were done mostly with the deuteron beam, because its range in the brain (about 2.5 mm) is twice as large as the ranges of available protons or alpha particles. Figure 5 illustrates the irradiation arrangement. The evacuated beam pipe brings the col-

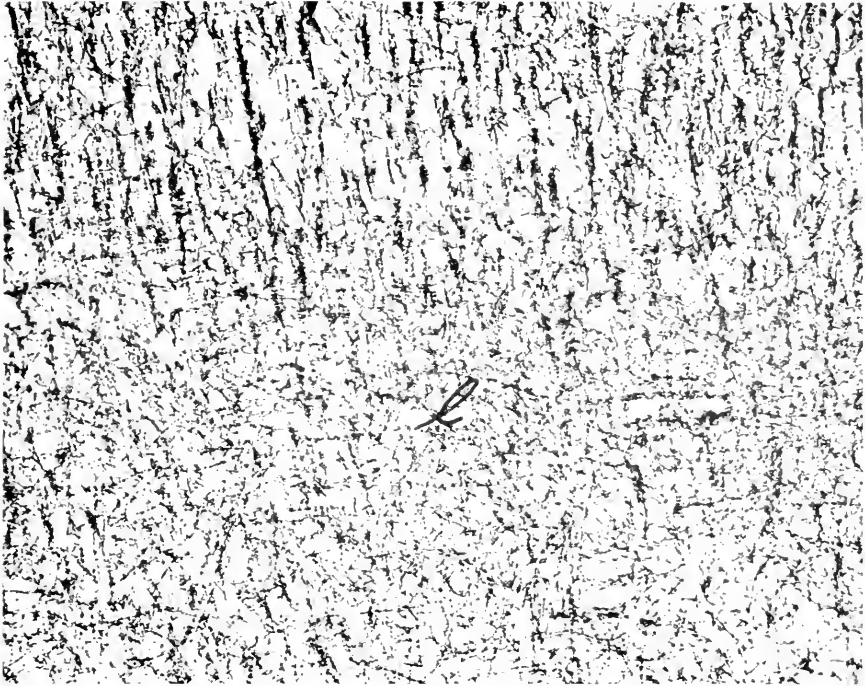


FIG. 4. Area enclosed by the rectangle in FIG. 3 shown under magnification of 150.

limited, "defocused" beam from the cyclotron. Measurements of the current in an argon-filled, transverse ionization chamber, placed at the end of the beam pipe, were made to calculate the radiation doses. After the beam left the ionization chamber, it traversed usually $3\frac{3}{4}$ in. of air before striking the target. A rifle telescopic sight with an illumination device and a mirror was mounted on a slide on the ionization chamber, so it could be moved with precision into alignment, and the target area centered in the cross hairs of the telescope. With suitable calibrations, the telescope served to center the target in relation to the beam and to bring the target to a predetermined distance from the ion chamber.

The aseptic operation consisted of removal of the bone, preparation of a bloodless field, and closure after irradiation. All irradiations were done with dura intact.

Figure 6 shows the well known Bragg curve. The curve, for 20 Mev deuterons, plots the relative ionizations after penetration of aluminum foil against the thickness of the foil. There is a sharp rise in number of ions produced just before the end of the range, and the value for the ionization peak is nearly 5 times higher than at the beginning of the range. This sharp

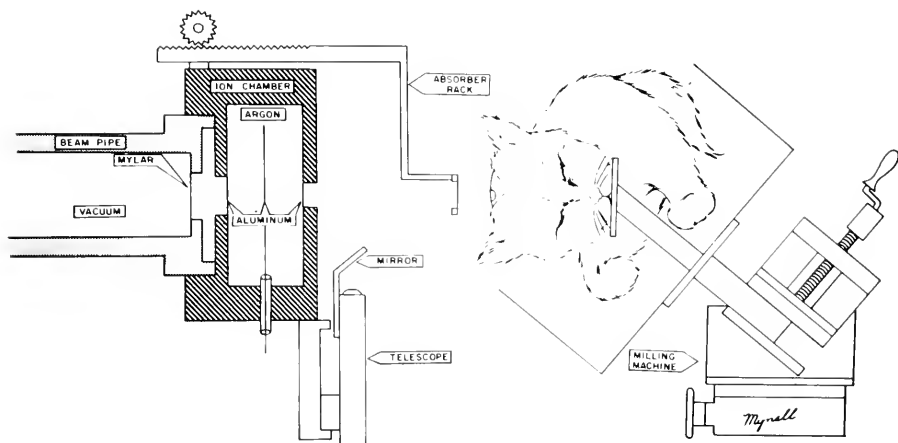


FIG. 5. Scheme of the irradiation arrangement. The telescope, which indicates the position of the target area in relation to the beam and the distance of the target from the ion chamber, is shown in the position it occupies during irradiation. An absorber (often used to shorten the range of particles in tissue) is advanced in the absorber rack to a distance of about 12 mm from the target to minimize the effects of scatter. The absorber does not touch the target in order not to compromise the sterility of the exposed cortex. The animal is positioned in relation to the beam in the milling machine.

rise in ionization near the end of the range makes possible the production of a laminar lesion. Considering that one mil thick aluminum foil is nearly equivalent in its stopping power to 50μ of brain, one can estimate the probable width of laminar destruction from the ionization curve. There is a reasonable, though not strict, relationship between the expected and measured thicknesses of the laminar lesions for light and moderate radiation doses. Since the zone of maximal ionization occurs near the end of the beam path, it is readily possible to shift this zone, and hence the locus of the laminar lesion, if the range of particles is shortened in tissue by introducing energy-degrading materials into the beam path. It follows, from consideration of the stopping power of brain and aluminum, that introduction of one mil thick aluminum foil elevates the zone of maximal ionization in the brain by about 50μ .

Several difficulties arise in the determination of the radiation dose. The first group of problems pertains to the determination of the actual number of particles which strike the target in a given irradiation arrangement. Further difficulties occur when one wishes to express the radiation dose in a manner which has biologic meaning, once the number of particles bombarding the target is known with reasonable accuracy.

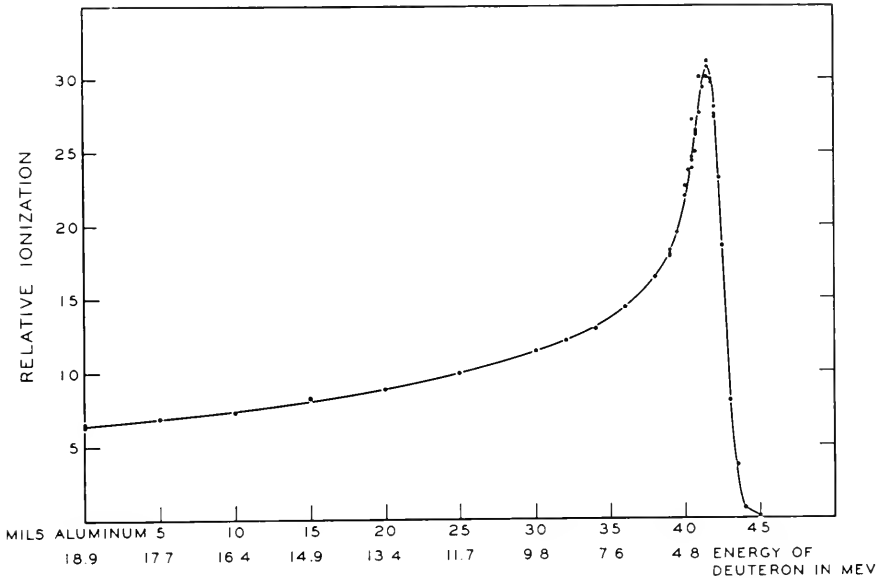


FIG. 6. Bragg ionization curve. Relative ionization produced by the beam of deuterons in a thin ionization chamber after passage through aluminum foil of varying thickness. The actual energy of the particles, as delivered by the cyclotron, was slightly higher than 20 Mev. Since, however, some absorbers had to be introduced into the beam path to make the determinations shown, the first value for relative ionizations is given for deuterons of 18.9 Mev energy. Ordinate: amount of ionization current in arbitrary units per constant number of incident deuterons. Abscissa: upper row of figures: thickness of aluminum foil in mils; lower row of figures: the remaining energy of deuterons in Mev after passage through aluminum foil of stated thickness.

The first group of problems is technical. We have used for calibration purposes measurements of radioactivity induced by irradiations of copper and lead discs. It was necessary to introduce correction factors for the non-linearity of the ion chamber and for the scatter of the beam.

Assuming that our calibrations are reasonably satisfactory, it is possible to express each irradiation dose as the *number of deuterons per cm²*. Since knowledge of the number of deuterons per cm² is essential for expressing the dose in any other way, it is apparent that this number is a straightforward measurement of the radiation dose. From a biologic point of view, the dose so expressed permits a reasonable estimate of the number of ions per unit area which may be relatively harmless, injurious, or lethal for a neuron or nerve fiber. Our data suggest that mild to heavy laminar lesions are produced by doses of 4×10^9 to 12×10^9 deuterons per cm², respectively.

The basic disadvantage in expressing the dose as a number of deuterons

per cm² is made clear by the consideration (see Fig. 6) that the number of ions produced per unit length of path varies for different sectors of the path and that it is the number of ions produced per unit length of path and not the number of particles which traverse the cortex which is responsible for the laminar destructions.

One does not avoid this essential disadvantage by computing the dose in rads, if the computation is made on the basis of number of particles, their energies, and their range in tissue. The figure which emerges from such calculation represents an *average dose* in rads, which would be an adequate measure only if the energy loss along the beam path were uniform. Since this is not the case, it is desirable to estimate the actual doses delivered at chosen points along the beam path. We have routinely computed such doses at two levels. The first is the dose in rads delivered to the *surface of the cortex* (*surface dose*); the second is the dose delivered at the *ionization peak* (*peak dose*). For calculation of the surface dose, the rate of energy loss must be known. For brain, this rate was considered equal to the appropriate values tabulated for water (Rich and Madey, 1954), since the rates of energy loss for brain and water cannot be significantly different. Once the surface dose is determined, the peak dose (or any other dose at a desired depth) can be estimated from the ionization curve (Fig. 6), if a reasonable assumption is made that the shapes of the ionization curves in brain and aluminum do not differ materially.

The peak dose is, we believe, the most appropriate measure of the radiation effect in our material, despite the fact that its estimate is technically less reliable than that of other measures. The peak dose is the highest dose delivered over a short distance in tissue. It can be argued from the ionization curve in Fig. 6 that values close to the peak dose can be expected to be delivered to a strip of cortex about 50 μ wide.

The peak dose, the surface dose, and the average dose have constant relationships to each other if the range of particles in tissue remains the same. If the range is shortened in tissue by introduction of an absorber into the beam path, the ratios change, since the value of the peak dose remains constant, while the average dose and the surface dose become higher. In our irradiation arrangements, the peak dose was usually almost 5 times larger than the surface dose and about 3 times larger than the average dose.

As is known, some time elapses before a radiation lesion manifests itself histologically. The latent periods tend to be long with marginal doses and become greatly reduced as the dose increases (Malis *et al.*, 1960). We will consider the dose necessary to produce a laminar lesion in rabbits which survived from 3 weeks to 18 months after irradiation. Table I assembles such data for 177 lesions. Each lesion was studied in serial sections cut at 30 μ , and every section was mounted.

TABLE I

RELATION OF PEAK DOSES TO HISTOLOGICAL FINDINGS

Peak doses ^b (in 1,000 rads)	Number and type of lesions ^a			
	No evidence of lesion	Light laminar	Heavy laminar	Necrotic foci
< 14	6	9	—	—
15-24	1	16	—	—
25-34	—	20	10	2
35-44	—	8	25	16
45-54	—	—	7	29
> 55	—	—	4	24

^a Findings for 177 radiation lesions in rabbits surviving from 22 to 548 days.^b Single irradiations with 20 Mev deuterons.

The lesion is defined as light laminar if the destruction is restricted to the zone of maximal ionization throughout the entire irradiated region. It is called a heavy laminar lesion if the band of destruction is broader and some changes are manifest for a short distance above the zone of maximal ionization. A lesion is classified as showing a necrotic focus if anywhere within the irradiated region there is a sign of vascular occlusion or its sequelae. Only some lesions in the last category (produced by very large doses) display signs of radiation necrosis throughout the entire irradiated region.

The table implies that each type of lesion can be produced by a large span of radiation doses, even if one assumes that some extreme figures in each category merely represent an apparent spread due to errors in determination of the delivered doses. Several conclusions are apparent. A peak dose of about 30,000 rads (which corresponds in our irradiation to a surface dose of approximately 6,000 rads and an average dose of about 10,000 rads) leads almost invariably to excellent laminar destruction. A peak dose up to 45,000 rads often produces heavy laminar destruction; on the other hand, a peak dose can be reduced to about 15,000 rads and still almost always result in a narrow, light laminar lesion. Peak doses below 15,000 rads may fail to produce lesions, at least for many weeks or months, while doses in excess of 45,000 rads can be expected to produce necrotic foci.

It appears that a peak dose of about 15,000 rads can be tentatively accepted as a reasonable approximation of a minimal dose, which must be applied over a short distance in the cortex to produce total destruction of nerve cells within a few weeks. It follows from this consideration that a *surface* dose of about 15,000 rads (which roughly corresponds to a peak dose of 75,000 rads) should be necessary to produce *total* necrosis of the irradiated cortex with our beam. This expectation seems in reasonable agreement

with our observations, since no peak dose in our material smaller than 65,000 rads produced total necrosis.

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Fluorescein as a Sensitive, Semiquantitative Indicator of Injury Following Alpha Particle Irradiation of the Brain*

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Soluble fluorescein U.S.P. (Uranine), a dye routinely used by physicians as a convenient and sensitive indicator of damage to corneal epithelium, has been found to be an equally convenient, semiquantitative, and sensitive indicator of irradiation injury to brain tissue. The use of this method as an indicator of damage to brain tissue is based on the same principle as its use as an indicator of corneal epithelial damage, i.e., normal brain tissue, like normal corneal tissue, does not stain, whereas injured tissue of both types readily takes up the dye. The method is quantitative, since the intensity of staining is dependent on the severity and number of injured cells. Permeability of the tissue to fluorescein represents a physiologic or biochemical alteration of the cell which precedes morphologic changes demonstrable by light microscopy.

The invasion of brain tissue by cells which are normally permeable to fluorescein can be recognized by the fact that they fluoresce following administration of the dye. This fact has been successfully used as a technique to locate brain tumor tissue at surgery (Moore, 1953).

This paper describes the use of fluorescein staining as a sensitive and semiquantitative indicator of injury to brain tissue following localized irradiation with a beam of alpha particles from the 184 in. cyclotron. The method has been used to demonstrate the difference in radiosensitivity of brain tissue in different species.

Material and Methods

Male rats of the Long-Evans strain, 28 days of age at the time of irradiation, were used, except when the effect of age difference was investigated. Young adult cynomolgus monkeys and rabbits of the New Zealand White

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strain were used in the comparison of the radiosensitivity of various species. During irradiation all animals were given light barbiturate anesthesia, and their heads were firmly mounted in specially designed holders (Fig. 1). Rigid fixation of the heads of all animals was accomplished by the use of ear plugs and a sharp pin into the gingiva and maxilla between the dorsal incisors. To keep the level of barbiturate anesthesia light, it was necessary to use xylocaine anesthesia locally in the ear canals and gingiva of the monkeys and rabbits.

Proper anatomic positioning of the beam was insured by having an x-ray source aligned with the path of the beam, so that a roentgenogram of the



FIG. 1. During irradiation all animals were given light barbiturate anesthesia, and their heads were firmly mounted in a specially designed holder. For proper positioning of the beam, a "Land" camera was placed in the path of the beam beside the animal's head.

skull with the image of the alpha particle beam superimposed could be made for each animal. For this purpose a "Land" camera was placed in the path of the beam beside the animal's head (Fig. 1). Figure 2 is such a "Land" camera roentgenogram of a rat's skull with the image of the 2.0×25.5 mm beam superimposed. Such pictures served as a permanent record for later comparison with the position of the band of fluorescence or the position of microscopic changes.

A 2.0×25.5 mm brass aperture 6 in. thick was used, except in the experiment where the effect of aperture size was specifically studied. The distance

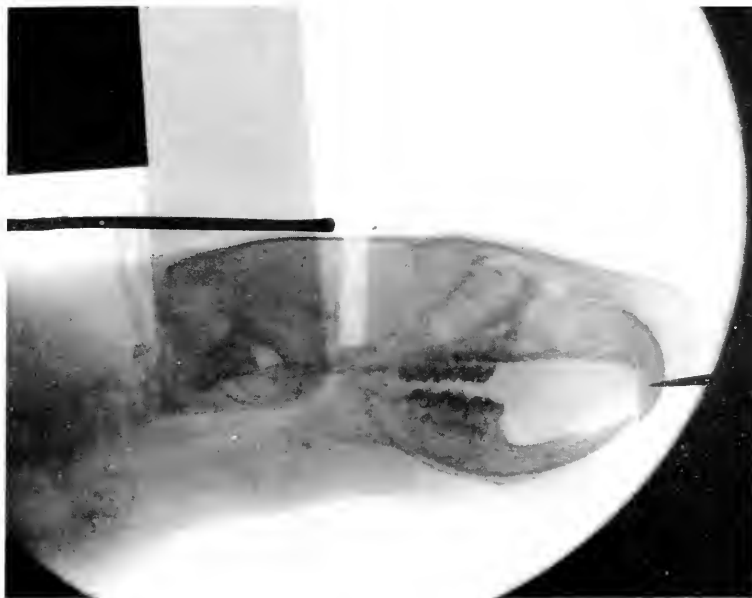


FIG. 2. A "Land" camera roentgenogram of a rat's head with the image of the 2.0×25.5 mm beam superimposed.

between the aperture and the area of the brain to be studied was kept constant (1.75 in.).

Fluorescein staining of the damaged brain tissue was accomplished by intravenous administration of fluorescein sodium (100 mg per ml, pH 8.2) under ether anesthesia of 10 mg per 60 gm body weight. After 45 minutes the animal was anesthetized with ether, and the head was removed by guillotine and immediately frozen in liquid nitrogen. The head was then sectioned on a high-speed bandsaw in the sagittal plane, which was 1.75 in. from the aperture at the time of irradiation. The cut surface was polished by holding it under running water until free of all dust fragments, but not long enough to melt the tissue. The two halves were examined in a dark room under ultraviolet light while solidly frozen. A visual estimation of the intensity of the fluorescent band was recorded. Evaluation of the fluorescein staining was made on the basis of an arbitrary scale from 1+ to 4+, with 1+ being faintly visible and 4+ being intense. When subsequent histologic examination was desired, the frozen brain was immersed directly in formalin and processed in the usual way. Rapid freezing with liquid nitrogen did not seriously interfere with later microscopic evaluation of the tissue. Microscopic examination was also made of brain tissue which had not been subjected to freezing to insure accurate interpretation. The skull was carefully

opened and the head immersed in 10% formalin. The brain was removed from the skull the following day and placed in fresh formalin. When thoroughly fixed, the irradiated area was blocked out, dehydrated in graded alcohols, and embedded in paraffin. Sections were cut at $6\ \mu$ and routinely stained with hematoxylin-eosin, van Gieson, 1% thionin solution for Nissl substance, and Palmgren's silver stain for fibers.

Throughout this study, 900 Mev alpha particles from the 184 in. cyclotron were used. All effects described resulted from a single dose given at a dose rate of 2,500 rad per min.

The beam was monitored with a parallel plate ionization chamber with aluminum foil electrodes and nitrogen atmosphere (Tobias *et al*, 1952; Birge, *et al*, 1956). For absolute standardization, this ion chamber was calibrated against a Faraday cage, which was used to measure the particle flux in the beam. The distribution of beam intensity after the beam passed through the aperture was calibrated with photographic emulsion dosimetry. By this technique, dose of beam measured by the monitor ion chamber was allowed to pass through the aperture and fall on a photographic film exposed in a phantom to correspond with the position of the irradiated animal's head. Densimetry of the photographic film yielded information for dose distribution. Dose values are in rads and refer to peak dose at the central plane of the knife-edge lesion.

Results

The minimum dose which would produce changes and the time of onset of demonstrable radiation changes in the brains of rats were studied by giving single doses from 5,000 to 26,000 rads and autopsying the rats at postirradiation intervals from 6 hours to 100 days. The results were evaluated by the presence of fluorescein staining and by morphologic changes seen with light microscopy. Figure 3 summarizes the results obtained in rats, using fluorescein staining as the criterion of damage. The time of onset and intensity of fluorescein staining is different for different doses of irradiation. At the lowest doses of 5,000 and 6,000 rad, no staining occurs until several weeks after irradiation, the intensity of staining is low, and the effect appears to be transient. Following doses of 26,000 rad, there was definite staining 24 hours after irradiation and liquifaction of the irradiated zone by the 3rd postirradiation day. Doses of irradiation differing by 20% between these two extremes can be clearly separated both as to time of onset and intensity of staining. Figure 4 is a log-log plot of the time of first appearance of fluorescence as a function of dose and serves to illustrate the definite relationship between dose and time of onset of recognizable changes (staining with fluorescein).

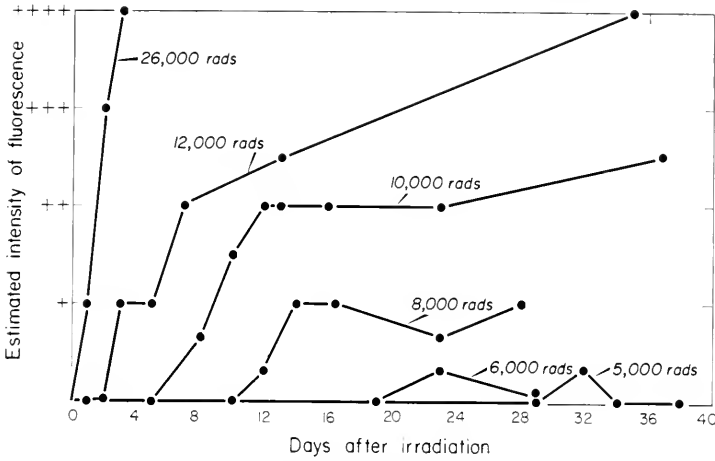


FIG. 3. Summary of results obtained using fluorescein staining as a criterion of damage in rats. The time of onset and intensity of fluorescein staining are different for different doses of irradiation.

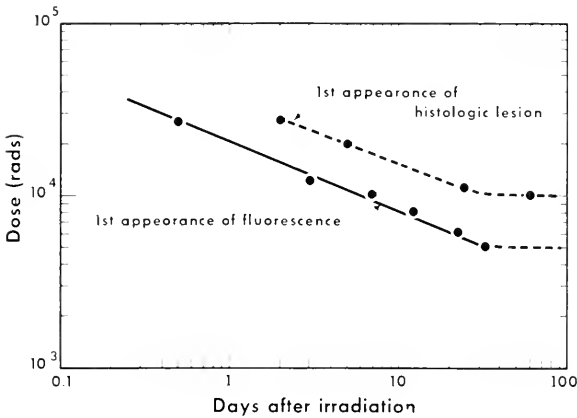


FIG. 4. A log-log plot of the time of first appearance of fluorescence as a function of dose, illustrating the definite relationship between dose and time of onset of recognizable changes (staining with fluorescein).

Microscopic examination of the same brains that were examined for fluorescence, as well as of brains from companion rats from whom the tissue was removed and fixed without previous freezing, demonstrated that a lesion is seen earlier and at lower doses with fluorescein staining than with light microscopy. After a dose of 8,000 rad, a fluorescein-stained lesion was clearly demonstrated during the 2nd and 3rd weeks postirradiation without subsequent clearly demonstrable microscopic evidence of damage.

After a dose of 10,000 rad, no microscopic lesion was recognizable during the first few weeks (none by 37 days), but eventually (84 days or more) a minimum lesion appeared in the irradiated area.

At doses above 10,000 rad, a microscopic lesion appeared earlier and at a dose of 20,000 eventually (6 weeks postirradiation) led to complete necrosis and removal of the irradiated zone with healing of the margins. At 20,000 rad, the first recognizable morphologic changes were seen by the 5th day.

Because doses of irradiation differing by 20% can easily be differentiated by the fluorescein technique, it was thought that this method might be useful in demonstrating whether such variables as age of animal, size of aperture, or type of particle would affect the result.

A dose of 10,000 rad appeared to be ideal for demonstrating any differences in effectiveness, because at this dose there is a definite time lag followed by a plateau in response which clearly differentiates it from the next highest or next lowest dose (12,000 or 8,000 rad).

The effect of age of the rat on fluorescein staining of the irradiated brain was studied using 22-day- and 120-day-old rats, a dose of 10,000 rad, and a postirradiation interval of 7 days (4 rats of each age). The results in the two groups were identical, 3 rats of each group being classed as 1+ and 1 from each group being classed as 2+, a response which could not be expected from a dose effect equivalent to 8,000 or 12,000 rad. Thus, the appearance of the fluorescent lesion is not affected by the age of the animal, within the range studied.

To determine the effect of size of lesion on fluorescein staining of the irradiated rat brain, a study was made 7 days after irradiation with 10,000 and 8,000 rad through a 4, 2, or 0.5 mm slit aperture (2 mm used in all other studies). The exact dosimetry for the different apertures was determined in the manner described. When the animals were autopsied, all brains given the larger dose showed a clear band of fluorescence corresponding to the position of the beam, and no brain given the smaller dose showed a lesion. Thus the dose-effect relationship appears to be independent of the size of the irradiated area within the limits studied, i.e., 0.5 to 4 mm.

Two experiments were done to compare the effectiveness of a given dose of alpha particle irradiation in different species. Rats, rabbits, and monkeys were given a single dose of 11,000 rad and examined at various postirradiation intervals. In the first series, the earliest time interval was 48 hours, by which time the monkey brain showed a 4+ fluorescein staining and beginning liquefaction of the irradiated area. The results from examination of fluorescein staining in the second series, in which the time intervals were shortened to get early observations on monkeys, are compared in Fig. 5. Five monkeys given 3,000 rad and autopsied at 3, 5, 8, 12, and 15 days showed no staining with fluorescein. By comparing the results from Fig. 5

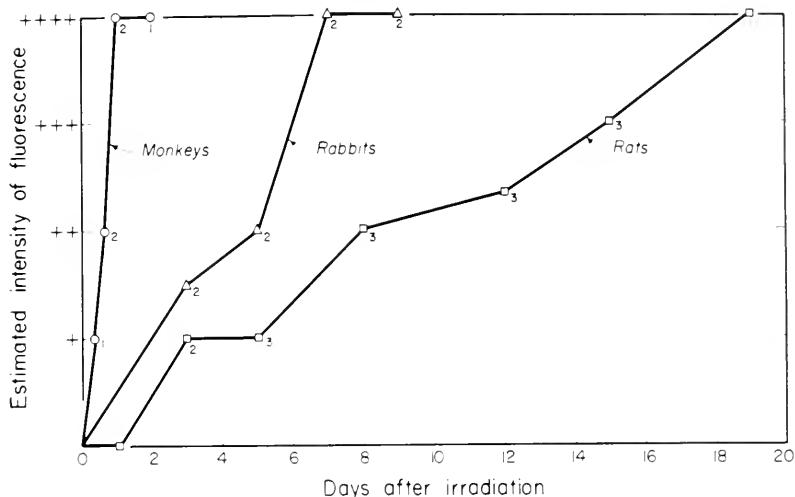


FIG. 5. A comparison of the development of a fluorescent lesion in the brains of rats, rabbits, and monkeys, all given the same dose (10,000 rad) through the same aperture on the same day.

with the dose-response curve in Fig. 3, one sees that the response obtained in the rats in this experiment corresponded to a dose of approximately 11,000 rad, whereas the response in rabbits corresponded to approximately 15,000 rad, and the response in monkeys corresponded to something greater than 26,000 rad. Figure 6A illustrates the intensity (4+) of fluorescein staining of the monkey brain 24 hours after a dose of 11,000 rad. The photograph was taken while the brain was still frozen, using ultraviolet light. Rats given the same radiation dose showed no fluorescein staining at 24 hours and showed only 1+ fluorescence at 5 days. The monkeys killed at 24 and 48 hours had generalized paralysis and periodic clonic convulsions, illustrating that not only their permeability to fluorescein but also the functional capacity of the tissue was altered. Figure 7 compares the morphologic changes seen in the monkey brain 24 hours after a dose of 11,000 rad with the morphology of the rat brain 24 days after the same dose delivered under identical conditions.

Figure 7A is a low power photomicrograph of the irradiated portion of the brain from a companion monkey to that shown in Fig. 6A. The animal

FIG. 6A. An illustration of the intensity (4+) of fluorescein staining of the monkey brain 24 hours after 10,000 rad. The photograph was taken while the head was still frozen, using ultraviolet light. Rats given the same radiation dose and killed at the same time showed no fluorescence.

FIG. 6B. "Land" camera picture taken at the time of irradiation, superimposed on a roentgenogram of a monkey's skull for orientation.





A

Fig. 7A. A low power (H. & E. $\times 6.5$) photomicrograph of the irradiated portion of the brain from a companion monkey to that shown in Fig. 6A. The animal was killed 24 hours after irradiation with 11,000 rad. The width and position of the beam is indicated. There was a 6-mm wide band of intense (+ +) fluorescence extending $\frac{3}{4}$ of the way to the bottom of the section in the fresh specimen. The photograph illustrates the isolated patches of lightly stained tissue seen in the gray matter and the mottled appearance of the white matter in the path of the beam.

Fig. 7B. Lightly staining patches in the gray matter enlarged 24 times. By 48 hours the entire irradiated area had become liquescent.

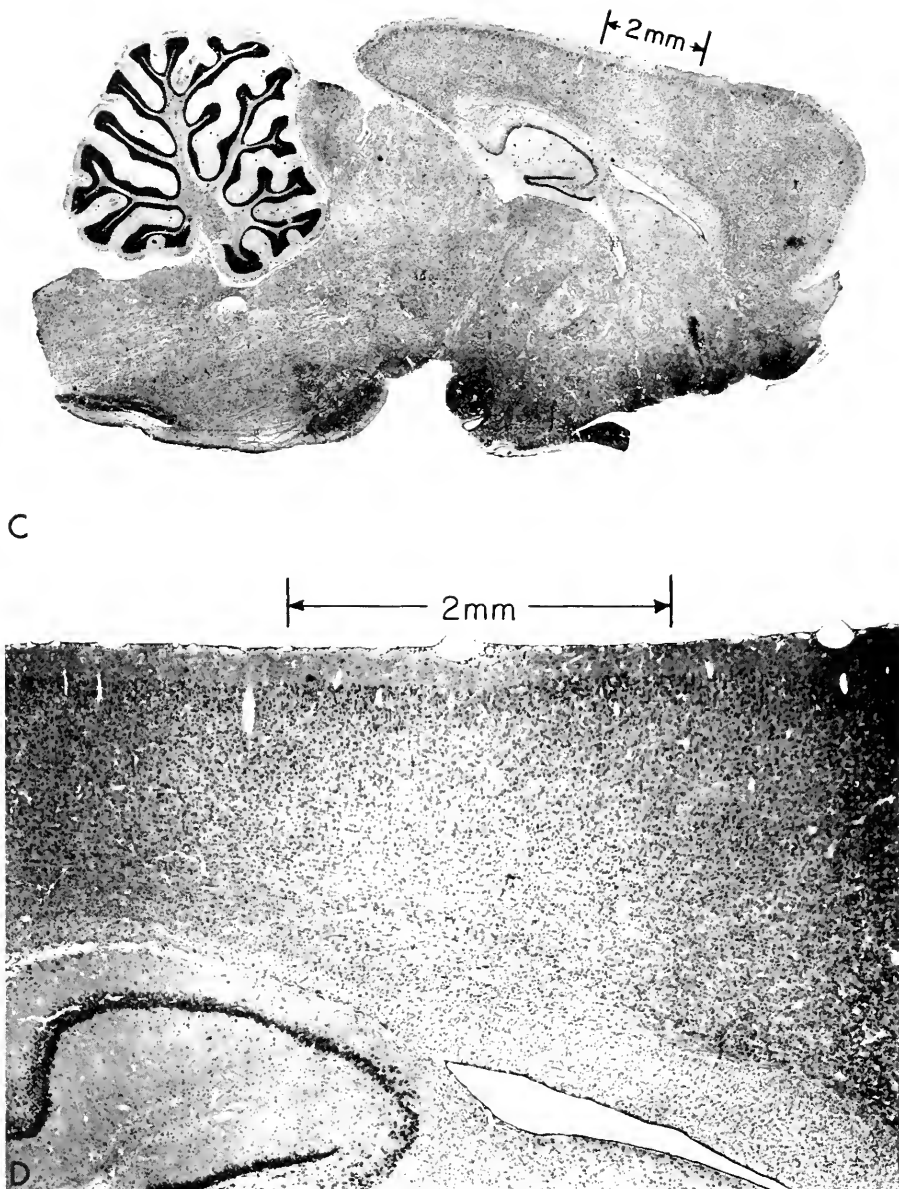


FIG. 7C. Rat brain (H. & E. $\times 6.8$) 24 days after the same dose and aperture as used in the monkeys in Figs. 7A and 7B. Without magnification or with the help of low magnification, it can be seen that in the cortex there is a decrease in the staining quality of the ground substance with some capillary dilatation. No changes were recognized in the white matter (corpus callosum).

FIG. 7D. Higher power magnification ($\times 26$) of the area of the cortex showing changes. Microscopic examination of the brains of rats from the same group autopsied at 20 days or less after irradiation showed no recognizable morphologic changes.

was killed at 24 *hours* after irradiation with 11,000 rad. There was a 6 mm wide band of intense (4+) fluorescence extending $\frac{3}{4}$ of the way to the bottom of the section in the fresh specimen. The photograph illustrates the isolated patches of lightly stained tissue seen in the gray matter and the mottled appearance of the white matter in the path of the beam. Figure 7B shows the lightly staining patches in the gray matter enlarged 32 times. By 48 hours the entire irradiated area had become liquescent.

Figure 7C shows a rat brain 24 *days* after the same dose and aperture as used in the monkeys in Fig. 7A and 7B. Without magnification, or with the help of low magnification, it can be seen that in the cortex there is a decrease in staining quality of the ground substance with some capillary dilatation. No changes were recognized in the corpus callosum. Figure 7D is a higher power magnification of the area of the cortex showing changes. Microscopic examination of the brains of rats from the same group autopsied at 20 days or less after irradiation showed no recognizable morphologic changes. By 44 *days* after irradiation, the irradiated area had undergone hemorrhagic necrosis with a lethal subarachnoid hemorrhage occurring in some animals, a degree of pathology grossly comparable to that found in the monkey after 48 *hours*. A detailed study of the histopathology of such lesions has been presented by Janssen *et al.* (1961).

Two methods of more accurately quantitating the extent of injury, using fluorescein as an indicator and as a method of evaluating the accuracy of the arbitrary visual grading of the lesions, have been suggested. Photographing the lesion, using standardized conditions under ultraviolet light, as in Fig. 6, and quantitating the film, using a micro-densitometer, could be done. Pieces of tissue of uniform size taken from the center of the lesion have been analyzed for fluorescein content using a fluorospectrophotometer in a few cases. The values obtained for tissue which had been graded 1+, 2+, and 4+ were 62, 78, and 460 μg fluorescein.

Discussion

The use of fluorescein staining as a criterion of brain injury following irradiation has been described in detail because it combines the advantages of being quick, simple, semiquantitative, and remarkably sensitive for brain, rather than because of any basic physiologic or biochemical significance of the property of staining *per se*. The simplest explanation for the fact that the tissue takes up the dye only after injury would seem to be that the membranes of injured cells became permeable to substances which they would normally exclude. However, when considering brain tissue, one cannot ignore the so-called blood-brain barrier phenomenon which has been the subject of so much controversy (Clemente and Richardson, 1961). This study does not

provide evidence as to the mechanism involved in the acquisition of stainability of brain tissue following irradiation.

It should be emphasized that the results presented in this paper both for fluorescein staining and morphologic changes represent acute changes following high doses of irradiation and are for the most part confined to observations during a 3 week postirradiation period. The possibility of profound changes occurring in the central nervous system many months or years after relatively low doses of irradiation has been adequately emphasized by other studies (Arnold *et al.*, 1954c; Lindgren, 1958) and has not been considered in this work.

The doses of alpha particle irradiation which must be given in order to produce changes in the central nervous system have been found to be relatively high (above 5,000 rad). Parallel studies done by Janssen *et al.* (1961) using alpha particle irradiation confirm the finding that a dose in excess of 5,000 rad (6,000 was the minimum effective dose found by these authors) must be given before morphologic changes are observed at any postirradiation interval in the rat. However, Hicks and Montgomery (1952) have shown that 6 to 24 hours after x-ray doses of as little as 1,200 rad to the head of rats, necrotic oligodendroglia cells were found scattered through the white and gray matter.

The greater radiosensitivity of the primate brain as compared to that of the rodent brain which was found in this study both by the fluorescein staining technique and gross or microscopic morphologic examination, has been observed and emphasized by Arnold *et al.* (1954a,b,c) and by Lindgren (1958). The work of these authors indicates that the greater radiosensitivity of the primate brain occurs not only in the early postirradiation period after high doses, as was the case in this study, but also after long postirradiation periods and relatively low doses. Lindgren (1958) has emphasized that the radiosensitivity of the brain appears to vary from one region to another, cortex and the medullary region immediately underneath it being less radiosensitive than deep-seated parts of the white matter (Markiewicz, 1935; Pennybacker and Russell, 1948; Zeman, 1950). The radiosensitivity of the white matter also appears to vary from region to region, as is suggested by the disseminated appearance of the lesions on uniform irradiation of the entire brain (Scholz and Hsu, 1938) and by lesions seen in the midbrain (Arnold *et al.*, 1954b). One must be cautious in generalizing about brain tissue as a whole, and cautious in comparing work done with different types of radiation, different species, and different postirradiation intervals, until more data have accumulated.

Monkeys given 11,000 rad showed a response greater than that found in rats after 26,000 rad, suggesting that at this dose the brain tissue of the monkey is almost 3 times more radiosensitive than the brain tissue of the

rat. However, monkeys given 3,000 rad showed no staining with fluorescein, suggesting that the dose response curve for monkeys would have a steeper slope than that for the rat when plotted as in Fig. 4, but that the monkey may not show a greater radiosensitivity of brain tissue at lower radiation doses. The work of Hager *et al.* (1961) using x-ray indicates that the hamster brain is probably quite radiosensitive.

In an attempt to rule out the possibility that the data collected in this study pertained to the age of animal and aperture size, age and size of aperture were investigated. Rats 22 days old showed the same response as did rats 120 days old. There was no demonstrable difference in biologic effectiveness with aperture sizes ranging from 0.5 to 4 mm. These studies tend to emphasize the species differences found and broaden their interpretation, but are not intended to imply that age and size of lesion are not important factors in more extreme situations. Hicks (1953) has demonstrated that the adult nervous system is radioresistant and the embryonic nervous system radiosensitive, and Lindgren (1958) has stated that in clinical practice it has proved advisable to reduce the adult dose by 25% for treating brain lesions in 5-year-old children and by 50% when treating 2-year-old children. Zeman *et al.* (1959) have demonstrated that in order to produce a lesion in the brain when the aperture is narrowed (25 μ), an extremely high dose must be delivered to the tissue. Hicks *et al.* (1958) has demonstrated differences in radiosensitivity between strains of mice and differences in pathologic and physiologic effects when the intensity of the radiation is varied (Hicks *et al.*, 1956).

Summary

Soluble fluorescein U.S.P. (Uranine), a dye routinely used by physicians as a convenient and sensitive indicator of damage to corneal epithelium, has been found to be an equally convenient, semiquantitative, and sensitive indicator of injury to brain tissue following localized irradiation with a beam of alpha particles from the 184 in. cyclotron. Permeability of the tissue to fluorescein represents a physiologic or biochemical alteration of the cell, which occurs before any morphologic changes demonstrable by light microscopy. The time of onset and intensity of fluorescein staining are different for different doses of irradiation. The minimum effective dose was 5,000 or 6,000 rad. A species difference in radiosensitivity of brain tissue was demonstrated both by the fluorescein technique and by histologic morphology. The brain of rabbits and monkeys is considerably more sensitive to alpha particle irradiation than is that of rats.

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Pathologic Changes in the Brain from Exposure to Alpha Particles from a 60 Inch Cyclotron *

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Introduction

This paper deals chiefly with time-dose relationships in the appearance of alterations in the cerebellum and cerebrum of rats following exposure to alpha particle radiation from the 60 in. cyclotron in Berkeley. Exposure of brains to protons was also done for comparison. We were concerned with (1) severity of the lesions in terms of energy given off along the course of the Bragg curve, (2) alterations in structural elements with respect to time-dose relationships, and (3) circulatory and vascular alterations, including permeability changes as assessed by the Pickworth-Lepchne stain for erythrocytes and by certain fluorescence indicators, i.e., fluorescein-labeled serum proteins (FLSP) and sodium fluorescein.

Methods

In earlier phases of the study with about 25% of the animals, the top of the skull was removed (in a 15 x 10 mm area) and the dura left intact

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(Simpson *et al.*, 1953), but this practice was discontinued when it was found that adequate lesions could be produced through the intact skull. Thereafter the operation consisted simply of incision and reflection of the scalp prior to irradiation. The cyclotron aperture was circular and 14.3 mm in diameter, sufficient in size to allow irradiation of most of the dorsal surface of the cerebellum and cerebrum bilaterally.

For morphologic studies, metallic impregnations for glia and Pickworth-Lepelne benzidine staining for visualization of the vascular tree were used. Usually the brains were fixed in Bouin's solution. Vascular permeability to serum proteins was studied by the FLSP technique (Klatzo and Miquel, 1960). In this procedure, the rats were injected intravenously with 2 cc of 8% fluorescein isothiocyanate-labeled albumin, usually 24 hours prior to sacrifice, and unstained, formalin-fixed, frozen sections were examined under the fluorescence microscope. For observations on vascular permeability to sodium fluorescein, animals irradiated at the same dose level were injected with 1 cc of 10% sodium fluorescein intravenously, usually 24 hours prior to sacrifice, and the gross observations for fluorescence were conducted as previously described (Klatzo *et al.*, 1958).

The alpha particles and protons to which the dorsal part of the brain was exposed had an energy of approximately 12 Mev per nucleon. These particles were first deflected away from the magnet and down the deflector channel, which contained a $\frac{1}{4}$ mil Al stripping foil and a slit system to exclude particles of unsuitable energy or charge-to-mass ratio. To obtain a homogeneous particle flux distribution over the target area, a defocused setting of a pair of quadropole magnets was used. The beam then passed through a monitor, called a "high vacuum ionization chamber" (Brustad *et al.*, 1960), and finally out of the vacuum system through a 1 mil Al window. A specially constructed parallel plate ionization chamber (Brustad *et al.*, 1960) was attached to the snout, and the animals to be irradiated were placed in a special holder about 5 mm from the end window of the ionization chamber. This air gap reduced the effective particle range in tissue by less than 5 μ . The ionization chamber could be detached and replaced by a magnetically guarded Faraday cup. The monitor upstream was always calibrated against the Faraday cup in terms of number of bombarding particles per cm^2 . When animals were exposed, the ionization chamber response and the calibrated monitor response were recorded independently and converted to surface dose in rad. These two different dosimeters generally agreed to within a few per cent.

Between the ionization chamber and the monitor, sets of calibrated Al-absorbers could be introduced with a remote controlled absorber changer. With the Faraday cup connected, accurate determination of particle range and beam-energy homogeneity could be performed routinely. With the

ionization chamber connected, Bragg curves could be measured and converted to tissue equivalent values. Depth-dose distribution in tissue was inferred from the measured surface dose by use of a Bragg curve derived in this manner (see Fig. 6). It was found that the effective range in tissue of the full energy protons and alpha particles (after allowance was made for the various absorbers, Al foils, and air gaps, which are permanently in the beam during exposure) was about 118 mg per cm², or 1.180 μ , if a tissue density of 1 is assumed. Homogeneity of the particle flux over the target area was checked from densitometer readings of exposed films or from "burn patterns" obtained on exposed ozalid paper. When the flux distribution was unsatisfactory, adjustments were made with the focusing magnets and, if necessary, in the entire alignment of the experimental setup. Details of dosimetric procedure and of particle properties are described elsewhere (Brastad *et al.*, 1960; Birge *et al.*, 1956).

The dose rate used during the exposure of the rats was approximately 10,000 rad per minute surface dose, which corresponded to about 4×10^9 particles per cm² per sec. Due to difficulties in the accelerator operation, a constant dose rate was sometimes difficult to maintain; fluctuations over a factor of 2 or more occurred.

Observations

The brain-surface doses of alpha particles and protons and the times of sacrifice of the animals are given in Table I. The brains were exposed to alpha particles at surface doses of 50 to 6,000 rad and to protons at a surface dose of 6,000 rad.

Whether produced by alpha particles or by protons, the basic lesion following irradiation consisted initially of a *zone* of cell damage which stretched horizontally across the cerebellar and cerebral cortex. The lower border of the zone was sharp; the upper border tended to be indistinct. Within the deepest part of the zone of damage a nerve-cell-poor or nerve-cell-free *band* eventually appeared. The width of the zone and of the band varied with the radiation dosage. In the text which follows, the terms "zone" and "band" are used in the sense indicated.

WIDTH OF THE LESIONS IN TERMS OF THE ENERGY GIVEN OFF ALONG THE BRAGG CURVE

Measurement of the width of the zone and the band of damage following exposure to 6,000 rad surface radiation dose was carried out in practically all 211 brains (Table I). The average maximal width of the zone and of the band, both in the cerebellum and the cerebrum, was virtually the same in the brains exposed to alpha particles as those exposed to protons (Table II).

TABLE I

NUMBER OF RAT BRAINS STUDIED AT DIFFERENT TIME INTERVALS FOLLOWING
IRRADIATION WITH ALPHA PARTICLES AND PROTONS ^a

Time of sacrifice after irradiation	Dosage to brain surface (in rad)							Totals
	50 A	250 A	750 A	1,500 A	3,000 A	6,000 A	P	
Hours 1- 6	—	—	—	—	—	8	2	10
14-18	—	—	—	—	—	7	—	7
20-28	—	—	—	—	—	13	4	17
36-48	—	—	—	—	—	12	4	16
Days 2.5- 5	—	—	—	3	3	38	12	56
6-11	—	—	—	—	—	35	25	60
12-18	3	3	3	—	—	17	5	31
20-46	—	—	—	—	5	3	6	14
64	4	3	3	3	3	9	—	25
120	—	—	—	—	—	1	—	1
150	—	—	—	—	—	1	—	1
216	3	2	3	3	3	2	—	16
	10	8	9	9	14	153	58	261

^a KEY: A, alpha particle radiation; P, proton radiation.

TABLE II

WIDTH OF REGION OF DAMAGE IN THE CEREBELLUM AND CEREBRUM FOLLOWING
EXPOSURE TO ALPHA PARTICLES AND PROTONS AT 6,000 RAD SURFACE DOSE

Site of irradiation	Alpha particles		Protons	
	Zone (μ)	Band (μ)	Zone (μ)	Band (μ)
Cerebellum	6 hr-5 days	7-216 days	6 hr-6 days	7-30 days
average width	110	41	123	45
Cerebrum	24 hr-7 days	8-216 days	60 hr-6 days	7-30 days
average width	236	110	291	119

On the other hand, the width of the zone and of the band was less for the cerebellum than for the cerebrum by a factor of around 2½. Due to the factor of tissue shrinkage as a consequence of irradiation, the width of the zone or band decreased with time. With respect to alpha particles, for example, the average width of the zone in the cerebellum during the 6 hour-5 day period was 110 μ , and at the 7-216 day period, 41 μ , at which time the "zone" had shrunk to form a "band." In the brains exposed to a 3,000 rad surface dose, the series was too small to obtain dependable averages. However, the width of the band was substantially less (Fig. 1-4).

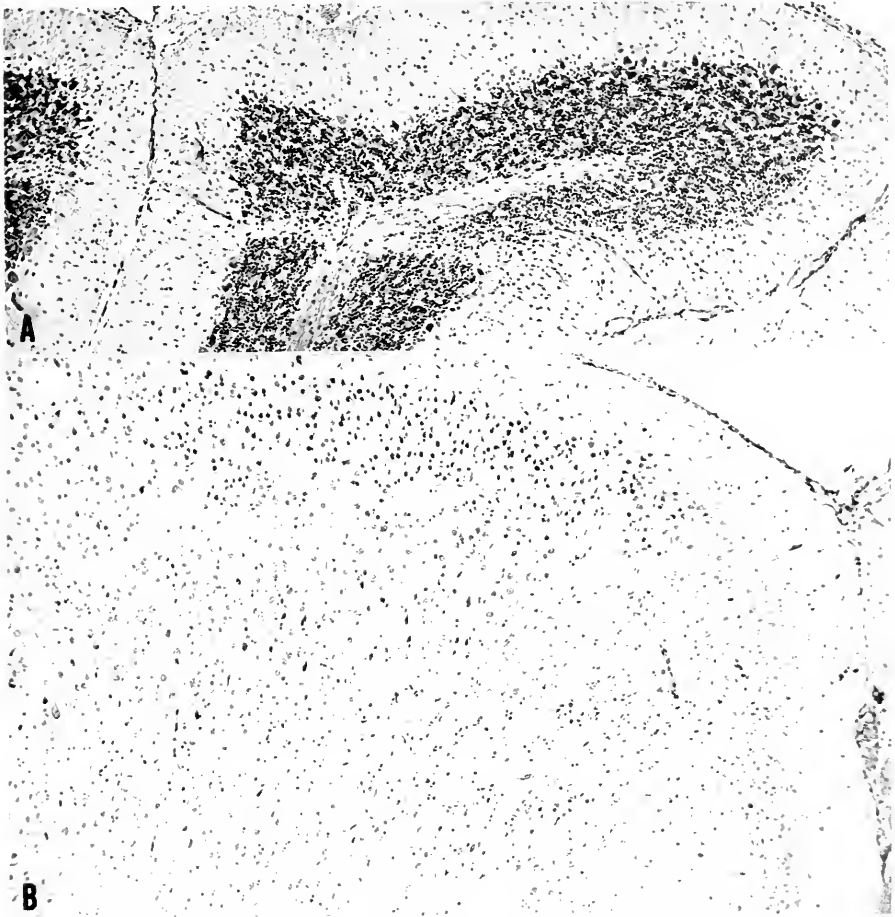


FIG. 1. Proton radiation, 6,000 rad surface dose; sacrifice at 10 days. *A*. A narrow cell-poor band extends across the cerebellar folia. Above the band the granular layer is rarefied, and some of the granule cells are atrophic. The intrafolial white matter is slightly disrupted and has reduced stainability. The Purkinje cells in the region of the band have disappeared. Bergmann cells show little alteration. Proliferated glia are present in the molecular layer, especially at the level of the band. *B*. In the cerebral cortex a wider nerve-cell-poor band is to be seen. Glia within the band have increased somewhat in number. Pyknosis of the nerve cells near the cerebral surface represents postmortem artifact. Both stained by Van Gieson-hematoxylin. Both $\times 75$.

At the 6,000 rad surface (Figs. 1 and 2) a wide nerve-cell-poor band was present in the cerebellum and cerebrum, but after 64 days both the band and the cerebellar and cerebral tissue above the band were greatly shrunken as compared with sections obtained at the 10-day stage. At the 3,000 rad surface dose, after 64 days (Fig. 3) the degree of damage was far less advanced. At



FIG. 2. Alpha particle radiation, 6,000 rad surface dose; sacrifice at 64 days. *A*. A narrow band poor in granule cells lies just above an intact granular layer. Above the band is a wide zone in which practically all granule cells are shrunken. Not only is there cellular shrinkage, but there is also gross atrophy of the granular and molecular layers in the irradiated region. Many Purkinje cells have disappeared. Glia in the molecular layer have multiplied, and at the level of the band near the middle of the photograph the cells of the molecular layer have disappeared. The white matter in the irradiated area is pallid, and its glia have increased somewhat in number. *B*. A nerve-cell-poor band, which contains an excess of glia, stretches across the cerebral cortex. The overlying cortex is greatly shrunken when comparison is made with that in Figs. 1 and 3 of the same magnification. Below the band are the white matter, ventricle, and hippocampus. *C*. From a field in the cerebral cortex, showing an atrophic band in which vessels are greatly dilated. The dark structures are vessels which have undergone advanced hyalinosis. All stained by Van Gieson-hematoxylin. *A* and *B*, $\times 70$; *C*, $\times 265$.

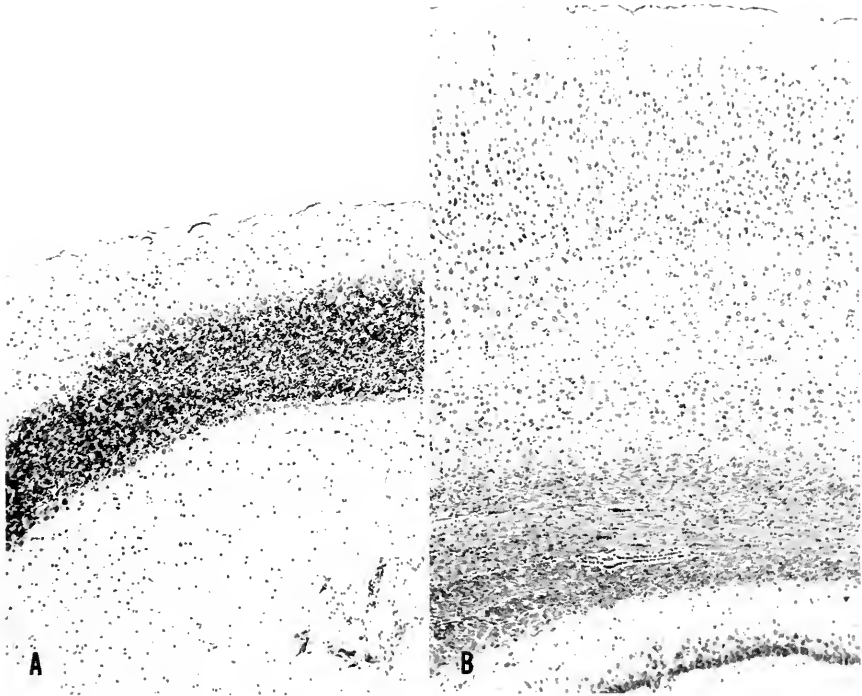


FIG. 3. Alpha particle radiation, 3,000 rad surface dose; sacrifice at 64 days. *A.* Cerebellum, showing a zone of shrunken granule cells marked off by a sharp lower border. Rarefaction of the granular layer is greatest in the region of maximal depth of the damage. To the left, Purkinje cells in the region of most intense irradiation have disappeared. Glia in the molecular layer have undergone slight proliferation. *B.* Cerebrum. The nerve-cell-poor band of damage is marked off by a sharp lower border. Above the band, scattered nerve cells have disappeared. Both $\times 65$. Van Gieson-hematoxylin stain.

the 1,500 rad radiation level (surface dose), the band of damage, present only in the cerebrum, was significantly narrower at 216 days (Fig. 4) than at 64 days after exposure to 3,000 rad.

One cerebellum was studied with a view to determining whether the width of the zone of damage was consistent with the stopping power of the particles as expressed by the Bragg curve. The cerebellum had been exposed to a surface dose of 12,000 rad, and the animal was sacrificed at 24 hours. The lower border of the granule cell pyknosis (Fig. 5) was 720 deep to the cerebellar surface. The pyknotic granule cells were counted in 8 different tissue depths within a 9×9.8 cm area on a photograph enlarged 225 times: the number of pyknotic cells for each of the 8 units within the area, when

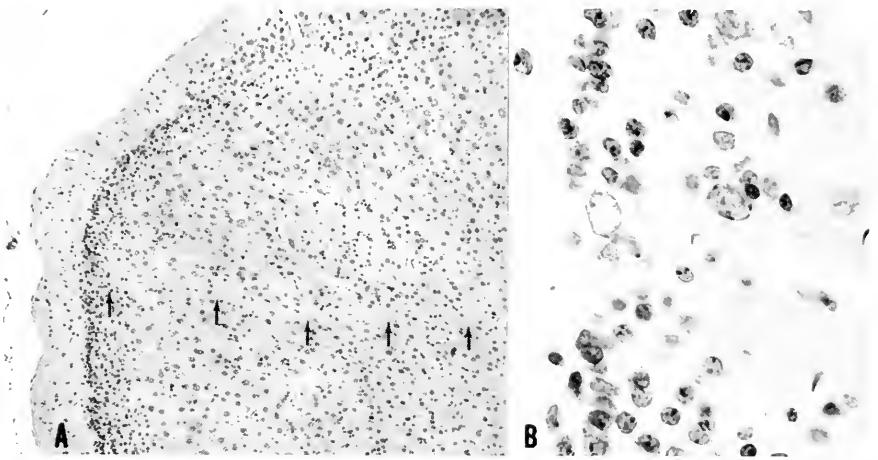


FIG. 4. Alpha particle radiation, 1,500 rad surface dose; sacrifice at 216 days. A. Cerebral cortex, illustrating a faint nerve-cell-poor band (arrows) extending laterally from the region of interhemispheric fissure. The meninges appear unaffected. $\times 65$. B. Lamina in the region of the interhemispheric fissure. Numerous nerve cells have disappeared and others are faded. Two nuclei, presumably those of nerve cells, are enormously enlarged. $\times 410$. Van Gieson-hematoxylin stain.

plotted against the Bragg curve, coincided with the energy given off along the slopes and at the peak of the curve (Fig. 6) if the assumption could be made that the cerebellum had undergone linear shrinkage by a factor of 35% as the result of processing. Measurements were made to test this assumption. The maximal width of the cerebellum in the stained section in which the pyknotic cells were counted was 7,590 μ ; when this figure was increased by 35%, the value was 11,660 μ . In a control rat of the same age as the experimental animal, the maximal width of a fresh cerebellum immediately after autopsy was 10,120 μ . Thus, the assumption was approximately correct. As to the cerebrum, its width on the stained section was 7,084 μ , and in the unfixed fresh brain, 8,602 μ . Thus, linear tissue shrinkage was relatively less for the cerebrum than the cerebellum.

ALTERATIONS IN STRUCTURAL ELEMENTS WITH RESPECT TO TIME-DOSE RELATIONSHIPS

Nerve cells

At surface doses of 50, 250, and 750 rad, observations up to 216 days (Table I) failed to reveal any neuronal changes. The lowest surface dose at which alterations were seen was 1,500 rad, and then not until the 216 day stage. In the cerebral cortex at this stage, a faint, narrow nerve-cell-poor band was detected (Fig. 4A). Adjacent to the interhemispheric fissure, the



FIG. 5. Alpha particle radiation, 12,000 rad surface dose; sacrifice at 24 hours. A fairly sharp border separates irradiated from nonirradiated granular layer. Above this border the density of pyknotic granule cells decreases progressively. The Purkinje cell (arrow) at the lower border of the damaged granular layer, i.e., in the most intensely irradiated region, shows an alteration in nuclear chromatin. Cells in the corresponding region of the molecular layer appear unaffected. Vessels are not dilated. $\times 175$.

nuclei of occasional cells, presumably nerve cells, were extraordinarily enlarged (Fig. 4B). By contrast, no evidence of damage was observed in the cerebellum.

At the 3,000 rad surface dose, with sacrifice at 4 to 216 days (Table I), two brains studied at 20 days had a narrow band of granule cell rarefaction

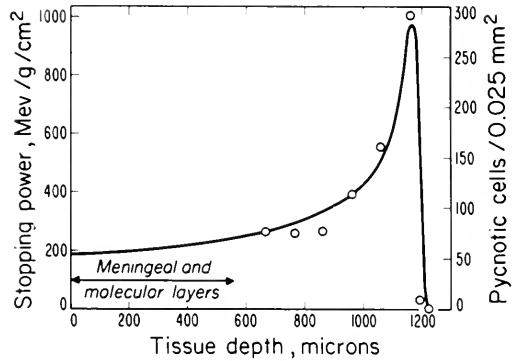


FIG. 6. Distribution of pyknotic cerebellar granule cells (illustrated in Fig. 5) as a function of depth, superimposed on the physically measured Bragg curve, assuming linear tissue shrinkage of 35%. This assumption was fairly well substantiated by direct measurements of fresh cerebellum and of the stained section.

in the cerebellum (and scattered pyknotic granule cells above the band) and a significantly wider band of nerve cell damage or loss in the cerebral cortex. In the cerebellum in the region corresponding to the Bragg peak, the nuclei of occasional Purkinje cells were chromatin-poor, while others had undergone the homogenizing type of necrosis; the molecular layer had a somewhat disrupted appearance.

At the 6,000 rad surface dose a distinct zone of nerve cell damage was first encountered in the *cerebellar cortex*, namely at 6 hours in 4 of 6 animals exposed to alpha particles or protons, and it consisted of a fairly even row of pyknotic granule cells in pale, somewhat loculated tissue (Fig. 7A). No alterations were seen in Purkinje or Golgi cells or in nerve cells of the molecular layer. The earliest time interval at which nerve cell damage was encountered in the *cerebral cortex* was also 6 hours, but only isolated nerve cells were affected, chiefly in the region adjacent to the interhemispheric fissure. Such changes were found consistently in the first 2 days, and were virtually limited to the region corresponding to the Bragg peak. Some of the affected nerve cells exhibited the homogenizing type of necrosis (Fig. 7B); others, fading of nuclear chromatin, often advancing to complete nuclear "skeletonization" (Fig. 7C). Cellular damage at these early stages made it difficult to determine whether some of the elements implicated were nerve cells or glia (Fig. 8). Both cell types appeared to be affected simultaneously. The earliest time at which a distinct zone of damage was found in the *cerebral cortex* was 42 hours, but such damage, which was in the form of fairly broad interrupted segments, was found in only 1 of 4 brains studied. Some areas within the zone were spongy, and nerve cells showed the homogenizing type of necrosis; in nonspongy areas within the zone of damage, the

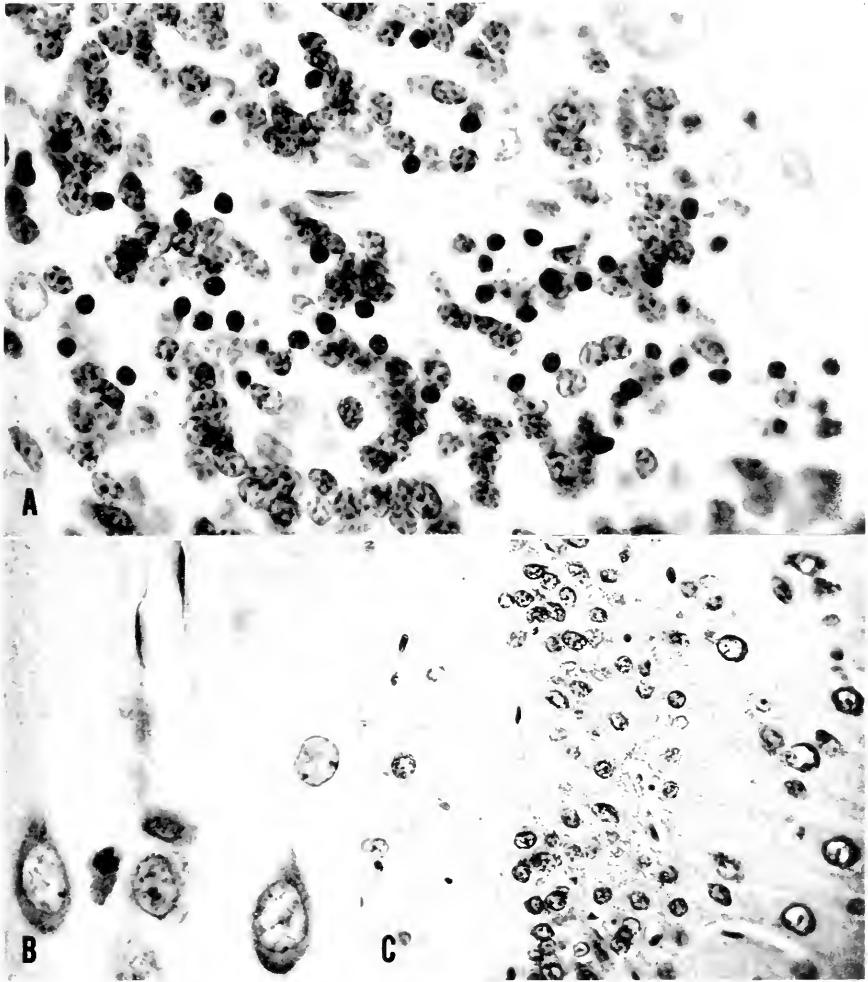


FIG. 7. Nerve cell changes at early stages following irradiation at the 6,000 rad surface dose level. *A.* (6 hr) Alpha particle radiation. Cerebellum, showing a fairly broad row of pyknotic granule cells in the region corresponding to the Bragg peak. Purkinje, Golgi, and Bergmann cells at this level appear unaltered. $\times 675$. *B.* (6 hr) Proton radiation. Cerebral cortex, showing a pyknotic cell, judged to be a nerve cell which has undergone advanced necrosis. $\times 675$. *C.* (24 hr) Alpha particle radiation. Cerebral cortex in the region of the interhemispheric fissure (which is to the left, outside the field illustrated). Some nerve cells have vanished, and those that remain exhibit a varying reduction in the amount of nuclear chromatin. Some of the nuclei are "skeletonized." $\times 375$. Van Gieson-hematoxylin stain.

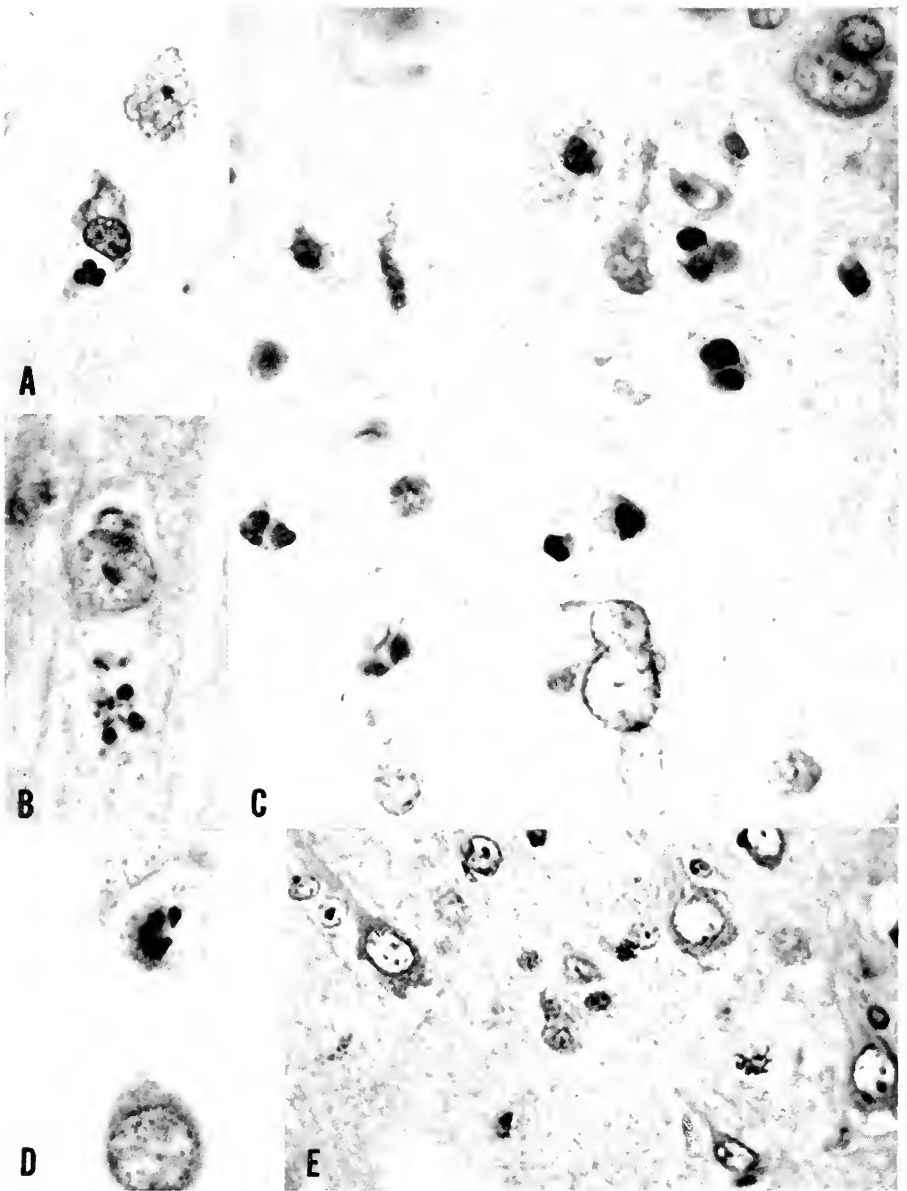


FIG. 8. Damaged cellular elements in the cerebral cortex at relatively early stages following alpha particle radiation at 6,000 rad surface dose level. *A*. (6 hr) Nuclear breakdown in satellite glial cell, $\times 720$. *B*. (12 hr) Chromatin particles of disintegrated cell of undetermined nature, $\times 1170$. *C*. (16 hr) Severely damaged nerve cells and glia in loosened tissue. Some of the pyknotic elements may have been as large as the nerve cell and its glial satellite in the upper right corner of the photograph. The large clear nucleus with the constriction is probably that of a damaged nerve cell, $\times 900$. *D*. (42 hr) Severely damaged cellular element, $\times 1170$. *E*. (8 days) Two mitotic figures are to be seen, $\times 440$. Van Gieson-hematoxylin stain.

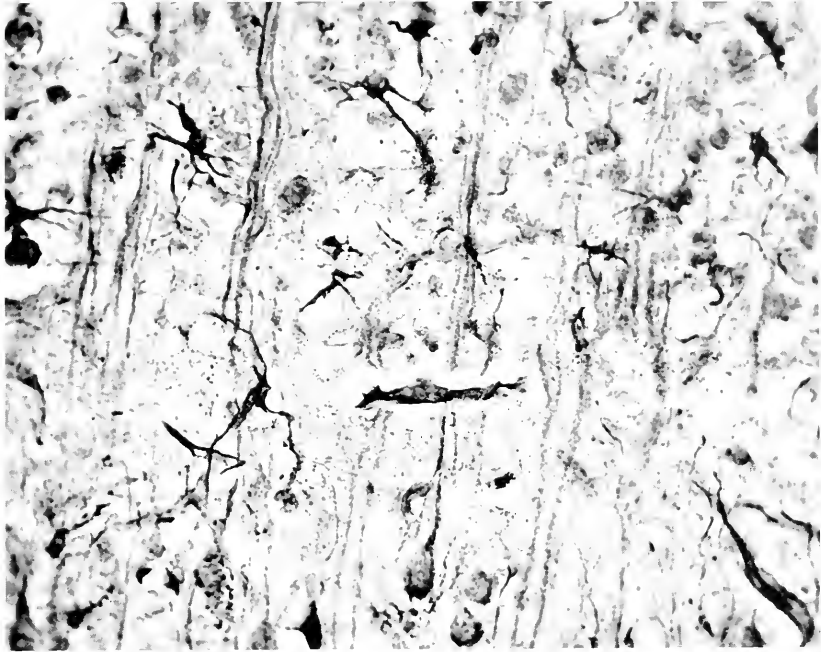


FIG. 9. Proton radiation, 6,000 rad surface dose; sacrifice at 30 days. Cajal impregnation of the cerebral cortex, showing a wide nerve-cell-free band through which apical dendrites are coursing. In the region of their passage through the band, some of the dendrites have decreased affinity for gold chloride and some appear somewhat swollen. Scattered reactive astrocytes are to be seen. $\times 220$.

"skeletonization" type of nerve cell necrosis prevailed. Nerve cells above the band had a strikingly decreased affinity for gold chloride, as did some dendritic processes traversing the band (Fig. 9).

From these data, a time-dose relationship in the development of lesions emerged (Table III). At the 1,500 rad surface dose the feature that stood out was the presence of a nerve-cell-poor band in the cerebrum, but not in the cerebellum. At a surface dose of 3,000 rad both the cerebellum and cerebrum contained a distinct zone of nerve cell damage at 20 days. Precise time of initial damage at this dose level could not be ascertained because of lack of material between the 4 day and 20 day stages. At a surface dose of 6,000 rad, necrotic nerve cells were observed in the cerebellum and cerebral cortex beginning at 6 hours after irradiation. In the cerebellum at the 6 hour stage a zone of granule cell pyknosis had been established, but it was not until the 42 hour stage that a zone of nerve cell damage was encountered in the cerebrum. At the 12,000 rad surface dosage, nerve cell necrosis was evident

TABLE III
 TIME OF APPEARANCE OF ABNORMALITIES IN THE CEREBELLUM
 AND CEREBRUM FOLLOWING ALPHA PARTICLE AND PROTON RADIATION^a

Surface dose (rad)	Nerve cell damage		"Zone" or "band" lesion (Van Gieson-Hematoxylin)		Vascular dilatation (Pickworth-Lepehne)		Barrier penetration		Sodium fluorescein (macro)
	Cerebellum	Cerebrum	Cerebellum	Cerebrum	Cerebellum	Cerebrum	micro	macro	
50	0	0	0	0	—	—	—	—	—
250	0	0	0	0	—	—	—	—	—
750	0	0	0	0	—	—	—	—	—
1,500	0	0	0	216 days	—	—	—	—	—
3,000	—	—	20 days	20 days	—	—	—	—	—
6,000	6 hr	6 hr	6 hr	42 hr	48 hr ^c	48 hr ^b	48 hr ^b	48 hr ^b	72 hr ^c
12,000	3 hr	3 hr	4 hr	4 hr	—	—	—	—	—

^a Alpha particle radiation at various surface doses including 6,000 rad; proton radiation at the 6,000 rad level only.

^b Vascular dilatation first become concentrated in the Brage zone at 60 hours.

^c Penetration of blood-brain barrier was first noted at 48 hours in the cerebrum and at 72 hours in the cerebellum, and was last seen at 36 days in the cerebrum and at 18 days in the cerebellum.

^d Fluorescence occurred both in the cerebrum and cerebellum at 72 hours and had practically vanished by the 13th day.

^e Fluorescence occurred both in the cerebrum and cerebellum at 72 hours and had practically vanished by the 14th day.

in the cerebellum and cerebrum at 3 hours and a zone of damage at 4 hours. At these two times many more nerve cells were necrotic at 12,000 than at 6,000 rad exposure.

Glial vulnerability and reactivity

Van Gieson-hematoxylin preparations were available from material exposed to all the dose levels; metallic impregnations, only from the 6,000 rad surface dose. For observations on astrocytes, Cajal's gold chloride method was employed, and for the study of oligodendroglia and microglia, Penfield's modification of Hortega's silver carbonate technique was used.

In Van Gieson-hematoxylin preparations from brains exposed to the 1,500 rad surface dose of alpha particles, no glial changes were evident (Fig. 4). At 3,000 rad, enlarged astrocytic nuclei were noted in the damaged zone of the granular layer of the *cerebellum* at the 20 day period, and, at the same level, occasional Bergmann glia were faded and a few hyperplastic glia were evident in the somewhat disrupted molecular layer. In the zone of damage in the *cerebrum*, glia had obviously increased in number, and focal collections of polymorphous reactive cells were seen here and there. At subsequent stages glial reaction was more pronounced.

At the 6,000 rad surface dose of alpha particles and protons, damage to glial cells was noticeable in the *cerebellum* in Van Gieson-hematoxylin preparations around the 4th day, consisting of chromatin loss in the nuclei of Bergmann cells and in cells of the molecular layer in the region corresponding to the Bragg peak. Subsequently, in the region of the upper slopes of the Bragg curve, glia in the molecular layer, and, in less measure, those of the Bergmann layer, underwent multiplication (Figs. 1A and 2A). Glia in the region of the Bragg peak sometimes disappeared. Glia had frequently multiplied in damaged intrafolial white matter as well. In the *cerebral cortex* in the region of the anticipated Bragg peak area, damage of isolated satellites and other glia was visible at various stages from 6 hours onward, and at later stages mitosis was evident (Fig. 8E).

In Cajal preparations from brains exposed to 6,000 rad surface dose the first changes in astrocytes in the cerebellum and the cerebrum were noted at the 48 hour stage. Astrocytes in their entirety were enlarged and had an increased affinity for the gold chloride. In the *cerebellum* these changes were visible in the irradiated region of the cortex and in the intrafolial white matter, and later beneath the band, where Bergmann cells as well as astroglia exhibited prominent hypertrophy (Figs. 10A and 10B). In the *cerebral cortex* in early stages, astroglial reaction was particularly conspicuous in the region adjacent to the interhemispheric fissure. Subsequently, the reaction increased in intensity, and around the 3rd or 4th day a nerve-cell-poor band

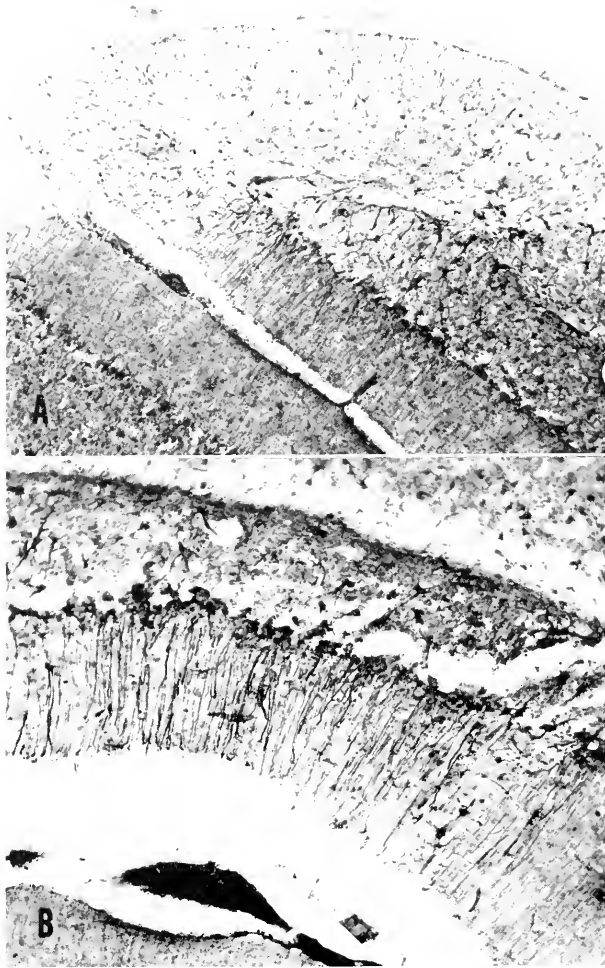


FIG. 10. Alpha particle radiation, 6,000 rad surface dose. Cajal preparations of cerebellum. *A.* (9 days) Above the line separating irradiated from nonirradiated cortex, the cerebellar tissue has lost its affinity for the gold chloride. Beneath this line, many enlarged astrocytes are to be seen in the granular layer, and Bergmann cells have undergone hypertrophy. $\times 80$. *B.* (10 days) The hypertrophied Bergmann cells are to be seen to better advantage. $\times 150$.

became apparent; within the band the astrocytes were undergoing distintegration. At 5 days, astrocytes had disappeared from the band and some above the band were in a state of advanced distintegration (Fig. 11A). Immediately beneath the band, the astrocytes were strikingly hypertrophic and

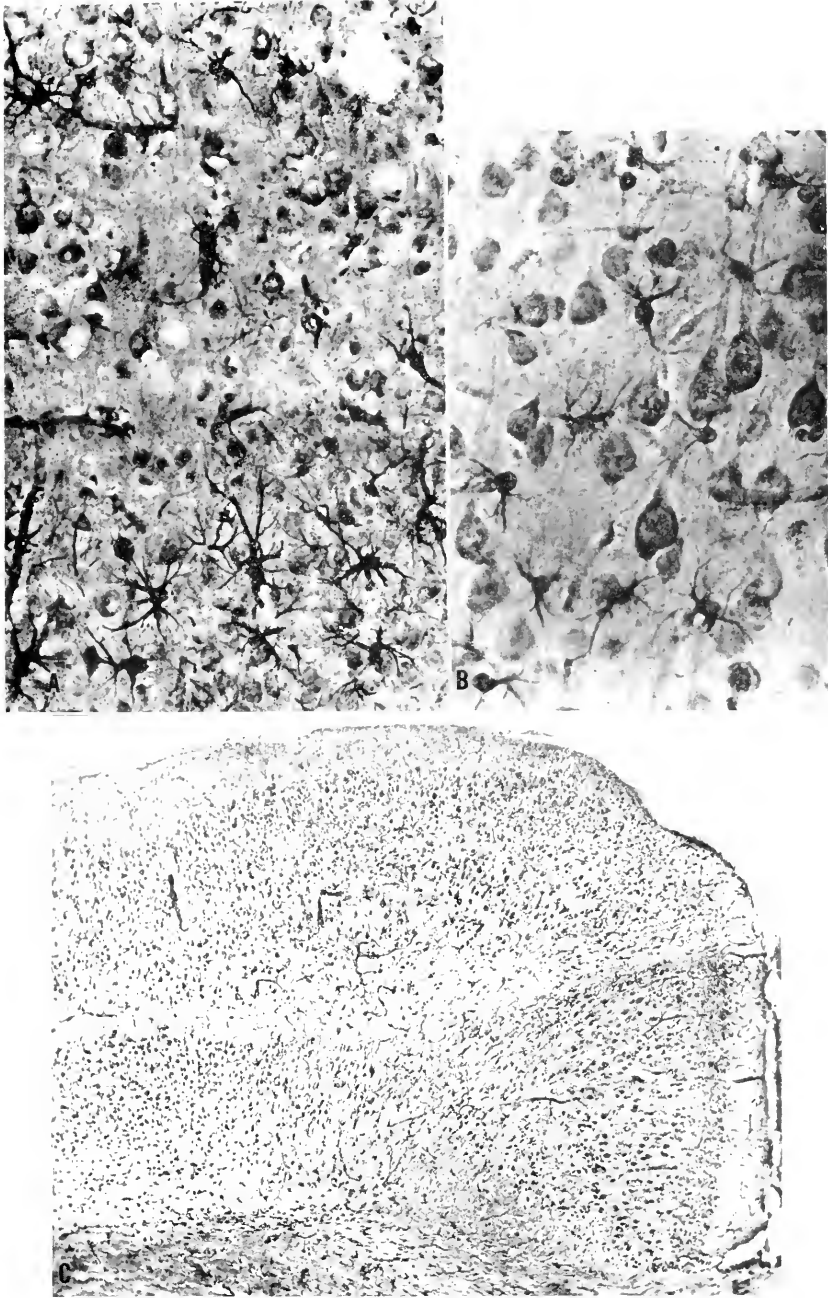


FIG. 11. Legend on next page.

had long processes which extended up to the lower edge of the band. Subsequently, the astroglial reaction was present in the subcortical white matter and corpus callosum, particularly in the region adjacent to the interhemispheric fissure (Fig. 11C). In later stages after irradiation (up to 5 months), the zone of tissue destruction became increasingly narrowed and was devoid of astrocytic elements; however, along the upper and lower surfaces of the zone hypertrophic astrocytes persisted.

Oligodendroglia in both cerebellum and cerebrum were difficult to impregnate. Data on their vulnerability are, thus, not available.

Reactive changes of microglia in the cerebellum and cerebrum in Hortega-Penfield preparations were first noted at 48 hours after irradiation. In the *cerebellum* at this time there were scattered reactive microglia in the cortex. Subsequently, they were noted over a wide irradiated area (Fig. 12A). A similar microglial response was observed in the *cerebral cortex*, being particularly evident in the white matter beneath the mesial cortex, i.e., in the region adjacent to the interhemispheric fissure. At earlier stages hypertrophied microglia were noted here and there at various levels of the cortex. With the development of a band of nerve cell damage, microglia appeared within the band as well as in the underlying cortex and white matter. Changes in the cerebral cortex at the 4 day stage are illustrated in Fig. 12B. Subsequently, most of the microglia were in a hypertrophic state and occasionally appeared amoeboid. Gitter cell forms were few. After the first week following irradiation, the zone usually contained microglial cells which, when their cell body was round, could be distinguished from oligodendrocytes by the short thorny spikes on their main cytoplasmic processes (Fig. 12C). In adjacent areas, most conspicuously just above the band, diffusely scattered hypertrophic cells, including rod cells, were noted.

Mesenchymal elements

In general, both histiocytic and fibroblastic reactions occurred in the

FIG. 11. Alpha particle radiation, 6,000 rad surface dose. Cajal preparations of cerebrum. *A.* (5 days) In the band (the clear region in the middle of the photograph), there are occasional necrotic nerve cells and astroglia. In the part of the zone about the band, nerve cells have vanished or are in a necrobiotic state; astrocytes, which are hypertrophic, are disintegrating. Beneath the band the astrocytes are hypertrophic and have prominent processes oriented toward the band. $\times 335$. *B.* Astrocytes in the corresponding area of a normal control rat. $\times 400$. *C.* (16 days) A fairly wide band extends across the cortex. In the middle of the photograph some nerve cells beneath the band are damaged, and hypertrophied astroglia are to be noted from the level just beneath the band down to, and including, the corpus callosum, where an area of the white matter is pallid. Vascular dilatation is most prominent in the region of the zone and the band just above the area of damage beneath the band. $\times 44$.

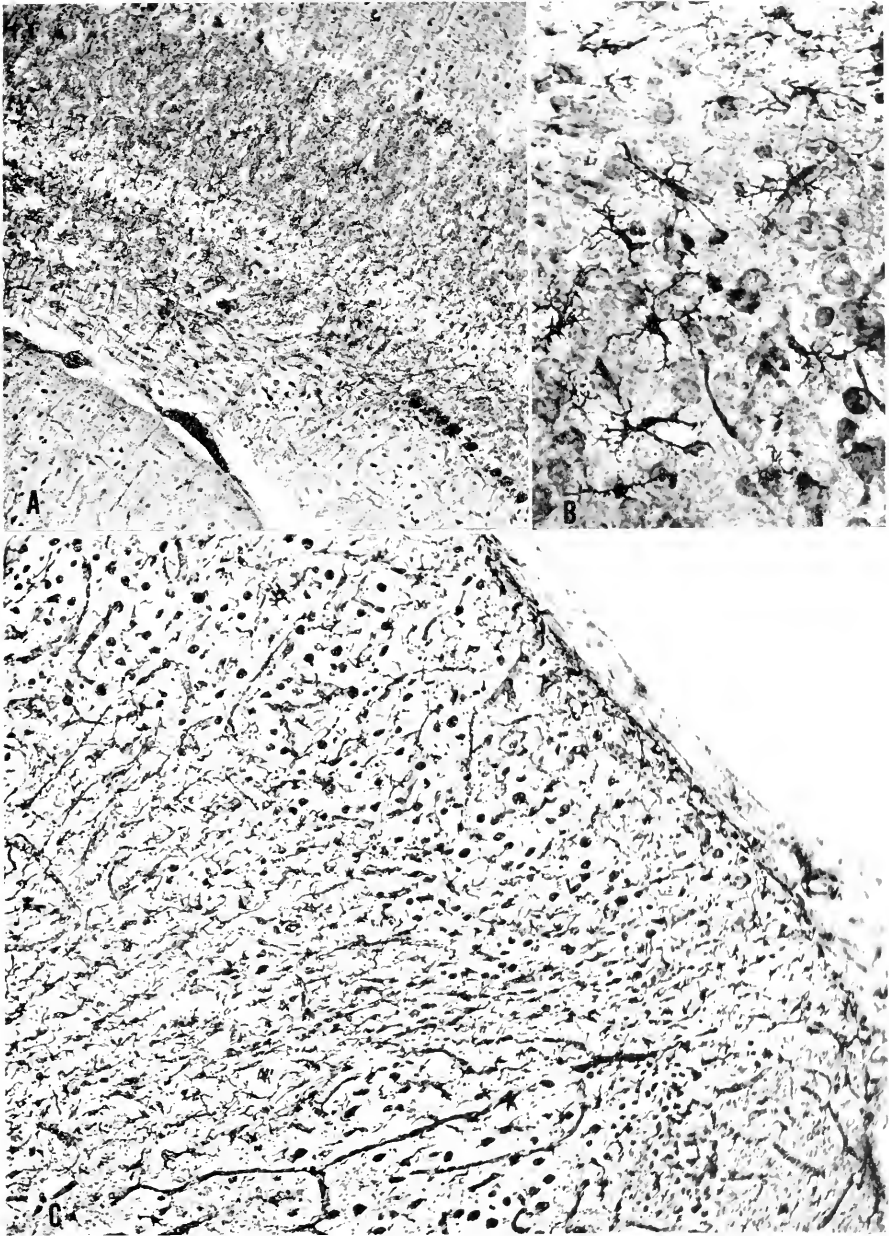


FIG. 12. Legend on next page.

meninges and in the sheaths of larger vessels in the meninges and in the cerebellar and cortical substance; the histiocytic phase occurred earlier and the higher the dose, the more pronounced it was; the reactions were usually most advanced in the region corresponding to the Bragg peak, and in time they advanced upward in accordance with the irradiation dose. After a considerable latent period following exposure to the 3,000 and 6,000 rad surface doses, blood vessel walls frequently underwent fibrohyaline change or hyalinosis (Fig. 2C).

CIRCULATORY AND VASCULAR CHANGES AS BROUGHT OUT BY STAINS

Sections from 83 brains exposed to alpha particles and from 30 exposed to protons, all at the 6,000 rad surface dose level, were stained by the Pickworth-Lepelne method from 1 hour to 5 months after irradiation.

In sections prepared by routine methods, no vascular changes, hemorrhages, or plasma transudates were encountered in brains exposed to surface doses of 50, 250, 750, 1,500, or 3,000 rad. At 6,000 rad surface dose, the various circulatory and vascular changes produced by alpha particle and proton irradiation were the same, whether in the vessels of the cerebellum or cerebrum. At various times through 18 hours, no vascular dilatation was observed within the region presumably irradiated. It was noted in a rather wide zone of irradiation in both cerebellum and cerebrum at 24, 36, and 42 hours.

In Pickworth-Lepelne preparations, the earliest dilatation of vessels observed in the cerebellum and cerebrum was at 48 hours. It was diffuse in the irradiated region from the pia downward. By 2.5 days, it had become concentrated in the region corresponding to the upper slopes and the peak of the Bragg curve. The lower border of the zone of vascular dilatation was fairly sharp and corresponded to the lower border of the zone of tissue damage, as seen in Van Gieson-hematoxylin preparations. At 3 days, the cerebellum and cerebrum in 1 of 3 rats contained tiny hemorrhages predominantly in the "Bragg peak" area. At 4 days, capillaries within the band had a suggestively swollen basal membrane, while others had collapsed. Within the

FIG. 12. Radiation, 6,000 rad surface dose. Hortege-Penfield preparations. A. (30 days) Proton radiation. Cerebellum. The maximal depth of irradiation is at the level of the highest Purkinje cell. Microgliosis has occurred in the granular layer, intrafolial white matter, and the molecular layer, and Bergmann cells are hypertrophic. $\times 110$. B. (4 days) Alpha particle radiation. Cerebral cortex. The light area at the top of the photograph is the band of damage. Beneath it are increased numbers of hypertrophic microgliaocytes. $\times 310$. C. (30 days) Proton radiation. Region of interhemispheric fissure of cerebral cortex, showing a fairly wide band of damage in which great numbers of microgliaocytes are congregated. $\times 145$.

band of damage there were minute hemorrhages and extravasates of globular proteinaceous material. Between 5 and 8 days, vascular dilatation was more evident (Fig. 13A), and a fair number of reactive cells had appeared in the sheaths of vessels in the nerve-cell-poor band and somewhat higher.

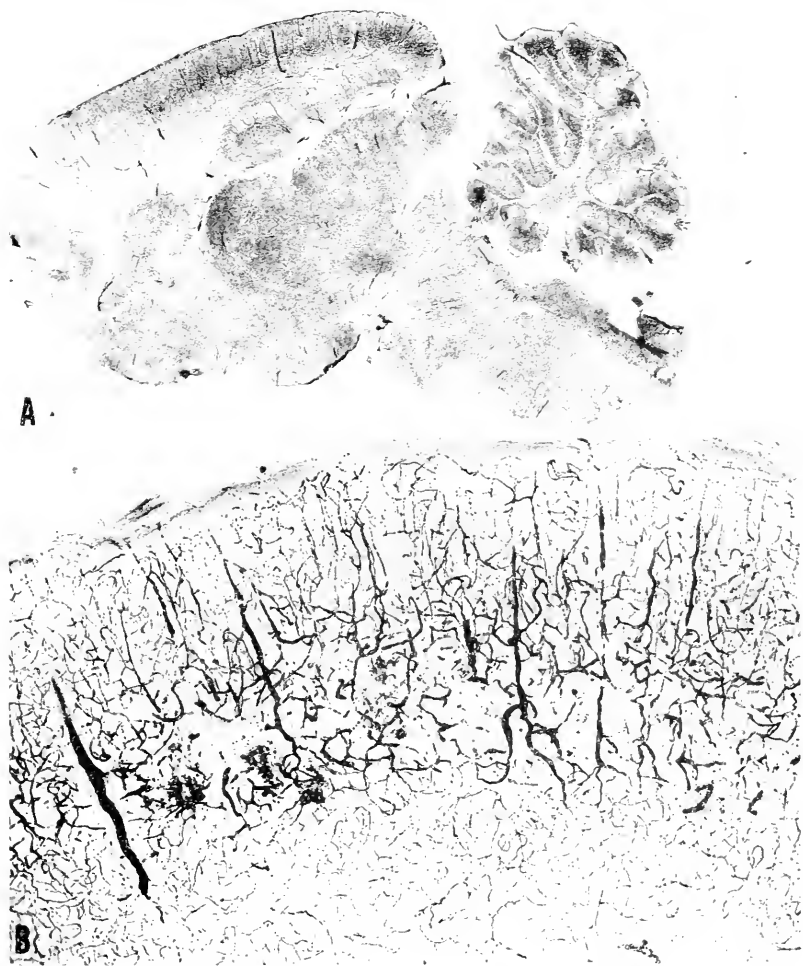


FIG. 13. Radiation, 6,000 rad surface dose. Pickworth-Lepelne preparations. *A.* (8 days) Alpha particle radiation. Vascular dilatation is to be seen in the irradiated part of the cerebellum and cerebrum, and in the latter is most apparent in the region corresponding to the peak and upper slopes of the Bragg curve. $\times 6$. *B.* (9 days) Proton radiation. Aneurysmal vascular dilatation and multifocal lack of erythrocyte staining are to be noted. Occasional hemorrhages have occurred. $\times 50$.

Between 9 and 16 days, there were more filling gaps in the vascular tree in the irradiated zone (Fig. 13B), more evident degenerative changes in the walls of smaller vessels, fibrinoid necrosis and swelling in the walls of occasional arterioles in the band, and fusiform and saccular microaneurysms. During these stages the hemorrhages increased in number and size and had a linear arrangement in the band of cerebellar and cerebral damage. Hemorrhages were occasionally seen below the band. After 18 days, they were rare. In the brains of 2 animals which survived 30 days, the capillary network in the band was as rich as in the adjacent cortical tissue. Vessels just above and just below the band were still dilated. At 4 and 5 months, at a time when the band was greatly shrunken, the same vascular conditions prevailed.

VASCULAR PERMEABILITY TO FLUORESCHEIN-LABELED SERUM PROTEINS (FLSP) AND SODIUM FLUORESCHEIN

Altogether 54 rats (38 for FLSP and 16 for sodium fluorescein studies) were exposed to a 6,000 rad surface dose of alpha particles. FLSP is innocuous and under normal conditions does not penetrate vessel walls. In the *cerebellum*, penetration of vessels by FLSP was first noted at 72 hours in 1 of 3 animals studied. The disturbance of vascular permeability was striking in that intrafolial white matter below the Bragg peak zone showed extensive green fluorescent mottling. In the granular layer within the irradiated zone and below the zone, widespread glial cells contained FLSP inclusions, and in the molecular layer at corresponding levels small FLSP globules were found about a few capillaries. The final stage at which increased permeation of FLSP was visible in the cerebellum was in an animal sacrificed 18 days after irradiation.

In the *cerebrum* the first microscopically detectable vascular penetration by FLSP was at 48 hours and consisted of small, bright-green fluorescent droplets of FLSP along the outer surface of a few capillaries in widely scattered areas of the exposed cerebral cortex (Fig. 14). At 72 hours the capillaries exhibiting increased permeability to FLSP were much more numerous in the cortex, and many neuroglial cells in the underlying white matter were studded with FLSP inclusions. One cerebrum showed an irregular green fluorescence throughout the underlying white matter. Subsequently, intraparenchymal FLSP was conspicuous around the blood vessels in the cortex, especially those in the zone. In the underlying white matter, protein inclusions were found in the glial cells, and in some regions the white matter exhibited irregular, mottled FLSP fluorescence. FLSP transport occurred toward the pial and ependymal surfaces, as judged by the collection in these regions of protein inclusions. In the cerebrum of 1 of 2 rats sacrificed at 36



FIG. 14. Alpha particle radiation, 6,000 rad surface dose; sacrifice at 48 hours. The animal was injected with 2 cc of 8% fluorescent albumin 24 hours before sacrifice. A capillary in the irradiated cortex, showing droplets of extravasated fluorescent albumin. Approximately $\times 400$.

days, the outer surface of a few small blood vessels in the cortex was still studded with FLSP droplets. The superficial leptomeninges contained numerous FLSP-laden macrophages. In animals sacrificed at 4 and 5 months, no abnormal permeation of FLSP was noted.

The first appearance of FLSP fluorescence in the *gross brain* was observed at 72 hours. At this stage the irradiated region of both cerebellum and cerebrum fluoresced. In coronally cut blocks of the *cerebellum*, the fluorescence was confined to the irradiated region of the folia. In the *cerebrum* a faint green fluorescence was noted in the irradiated part of the cortex and a brighter green fluorescence in the underlying white matter, including the corpus callosum. In the *next few days* the white matter below the irradiated medial cerebral cortex exhibited intense green fluorescence, particularly in the region close to the interhemispheric fissure. The cortex showed much less intense green fluorescence. In both cerebellum and cerebrum grossly visible fluorescence persisted for approximately a week and then diminished. Faint fluorescence was last seen at 13 days after irradiation.

The study with *sodium fluorescein* was limited to gross observations because it does not localize microscopically. Parenchymal fluorescence was first seen at 72 hours, and resembled that observed with FLSP, except that the contrast in intensity in grey and white matter was much less distinct. The final stage at which fluorescence in both cerebellum and cerebrum was seen was 14 days after irradiation.

Discussion

The foregoing observations supplement in various ways those reported by Malis and his associates (1957) dealing with the effects of protons (10 Mev per nucleon) on the cerebral cortex of 2 cats. Although, in terms of energy transfer, comparison of results meets obstacles because of the difficulties they encountered in establishing tissue irradiation dosage, the pseudolaminar cerebral cortical lesion they produced was highly similar to that observed in our animals.

One of the problems with which we were concerned was whether or not, at a given radiation dosage, alpha particles produced the same pathologic changes as protons in the cerebellum and cerebrum. Although alpha particles and protons with the same energy per nucleon (in the present investigation, about 12 Mev per nucleon) have the same range, the stopping power, or linear energy transfer (LET), of the alpha particle is 4 times that of the proton. It was therefore of interest to determine whether the fourfold difference in LET was reflected pathologically. At the 6,000 rad surface dose, the changes produced by these two types of particles were highly similar in the time of appearance of lesions, width of the band of most intense damage (Table II), extent and severity of nerve cell damage in the tissue above the band after a given latent period, and time of appearance of vasodilatation in sections stained by the Pickworth-Lepehne method (Table III). Thus, pathogenically, alpha particles and protons had much the same effect despite the fourfold difference in particle LET.

In animals exposed to the lowest effective radiation dosage (1,500 rad at the brain surface), a band lesion was evident in the cerebrum at 216 days (Fig. 4), when no changes were observed in the cerebellum (Table III). This was considered as evidence that the cerebral cortex was the more radiovulnerable. At 6,000 rad cerebellar granule cells and nerve cells and glia of the cerebral cortex seemed equally radiovulnerable from the standpoint of the time of appearance of damage, but a zone of damage was evident earlier in the cerebellum (6 hours) than in the cerebrum (42 hours). Invariably the band of nerve cell loss in the cerebellar granular layer was much narrower than that in the cerebral cortex (Table II). Whether this means that nerve cells of the cerebral cortex were more radiovulnerable than

the granule cells of the cerebellum is not clear to us. A further point is that with the passage of time the tissue above the zone of damage in both cerebellum and cerebrum underwent profound atrophy (Figs. 1 and 2). Measurements indicated that reduction in the width of the irradiated part of the cerebellum was relatively somewhat greater than that in the cerebrum (Table II). This does not necessarily mean that the cerebellum was relatively more radiovulnerable, for the cerebellum underwent greater shrinkage than the cerebrum during processing of the brain.

Nerve cells and neuroglia of the *cerebral cortex* appeared equally vulnerable at early stages, as indicated at the 6,000 rad surface dose levels by necrosis of isolated nerve cells and glia (Figs. 7 and 8), and at subsequent stages, as indicated by necrosis of astrocytes and nerve cells (Fig. 11A). In the *cerebellum* the granule cell seemed the most radiovulnerable. Depending on dosage and time, varying numbers of these cells in the more intensely irradiated part of the granular layer subsequently underwent necrosis. Purkinje cells situated at comparable levels in the cerebellum also suffered, but the tempo at which the damage occurred seemed slower than that in granule cells. Purkinje cells sometimes appeared intact at a time when granule cells were pyknotic (Fig. 7A). Since at later stages following irradiation the width of the zone of Purkinje cell damage was often not much different from that of the pyknotic granule cells (Figs. 1A, 2A, 3A, and 12A), it was concluded that relatively little difference in radiovulnerability of these two cell types existed. Less radiovulnerable than granule and Purkinje cells were Bergmann cells, nerve cells and glia of the molecular layer, and Golgi cells in the granular layer, in that order of decreasing radiovulnerability. At fairly early stages, damage was also incurred by intrafolial white matter, and the reactivity of its glial cells was about the same as that elsewhere.

Examination of material exposed to the 1,500 rad surface indicated that nerve cells had been destroyed and that blood vessels were not morphologically altered (Fig. 4). The same was true at the 3,000 rad level at 20 days when a zone of cytologic damage was encountered. At 6,000 rad, nerve cell and glial necrosis occurred as early as 6 hours after irradiation, but it was not until 48 hours that vasodilatation as brought out by the Pickworth-Lephehne method appeared and not until 60 hours that vasodilatation was concentrated in the "Bragg zone." Clearly, nerve cell and glial damage occurred before circulatory disturbances sufficient to cause vasodilatation were evident. Moreover, only at 48 hours did the blood-brain barrier become permeable to FLSP, indicating that during the preceding hours any vascular change that might have occurred was not functionally evident. Further, the presence of hemorrhages, taken as evidence of vascular damage, did not occur until the 3rd day after irradiation. The conclusion seems inescapable that, in earlier stages at least, nerve cells and glia were primarily damaged by particle radiation. Damage of nerve cells and glia concurrently is most

clearly shown in Fig. 8C. From their study of the brains of mice exposed to a 25- μ -wide beam of deuterons with an energy of 22.5 Mev per nucleon, in which the width of the track corresponded to the width of our bands, Zeman *et al.* (1959) reached the conclusion that damage to nerve cells and glia represented a direct irradiation effect.

Further evidence pointing to primary cellular damage in our animals, which would not be expected if circulatory disturbance and an edematous process were the sole factors, was the close correlation of the granule cell pyknosis with the magnitude of the energy given off along the slopes and at the peak of the Bragg curve (Figs. 5 and 6) and the straightness of the lower border of the irradiated zone. In later stages following exposure to particle radiation (i.e., from 5 or 6 days onward), it would be difficult to judge to what extent circulatory disturbances contributed to the extent of the damage. There were two pieces of evidence that circulatory disturbances did contribute to further advance of lesions. In sections stained by the Pickworth-Lepehne method (6,000 rad surface dose), collapse of occasional small vessels in the band of cellular damage was noted at 4 days, and numerous filling defects were found in the vascular tree from the 9th day to about the 24th day. Secondly, lesions in the cerebral cortex were particularly striking in the region of the interhemispheric fissure (Figs. 4, 7C, and 11C), and cortical nerve cells *below* the band in this general region were sometimes damaged in association with astrogliosis and microgliosis, which extended down into the subcortical white matter and corpus callosum. Such effects were considered to be related to greater circulatory disturbances in this region than elsewhere, perhaps because in this "angle" the vascular tree is more prone to be constricted by a generalized edematous process than elsewhere.

With regard to the blood-brain-barrier permeability studies, the FLSP technique had the advantage over the sodium fluorescein indicator in that the labeled proteins could be demonstrated microscopically both before and after the gross fluorescence was perceptible (Table III). Extension of the fluorescence to regions far beyond the irradiated area was considered to have been due to disturbance of vascular permeability initiated in the irradiated area. Even ependymal epithelial cells lining the upper wall of the ventricle contained large quantities of fluorescent protein.

Summary and Conclusions

This article deals with the effects on the cerebellum and cerebrum of alpha particles with an energy of about 12 Mev per nucleon at surface doses of 50 to 6,000 rad, and of protons of the same energy at a surface dose of 6,000 rad. The dorsal surface of much of the cerebellum and cerebrum was

exposed. The maximal tissue dosage was approximately 5 times that at the brain surface.

No essential differences were observed in the alterations produced by alpha particles and those produced by protons.

The width of the zone of damage in the cortex of both the cerebellum and cerebrum corresponded to the amount of energy given off by the particles as expressed by the Bragg curve.

Granule and Purkinje cells of the cerebellum and nerve cells and glia in the cerebral cortex were highly radiovulnerable. The greater width of the band of damage in the cerebrum than in the cerebellum and the observation that at the lowest effective radiation dose (1,500 rad at the brain surface) a band of nerve cell loss was found in the cerebrum but not in the cerebellum suggested that the cerebral cortex was the more radiovulnerable. From other standpoints, however, the cerebellar granular layer seemed the most radiovulnerable.

Nerve cell and glial damage was incurred before circulatory or permeability disturbances were evident, strongly suggesting direct irradiation of cellular elements as the primary factor in pathogenesis. At subsequent stages circulatory changes and increased vascular permeability contributed to the extent of the lesions.

No lesions were encountered after as long as 7 months at surface doses of 50, 250, and 750 rad. The earliest times at which damage to cellular elements was observed following exposure at other surface doses were as follows: at 1,500 rad, 7 months (in cerebrum alone); at 3,000 rad, 20 days; at 6,000 rad, 6 hours; and at 12,000 rad, 3 hours.

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Some Observations on Radiation Effects on the Blood-Brain Barrier and Cerebral Blood Vessels*

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For many years it has been supposed that the adult nervous system is relatively resistant to radiation dosages capable of injuring or destroying cells of other tissues and organs. The primary reason usually forwarded to explain this phenomenon has been that the primitive cells in the body are more radiosensitive than cells differentiated to a high order of specialization. In this regard observations seem to suggest the thesis that cellular radiosensitivity depends somewhat on the rate of nucleic acid synthesis. It is usually stated that lymphocytes, developing germ cells, and maturing neuroblasts are among the most radiosensitive of cells, whereas the adult neuron is among those most resistant.

When assessing the effects of radiations on the brain, it seems difficult to speak in generalizations as to the radiosensitivity or radioresistance of the entire organ. For in the central nervous system, as in other organs, the pathology which develops with ionizing radiations can be considered to result from several sources or a combination of them. We describe parenchymal, stromal, and intercellular effects of radiation in most other organs in the body. Therefore, it seems wise not to have our opinions of the radiosensitivity of the brain as an organ unduly influenced, just because the adult neuron has been described as being relatively radioresistant. As a matter of fact, even this latter generality has been questioned recently (Grigoriev and Tsy-pin, 1957; Lebedinsky *et al.*, 1959; Livanov and Biryukov, 1959;

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Gangloff and Haley, 1960), and we have little information of subtle changes that radiation can produce in neuronal function or of neurochemical observations.

We would like to slant this report away from the direct effects of radiation on the neuron and examine more closely our research results, and those of other investigators, on the cerebral capillary and neuroglial systems. This we feel has an important place in such a conference because it has been shown that brain radiations can result in edema and inflammatory reactions around cerebral capillaries and because neurons are elaborately sensitive to ionic and other chemical changes in their environment; their functional integrity can be altered by the slightest alteration in its metabolic medium.

The Concept of the Blood-Brain Barrier

That a selective barrier exists between certain circulating elements in the blood stream and brain tissue has been known since Ehrlich (1885) found that the brain remained unstained following the intravenous injection of acidic dyes, which brilliantly stained most of the other organs. This peculiarity in regard to the central nervous system has given rise to the concept of the existence of a blood-brain barrier, and in general it is observed that transvascular permeability characteristics tend to be manifested by a slower exchange rate of substances passing between blood and brain (in comparison to other organs) rather than a faster rate of exchange.

The acidic aniline dye, trypan blue, has been classically used by many investigators to demonstrate the blood-brain barrier phenomenon. More recently, certain radioactive tracers (notably P^{32} , Br^{82} , and Na^{24}) have been used. Although the use of dye techniques has been valuable in the formulation of the barrier concept, these methods are not above criticism. It should be pointed out that acidic dyes (such as trypan blue) tend to bind more completely with plasma proteins than basic dyes, which tend to stain brain structures following systemic administration (Bennhold, 1932). Were we to depend on dye studies alone, it could be argued that the blood-brain barrier is merely a display of the impermeability of the cerebral vasculature to plasma proteins, a reasonable and predicible situation.

With the advent of radioactive tracer methods, however, it has been shown that following the systemic injection of certain ions the tracer becomes distributed throughout all the organs of the body uniformly and rapidly, whereas the relative concentration of the tracers in the brain are significantly lower (Manery and Bale, 1941; Greenberg *et al.*, 1943; Wang, 1948). Although the rate of passage of radioactive tracers from blood to brain differs with respect to the particular ion being studied, curves can be obtained which reveal the amount of tracer in the brain at any given time

after the systemic administration of the radioactive ion, and one can compare the amount of radioactivity in brain tissue with respect to other organs in the body such as liver, spleen, or kidney. Thus, it is generally accepted today that the phenomenon of a blood-brain barrier exists.

How the blood-brain barrier functions and the anatomic locus of the blood-brain barrier are more hypothetical. These two questions are, by their nature, related. At present two groups of structures are considered by various investigators to be the possible anatomic sites of the blood-brain barrier: the capillary endothelium of the central nervous system and the perivascular sheath comprised of the membranes of the neuroglial cells.

The proponents of the capillary wall theory point to many experiments performed with vital dyes. At first it was felt that the endothelial cells of cerebral vessels were different in their permeability characteristics because they remained unstained with vital dyes (Spatz, 1933). Broman (1937, 1940a,b) also emphasized the vascular walls as being the site of the blood-brain barrier and noted that vitally stained vessels in the choroid plexus were in communication with branches within the brain which did not stain.

Hauptmann and Gärtner (1932), Hoff (1933), and Tschirgi (1952) have expressed a preference for the perivascular glial membrane (Figs. 1A and 1B) as the more probable locus of the selective vascular-permeability characteristics encountered in the brain. Although the capillary wall enthusiasts today still far outnumber the neuroglial membrane supporters, Tschirgi (1958) offers a most interesting theory involving an active mechanism of transport of substances from blood to brain and metabolites from brain to blood, rather than a passive mechanism of diffusion. For an excellent review, see the chapter written by Tschirgi (1960) in the "Handbook of Physiology."

Radiation and Blood-Brain Barrier

It is well known that certain permeability changes in the cerebral vasculature can occur following direct trauma to the brain, during simple exposure of the nervous system (Macklin and Macklin, 1920; Prados *et al.*, 1945; Grenell and McCawley, 1947), or following intravenous injection or topical application of certain chemical substances such as Diodrast (Broman and Olssen, 1948, 1949; Bassett *et al.*, 1953), epinephrine (Friedemann and Elkeles, 1932), and hydrogen peroxide (Givré and Raxed, 1948). Thus, it is not surprising that another mode of physical injury to the brain, exposure to ionizing radiation, is also capable of producing permeability changes in cerebral vessels. This fact has been recognized since Rachmanow (1926) and Mogilnitzky and Podljaschuk (1930) showed that ionizing radiations caused the perivascular neuroglia of mice and rabbits to be stainable with trypan blue.

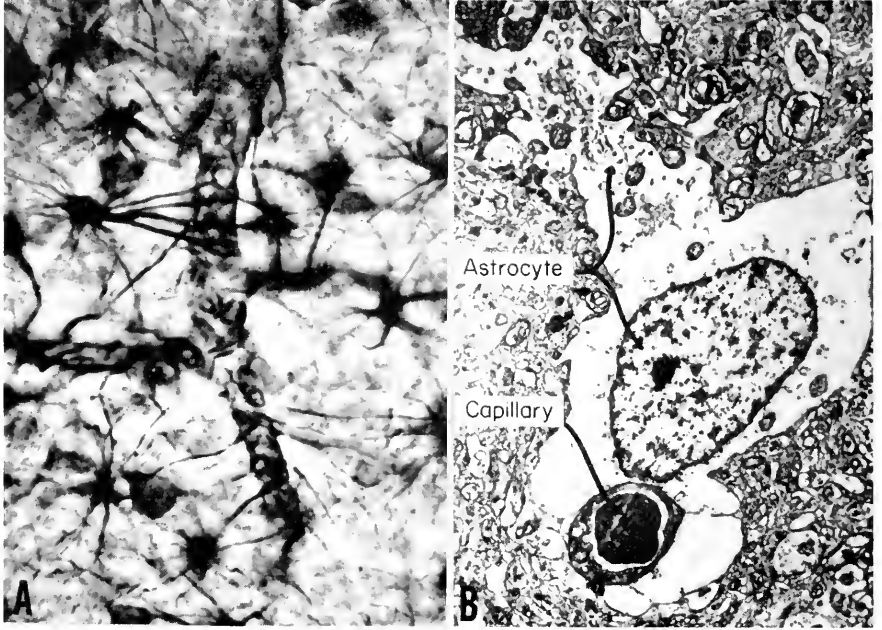


FIG. 1. (A.) A section of monkey parietal cortex impregnated to show astrocytes by the Cajal gold sublimate method. Note the myriad numbers of astrocytic processes surrounding the capillary forming the so-called perivascular glial membrane. $\times 150$. (B.) An electron micrographic demonstration of the perivascular astrocytic processes. Notice the virtually complete encasement of the capillary by the watery-looking cytoplasmic projections of the nearby astrocyte. Approximately $\times 1,500$. (Figure 1-B was loaned to the authors by Drs. D. Pease, E. Maynard, and R. Schultz.)

As is the case with the blood-brain barrier, pathologic, chemical, and physical insults are capable of breaking down the blood-aqueous humor barrier and the blood-cerebrospinal fluid barrier. The most obvious manifestation of a breakdown in the blood-fluid barriers is an increase in protein in the central humor (Davson, 1960). Again, it has been shown that exposure to x-rays affects the permeability characteristics of these blood-humor barriers. An increase in permeability of the blood-cerebrospinal fluid barrier was observed by Tatsumi as early as 1933 and by Hsu *et al.* in 1936.

In a series of 37 monkeys, Clemente and Holst (1953, 1954) observed changes in permeability of cerebral vessels following single doses of x-irradiation ranging from 1,500 to 6,000 r. Trypan blue injected intraperitoneally demonstrated a profound functional impairment of the blood-brain barrier, especially in the brain stem and hypothalamus, and was best visualized after doses of 4,500 and 6,000 r (Fig. 2). Neuroglial, neuronal, and vascular dam-

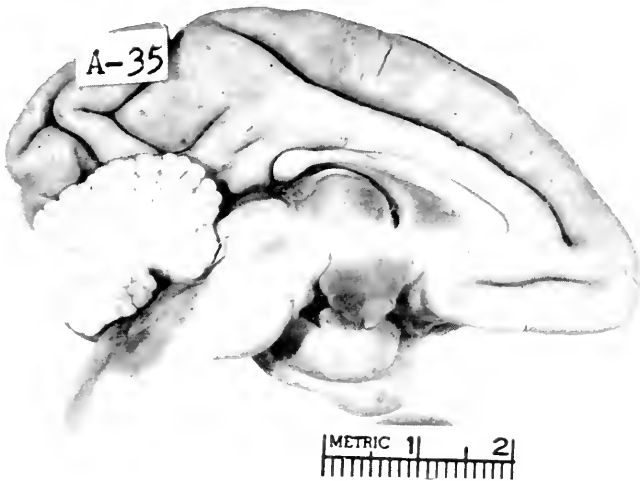


FIG. 2. A midsagittal view of the brain of a monkey which had received 4,500 r of x-irradiation (250 kv, 118 r per min) to the head and which survived for 21 hours. Notice the trypan blue discoloration in the medulla, hypothalamus, thalamus, and septum brought about by the radiation effects on the permeability of the blood-brain barrier. (Taken from Clemente and Holst, 1954.)

age (Figs. 3A-E) was observable microscopically, and the areas of trypan blue penetration into the brain were characteristically those regions in which astrocytic damage was most intense.

Monkeys subjected to a 1,500 r head x-radiation dose and sacrificed 4 to 8 months after irradiation showed pinpoint areas of degeneration surrounding blood vessels in cortical and subcortical white matter (Fig. 4). The lesions were characterized by axon swelling, myelin breakdown, phagocytosis, and astrocytic scar gliosis. With higher doses of x-ray radiation to the brain in adult rats (20,000 to 23,000 r), Brightman (1959) likewise found vascular changes characterized by petechial hemorrhagic foci, and "the hemorrhagic areas were stained a dark blue in those rats given trypan blue."

Although obvious early changes in the permeability of the blood-brain barrier were difficult to observe with trypan blue in monkeys given 1,500 r

(Clemente and Holst, 1953, 1954), it was felt that a more sensitive method of evaluating increases in the permeability of the blood-brain barrier might reveal such alterations. The study by R. G. Rose (1958) seems to have substantiated these suspicions. He utilized the rate of passage of intravenously injected radioactive sodium into the brain and cerebrospinal fluid of radiated and nonradiated rabbits to determine the influence of lower x-ray doses (100 to 1,500 r) on cerebral capillary permeability. His data indicate an

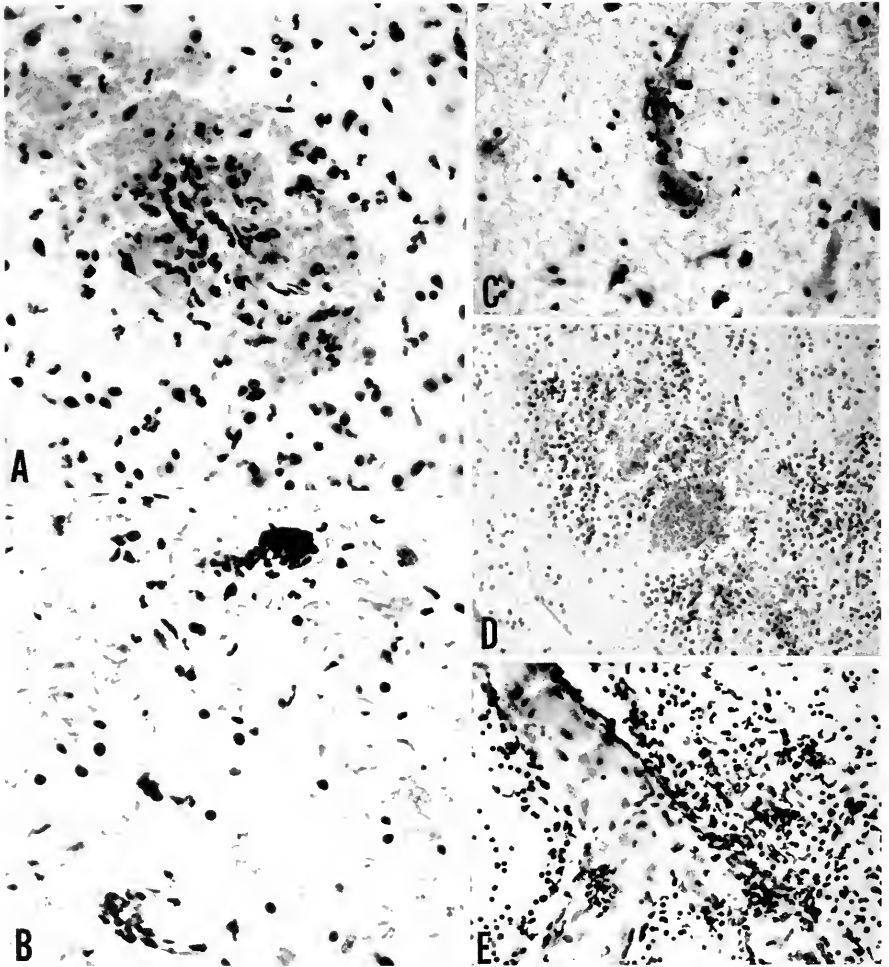


FIG. 3. In these 5 pictures are examples of some acute vascular and perivascular reactions in various areas of the brain after head x-irradiation in monkeys. (250 kv, half-value-layer for filtration of 0.5 mm Cu and 1.0 mm Al was 1.4 mm Cu; mid-cranial focal distance 35 cm; Dosage rate 118 r per min.) Thionin, H. and E., and Van Gieson stains. (A.) Petechial hemorrhage in white matter of occipital cortex, 8 hours after 4,500 r. \times 175. (B.) Perivascular leucocytic reaction in medulla 36 hr after 6,000 r. \times 175. (C.) Perivenous reaction in the lateral hypothalamus 8 hr following 4,500 r. \times 140. (D.) Red and white blood cell diapedesis in the caudate nucleus in a monkey 8 hr following 6,000 r. \times 85. (E.) A severe perivascular leucocytic infiltration in the parietal cortex 6 hr following 4,500 r. \times 85.

accelerated penetration of Na^{24} into the brain and cerebrospinal fluid in the radiated animals, even though histologic changes were minimal even at 1,500 r.



FIG. 4. This figure demonstrates delayed white matter degeneration (arrows) seen in the internal capsule of a monkey given 1,500 r to the head and sacrificed 8 months after x-irradiation. These regions showed areas of demyelination and neuroglial scar formation. Cajal-gold sublimate impregnation. $\times 30$.

One is left to wonder whether our methods of determining the effects of lower x-irradiation doses on the adult brain have been, to this time, sharp enough to detect the more subtle changes which may be taking place. The axiom that the brain is relatively resistant to radiation might be questioned purely on the lack of sensitive methodology. When Rose (1958) is able to detect vascular permeability changes utilizing only several hundred roentgens, and Gangloff and Haley (1960) show that only 200 to 400 r are required to alter spontaneous and evoked electrical activity in certain parts of the brain, it is time to reconsider our opinions of the radiosensitivity of the

central nervous system. In light of the fact that morphologic changes in cells and fibers at these low dose levels are unobservable, perhaps the functional alterations observed are the result of an altered neuronal milieu or changes in the ionic medium of neuronal pools which may be transient at lower doses or may result in neuronal damage or neuron death at somewhat higher doses. It seems evident that at extremely high doses of radiation, vessels, glia, and neurons all suffer damage. There may be a level of radiation, however, at which the neuronal damage is secondarily brought on by damage to the functional integrity of the capillary system.

Radiation Effects on Cerebral Vessels

Doses of ionizing radiation to the brain which have been reported capable of producing radiation lesions have, in virtually every instance, involved not only nerve cells and fibers, but also the vascular system. The vascular reaction to radiation in the brain is characterized by generalized vasculitis, brain edema, and vascular fragility (Lyman *et al.*, 1933, 1952; Davidoff *et al.*, 1938; Russell *et al.*, 1949; Colwell and Gladstone, 1937; Haymaker *et al.*, 1958). Similar vascular reactions following irradiation have been described in skin (Wolback, 1909, 1925; MacKee, 1938; Miescher, 1930; Snider, 1948), lung (Englestad, 1934; Warren and Spencer, 1940), and kidney (Warren, 1936).

Observations on the fate of capillaries in radiated skin and in developing granulation tissue reveal that endothelial protoplasmic buds retract and begin to become obliterated at about 400 r, even though inhibition of granulation tissue does not occur until 2,000 r is administered. Under these circumstances there is swelling of the endothelium, producing first a narrowing of the lumen and then a dilatation which results in a primary radiation erythema. Edema ensues, apparently being dependent on the reactivity of the capillary endothelium and of the surrounding parenchyma (Van den Brenk, 1959; Borak, 1942a,b,c). Following the edema, diapedesis of cellular elements through the vessel wall occurs, involving lymphocytes, red blood cells, and polymorphonuclear leucocytes. This results in the commonly observed inflammatory response of the perivascular tissue to radiation.

One of the reported effects of ionizing radiation on cells is the intracellular disintegration of large protein molecules into those of smaller size. This results in an increase in osmotic pressure leading to imbibition of fluid by the cell (Heilbrunn and Mazia, 1936; Borak, 1942a). Endothelial cells in the brain prove no exception. An increase in basophilia and endothelial cell swelling were among the first reactions observed by Clemente *et al.* (1959, 1960) in cerebral capillaries of neonatal rats subjected to 500 r doses of

x-rays. With higher doses, these vascular reactions were followed by petechial hemorrhages at the capillary level and polymorphonuclear perivasculitis.

A pattern of cerebral vascular damage following radiation could be observed. The earliest and commonest change with the lowest radiation threshold was a simple swelling of the endothelial cell with an increase in nuclear basophilia. With a longer survival time this process either was arrested and reversible (with lower radiation dosages) or progressed to perivasculitis or the development of petechial hemorrhages (with higher radiation dosages, Clemente *et al.*, 1960). Russell *et al.* (1949) described similar findings in the brains of rabbits.

Radiation damage in larger sized arteries and veins becomes manifested in the pathologic changes observable in vessel walls. The inflammatory radiation reaction in the vascular tunics of the brain seems indistinguishable from arteritis or phlebitis observed during inflammation in other tissues. Incompetency of the vessel walls leads to plasma leakage and cellular migration. These degenerative and subsequent reparative phenomena in cerebral vessels of radiated brain contribute to narrowing of the lumen and at times to complete occlusion of the vessel with apparent cessation of blood flow. Characteristically observed is intimal fibrosis and hyalin degeneration of fibrous and muscle tissue. Of prime importance is the fact that a radiation dose capable of producing exudation and leucocytic emigration through the endothelium of capillaries is not especially sufficient to produce a generalized inflammatory reaction in the walls of larger arteries and veins (Borak, 1942a,b,c). A considerable increase in dose is necessary to produce the generalized vasculitis often described. We wish to stress the fact that low doses to the brain (under 1,000 r) are capable of interfering with the competency of the cerebral capillary system and that this may eventually be manifested by localized or more widespread effects on the electrical activity of neuronal pools, even though histopathologic effects have not often been detected at these dosage levels.

The delayed white matter necrosis observed (Fig. 4) by Clemente and Holst (1954) in monkeys whose heads had been radiated with 1,500 r (4 to 8 months before sacrifice) may have been a chronic manifestation of nerve fiber pathology due to small blood vessel damage. This seems especially likely since the areas of degeneration were close to small vessels whose walls revealed intimal thickening. Animals in this group which were administered trypan blue just before sacrifice showed a blue staining of the brain in the degenerated areas only, another indication of a localized incompetent vasculature at the lesion sites.

The phenomenon of delayed cerebral necrosis due to radiation is difficult, if not impossible, to explain except by implication of vascular pathology. The necrotic areas are limited to the regions irradiated and the immediate

surrounding zones (Courville and Myers, 1958). For the most part, authors reporting this process describe vascular changes of such a nature as to account for the necrosis either on a hemorrhagic or ischemic basis (Marburg *et al.*, 1945; Pennybacker and Russell, 1948). Analysis of vascular reactions to irradiation in other tissues revealed that following irradiation it takes about a month to produce an ischemic ulcer in the skin (Borak, 1942a). Once a segment of skin has been irradiated, it continues to be more susceptible to noxious influences such as heat, cold, infection and trauma. Dynes and Smedal (1960) report a case of radiation necrosis precipitated by trauma, and other authors suspect a progressive deterioration of blood vessels following high doses of radiation (Russell *et al.*, 1949; Berg and Lindgren, 1957; Bailey *et al.*, 1957).

More unique experiments have been reported in which brain destruction does not seem to be a direct result of vascular damage. Malis *et al.* (1957) reported the production of laminar lesions in the cerebral cortex with a minimum of 15,000 rads peak dose. This is 2 to 3 times the minimum dose necessary to produce a lesion when a large volume of brain is irradiated with a uniform dose. Even under these conditions, capillaries appeared to dilate early, and occasionally extravasation of blood elements occurred. Older "line lesions" showed thickening of capillaries (J. E. Rose *et al.*, 1960). Zeman *et al.* (1959) irradiated mouse brains with beams of deuterons of varying diameters. The beam diameters ranged from 1 mm to 25 μ . They found that 15,000 rad were below a minimal effective dose for a 1 mm diameter beam, 75,000 rad were below the minimal effective dose for a 75 μ beam and 5.5×10^5 rad were not minimally effective with a 25 μ beam. With the smaller beams, little or no involvement of the vasculature occurred, presumably because little or no vasculature was in the field; hence, under these conditions, the production of a lesion seemed dependent on the "direct killing" of brain parenchyma.

In 28-day-old rats, the effects of alpha particle radiation on the vasculature and parenchyma of the central nervous system were studied. The radiation was delivered to the lower bulbar or upper cervical spinal cord region from the 184 in. cyclotron at Berkeley, California. The beam was directed through 1×5 mm slit aperture and delivered at approximately 2,000 rad per min with doses of 10,000 or 12,500 rad. In other animals, the beam was directed through a 3×4 mm aperture over the cerebellum. Animals were sacrificed at varying periods up to $4\frac{1}{2}$ months after irradiation. Often a rather large cyst developed in the central nervous system at the radiation site (Fig. 5A), but perhaps the most consistent observation was thickening, hyalinization, and often occlusion of the blood vessels. Both surface vessels (Figs. 5B and 5C) and deep vessels (Figs. 5D-F) were affected. In certain areas (Figs. 6A and 6B), virtually all smaller vessels were hyalinized and

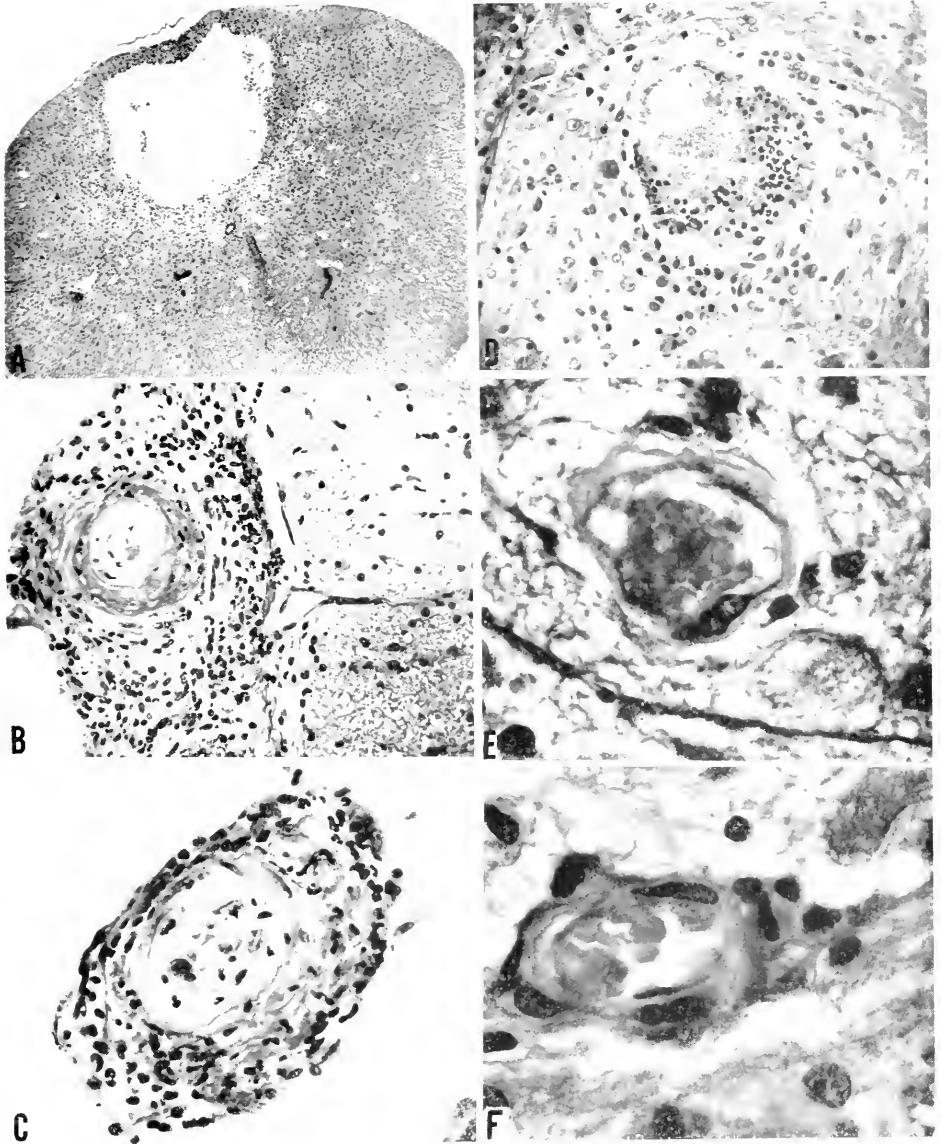


FIG. 5. These 6 pictures demonstrate histologic material taken from the brain of rats subjected to 10,000 rad alpha particle radiation delivered through a slit aperture from the 184 in. cyclotron at Berkeley, California. Van Gieson's vascular stain. (A.) Low power view of cervical spinal cord (level C-1) 2 months after radiation. Note the pronounced cyst formed in the dorsal funiculi. $\times 27$. (B and C.) Examples of perivascular infiltration, vascular lumen thickening and almost complete occlusion of the left (B) and right (C) dorsal descending spinal arteries, surface vessels taken from the same level as shown in Fig. 5A. $\times 310$. (D, E, and F.) Three examples of perivascular infiltration, thickening, and hyalinization of deeply situated vessels in the brain. Rat sacrificed 3 months after radiation. These vessels were situated in the nucleus gracilis, (D) $\times 250$; medullary reticular formation, (E) $\times 480$; and central medulla at the level of the pyramids, (F) $\times 480$.

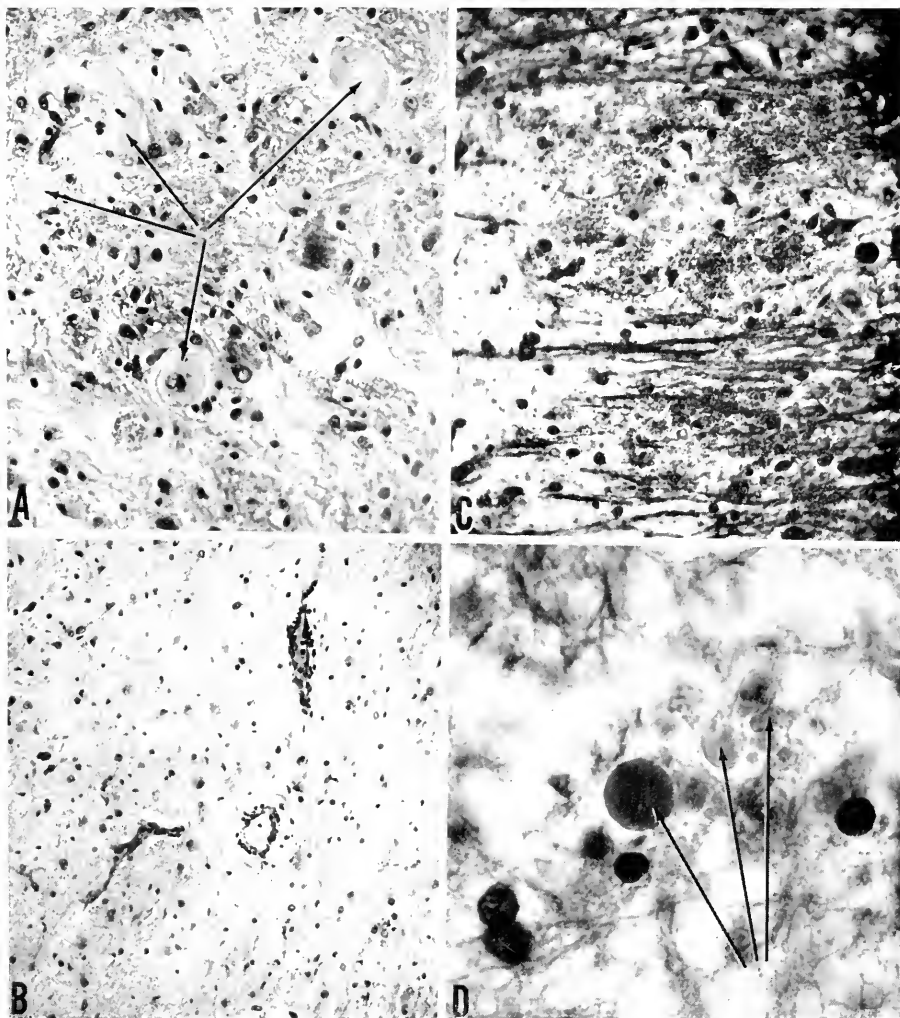


FIG. 6. (A.) Medium power view of the nucleus of the spinal tract of the trigeminal nerve in same rat as 5D.E.F, sacrificed 3 months after irradiation with 10,000 rad alpha particles. Note the widespread vascular hyalinization (arrows) and the absence of normal-looking neuron cell bodies. Van Gieson's stain. $\times 360$. (B.) Low power view of 3 vessels in the white matter in the occipital cortex of a cat 6 weeks after irradiation with 10,000 rad alpha particles. $\times 250$ (C and D.) Sections at the mid-pontile level in rat given 12,500 rad alpha particles and sacrificed 10 days after radiation. Note the frank hemorrhages (C) $\times 360$, and the perivascular globules (arrows) described by Brightman (1959) (D) $\times 560$.

partially or completely occluded. In these regions, few, if any, nonal-looking neurons could be found. In another animal, sacrificed 10 days after the beam was directed at the brain stem, there were frank hemorrhagic areas in the midbrain (Fig. 6C) and perivascular globules (Fig. 6D) in the brain stem, similar to those reported by Brightman (1959).

Vascular and parenchymal damage was also observed in the cerebellum following alpha particle radiation. This was characterized by spongy loss of cerebellar cortical tissue, thickening of the meningeal and invaginating vessels, and vacuolization of the Purkinje cell layer (Figs. 7A-D).

In these higher radiation dose experiments, it is difficult to say that the entire neuropathology observed is the result of an altered circulatory system. On the other hand, it is impossible to believe that the neurons in a portion of the neuraxis with such badly damaged vessels could react physiologically.

Summary

In this communication we wished to slant our attentions to the reactivity of the vasculature in the brain and spinal cord to ionizing radiations. Studies dealing with the influence of x-rays on the blood-brain barrier were reviewed, and pathologic changes in central nervous system blood vessels due to x-rays and to alpha particle radiation were described.

It is felt that x-radiation dosages under 1,000 r administered to the brains of adult animals fail to reveal marked histopathologic findings, even though these have been observed following this radiation dosage in newborn animals. Nevertheless, the effect of these relatively low doses may become manifested in neural dysfunction through changes in the surrounding medium of neuronal pools. Thus, we feel that one of the effects of low dosage radiation is an increase in the permeability of cerebral vessels.

With higher x-ray doses (4,500 or 6,000 r) and with alpha particle radiation (10,000 rad) to the central nervous system, histopathologic findings have been observed not only in the cerebral vasculature, but also in neurons and neuroglia. Vascular damage was evident by thickening and hyalinization of the vessel walls. Perivascular infiltration of leucocytes and complete occlusion of larger and smaller vessels were often observed. Neuronal damage was evident by morphologic changes in the cytoplasm and nuclei of remaining cells and by spongy loss of neuronal elements in the areas irradiated. Neurons showing vacuolization and ghost cells were frequently observed. Although at these higher dosages it was difficult to maintain that the neuronal pathology was only the direct result of altered circulation, it is felt that vascular pathology cannot be underestimated in an analysis of the response of the central nervous system to ionizing radiations.

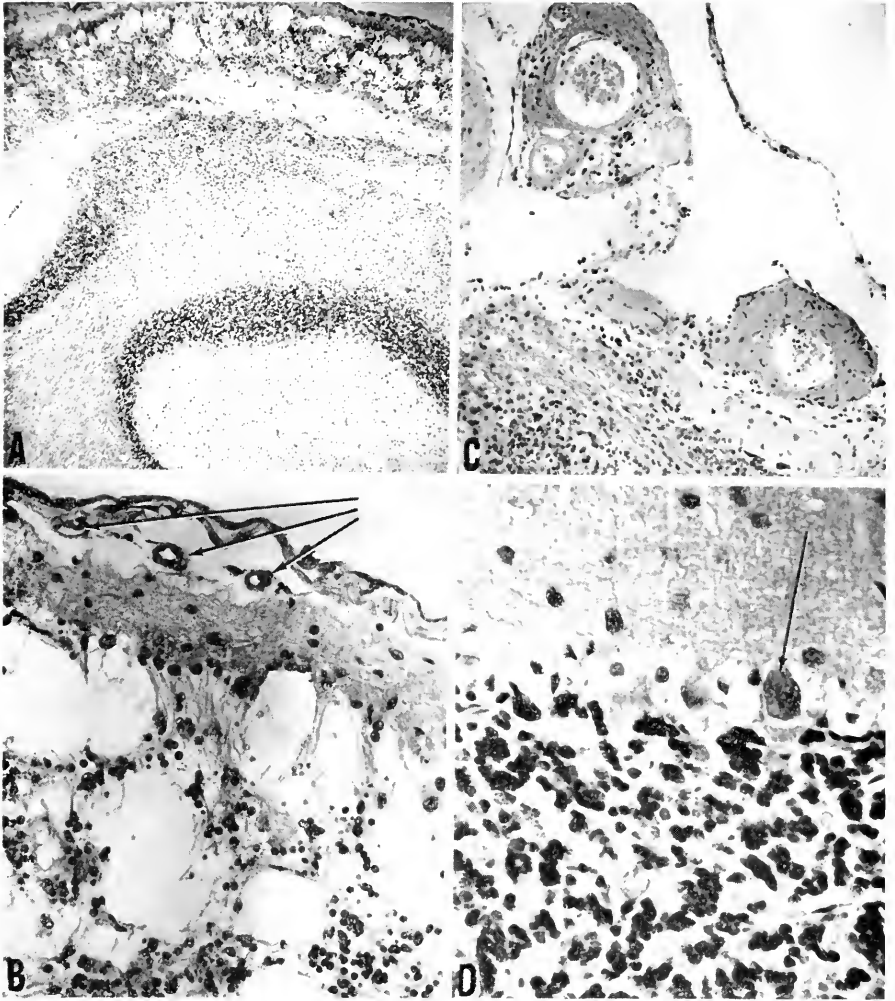


FIG. 7. Sections of cerebellum in rats administered 12,500 rad alpha particles. (A.) Rat sacrificed 7½ weeks after radiation. Note the spongy degeneration of the cerebellar cortex. $\times 63$. (B.) Higher power view of cerebellar surface pictured in 7A, showing degenerative vacuolization, meningeal thickening, and vascular hyalinization (arrows). $\times 360$. (C.) Another rat 10 weeks after radiation. Note the greatly thickened surface cerebellar vessels. $\times 175$. (D.) High power view of cerebellar cortex in rat described in 7C. Note the extensive Purkinje cell loss just under the cerebellar molecular layer. One pyknotic cell (arrow) still remains while a few nuclei of other vacuolized Purkinje cells can also be seen. $\times 500$.

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Chemical and Enzymatic Changes in Nerve Cells Irradiated with High Energy Deuteron Microbeams *

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In previous studies on the mouse brain, it was found that high energy deuteron microbeams with a diameter of approximately 25 and 75 μ will produce nerve cell necrosis without any definite permanent damage to interstitial elements (Zeman *et al.*, 1959). The first stages of this process can be observed in histologic preparations at approximately 4 days after a surface dose of about 400,000 rad delivered by a beam 0.025 mm in diameter. After 24 days, a complete loss of nerve cells has occurred, located strictly within the area of the beam path. In other words, the lesion forms a cylinder, the dimensions of which are determined by the reducing apertures and by the depth range of the particles (Fig. 1). Nerve fibers, myelin sheaths, and vessels do not appear altered.

These findings are in contrast to lesions resulting from wider beams. With beams of 1 mm diameter, it is impossible to produce only a selective nerve cell necrosis within the beam path. With a surface dose of up to 4,800 rad, nothing happens to the irradiated cortical neurons. With higher doses, there is focal tissue necrosis resulting in small cystic lesions, which occur predominantly at the end of the depth range of the deuterons (Fig. 2). To destroy all nerve cells within the path of a 1 mm beam, about 14,000 rad is required, but this dose destroys all the other tissue elements as well.

Since there is apparently no dosage using beams of 1 mm in diameter which would selectively destroy nerve cells within the path of the beam without also destroying the interstitial elements, microbeam irradiations seem to afford a unique opportunity to study one of the two different dose-dependent

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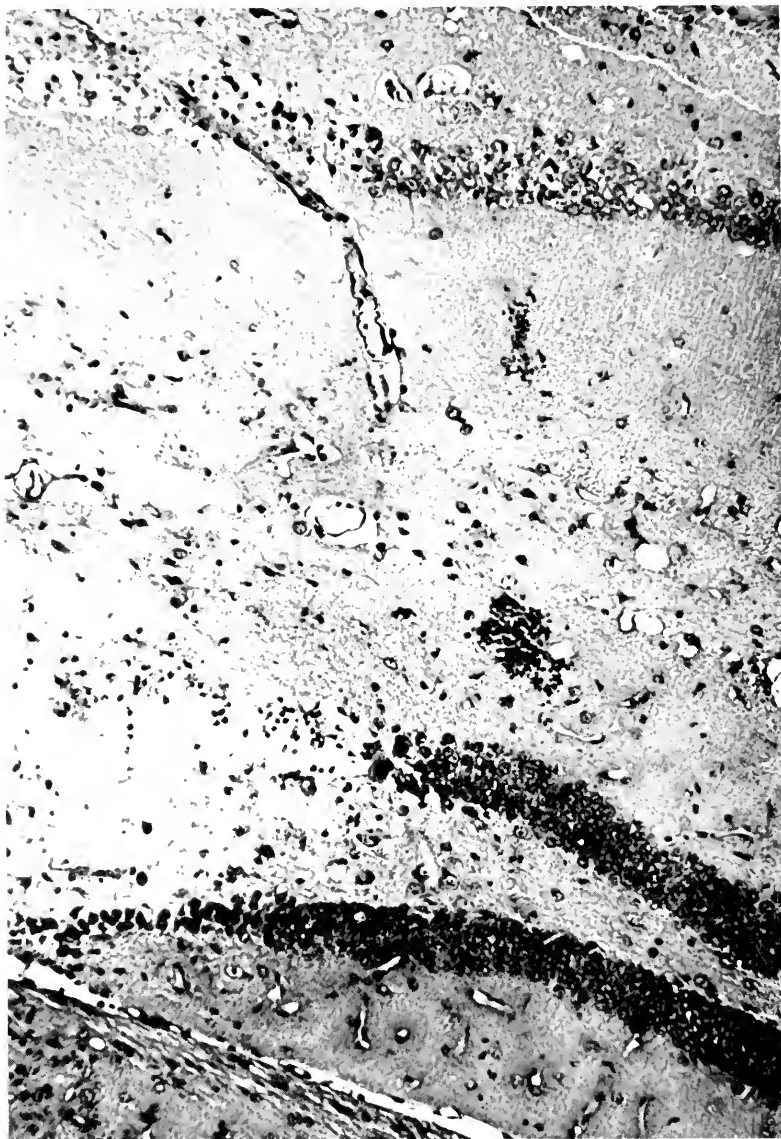


FIG. 1. 0.075 mm beam: 93,000 rad. 17 days survival. Visual cortex. The beam path is characterized by the loss of nerve cell bodies which are frequently represented by holes. Note the apparently normal capillary traversing the lesion. PAS-Gallocyanin.

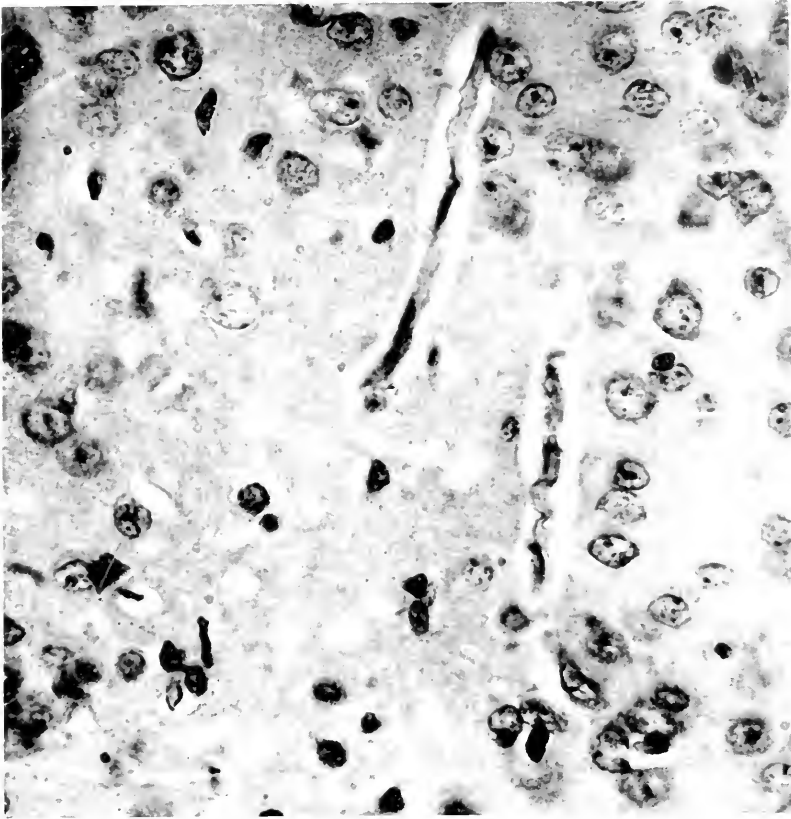


FIG. 2. 1 mm beam: 16,600 rad. 7 days survival. Hippocampus. Note complete tissue necrosis with cavity formation, hemorrhages in molecular layers and beginning formation of compound granular corpuscles. PTAH.

pathogenic mechanisms of radiation damage of the brain tissue observed by Schümmelfeder (1959), namely direct radiogenic nerve cell necrosis.

Preliminary experiments were designed to elucidate some chemical events incident to this necrosis.

Methods

Carworth CF₁ female mice, 6 to 7 weeks old, weighing 25 to 35 gm, were irradiated with different doses and beams and were sacrificed after 1, 2, 3, 4, 5, and 7 days. The animals were anesthetized and the brains were fixed by *in situ* perfusion with Heidenhain's Susa fluid. After being embedded in paraffin, the brains were cut in serial sections, every second section being

stained with PAS-galloycyanin. After the radiation-induced lesions has been identified on these slides, the alternate sections were stained for cytoplasmic proteins with Fast Green at pH2, for cytoplasmic RNA with Azure B at pH 4, and for nuclear DNA according to Feulgen's technique after digestion with RN-ase.

On these preparations, the following observations could be made. Depending on dose, the development of nerve cell necrosis begins at 2 to 5 days after irradiation; higher doses usually resulting in a shorter latency. Using beams of 75 μ and less, and doses of 100,000 rad and more, most nerve cells within the beam path show a synchronous pattern of necrosis, while wider beams and doses of less than 100,000 rad produce nerve cell necrosis in a random distribution, i.e., some neurons are in a state of complete disintegration, while others are still unaltered. The first stage of this radiation-induced nerve cell necrosis consists of disintegration of the cytoplasm. About the same time, the cytoplasmic DNA disintegrates. The nerve cell nuclei, however, remain unaltered and appear naked. It is difficult to estimate how long the nuclei rest in this state. They eventually become pyknotic, disintegrate, and disappear completely. It is not clear which cellular elements finally phagocytize the debris from the necrotic neurons; however, it appears that the nerve cell necrosis takes place without any demonstrable evidence of microglial proliferation or activation.

From these observations, it was assumed that radiation-induced nerve cell necrosis might conceivably be an autolytic process generated by the activation of proteolytic enzymes within the irradiated nerve cells. To test this hypothesis, the following studies were undertaken.

Fourteen mice received multiple beam irradiations over the visual cortex and the cerebellum. Each animal of the 1st group received 4 irradiations with 0.250 mm beams at 30,000, 60,000, 120,000, and 240,000 rad. Those of the 2nd group were given 8 irradiations with 0.075 mm beams at 90,000, 180,000, 270,000, and 540,000 rad. These doses were measured in the ionization chamber, thus indicating relative, but not necessarily absolute, tissue dosages. The beams were directed to the visual cortex and the cerebellum by means of telescopic focusing. They were arranged in a frontal plane, spaced 60 mils from center to center. The animals were sacrificed by neck dislocation at 4, 7, 10, and 30 hours after irradiation.

The brain was immediately removed and fixed in formol-calcium at 4°C. After 24 hours fixation, the brains were cut in serial sections at 15 μ on a standard freezing microtome. The sections were incubated in a medium prepared after Holt (1958) at pH 5 for demonstration of esterases. To block carboxylic esterases of the acetylcholinesterase, pseudocholinesterase, and lipase type, the medium contained a 10^{-6} M concentration of diisopropyl-fluorophosphate. Thus, only A-type cathepsins were believed to be stained.

The frozen sections were counter-stained with a variety of methods, such as Oil Red "O", Nuclear Fast Red, or azocarmine, or were impregnated with silver carbonate, and mounted.

Results

Although extreme care was exercised in processing the sections, radiogenic lesions were discovered and adequately demonstrated in only 5 brains. A variety of factors, such as the minute size of the lesions, the tremendous number of sections (over 2,500), and possible errors in the placing of the lesions, probably accounts for the high rate of failure. Nonetheless, the material available for study yielded interesting results.

Animal No. 4 was subjected to 4 irradiations with 0.250 mm beams and was sacrificed 5 hours later. Only the lesions produced with 120,000 and 240,000 rad, respectively, were found histologically. The irradiated nerve cells appeared shrunken and hyperchromatic in silver-carbonate impregnations. No abnormal enzyme activity was noted.

Animal No. 5 received 4 irradiations with 0.250 mm beams and was permitted to survive 10 hours. The lesions produced with 120,000 and 240,000 rad, respectively, were found in the visual cortex. The nerve cells were shrunken and hyperchromatic. They did not show any enzyme activity. On the other hand, the vascular endothelial cells exhibited considerable activity in the form of densely packed blue granules in the cytoplasm.

Animal No. 6 received 4 irradiations with 0.250 mm beams and was sacrificed after 31 hours. Only the lesions in the visual cortex, produced with 60,000 and 240,000 rad, respectively, were found. The nerve cells were shrunken, and a few of them contained blue granules in the cytoplasm. In the dentate ligament of the Ammon's horn, the enzyme appeared to be located predominantly in endothelial and proliferating microglia cells and in astrocytes. The nerve fibers of the callosal radiation were unaltered, while those of the cortical radiation exhibited retraction bulbs.

Animal No. 8 received 8 irradiations with 0.075 mm beams and was sacrificed after 10 hours. Only one lesion in the visual cortex, produced with 540,000 rad, was found. All nerve cells in the beam path exhibited considerable enzyme activity in their cytoplasm. The nuclei were shrunken and stained a deep red with azocarmine.

Animal No. 9 received 8 irradiations with 0.075 mm beams and was killed 30 hours later. Six lesions were identified, produced with 180,000, 270,000, and 540,000 rad, respectively. By sheer chance, in one section of the cerebellum, 3 lesions were cut on the same plane (Fig. 3). In these lesions, the enzyme activity was almost exclusively restricted to the Purkinje and granular cell layers. In contrast, the white matter showed no activity at all, and

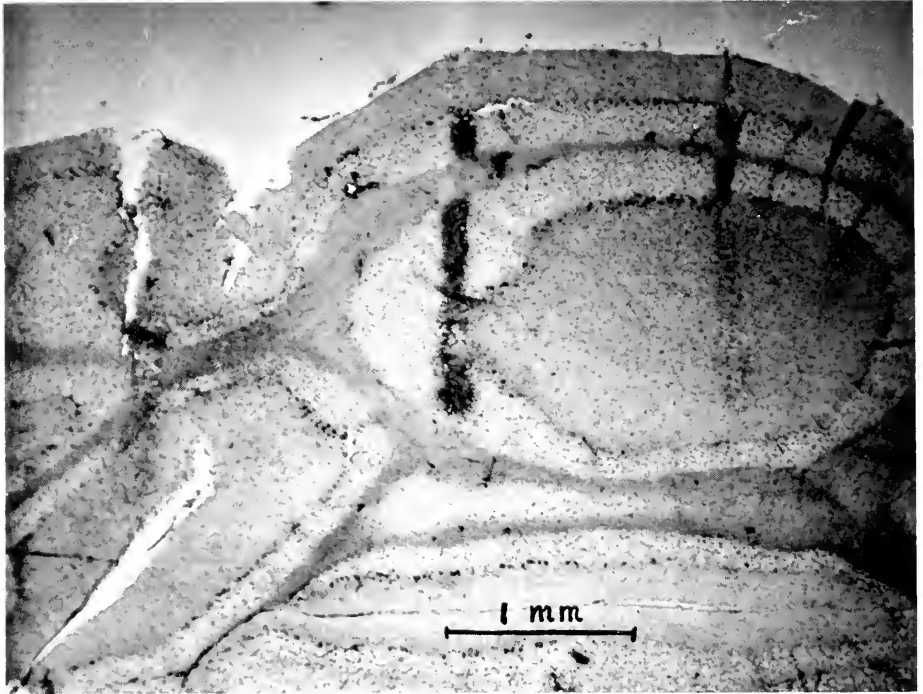


FIG. 3. 0.075 mm beam: (from left to right) 540,000 rad, (site of lesion is torn), 270,000 rad, and 180,000 rad. 30 hours survival. Cerebellum. Note that the path of the beam is outlined by the dark staining blue indigo granules developed by the activity of the cathepsin-like enzymes. Frozen section, Holt's technique for esterases, blocking with DFP, counter-stained with Oil Red "O".

the molecular layer contained only a few spots of activity (Fig. 4). Much of the latter appeared to be in capillary endothelial cells and astrocytes, but some of the superficial stellate cells also exhibited blue granules in their cytoplasm.

Owing to the thickness of the sections, it was extremely difficult to properly localize the site of enzyme activity in the Purkinje and granular cell layers. In some instances, however, chance permitted us to make more definite statements. In Fig. 5, one can readily discern the emergence of compound granular corpuscles, loaded with blue indigo granules from a capillary wall. Figure 6, on the other hand, shows 2 Purkinje cells, irradiated with 540,000 rad, having a deep blue cytoplasm. It can also be noted that their baskets are apparently unaltered. Some of the adjacent granule cells are similarly affected. Figure 7 depicts the granule cell layer of the hippocampus where it was hit by a beam of 180,000 rad. Here, the cytoplasm of many neurons is stained a deep blue.

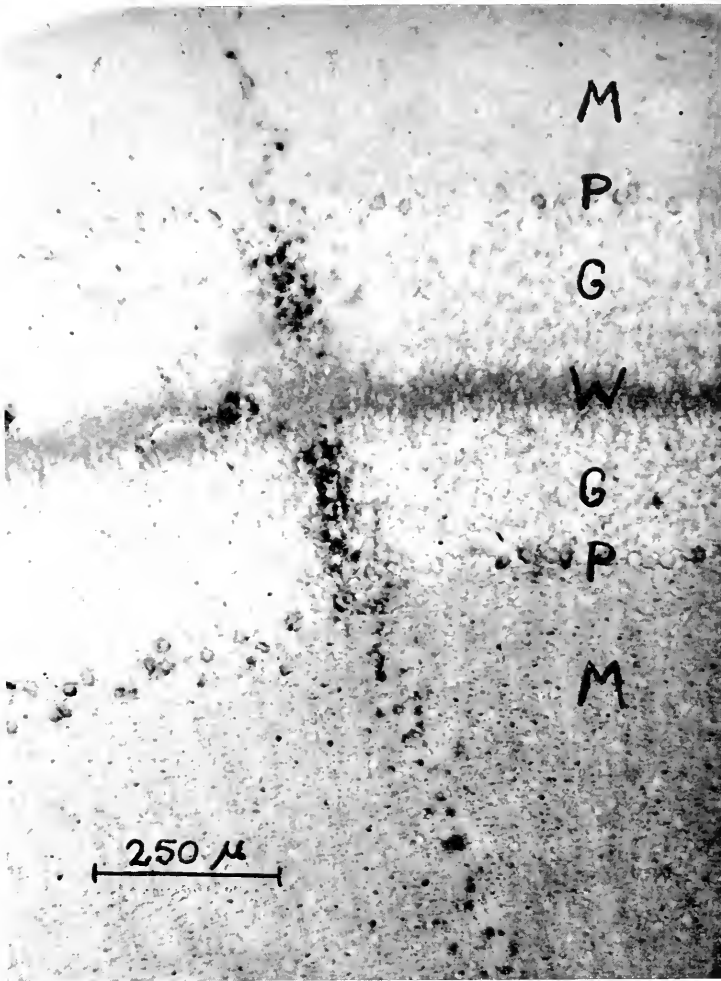


FIG. 4. 0.075 mm beam: 130,000 rad. 30 hours survival. Cerebellum. Note the degree of enzyme activity in the different layers. (M) molecular layer, (P) Purkinje cell layer, (G) granule cell layer, and (W) white matter. Frozen section. Holt's technique for esterases, blocking with DFP, counterstained with Oil Red "O".

Discussion

One cannot overlook the marked difference between the activity of A-type cathepsins as elicited by small and large diameter beam irradiations. While the path of 0.075 mm beams is sharply outlined by the blue indigo formed by the enzymes, the 0.250 mm beam path, after an almost identical dose and survival time, displays only sporadic evidence of increased enzyme activity. Furthermore, in the lesions produced by the small beams, the enzyme activity is found just as often in the nerve cell cytoplasm as in the interstitial elements,

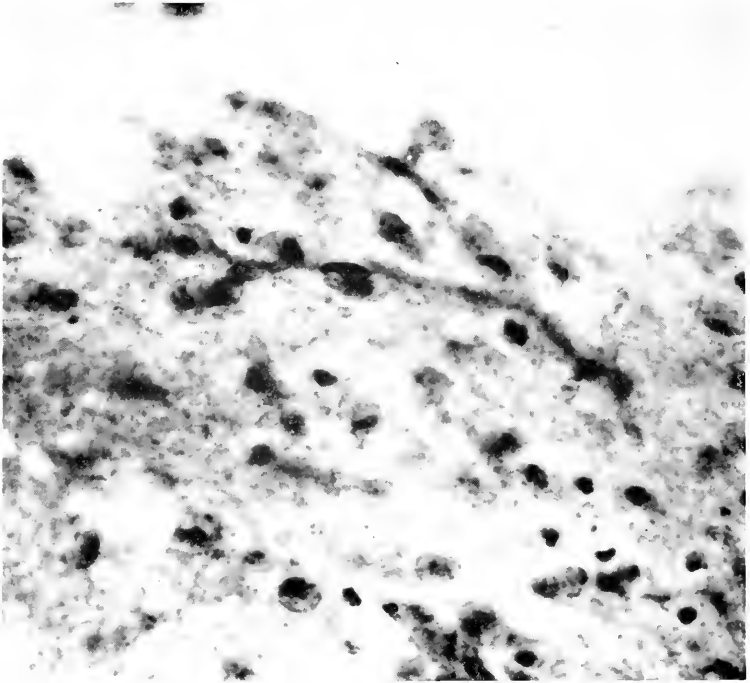


FIG. 5. 0.075 mm beam: 540,000 rad. 30 hours survival. Cerebellum. Capillary with emerging compound granular cells. Frozen section, Holt's technique for esterases, blocking with DFP, followed by silver-carbonate impregnation.

while in the lesions produced with 0.250 mm beams, the enzymes are predominately active within the interstitial cells. The degree of enzyme activity is in direct proportion to the density of the nerve cell population. Thus, in white matter, and to a lesser extent in the molecular layers, radiation-induced activity of cathepsin-like enzymes is practically nil.

The difference between the enzyme activity as elicited by beams of two different sizes might be somehow related to dose rates. All lesions were produced with a high dose rate of 63,000 rad per sec. While such a dose rate permits the enzymatic changes to develop with small beams, it appears to introduce physico-chemical changes in the path of wider beams which hinder the activation of cathepsin-like enzymes. Support for this assumption is derived from previous studies in which one hemisphere of the mouse brain

FIG. 6. 0.075 mm beam: 540,000 rad. 30 hours survival. Cerebellum. Note 2 Purkinje cells with darkly staining cytoplasm. Some of the granule cells are similarly affected, but this is difficult to appreciate on the photograph. Frozen section, Holt's technique for esterases, blocking with DFP, followed by silver-carbonate impregnation.

FIG. 7. 0.075 mm beam: 180,000 rad. 30 hours survival. Hippocampus. Cathepsin activity in the cytoplasm of the granule cells. Holt's technique for esterases, blocking with DFP, counter-stained with azocarmine.

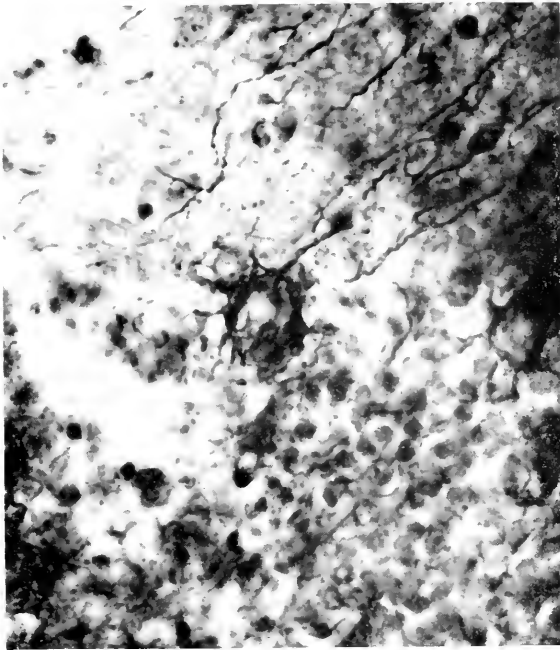


FIG. 6



FIG. 7

received 8,000 rad with 5 mm beams at a rate of 1,600 rad per sec. At 72 hours after irradiation, numerous nerve cells showed cathepsin activity in the cytoplasm. The active nerve cells were randomly distributed.

The occurrence of cathepsin-like enzymes in the nerve cell cytoplasm after irradiation is difficult to explain. Numerous controlled studies have shown that, except for certain groups of neurons located in the hypothalamus and the motor and reticular nuclei of the brain stem, the nerve cell bodies do not contain cathepsin-like enzymes as demonstrated with the technique used in this study. This would mean that ionizing radiation can either activate the precursor of such enzymes or liberate them from the lysosomes in which they are locked up, according to de Duve (1959). That such submicroscopic particles are abundantly present in nerve cell cytoplasm has been shown by Scarpelli and Zeman (1960) in electron micrographs. Further studies to test the latter hypothesis are underway.

The occurrence of radiation-induced proteolytic activity in nerve cells might help to explain radiation-induced central nervous system excitation consistently being observed and described by Russian investigators (Stahl 1959). Evidence for the causal correlation of cellular excitation and proteolytic activity has been amply provided by the work of Nassonow and his school (discussed by Troschin, 1958). It could also be entertained that some of the early clinical manifestations of radiation, such as shock and radiation sickness, might be related to a release of proteolytic enzymes into the circulating blood.

In conclusion, it can be said that the present studies, although preliminary and incomplete, might yield new knowledge in regard to certain phenomena of radiation biology.

Summary

It has been shown that the direct radiation injury of nerve cells is associated with the occurrence of proteolytic enzymes within the nerve cell cytoplasm.

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Tolerance of Central Nervous System Structures in Man to Thermal Neutrons*

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During the past several years at Brookhaven National Laboratory, the experimental procedure of neutron capture therapy of intracranial neoplasms has been investigated utilizing a nuclear reactor as a neutron source (Farr *et al.*, 1954-1960). In neutron capture therapy there are two main components to the therapeutic system: the capture or target atom and the thermal neutron or triggering component. As a result of thermal neutron capture, an atomic transformation occurs with release of energy. When the target atom is boron-10, the thermal neutron capture triggers an immediate disintegration of the B^{10} into an energetic lithium-7 atom and an energetic alpha particle with a modest gamma ray emission, totaling an energy release of 2.8 Mev. The restricted distribution of the energy release to a volume of one average cell provides a selectivity of action that, in principle, will affect one cell suitably primed and loaded, while the adjacent cell is totally unscathed. It is of importance in the development of a new therapeutic modality to determine not only the effect on the disease process, but also the possible effects on residual normal cell structures. Since large numbers of thermal neutrons must penetrate tissue within a few minutes at most, the tolerance of the central nervous system to thermal neutrons becomes a matter of great practical, as well as theoretical, interest. Fortunately, the conditions of therapy permit a study of neutron effects to be made relatively independent of the B^{10} capture reaction, since the distribution of the two components of the reaction differ widely at the time of exposure.

This presentation deals with observations made on patients so treated, with one patient receiving only thermal neutrons. In addition to the clinical observations, a combined topographic and histopathologic survey was made of the irradiated brains.

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The underlying purpose has been to determine whether the reaction was capable of destroying neoplastic cells and whether surrounding and distant structures were affected by the procedure. To make this evaluation, a comparison series of cases was collected to determine the spontaneous alterations which occur in untreated cases, e.g., the amount of necrosis occurring in the development of a neoplasm of the glioblastoma multiforme type, the most commonly investigated neoplasm in this study. In addition, forms of irradiation other than neutron capture therapy, such as that occurring in x-irradiation, have been considered for comparative purposes. Thus, in the nonirradiated and irradiated brain, the whole brain was embedded in celloidin, with whole brain sections being cut in coronal, horizontal, or sagittal planes at $25\ \mu$ and mounted on slides for low power survey. In addition, representative sections were cut at $7\ \mu$ and mounted with thin cover slips for detailed microscopic examination. A variety of special stains designed for studying the cellular details of the central nervous system with the nerve tracts were used for detailed observations.

The major series comprised 16 patients to whom neutron capture therapy was given in 1 to 4 exposures with, in the various patients, a total thermal neutron exposure ranging from 0.44×10^{12} to 6.31×10^{12} per square cm at the skin surface. An additional patient was treated at the new medical research reactor in a single exposure with a total thermal neutron penetration of 1.73×10^{12} per square cm at the cortical surface. Studies are still in progress on another patient who, in a similar fashion, received over 10^{13} neutrons per square cm on the cortical surface. In contrast to these cases with neutron capture therapy, one patient received thermal neutrons only, and this data provides comparison material.

Neutron capture therapy is accompanied by radiation other than that specifically provoked. Thus, it becomes necessary to establish the levels of these ancillary radiations and to evaluate whether they contribute to or detract from the clinical picture. The several contaminating radiations include (1) gamma radiations from the reactor core and gamma rays induced when neutrons are lost through capture in the shielding and reflecting materials, (2) the remaining small fraction of the emergent neutrons which retain energies in the kilovolt range which may possibly cause tissue damage in their own right, and (3) the effects of the passage of the great quantities of slow neutrons through tissue, wherein, through thermal neutron capture by hydrogen and carbon, there is produced appreciable induced gamma emission; and by nitrogen, energetic protons. The first two factors are approached chiefly as engineering design problems; the third is the subject of this essay.

Neutrons are elementary nuclear particles which have essentially unit mass, about the size of an hydrogen nucleus or proton, but no electrical

charge. We know that neutrons of high energy, from 0.1 or 0.2 Mev and up, do have an extensive damaging effect on tissue. These neutrons are able to disrupt molecular relationships and otherwise cause disturbance of the normal life pattern. These effects arise largely from the mechanical impact of fast neutrons on atomic nuclei. It is the lack of such high levels of kinetic energy which make the slow neutron innocuous in this respect.

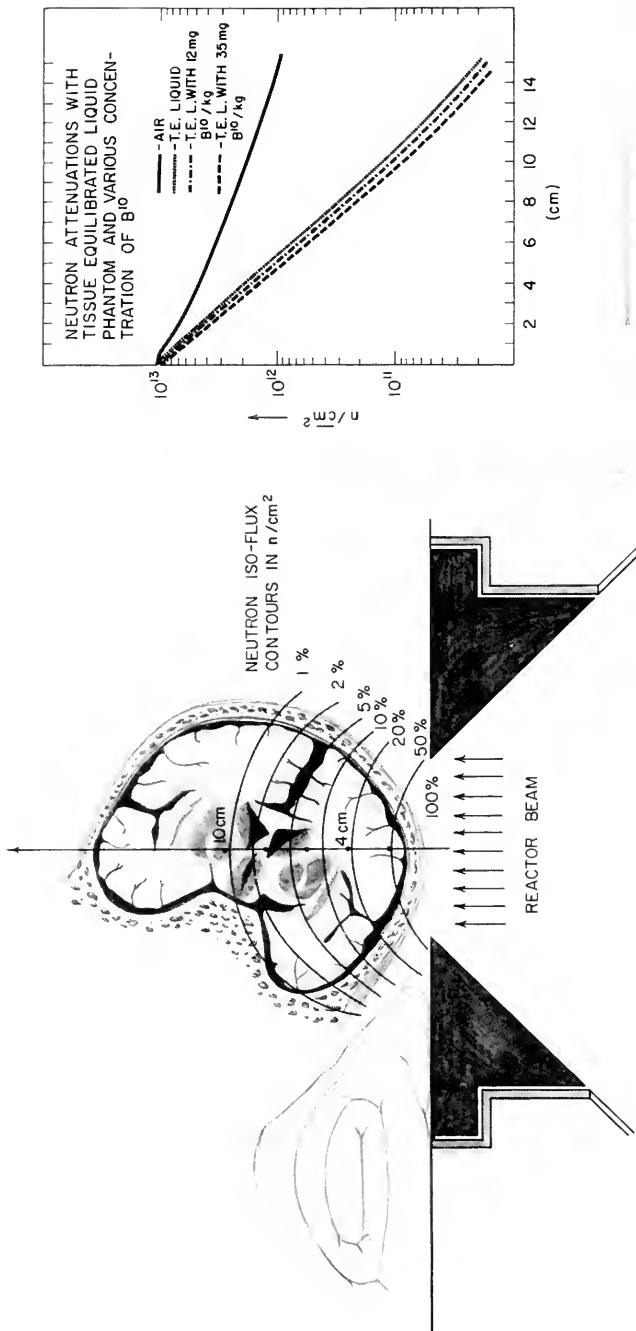
When neutrons of thermal energy, namely 0.025 ev, penetrate into tissue, they do not directly cause damage by their passage; rather, they pervade the tissue as a gas pervades whatever space it is released into. However, with increasing penetration their numbers significantly and predictably decrease as they travel from the surface through solid tissues. The pattern of attenuation is shown in Diagram I. Any effects may be expected to result only from capture with atomic transformations, radioactivity resulting therefrom, or both.

Radiations coming from the induction of activity in tissue components have to be recognized in assaying the total effects of neutron exposure of tissue. Chief among these reactions are the capture of slow neutrons by hydrogen, resulting in an immediate gamma ray emission at over 2 Mev, the capture of slow neutrons by nitrogen, giving an instantaneous proton of energy 0.6 Mev, and the capture of slow neutrons by carbon, yielding gamma rays of up to 8 Mev. Other constituents in tissue, particularly sodium and chlorine, may contribute readily measurable additional radiation.

In neutron capture therapy, a much greater intensity of reaction is induced in the treated tissue by providing thermal neutrons in large numbers at such a time after the target atom injection as we empirically have shown to be effective in experimental tumor destruction and sparing of adjacent normal tissues. The mechanisms of the cytotoxic effect are under intensive study, as no explanation is conveniently provided by chemical analyses of target atom species for the differently affected tissues.

In the experimental investigation of neutron capture therapy at Brookhaven, two reactors have been used as sources of neutrons. The work was begun at the large graphite research reactor when it first reached its full operating power in 1951. A part of its biologic shielding was modified to provide a hollow cone delivering neutrons through a treatment aperture. Subsequent improvements in this arrangement provided a shutter and a higher flux of neutrons. Some 40 experimental procedures with patients, together with studies on mice, rabbits, and swine at the graphite research reactor, gave experience and background for the design and construction of the medical research reactor, a reactor entirely oriented toward studies in man. The details have been published previously (Farr, 1959).

Dosimetry of neutron capture-induced gamma radiations in tissue is not a straightforward measurement, since there is no detector which, in a gamma



field, is completely sensitive only to specific particle radiations resulting from the reaction. Consequently, we must rely largely on calculations with such experimental measurements and observations as can be appropriately carried out to establish the validity of our conclusions. Since the particle energy released is proportional to the concentration of target atoms and total number of neutrons, our measurements are directed to these ends. The concentration of the neutron capture target element is assayed by chemical means, while the total number of neutrons passing a plane is determined by activation of gold foils or wires inserted into the neutron penetrated area. Gamma ray levels have been calculated and roughly measured by ordinary ionization chambers covered by neutron filters and, experimentally, by chemical dosimeters, glass needles, and solid state ionization devices. Fast and intermediate energy neutrons in various regions are evaluated insofar as possible by characteristic energy activation foils and fission chamber techniques. Measurements given in this paper were derived by one or more of these procedures.

Results

Effects on nontumorous neuronal structures were studied in 16 patients receiving neutron capture therapy for gliomas and sarcomas, with a 20–40 minute exposure to thermal neutrons. The material discussed is being reported in detail elsewhere (Farr *et al.*, to be published).

Whole brain sections were prepared from 16 cases in which the total surface neutron exposure varied from 0.44×10^{12} to 6.31×10^{12} per square cm and in which the total dose of tetraborate and pentaborate salts of sodium, ranged from 26–50 mg per kg body weight when calculated as B^{10} per dose. Experimental evidence has firmly established a correspondence between tissue concentration and total dose for immediate distribution. Borate salts appear to distribute uniformly in body water, eventually reaching a single equilibrium concentration. In 3 cases of glioblastoma multiforme tumor, necroses appeared to be present in the region of the emergent neutron port. In a 4th case of cerebellar angiosarcoma and ependymoma, there may have been an irradiation effect, but the presence of a fungal lesion did not lead to a clean-cut evaluation. In a 5th case of sarcoma at the site of

DIAGRAM I. On the left is shown the thermal neutron approximate iso-flux contours in neutrons per square cm with the maximum intensity exposure from the neutrons cloud at the port of entry. The direction of movement of the neutron fog is shown, and the fall off in neutron intensity from the port of entry to the midbrain. To the right is a graph indicating effects on thermal neutron distribution in tissue with different B^{10} concentrations. Surprisingly little effect is noted therefrom in these phantom studies.



FIG. 1A. Patient 5972 r. Whole brain section (myelin stain) showing destruction of most of the right temporal lobe in which the tumor was located. The areas not affected by the tumor, but receiving the maximum irradiation, show intact myelin.

maximum neutron intensity, destructive effects to the neoplastic area occurred. In the other cases, no alteration in the neoplasms, attributable to neutron capture therapy, could be established. Microscopic studies of nontumorous and adjacent neuronal structures in all these cases, carried out with various staining techniques, gave no suggestion that damage had been done to the nonneoplastic central nervous system structures by the irradiation procedure. A typical section is shown in Fig. 1A. By one set of assumptions, in which a uniform neutron exposure is postulated to permit simple calculation, the computed dose from tissue-component capture gammas alone (administered to the cerebral cortex) ranged from 78 to 1128 rad in these patients. These and other calculations were made by Dr. J. S. Robertson of our department.

Effects due solely to thermal neutron exposure were studied in a man, aged 53, with glioblastoma multiforme in the right frontoparietal region, who was admitted for neutron capture therapy. He had had operative removal of part of the lesion and no subsequent x-ray therapy. Due to a technical difficulty not appreciated or known at the moment, the patient did not receive the injected sodium pentaborate into the carotid artery; thus, when

exposed to thermal neutrons, only the tissue components were present for interaction. The thermal neutron flux at the skin level was 1.875×10^9 per square cm per sec. The irradiation time was 502 sec. The region of the tumor and the adjacent cortical surface were exposed to a total of 4.7×10^{11} thermal neutrons per square cm. The port was 3.5 in. in diameter. Intrinsic gamma irradiation from the reactor over this period amounted to 40 r. Death occurred 55 days after irradiation.

Microscopic examination of the whole brain sections indicated that the residual neoplasm had essentially the same histologic appearance as that noted in the original biopsy. Thus, as far as could be ascertained, the thermal neutrons had no effect on the neoplastic cells.

The area of the brain adjacent to, and at various levels distant from, the neoplasm was examined microscopically. In the region at the level of the tip of the frontal lobe, a stain for myelin (Fig. 1B) shows that there is no loss of this material in this region. In contrast, with the same stain (Fig. 2), at the level of the genu of the corpus callosum, there is extensive demyelination, in all probability due to the presence of the adjacent tumor. In Fig. 3 a large mass of neoplasm is shown with partial demyelination in the adjacent area in the right occipital lobe. In contrast, the left occipital lobe

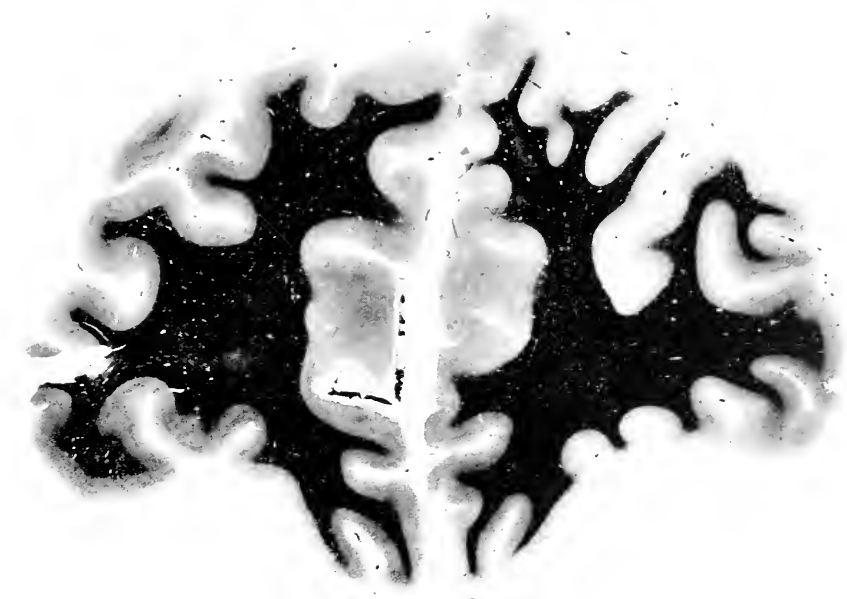


FIG. 1B. Whole brain section of case 8180 r at the level of the frontal lobes showing a normal myelin content and architecture.

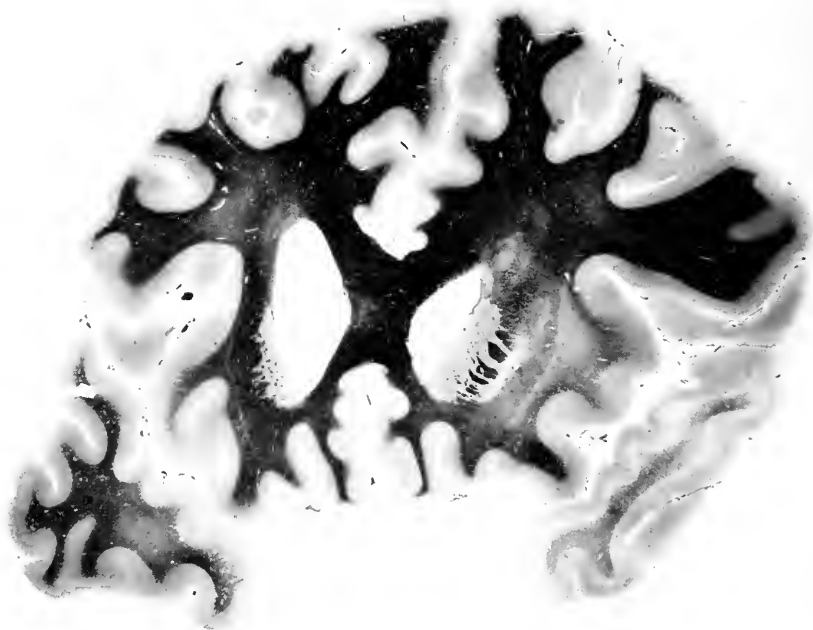


FIG. 2. Whole brain section of case 8180 r at the level of the genu of the corpus callosum, showing demyelination of the right temporal lobe and the insular region of the same side in the vicinity of the tumor. The frontal lobe has a normal myelin content.

appears completely normal. In the same section the cerebellum may be seen with an entirely normal-appearing pattern of myelin. The Nissl substance and the neurofibrils show no alteration in a section taken from the cortical region receiving the maximum neutron exposure (Fig. 4).

Effects on nonneuronal structures were studied in one patient receiving neutron capture therapy with high exposure achieved in 200 sec. A man, aged 44, who had a left frontal craniotomy for oligodendroblastoma, was admitted after x-ray treatment following surgery. The surgical wound was infected, and there was a brain abscess. Following treatment for the latter, it was observed that there was a recurrent deep lying neoplasm in the left frontal lobe. He was given 35 mg B^{10} per kg body weight in the antecubital vein. At 31 minutes after the boron infusion, the left frontal area was irradiated for 200 sec with 1.73×10^{13} thermal neutrons per square cm at the cortical level. Death occurred 30 days after neutron capture therapy.

Figure 5 shows the whole brain as removed at autopsy. The defect in the frontal lobe indicates the area of original operative interference and the site of irradiation with thermal neutrons. Figure 6 is a coronal section 3 cm from



FIG. 3. Whole brain section of case 8180 r showing a neoplasm producing enlargement of the occipital lobe and demyelination of the adjacent area. The opposite occipital lobe and the whole cerebellum are intact.

the tip of the frontal lobe and shows part of the scar viewed in Fig. 5. In Fig. 7 the photomicrograph shows a section from the scar seen in Fig. 6 and is from the outer surface of the brain. The connective tissue reaction with fibrocytes and the glial reaction are both predominant. Of great importance is the fact that within the effective depth no residual neoplastic cells are observed in this region.

Figure 8 shows a photomicrograph from the region about 3 cm deep to the gross area of scarring. The neurons are intact, and there is no destructive effect of the thermal neutrons in the non-neoplastic area adjacent to the site of the previous primary neoplasm. Figure 9 is taken from the meninges at the site of the arrow seen in Fig. 6 and within the port of irradiation. The meninges are not thickened, although occasionally phagocytic cells with blood pigment are present. In general, this is a mild reaction, and there is no evidence of fibrosis from irradiation. Just below this area, Fig. 10 a high

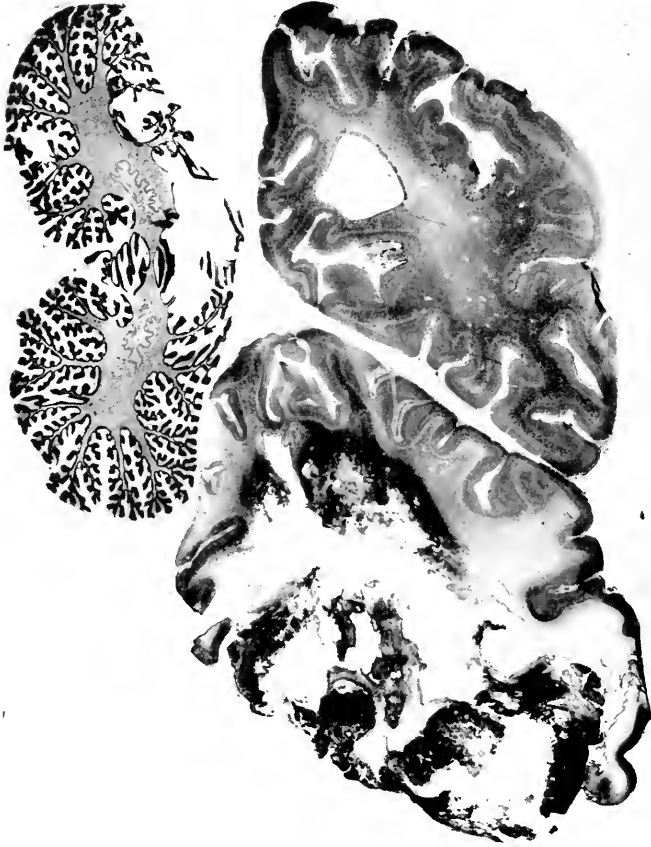


FIG. 4. Whole brain section of case 8180 r stained to show the Nissl substance. The neurons show tigrolysis only in the limits of the neoplasm.

power magnification with the Nissl stain) shows intact neurons as further evidence of a lack of irradiation effect. The cortical areas receiving maximum exposure received 1,905 rads from capture gamma. This is calculated on the basis of the same set of assumptions as the previous values.

Discussion

In assessing the role of thermal neutrons as a potentially noxious agent when administered to the central nervous system, certain assumptions are implicit. Among these is the assumption that the only effect which need be considered in the exposures of the size discussed is that which results in a gamma emission as a result of thermal neutron capture by tissue components.

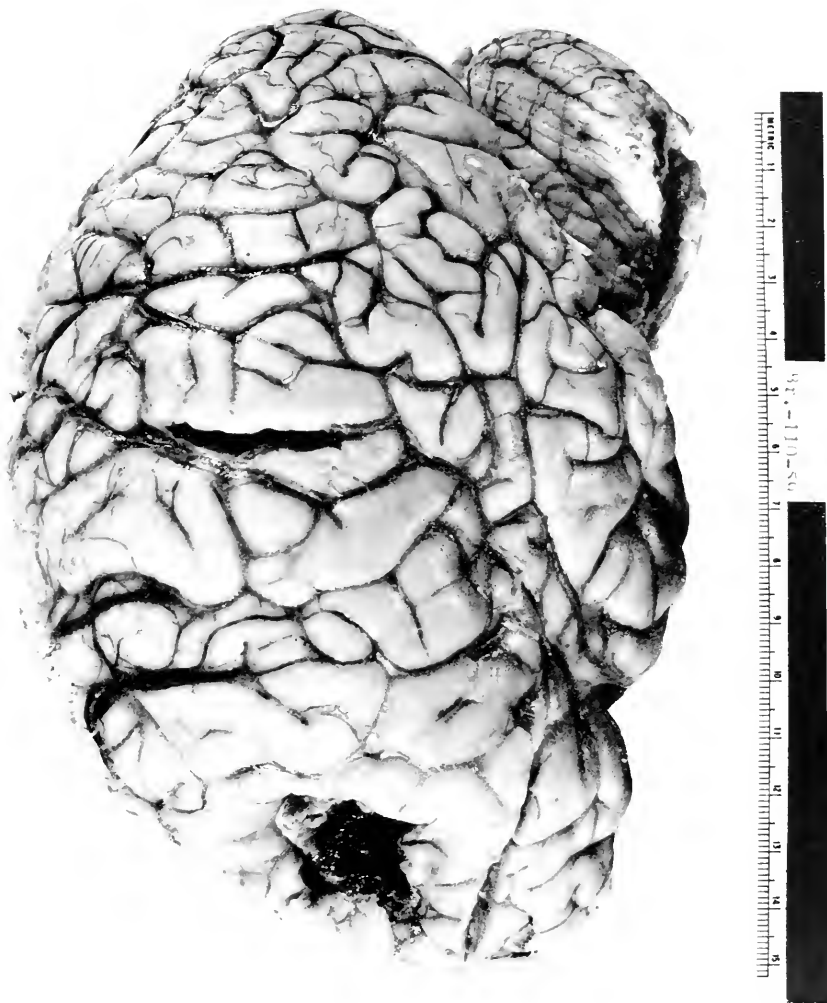


FIG. 5. Photograph of brain at autopsy showing a frontal scar at original operative and irradiation site. Case 8-00-83 r.

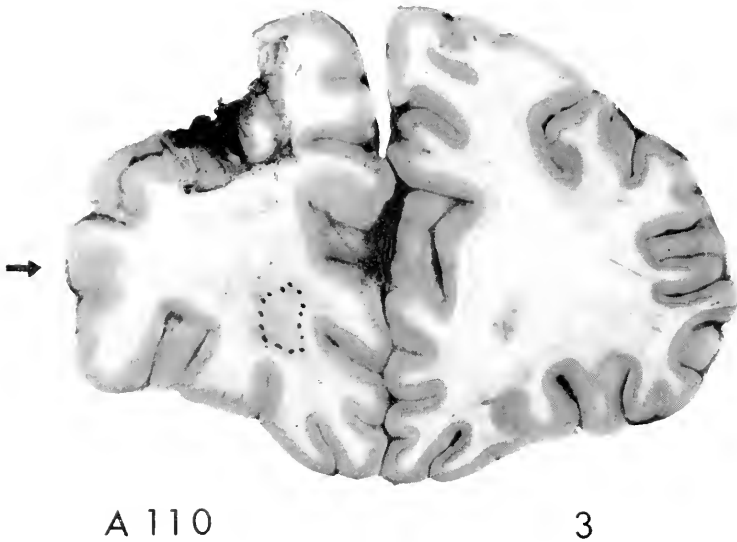


FIG. 6. Coronal section of gross brain, case 8-00-83 r, at 2 cm from the tip of the frontal lobe across the scar partially seen in FIG. 5. There is no tumor growing in the region of marginal irradiation.

While in a few instances the capture reaction will result in an atomic transformation and a beta particle emission, these are so infrequent that in the aggregate no significant contribution to the total exposure is provided by them. It then becomes of major importance to determine the formulation by which the capture gamma dose is computed. It is here that both the major uncertainty and the major difficulty are encountered. Since the neutron exposure is not uniform, precise description of the attenuation becomes paramount. Yet difficulties in measurement and alterations in pattern by geometry or port of entry make this factor one of approximation and not certainty. In similar fashion, the formulation of a suitable integral which can be solved to give the summation of the dose and volume of maximum intensity has developed into a problem which can be handled only by a computer, and we have not obtained a final answer, though work is continuing. Therefore, in this discussion we have used the values computed by Robertson for the rad dose derived from capture gamma on the assumption of a uniform distribution of thermal neutrons at the maximum observed intensity of exposure. It is clear that the dose values given in this paper may be greater than the maximum expected value to be obtained by a more precise estimation, but the volume of maximum intensity in the latter case may equal or exceed the approximate value herein reported. Whatever the error and

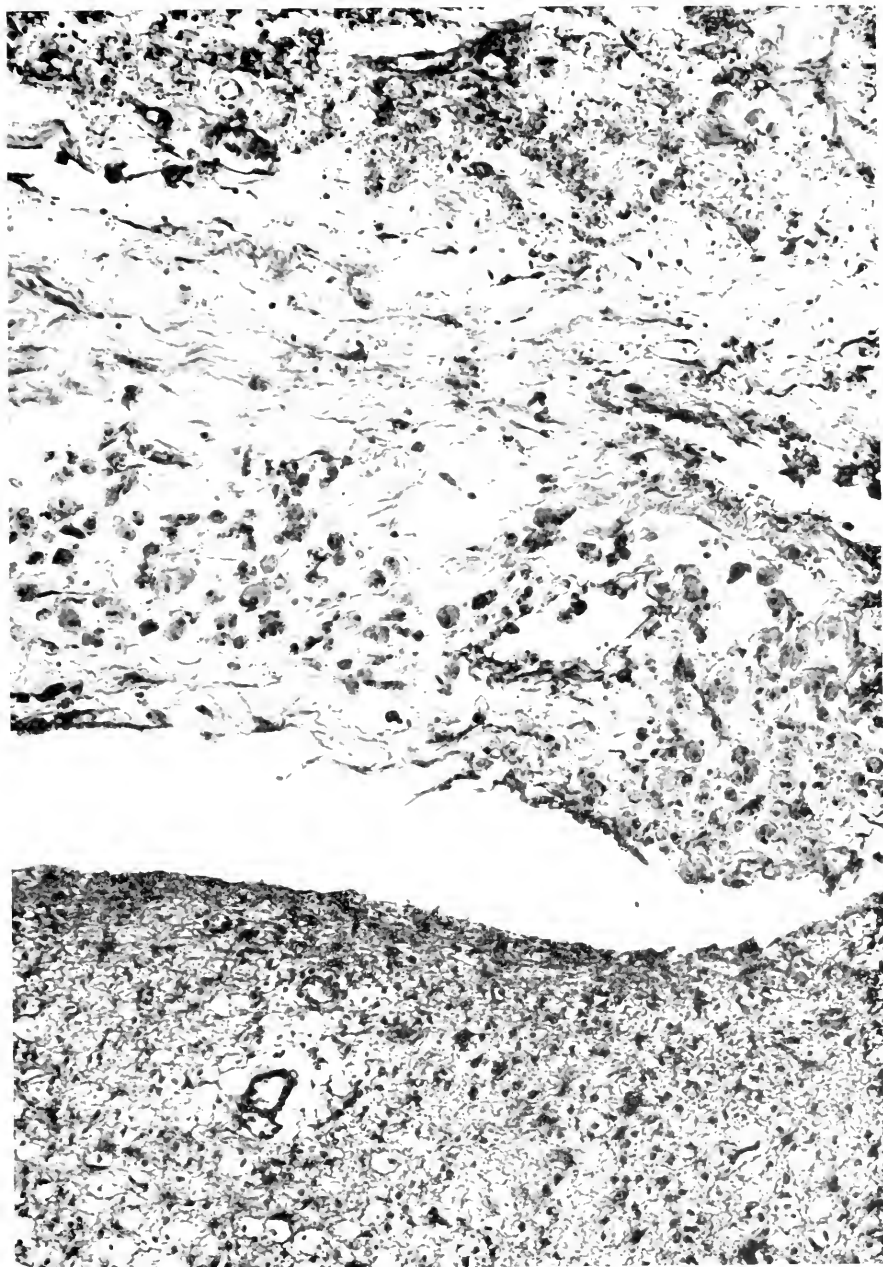


FIG. 7. Photomicrograph ($\times 181$) of the scar located in the left frontal lobe, case 8-00-83 r, showing the thick fibrotic tissue in which there are numerous phagocytes. At the left of the brain tissue, lying below the connective scar there is marked gliosis.

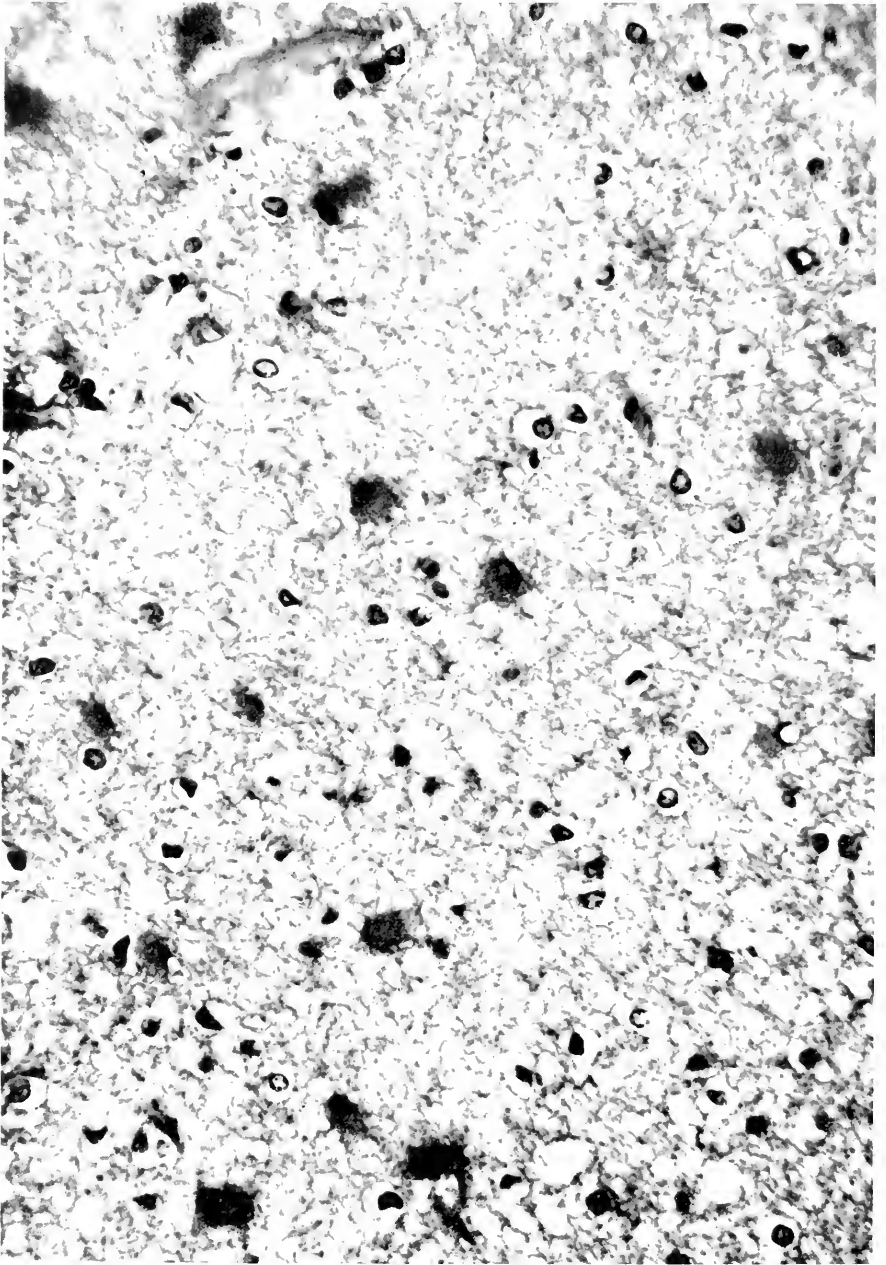


FIG. 8. Photomicrograph ($\times 480$) of an area of gliosis located in the white substance at a deeper level than FIG. 7. Case 8-00-83 r.

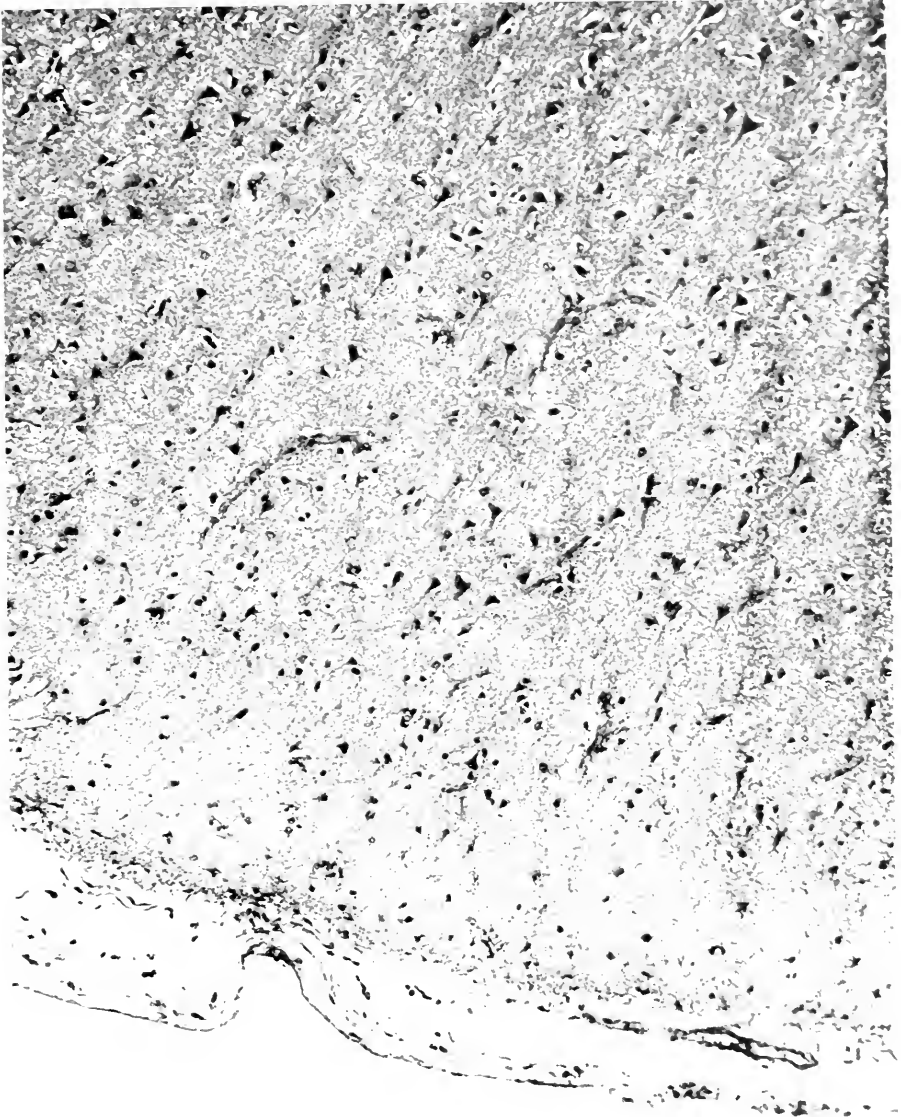


FIG. 9. Photomicrograph ($\times 150$) of the cortex of case 8-00-83 r taken from the zone of maximal irradiation on the frontal lobe below the scar. The cortex appears normal. The leptomeninges are not thickened by fibrous tissue.



FIG. 10. Photomicrograph ($\times 600$) of cortex, case A110, taken from the same area as FIG. 9, showing that the Nissl substance is intact in this zone of maximal irradiation.

difficulty in calculating the rad equivalent of thermal neutron exposure, we have had sufficient experience with measurement of thermal neutrons by foil activation to place a high degree of confidence in the total thermal neutron exposure indicated. Moreover, the observations, as made, are dependent on their calculations only for further interpretative elucidation and not for primary validity. Preliminary observations on animals and patients, in which the maximum intensity reported in these studies has been exceeded, are pointed-to only to emphasize that beyond the exposures discussed here there still remains a margin of safety. We have been surprised that none of the structures, neuronal, supporting, or vascular, have shown any changes under the exposures noted. To achieve greater exposures, further change in the moderator-reflector system of our reactor must be accomplished, so that gammas resulting from materials capture of wayward neutrons may be further diminished in a significant manner.

Since we have observed no evidence of a limit to the tolerance to thermal neutrons, we cannot speculate on these data. They are presented as observations made repeatedly (in animals) to establish their reproducibility. The practical significance of this may be considerable in the consideration of a reactor for diagnostic purposes, but until limits are set, this must remain largely an area for discussion only.

It must be emphasized that our observations were not limited to an histologic examination of exposed tissue. Functional observations were carried out on all patients receiving neutron capture therapy. These included specific visual acuity and perception tests, audiometric examinations, and complete neurologic examinations at frequent intervals. Perhaps the most significant was day to day observation of the patient, his general behavior, and his reaction to situations frequently new to him. By all these criteria, no deleterious effects were seen. Complex movements were unaffected, and both logical and emotional responses showed no changes. Patients were observed closely up to $1\frac{1}{2}$ years, which probably is adequate time to permit the development of usual postradiation sequelae. In regard to visual acuity, it should be pointed out that frequently the retina itself received considerable exposure to thermal neutrons, but no changes in function resulted.

Summary

In the histologic studies of serial sections of the brains of 17 patients treated by neutron capture therapy for intracranial neoplasms, no changes consistent with and attributable to exposure with thermal neutrons were found in the nervous tissue. A maximum thermal neutron exposure of 1.73×10^{13} thermal neutrons per square cm occurred over a 200 sec interval. One additional patient receiving only thermal neutron exposure (approx-

mately 4.7×10^{11} thermal neutrons per square cm to the brain surface) showed no histologic changes attributable to the neutron exposure in either neoplastic or normal brain tissue.

The difficulties in transposing the observed neutron exposure intensities into rad units are discussed.

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GENERAL DISCUSSION

JERZY ROSE (*Johns Hopkins University, School of Medicine*): Many papers were concerned with the dose of heavy ionizing radiation, the consideration of species differences, individual differences, differences in different portions of the brain, differences in time of application and examination of the tissue, and more sensitive methods to detect radiation damage before pathologic changes are apparent. Perhaps we need to define a little better what we mean by "minimal radiation dose," "lethal dose," and other terms.

F. A. METTLER (*New York, New York*): Dr. Malis showed a section in which there was irradiation of the Bragg peak and elimination of fibers. Later, on a subsequent slide, we saw fibers present in excess. There are three possibilities that might explain this. Some of them are rather significant in terms of a possible explanation with regard to physiologic experiments in general. The first possibility is perhaps trivial, but Dr. Zeman showed a picture in which there was passage of the beam and no fibers degenerated. So, the first possibility would be that these fibers were not degenerated at all. If one considers the paper by Drs. Janssen, Tobias, and Haymaker and several other papers in which there was considerable damage in the capillaries in the level of the Bragg peak, the second possibility would be that the fibers seen in the second slide of Dr. Malis were really not nerve fibers, but were fibers of connective tissue, perhaps fibers of glia. The third possibility would be that the fibers seen in the second slide were nerve fibers. If so, where did they come from? They could come from two places. There was a paper presented earlier which was of remarkable importance to the question of regeneration of the nervous system. There was transection of the cord with no interference with the external meninges, an ideal situation for determination of regeneration in the cord. So, these fibers might be regenerative. If this were an area of peripheral degeneration or denervation, it would be possible to conceive of another possibility, for we know that in peripheral areas of denervation other fibers have a tendency to invade. So, if regeneration occurred, it could occur as regeneration from a nerve fiber, or it could occur from the invasion of other fibers in the vicinity. Such a situation has never been implied in the central nervous system. If it does exist, it is of considerable importance in the interpretation of chronic neurologic preparations. I would like to ask the chairman his opinion.

JERZY ROSE: We did not present the material completely, because giving evidence required many slides and there simply was not enough time, so we presented only a few slides which illustrated that damage was done. There is no question that these are nerve fibers. I think we have rather large material on this aspect. If we skip the unessential modification, we have done a sufficient number of a variety of these things, and as long as we are going to believe that a silver stain or any other accepted classic neurologic stain means a nerve fiber, it is a nerve fiber. Not only that, but it is easy to demonstrate in the older lesion that apical dendrites

are certain because they are in connection with the cell; of course, it is not always easy to be sure it is not an accident. I would say there is no question at all that there are nerve fibers and dendrites within the lesion. What are they due to? This is an open question. Some people may say that these are preserved fibers. No doubt, with a light lesion some fibers may be preserved, but there are many more fibers which appear. So, even if you wish to assume that there are no fibers at all regenerating, we still have to account for an apparent large increase of the fibers. The argument does not rest on the fact that the fibers first degenerate, even though there is evidence that they do. There is positive evidence of sprouting fibers, we believe. Whether this is regeneration or, as we think, a perfectly normal growth which only becomes apparent with light irradiation remains to be determined.

LAWRENCE KRUGER (*University of California, Los Angeles, California*): Having switched teams, oceans, and cyclotrons, it is a particular pleasure for me to be able to say that with lesions of the same size as we had with the Brookhaven series, in another species, the rat, and in another laboratory—Dr. Tobias' laboratory at Berkeley—Dr. Clemente and I have had excellent correspondence of dosage. It occurred to me, however, in listening to Dr. Haymaker, that there was a slight discrepancy—perhaps an unimportant one. The lowest dosage in the series of Dr. Haymaker and his co-workers appears lower than the first animals we irradiated in Berkeley which were treated with smaller dosage. We had one animal with 4,000 rad at 28 days without a lesion, and yet these animals were irradiated on the same run. It would seem reasonable to suggest that the size of the lesion could explain the discrepancy, since this is the only likely difference in the conditions of both sets of experiments. Size is probably important, because Dr. Tobias did show an excellent slide of dosage times volume as being an important variable. This is also important in discussing the vascular problem in relation to a neuronal lesion. Dr. Sourander stated that, with a slit of 1.5 mm and 10 mm, the dosage for neuron damage was still 20,000 rad although the appearance was different. I wonder whether he would care to comment upon the difference in a general way to explain the nature of the dosage volume relationship. Some of the discrepancies that might have been apparent at first must be solved in this way. As for species difference, it is remarkable that we have a good correspondence now for line lesions of the same size for rat, rabbit, and cat. This is now true for independent cyclotrons, too. This good agreement in different species does not appear to exist for vascular damage, suggesting that neuronal and capillary damage might be independent variables. How reasonable is it to assume that the destruction of neurons is concerned with the destruction of capillary circulation? I should like to join the Drs. Bailey in stating that it does not seem reasonable to say that *all* neuron destruction is the result of capillary damage. To me, the most convincing argument is probably the appearance of a line lesion. The sharpness of the lesion would in itself discourage the interpretation that this is vascular. However, this is certainly not a proof. The collateral circulation might be extensive, and here is where the dose-volume problem might solve the question to some extent. A recent observation that Dr. Clemente and I have made is that the gross observation of capillaries would appear to indicate that the time relationship of the vascular change and the neuronal changes in an India ink injected preparation are vastly

different. One can see a marked surface change within a time as long as 2 months. This at least seems likely at present for a surface dose of 9,000 rad, which is not a dose for total necrosis of neurons, and can produce a line lesion in depth. The presence of gross capillary changes in a region where neural change is not apparent also suggests a possible dissociation of vascular and direct neural damage. The gross discrepancy between the time relations, the distribution in space in vascular changes and the neuronal changes, the gross discrepancy between species differences and susceptibility of vessels, and the lack thereof for neurons would seem to me a convincing argument that neurons are susceptible to ionizing radiation without necessarily involving capillary damage.

HORACE W. MAGOUN (*University of California, Los Angeles, California*): I want to ask another question related to this same program of study, which in addition to its importance for central neural regeneration would seem to be a great potential for contributing to the neurophysiology of the lamina of the cerebral cortex. Here is the first time it has been possible to interfere morphologically with individual cortical laminae. One of the most interesting developments that has come in this area recently is the concept that one can identify in neurons two types of excitation: the classic conducting all-or-none mechanism in the axon and a graded response mechanism with a longer time course and excitable only to chemical transmitters at the synapses on dendrites. I judge that these laminar sections sever the apical dendrites, at least, of deep-lying cortical pyramidal cells; and for a time before they regenerate (if that is what they do), the distal apical dendrite must be destroyed. A whole category of evoked potentials recorded from the surface of the cortex have been attributed to this graded response mechanism of the dendrites, and they are called, perhaps loosely, "dendritic potentials." Among these are the surface-negative local cortical response, the recruiting response from exciting the nonspecific cortical projection, the surface chemical features of the augmenting response to the repetitive excitation of specifically projecting terminal cortical connections, and features of the transcollosum potential. Because each of the co-authors of this remarkable contribution is a sophisticated neurophysiologist as well as morphologist, I wonder if one of them would tell us what is being done in the study of electrophysiology of this situation, which it seems to me is far more interesting than simply the morphologic observation, and in particular what is happening to what is being called today "cortical dendrite potentials."

LEONARD I. MALIS (*Mount Sinai Hospital, New York, New York*): Again as in the growth of nerve fibers, the story is long, and we cannot go into detailed presentation. Briefly, we have prepared 75 or so cats with unilateral right striate cortex laminar lesions at various layers and at various times after the irradiation have studied them with surface maps and with microelectrode punctures for the evoked responses in striate units above and below the laminar lesions to light flashes in optic stimuli. Unfortunately, the study required a great deal of correlation with the various times of the units, and with silver stains to show us the stages of fiber regrowth. We are in the process of analysis now, and some of the changes have been somewhat surprising. One of the most difficult to do was to get the units to stop firing on top of the lesion. They have a remarkable tendency to continue this. The surface maps showed almost consistently, whenever the laminary lesion was fairly

decent and not totally necrotic, a marked increase in the evoked response just anterior to the lesion from a band of several millimeters, as compared to the normal side. Relatively thin lesions may have an increase of activity to the evoked response technique even within the lesion. The heavier lesions may have (in the earlier phases at least, before the nerve fibers are all back) a considerable decrease within the lamina, but still will have a hyperactive zone in front.

LAL HARBANS (*University of Chicago*): It was pointed out in various papers that radiation produces changes in the blood-brain barrier, and an increase in the mobility of the blood vessels can be produced. In studies on various effects on nerve and other tissues, it is possible that the effects were altered by an animal kept for observation which is eating toxic substances which reach the nerve cells and other tissue in greater concentration because of the barrier changes. Thus, we are exposing the tissue to greater toxicity. In one of the papers on the blood-brain barrier, a dye was used, and it was only possible to show changes with a dose of about 5,000 r in monkeys and only in those areas which already have soft barriers, such as those close to the cerebrospinal fluid and around the hypophysis. The sodium level was changed in doses as small as 15 r. When dyes are used as indicators, they have a strong tendency to bind themselves with plasma proteins which are large molecules. You should be able to make really big holes in the blood vessels so that these proteins, along with the dyes, should be able to permeate and show on the slides. Using sodium and other smaller organic molecules, as we use in labeled form, we keep in mind their various degrees of binding with plasma proteins. If one can decrease the binding and let the molecule be small enough to go back and forth by itself, one may be able to pick up smaller changes, which will not be possible if you have not pushed up the plasma proteins through the blood vessel to indicate the changes.

LEO E. LIPETZ (*Ohio State University*): In regard to the question Dr. Kruger raised earlier about how a vascular change could cause such sharp destruction of the neurons, I would like to do a little speculating in terms of the retina. In the retina, every neuron is surrounded by glial cells, and apparently it has to get all its nutrition via the glial cells. The glial cells are not large, so by injuring them you could produce a localized destruction or interference in the nutrition of a localized band of neurons. This might be a means of producing local neuronal damage. I wonder whether this could apply to the rest of the central nervous system.

ORVILLE BAILEY (*University of Illinois*): I would not think that these lesions could be well explained on neuroglial injury alone. Dr. Rose gave me the opportunity of spending some time on his preparations. The destruction of neuron elements in the band is complete, and it is incredibly sharp. Along the edge of the band one occasionally can see a cell body minus its apical dendrite in the more acute phase. In the reparative phase, similar cells are so placed that it seems necessary to conclude that the dendrite has grown back. From the standpoint of general neuropathology, the evidence of true regeneration of neurons is of two types. There are too many nerve fibers in the area. As a general pathologic phenomenon, repair tissue ought not to be exactly in the expected normal proportions. It is quite often too much, a criterion fulfilled admirably here. Second, the orienta-

tion of fibers ought not to be quite according to the normal pattern. And here the striking thing is the orientation at an angle—often at a right angle—to the expected one. The histologic technique is such that it leaves no question that these are truly nerve fibers. In fact, the technique is so good as to give one every confidence in the care with which the whole investigation has been carried out.

WEBB HAYMAKER (*Armed Forces Institute of Pathology*): One of the questions was directed to me with regard to the fluorescent technique data by Dr. Calvo. It was suggested that there perhaps were some holes knocked in some of these vessels, and the extrusion of the labeled albumin came through these holes. To that I would say this: This was a 6,000 r surface dose, and the first changes were seen in two days. At that time, under the light microscope, we could find no morphologic alterations in the blood vessels. Secondly, there were no hemorrhages. On the basis of this and other data, I believe that Dr. Calvo would feel that this is really a matter of permeability to a rather high molecule. I do not think there is any way of comparing the experiments of Dr. Van Dyke, using soluble fluorescein, with the technique which we use. His lesions were made with a 184 in. cyclotron with far greater energy. I would suspect that if he had used 6,000 rad and duplicated our experiment the soluble fluorescein would come through much more rapidly than the protein molecules, the data on which we presented.

CORNELIUS A. TOBIAS (*University of California, Berkeley, California*): First, I wish to comment on the remarks Dr. Kruger made with respect to the dose-effect relationship. You could see from the curve I presented, which had the dose volume, that not all the points were exactly on the curve. Actually the relationship is probably much more complicated than that. If you keep the volume the same and give different doses, you have one relationship in which with lower doses, the lesions appear later. Also, as Dr. Sourander has done, you can use the other approach, keeping the dose constant and changing the volume. This will give rise to another curve. So we have actually more than one parameter to deal with. Moreover, I believe now that different parts of the brain are somewhat different. For example, in the cerebellum you get a slightly different dose-effect relationship from that in the cerebrum. However, when you try to plot the relationship to the time, the time slope of the curves obtained is about the same; whereas, the absolute position of the curve in the dose-effect relationship is somewhat displaced. I think we have heard possibly enough evidence today to realize that there must be at least two different relationships. The relationship where the dose and time both enter makes one strongly suspect the interdependency or the importance of circulation, and of the capillarity and perfusion by blood, of vascular accident, like hemorrhage that may occur on a statistical basis. On the other hand, some of the data presented with respect to neurons have a more or less direct inner dose-effect relationship, as in the nuclei that Dr. Janssen counted. It may be due to a direct effect, perhaps possibly even a direct effect of particles which hit the nuclei of the cells. Dr. Clemente of U.C.L.A., Dr. Richards of our laboratory, and Dr. Gaffey have undertaken ambitious programs, in which electrical activity will be studied as the function of the location of the irradiation and the dose. The technique used is imbedding electrodes in various locations, then placing the lesion with the high energy beam at the various locations. For example, at the top of one of the sets of electrodes. Then it probably is possible to stimulate and study electrical activity

in various parts of the brain with regard to accessibility. The Swedish investigators, particularly Dr. Anderson, have for many years been studying the hypothalamus with electrodes which reach in the same direction. And the Russian investigators, in total brain irradiation studies, have for some time been realizing the inaccessibility of the hypothalamus which follows irradiation.

JERZY ROSE: It seems to me that as far as protons, alpha particles, and neutrons are concerned there seems to be agreement among all hands as to the minimal dose, properly defined, with a proper relationship of latent periods and without fluorescein. Minimum lethal dose seems to be about 50,000 rad. The total destructive dose is around 75,000, an unsatisfactory figure in detail, but of the proper order. Perhaps our Swedish guests would comment on whether they agree with this conclusion and Professor Grashchenkov would comment on what the investigators in the Soviet Union found.

N. I. GRASHCHENKOV (U. S. S. R.): We have some figures which indicate that part of the brain connection with the hypothalamic region was damaged by a small dose of irradiation. At the same time we have some investigations—morphologic as well as postphysiologic—which indicates the role that the brain played. In our country, Professor Stamm and his large school with many collaborators have dealt with this problem. In this last period, his studies indicated that this formulation played an important part in the mechanism of hyalinization.

PATRICK SOURANDER (University of Gothenburg, Goteborg, Sweden): When the spinal cord was irradiated with the 1.5 mm beam, there was little vascular damage seen. Of course, there was irradiation of red blood corpuscles in the capillaries, but no damage of the bigger cortexes. If a broader beam was of 10 mm, after a few days considerable damage of blood vessels was seen with massive hemorrhages. One explanation would be that with the 1.5 mm beam only a relatively small volume of capillaries are destroyed, and there might be compensatory mechanisms in the circulation. When a bigger volume is destroyed, this compensatory mechanism fails. I do not know if this is the right explanation, and I would like to ask the specialists what they think about such an explanation. The other phenomenon observed, both in the thin beam and the broad beam, was that some fibers were always affected. One possible explanation would be that the situation as to the blood supply might be better for the periphery or the spinal cord than the central parts. Concerning changes in the nerve cells, I would like to mention a general point of view. I doubt the reliability of the methods used for studying the nuclear proteins and nucleic acids in nerve cells after irradiation, whether used in fluorescein stains or aniline stains at a certain pH. Certainly, there are more reliable methods, such as the ultraviolet absorptions at 2,600 Angstrom, both before and after digesting with ribonuclease or desoxyribonuclease. There are recently published methods from Goteborg concerning nucleic acids seen in the glial cells. They were studied by means of electrophoresis on a micro scale with single cells. It is by no means clear that there should be initially a depletion or reduction of nucleic acids in degenerating neurons. As was recently shown by Ingstrom, after the axon is cut, there is an increase of nucleic acids and later a decrease of nucleic acids. I think these newer methods should be used when studying the effects of irradiation on nerve cells.

HERBERT LOCKSLEY (Iowa City, Iowa): I have been appreciative of the progress

reported to us by Dr. Haymaker, Dr. Tobias, Dr. Van Dyke, and Dr. Clemente on the difficult problem of trying to quantitate irradiation changes in the blood-brain barrier. To emphasize the point made by one of the discussants, most of the dyes that are used are bound by serum protein, and therefore changing permeability to them represents a gross breakdown in the barrier. One question for Dr. Clemente: In the classic descriptions of the use of trypan blue, it has been observed that normally some areas of the brain are permeable to trypan blue, namely, the superoptic and infundibular regions, the area postrema and the locus cinereus in the fourth ventricle. It seemed to me from your slides that these areas were strongly represented in those shown after low dosage radiation. I had the privilege of participating with Dr. Lee Farr and Dr. William Sweet in the second series of neutron capture radiation at Brookhaven, a series of 10 radiations, to study intracranial neutron flux during treatment and to make some physiologic studies of possible changes in the blood-brain barrier. This is of crucial importance in that type of treatment because its success is contingent on a ratio of concentration of boron or other capturing agent within the tumor cells and in normal tissue. With boron, this has been established to be about 3 or 5 to 1. The high ratio is maintained for a short time, perhaps half an hour, which limits the duration of radiation. The question that arose was this: Does the patient after one treatment have sufficient changes in the blood-brain barrier to reduce the therapeutic ratio, that is, to reduce the concentration between tumor and brain, which might compromise subsequent treatments in the same patient? We considered the possibility of using dyes, but felt this would represent too gross a test. Extending some techniques for studying the dynamics of cerebrospinal fluid formation and absorption developed by Dr. William Sweet and myself, simultaneous isotopic tracer studies were made in patients with tumors before and after neutron capture radiation using isotopes K-24, sodium-24, heavy water, P-32 and chloride-38. In patients who had received possibly 1,000 to 3,000 rep of neutron capture therapy, there was no consistent detectable change in the blood cerebrospinal fluid barrier to these isotopic tracers.

RAY S. SNIDER (*Northwestern University*): I found this afternoon's group of papers stimulating. I don't think I have ever sat through a half-day session among the radiologists and found it more rewarding from the standpoint of getting new ideas for future experiments. That is a bold statement, even for a chairman to make. I would like to point out that we are dealing here with an exquisite microradiologic tool that can destroy not only single cells, but even parts of cells. I would like to ask what happens when this radiation goes in a cell and tickles it gently without destroying it. I ask that because of the microchemical methods now at our disposal, especially those of Lowry and associates, where they do chemical reactions on single cells and parts of cells. I also ask it because of the newer electron microscopic methods that we have, especially in relation to the study of the structure of protein membranes. I ask it because of Dr. Magoun's question on neurophysiologic activity, where we are measuring activities of single cells, parts of cells, and the cellular environment. I ask it from the standpoint of regrowth of the cell and emphasize again a point made by Dr. Mettler. I would like to know what is happening to the behavior of this cell not only electrically,

but also from the standpoint of the chemical environment. I could not help but agree with Dr. Lipetz when he raised the question—what is happening to the neuroglia here? We have long wondered whether or not they may help to conduct the nervous impulse. This is an excellent method, it seems to me, for answering this old question. It is also a good method to us for the study of environmental vascular changes.

PART IV

Functional Changes in the Nervous System Resulting from Radiation Exposure

Review of Neurophysiologic and Psychologic Research on Irradiation Injury in the U.S.S.R.*

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One often hears the statement, "Using conditioned reflex techniques, Soviet scientists have found the central nervous system to be very sensitive to ionizing radiation." The goal of this analysis is to demonstrate that such a statement is an oversimplification; it requires considerable elaboration before a clear interpretation of the implied claim is possible.

Most of the illustrations to be cited are drawn from the Soviet radiobiologic literature and cover materials through August, 1960. An extensive bibliography has already been presented in two recent review papers (Stahl, 1959, 1960). However, several sources for the scientist who does not read Russian have been cited, such as reports for the United Nations Scientific Committee on the Effects of Atomic Radiations (Brazier, 1959; Bykov, 1957; Gubin and Moskalev, 1960; Livanov and Kondrat'yev, 1960; Pavlov, 1959).

This review attempts to distinguish several fundamentally different nervous system reactions which may take place after irradiation. These include (1) minor damage to neurons, not inconsistent with the phenomenon of "radiation aging," (2) specific or selective damage to the central nervous system (CNS) causing loss of particular nerve functions, (3) perception of radiation by the CNS without any pathologic consequences, (4) abnormal CNS functioning, resulting from radiation, which causes or contributes to radiation sickness, (5) somatic mechanisms which alter the response in a test of CNS activity in a misleading manner, and (6) certain general reactions, such as alterations in levels of circulating biochemical mediators which result in altered nerve functioning.

In addition, it is always necessary to bear in mind the age, species, and sex of experimental animals, the size of the radiation dose, whether radiation sickness actually developed, how long after exposure the CNS test was done, possible disturbances in the general state of the animals, such as change in appetite and normal motor activity, and validity of the result, statistically.

* This paper was presented at a dinner meeting between the third and fourth sessions.

Radiation Damage which is Histologically Apparent

As is well known, beyond a certain dose nerve tissue shows major damage with cytolysis, fiber degeneration and eventually scarring. In the Soviet literature, such pathology has been reported to occur after 1,500 r in some cases, though doses of 5,000 r are often mentioned also as the threshold for really major damage (Livshits, 1956; I. A. Brodskaya and Merkulova, 1956). A demyelination syndrome following several thousand r was cited recently by Bibikova (1959).

However, many Soviets have found injury at levels far below these. For instance, Lebedinsky (1959a, b; Lebedinsky and Moskalev, 1959) describes rather selective injury to the cortex, hypothalamus, certain autonomic structures, and elsewhere following near-lethal mammalian doses. Selective damage to afferent neurons of the spinal cord has been reported by several workers, such as Shabadash *et al.* (1959) who used a special RNA isoelectric-point staining technique. Others have found autonomic, and especially sympathetic peripheral structures, to be most sensitive (Smirnov, 1960). Spinal synapses were found partially damaged following exposures to 450–600 r (Lev, 1957).

In these studies, injury was fairly general in the structures cited. Scattered, random injury with quite low doses has been reported by Soviet workers. For instance, Aleksandrovskaya (1958) found an increase in numbers of pathologic cells after only 50–150 r, with 150 r also damaging a few glial cells. Spotty damage to peripheral nerve has been cited after a few hundred r by Garvey (1960), Anisimova-Aleksandrovskaya (1959), and others.

Many Russian papers have dealt with postirradiation injury to nerve structures within specific organs. For instance, Zarat'yants (1960) states that only 60–90 r of chronic exposure induces alterations in the nervous structures of the gastrointestinal tract; this pathology is said to be the first seen under the cited exposure conditions. The extent of injury was small. Levinson *et al.*, (1957) could find only slight injury in nerve structures of the gut following 3,000 r. In skin structures of rats exposed to 700 r (Garvey, 1960), changes were slight during the latent period of the radiation syndrome, marked at about 5–20 days, and still evident as late as 60 days after exposure. Damage to cutaneous nerve structures only 30 minutes after irradiation is noted by Oleynikova (1959). Gromada and Polachek (1959) found significant injury to receptor endings in heart muscle, fascia, and elsewhere after many hundreds of r. Major injury of nerve endings in the spleen and lymphatic organs following near-lethal doses have been reported by various Soviet workers such as Alekseyeva (1958).

Scattered, low level pathology is not easy to demonstrate. It is evident that the researcher must use extensive controls and analyze his results statistically.

Allowance must be made for possible tissue autolysis after spontaneous deaths and for intercurrent intoxication, focal or general infection, hemorrhagic diathesis, fever, and other well known concomitants of acute radiation sickness. Thus, a clear and explicit statement must be made about when the injuries were seen in respect to occurrence of an acute radiation syndrome. Changes in the nutritional state of the animal and use of any drugs must be considered. Development of low level viremia is entirely possible after substantial radiation exposures and would be expected to cause CNS reactions. Interpretation of nerve injury in specific organs, such as the spleen, is complicated by the extensive cellular changes known to take place in this structure after radiation. The extensive cellular restructuring seen after exposure may cause reactions in nerve endings. In general, most of these papers find much more significant damage during the acute radiation syndrome, with only reactive or irritative changes in the latent period. Effects on receptors are of great interest from the standpoint of possible abnormal afferent flow to the CNS during developing radiation sickness.

Soviet and Western workers agree that the very young CNS is much more radiosensitive than the developed CNS. By way of illustration, Olenov and Pushnitsyna (1952) find extensive neuronal injury in young animals after only 40–120 r. Kosmarskaya and Barashnev (1958) describe reactions in neonatal rats exposed to 250–500 r. Others have described radiation-induced congenital anomalies in the CNS.

In regard to histologically apparent findings, one can say that while scattered, random injury to some nerve structures would not be too surprising following several hundred r, there is a heavy burden on the experimenter to prove whether this is direct or due to somatic radiation injury. He must also control and eliminate all possible secondary mechanisms known to injure neurons, such as fever, viremia, hemorrhage, and general cachexia. In the absence of full details on control measures, reports on low level neuronal injury are hard to interpret.

Functional Damage to Nerve Structures which is Not Apparent Histologically

Important changes may take place in synapses, neuronal membranes, and elsewhere, but not cause any notable alteration in histologic preparations. Because of well known difficulties in the electron microscope study of the nervous system, the discovery of random minimal injury is not going to be easy. One can cite a great many possible mechanisms of nonmorphological radiation damage.

Several papers have dealt with direct radiation effects on simple nerve-muscle preparations. For example, Pshennikova (1958) reported both local

damage and spreading axonal effects from a small segment of frog sciatic nerve exposed to 10 kr and more. Lebedinsky (1959b) assessed synaptic transmission in the irradiated animal under physiologic conditions by testing blinking of the eye following cervical sympathetic stimulation; alterations occurred after doses of a few hundred r.

In another synaptic study, using a leg withdrawal reflex in the rabbit, Kudritsky (1957) found that 10 r will alter some of the timing parameters, especially the variability of the latent period of response. Similar types of alterations have been reported following 500–1,000 r (Gvozdikova, 1957). Kudritsky has also suggested that there may be adaptation of the organism to radiation and found evidence that pretreatment of a rabbit with several hundred r eliminated its response (noted previously) to a subsequent 10 r test.

Many indirect factors can enter into an altered CNS response following irradiation such as changes in basic metabolic processes in nerve cells involving DNA metabolism (Levinson *et al.*, 1957) or cerebral amino acid metabolism (Minayev and Skvortsova, 1957). No final conclusions seem possible on this point; nucleic acids are known to be fairly radiosensitive in some biologic systems, but maintenance of the membrane potential is expected to be quite stable to slight biochemical damage.

Many Soviet studies have dealt with various vascular reactions to radiation exposure. Lyubimova-Gerasimova (1960) found significant alterations in tone of cerebral vessels following 1,000 r in the rabbit. Several studies discuss blood-brain barrier permeability changes and alterations in capillary properties; these may well play a role in some of the reactions seen after exposure.

The possible importance of circulating toxic or physiologic substances in radiation reactions has been stressed in many works. Working under Lebedinsky, Maslova (1958) and others have produced evidence indicating fluctuations in circulating sympathin levels following near-lethal exposures in cats and rabbits. There seems little doubt that under some conditions sympathins are increased and that they fluctuate considerably. These substances may well exert a role on almost any aspect of CNS activity. The possible presence of sympathins should be given serious thought in experimental results without a ready interpretation, such as atypical subcortical EEG reactions. Mozzhukin and Pevsner (1959), working under Zedgenidze, showed important changes in pressor reflexes following radiation and suggested that altered adrenal medullary activity was responsible. Hypotonia or other vascular reactions may alter reactions in CNS tests.

Direct radiation injury to an effector organ needs serious thought with CNS studies involving secretory organs. For instance, Lomonos (1957) and Ye. A. Brodskaya (1958) showed that there are changes in the uncondi-

tioned responses of the salivary glands and gut secretory organs following radiation, a fact which would be anticipated from the known postexposure histologic reactions of these organs. Thus any conditioned reflex study using a secretory response is difficult to interpret.

One would hope to eliminate such difficulties by using motor studies in experimental animals. These are usually based on feeding reflexes, however, and it is well known that irradiated animals show alterations in appetite and spontaneous motor activity. They may develop an aversion to certain foods for no known reason and may specifically avoid foods eaten shortly before irradiation (Kimeldorf, 1961). Following anorexia, irradiated animals may develop an appetite which is stronger than normal, and this may lead to important alterations in some psychologic testing situations.

Conditional Reflex Studies

The term "conditional" rather than "conditioned" is used in this report because it represents a better translation of the corresponding Russian word; it has been advocated by a good many U.S. conditional reflex (CR) researchers because it stresses the temporary or "noninnate" nature of CRs (Brazier, 1959).

Some assumptions concerning CRs which are generally taken for granted by Soviet physiologists may be little known to Western radiobiologists. The serious student of CR testing is urged to read Pavlov (1959)—there is now available an excellent translation by Anrep—and the recent review of CR studies by Grashchenkov (this volume). At present Soviet physiologists often use motor-feeding reflexes and include an instrumental response in their analysis. Thus, the typical modern CR test technique may be quite different from that using a dog in a harness and approximates a total-observation situation of activity in an animal, as presented with a sequence of stimuli. In some cases work has been done with dogs running free in a room, but more commonly rodents in a small sound-proof chamber are used. Under these conditions one can get information about the spontaneous motor activity of the animal. However, CR studies do not lend themselves to tests of spontaneous preference, a technique which Western radiation researchers have shown to be sensitive in postradiation experimentation.

The magnitude of the response to the presented stimulus is of cardinal importance. Pavlov demonstrated the important basic fact that a strong stimulus produces a quantitatively larger response, as exemplified in measurement of drops of saliva. The same holds true for motor reactions, as shown in the force exerted by the animal. Typically, the animal is presented with a fixed sequence of stimuli of varying strength (a "stereotype" of stimuli). For example, one can use a sound of a certain frequency as the "strong"

stimulus, a weak light as the "weak" stimulus, and a sound of the same intensity, but different frequency, as the "differentiating" or inhibitory stimulus.

In a normal animal with a well developed CR pattern, there is a clear parallelism between the strength of stimula and response, and differentiation is distinct and complete. Possible alterations in CR stereotypes include equalization of reactions to varying stimuli, or a strong reaction to a weak stimulus and none at all to a strong one, failure of differentiation, weakening of all responses, residual inhibition following the differentiation stimulus, or increased errors or variability of response. All of these changes have been stated to occur after radiation, in Soviet studies.

Pavlov demonstrated that animals differ in their reactions to a stereotype. He classified several higher nervous types (CNS types), namely, strong-balanced, strong-unbalanced, weak, and poorly balanced types, the last with a predominance of excitation or inhibition. In all cases, one is referring to the occurrence of excitation and inhibition, as judged by strength, balance, and mobility. Objective methods for classifying animals are well known in the Soviet Union and involve tests of reactions to strong stimuli, reactions to inhibitory stimuli, responses following caffeine, etc. Typing is not always easy or clear-cut, and many animals may not fall into a distinct type, but nonetheless this classification is helpful for assessing reactions. In higher animals the complexities are still greater, but human typing has been attempted. As a simple illustration, persons react differently to a strong, frightening stimulus—some tend to respond with activity (excitation) and others with inactivity (inhibition); moreover, the time needed for restoration of normality varies (being a function of CNS mobility and balance). The occurrence of CNS types adds another degree of freedom to CD studies and complicates their interpretation further.

Pavlov did not attribute any morphologic or biochemical basis to excitation and inhibition but felt the phenomena were purely functional. Basically, one starts with the view that salivation is preceded by excitation in the "salivatory center," and that there are antecedent foci of excitation before the latter is stimulated. If salivation fails to occur when expected, then inhibition occurred somewhere in the cerebrum. The quantitative nature of CR experiments places a strong emphasis on the relative balance of excitation and inhibition. Possibly the terms excitation and inhibition have been identified too fully with the identical words used in Western psychology, although in the original Pavlovian context they were different and perhaps simpler.

"Internal inhibition" often enters into the discussion in studies of radiation effects on CRs. Pavlov originally distinguished between types of inhibition, such as that exemplified by failure of a response due to frequent

monotonous repetition of the stimulus and that due to a sudden distracting stimulus, as a door slamming. Later, however, he expressed doubts about a precise classification of inhibitory effects. Internal inhibition is typically innate inhibition which develops in the normal course of training, as with improving differentiation. It is said to be often the first function to show damage following any noxious influence, including radiation exposure. In my view, internal inhibition can be compared with a multiplicity of cybernetic functions in the CNS, functions which refine the accuracy of CNS reactions and control them.

Finally, statistical analysis of CR studies is rather complex. Theoretically, it typically involves the interpretation of a 8-dimensional vector (the stereotype) in all its possible alterations, a problem which does not lend itself to simple treatment. One may try to isolate certain elements, such as the strength of the strong positive response or the degree of differentiation, and test them statistically, but this would not necessarily prove or disprove that some subtle change has occurred in the entire stereotype. Under these conditions, it is easier to understand that the opinion of the experimenter, who has typically done hundreds of studies on each of a small group of animals, is accepted at face value by most Soviet physiology laboratories. However, more objective methods of evaluation will clearly have to be developed, and their design represents a considerable challenge for probability theory.

In studies of radiation effects on CRs, Soviet physiologists have found differing reactions, and no final opinion concerning the radiosensitivity of the CR mechanism ought to be reached at this time.

Some workers have observed subtle changes in CR patterns following very low exposure. It was reported to the UNSCEAR that 50 mr had some effect on an inhibitory CR, interpreted as being due to a change in internal inhibition. Several papers by Piontkovsky (1959) and Khozak (1958) state that 0.5–20 r single exposures have a stimulatory effect on conditioned responses lasting days or even months. Cherkasov (1960) asserts that a single dose of 30 r or chronic exposure at the rate of 0.1 r per day may cause disappearance of all CR responses in certain experiments. Others state repeatedly that CRs show changes after only low radiation exposures. In rats having a motor-feeding CR to successive 50 r doses, with intervals of 1–3 weeks for a total of 350 r, Khozak (1958) found marked variability in the particular animal following only 50–100 r total dose (Fig. 1). He interprets this result as primarily a stimulatory and disinhibitory action on higher nervous activity. It is unfortunate that only one control graph is shown, since the variability of the animal response prior to irradiation is critically important.

Another group of experiments deals with near-lethal exposures in rodents

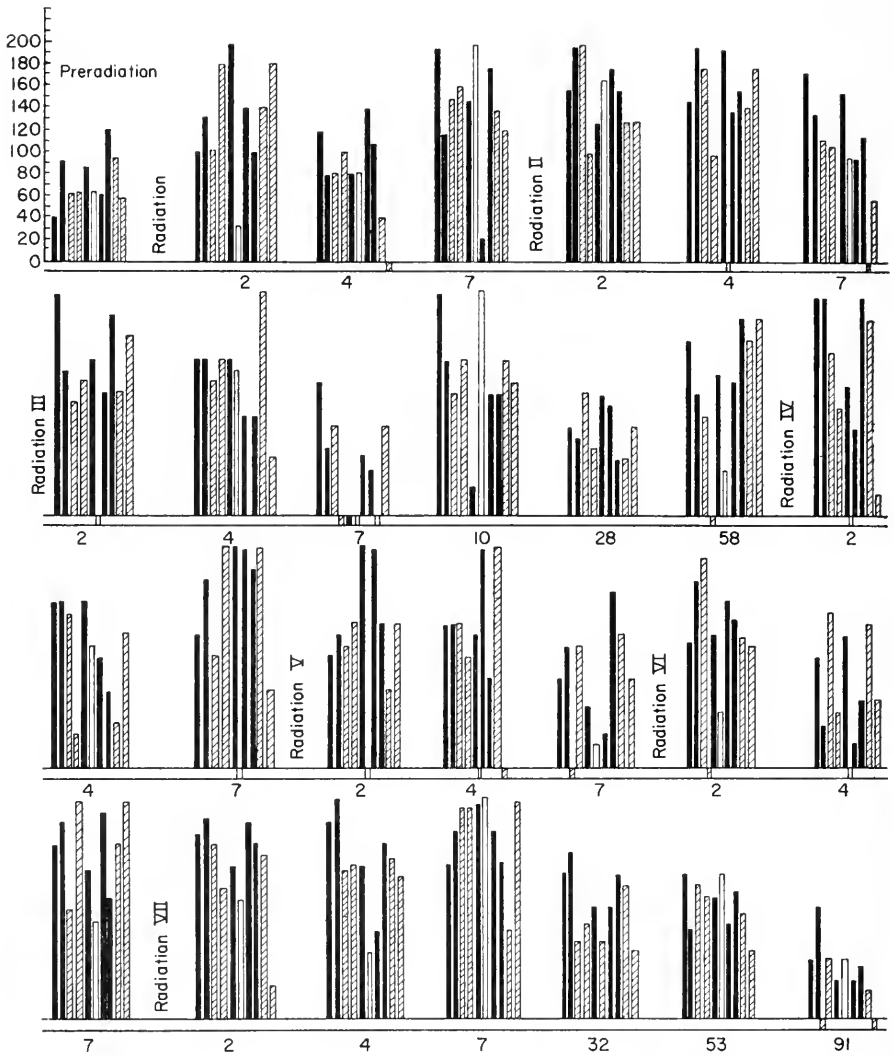


FIG. 1. Alterations in conditional positive and inhibitory reflexes following exposure to 7 doses of 50 r each. The first graph gives control data: irradiation was administered as shown by the word written vertically, and the time scale is in days following each day of administration. This animal is considered strong and well balanced, and the upset of strength relations and frequent increase in the motor-feeding reaction is noted particularly. Redrawn from Khozak (1958).

and dogs and is typified by the work of Livshits (1956), Yarullin (1959), and Lomonos (1957, 1959). These experiments may show a slight stimulatory effect initially, but more commonly they show depression of the CRs a few weeks after exposure. The possibility of an indirect somatic effect, such as suppression of salivatory activity, must enter into the interpretation of these findings. Most studies suggest depression of CRs after several hundred r, at some time after exposure.

However, quite a few Soviet authors, such as Korol'kova (1958) and Biryukov (1957), noted slight or insignificant alterations in certain CR experiments after 1,300–5,000 r (chronic exposure, usually). It is doubtful that these authors would deny any effect on a CR with low doses, but in some studies they were able to produce quite normal new CRs even after thousands of r.

Thus, it is apparent that there is no unanimity at present as to whether the CR method shows very low level radiation alterations or whether it is a highly sensitive indicator of damage. More work will have to be done on this matter, and it will have to be fully controlled statistically; the interpretation must differentiate between direct effects on the CNS and indirect actions through somatic organs.

Other types of tests of general higher nerve functioning after radiation include studies on experimental neuroses following exposure. Korol'kova (1958), workers at the Sukhumi primate laboratory in the U.S.S.R. (personal communication), and others indicate that experimental neuroses develop quicker, are more severe, and last longer after irradiation. In a general way, some researchers indicate a "weakening" of higher nervous activity, particularly in regard to inhibitory control. Livanov (1957, 1959; Livanov *et al.*, 1960a, b) has stressed repeatedly that such injury may be hard to detect because the CNS compensates for the insult almost immediately; he feels CNS radiation damage is nonthreshold and cumulative.

Substantial portions of the cortex have been removed in experimental animals without drastic, sometimes without any discernible, effects on CR and behavioral tests. These techniques would hardly be expected to reveal damage to a small percentage of neurons in the cortex or elsewhere. One can probably speculate that negative feedback, compensatory mechanisms are present in all parts of the CNS, as are nonlocalized pattern transfer systems. As pointed out by von Neumann, one can make reliable devices out of unreliable components by multiplexing circuits, and, therefore, it seems that slight damage in the CNS will be exceedingly hard to demonstrate by ordinary methods.

Special test methods may turn out to be of the greatest interest from this viewpoint. For example, one may mention the report on audiogenic seizures in mice, presented at this conference, and work by Biryukov (1957) showing

that 100 r to the head of fowl in a state of catalepsy would reproducibly arouse it in a condition of agitation. Elsewhere it has been observed that sleeping animals may be awakened by modest radiation doses. Humoral mechanisms should not be ruled out in these cases, and indirect actions, such as variations in blood-brain barrier, should be kept in mind when interpreting the results.

Certain types of CR studies are infrequent in the Soviet literature. There seem to have been no tests reported in which radiation was the conditioned stimulus (e.g., a signal of impending shock) which might indicate the lowest perceptible dose or subliminal effects. Work on conditioning of somatic effects is limited. Some older articles indicate the possibility of conditioned leucopenia, hyperglycemia, and other reactions, but the results do not seem to have been confirmed. The possibility of humoral changes would have to be kept in mind with such studies. Radiation has been used as an unconditioned stimulus by Kimeldorf (1961), but in this paradigm it served as an accessory factor modifying the thirst reaction.

Much interesting speculation is possible as to the basic mechanisms which may be involved in the typical CR and behavioral tests. CR trials typically do not involve learning; the animal knows what the signal means, and the response to the known stimulus is the matter of critical importance. Even in differentiation trials, it is normally not a question of learning, but of discriminating between two known alternatives. Nor do CR studies deal with frequency of response, which is determined by the experimenter. They might be loosely considered to be primarily motivational tests, but with the added important factor of residual influences from the previous elements of the stereotype. The full analysis of correspondence between various types of psychologic tests used in the U.S., Europe, and the U.S.S.R. is a matter that deserves more attention.

Nervous Mechanism in Radiation Sickness

Livanov (1957, 1959; Livanov *et al.*, 1960a, b) has been the leading Soviet protagonist of the idea that following radiation exposure there is abnormal afferent activity which leads to some of the well known pathology in radiation sickness. This idea would be considered novel by many Western investigators and has had few protagonists outside the U.S.S.R. The outlook that the CNS play an important role in the *production of radiation pathophysiology* follows directly from the theoretical Soviet position that disease is the response of the organism to noxious agents in the environment. The CNS mediates such responses and, moreover, attempts to adapt or adjust the organism to the disturbing influence.

The CNS is thought by many Soviets to analyze the internal milieu in

much the same way it analyzes the physical environment. Pavlov advanced such a viewpoint after finding that CRs were possible for introceptive stimuli. Much later work, such as that of Bykov (1957), stressed introceptive stimuli and introceptive reflexes. Presumably, then, the CNS should analyze the complex internal derangements which follow irradiation and adjust the organism to them. This view is tenable for pathology which affects known introceptors, but is somewhat doubtful in matters such as depression of cell mitosis in the gut, testes, or bone marrow, for which no introceptors or central regulatory centers are known.

It has also been thought by some Soviet research workers that nerves have a direct controlling influence over mitosis, local regeneration, and inflammation. Such mechanisms are usually included under the concept of "trophic nerves," which was developed by Botkin, Speransky, and other older Russian physiologists. This theory is not completely without support in the Western world (Wyburn-Mason, 1950) but a great deal of the evidence is contradictory. A recent Soviet report for the U.N. (Mastryukova and Strzhizhovskii, 1960) summarizes considerable research on the matter and concludes that altered levels of epinephrine and adrenal corticoids may be the most important factor in the mitotic depression known to follow various insults to the nervous system.

With this background in mind, it is easier to understand Livanov's viewpoint, though one may not agree with it. In a number of papers Livanov (1960) states that radiation with a few hundred r causes abnormal afferent inflow, reflected in altered cortical and subcortical encephalograms. There follows a complex series of events involving subcortical and cortical inhibition, leading to altered spinal and autonomic activity, and then gradual disinhibition occurs at different times. Livanov states that single doses of 5-1,000 r commonly cause a stimulatory effect, which may be repeatable, but that after total doses of 200-1,000 r there is a general depression of EEG activity. EEG reactions to radiation have been described by Livanov *et al.* (1960b) with doses down to 50 r per minute, and such effects are said to be predictable with 10-15 r single exposures.

Grigoryev published a book (1959) which deals with EEG studies on humans receiving radiation therapy for tumors of the head or other parts of the body. He frequently cites a stimulatory action during the first few sessions, then a depressant effect. The precise origin of the claimed changes of EEG activity remains obscure, but it will be necessary to rule out stimulation of the retina, humoral mechanisms, biochemical shifts, and numerous other factors before one can conclude with assurance that the EEG change indicates an alteration in afferent flow which is communicating information about the somatic radiation pathology to the higher nerve centers.

If significant purposeful, or damaging purposeless, afferent activity oc-

curred after radiation, one might expect that nervous system drugs or CNS surgery would radically alter the course of radiation sickness. While many agents, including some with actions on the nervous system, can alter radiation dose-effect curves slightly, there is no evidence that they alter them in major ways. It is of basic importance that the most significant delayed radiation effects, namely genetic mutations, simulated aging, somatic mutations, and cancer induction, are all mechanisms in which the organism does not adapt to a noxious environmental influence, but suffers innate injury. On the other hand, Soviet scientists take a broad view of nervous control and may include any neurohumoral and all hormonal mechanisms in the category of CNS adaptive responses. Thus, pituitary hormonal changes have been scrutinized for importance in radiation sickness.

The possible role of nerve damage in aging phenomena and in production of cancer becomes of special interest from this viewpoint. It is well established that tumors do not have a normal innervation, but it is by no means clear that this is of causal significance. Zubareva (1959) reported on the detailed histology of nerves in spontaneous breast tumors in mice, and Sheveleva (1959) studied nervous influences on the development of a Brown-Pearce implanted tumor in rabbits. Similar work is often found in the *Soviet Bulletin of Experimental Biology and Medicine*.

Dzharak'yan (1957) has suggested that postirradiation leucopenia is in part due to faulty splenic nerve activity resulting from radiation damage to nerve endings. Many studies have dealt with vascular mechanisms in irradiated animals and commonly report major alterations in tone following several hundred r; this may in part be due to altered levels of circulating sympathins, but direct damage to the vessels and their innervation is also proposed. Cyclic alterations in the secretory activity of the gut are reported by Uspensky (1959) and are said to be due to altered "trophic control" predominantly. This will have to be qualified on the basis of known direct radiation injury to mucosal cells.

Many of these studies implicate the autonomic nervous system in radiation injury. Soviet authors, especially Lebedinsky, have pointed out that the radiation syndrome involves changes in vascular phenomena, thermoregulation, cardiac activity, respiration, capillary permeability, and numerous other autonomic phenomena. He observed changes in hypothalamic stimulation thresholds for these after irradiation. Studies by others have shown changes in the excitability level of the vagal centers after irradiation (Gromovskaya, 1959) and similar phenomena.

Still another aspect of radiation sickness is the specific role of the pituitary-adrenal axis after irradiation. Results remain contradictory, but some Soviet workers feel they have shown a distinct stress reaction to radiation injury. Possibly this reaction obtains only in some species and at some doses,

because much Western work does not support the hypothesis. However, the possibility of an adrenal cortical mechanism must be kept in mind.

Alterations in Afferent Activity

Some stimulatory mechanisms are not pathologic. This sort of reaction is typified by the radiation phosphene, covered thoroughly at this conference by Lipetz. Recently the Soviet literature also carried a detailed article on radiation phosphenes by Gurtovoy and Burdyanskaya (1960), who showed this phenomenon in humans receiving 0.2–3 mr. Using blinded subjects, it was established that as much as 3 r to the occipital lobe did not produce the phosphene, and the evidence is clear that it originates in the retina.

There are several Soviet reports on electroretinograms. Lebedinsky (1959a) cites evidence for changes in frog preparation after 10 r. He has discussed alterations in human EEG responses to an increasing light level, and it seems entirely possible that subliminal retinal changes are involved in the observed alterations.

Receptors other than the retina may be affected by radiation. One possibility is a change in the sensitivity of an undamaged receptor; this seems to have been involved in the work of Gorbunova and Rikotova (1958), who found that 2 rad of beta rays given locally to the intestinal mucosa led to an alteration following standard surface stimulation. Similar findings were noted with much higher doses to the skin by Delitsyna (1959a, b), who measured afferent activity from the rabbit paw after doses of 500–5,000 r. With the larger values, one would expect frank ulceration and changes in receptor threshold.

Other Soviet work suggests that there are changes in afferent inflow from the gut, spleen, bladder, lymph nodes, and elsewhere following irradiation. Livanov (1959; Livanov *et al.*, 1960a) has stressed central change due to this activity, as noted, and he finds alterations in the firing rates of subcortical structures, such as the reticular formation and hypothalamus of irradiated rabbits, using implanted electrodes. The possibilities of a humoral mechanism and retinal effects need consideration. It does not appear to be clear whether radiation may cause changes in the spontaneous firing rates or stimulation thresholds for the neurons themselves, as opposed to receptor reactions.

Damage to the Nervous System with Antenatal Irradiation

Histologic findings after much exposure have been noted. In general, production of CNS congenital anomalies has been given some attention in the Soviet literature, but not nearly so full a treatment as seen in the

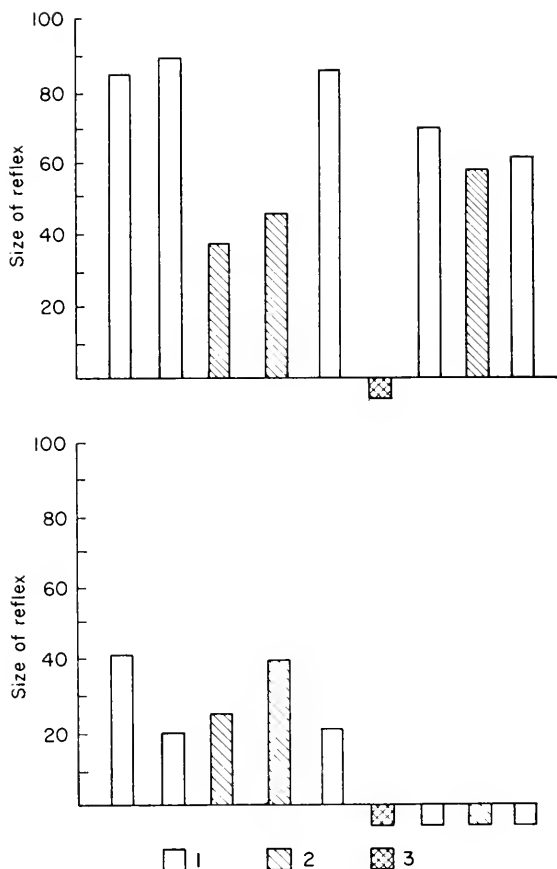


FIG. 2. Effects of 200 r antenatal irradiation (18th day) on the conditional reflex stereotype of white rats. Top series are controls; bottom, irradiated; (1), sound (400 cycles per sec) as a strong positive stimulus; (2), light from small bulb as a weak stimulus; (3), sound (800 cycles per sec) as differentiation stimulus. The equalization of reactions to stimuli of various strengths and inhibitory after-effect are noted. Redrawn from Piontkovsky *et al.* (1959).

works referred to by Hicks, Rugh, and others at this symposium. Piontkovsky and his associates found that CRs in animals antenatally exposed to 50–200 r showed certain characteristic forms of injury (Fig. 2).

Figure 3 provides data on a typical stereotype in control and irradiated rats. The experimental animal had an equalization of reaction to strong and weak stimuli, showed a marked inhibitory after-effect, and displayed considerably greater than normal spontaneous motor activity and erratic CR learning performance. They often gave a correct response sooner than

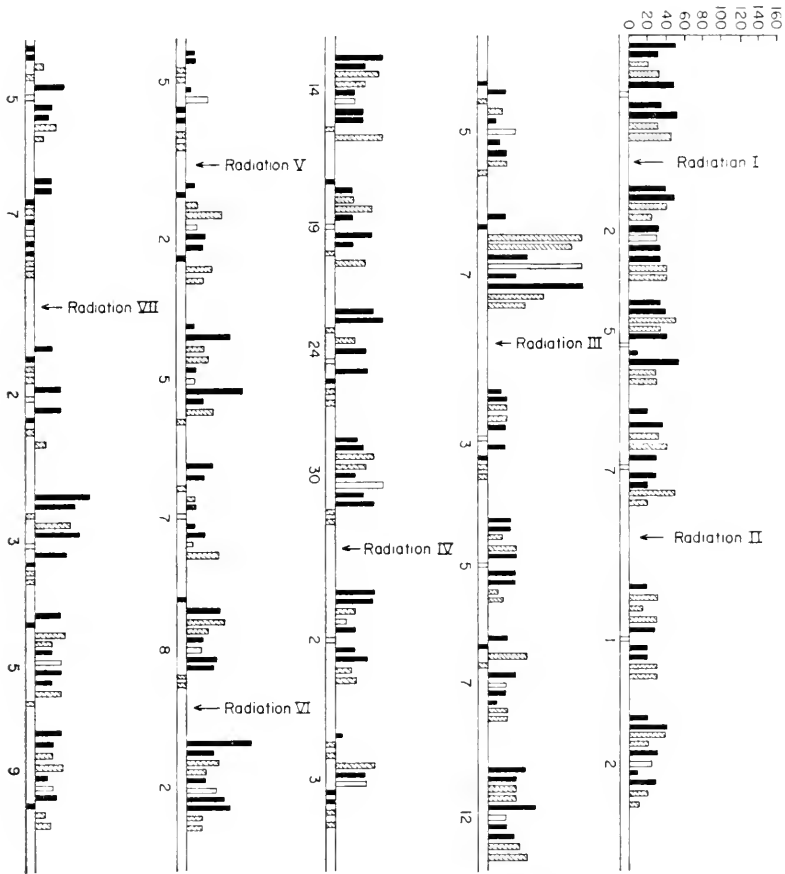


FIG. 3. Similar to figure 1, except that the animal was classified as a weak CNS type. Failure of some responses is noted. Redrawn from Khozak (1958).

controls but would not show a fully dependable reflex until many trials more than the latter. All these reactions are indicative, in the opinion of Soviet scientists, of a failure of internal inhibition. I suggest that the effect can be described in other terms as a loss of fine negative feedback or inhibitory control. Such a loss would perhaps be expected as the first evidence of diffuse, mild injury and would account for the pseudo-stimulatory action of radiation.

With *in utero* irradiation of animals with different CNS types, the specific reaction seems to depend in part on this parameter. There is no question of injury with these doses, and the animals show major weight loss and

histologic brain atrophy. No Soviet papers demonstrate such changes following small antenatal doses.

The fact that a CR stereotype could be produced at all under these conditions raises the question of the sensitivity of the CR method. Pavlov noted that the conditional reflex is a basic type of reaction which can occur in primitive animals and after extirpation of substantial portions of the cortex. After radiation the question is clearly not one of complete failure of CR production, but rather of subtle disturbances in higher nervous activity which are nearly, but not quite fully, compensated by feedback mechanisms.

Antenatal irradiation is of particular interest in that it makes possible the production of nonspecific functional brain damage. The work on CRs suggests that inhibitory control is damaged particularly, and one might speculate that psychopathic personalities also lack normal inhibitory control. Further experimentation on emotional or motivational control following antenatal irradiation appears promising.

Conclusion

It does not seem possible to provide a simple, satisfying summary of the multitude of complex experiments discussed. Clearly any definitive statement about CNS sensitivity must go into considerable detail concerning the mechanism involved and the form of sensitivity under consideration.

A final conclusion concerning CNS radiosensitivity must also take cognizance of certain clinical observations on radiation exposures, namely: persons receiving even high doses feel no pain and only equivocal mild sensations; humans getting doses of 500–1,000 r do not ordinarily show any obvious neurologic disturbances, and treatments directed at the nervous system have not been helpful in combatting radiation sickness. Many ordinary virus infections appear to produce more obvious encephalopathy than radiation, and numerous chemicals now used in the environment might evoke CNS reactions as great or greater than those seen after radiation exposures at moderate levels.

Not all Soviet radiobiologists place special stress on CNS mechanisms in radiation sickness. They point out, however, that the nervous system is a major organ system and ought to be given attention in radiation studies. During the last two years, there has been a relative decrease in the Soviet literature of papers stressing CNS effects, particularly those works citing the formal Pavlovian view on the pathophysiology of radiation sickness.

Following exploration for damage from radiation, certain interesting questions of basic biology have now become apparent in connection with CNS radiation exposures:

1. Is radiation damage to the CNS similar to natural aging in this system?
2. Are the feedback, multiplexing, and other cybernetic mechanisms in the CNS such that complete or nearly complete compensations for quite massive diffuse neuronal injury can take place?
3. What tests can be used to bring out diffuse functional damage which is well compensated?
4. Is the role of the nervous system in tumor induction absolutely passive, i.e., are the abnormal nervous structures seen in tumors simply the result of cells growing out of control?
5. Does the nervous system exert any direct local control over mitotic activity, even of a limited magnitude, or are cell division and differentiation controlled wholly by local and circulating biochemical factors?
6. Is there a complete overlap between the innate mechanisms which are tested by the totality of usual behavioral studies and conditional reflex techniques?
7. Does antenatal radiation make it possible to produce functionally damaged nervous systems comparable to those found in psychopathic or otherwise mentally disturbed humans?
8. What is the role of direct autonomic system damage in such proven postirradiation effects as vascular reactivity changes?

These questions differ considerably from the problem of central nervous radiosensitivity as posed originally and emphasize the continuing need for radiological research at the basic scientific level.

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General Survey — Functional Changes in the Nervous System Induced by Ionizing Radiations

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This session deals with functional alterations in the nervous system that result from exposure to ionizing radiations. In previous sessions, functional changes have been touched on, but more in connection with particular parts of the system. Here, we will devote attention to functional changes involving the nervous system as a whole, and, thereby, performance of the entire organism and even of population groups.

In this survey I shall consider the effects of different levels of radiation exposure on the nervous system as an *information processing device*, and then take into account features which seem to have particular meaning in relation to functional changes.

Range of Adaptiveness

Adaptiveness—vigor, stamina or fitness—characterizes nearly all living systems. All *ecoentities*, such as cells, tissues, organs, organ systems, organisms, and population groups possess an *ecoforce* which enables them to function and thus to survive, assuming availability of substrate or nutrient materials.

Figure 1 is drawn as a scale to represent adaptiveness. On such a scale, it is logical to think of three levels of capability: one of maximum performance, one of normal or usual range performance, and one of minimum performance or the level at which failure occurs.

With respect to radioneurology and radiobiology, let us consider different kinds of ecoentities, taking first hemopoietic tissues, among the most radiosensitive. We can visualize a level of hemopoietic performance so low that the organism cannot survive, an anemia so severe that death is inevitable. Similarly, we can visualize a level of performance of blood cell output sufficient to maintain the normal peripheral blood picture, and one at a much

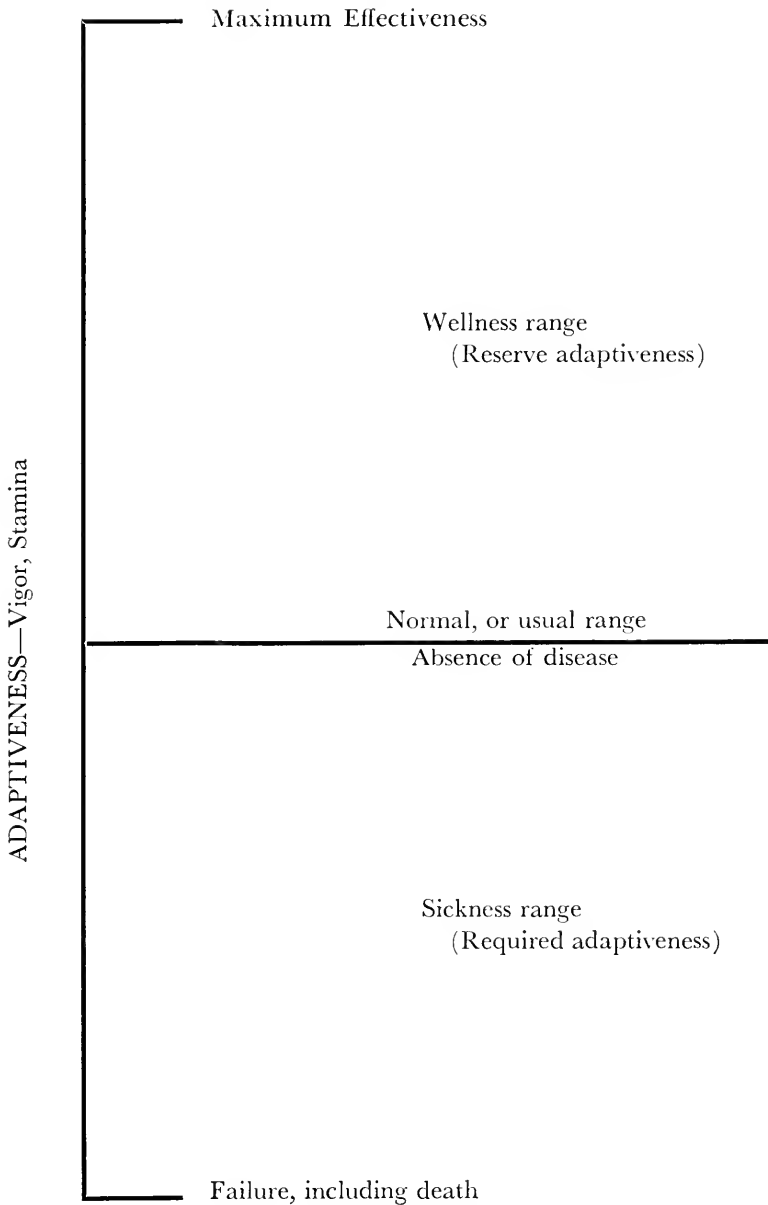


FIG. 1. Scale of fitness.

higher level such as required in extreme emergency (e.g., sustained heavy hemorrhage). In the same way, we can think of the adaptiveness of kidney, liver, intestinal, epithelium, pituitary, testes, or skin, with performance function at the normal level or at one of the extremes. Failure does not always result in death. Failure of the testes, for example, results in sterility; failure of the skin, in an open lesion or ulcer.

Components of the nervous system and the system as a whole can manifest different degrees of performance capability. As in other eocentities, we have impressions of what is meant by superior mental abilities, by "normal" mental function, and by mental failure. We are aware that a certain level of performance efficiency must be maintained for the nervous system to provide for the usual needs of the organism and that the nervous system, like other systems, has a reserve of capability which can be drawn upon when requirements are excessive. In dealing with the influence of radiation, attention must be given to the entire range of adaptiveness, if considerations are to be at all comprehensive. There is, thus, a range of "wellness" or reserve fitness, as well as a range of "sickness" or deficient fitness, separated by a line designating the level at which there is no clinical evidence of disease or abnormality.

Determination of normalcy or satisfactory performance in the usual range of freedom from disease is not in itself sufficient reason for saying that a biologic system has not been affected by an agent such as radiation. Reserve capacities must also be considered, if any analysis is to have full meaning. This has particular significance in connection with the nervous system as an information processing device.

Effects on the Nervous System as a Whole

Figure 2 shows a range of radiation dosages we wish to consider in relation to treatment of the nervous system with penetrating radiations such as x-rays or gamma rays. Each number is different from the one above or below by a factor of 10. The digit 2 is used because it applies most satisfactorily, especially at the extremes.

Doses of 200,000 r applied to the head or the whole body of laboratory animals usually result in spastic seizures and death in a matter of minutes. Such killing has sometimes been called *nervous system death*, inasmuch as death resulting from other kinds of irradiation damage requires a significantly longer time. In relation to other systems, 200,00 r will interfere with muscular performance, stop sperm motility, and cause measurable biochemical changes.

Doses of 20,000 r will cause rapid loss of motor coordination and seizures of different kinds, leading to death in a matter of hours. The effects appear

200,000	r
20,000	r
2,000	r
200	r
20	r
2	r
0.2	r
0.02	i
0.002	i
0.0002	r

FIG. 2. Levels of exposure for consideration.

to be of the same type as those resulting from the larger doses, but less precipitous.

Doses of 2,000 r applied to the whole body of mammals leads to death in 2 to 3 weeks, but it is more due to damage in intestinal epithelium and hemopoietic organs than in the nervous system. Doses of 2,000 r are especially interesting, since they are below the level required to cause the so-called nervous system death, yet are sufficient to cause death in days or weeks. The key to the situation appears to lie in the fact that this level of dosage causes extensive damage, and in some cases complete destruction, of the growing or progenitive tissues.

This is illustrated most diagrammatically in the skin (Fig. 3). Skin epithelium has at its base a germinal layer (stem cells), which is the source of cells giving rise to functional squama, the dry protective outer layer. Cells originating in the germinal layer mature or differentiate as they move outward to form the squama. True to a general finding in radiobiology, the more primitive proliferating cells of the germinal layer are the most sensitive to radiation. When cells of this layer are damaged, the renewal or replacement process is impaired. When all are destroyed, stopping replacement altogether, skin breakdown with development of an open lesion is inevitable. This arises as surface cells wear away and disappear.

The germinal layer of the testis involves similar renewal and maturation processes, the renewal elements being the most sensitive to radiation. The same situation exists with respect to intestinal epithelium, hemopoietic tissues, and the lens of the eye (Henshaw, 1958), each tending to show failure of some kind (sterility, anemia, poor assimilation, cataracts) following 2,000 r of exposure.

When doses are somewhat less than 2,000 r or when only parts of the body are exposed, death may not occur, and often there is recovery in the form of regrowth of depleted progenitive elements, the regrowth stemming

SKIN

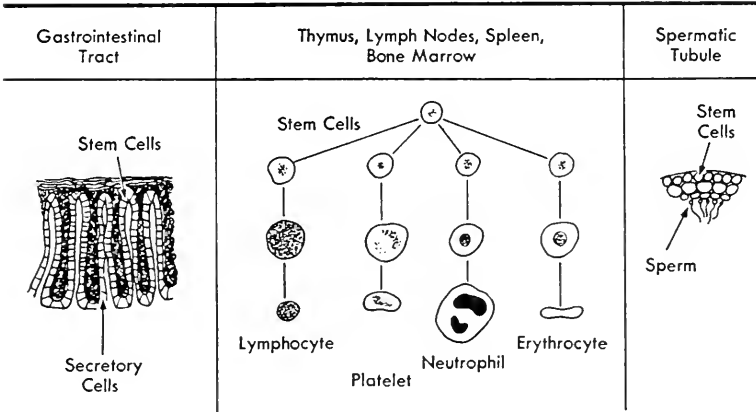
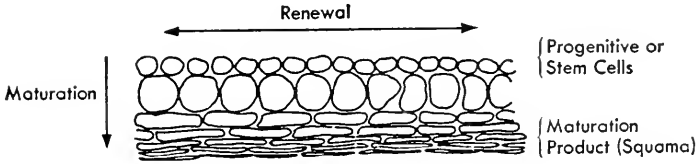


FIG. 3. Progenitive tissue renewal and maturation.

from irradiated cells that survived. Whereas regrowth of progenitive elements may be essentially complete, it has been found that the surviving tissues do not perform as well in later life as do similar tissues which did not receive irradiation. In this connection, it is important to consider whether nervous system components, while nonproliferative in adult life and less affected by radiation so far as cell-killing is concerned, are able to perform as well after irradiation.

The 200 r dose may cause significant destruction of progenitive tissue elements, but damage is rarely (if ever, in humans) sufficient to cause death. In the descending scale of exposure, therefore, the range between 2,000 and 200 r is of particular significance, inasmuch as we become confronted with organisms that have recovered from substantial proliferative tissue loss, but nevertheless show earlier deterioration of function in later life. Studies to detect modified nervous system function following 200 to 2,000 r exposures are in preliminary stages, but some positive findings have been reported.

A point of departure has been the investigation of effects that follow prenatal exposure, when the nervous system is progenitive. As seen from reports by Hicks and co-workers (see Hicks, 1954, 1958; Hicks *et al.*, 1957), Russell (1954), Rugh and Grupp (see Rugh, 1959a,b; Rugh and Grupp, 1959a-c, 1960), and others at this meeting, extensive changes are produced in the developing morphology of the peripheral and central nervous system by radiation dosages in this range. Along with these distinctive findings has been the work since 1952 (Levinson, 1952; Tait *et al.*, 1952; Furchtgott and Echols, 1958a, b; Furchtgott *et al.*, 1958; and Sikov *et al.*, 1960) correlating *in utero* exposures with performance abilities in postnatal life. Results indicate that a few hundred r have adverse effects on learning ability, emotionality and locomotor coordination. In this session work will be reported which deals with modifications in behavior stemming from irradiation of adult organisms.

There are reports by Eldredge and Trowbridge (1959), Arnold *et al.* (1954), Ross *et al.* (1954), Lee *et al.* (1955), and Gangloff and Haley (1960) showing electroencephalographic changes within minutes, hours and days following whole body exposures below the LD-50 range. Work by Garcia and co-workers (see Garcia, 1960; Garcia *et al.*, 1955, 1956a-c) that conditioned aversion to saccharine can be established in rats by exposure to a few hundred r of x-rays, and work by Lynn Brown and associates (Brown *et al.*, 1959, 1960; White and Brown, 1959; Overall and Brown, 1959) indicates that the effectiveness of peripheral cues in discrimination learning tasks in monkeys is reduced by irradiation.

Dosages from a few r to a few tens of r are in the range of threshold levels of exposure, as far as presently measurable nervous system effects are concerned. Rugh and Grupp (1960) reported certain brain anomalies in mice exposed *in utero* to 5-25 r from 0.5 to 1.5 days after fertilization, and foreign investigators, mostly Soviet (Lebedinsky, 1956; Lebedinsky *et al.*, 1959; Krabbenhoft, 1955; Grigoryev, 1954, 1956; Kudritsky, 1955, 1957; Nemenov, 1944; Livanov and Diryukov, 1959) have reported changes in cognitive functions correlated with radiation exposures at 1 r and at 0.05 r (Kudritsky).

Except for the findings of Soviet investigators, exposures of 0.2 r, as far as we know, are below the range found to have effects on nervous system functions. It would be an important result of this conference to ascertain if the specialized techniques and the work on the more complex nervous functions in higher forms have enabled the lower dose findings.

On our scale of exposures 0.02 r is in the range of permissible dose levels, which is only a factor of 100 above the range of natural or background radiations in which all life on the earth arose and evolved.

Effects on Component Parts of the Nervous System

We will consider the performance of certain component parts of the nervous system by giving attention to the functional elements in relation to information processing:

- Perception (transducer organs)
- Impulse transmission (nerves, axons)
- Information storage and utilization (brain)
- Coordination (motor organs)

Only a small amount of work has been done (Girden, 1935) dealing with the effects of radiation on the tissues and organs by which external stimuli are transformed into action currents. The work of Bachofer (1957) and others deals with the ability of receptor organs to detect ionizing radiation. It is significant that in receptor parts of the eye an agent such as radiation, which causes ionic rearrangement by displacing electrons and exciting molecules, should generate an impulse that is measurable as it is transmitted along nerve pathways.

Impulse transmission itself appears to involve the movement of free electrons. To modify this process with ionizing radiation would be somewhat comparable to affecting an electric current in a conductor. Since in a good conductor there is an abundance of free electrons, it is to be expected that the comparatively small additions provided by even large doses of radiation would have virtually no effect. Perhaps it should not seem surprising, therefore, to learn (Gerstner, 1956; Nachmanson, 1957) that enormously large doses of radiation (10,000–100,000 r are required to produce significant changes in transmission abilities of nerves and axons—doses in the range that produce measurable biochemical modifications.

After impulse transmission in the information processing system comes information storage and utilization—memory, concept formation, and decision making. Such action occurs in the brain primarily and apparently in the neurons that, to a large extent, comprise the brain. How information is stored and redistributed in the processes of mental function is only slightly understood, but *a priori* analysis suggests that it is accomplished by ionic or molecular rearrangements, the level of activity at which ionizing radiation exerts a strong effect. Impressions are that storage of genetic information, which is inherited and existent in all living cells, is by sequential arrangements of atoms and side chains attached at particular locations along protein molecules. Whether the information transmitted by nerve impulses is stored by means of side chains, by passages of valence electrons from one orbit to another in a low energy lattice system, or by right and left or up or down orientation is little more than speculation.

However, insight can be gained by comparing the information utilization process of the nervous system with that of other systems. In a television system, for example, information is transmitted along cables by free electrons in pulses with varying frequency and amplitude, and in the receiving set such pulses are sorted, transformed, and projected in such a way as to yield shades of light contrast and precise organization for picture formation. In a computer system, the features of information storage, reassociation, and utilization have been added. Storage, in some cases, is accomplished merely by orientation of iron molecules with respect to each other on a plastic tape or disc, a process so accurate that it can preserve and return all the attributes of beauty in a character portrait or in a complex orchestral rendition. Information storage can be accomplished as well by a groove on a vinyl disc or by a printed page on which the single medium used for transmitting details of profound logic or poetic beauty is black characters. Information of great complexity can be acquired, stored, and reassociated in an organized manner by means of one or a few unit mechanisms. In the effort to determine how mental functions take place in cellular systems, attention is being given to the nature of information-storing molecules (polymers) contained in neurons, the kinds of structures affected directly by ionizing radiation. It is of real practical and scientific significance to determine whether radiation affects memory the way a magnet affects information stored on a magnetic tape, causing dulling or erasure. Surely it is to be expected that, in time, the information storage and utilization processes of the nervous system will be reasonably understood and that radiation may have an important role in the development.

Indirect Effects

While direct action may be of greater interest from the standpoint of information handling, equally important changes may result from indirect action. Vascular damage may result in injury or failure of a local part or of the entire nervous system. Recovery from vascular or traumatic injury can result in scarification and, thereby, permanent impairment. Alteration of membrane conditions and biochemical differentiations conceivably could have more far-reaching effects in the nervous system than in other systems. Before concluding that radiation is having effects directly on the nervous system, indirect effects must be ruled out.

Effects on the Intellect

From both practical and scientific standpoints, there is also the *mind* to be considered, the device by means of which one thinks and stores information, and beyond this there is the aggregate or mass mind. The underlying

practical problem, and one of particular concern in the field of radiobiology, is whether low levels of environmental radiation affect in any way *powers of the intellect*.

Effects on the nervous system or any of its parts, including effects on storage and use of information, affect the intellect, and we can develop some ideas about effects of radiation on the mind by examining the fragmentary information at hand.

If by low level environmental radiation we mean intensities only a few times natural levels, there is no information at all, so far as the writer is aware, indicating whether there is an effect or not. If, by low level, we mean exposures below those required to produce clinical symptoms, say 0.01–1 r (100–10,000 times background, such as might exist in accidents), we have mostly the Soviet evidence indicating detectable changes. If by low level we mean exposures just below the lethal range, say 100–500 r (something over 100,000 times background, such as might occur in nuclear catastrophe), we are, with refined techniques, beginning to see some changes, mainly of the prompt response type.

On the basis of such evidence, we might be tempted to conclude that environmental radiation is not a very serious practical problem, so far as effects on the power of the intellect are concerned, and with further investigation this may prove to be the case. However, on the basis of the same evidence, one gains the impression that the more refined the techniques and the more advanced the specialization of the nervous system in intellectual power, the easier it is to demonstrate effects.

If this impression is to be regarded as having merit, we would scarcely be justified in concluding at this time that levels even a few times background are inconsequential—particularly, if we take into account the still more complex intellectual powers of population groups. Group intellect involves moods, emotional tone, and temperament, as well as ability to think. At this stage in human history, when so much depends on such limited individual and group intelligence, it is important to ask whether even a small loss in mental fitness, due to a lack of learning abilities, or abilities to think, may not be a serious loss.

Evaluation of Low Level Effects

I wish to outline a philosophy and approach for dealing with effects of low level environmental radiation in the general situation and to identify some important underlying radioneurologic problems.

At the beginning of this presentation, attention was called to the *adaptiveness of ecoentities*, the abilities of cells, tissues, organisms, and population groups to perform, and to the idea that each ecoentity possesses an *ecoforce*

or inherent driving power which, as a minimum, is sufficient to keep it alive. This situation is characterizable by a formulation similar to or resembling Ohm's Law as it applies to electric currents. Ohm's Law states that the current (A) is directly proportional to the electromotive force or voltage (V) provided by the source and inversely proportional to the resistance (R) of the circuit:

$$A = \frac{V}{R}$$

In an ecosystem, A can be identified as adaptiveness or the ability to perform; V , as ecoforce or maximum performance potential; and R , as the composite of deterrent influences—though at the present state of understanding there is no basis for indicating a proportionality relationship, only a ratio. On the adaptiveness scale (Fig. 1), the maximum value on the scale would be the maximum achievable potential with respect to any given function of any particular ecoentity at any given time. With respect to whole mammalian organisms, this is the situation at the moment of conception. During most of the life period of whole organisms or parts of organisms, ecoforce (V) is something less than maximum, and eventually it disappears at death. Throughout the course of life, V travels the entire length of the fitness scale from top to bottom. Ecoforce is not readily measurable, even assuming the ability to specify tangible units, since it is defined as potential apart from the ever present deterrent forces (R). Adaptiveness (A), on the other hand, is measurable (again assuming tangible units), inasmuch as it is by definition determined by the strength of R acting against the strength of V . In this picture, V is determined by the interaction of numerous organizational forces and R by the interaction of numerous disorganizational forces. Key to this concept is the driving force, V , the same as in an electric current. The driving force (ecoforce) appears to be determined largely by genetic organization fixed at conception.

Many models have been developed by analysts as an aid to thinking about biologic mechanisms. The one I have just presented has been developed in greater detail elsewhere (in preparation). It has the advantage of being simple, gives a perspective view of the process being considered, aids in defining requirements for dealing with the problem at hand, and provides an approach for dealing with certain radiobiologic capability problems.

Application

The Ohm's Law adaptation, which states in effect that potential for performance of any ecoentity (nervous system as well as other systems and in-

cluding population groups) varies directly with ecoforce and inversely with deterrent influences, aids in understanding the nature of community-wide situations and problems that follow environmental irradiation. It also aids in understanding something of the requirements for experimental designs.

First, the formulation points up the logical and obvious feature, that when deterrent forces (R) are great, failure of the ecosystem will occur when the ecoforce is diminished only slightly; conversely, it indicates that when the deterrent influences are of low order, failure will not occur until the ecoforce has diminished to a low level—as is the situation in late life. Recognition of this situation suggests a useful experimental approach. When loss of capability is the effect being studied or analyzed, evidence of change is revealed more readily and earlier by applying additional stress sufficient to cause failure in the upper range of fitness, that is, by requiring the ecoentity to exceed more quickly the capacity of the fitness reserve.

Second, the formulation points the way for dealing meaningfully with changes in ecoforce, even though ecoforce cannot be measured directly. By keeping the environmental deterrents constant (i.e., by keeping uniform control conditions) and by measuring adaptiveness at different times, changes in rate of ecoforce deterioration are obtained. Then, by comparing rate changes in experimental and control samples, information is obtained indicating how an affecting agent, such as radiation, modifies ecoforce—a central objective in studies of residual capabilities.

Third, the formulation gives a perspective of the deterrent factor, R . In nature, this factor is comprised of many elements. To determine the effects of a single deterrent element, such as low level environmental radiation, its influence must be considered in relation to other deterrents that are inherent in the situation, such as parasitism, infectious diseases, air pollution, nutritional deficiencies, toxicities, oppressive climates, intemperances, tensions, antagonisms, insecurity, and suppression. To be worthy of concern as an impediment to life capabilities, radiation effects must be significantly large compared with the effects produced by other deterrents.

Summary

Attention has been called to biologic processes as energy mechanisms.

Central components of life mechanisms have been designated as: *adaptiveness*, meaning capability of ecoentities to perform; *ecoforce*, meaning degree of organization for biologic function; and *deterrent influences*, meaning all forces acting to reduce the organization for life.

As a theorem, it is stated that adaptiveness varies directly with ecoforce and inversely with deterrent influences.

By relating the central components of biologic mechanisms logically and

taking into account the precise nature and relative influence of each, a ground work is laid for systematic assessment of residual capabilities in eco-entities of any level of organization, with or without irradiation.

Analysis of the effects of low level environmental radiation on the nervous system yields a double return; in addition to contributing to an understanding of the practical problem of irradiation hazards to individuals and population groups, it provides fundamental information about the operational character of the nervous system.

Preliminary findings that appear to have meaning are: (a) the older the fetus, the greater the dose required to produce decrements in learning, suggesting that since cerebral cellularity appears to vary correspondingly, learning must somehow be associated with the number of cells in the adult brain; (b) improvements in certain performance capabilities following irradiation, upon careful analysis may be found to stem from reduced distractability, a deleterious rather than a beneficial effect; (c) the more refined the techniques used and the more complex the nervous system studied, the easier it is to demonstrate a radiation-induced modification, suggesting that the advanced human nervous system may be more easily affected by radiation than the nervous systems of other species; and (d) late effects, consisting mainly of altered rates of degenerative changes, are difficult to demonstrate, requiring time-consuming and costly techniques.

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Acute Central Nervous System Syndrome of Burros*

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Introduction

Nerve tissue is usually listed among the tissues most resistant to ionizing radiation. Experimental data from the animals most commonly used in the laboratory give considerable support to this contention. Very high doses (1,000 r and above) are required to produce pronounced derangement and lesions of the central nervous system in dogs (Peng *et al.*, 1958), monkeys (Arnold *et al.*, 1954; Vogel *et al.*, 1958; Tonnis, 1959), rabbits (Hicks *et al.*, 1956), and mice (Hicks *et al.*, 1958; Gleiser, 1954). However, data (Gangloff and Haley, 1960; Lebedinsky, 1956) on functional changes of the brain indicate effects at relatively low dose levels. Consistent changes in the conditioned reflex response in the dog have been reported with doses as low as 20–150 r (Girden, 1935).

The burro seems to be unique in that pronounced symptoms of central nervous system disturbance appear following exposure to relatively low doses of radiation. The dose rate and type of radiation appear to be important factors in this response. Trum *et al.* (1952) observed deaths with central nervous system symptoms in their early studies with burros exposed to total body gamma radiation with dose levels of 600–900 r delivered at 50 r per hour. This paper is a review and discussion of data from recent experiments in which pronounced central nervous system disturbances and lesions were observed.

Experimental Procedure

Adult male and female burros (*Equus asinus asinus*), 2 to 7 years old, were used.

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In the first study at the Nevada Test Site in 1957, which was reported in detail (Kuhn and Kyner, 1958), 88 burros were exposed to prompt neutron-gamma radiation from a nuclear detonation. They were divided into groups of eight and placed in steel culvert shelters, 5 feet in diameter and 13 feet in length, designed to hold 2 animals placed nose-to-nose and broadside to the nuclear device. Four shelters each were placed in 11 regularly spaced rows at a distance from ground zero calculated to bracket the median lethal dose of prompt radiation. The dose range (air) was 250 to 780 rep with a neutron/gamma ratio of approximately 1.

In the second study (Kuhn and Brown, 1960) on head irradiation, 25 male burros were exposed to a single Co^{60} source with the brain as the principal target. The dose rate was approximately 100 r per minute. The animals were lightly anesthetized and placed in a lateral recumbency with the head positioned so that the midpoint of the brain was 9 cm from the source, as determined by topical reference points. The animals were exposed in groups of five in a sequential dose pattern (dose range 150–1,200 r) described by Mewissen (1958).

In the third study (Thomas and Brown, 1960), which was designed to study sodium activation in the blood, 7 male burros were exposed unilaterally to Godiva II (bare reactor) at the Los Alamos Scientific Laboratory. The animals were positioned with their right side 3 meters from the center of the reactor. Each animal was restrained in a crate to maintain constant position. The dose of 180 rad was delivered at 6 rad per minute.

All tissues were fixed in 10% buffered formalin, imbedded in paraffin, sectioned at 5 μ , and stained with hematoxylin-eosin. Sections from the central nervous system included cerebral hemispheres, pituitary, hypothalamus, thalamus, portions of the ventricles, corpora quadrigemina, medulla, cerebellum, and hippocampus.

Results

MORTALITY

Burros in each experiment died early. All deaths occurring within 120 hours after exposure were associated with pronounced neurologic symptoms. Forty-five of the 55 acute deaths in the Nevada study and all deaths in the head-irradiation and Godiva studies occurred within 120 hours after irradiation.

NEUROLOGIC SYMPTOMS

Following irradiation the animals appeared normal for 2–10 hours; then an abnormal behavior pattern ensued.

Disturbance of consciousness was manifested throughout the central nervous system syndrome. An initial period of depression was followed by irritability, delirium, and mania. Irritability was manifested by excitement, biting, and kicking in response to minor provocation and general resentment of any external stimuli; delirium, by aimless wandering and continuous tail switching, as if molested by flies; and mania, by violent and purposeless movements. If an animal progressed to a state of mania, eventually coma and death ensued.

There was also pronounced motor irritation, characterized by clonic spasms (trembling, muscle tremors, and bobbing of head) and forced movements [circling, pressing forward (Fig. 1B)], and grinding of teeth or chewing motion], usually with excessive salivation.

Derangement of sensation was indicated by hyperesthesia and disturbance of sight and equilibrium.

Paralysis of lower lip and tongue was common. Incoordination, although somewhat difficult to recognize in the presence of disturbed equilibrium, was definitely observed in many animals.

Other observations were laceration in all animals, swelling of the neck in some, and anorexia, which began with the onset of depression.

The onset and duration of neurologic symptoms varied with dose and dose rate. In the Nevada group, 19 deaths occurred within 24 hours. The first death (625 rep) occurred 5 hours after exposure. In the head-irradiated burros the first death (1,200 r) occurred at 15 hours; in the Godiva burros, at 71 hours after exposure. The severity of neurologic manifestation in respect to dose appeared to vary with the individual. Some animals in the low dose groups exhibited violent response, even though there was a prolonged postirradiation period of apparently normal behavior.

PATHOLOGY

The most frequent gross lesion in the head-irradiated burros was diffuse hemorrhage into the subarachnoid space on the posterior dorsal and/or ventral aspect of the medulla and cerebellum (Fig. 2). Occasionally the meninges were congested and contained small hemorrhages. A similar pattern of lesions was noted in the Godiva burros. The Nevada burros exhibited few gross lesions.

Perivascular cuffing (Fig. 3) was the most frequent lesion seen in all three groups. It was characterized by clusters of neutrophils around the parenchymal and pial vessels, as well as moderate to pronounced infiltration at other sites (Fig. 4). In the head-irradiated animals, these clusters contained many eosinophils.

Hemorrhages were seen frequently in all groups, primarily around the

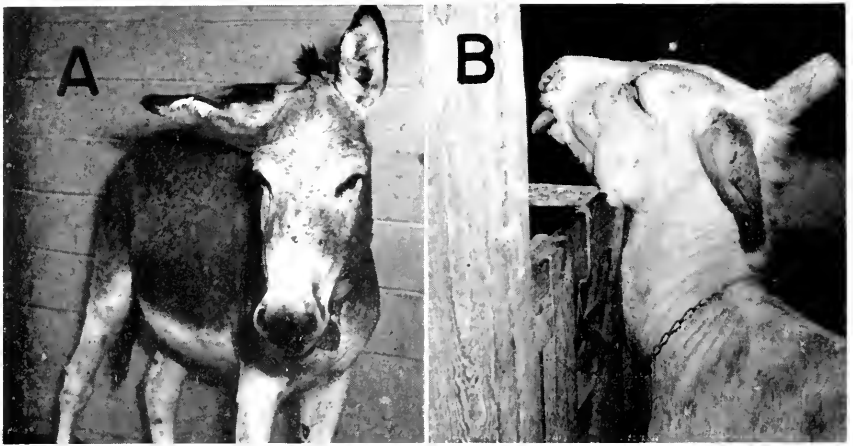


FIG. 1. A. Godiva burro, 180 rad, loss of motor control of right ear 24 hours after irradiation. B. Head irradiated, 485 r. pressing forward, tongue paralyzed, animal survived 30 hours.

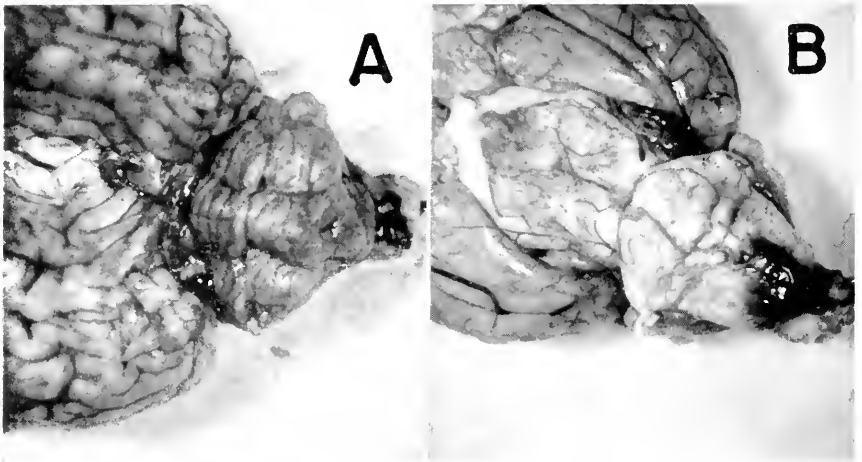


FIG. 2. Brain from head-irradiated burro, 485 r. Hemorrhage in subarachnoid space in area of medulla and at base of cerebellum. A. Dorsal view. B. Ventral view.

blood vessels, principally within the space of Virchow-Robin, but also extending into the parenchyma. Hemorrhages in the subarachnoid spaces of the cerebrum and cerebellum (Fig. 5) were diffuse and extended into the perivascular spaces.

In many head-irradiated animals there was considerable extravasation of plasma into the Virchow-Robin space, often with extensions into the parenchyma forming large irregular "plasma lakes" (Fig. 6).

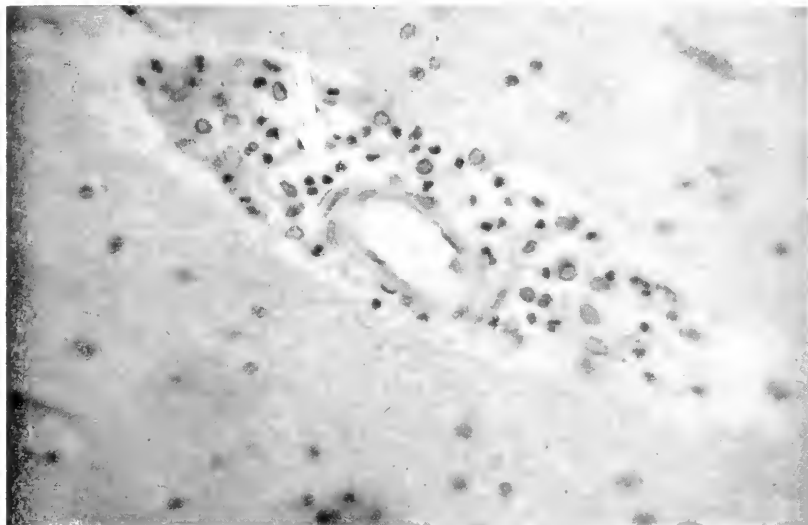


FIG. 3. Head-irradiated burro, 485 r, survival time 28 hours. Perivascular cuffing (neutrophils, arrow) in the cerebral white matter. $\times 700$.

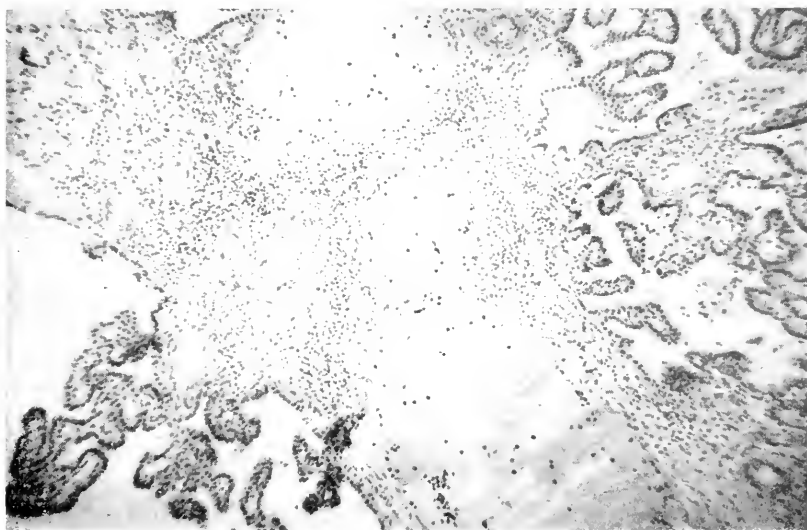


FIG. 4. Head-irradiated burro, 485 r, survival time 24 hours. Congestion, hemorrhage, and neutrophilic infiltration (arrow), choroid plexus, 4th ventricle. $\times 175$.



FIG. 5. Nevada Test Site burro, 625 rep, survival time 25 hours. Massive subarachnoid hemorrhage (arrow), cerebellum. $\times 75$.

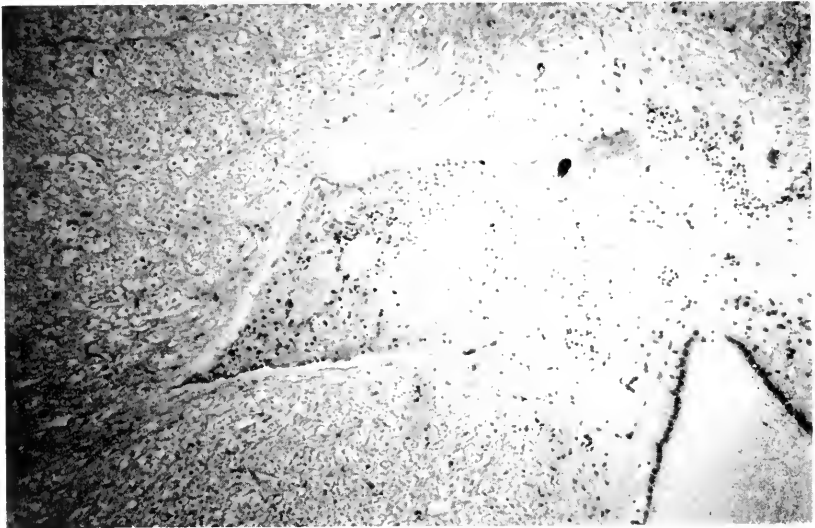


FIG. 6. Head-irradiated burro, 485 r, survival time 24 hours. Large plasma lake surrounding blood vessel (arrow). Other lesions in the pons are perivascular cuffing and hemorrhage. $\times 175$.

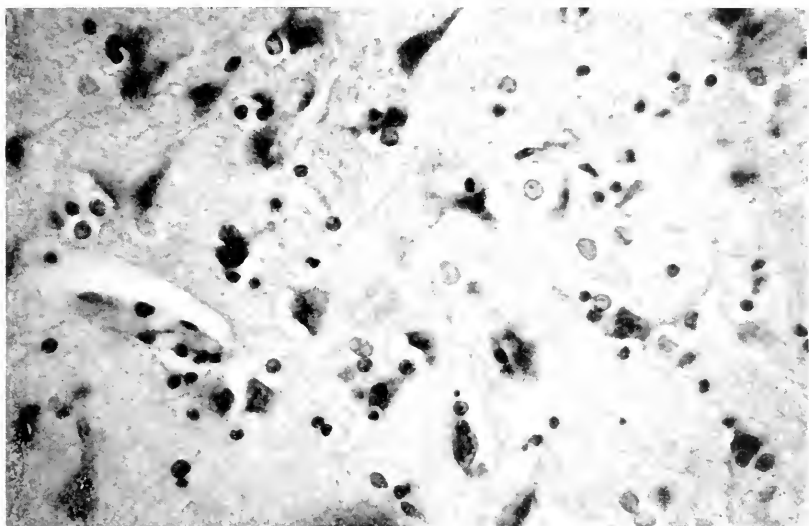


FIG. 7. Nevada Test Site burro, 785 rep, survival time 24 hours. Satellitosis (arrow) and neuronophagia, cerebrum, $\times 700$.

Neuronophagia and early satellitosis (Fig. 7) were observed in the head-irradiated and Godiva animals, but were not distinct in the Nevada group.

The portions of the central nervous system most severely involved were the cerebral hemispheres, the cerebellum, and meninges, in that order. All groups had a similar pattern. Vascular congestion and increase in size of the perineuronal, periglial, and Virchow-Robin spaces were most prominent in the head-irradiated animals.

Lesions affecting the spinal cord, which involved both the parenchyma and meninges, were infrequent and mild. These lesions were perivascular cuffing, hemorrhage, and congestion.

Minor changes seen in the pituitary gland were infrequent perivascular cuffing and hemorrhage in the pars nervosa.

Lesions in other areas of the body were few and apparently of little consequence, with the exception of the Godiva and some Nevada burros, in which there were multiple disseminated hemorrhages, pulmonary edema, and liver changes.

Lymphadenitis and atrophy were noted in the lymph nodes of the head and cervical region in the head-irradiated animals. In the animals exposed to total body radiation, there was generalized atrophy of all lymphoid tissue.

Discussion

There are many questions which must be answered before the significance of the central nervous system syndrome in burros is fully realized. Why is the

central nervous system of burros more sensitive to radiation than that of other species? What are the physiologic aberrations leading to the central nervous system response? Is the neurologic syndrome a direct or indirect effect?

We do not believe that the dose rate should be emphasized, because the head-irradiation experiment suggests a threshold for dose rate effect. The Nevada and head-irradiated animals responded in a similar manner except deaths occurred earlier in the Nevada burros, and mortality was slightly higher in the head-irradiated animals at equivalent dose levels, possibly a result of anesthesia. A recent report (Zauder, 1959) indicates that anesthesia enhances the mortality effect of irradiation.

The data presented suggest that vascular changes contributed greatly to the central nervous system effects observed. According to Udall (1946), the symptomatology described is related to conditions which cause an increase in intracranial pressure, such as cerebral congestion and hemorrhage, meningitis, and encephalitis. Extravasation of plasmatic fluid is often concomitant, and hemorrhages are prominent. It seems likely that the perivascular cuffing with neutrophils is a response to the plasmatic fluid extravasations.

Alterations in the blood-brain barrier may be an important factor in the burro's response to irradiation. Rose (1958) reported an accelerated penetration of electrolytes into the cerebrospinal fluid and brain of rabbits following irradiation. This could possibly contribute to an increase in cerebrospinal fluid pressure. Two manometric readings of cerebrospinal fluid pressure taken in moribund, recumbent head-irradiated burros at the lumbrosacral fossa approached 800 mm water. Less than 300 mm is normal.

Although the primary lesion is vascular, satellitosis and neurophagia are evidence of neuronal damage. Whether these lesions are results of a direct effect on the neurons, a sequela to vascular damage, or metabolic disturbances is not known.

Summary

Three irradiation experiments with burros showed pronounced central nervous system disturbances and death at relatively low dose levels. The data suggest that vascular changes contributed greatly to the central nervous system effects observed.

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Effects of Low Level Radiation on Audiogenic Convulsive Seizures in Mice *

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Much experimental evidence has led to the conclusion that nervous tissue is peculiarly resistant to the influence of ionizing radiation. It has been generally reported that doses as high as 75,000 r are required to affect the response of isolated nerve fibers of various animals. The relevant literature is summarized in a recent paper by Rosen and Dawson (1960), in which they report minor changes in the frog nerve-muscle preparation following x-irradiation at 20,000 r.

Fewer studies have been concerned with the effects of lower doses of radiation on the functional activity of the nervous system in the intact animal. Caster, *et al.* (1958) reported that following 700 r total body x-irradiation in the white rat, a significant decrease in brain DNA occurred within 12 hours; electrocortigrams also revealed fluctuations in both slow and fast rhythms, with low frequency activity paralleling changes in DNA. Minaev (1954) reported alterations in conditioned reflex activity in rats, cats, and dogs, following localized x-ray doses of 100 r to the central nervous system. Schwartzbaum *et al.* (1958) found that following a single whole body exposure to 500 r x-radiation, the electroconvulsive threshold of the rat was reduced, although the incidence of electroshock seizures was not affected.

Studies related to the influence of ionizing radiation at dosages below 10 r have been done primarily by Russian investigators. Lebedinsky *et al.* (1958) have reviewed the Russian literature, including reports that nervous function may be altered following doses as low as 0.1 r. Garcia and Kineldorf (1960a, b) reported the establishment of a conditioned avoidance response in rats following exposure to gamma radiation at 10 r and to fast neutrons at 7.5 r.

We appear to have convincing evidence that the frequency and severity of audiogenic seizures in mice is markedly influenced by less than 1 r gamma radiation. Our experimental approach was not based on any prior conviction or working hypothesis, but on the necessity of explaining a mass of unexpected data.

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Materials and Methods

We have been working for several years with sound-induced convulsive seizures in inbred mice, with particular reference to the mode of inheritance and the physiologic basis of the trait (Miller *et al.*, 1952, 1955). Our material consists of two pure lines of mice unusually sensitive to sound (DBA lines 1 and 2) and two sublimes of sound resistant mice (C57BL6 and C57BL10). The animals are tested for seizure susceptibility by being exposed to a bell at 100 decibels for 2 minutes on four successive days, starting when the mice are 30 days old. In DBA/1, more than half of the animals suffer tonic-clonic seizures, usually fatal. In DBA/2, the frequency of seizures is close to 100%, mostly fatal. Seizures are rare in the C57 strain.

The most obvious criterion of seizure susceptibility is the incidence of seizures. Other measures helpful in determining the degree of susceptibility are severity of seizures, latency, and percentage of seizures on the first trial.

In DBA mice, the most severe and most common convulsive pattern is the fatal tonic-clonic seizure. In a few cases, animals survive the tonic-clonic seizure after respiratory arrest. The tonic seizure is rarely fatal, and the least severe response, the standing spasm, is never fatal.

Latency, or the number of seconds after the onset of sound stimulation when the seizure occurs, is most commonly between 35 and 45 sec. Highly susceptible mice tend to convulse early, and in less susceptible animals the latency is longer. Premature seizures (before 16 sec.) are observed only in very susceptible animals. Delayed seizures (after 60 sec.) are associated with lower susceptibility.

Very susceptible mice tend to convulse on the 1st exposure, while in less susceptible mice, the 1st seizure may occur on the 3rd or 4th trial. Therefore, in comparing groups of moderate susceptibility, the 1st trial data often furnish a more accurate measure of relative susceptibility; actual differences may be masked within the 4 trials.

The Problem

In July, 1952, our mouse colony was moved from Whitman Laboratory into a frame building behind the laboratory. At that time we were completing the genetic analysis of seizure susceptibility through crosses of both sound-susceptible lines to both sound-resistant sublimes. In each cross, a fairly predictable incidence of seizures had been observed. Immediately after moving the colony to the new location, there was a striking increase in the frequency of seizures in all groups.

Table I summarizes the change in the percentage of fatal and total seizures in those groups with adequate numbers for valid comparison. The

TABLE I

COMPARISON OF AUDIOGENIC SEIZURE INCIDENCE IN VARIOUS GROUPS OF MICE BEFORE AND DURING LOW LEVEL RADIATION IN ANIMAL QUARTERS^a

Cross and generation		Incidence of seizures (%)					
		Number		Fatal		Total	
		Whit ^b	Room R ^c	Whit	Room R	Whit	Room R
DBA/1 × C57/10	F ₁	92	80	41.3	68.8	52.2	72.5
	BxC ^d	178	34	11.2	23.5	14.1	26.5
	BxD ^e	152	140	40.8	80.0	50.7	84.3
DBA/1 × C57/6	F ₁	121	222	31.4	87.8	47.9	91.9
	BxC	229	138	17.5	41.3	22.7	45.7
	BxD	144	133	45.6	81.2	62.3	85.7
DBA2/ × C57/10	F ₁	91	97	64.8	81.4	82.5	84.5
	F ₂	120	263	31.7	52.5	55.0	76.1
	BxD	45	206	64.4	85.0	80.0	95.2
DBA2/ × C57/6	F ₁	151	181	58.3	93.4	92.1	98.9
	F ₂	198	66	44.4	68.7	67.2	81.8
	BxD	58	191	62.1	91.1	84.5	95.3

^a Data based on four daily trials.^b September, 1949, through June, 1952, in Whitman Laboratory.^c July, 1952, through August, 1956, in Room R.^d BxC = backcross to C57.^e BxD = backcross to DBA.

columns headed "Whit" are data collected from September, 1949, through June, 1952, in Whitman Laboratory. The columns headed "Room R" are data collected from July, 1952, through August, 1956, in the frame building. In each generation within the 4 basic crosses, the mice had become significantly more seizure prone while in Room R. In no group was this trend reversed. In addition to the greatly elevated seizure frequency, the proportion of fatal seizures was also higher.

Painstaking examination failed to reveal any change in food, water, disinfectants, background sound level, personnel, or procedure which might be responsible for the increase in seizure susceptibility. After the second coat-color mutation (Miller and Potas, 1955) appeared in the colony, we had the mouse quarters monitored for possible radiation. A level of 5-10 times background (0.1 to 0.2 mrad per hour) was detected, supplied from a Co⁶⁰ source in a neighboring laboratory, unshielded on the side facing our windows. By chance, the rack directly in front of these windows was used to house the developing litters, which were therefore exposed to the maximal dose in the room. Within the 30 day period before being tested for seizure susceptibility, each mouse received at total of approximately 0.15 r.

In September, 1956, the colony was moved to new quarters, Room G, which was free of radiation. The unusual seizure frequency immediately subsided. There was little doubt that Room R was implicated in increasing the susceptibility to audiogenic seizures. Further tests were done to determine whether the radiation level in the room was the causative factor.

Genetic and Environmental Effects

DBA/1. The shifts in seizure susceptibility in untreated DBA/1 mice under the several environmental conditions are summarized in Table II. All the litters were derived from our inbred stock, which had been maintained by brother-sister matings since 1948. Originally the seizure incidence was moderate, with males more susceptible than females. Under low level irradiation in Room R, the frequency of seizures and of deaths on the 1st trial rose in both sexes, with a greater increase in females. Within 4 trials, the sex difference was reversed, with females significantly more susceptible than males. The transition group was nonhomogeneous, including litters born in Room R and moved to Room G before being tested at 30 days of age. In this brief period, the frequency of seizures on the 1st trial declined toward

TABLE II

INCIDENCE OF AUDIOGENIC SEIZURES IN UNTREATED DBA/1 MICE REARED UNDER DIFFERENT ENVIRONMENTAL RADIATION LEVELS

Period and level	First trial				Four trials			
	Deaths (%)		Seizures (%)		Deaths (%)		Seizures (%)	
	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀
1949-50 (background r)	61.8	47.3	68.7	49.7	81.8	71.6	81.8	77.1
1954-55 (5-10 × backg.) Room R	65.2	69.0	72.3	78.4	86.8	90.5	90.0	93.0
Jan.-Sept. 1956 (5-10 × backg.) Room R	73.2	69.6	78.1	76.1	76.9	90.0	80.8	93.0
Sept. 1956 (transition) Room R → G	59.4	40.0	59.4	47.6	75.0	90.5	75.0	90.5
Oct. 1956- May 1957 (background r) Room G	25.2	27.1	31.5	34.1	56.0	52.4	57.6	58.5

the normal DBA 1 level. The rapid drop in susceptibility is strong evidence for an environmental influence of radiation on the seizure response. In those litters born and reared in Room G during the ensuing 8 months, the seizure frequency was significantly below the normal DBA 1 level, with no sex difference in susceptibility. Many litters included in the last figures were offspring of the same matings which had produced the high susceptibility group earlier in the year.

In addition to the lowered incidence of seizures, two other measures indicate that the DBA 1 line had become genetically less sound susceptible while exposed to chronic low level radiation. The latency of fatal seizures was significantly longer, with many delayed seizures, and the proportion of the fatal seizures which occurred on the 1st trial declined from 96% to 49%. We conclude that while the low level radiation exerted a physiologic effect in increasing the frequency and severity of seizures, the line had undergone genetic change in the direction of lowered susceptibility to audiogenic seizures.

C57BL6. Although the chief influence of low level radiation on the seizure response appears to be physiologic, there was further evidence of genetic change in the colony during chronic exposure. C57BL6 mice are normally almost completely resistant to sound stimulation. After the colony had been maintained in Room R for 1 year, the C57BL6 mice were tested systematically, and a seizure frequency of 16% was observed.

To determine whether this unusual seizure susceptibility had a genetic basis, a selective breeding experiment was undertaken. Males and females which were negative on 4 trials, and had no littermates or close relatives which had convulsed, were bred together. Likewise, males and females which convulsed were bred to each other. It is possible to save for breeding some animals that suffer fatal seizures by applying prompt artificial respiration. Among the litters of the negative animals 3% had seizures, while 19% of the progenies of convulsers had seizures. The incidence of seizures did not change in subsequent litters of these selected matings after the colony was moved from Room R to Room G. The increase in seizure susceptibility of the C57BL6 mice is attributed to mutations of "seizure" genes under safe radiation levels.

Tests for Influence of Radiation on Seizure Response

Since it was apparent that both DBA 1 and C57BL6 stocks had undergone genetic change affecting their seizure susceptibility, we obtained new stocks of mice from Jackson Laboratory and restored 2 crosses in which there had been a dramatic increase in susceptibility while in Room R: the F₁ generation of DBA 1 by C57BL6 and the background of this F₁ generation to C57BL6.

Through the cooperation of Dr. Eric Simmons of the Argonne Cancer

Research Hospital, we were able to irradiate young mice before testing them for seizure susceptibility. F_1 and backcross litters were exposed to a small Co^{60} source which delivered 350 mrad per day. Since it was not advisable to expose the mothers, and since the litters are tested at 30 days of age, the exposure was limited to from 5 to 7 days after weaning. The total dosage was 1.5–2 rad during the ages of 23–30 days. Parallel control litters were weaned on the same day.

Incidence of Seizures in F_1 Generation of DBA/1 by C57BL6

Table III shows the frequencies of total and fatal seizures in the F_1 generation. There were no significant sex differences. The first two groups, derived from our original stocks, show the enormous increase in susceptibility which occurred in Room R. The 1956–57 population was derived from new stocks of mice. This DBA/1 pure line exhibited a seizure incidence 10% higher than our original DBA/1. While the higher susceptibility of the DBA/1 parents is reflected in the incidence of their F_1 progeny, the seizure incidence in the F_1 generation reared in Room G declined toward the

TABLE III

INCIDENCE OF AUDIOGENIC SEIZURES IN F_1 GENERATION (DBA/1 \times C57BL6)
UNDER DIFFERENT ENVIRONMENTAL RADIATION LEVELS

Period	N	First trial		Four trial	
		seizures (%)		seizures (%)	
		Total	Fatal	Total	Fatal
1949–52 (background r)	121	26.4	18.2	47.9	31.4
1953–56 (5–10 \times background r) Room R	215	85.1	71.6	91.9	87.8
Sept. 1956–May 1957 Room G (background r)	166	27.1	25.3	56.0	53.0
Irradiated (1.5 to 2 r)	194	61.3	53.1	74.2	71.1
May 1957–Sept. 1957 Room G (high background)	182	46.2	41.8	56.0	52.2
Irradiated (1.5 to 2 r)	103	57.3	53.4	70.9	68.0

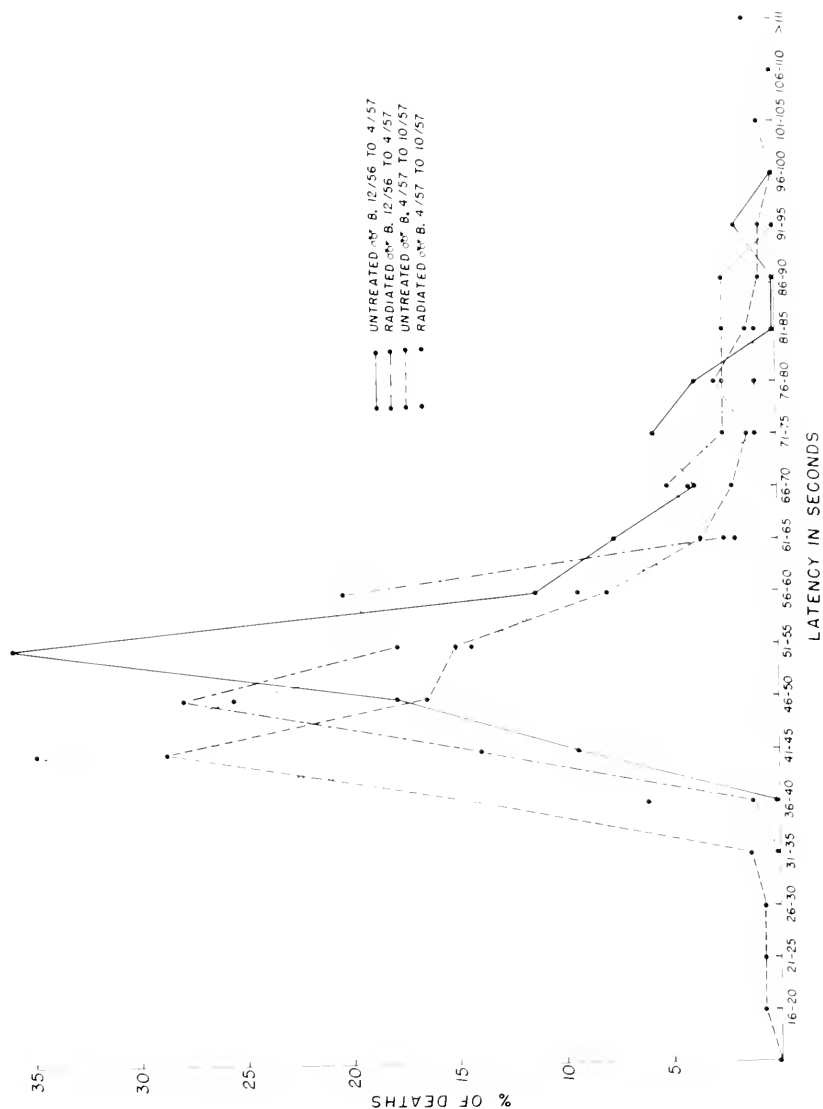


FIG. 1. Latency of fatal audiogenic seizures in F₁ male mice. Comparison, during two periods, of latency in controls and groups irradiated at 350 mrad per day. Mice were born on the dates indicated and were tested 30 days later. Low background prevailed from December, 1956, to April, 1957. From April, 1957, to October, 1957, the background was considerably higher.

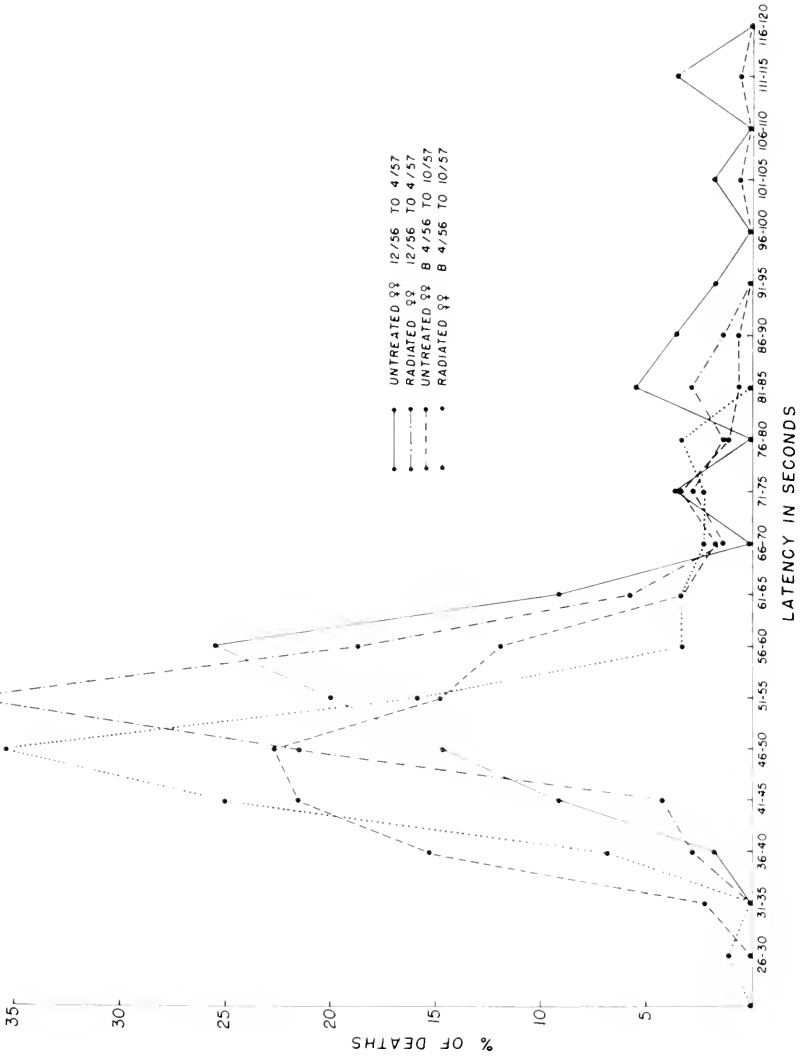


FIG. 2. Latency of fatal audiogenic seizures in F₁ female mice. Comparison, during two periods, of latency in controls and groups irradiated at 350 mrad per day. Mice were born on the dates indicated and were tested 30 days later. Low background prevailed from December, 1956 to April, 1957. From April, 1957 to October, 1957, the background was considerably higher.

original level. In the litters irradiated at 1.5–2 rad, the frequency of seizures and of fatalities was significantly greater than in controls. Moreover, the proportion of the fatal seizures occurring on the first trial was 75% in the irradiated group, in contrast to 47% in the controls.

This dosage of gamma radiation also influenced the latency of fatal seizures. The graphs in Figs. 1 and 2 present the percentage of fatal seizures occurring at each time interval in F_1 males and females. The two curves at the right in each graph compare the latency in the control and irradiated mice during this period (12/56 to 4/57). In the irradiated mice, the latency was shorter, with an earlier peak and few delayed seizures. The three measures (increased seizure frequency, higher proportion of fatalities on the 1st trial, and shorter latency of fatal seizures) all indicate heightened susceptibility to audiogenic seizures following gamma irradiation at 1.5–2 rad.

Backcross to C57BL6

The F_1 generation derived from new stocks of DBA/1 and C57BL6 was crossed to the new C57BL6 line, and the resulting backcross was maintained in Room G. After the seizure incidence in this low background environment was established, some of the backcross matings were returned to Room R. To control all other factors except radiation level in the room, part of the backcross matings and their litters were placed behind lead and steel shields in Room R.

Table IV summarizes the percentage of seizures in the backcross generation under these different conditions. In the 1954 group, there had been an unsuspected genetic contribution to the high susceptibility: the C57BL6 parents were from our original stock, in which the susceptibility had risen above the normal low level. The 1956–57 populations were derived from the new, low incidence C57BL6 stock. The difference in response between the unshielded and shielded groups in Room R constitutes strong evidence for the influence on seizure susceptibility of this astonishingly low level of chronic irradiation (0.2 mr per hour).

Several additional series of backcross mice were maintained in Room R during 1957 and 1958 while the Co^{60} source in the adjacent laboratory was in operation. Table V compares the seizure incidence in backcross litters reared in Room R and in Room G. During 2 periods of several months each, we had an unexpected control on the influence of the radiation level in Room R. In May, 1957, backcross matings were moved into the room, in the belief that the source was to be up for the next 3 months. At the end of 3 months, we learned that the source had been up for only 3 days. The seizure incidence was almost identical in the groups in Room R and Room G (Table V). During the ensuing 3 months, while the source was up, the fre-

TABLE IV

INCIDENCE OF AUDIOGENIC SEIZURES IN C57-BACKCROSS GENERATION^a MICE
UNDER DIFFERENT ENVIRONMENTAL RADIATION LEVELS

Period	N	First trial seizures (%)		Four trial seizures (%)	
		Total	Fatal	Total	Fatal
1950-52 (background r)	229	12.2	7.8	22.7	17.5
1954 (5-10 × background r) Room R	138	28.3	24.6	45.7	41.3
1956-57 (back- ground r) Room G	120	8.3	7.5	21.7	18.3
1957 (5-10 × back- ground r) Room R	243	21.4	15.6	25.5	19.8
1957 (shielded by lead) Room R	105	11.4	9.4	13.3	10.4

^a F₁ generation derived from DRA/1 by C57BL6.

TABLE V

FIRST TRIAL INCIDENCE OF AUDIOGENIC SEIZURES IN MICE OF BACKCROSS
GENERATION (F₁ × C57BL6) UNDER VARIOUS ENVIRONMENTAL CONDITIONS^a

Period	r dosage	N	Incidence of seizures (%)			
			Fatal		Total	
			♂ ♂	♀ ♀	♂ ♂	♀ ♀
5/57 to	background	202	12.1	5.8	1.14	6.8
8/57	<i>no source</i>	276	<i>12.1</i>	<i>8.1</i>	<i>1.29</i>	<i>9.6</i>
9/57 to	background	147	14.9	11.7	25.3	18.3
11/57	<i>0.2 mr/hr</i>	<i>380</i>	<i>28.6</i>	<i>23.4</i>	<i>38.8</i>	<i>34.2</i>
	1.5r - 2 r	222	24.1	30.7	27.8	35.1
4/58 to	background +	560	13.7	19.6	17.6	22.5
9/58	<i>no source</i>	477	<i>15.6</i>	<i>18.6</i>	<i>22.3</i>	<i>19.9</i>
9/58 to	background ++	148	23.4	29.6	23.4	31.0
11/58	<i>0.2 mr/hr</i>	<i>112</i>	<i>45.8</i>	<i>37.5</i>	<i>47.9</i>	<i>43.8</i>

^a KEY: Regular type, backcross in Room G; *Italic* type, backcross in Room R; **Boldface**, backcross irradiated at Argonne.

quency of total and fatal seizures was significantly greater among the Room R litters than in the Room G controls. At this time, a series of backcross litters were irradiated at Argonne Hospital, receiving 1.5–2 r between days 23 and 30. While the incidence of seizures was close to that observed in Room R, the elevation was greater in females than in males. Another unexpected control period occurred in 1958 when the source was not in use, followed by a period of exposure to 0.2 mr per hour.

The higher frequency of seizures among the controls in Room G beginning in September, 1957, coincided with increasingly high background from radioactive fallout (to be discussed below.) The data summarized in Table V lend further support to the belief that the radiation level in Room R was responsible for the elevation of the incidence of audiogenic seizures among the mice reared in this environment.

Glutamate Protection in DBA/2

In DBA/2 mice, the incidence of audiogenic seizures is so high, approaching 100%, that any increase in susceptibility would ordinarily not be detectable. However, in a series of mice injected with glutamic acid, we have evidence that low level radiation also influences the sound susceptibility of these mice. Glutamic acid, injected as monosodium glutamate 30–45 minutes before the sound test, gives almost complete protection from seizures in DBA/1.

In 1954, Dr. Benson Ginsburg at Jackson Laboratory, found that one injection of glutamic acid (20 mg per 10 g body weight) confers significant protection from audiogenic seizures in DBA/2 mice, with the seizure frequency reduced more in females than in males. In the same summer, we attempted to enlarge his series, using the same dosage in our DBA/2 line. These mice, derived from Jackson Laboratory stock, had been maintained by brother-sister matings in our laboratory since 1951. Twenty mg of glutamic acid gave little, if any, protection from seizures in mice kept in Room R (Table VI). With 30 mg, protection was only moderate. In 1957, with the mice maintained in Room G at background, the glutamic acid series was repeated, using DBA/2 mice which were direct descendants of those in the 1954 series. In the absence of radiation, 20 mg glutamic acid reduces significantly the frequency of seizures and deaths; at 30 mg, the protection is excellent. Contrary to Ginsburg's findings, however, we observed no sex difference in the response to glutamic acid, possibly an indication of genetic change in our DBA/2 line.

Additional evidence of a difference in response to glutamic acid in the presence and absence of low level radiation is seen in the latency of fatal seizures during the two periods. Figure 3 shows the latency curves of control

TABLE VI

EFFECT OF MONOSODIUM GLUTAMATE IN PROTECTING DBA/2 MICE FROM AUDIOGENIC SEIZURES UNDER DIFFERENT ENVIRONMENTAL RADIATION LEVELS

Group	Dose (mg)	N	Total seizures		Tonic-clonic seizures		Fatal seizures	
			♂	♀	♂	♀	♂	♀
Jax Lab, 1954	00	92	96.1	95.1	94.1	85.3	84.3	73.2
	20	70	74.2	54.3	57.1	25.7	28.6	20.0
Room R, 1954 (5 to 10 × background)	20	38	100.0	100.0	81.8	81.3	72.7	75.0
	30	200	84.7	87.3	70.3	60.7	65.8	58.4
Room G, 1957 (background)	20	81	79.5	83.3	53.9	50.0	41.0	42.9
	30	149	59.5	57.1	30.4	24.3	26.6	21.4

DBA/2 mice and of those injected with glutamate. The bimodal curve is typical of uninjected DBA/2 mice, which show many premature seizures. In the injected group maintained in Room R with low level radiation, there was a reduction in the frequency of early seizures and a 10 sec delay in the second peak. In the injected series maintained at background level, the curve was displaced far to the right, with no premature seizures and the second peak 25 sec later than in controls.

Both the reduction in frequency of seizures and the longer latency of fatal seizures indicate that protection by glutamic acid is much more effective in the absence of extra radiation in the environment.

Implication of Radioactive Fallout in Increasing Seizure Frequency

After the colony was moved to Room G, our data appeared to be stabilized, with repeatable results in all groups. However, a puzzling development appeared in May, 1957, with a sudden, dramatic new increase in seizure susceptibility for the following 6 months (Table VII).

We investigated the possibility of a new radiation source, which was not found. Records of local radioactive fallout, supplied 1 year later by the New York Operations Office of the Atomic Energy Commission, showed that the sudden rise in seizure frequency coincided exactly with a sharp increase in fallout level. The first litters which showed unusual sound sensitivity were born on April 8; on April 10, an 80-fold increase in beta and gamma radiation was recorded by the AEC. There were similar bursts on April 13 and 15, and a higher level persisted for several months.

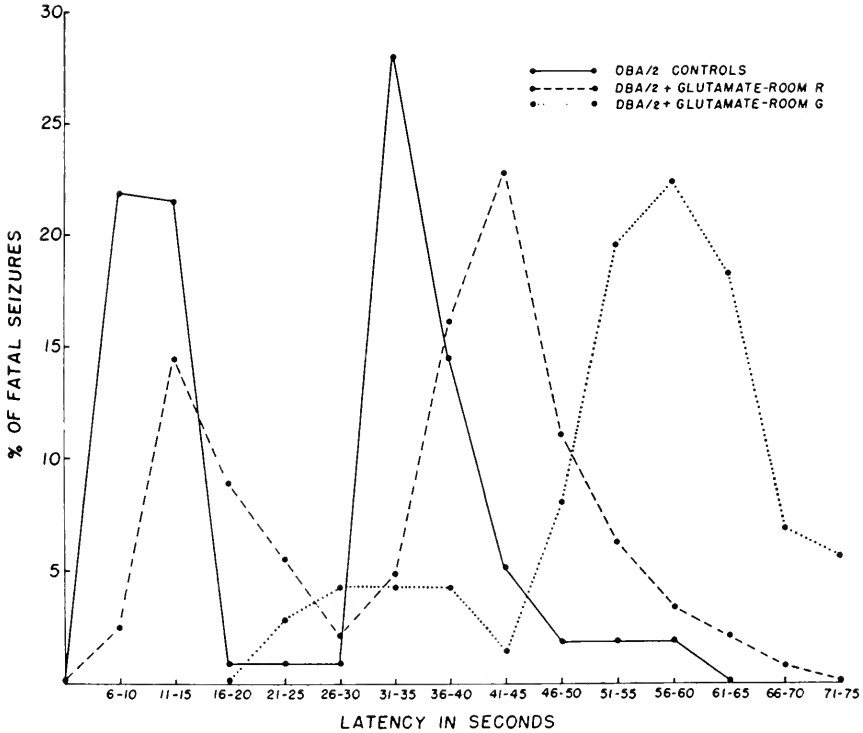


FIG. 3. Latency of fatal audiogenic seizures in DBA/2 mice, control and injected with glutamate, 20 mg per 10 gm body weight. In Room R, the mice were exposed chronically to gamma radiation at 5 to 10 times background. Room G was at low background level.

In addition to the striking increase in seizure frequency, the latency of fatal seizures also reflected the higher susceptibility of the mice in response to the higher background level. Figure 1 compares the latency of fatal seizures in F_1 males, controls and those irradiated at Argonne Hospital, during the two 6-month periods before and after May, 1957. In each period, the latency was shorter following irradiation at 1.5–2 r, with an earlier peak and few delayed seizures. However, during the high background period (May to October, 1957), the latency of seizures in the controls was shorter than in the earlier irradiated group. Irradiation at this time pushed the curve even farther to the left, in the direction of earlier seizures. Figure 2 presents similar comparisons in F_1 females. In all groups, the latency was shorter in males than in females.

Following the sudden rise in radioactive fallout in April, 1957, the back-

TABLE VII

CHANGES IN INCIDENCE OF AUDIOGENIC SEIZURES IN FOUR GROUPS OF MICE BEFORE AND AFTER MAY, 1957^a

Group	N	First trial		Four trials	
		Deaths (%)	Seizures (%)	Deaths (%)	Seizures (%)
DBA/1 (original stock)	217	26.7	33.2	54.7	58.0
	<i>165</i>	<i>62.4</i>	<i>66.7</i>	<i>81.7</i>	<i>87.2</i>
DBA/1 (new Jax stock)	234	30.3	32.9	73.8	74.7
	<i>82</i>	<i>78.0</i>	<i>84.1</i>	<i>87.7</i>	<i>91.4</i>
F ₁ (our DBA/1 by Jax C57BL6)	213	22.1	30.1	43.2	51.6
	<i>378</i>	<i>39.9</i>	<i>52.4</i>	<i>51.8</i>	<i>64.2</i>
F ₁ (Jax DBA/1 by Jax C57BL6)	166	25.3	27.1	53.0	56.0
	<i>156</i>	<i>57.1</i>	<i>62.8</i>	<i>66.5</i>	<i>69.7</i>

^a Figures in ordinary type: tested October, 1956 to May 1, 1957. Figures in *Italic* type: tested May, 1957 to October, 1957.

TABLE VIII

CHANGES IN INCIDENCE OF FIRST TRIAL FATAL AUDIOGENIC SEIZURES IN THREE GROUPS OF CONTROL MICE DURING 27 MONTHS OF CHANGING RATE OF RADIOACTIVE FALLOUT^a

Interval ^b	Relative ^c fallout	Incidence of fatal seizures (%)					
		F ₁		Backcross		DBA/1	
10/56 to 4/57	0.6	(379)	23.8	(120)	7.5	(451)	28.6
4/57 to 10/57	2.2	(534)	44.9	(202)	8.9	(247)	67.6
1/58 to 5/58	2.7	(433)	58.2	(246)	24.9	—	—
5/58 to 9/58 ^d	4.1	(548)	42.6 ^d	(560)	16.6 ^d	(128)	74.6
9/58 to 11/58	3.6	(185)	58.4	(148)	26.4	(64)	67.9
11/58 to 2/59	3.8	(218)	78.0	(241)	34.8	(60)	67.7
2/59 to 5/59	4.5	(163)	74.6	(205)	30.5	(66)	69.7
6/59 to 8/59	1.4	(138)	53.6	(161)	14.3	(96)	58.1
9/58 to 12/59	0.2	(768)	42.7	(313)	12.8	(86)	51.2

^a Numbers in parentheses are numbers of mice.

^b Mice were born during this interval and tested 30 days later.

^c See text for explanation of arbitrary relative figure.

^d Mouse quarters were scrubbed thoroughly in June, 1958.

ground level fluctuated, but remained high, reaching a peak from October, 1958, through April, 1959, then gradually declining. Table VIII shows the percentage of fatal seizures on the 1st trial in 3 groups of untreated mice (F_1 , backcross, and DBA/1) during 9 intervals of several months each. The figures extend through the 27 months during which the background level was changing. A "relative fallout" figure is shown for each time interval, based on fallout records supplied by Health Physics, the Atomic Energy Commission, and Argonne National Laboratory.

The 3 populations of control mice responded differently to the changing background level. The F_1 group, with intermediate seizure susceptibility, shows the closest correlation with fallout level. In the backcross generation, a low susceptibility group, the response did not change significantly until the second increase in background, after which the incidence of fatalities increased. The DBA/1 mice, the most seizure prone, appear to have a low threshold for radiation effect. The incidence of fatalities was stimulated maximally at the first rise in fallout and showed little further change until the extreme decline in background level late in 1959. Some lag in the effect of fallout rate was observed; this would be expected on the basis of the contribution of fallout to food content and to dust in the animal rooms. (The lower incidence of fatalities during May to September, 1958 almost certainly is related to lower background in the mouse quarters despite the high fallout rate. At that time the whole installation was thoroughly scrubbed and disinfected because of an infestation of mites.)

It should be emphasized that the changes in seizure frequency were observed first, and the figures on fallout were obtained later in an effort to explain the shifts in our data.

A series of DBA/2 mice, maintained in Room G at background, were injected with 20 mg glutamic acid between June, 1959, and February, 1960. The protective effect of glutamate on the incidence of seizures was moderate, with a slight reduction in total incidence, but a higher proportion of non-fatal seizures. However, the curve representing the latency of fatal seizures in this group was unlike that obtained in Room G during the earlier period of low background (Fig. 3). Figure 4 shows the latency curves of DBA/2 control mice and those injected with glutamate. For comparison, we have added the curve showing the latency of glutamate-injected mice maintained in Room R under chronic low level gamma radiation during 1954. In the uninjected controls, the early peak was exaggerated over the typical DBA/2 curve, with an unusual proportion of the mice convulsing between 6 and 10 sec after the onset of stimulation. In the mice injected with glutamate, the latency figures were similar to those of the earlier group reared in Room R; the difference shown in the high background group is in the direction of shorter latency, indicating greater seizure susceptibility. These curves suggest

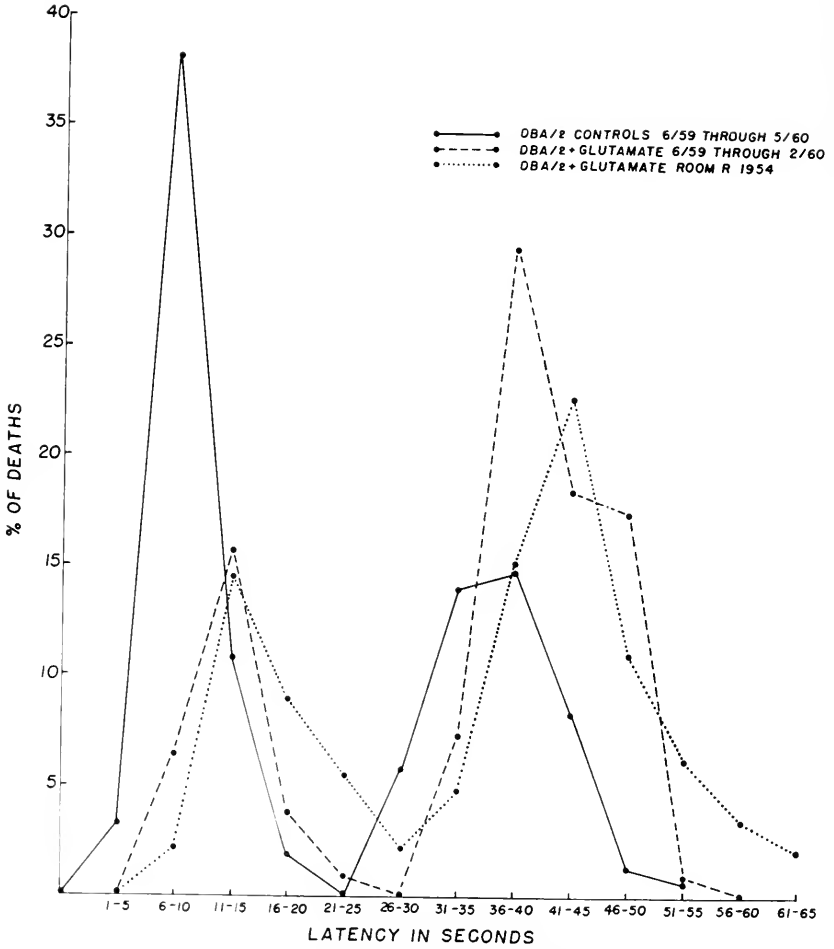


FIG. 4. Latency of fatal audiogenic seizures in DBA/2 mice, control and injected with 20 mg glutamate during elevated background radiation level. For comparison, curve (from FIG. 3) obtained in Room R during chronic exposure to low level gamma radiation has been repeated.

that the recent group reared in Room G received as much radiation from fallout as the mice in Room R received from the Co⁶⁰ source.

Discussion

In these studies we have observed a significant increase in susceptibility to audiogenic seizures following surprisingly low doses of ionizing radiation.

The time when radiation is applied appears to be important. If the mice were exposed from birth throughout the 30 day period before being tested for sound sensitivity, doses of gamma radiation as low as 4.8 mrad per day (total dose 0.14 rad) increased the frequency and severity of seizures. When the period of exposure was between days 23 and 30, a dosage of 350 mrad per day (total 1.5 to 2 rad) was required to induce a significant rise in seizure susceptibility. In one series of 150 F_1 mice irradiated at $\frac{5}{8}$ of this dosage (220 mrad per day), the incidence of seizures was elevated in females, but not in males. In another group of 100 F_1 mice, this dosage was without effect in either sex.

Some fragmentary evidence suggests that radiation during the first few days of life is especially effective in increasing seizure susceptibility; the first 11 days may be critical. Backcross litters exposed for only the first 3 days to the remote Co^{60} source in Room R showed a slight increase in seizure-incidence. In the transition group of DBA/1 mice, born in Room R and moved to Room G before being tested, those litters which had been in Room R for 7-10 days were as seizure prone as those which had been in the room for 17-19 days. From March to August, 1960, we had access to an insulated cave in the hope that this would constitute a low background control environment. A population of F_1 matings and litters was moved into the cave. However, the incidence of seizures in this group was high rather than low. In monitoring the room, Health Physics found a spot on the concrete floor which emitted 7.5 mr per hour of mixed beta and gamma radiation: the intensity dropped to 0.4 mr per hour at a distance of 6 in. The cave, therefore, served as an unexpected low level exposure situation, rather than the reverse. Among the first litters tested in the cave, those that were older than 11 days when moved to the cave showed low seizure incidence. In the litters that were 3-11 days old when introduced into the cave, a high proportion suffered fatal seizures on the first trial. The fact that neonatal mice are small and naked may explain why they are unusually radiosensitive. The influence of exposure to radiation at various intervals in the life span has not been tested systematically.

An interesting contrast was observed in regard to the interaction of the high fallout level with other sources of radiation in influencing seizure susceptibility. In the backcross generation maintained in Room R, the effects of increased background from fallout and of chronic low level gamma radiation appeared to be additive (Table V). Both factors were operative throughout the 30 day period before the sound test. In the F_1 generation, 1.5-2 rad given after weaning resulted in no greater incidence of seizures when the mice were reared in a high background environment than at low background (Table III). This difference is a further indication that very low doses of

radiation applied early are especially effective in influencing the audiogenic seizure response.

We have no reasonable explanation for the basis of the heightened seizure susceptibility following low level radiation. Several lines of evidence suggest that seizure susceptibility may be related to a defect in carbohydrate metabolism in brain tissue. Insulin and glutamic acid, which are among the most effective protective agents, may compensate in part for this defect. Ginsburg (1954) has screened many metabolically active substrates and antimetabolites for their influence on audiogenic seizures in DBA mice. Some of these compounds reduce and some enhance the number and severity of seizures. Ginsburg postulates that deficiencies in particular enzymatic reactions leading to normal, controlled energy release in the nervous system are the basis for the seizures. If this metabolic hypothesis is correct, the action of radiation in increasing the incidence of seizures may be at the enzymatic level. At present, this suggestion is speculative.

Conclusion

The audiogenic seizure response appears to be remarkably radiosensitive in DBA mice and in hybrid generations derived from crossing DBA with C57 mice. A striking increase in susceptibility to audiogenic seizures has been observed following exposure to very low doses of ionizing radiation. Heightened susceptibility is indicated by an increase in frequency and severity of seizures, by a higher percentage of total and fatal seizures on the first trial, and by shorter latency of fatal seizures.

In these mice, which are tested for sound sensitivity at 30 days of age, chronic exposure to gamma radiation from birth throughout the 30 days, at a total dosage of 0.14 r, caused a significant increase in seizure susceptibility. In mice exposed to Co^{60} between days 23 and 30, a dosage of 350 mrad per day (total, 1.5–2 rad) resulted in heightened susceptibility.

In DBA/2 mice, the effect of glutamic acid in protecting them from seizures was negated when the animals were maintained in an environment of 4.8 mrad per day (5–10 times background).

Evidence was presented indicating that the level of radioactive fallout from April, 1957, through 1959, was sufficient to enhance the frequency and severity of seizures.

In addition to the primary physiologic effect on the seizure response, evidence of genetic change affecting seizure susceptibility in both DBA/1 and C57BL6 was noted following chronic exposure to gamma radiation at 5–10 times background.

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Effects of Ionizing Radiation on Visual Function

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Research workers studying the visual system have measured spectral sensitivity (or, in the jargon of radiobiology, relative biologic effectiveness) qualitatively for nearly 300 years and quantitatively for over 100 years (Duke-Elder, 1938). As a result, a great deal of experience has been obtained with problems similar to those now of interest to radiobiologists.

The clearest single principle that has emerged from these centuries of study of the effects of radiation on the visual system is the importance of the organization of that system in determining the effect observed. It has turned out that the organization of the biologic system is crucial at all levels from the molecular through the organismic.

Consider the finding that 7 photons (each of at least 1.8 ev energy) absorbed simultaneously, one each in 7 human photoreceptor cells, can cause the following sequence of events before a subject pushes a button to indicate he has seen a flash of light: activation of the 7 photoreceptor cells; activation of at least one bipolar cell each of the retina, ganglion cell of the optic nerve, neuron of the lateral geniculate body, and neuron of the optic cortex; and a change in activity of at least several hundred optic cortex neurons, an unknown number of motor cortex neurons, several hundred motor fibers, and several thousand muscle fibers (Adler, 1953; Fulton, 1955; Hecht *et al.*, 1942; Polyak, 1957; Walls, 1953).

Consider further that the action potential produced by each of the activated neurons and muscle fibers involves the movement out of the cell of about 4×10^{12} ions per cm^2 of nonmyelinated cell surface (Hodgkin, 1951). So at least 10^{13} ions have been caused to move as a result of the absorption of only 7 photons with an energy content of about 13 ev. Since each ion moves against an electric potential of about 0.1 volts (Hodgkin, 1951), this is an energy change of about 10^{12} ev. The chance of such a small energy input causing such a large energy change in a random ensemble of atoms is infinitesimal. It is apparent that the biologic system must be highly organ-

ized and must contain local concentrations of potential energy for such a sequence of events to occur.

High energy radiation can act on the visual system to produce the same sensation as light (Lipetz, 1955b), and the evidence is consistent with the idea that radiation does so by the same mechanisms as does light (Lipetz, 1953, 1955a). X-rays can evoke a sensation of light with a dose as low as 0.5 mr (Bornschein *et al.*, 1953; Pape and Zakovsky, 1954).

At each structural level of the visual system there are examples of energy transfers which would be most improbable were it not for the organization at that level. *At the molecular level*, there is strong evidence (Granit, 1947) that scotopic (dim light) vision is initiated by the absorption of photons by a single kind of molecule, rhodopsin (visual purple). This molecule consists of a lipoprotein, opsin, to which is attached a pigment group (chromophore) called retinene, which is the aldehyde of vitamin A. Only one stereoisomer of retinene, the 11-cis, a bent chain form, can unite with the opsin to form rhodopsin. When a photon of sufficient energy is absorbed by a rhodopsin molecule, the retinene changes to a different stereoisomer, the all-trans, or straight chain form (Kropf and Hubbard, 1958). This is the primary action of light on the visual system.

It appears that this action occurs only if the photon is absorbed in the retinene, not in the opsin, and only if the retinene receives at least 1.8 ev energy. If the photon absorbed by the retinene has energy less than 1.8 ev, it is still possible that thermal energy sufficient to bring the total to 1.8 ev would be transferred from the opsin to the retinene and cause the retinene to change its shape. In this instance, an *irreversible intramolecular energy transfer* has resulted from the particular organization of the rhodopsin molecule (St. George, 1952).

A mechanism has been suggested (Kropf and Hubbard, 1958) by which the primary action of light, this stereoisomeric change in the retinene, can lead to further changes in the visual system. It is postulated that the 11-cis isomer of retinene is the only one that can combine with opsin to form rhodopsin because that isomer has a shape that permits its entire length to fit closely to a surface of the opsin group, close enough so that interatomic bonds can join the retinene to the opsin at several points. When the absorbed photon causes the retinene to change its shape, sections of the retinene lift away from the opsin, breaking the bonds to opsin along that section. This frees broken bond sites on both the retinene and opsin, allowing them to react with other molecules.

In support of this mechanism are the findings that the stereoisomeric change of the rhodopsin chromophore by light will occur even at low temperatures and without water, but that further changes in the rhodopsin (such as bleaching) and further activation of the visual system occur only

if the rhodopsin is in the presence of water and heat (Wald *et al.*, 1950). Presumably, the water and heat permit ions and molecules to move more rapidly to their reaction site, the freed bond sites on the retinene and opsin, and even furnish some of the energy needed for the reactions. It is suggested that these reactions catalyze other reactions and so make large numbers of reacting particles available for further reactions.

Rhodopsin is found only in the outer segment of the rod cell, where it forms part of a *submicroscopic* lattice. The outer segment has an outer membrane consisting of a layer of aqueous protein molecules next to a layer of lipoprotein molecules with the rhodopsin molecules lying in between (Wolken, 1958). This membrane is stacked up in folds for the entire length of the outer segment and continues into tubules (like those found within cilia), which extend into the rod's inner segment (De Robertis, 1956; Sjöstrand, 1960). It can be estimated from data on rods taken from cattle that the human rod's outer segment contains several million rhodopsin molecules (Hubbard, 1954). The remarkable thing is that light absorption by *any one* of these molecules, whatever its position, can cause activity to spread from that rod to adjoining neurons and on through the visual system.

The spread of activity, even just along the rod's outer segment, is too fast to be attributed to diffusion, so another mechanism must be found. There is no doubt that the peculiar structure of the outer segment membrane is concerned. In vertebrate and arthropod eyes and in chloroplasts of plants, photopigments have always been found arranged in similar repeatedly invaginated continuous membranes (Wolken, 1958). My guess is that the isomerized rhodopsin molecule, which in every case lies at the boundary between the inside and outside of the outer segment, catalyzes the formation of free electrons or electron holes. These may then be rapidly transferred in crystal fashion along the membrane lattice to the inner segment. In support of this suggested mechanism is the finding that illumination of chloroplasts causes the formation of electron spin resonances (Tollin *et al.*, 1958). Whatever the actual mechanism, it is apparent that the submicroscopic organization of the cell has made possible the rapid transfer of energy along large portions of the cell.

At the *cellular level* little is known about the activity transfer mechanisms of the vertebrate. The mechanisms of the somewhat simpler compound eye of the horseshoe crab, *Limulus polyphemus*, probably acts as follows (Lipetz, 1960b). When the photoreceptor is illuminated, it releases a substance that changes the surface membrane's permeability on the adjoining dendrite of a neuron. This changed permeability permits positive ions to flow into the dendrite from the higher concentration outside the neuron, and this in turn causes a net outward flow of similarly charged ions through the cell membrane at the neuron's cell body and axon. This ion flow through the axon

changes the properties of its surface membrane and causes the generation of nerve impulses.

Such mechanisms require that stored energy be available; in this case, in the form of ion concentration gradients. The metabolic processes that built up such concentration gradients are made possible only by the organization of the cell. At the same time, this organization also limits the number of mechanisms by which stored energy can be discharged.

At *tissue and organ levels*, the importance of organization is obvious. The visual system no longer responds to light if the retina is replaced by a piece of dermis, for example.

At the *organismic level*, subtle changes in organization can produce large changes in response. A human subject might be repeatedly exposed to a flash of light and never once push the button. But, if the experimenter now merely says to him, "When you see a light, push the button," the subject will then push the button after every flash. The structural change that occurred in the organization of the organism is presently beyond our powers to specify, yet the change in the effect of a given radiation stimulus on the system was overwhelming.

As these examples show, the visual system is so organized at all levels as to be extremely sensitive to light, that is, to give readily detected responses on exposure to low doses of light. It does this by using the energy of the light to trigger the release of a larger amount of energy, and this to trigger the release of still larger amounts, so that even within the visual system proper an energy amplification of 10^9 may be obtained.

This sensitivity need not be limited to light. Anything that could trigger the initial energy release in the sequence would give the same effect. Ionizing radiation can do this, apparently by breaking up the visual pigment molecule (Lipetz, 1955a), with the result that normal visual responses can be obtained with as little as 0.5 mr of x-rays. Table I lists the quantitative studies that have been made of normal visual responses evoked by high energy radiations.

The great sensitivity of the visual system depends on the high specificity of its organization. Since that specificity can be disturbed by comparatively low energy agents, very little energy should be required to produce reductions in the sensitivity of the visual system. Indeed, objectively observed decreases have been produced with as little as 10.5 r. Table II lists the quantitative studies of damaging effects of high energy radiations on visual function.

A final word of warning: the second clearest principle that has emerged from the study of vision is that the organization, at all levels, is in a state of flux. For some time workers in visual research have been familiar with the constant exchange of atoms in a molecule, the slow growth and development

TABLE I
VISUAL RESPONSES TO HIGH ENERGY RADIATION

Threshold	Effect	Subject	Radiation	Reference
160-180 r/min	Swim downward	Daphnia	115 kv x-ray	Baylor and Smith, 1958
24 β + 3 γ mrep? estimate	Flash ERG fluorescence	Frog	60 mg radium at 10 cm 3.15 Mev β 1.3 Mev γ	Thier, 1933
24 β + 3 γ mrep/sec? (estimate)	Continuous ERG fluorescence	Frog	3.15 Mev β 1.3 Mev γ	Thier, 1933
< 5r	Decrease of light threshold	Lamulus	79 kv x-ray	Dawson and Smith, 1959
1-10 r	Flash ERG	Frog	2 kv x-ray	Bankman, 1960
156 mr + 35% γ	Discharge of optic neuron	Frog	65 kv x-ray	Lipetz, 1953, 1955a
16 γ mrep/sec	Continuous light	Human	1.3 Mev γ	Thier, 1933
8 to 24 mr/sec	Continuous light	Human	76 kv x-ray	Newell and Borley, 1941
1 mm ² retina	Continuous light	Human	40-80 kv x-ray	Bornschein <i>et al.</i> , 1953
1.6 to 3.7 mr/sec	Flash of light	Human	65 kv x-ray	Lipetz, 1953, 1955a
0.5 to 1.0 mr computed from frog	Flash of light	Human	135 kv x-ray	Gantovoi and Burdianskaja, 1959
< 0.7 to 2 mr	Flash of light	Human	40-180 kv x-ray	Bornschein <i>et al.</i> , 1953
0.5 mr	Flash of light	Human		

TABLE II
 DAMAGE OF VISUAL FUNCTION BY HIGH ENERGY RADIATION

Threshold	Effect	Subject	Radiation	Appears in	Reversible	Reference
< 2400 r in air at skin	Reduced rate and extent of dark adaptation	Human	x-ray, therapeutic	< 30 days	?	Lenoir, 1944
6000-8900 rad	Reduction in ERG to light	Rabbit	100 kv x-ray	3-4 min	No	Baily and Noell, 1958
4400-5500 rad	Reduction in ERG to light	Rabbit	250 kv x-ray	3-4 min	No	Baily and Noell, 1958
3100-4100 rad	Reduction in ERG to light	Rabbit	2000 kv x-ray	3-4 min	No	Baily and Noell, 1958
?30 to 300r? (estimate)	Shooting star flashes Pain in orbit and globe	Human	? kv x-ray	? minutes 1/2 hour	Yes ?	Kolle, 1897
50 β + 3 γ rep? (estimate)	Loss of Ra phosphene	Human	1.8 Mev γ 3.15 Mev β	1 min	?	Himstedt, 1900
4 β + 6 γ rep? 10 r	Reduction in ERG Increase, then decrease in ERG	Frog Frog	3.15 Mev β 180 kv x-ray	15 min 20 min	No No?	Thier, 1933 Avakyan, 1958
120 r	Rise in x-ray vs light threshold for optic neuron response	Frog	65 kv x-ray	Cumulative minutes to hours	Yes	Lipetz, 1953, 1955a
11 r	Rise in light threshold	Frog	65 kv x-ray	< 20 sec	Yes	Lipetz, 1953, 1955a
0.7 r	Rise in x-ray threshold	Frog	65 kv x-ray	< 10 sec	Yes	Lipetz, 1953, 1955a
0.5 r	Reduced voltage of action potentials at optic neuron	Frog	65 kv x-ray	< 10 sec	Yes	Lipetz, 1953, 1955a
0.1 to 0.4 r	Increased difference in thresholds for appearance vs disappearance of electric phosphene	Human	70 kv x-ray	10 to 15 min	Yes	Umetzu, 1956; Motokawa <i>et al.</i> , 1956
0.4 mr	Increased variation in the above difference	Human	70 kv x-ray	same ? Cumulative	Yes	Motokawa <i>et al.</i> , 1957

of cells, the changes of the adaptation of the retinal and neural tissues, the movements of the eye, and the many subtle changes in the state of the organism. Recent electron microscope studies have shocked visual researchers by showing that, in addition, the submicroscopic organization of the photoreceptor can and does change greatly during light adaptation (Fernandez-Moran, 1960).

In summary, the visual system is an example of how the response of a biologic system to a stimulus is determined by the organization of that system, and this was shown for all structural levels of organization. Light energy absorbed in the visual system triggers the release of greater amounts of energy, and the latter the release of still more, etc., in a sequence of energy-amplifying processes. High energy radiations can also trigger such processes. The great sensitivity of a biologic system, such as the visual system, is achieved by the great specificity of its organization, and, as a consequence, small changes in the organization can produce large changes in the sensitivity. Studies have shown such changes in the visual system produced by comparatively small doses of high energy radiations.

In conclusion, the author suggests, in view of the principles evidenced in the above discussion, that radiobiology be defined as the study of the organization (at the molecular, submicroscopic, cellular, tissue, organ, and organismic levels) of the ensemble of atoms in part of which the radiation energy is absorbed and in which the response is observed.

Appendix: Effects of High Energy Radiation on Visual Function

The known stimulating and damaging effects of high energy radiation on visual function are listed in Tables I and II, respectively. Most of the studies listed have been discussed in earlier reviews (Lipetz, 1955b, 1960a), so only the newly added studies will be discussed here.

Baylor and Smith (1958) found that a small crustacean, *Daphnia magna*, showed positive geotaxis (swimming downward) and negative phototaxis (swimming away from a red light source) when exposed to sufficiently intense x-rays. The threshold dose rate was between 160 and 180 r per min. This behavior is typical of light stimulation of the violet receptor of its naupliar eye. The heat and fluorescence produced by x-rays were insufficient to account for this effect. The effect could be duplicated with reducing compounds added to the water bathing the animals.

Dawson and Smith (1959) recorded action potentials from a single optic nerve fiber leading from the compound eye of the horseshoe crab, *Limulus polyphemus*. It was found that as little as 5 r of x-rays caused a significant decrease in the threshold intensity of a light flash capable of evoking such a response in a fully dark-adapted eye. A cumulative lowering of the light

threshold was caused by further x-irradiation up to 150 r. After x-irradiation, this lowered threshold remained constant in the dark for at least 6 hours. When the eye was light-adapted and then dark-adapted after the x-irradiation, the light threshold always returned to the normal level, indicating that the x-irradiation had acted on the photochemical system of the eye. The visual pigment of the *Limulus* eye is known to be similar to that of the vertebrate eye (Hubbard and Wald, 1960).

Brinkman and Lamberts (1960) measured the over-all change in electric potential appearing across the whole eye (electroretinogram or ERG). He found that a detectable ERG could be produced in the intact frog's dark-adapted eye by momentary exposure to as little as 1 r x-irradiation.

Gurtovoi and Burdianskaia (1959) determined the threshold dose to the dark-adapted human eye to produce a sensation of light. They found that the threshold varied from person to person, but was constant for months for a given person. For a 0.14 sec irradiation the thresholds of 5 subjects lay somewhere between no response at 0.3 mr and 100% response at $3.0 \text{ mr} \pm 20\%$. For shorter durations, the threshold dose was somewhat reduced. Their results are in good agreement with the 0.5–1.0 mr threshold found by Bornschein *et al.* (1953) for a 0.020 sec duration.

Lenoir (1944) measured the course of dark-adaptation (to light) in cancer patients before and after many days of therapeutic x-irradiation. In every case the rate of initial dark-adaptation was slowed, and in most cases the final light threshold was raised.

Baily and Noell (1958) measured the doses of 100, 250, and 2,000 kvp x-rays needed to produce acute and irreversible reduction of the rabbit's ERG response to light. The doses agreed closely with those that produced death (lysis) of the visual cells. The relative biologic efficiency was greater for x-rays with lower linear energy transfer. This suggests that in the visual cell there are only a few critical sites of rather large extent which need be damaged to interfere irreversibly with the metabolism, causing reduction of the ERG amplitude in a few minutes and cell death in a few days.

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X-Irradiation Studies on the Mammalian Retina*

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Our studies on the effects of x-irradiation originate from our concern with mammalian retinal cells and their susceptibilities to poisons as related to the function of the retina and to hereditary retinal diseases (Noell, 1951-1959).

The retinal cells of main interest are the visual cells, particularly the rod cell, which in Fig. 1 is depicted as elaborated by Sjöstrand (1956), De Robertis (1956), and others. It comprises a sensory ending, the rod, which has an outer and an inner segment, a nuclear region, varying somewhat in location, and a fiber which terminates in a synapse of unusual morphologic arrangement with the bipolar cell. The outer segment contains the photosensitive pigment, rhodopsin, and is the site of the primary visual events. It consists of closely packed thin discs, which probably derive from foldings of the plasma membrane, and lacks cytoplasm and the enzymes of the respiratory and glycolytic systems (Lowry *et al.*, 1956). The inner segment seems to be the site of high metabolic activities. It is rich in enzymes, and its distal half is filled with mitochondria. In the rabbit, the distal part of the inner segment is the only region of the visual cell which contains mitochondria and, therefore, is the principal, and probably only, region capable of reducing oxygen. In the mouse and rat, one additional mitochondrion is located within the synaptic ending. Hence, glycolytic reactions may generally be of greater significance for the visual cell than for other cells. Indeed, the mammalian retina is known for its high glycolytic activity in the presence of oxygen. Its metabolism resembles that of cancer tissue much more than that of the brain, of which it is ontogenetically a part.

X-irradiation affects the rod cells of the mammalian retina selectively, killing them, but not other retinal cells. This selectivity has been observed with rabbit, rat, dog, and monkey. Predominant effectiveness on the rod cells is not an exclusive property of x-irradiation, but is characteristic of intravenous iodoacetate, a poison well known for its inhibitory effect on

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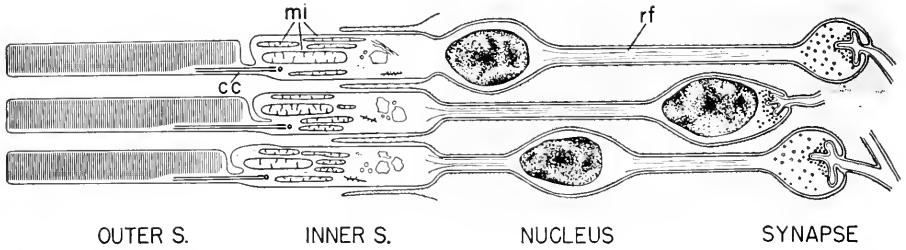


FIG. 1. Rod cell of the mammalian retina. Outer S., outer segment; Inner S., inner segment; cc, connecting cilium (De Robertis, 1956); mi, mitochondria; rf, rod fiber. The shaded area denotes Müller cells, which, like the glia cells of brain, fill the spaces between the neuronal elements of the retina.

glycolysis, and of oxygen at high pressure. The histopathology of x-irradiation is principally the same as observed in these cases; however, in several other respects, the retinal effect is very different.

Our x-irradiation studies, principally on the adult albino rabbit, were performed mainly with Dr. Norman Baily, now of Los Angeles. They will be presented more extensively in the near future. The action of various forms of x-irradiation on the retina was followed by electroretinography, and a correlation was attempted between effects on functional capacities, structure, and metabolism.

Procedure

The main methods used have been outlined by Baily and Noell (1958). The x-ray beam passed through the head of the rabbit in the main axis of both eyes, entering through the cornea of the close eye and passing out through the cornea of the distant eye. To minimize radiation damage to surrounding tissues, the smallest field size consistent with the necessity for uniform radiation intensity over the entire entrance region was employed. Eyes and x-ray beam were carefully aligned, so that every experiment was identical. The x-ray beams were produced by self-rectified generators operating at 100, 250, and 2,000 kv peak potential (kvp).

Because of the peculiar geometry of the eye, precise experimental determination of the distribution of the absorbed dose throughout the retina was not possible. It was felt that the best solution was to combine the knowledge of standard depth dose curves with certain experimental determinations described by Baily and Noell (1958), to obtain the dose at the various regions of the retina.

The retina covers the major portion of a spherical shell, thereby conditioning different doses for different regions. Our results concerning effects of

x-irradiation on the electroretinogram—a “mass response” of the retina—were evaluated in terms of the dose delivered to the anterior rim of the retina, the posterior pole, and an imaginary circle dividing the retina into an anterior half and a posterior half. The dose delivered to this imaginary circle (called the medium retinal dose) served as the principal reference. The minimal doses for visual cell death were determined histologically, usually after 10 to 14 days. The rate at which the medium retinal dose was delivered to the eye closest to the beam was, in rads per minute, 174 for 100 kvp, 482 for 250 kvp, and 302 for 2,000 kvp. Usually, the eyes were irradiated once; and, usually, results refer to irradiation at 250 or 2,000 kvp.

The electroretinogram (ERG) was recorded continuously during irradiation in response to brief flashes of light throughout irradiation and at predetermined times after irradiation.

Histologic Effects

The typical appearance of the rabbit retina after a dose which produced visual cell death is illustrated in Fig. 2. One medium retinal dose of 5,300 rads had been delivered in 11 minutes. Ten days after irradiation, the visual cells have disappeared. The pigment epithelium is preserved, and ganglion and bipolar cells are not measurably decreased in number.

Using 2,000 kvp x-rays, death of the rabbit's visual cells resulted when, on the average, 4,000 rads had been delivered to the area. As described by Baily and Noell (1958), the efficiency of radiation in producing the retinal changes seems to increase with decreasing linear energy transfer. The average lethal cell dose for 100 kvp irradiation was almost 8,000 rads.

Pyknosis was the first sign of visual cell death, appearing in 24–48 hours, and was invariably followed within 1 or 2 days by chromatolysis and cell disintegration. Ganglion cells and bipolar cells withstood a dose twice that which killed the visual cells.

In the monkey x-irradiation destroyed primarily the rod cells, thus differentiating between rod and cone cells (Cibis *et al.*, 1955). The rabbit retina is mainly one of rod cells, and its cone cells seem to differ only slightly in susceptibility.

Visual cells which survived a single dose slightly below the cytotoxic dose showed characteristic degenerative changes of their rods, with thinning and partial or complete disintegration of the outer segments and swelling of the inner segments (Fig. 3). Lesions of this kind were found after a single dose of 250 or 2,000 kvp irradiation, but only near the area of visual cell death. Widespread degenerative changes without cell death were evident after repeated x-irradiation with small doses (see Fractionation of Dose). In these experiments, the ERG became severely affected, as if a large fraction of the visual cell population had died.

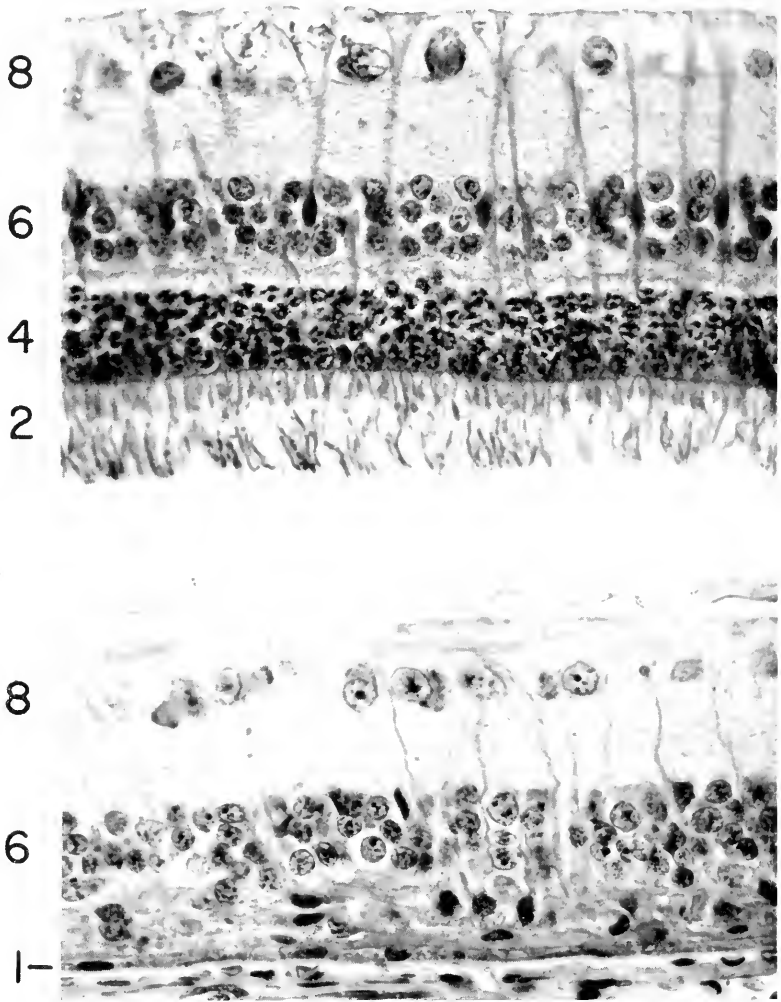


FIG. 2. Sections of the rabbit's retina. Hematoxylin-eosin. Objective (oil) $\times 50$. Top: Control retina which is detached from pigment epithelium. 2, layers of the rods and cones with the outer and inner segments; 4, outer nuclear layer comprising the nuclear regions of the visual cells; 6, inner nuclear layer, the nuclei of bipolar cells (round) and of the Müller cells (oval, dark); 8, layer of the ganglion cells. Bottom: Section from an equivalent retinal area 10 days after x-irradiation. 1, pigment epithelium, which after the disappearance of the visual cells makes contact with the remaining retina. The space between pigment epithelium and inner nuclear layer is invaded by Müller cells.

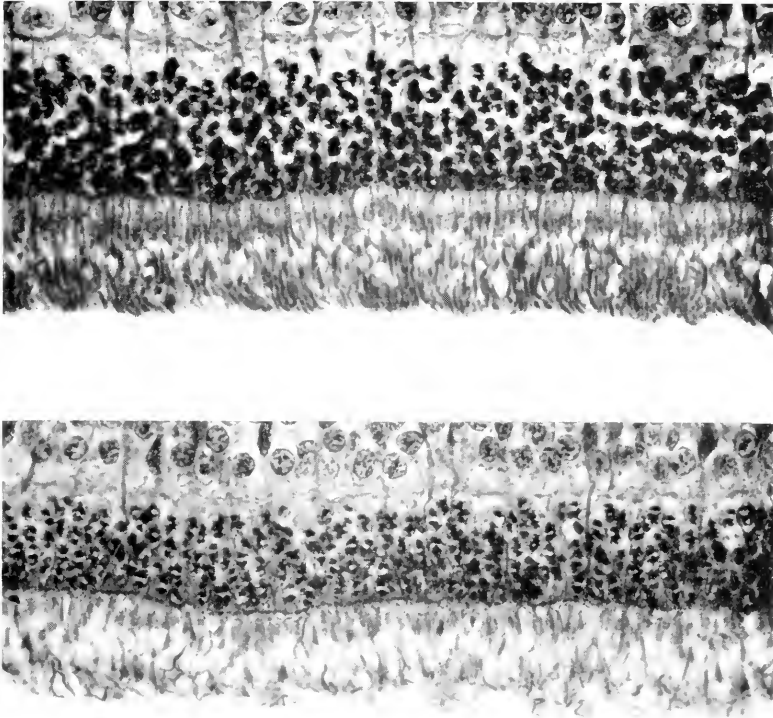


FIG. 3. Top: Normal visual cell layers of a control retina. Bottom: Retinal section showing rod degeneration.

Effects on the Electroretinogram

The most significant features of the x-irradiation effect were revealed by the electroretinogram (ERG) (Fig. 4), a "mass-response" of the retina to diffuse light stimulation. The ERG is recorded by an electrode in contact with the cornea or the fluid of the anterior eye chamber. It depends in form and amplitude on the various parameters of light stimulation and on the state of retinal adaptation. Its origin is still a matter of dispute. It does not simply compound the activities of all the elements of the retinal pathway. It does not record the excitation of the retinal ganglion cells but may contain components which depend on the function of the second neuron, the bipolar cell. However, its greatest dependence is on the first neuron of the pathway, the visual cell.

During exposure to x-radiation, an ERG of a rather simple form was

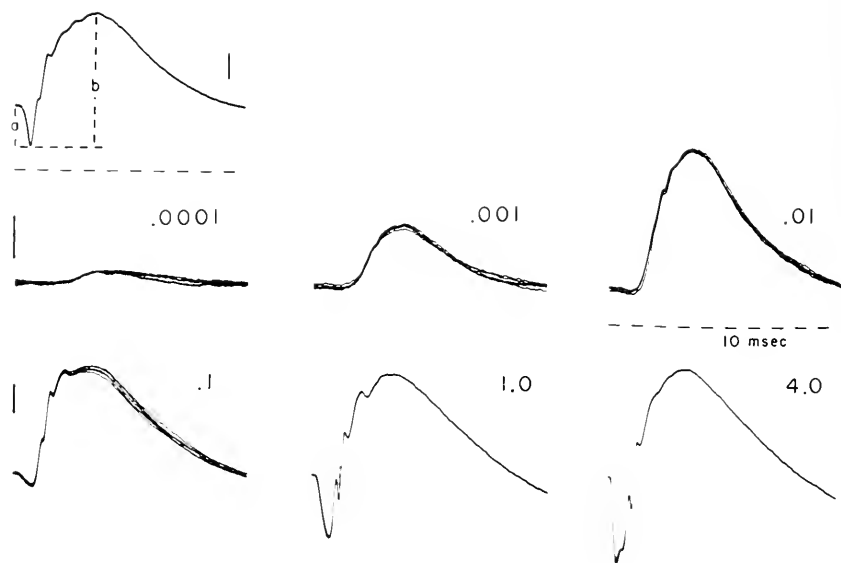


FIG. 4. Normal ERG from dark-adapted rabbits. The number beside the tracings denotes stimulus intensity in arbitrary units. The flash of light triggers the cathode ray sweep. Vertical lines denotes $100 \mu\text{v}$ and refer to the amplification of the corresponding rows. Each dash of the time marking represents 10 msec. A stimulus intensity corresponding to that of .01 was used for eliciting the ERG during x-irradiation.

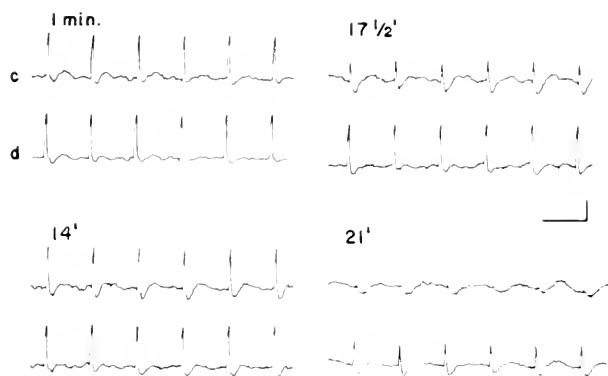


FIG. 5. Examples of the ERG during 2,000 kvp irradiation. c. ERG of close eye; d. ERG distant eye. Irradiation begins at zero time. The horizontal line denotes 2 sec; the vertical, $100 \mu\text{v}$.

recorded by an inkwriter in response to brief flashes of light delivered continuously at 0.5–1 per sec. In the experiment from which Fig. 5 is taken, the eyes were exposed to 2,000 kvp radiation. No significant changes occurred between the 1st and the 14th minute of exposure, but shortly thereafter the ERG of the eye at the point of entry of the x-rays (close eye) suddenly began to fall in amplitude. At 17 minutes it was less than half the size recorded 3 minutes earlier, and at 19 minutes it had become very small. At about this time, the ERG of the distant eye diminished in amplitude at a rate almost as fast as that of the close eye.

The disappearance of the ERG, which we believe is a manifestation of visual cell failure, was associated with extinction of the optic nerve response (Fig. 6), proving that the ERG decline indicated the failure of retinal excitability to light stimulation.

The sudden ERG decline, graphically depicted in Fig. 7 (200 kvp) and Fig. 8 (250 kvp), was consistent with regard to dose required and time of occurrence. It needed a precise minimal dose, and it started with a time lag of no more than 5 minutes after the application of this dose at a high dose rate. Higher total doses, delivered at the same rate, caused this decline to appear only slightly earlier. The amount of dose in excess of the minimum one—expressed in terms of the dose delivered to one of the reference points used for dosage evaluation—determined the level to which the decline proceeded in accordance with the distribution of absorbed dose over the retina. It is clear that one is dealing with effects on a homogeneous population and with doses of maximal effectiveness.

The ERG continued to fall slowly when the sudden change had not resulted in its disappearance (Fig. 9). Similarly, when the dose was below that required for the sudden ERG decline, a slow fall in ERG developed which, however, never lead to a striking amplitude reduction. A significant ERG

2000 kvp.

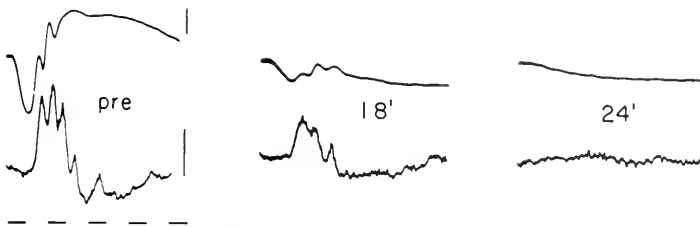


FIG. 6. ERG (upper tracing) and optic tract potential (lower tracing) in response to an intense flash during 2,000 kvp irradiation. The optic tract response disappears slightly later than the b-wave.

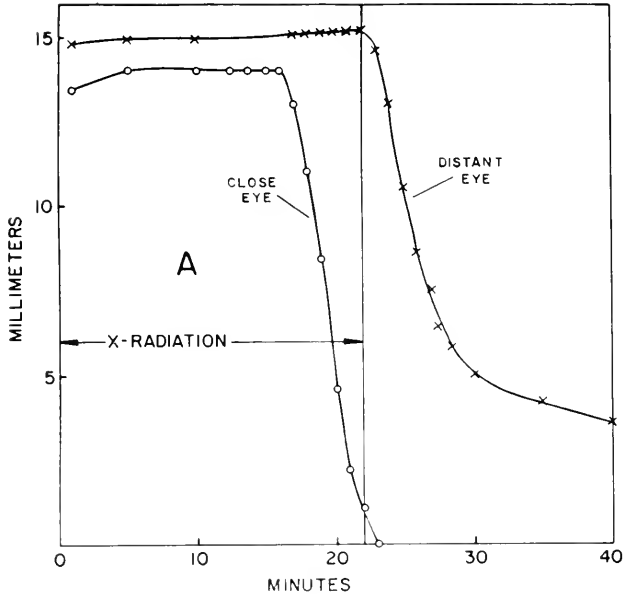


FIG. 7. Decline of the ERG of close and distant eye with 2,000 kvp irradiation. The ordinate gives the ERG amplitude in millimeters of deflection as recorded by ink-writer. The ERG decline of the distant eye starts at the time of termination of the irradiation. The dose delivered to the distant eye is insufficient to produce complete ERG disappearance, in the early postirradiation period.

diminution, if it occurred at all, became apparent within 2 hours after irradiation. In relation to the fairly even distribution of absorbed dose of 2,000 kvp irradiation over the retina, the effectiveness of the x-rays tended to follow the all-or-none rule; that is, if effective at all, 2,000 kvp radiation generally produced a sudden decline of the ERG to at least the 50% level. In contrast, 250 kvp radiation produced graded amplitude reductions, ranging from slight to severe, in accordance with the steeper gradient in absorbed dose of this radiation over the retina.

There was no recovery from any ERG decline which developed within 30 minutes after irradiation. If the ERG disappeared immediately or within a few hours after exposure, reappearance of a measurable response did not occur (Fig. 9). This lack of recovery differentiates the x-ray effects from those of iodoacetate and oxygen at high pressure. Recovery from ERG disappearance is observed following these poisons, unless their doses are excessive (Noell, 1959).

Transient effects in our experiments were a slight fall in ERG amplitudes, developing slowly within 2 hours after irradiation, and an accelerated b-wave

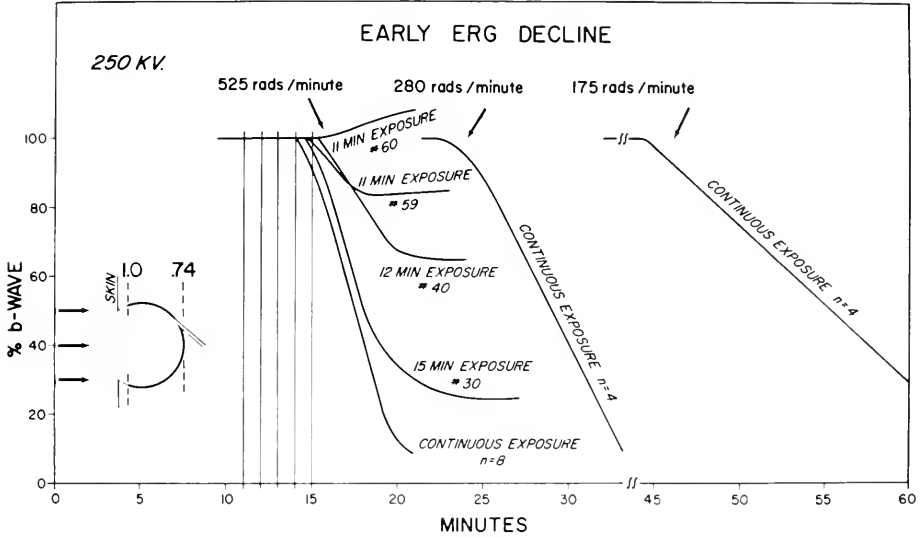


FIG. 8. The ERG decline resulting from 250 kvp irradiation. The dose delivered to the posterior pole of the eye is 74% of that to the anterior rim of the retina, to which the indicated dose rates refer. Start and rate of the ERG decline during continuously applied irradiation are almost linearly related to dose rate. Termination of x-irradiation prior to the start of the ERG decline, results in an incomplete effect occurring with a maximal time lag of 4 to 5 minutes after the end of irradiation.

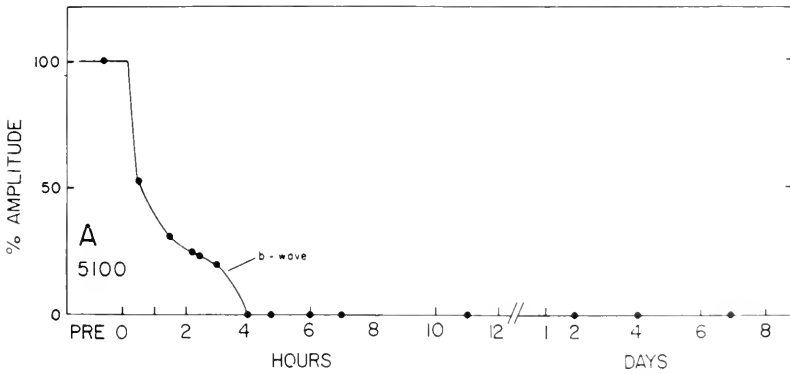


FIG. 9. ERG (b-wave) decline after 5,100 rads (medium retinal dose: 250 kvp). Immediately following radiation, the b-wave becomes reduced by 50%, a slower decline thereafter leads to its disappearance.

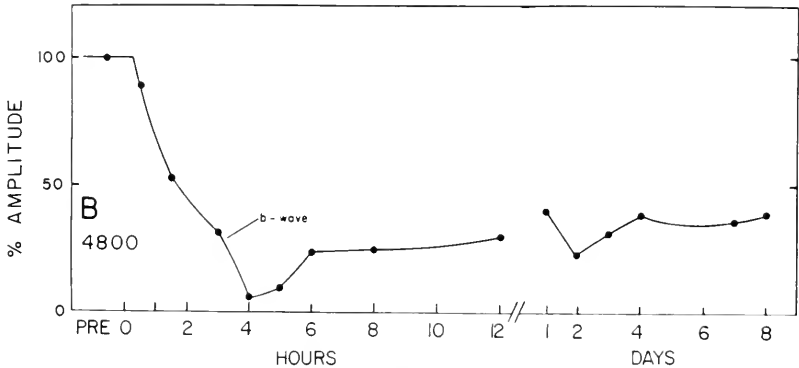


FIG. 10. ERG (b-wave) decline after 4,800 rads (medium retinal dose, 250 kvp).

decline between 4 and 8 hours after irradiation (Fig. 10). This transient b-wave change coincided with an a-wave increase, whereas during the early postirradiation period both waves diminished almost simultaneously.

A rapid ERG decline immediately following irradiation was consistently associated with visual cell death. In fact, death of the visual cells in the most exposed retinal region occurred when irradiation was insufficient to produce a sudden ERG decline but effected a more slowly developing ERG reduction. Using 2,000 kvp radiation, visual cell death was found when the absorbed dose was 4,000 rads, whereas a rapid ERG decline required an average minimal dose of 4,300 rads to the most exposed retinal region. On the other hand, a slight ERG reduction developing slowly within 1 to 2 hours after irradiation was generally not associated with visual cell death. The minimal dose required for such a slight ERG change was only 10–20% lower than for visual cell death. Obviously, cell deaths and ERG effects are very closely related.

The preservation of an ERG, whatever its size, 24 hours after irradiation was indicative of the survival of a fraction of the visual cells for the whole postirradiation period, i.e. up to about 2 weeks. Our observations suggest that visual cells which still function 24 hours after irradiation survive, whereas those which do not function at this time have died.

Effects on Metabolic Activities

The metabolic activities of retinas isolated from eyes after x-irradiation were measured in the Warburg flask (Cohen and Noell, 1960). The eyes had been exposed to 2,000 kvp irradiation of varying total dose applied up to 20 minutes. Their ERGs were recorded throughout irradiation and until 5 minutes before eye removal. The incubation medium was routinely Krebs-

TABLE I

EFFECTS OF X-IRRADIATION ON IN VITRO ACTIVITIES IN PER CENT CHANGE FROM CONTROLS^a

<i>Medium retinal dose (rads)</i>	<i>ERG^b b-wave</i>	<i>O₂-uptake</i>	<i>Glucose oxidation</i>	<i>Lactic acid aerobic</i>	<i>Lactic acid anaerobic</i>
<i>0 Control</i>	—	470 ^c (16)	310 ^c (12)	644 ^c (16)	960 ^c (4)
1300	—	-6 (2)	0 (2)	+5 (2)	—
2600	+5 (4)	-8 (4)	-6 (4)	-2 (4)	—
3400	+12 (6)	-3 (6)	-11 (6)	-3 (6)	—
4200	-55 (2)	-32 (2)	-51 (2)	+1 (2)	—
5000	-82 (10)	-52 (6)	-71 (5)	-2 (6)	-24 (4)
6000	-96 (4)	-57 (4)	-74 (4)	-5 (4)	—

^a Measured for 1 hour, starting 30 minutes after irradiation. Number of experiments is given in parenthesis.

^b Measured 20 minutes after irradiation.

^c Expressed in millimicromoles per milligram dry weight per hour.

Ringer-phosphate and occasionally a bicarbonate medium according to Ames and Hastings (1956).

The results obtained with Krebs-Ringer-phosphate are listed in Table I. No significant metabolic changes were apparent after doses which failed to reduce the ERG. At higher doses and in almost perfect relation to the ERG effect, respiration and glucose oxidation measured with C¹⁴-glucose were reduced, the latter more than the former. After doses which abolished the ERG, the inhibition of respiration was 50%; of glucose oxidation 70%. At this level of reduced metabolic activities, 2,4-dinitrophenol added to the medium was almost ineffective in stimulating these activities. Aerobic lactic acid production was neither reduced nor increased. Anaerobic lactic acid production was at most 25% inhibited. No different results were obtained in the bicarbonate buffered medium.

These data relate to the whole retina, and it is impossible to assess to which extent the various retinal cell population contribute to the metabolic changes. Nevertheless, these measurements clearly indicate that x-irradiation affects a cellular system on which respiratory activities, visual cell viability, and visual cell function intimately depend.

Recovery from the Latent Effects

In a series of experiments, the eyes were exposed to a dose of 4,150 rads (250 kvp, medium retinal dose), which was about the average threshold dose for a slight ERG change after irradiation. Recovery from the latent

effect of this dose was measured by determining the dose of a 2nd irradiation sufficient to produce the sudden ERG decline. This 2nd irradiation was applied continuously and the dose delivered at the time the ERG decline started or reached a certain magnitude was used as a measure of its effectiveness. Dose rate was the same for both irradiations. When the 2nd dose followed the first without interruption, 2,150 rads were needed to start the decline. After complete recovery from the latent effect of the 1st dose, 6,300 rads were required.

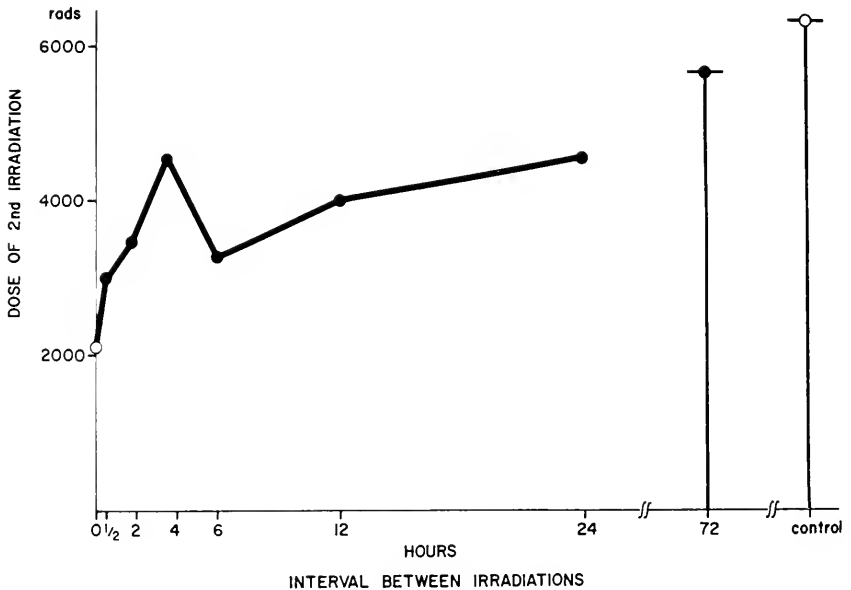


FIG. 11. Recovery from the latent effect of irradiation. For explanation see text.

The results are plotted in Fig. 11, each value representing the average of 3-5 animals. Although recovery started immediately and reached a significant level 30 minutes after irradiation, the effectiveness of the test irradiation was still increased (in a statistically significant manner) after 24 hours, and even after 3 days it differed from that in the control experiment.

Fractionation of Dose

Various types of dose fractionation were tested in our studies in order to gain information which might be applicable to problems of clinical radiology. A medium retinal dose of 460 rads of 250 kvp radiation was applied for 1 minute 5 times a week. Three animals were carried through 23 exposures.

After these exposures and a total medium retinal dose of more than

10,000 rads, the b-wave amplitudes were about 30% that of preradiation control (Fig. 12). The eyes were removed 3 days after the last dose. None showed visual cell death, although the total dose delivered was almost twice the cytotoxic one, as determined with a single dose. There were, however, widespread signs of rod degeneration (Fig. 3), which presumably were responsible for the b-wave reduction.

This b-wave reduction was associated with a proportionately greater a-wave reduction, illustrated (Fig. 12) by the increase in the ratio between b-wave and a-wave amplitudes (b/a ratio). Preferential a-wave reduction seems to be characteristic of rod degeneration; it has been described for rod degeneration following intravenous injection of iodate. (Noell, 1958) and during severe vitamin A deficiency (Dowling and Wald, 1958). Measured by the b/a ratio, it would appear that rod degeneration occurred in our fractionation experiments after about the same total dose which was effective in a single application. Without doubt, fractionation influenced the effectiveness of irradiation with respect to rod degeneration much less than with respect to visual cell death. This degeneration, however, may not be caused by direct action of x-radiation on the rods; it is equally probable

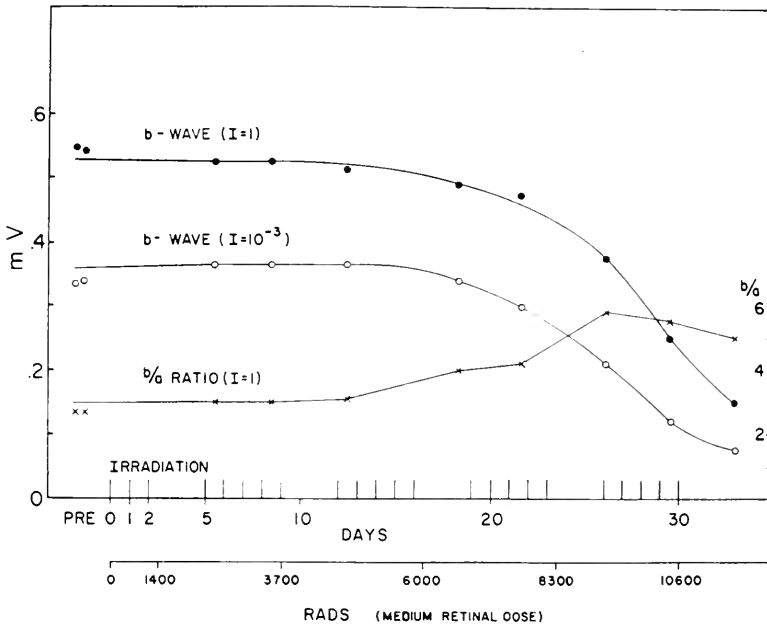


FIG. 12. Effects of irradiation applied intermittently. With a single irradiation, 460 rads (250 kvp) are delivered in terms of medium retinal dose. The b-wave is measured for responses to 2 different intensities ($I=1$, $I=10^{-3}$).

that it results from damage to other eye structures, such as chorioidal blood vessels and pigment epithelium.

Photoexcitation by x-radiation

As reviewed by Lipetz (this symposium), x-rays are known to produce a light sensation when the patient is well adapted to darkness. In our experiments photoexcitation by x-radiation revealed itself by ERG changes of the same kind as resulting from the application of a weak steady light upon which the test flashes are superimposed.

Figure 13 shows that the b-wave during application of x-radiation is

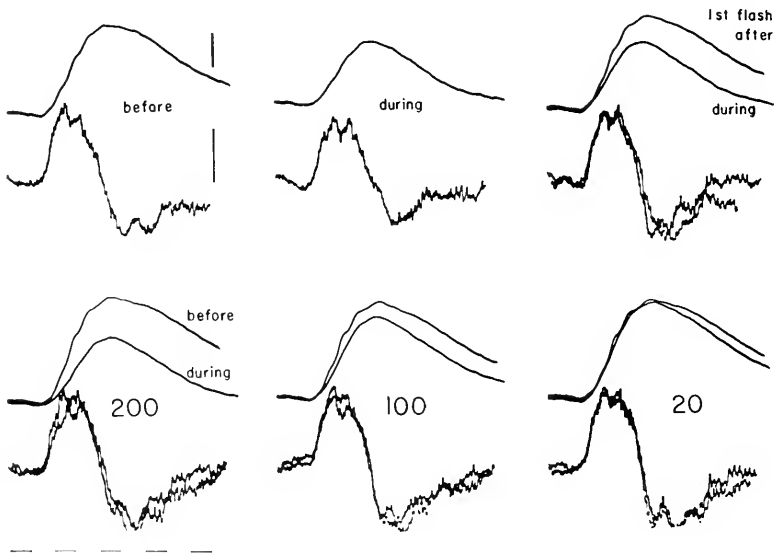


FIG. 13. The photoexcitatory effect of x-rays on ERG and optic tract response in the dark-adapted state. In the top row irradiation was at a rate of 300 rads per min (medium dose), in the lower row 200, 100, and 20 rads per min, respectively. For better demonstration of the x-ray effect the response to two flashes of light are photographed on the same film in some of the records. Calibration: 100 mv. Time: 10 msec equals one horizontal dash.

lower in amplitude and somewhat shorter in duration than during the control period, the magnitude of this change being clearly related to the intensity of x-radiation. The lowest x-ray intensity of 2,000 kvp radiation at which this effect was still evident was about 10 rads per minute expressed in medium retinal dose (Fig. 14). Throughout the x-radiation, the test flashes of light, 1 every 4 sec, provoked b-waves of the same reduced amplitude as those

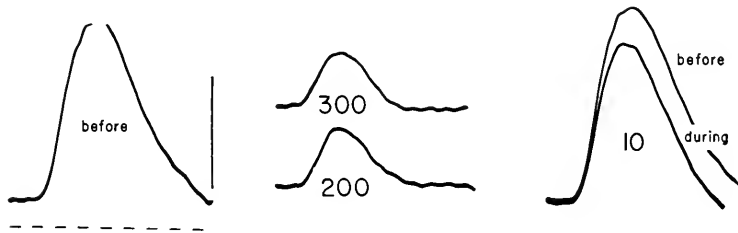


FIG. 14. Similar experiments as in Fig. 13, except the ERG is evoked by a weaker light stimulus and cathode ray sweep speed is slower. The responses in the center were obtained during irradiations of 300 and 200 rads per min, respectively. Irradiation was 10 rads per min for the reduced ERG to the right. Calibration: 100 mv. Time: 10 msec equals one horizontal dash.

seen immediately after the start of x-irradiation. When x-radiation was discontinued, the b-wave returned to the control level within the interval between two test flashes or slightly later. The effect also was tested after various total x-ray doses delivered to the eye. As long as the total dose was below that needed to produce the sudden irreversible ERG decline, changes of the same magnitude as at the start of the experiment were readily demonstrated by terminating radiation and resuming it.

Further tests sought to determine whether or not x-radiation by itself provokes an ERG if suddenly presented. A 34-in.-long lead brick in front of the 2,000 kvp machine served as a "shutter" and by its fall, at the desired moment, opened the x-ray beam to the eye so that within less than 10 msec the whole eye was exposed once the beam had begun to strike it. The animal, in a completely light-shielded enclosure, was dark-adapted. A response similar to that seen after the application of a very weak flash of light was recorded following the "on" of 2,000 kvp x-irradiation (Fig. 15). The lowest dose rate at which a small response was still provoked was 10^{-2} rads per 100 msec.

The photoexcitatory effect of x-radiation is clearly different from the effect with which we were mainly concerned and which is closely related to

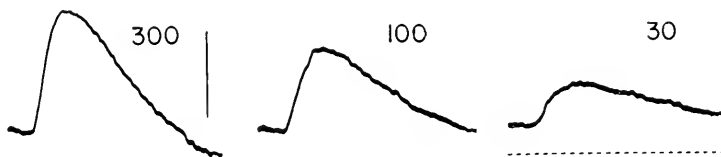


FIG. 15. Response to the sudden "on" of 2,000 kvp at the indicated rads per min. The electrode was a needle inserted into the anterior chamber. Calibration: 100 mv. Time: one dash corresponds to 10 msec.

the destruction of visual cell life. These two effects of x-radiation on the visual cell probably have no more in common than that both are the consequence of the basic action of x-radiation, in the physical sense. It would appear, however, that the simple ERG technique used for detection of the photoexcitatory effect (Figs. 13 and 14) might provide a sensitive tool for the evaluation of interactions of x-radiation and biologic matter.

Conclusion

The outstanding result of these studies is the close association of visual cell death from x-irradiation with the effects on the sensory function of this cell and on energy-yielding metabolic activities. With the exception of the photoexcitatory action of x-rays, all effects required about the same critical dose. They all seemed to develop at about the same time, as if the whole cell in all aspects of its function suddenly failed. It thus would appear that x-irradiation affects a critical region of the cell, a region on which all the measured activities intimately depend.

The visual cell characteristically comprises several distinct compartments, each probably having its own distinct function. If this critical region of x-ray action were the nuclear region, then the fact that the ERG disappeared within a few minutes (its main components disappearing simultaneously) would be difficult to understand, since it is well established that the a-wave is closely related to the integrity of function of the outer segment, and not so much to that of the more proximal parts of the visual cell.

On the other hand, were the outer segment the essential site of the action of x-irradiation, a close association of the ERG effect with cell death would be improbable because destruction of the outer segment is compatible with visual cell survival.

The only region of the visual cell which intimately might support all other parts of the cell and their functions is the distal portion of the inner segment to which the mitochondria of the cell are confined. The difficulty in assuming that this is the "critical" region derives from the well supported finding that poisons which affect the respiratory chain, and anoxia, have notably little capacity to produce visual cell death. The visual cells of the rabbit, for instance, survive ocular ischemia lasting 75 minutes.

In view of these difficulties in defining a "critical" region, it seems more probable that x-irradiation affects a dynamic system which is essential everywhere in the cell and which structurally may be represented by the plasma membrane or cytomembranes. In any case, I believe that whatever the system or cell component which x-irradiation affects in the visual cell, a similar or the same system must be an important site of action of x-irradiation throughout the nervous system.

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The Effects of Ionizing Radiation on Spinal Cord Neurons*

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There are numerous reports in the literature on the acute effects of radiation on conditioned reflexes and on the electroencephalogram (Yanson, 1959; Gorizontov, 1959; Bryukhanov and Lomonos, 1954). Most of these state that after single whole body irradiation (Livanov and Biryukov, 1959; Tsipin and Grigor'yev, 1960), or head irradiation (Sercov, 1955), there was an initial increase and subsequent depression of "cortical bioelectric activity." Brooks (1956) found depression in the EEG of monkeys after one minute of whole body irradiation at 1,000 r per minute. Irradiation of rat peripheral nerves at 6,000 r per minute gave increased amplitude and conduction velocity after 5 minutes of exposure (Lott, 1956). Conduction velocity in frog nerve (Gerstner and Orth, 1955) was found slightly depressed only after 75 kr. and not in less than 2 hours. The only work found on single nerve fibers was by Bachofer and Gautreaux (1959) on the effects of acute irradiation on single giant nerve fibers of the earthworm *in vitro* during exposure at 6 kr per minute. During the first 15-20 minutes, there was a significant increase in amplitude, and thereafter the amplitude progressively fell below normal.

From these reports it is evident that there is varied opinion as to the acute effects of small doses of irradiation. For this reason, we have undertaken to investigate the effects of such irradiation on the spinal cord neurons of the cat.

Methods

Decerebrated cats were fixed on a mounting rack and maintained under artificial respiration. For further immobilization, 3% *d*-tubocurarine was

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injected intravenously, 0.2 cc per hour. Dorsal and ventral roots of 6th and 5th lumbar segments were cut intradurally and used for orthodromic and antidromic stimulations, respectively. A tiny hole was made in the pia mater approximately 2 mm lateral from the entry of the stimulated dorsal root, using jeweler's forceps under a binocular microscope. A glass pipette microelectrode, filled with 2.75 MKCl solution, was used for both extracellular and intracellular recordings from the neural elements in the spinal cord, being inserted through the hole made on the surface of the cord. Gross electrodes of silver wire, 1 mm in diameter, were used for recordings of cord potential and monosynaptic reflex, and a large platinum plate, 3×3 cm, was placed between the skin and subcutaneous tissues of the back as an indifferent electrode. Two different microelectrode tip sizes, 5μ and less than 0.5μ in diameter, were used for extracellular and intracellular recordings, respectively.

The maximum rate of x-irradiation used was 1,270 r per minute at 10 cm from the x-ray tube. The x-ray tube, made by General Electric Co., was set 10 to 15 cm above the surface of the spinal cord at the lumbar segment, and in most cases a thin aluminum filter 0.25 mm thick was used for homogeneous irradiation. As the main flux of the x-radiation from the tube had a stereoangle of 35 degrees, the irradiated area of the cat 1,270 covered some of the thoracic segments as well as the sacral and caudal segments.

A cathode follower input preamplifier was used with a positive feedback system for improvement of frequency characteristics. In addition to this, a rectangular pulse of 1 msec (Lettvin *et al.*, 1958) was used as a monitor to see the resistance of the microelectrode and the adjustment of the positive feedback during recordings, being synchronized with the sweep of the oscilloscope.

Results

CORD POTENTIAL AND THE MONOSYNAPTIC REFLEX

The stimulus strength and amplitude relationship was investigated for each element of the cord potential and monosynaptic reflex. As shown in Fig. 1, the cord potential did not show any significant change in the shape of the curves after irradiation, while the monosynaptic reflex showed a remarkable increase in maximum amplitude after irradiation. There was a slight decrease in threshold strength of the stimulating current for all responses after successive irradiations. Figure 2 shows the responses of each element during a series of irradiation experiments. Each point represents a mean value of 7 measurements of amplitude. The control values of each

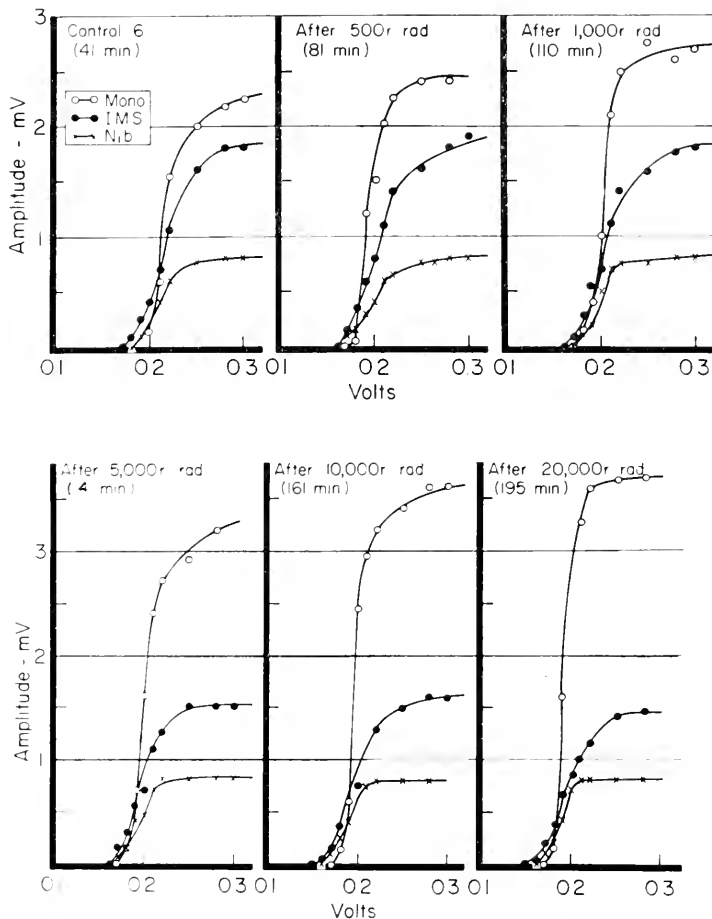


FIG. 1. Stimulus strength and amplitude relation. KEY: Mono, monosynaptic reflex; I.M.S., intramedullary spike; N₁b, interneuronal component of cord potential.

response remained almost constant for more than 1 hour before irradiation. After an initial small dose of irradiation, the monosynaptic reflex began to increase with a considerably steeper slope, while the intramedullary spike which is interpreted as revealing the activity of sensory fibers ascending the dorsal column, started to decrease in amplitude. N₁b, which is known to represent interneuronal activity, remained constant in amplitude until the end of the experiment.

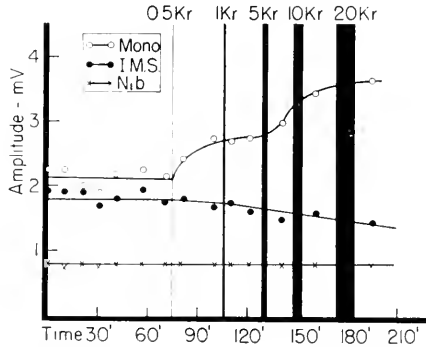


FIG. 2. Amplitude versus the time course (in minutes) of successive irradiation. Key for curves is the same as in Fig. 1.

EXTRACELLULAR RESPONSE FROM THE AFFERENT FIBERS AT THE DORSAL HORN

The maximum amplitude of the extracellular response from the dorsal horn at 10μ in depth from the surface of the cord was taken as a criterion for the effect of the irradiation on the afferent fibers. Usually a stimulating voltage twice the height of threshold was enough to elicit the maximum amplitude response. Figure 3 shows the responses recorded during and after the successive irradiations. The amplitudes showed a gradual decrease with successive irradiations. This corresponded to the decrease in amplitude of the intramedullary spike of the cord potential.

INTRACELLULAR RESPONSE FROM AFFERENT FIBERS

Although we did not systematically investigate the intracellular response from the afferent fibers, we could often record normal responses intracellularly even after irradiation with 10 kr.

INTRACELLULAR RESPONSE FROM INTERNEURONS

Because the amplitudes of the intracellular responses from the interneurons varies greatly, depending on the cells, it was difficult to estimate the effect of irradiation on the amplitude. However, no significant change in time course of the response or in repetitive activity could be seen even after irradiation with 10 kr.

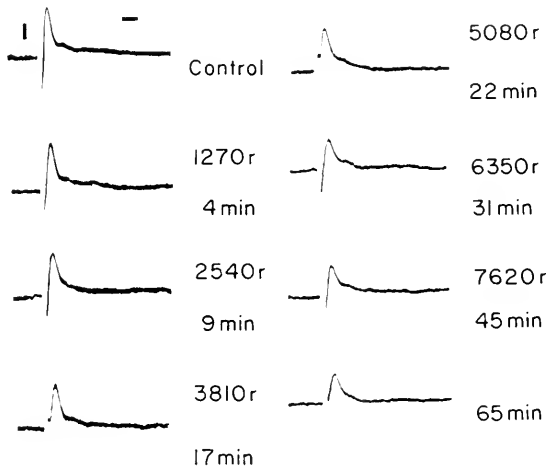


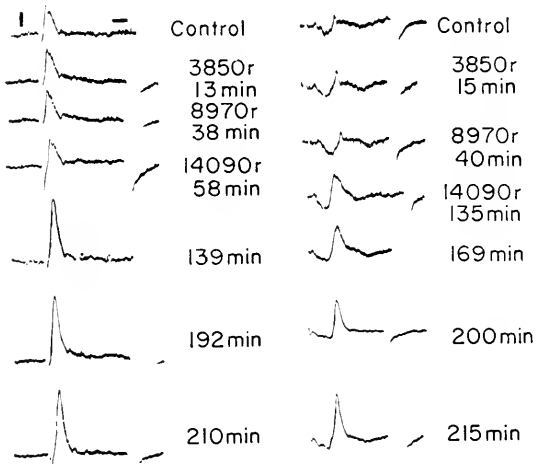
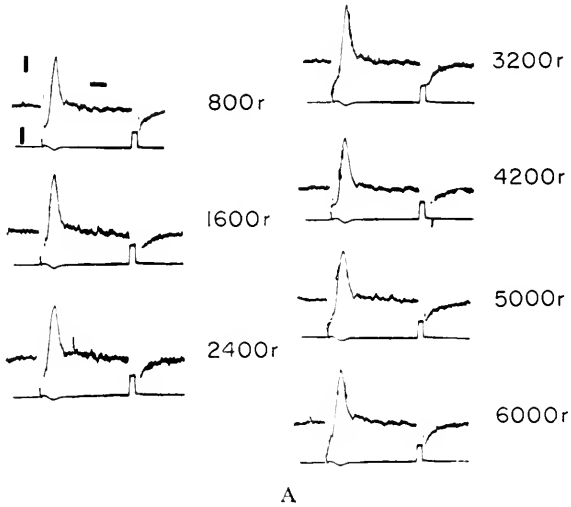
FIG. 3. Extracellular response from afferent fibers at the dorsal horn, during and after successive irradiation. Vertical and horizontal bars represent 1 mv and 1 msec, respectively. The total doses of irradiation and the time from the initial irradiation are shown.

EXTRACELLULAR RESPONSE FROM MOTOR NEURONS EVOKED BY ORTHODROMIC AND ANTIDROMIC STIMULATION

As the tip of the microelectrode approaches the motor neuron, the amplitude of the extracellular response increases gradually. However, the maximum amplitude that can be recorded just at the surface of the cell membrane is approximately the same for every cell under the same conditions. This extracellular response is satisfactorily stable, so it can be recorded for more than 2 hours without showing any distortion in the shape of the response. Figure 4 shows the maximum amplitude response from a motor-neuron during and after successive irradiation, with observation continued for about 3 hours after initial irradiation. The response did not show any significant change in shape within the first 2 hours, despite high doses of successive irradiation. Thereafter, however, it showed an unexpected increase in amplitude for both antidromic and orthodromic stimulations.

INTRACELLULAR RESPONSE FROM MOTOR NEURONS EVOKED BY ORTHODROMIC AND ANTIDROMIC STIMULATIONS

To investigate the acute effect of irradiation on the intracellular response from the motor neurons, an intracellular recording was made on the same



B

FIG. 4. (A) Antidromic extracellular responses from motoneuron during exposure to radiation. This observation was done within 1 hour after initial irradiation. Upper vertical bar represents 2 mv; the lower, 20 mv. Horizontal bar is 2 msec. (B) Extracellular response from motor neurons during and after successive irradiation. Left and right columns are antidromic and orthodromic responses, respectively. Vertical and horizontal bars represent 1 mv and 2 msec, respectively. Total doses and time from the initial irradiation are shown.

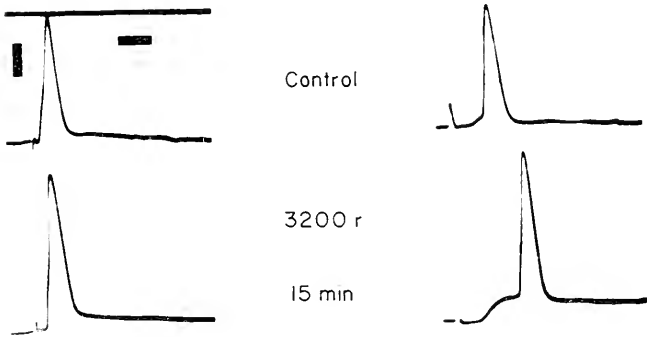


FIG. 5. Increased intracellular response from the motor neuron after irradiation. Vertical and horizontal bars show 20 mv and 2 msec, respectively. Left and right columns are antidromic and orthodromic responses, respectively.

cell for more than 20 minutes, including the periods of irradiation. In this experiment, 3 out of 10 showed a slight increase in amplitude of both the antidromic and orthodromic responses and in threshold strength of the stimulation (Fig. 5). The remainder showed no significant change in amplitude of responses although they showed a slight increase in threshold (Fig. 6). When the cell seems to have deteriorated, the amplitude becomes lower and the duration becomes longer, even without irradiation. As we often experienced such an increase in amplitude of the intracellular response, even in the control experiments, we interpreted this increase as possibly due to an incidental change in resting membrane potential caused by a slight change in contact between the microelectrode tip and the cell membrane.

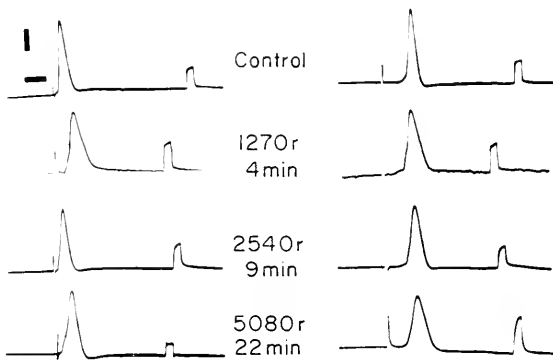


FIG. 6. Intracellular recordings from a motor neuron during successive irradiation. Vertical and horizontal bars show 20 mv and 2 msec, respectively. Left and right columns are antidromic and orthodromic responses, respectively. Total dose and time from the initial radiation are shown.

To exclude this effect, we recorded intracellularly from several cells immediately after irradiation in order to take an average value of the amplitudes. As a result, no significant change in amplitude or time course could be found during or following irradiation with 12 kr or even 2 hours after the initial irradiation (Fig. 7). The membrane potential showed no significant change in amplitude throughout the experiment.

Discussion

SITE AFFECTED BY IRRADIATION IN THE SPINAL CORD

Since the amplitude of the response from the cell is attributable to the magnitude of the ion shift across the membrane and is closely related to the ionic permeability of the membrane, we may expect some change in the excitatory process of a single neural element. Rothenberg (1950) has reported an increase in the uptake of Na^{2+} by irradiated squid axon. Bachofer and Gautereaux (1959) have reported an initial increase in amplitude of the response from the dissected giant axon of the earthworm immediately after irradiation with 30 kr. The doses used in our experiments were smaller than theirs and possibly were not large enough to cause the change in excitability.

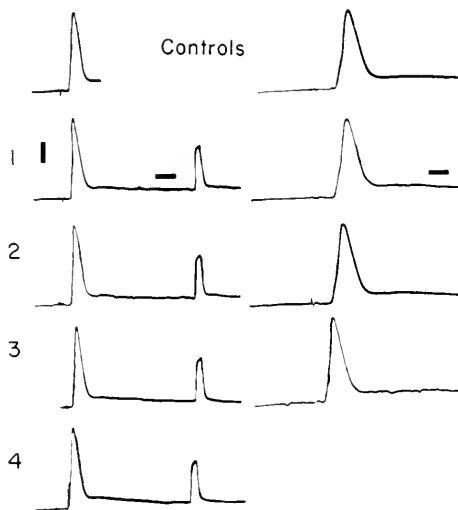


FIG. 7. Intracellular responses from different motor neurons evoked by antidromic stimulation, during and after successive irradiation. Vertical bar shows 20 mv and horizontal bar shows 2 msec and 1 msec in left and right columns, respectively. Recordings 1, 2, and 3 were made after irradiation at 3,850, 8,970 and 14,090 r, respectively; recording 4, 1 hour after the last irradiation.

From our preliminary experiments, it is difficult to discuss the ionizing effect of irradiation on the medium across the membrane. However, it can be inferred that in mammals the myelinated nerve fibers or cell soma have a great resistance to acute ionizing irradiation up to 14 kr.

On the other hand, the fact that the extracellular response from the afferent fibers and its intramedullary spike showed a slight decrease in amplitude, while most intracellular responses from afferent fibers did not show any significant change in amplitude, suggests that there might be some sensory fibers which are more sensitive to irradiation and are damaged by doses as low as 200 r. However, most myelinated fibers did not show an acute effect (within 2 hours) from irradiation up to 10 kr.

The increase in amplitude of the monosynaptic reflex immediately after the initial 500 r irradiation, while both extracellular and intracellular responses from the motor neuron did not show a significant increase in amplitude within 2 hours after initial irradiation, is difficult to explain. It suggests that some interneuronal inhibitory passway might be primarily or secondarily affected by irradiation and, accordingly, the number of motor neurons excited by an afferent volley would increase.

Although the amplitude of the extracellular response from the motor neurons showed an increase 2 hours after the initial irradiation, it is difficult to interpret this as a primary effect of irradiation on the spinal neural elements. This finding might be due to secondary effects of irradiation, such as damage to capillary blood vessels causing hypoxia or asphyxia. This effect might secondarily cause such a change in excitability of the neuronal network around the motor neurons.

Bakin (1946) and Lifshits (1956) concluded from different experiments on the frog's spinal cord that the sensory nerve is much more easily affected by irradiation than the motor nerve. Our results seem to support their conclusion, since our doses were of a similar range to theirs.

As for the exceptional neural elements which might be relatively sensitive to acute small doses of irradiation, and for the effect of higher doses of irradiation, we must await further experiments.

Summary

Using decerebrated cats, the acute effect of ionizing radiation on the neural elements of the spinal cord was investigated preliminarily.

Monosynaptic reflex showed a considerable increase in amplitude immediately after the initial irradiation with 500 r, while the intramedullary spike activity of sensory fibers ascending dorsal column decreased gradually in amplitude with successive irradiation. N₁b wave (interneuronal activity) did not show a significant change in amplitude.

Extracellular response from the afferent fibers showed a gradual decrease in amplitude immediately after an initial dose of 1,270 r.

Intracellular response from afferent fibers did not show a significant change in amplitude even after irradiation with 10 kr.

Intracellular response from interneurons did not seem to be affected even by irradiation with 10 kr.

Extracellular response from motor neurons did not show a significant change in amplitude or in time course for either orthodromic or antidromic stimulations, at least within the first 2 hours after the irradiation at 3,850 r. Thereafter, however, it showed a remarkable increase in amplitude and stayed almost constant more than 1 hour.

Intracellular responses from motor neurons evoked by orthodromic and antidromic stimulations were not affected significantly, even by irradiation of up to 12 kr, except for a few cases which showed a slight increase in amplitude.

From these results, it is inferred that myelinated nerve fibers and cell somata in the mammal have a considerable safety factor for the acute effect of irradiation up to 14 kr.

It is inferred that the sensory nerve is more sensitive to irradiation than the motor nerve, and it is suggested that interneuronal inhibitory pathway might also be relatively sensitive to irradiation.

Acknowledgments

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Radiation Effects on Bioelectric Activity of Nerves

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Introduction

The objective of this presentation is to consider the effects of ionizing radiations on the bioelectric activity of nerve fibers *in vitro* and *in vivo*. The immediate effects of irradiation on responses of nerve fibers were determined by recording from the nerve before, during, and, in some cases, after irradiation. After the basic patterns of activity were established with mammalian nerve trunks, the findings were supplemented with results obtained from the irradiation of single nerve fibers of invertebrates, making it possible to determine more precisely the basic pattern of activity of the individual fiber. To determine to what extent the results observed with x-rays could be attributed specifically to the ionization process itself, the results due to x-irradiation were compared to those obtained with nonionizing radiation.

Previous reports (Gerstner *et al.*, 1955; Gerstner, 1956) have considered chiefly the destructive effects of irradiation on nerves previously subjected to radiation. For references to earlier studies, the Gerstner articles may be consulted.

Booth *et al.* (1950) found that ultraviolet (UV) irradiation of the node of Ranvier of the frog sciatic nerve produced an increase in threshold and ultimate block; irradiation of the internode produced an immediate decrease in threshold followed by a steady increase. Pierce and Giese (1957) reported a decrease in spike amplitude and sensitivity of frog and crab nerves exposed to UV, but found no detectable effect on the refractory period. Boyarsky (1952) noted a fall in action potential, a prolongation of refractory period, and only a slight change in threshold of frog sciatic nerves. Lüttgau (1956) found that UV adversely affected action potential and threshold of single nodes of Ranvier of frog sciatic nerves considerably before it affected resting potential and electrotonic potential. All of these studies, with certain minor discrepancies, showed the ability of radiation to destroy the responsiveness of nerves, with an occasional suggestion of an increase in excitability.

Methods

The ventral caudal nerve of the white rat was selected for studies involving the effects of x-rays on mammalian nerve trunk. This is a long, clean nerve with minimal branching and a uniform fiber spectrum, giving compact, sharp action potentials on oscillographic traces. Mature white rats of the Sprague-Dawley strain, descendants of a single pair bred in this laboratory in 1954, were used. Two types of experiment were undertaken. Excised nerves were irradiated *in vitro* in a nerve chamber which permitted remote recording of activity during irradiation (Bachofer, 1957; Bachofer and Gautreaux, 1960b). Recordings were also made from nerves *in situ*, with the nerve trunk disturbed only at limited regions where electrodes made contact with the nerve (Bachofer and Gautreaux, 1960a). Details of the preparation and shielding of the animal are shown in Fig. 1.

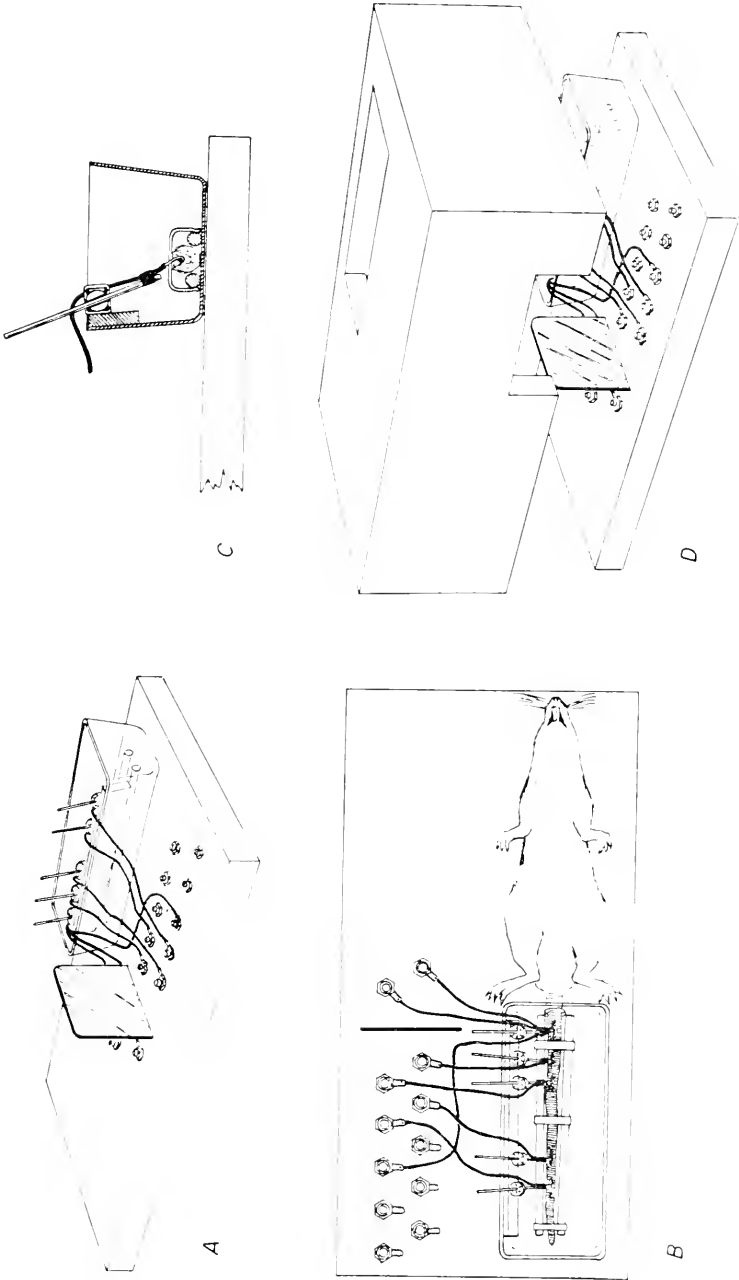
Studies involving radiation effects on giant nerve fibers were carried out on the nerves of the earthworm, *Lumbricus terrestris*, and of the lobster, *Homarus americanus*. Recordings were made of the bioelectric activity of the median giant fiber and of the lateral giant fibers of the earthworm. Complete details of the experimental procedure have been presented (Bachofer and Gautreaux, 1959).

The results presented here are based on the responses of the giant fibers of the earthworm, although the basic phenomena have been confirmed in experiments involving the median giant axons of the lobster in the circum-esophageal, thoracic, and abdominal regions.

Standard electrophysiologic procedures were followed in recording responses, the quantitative parameters of which were subsequently obtained from photographic reproductions of oscillographic traces, projected for accuracy of measurement and analysis.

A Picker Vanguard deep-therapy x-ray generator, operated at 280 kv and 20 ma, was the source of x-rays, given at 6 kr per min. This gave a reason-

FIG. 1. Arrangement used in recording bioelectric responses of rat caudal nerves *in situ*. A. Electrode board with Plexiglas tail holder, to which macromanipulators are attached. Perpendicular copper shield isolates electrostatic fields produced by stimulating electrode connections. B. Superior view of electrode board, showing rat in place for beginning of recording. Body and legs of rat were restrained as needed. C. Sectional view of Plexiglas tail holder. The macromanipulator with special platinum electrode is shown in contact with nerve. Confining rods along tail and plastic clips over tail reduce movement. D. Box constructed of lead 1 in. thick with opening in top for x-irradiation of tail and opening in side for cables, thermocouple leads, etc. When grounded, lead box shields the preparation and connections against electrostatic fields.



able enhancement of activity without undue prolongation of the experiments. For UV irradiation, low pressure mercury-vapor bulbs, emitting UV chiefly at 2,537 Å, were utilized at various dose rates.

Spike amplitudes were measured from both monophasic and diphasic recordings, with care being taken to see that stimuli were maximal or supramaximal, depending on the experiment. All conduction velocities were computed from the time interval between spike peaks recorded from two pairs of electrodes. This obviated any possible errors due to variable latent period of responses. Sensitivity to stimulation—to be distinguished from sensitivity to x-rays, which was determined by the dose of x-rays required to produce a given effect in the nerve—was arbitrarily defined as the reciprocal of the voltage just necessary to elicit a response. This threshold stimulus applied to the nerve was a single square-wave pulse of 10^{-5} sec duration. The rate of spike rise—the rate of voltage change of the action potential in volts per sec—was determined for the linear portion of the rise of the spike. The somewhat slower initial voltage change, corresponding to the period of electrotonic invasion preceding the impulse proper, was ignored in these measurements.

The determination of the refractory period should be explained in some detail. When a nerve fiber produces an action potential in response to a stimulus, there is a period within which the nerve will not respond to a second stimulus. This refractory period actually has two parts: the first, in which the nerve will not respond regardless of the magnitude of the stimulus (the absolute refractory period), and the second, in which the nerve will respond only if the stimulus is greater than that of normal threshold strength (the relative refractory period). The relative refractory period varies greatly in duration, the duration showing an inverse relationship to the magnitude of the stimulus. As the magnitude of the stimulus increases above threshold, the relative refractory period decreases until the nerve becomes absolutely refractory. In all cases the refractory period corresponds to the time within which the nerve recovers from the events associated with the production of the action potential. Since the duration of the refractory period is inversely dependent on the magnitude of the stimulating voltage, it was necessary to use a stimulus of a definite magnitude. For uniformity of results, the stimulating voltage was set 50% above the threshold at zero time. Pairs of square-wave pulses of 10^{-5} sec duration delivered at 1 sec intervals, were used to stimulate the nerve. Six pairs of stimuli, on the average, were required to determine the refractory period at each point; the nerve was not otherwise stimulated. The interval between the two members of the pair of stimuli was adjusted to the point where the nerve just failed to respond to the second stimulus. It was thus possible to determine with precision the refractory period of the giant fiber for the particular

stimulus utilized. Studies on refractory period were not conducted for compound nerve trunks.

Results

X-irradiation of rat caudal nerves in vitro. Irradiation was begun after a period of stabilization during which the output of the nerve was shown to be constant for a period of time exceeding that of the period of irradiation. Most of this period of stabilization is not shown in the accompanying figures, since the nerves were sufficiently stable after the initial adjustment on the platinum electrodes to make the curves appear as straight lines at the value of 1.0, with little deviation for well over an hour. The values on the ordinates, designated A/A_0 , represent the ratio of the activity, A , at a given time, to the activity, A_0 , at zero time. The activity at zero time, which constituted the point of reference in all figures, unless otherwise noted, was the activity at the beginning of irradiation. The abscissas are given in minutes, from which the dose can be calculated from the dose rate. The plotted values were calculated by determining the average time at which the responses reached their maximum value as well as various fractions of the maximum value. The abscissas, therefore, represent mean time values for various definite relative activities, which are plotted on the ordinate.

An immediate effect of x-irradiation on electrophysiologic responses is evident in Fig. 2. An enhancement of activity, attributable to x-irradiation, precedes the suppression of activity. The curves show three degrees of enhancement of activity, spike amplitude showing the greatest, and conduction velocity the least. Not only were there different degrees of enhancement for the various factors considered, but the peak of enhancement manifested itself at different times for the various factors. During most of the period of increasing spike amplitude, conduction velocity decreased, while sensitivity occupied a mid-position. The values shown in Fig. 2 are based on nerves which were essentially unstimulated, since they were stimulated only once each time a recording was made. Figures 3 and 4 show the effect of stimulation at 50 per sec and 100 per sec on the response to x-rays. Square-wave pulses of 10^{-5} sec duration and of amplitude just sufficient to elicit a maximal response were administered to the nerve after the usual initial period of stabilization. The activity of the nerves dropped under such repeated stimuli. The nerves were therefore stimulated for 20 minutes before irradiation in order to move into a period of relatively stable activity. The initial drop in activity shown in Figs. 3 and 4 represents the fall in activity due to stimulation. After 20 minutes of stimulation, the nerve was irradiated, with stimulation and irradiation continuing until the nerves failed to respond. A comparison of the effects of x-irradiation on nerves without stimu-

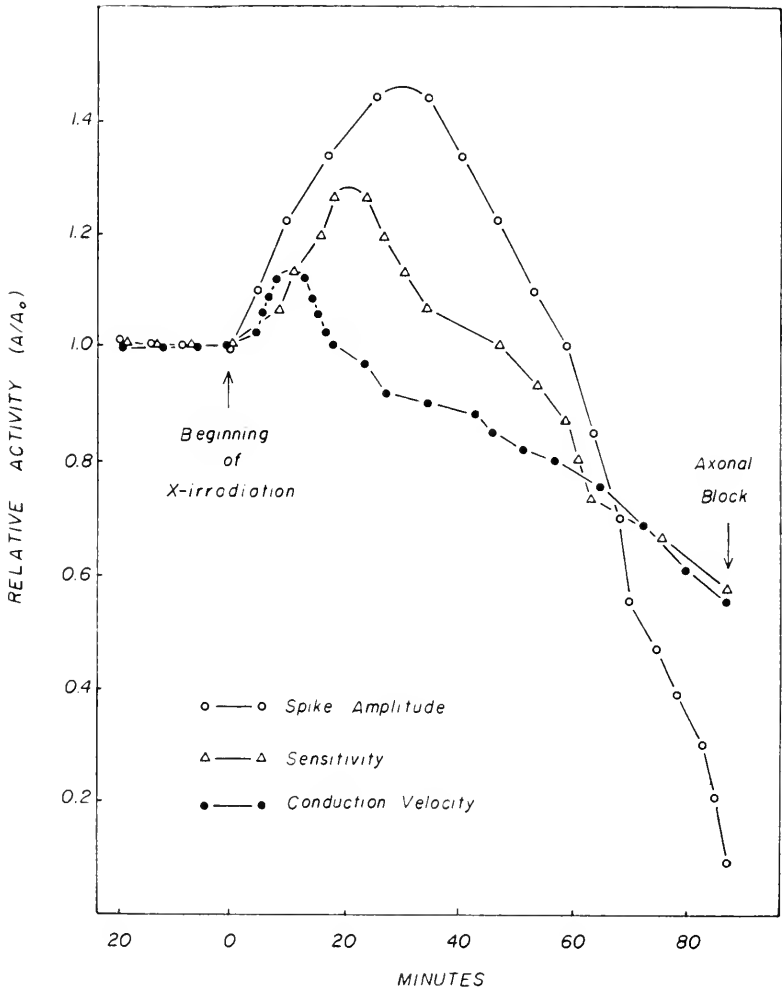


FIG. 2. Effect of x-rays on bioelectrical responses of rat caudal nerves irradiated *in vitro*. Mean relative activities are plotted as a function of time of x-irradiation. The values represent the ratio of the activity at a designated time relative to the activity at the beginning of x-irradiation. In this and other graphs, irradiation continued until nerves failed to respond, unless otherwise indicated. Dose rate: 6 kr/min.

lation with those under stimulation at 50 per sec and 100 per sec shows that the degree of stimulation influenced the response to x-rays. Higher frequencies of stimulation reduce the enhancement of spike amplitude, have a slight effect on sensitivity, and have no observable effect on conduction

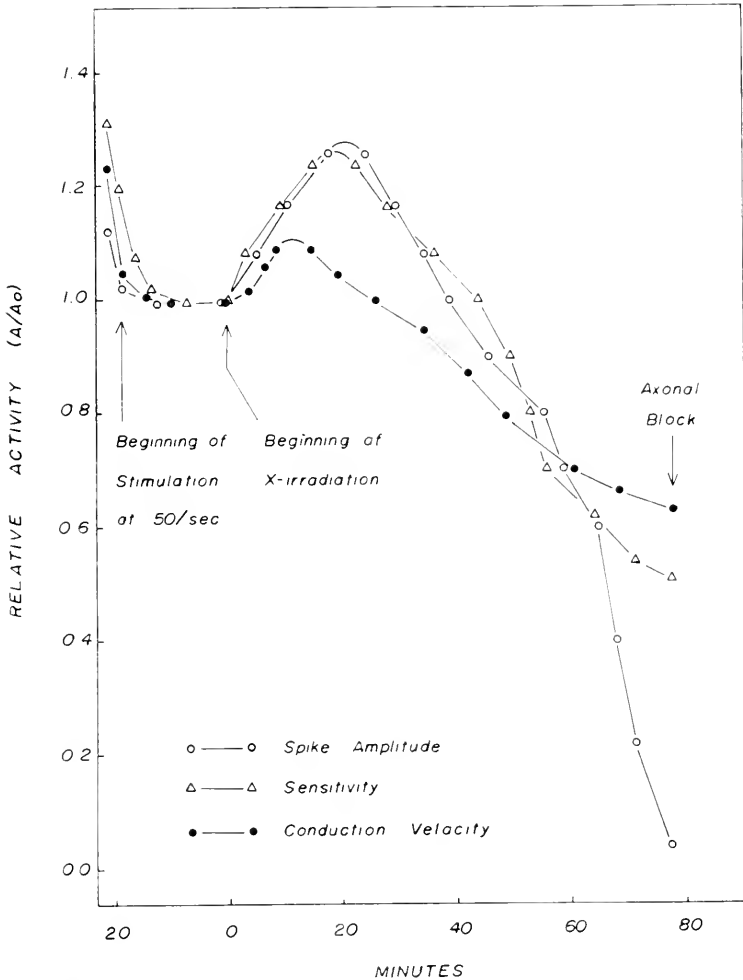


FIG. 3. Mean relative responses of 10 caudal nerves stimulated at 50 per sec for 20 minutes before and during x-irradiation. The stable values preceding stimulation, shown in Fig. 2, are omitted from this graph. The initial drop in activity represents a fall in response due to stimulation. Dose rate: 6 kr per min.

velocity. Under x-irradiation, nerves subjected to frequent stimuli failed to respond as long as unstimulated nerves.

Figure 5 shows the response of a typical nerve which, after a period of stabilization, was irradiated until a definite increase in spike amplitude was observed. The x-ray beam was then turned off, and for 5 hours the response of the nerve was followed, with stimuli only at the time a recording was

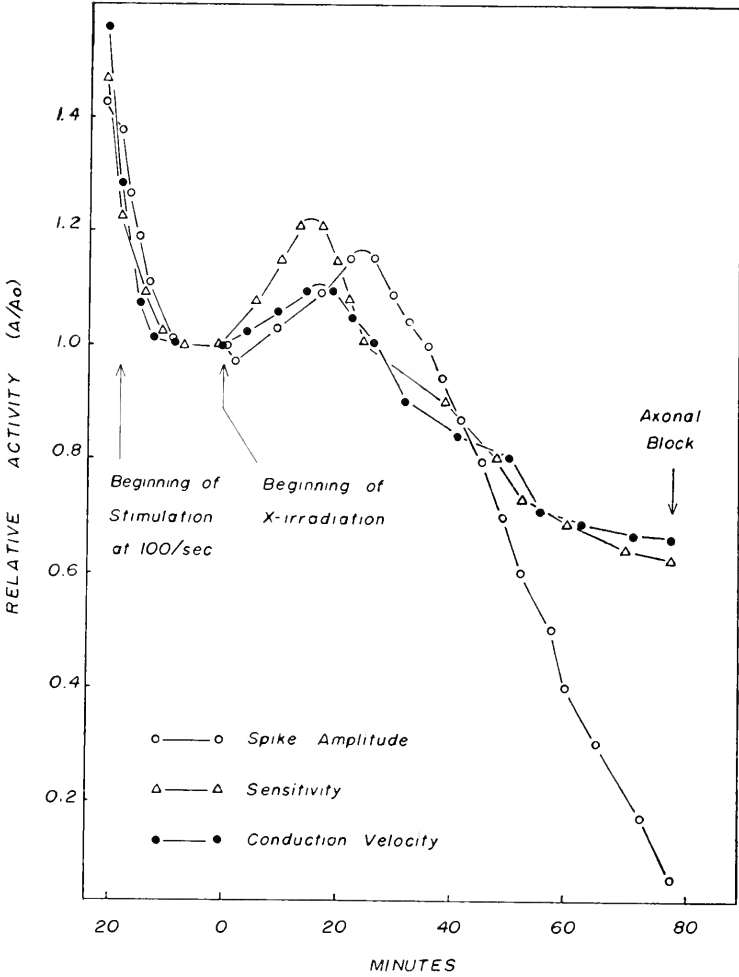


Fig. 4. Mean relative responses of 10 caudal nerves stimulated as in Fig. 3, except at 100 per sec. Higher frequency of stimulation reduced enhancement of spike amplitude, but had less effect on other factors. Dose rate: 6 kr per min.

made. Spike amplitude, which was in the rising phase of enhancement due to x-irradiation, continued to increase; sensitivity and conduction velocity, which were in the falling phase, having passed the peak of enhancement, continued to decrease.

X-irradiation of rat caudal nerves in situ. This phase of the research was undertaken to determine whether the effects observed in nerves irradiated *in vitro* might be attributed to the artificial environment in which the nerve

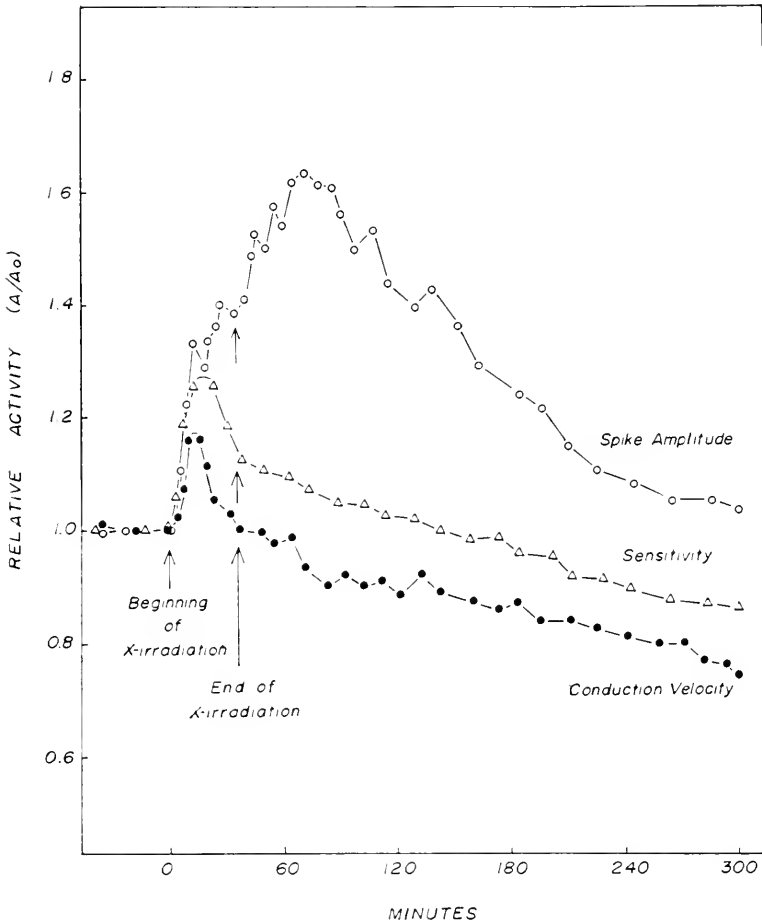


FIG. 5. Typical response of a rat caudal nerve trunk exposed for a limited period to x-irradiation. X-ray beam was turned off at a time when spike amplitude was increasing and sensitivity and conduction velocity were decreasing. The response was followed for 5 hours.

was placed, involving as it did a synthetic medium, the ionic relationships of which could never reproduce exactly the complex ionic environment of the nerve in the living animal. The question posed was whether these effects might be expected in the living animal. To answer this question, experiments involving x-irradiation of the caudal nerve were carried out, not merely *in vivo*, but also *in situ*.

From the results of 28 experiments, mean values were calculated for amplitude of the spike potential and for conduction velocity of the impulse

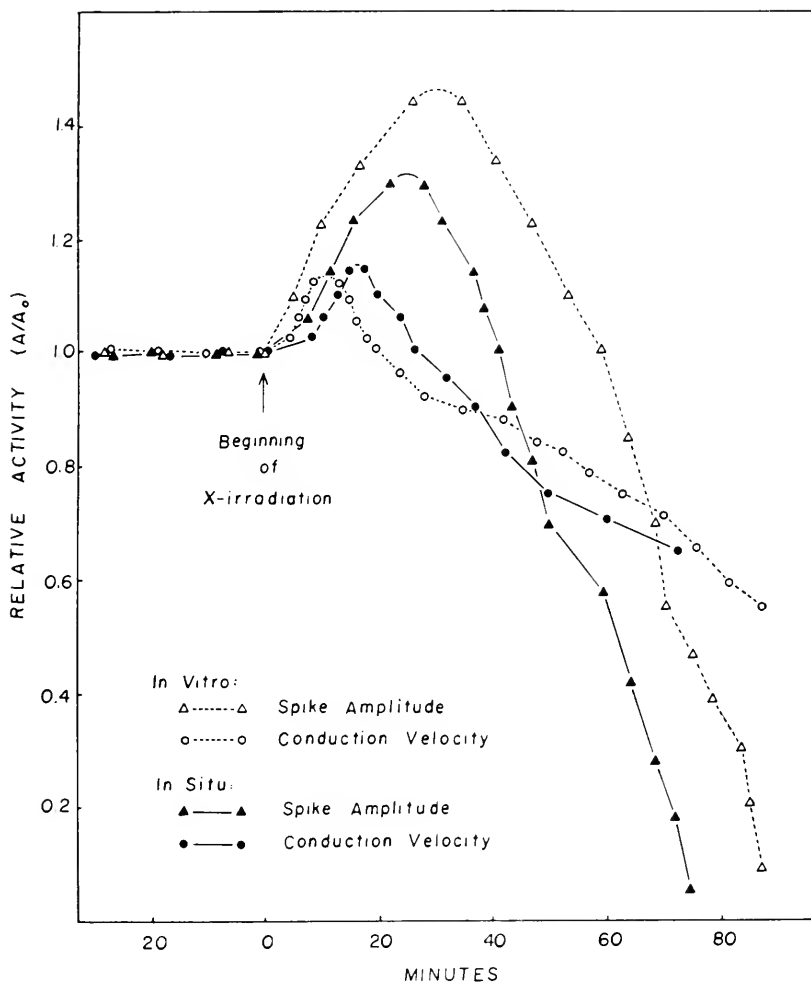


Fig. 6. Comparison of effect of x-irradiation on rat caudal nerves *in vitro* and *in situ*. Values are based on 25 *in vitro* and 28 *in situ* experiments. Dose rate: 6 kr per min.

in response to supramaximal square-wave stimuli of 10^{-5} sec duration at 2 minute intervals (Fig. 6). The mean absolute values at zero time for spike amplitude were 4.27 mv for *in vitro* preparations and 4.85 mv for *in situ* preparations. The absolute values at zero time for conduction velocity were 26.2 m/sec for *in vitro* preparations and 27.5 m/sec for *in situ* preparations. A comparison of the curves shows a striking similarity between *in vivo* and *in vitro* preparations. The minor differences were, chiefly,

failure of the nerves *in situ* to maintain the increased amplitude as long as those *in vitro* and a somewhat later increase in conduction velocity on the part of the nerves irradiated *in situ*.

X-irradiation of giant fibers of earthworm. Since a stable, predictable response on which to superimpose the effects of x-irradiation was necessary, 20 controls were observed for 10 hours for spike amplitude, sensitivity, conduction velocity, and relative refractory period (Fig. 7). Zero time corresponded to the beginning of x-irradiation, 90 minutes after the nerve cords were placed on the recording electrodes. The time at which x-irradi-

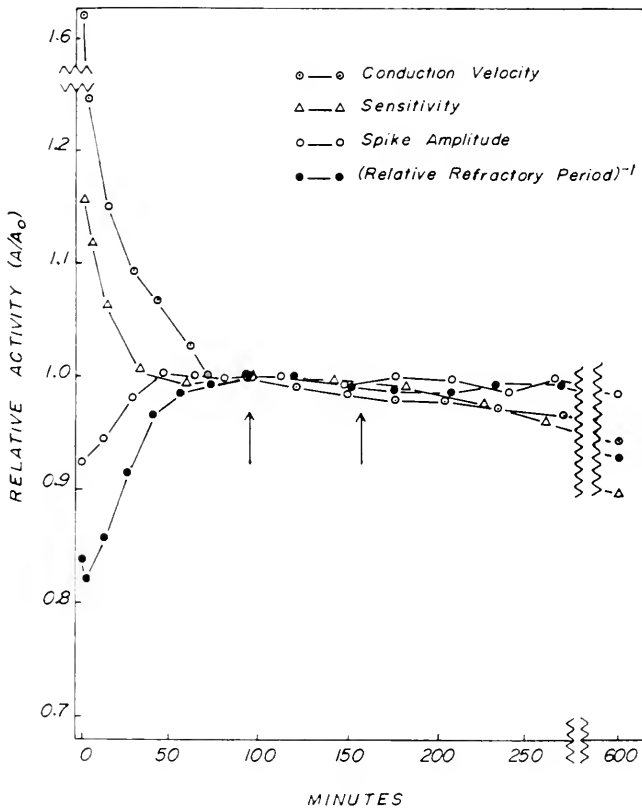


FIG. 7. Relative activities of giant nerve fibers of earthworm, plotted as a function of time. Curves represent mean values of 20 nonirradiated controls. Arrow at 90 minutes indicates time at which, in subsequent experiments, x-irradiation was begun. Second arrow indicates average time at which x-irradiation was terminated. Values plotted represent ratios of activities as designated times relative to the activity at 90 minutes. In most experiments involving UV irradiation, the right arrow would be moved to approximately 210 minutes.

ation was begun is indicated by the first arrow at 90 minutes, and the average time at which x-irradiation was terminated is indicated by the second arrow. (For UV irradiation, the second arrow would be moved somewhat to the right.) No irradiation was involved; the arrows simply indicate the times at which the nerve cords were x-irradiated in subsequent experiments. The relatively flat plateau during irradiation should be noted. No nerve was irradiated until it had passed through a 90 minute period of stabilization on the recording electrodes. The effects of x-irradiation on

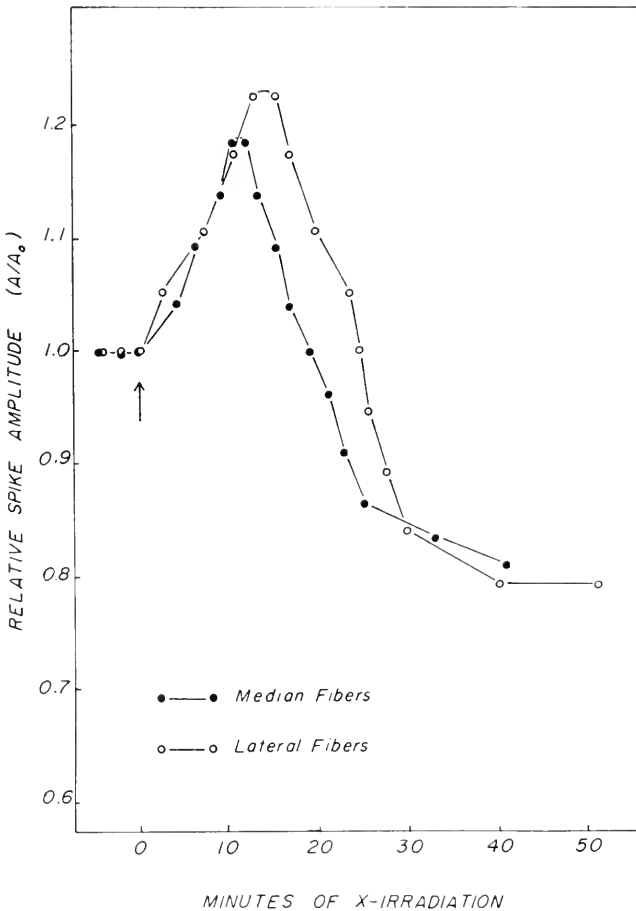


FIG. 8. Relative spike amplitude of giant nerve fibers of earthworm, plotted as a function of time of x-irradiation. Values plotted represent ratios of activities at designated times, relative to activity at beginning of x-irradiation, designated by arrow. Dose rate: 6 kr per min. (46 experiments).

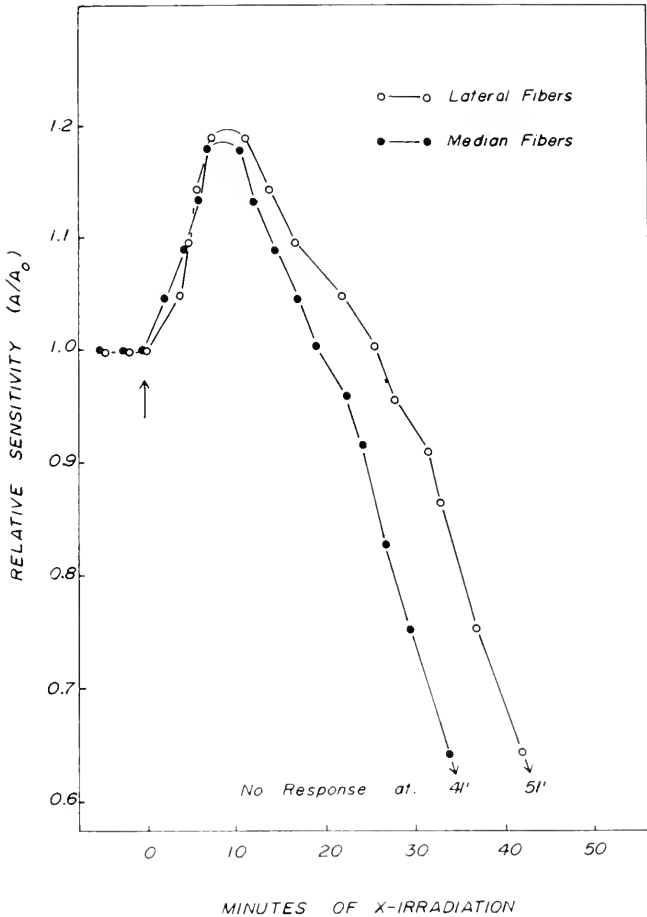


FIG. 9. Relative sensitivity (reciprocal of threshold stimulus voltage) of giant nerve fibers of earthworm, plotted as a function of time of x-irradiation, as in Fig. 8. (24 experiments).

responses of giant fibers are shown in Figs. 8 to 11. All experiments involved records of activities on over 90 different nerve fibers. An almost immediate response to x-irradiation is evident; an enhancement of activity preceded the deleterious effect of x-rays, which was manifested in suppression of activity and ultimate block of activity.

The median giant fibers were somewhat more sensitive to x-rays than were the lateral giant fibers, as shown by the fact that the last activity of the medians was recorded at 41 minutes, corresponding to 246 kr of x-rays, whereas that of the laterals was recorded at 51 minutes, corresponding to

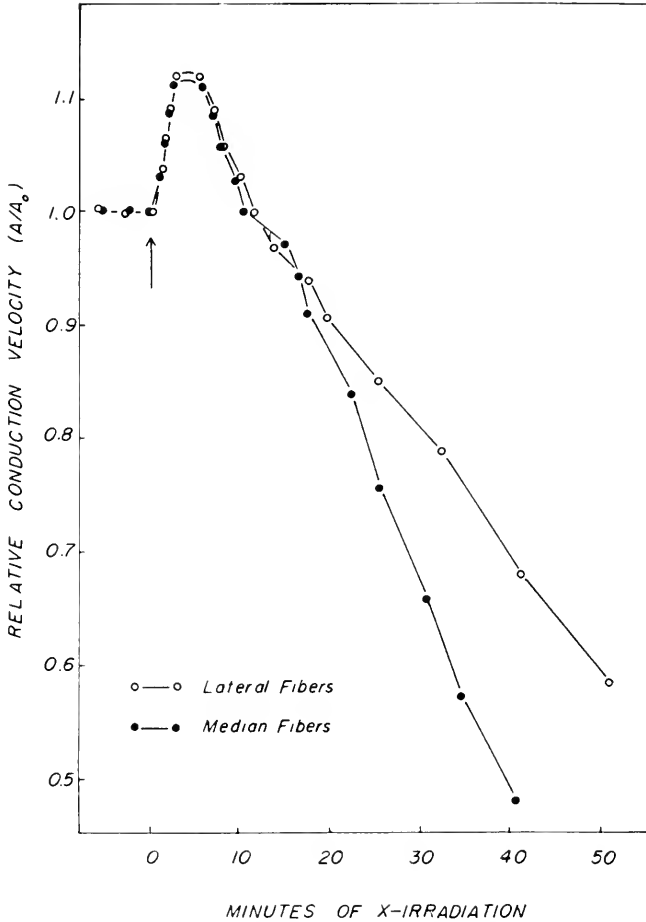


FIG. 10. Relative conduction velocity of giant nerve fibers of earthworm, plotted as a function of time of x-irradiation, as in Fig. 8. (20 experiments).

306 kr of x-rays. Figure 11, a composite graph in which the values obtained from median and lateral fibers are combined, shows much the same pattern as that observed in x-irradiation of mammalian nerve trunks. The refractory period, not investigated in the experiments involving mammalian trunks, showed a pattern of slight initial enhancement due to x-irradiation, followed by a deterioration more rapid than that found in the other factors considered. Of particular interest, in view of results to follow, is the fact that the refractory period showed very slight enhancement.

UV irradiation of giant fibers of earthworm. As in x-irradiation of giant fibers of the earthworm, 90 minutes of stabilization preceded irradiation.

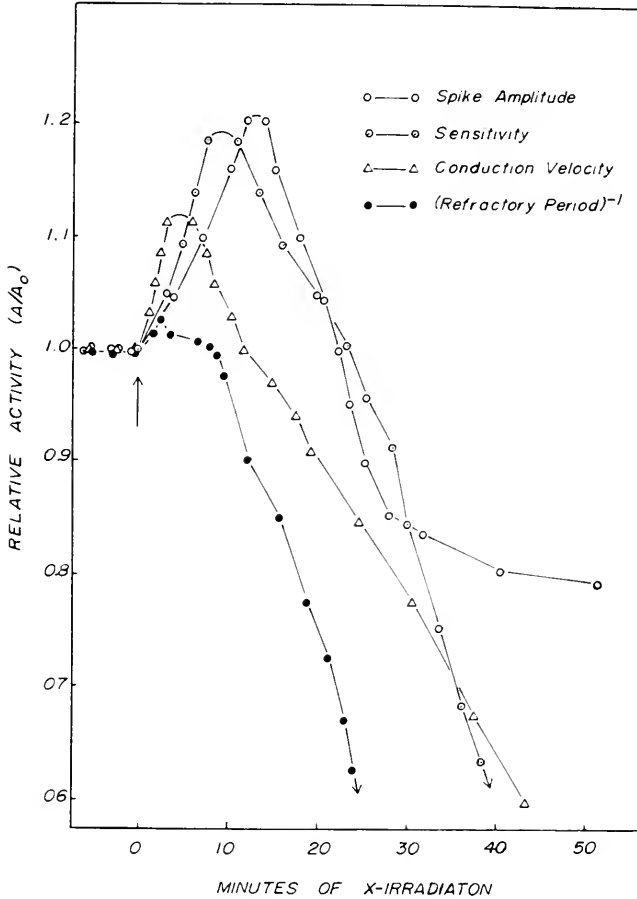


FIG. 11. Relative activity of giant nerve fibers of earthworm, plotted as a function of time of x-irradiation. Mean values of median fibers and of lateral fibers are combined.

The modification of the basic pattern in Fig. 7 is shown in Figs. 12 to 15. Figure 12 shows the increased sensitivity to external stimulation which preceded the precipitous drop in sensitivity as UV irradiation continued. The average threshold voltage of the stimulus at the beginning of irradiation was 0.24 v for the median fibers and 0.72 v for the lateral fibers. Figure 13 shows the relative increase in conduction velocity; the enhancement was slight and was not observed in 26% of the cases. Figure 14 shows the relative increase in spike amplitude; increases were observed in all cases. Absolute values for the amplitude of action potentials at zero time were 1.43 mv for the median fibers and 2.29 mv for the lateral fibers. The rela-

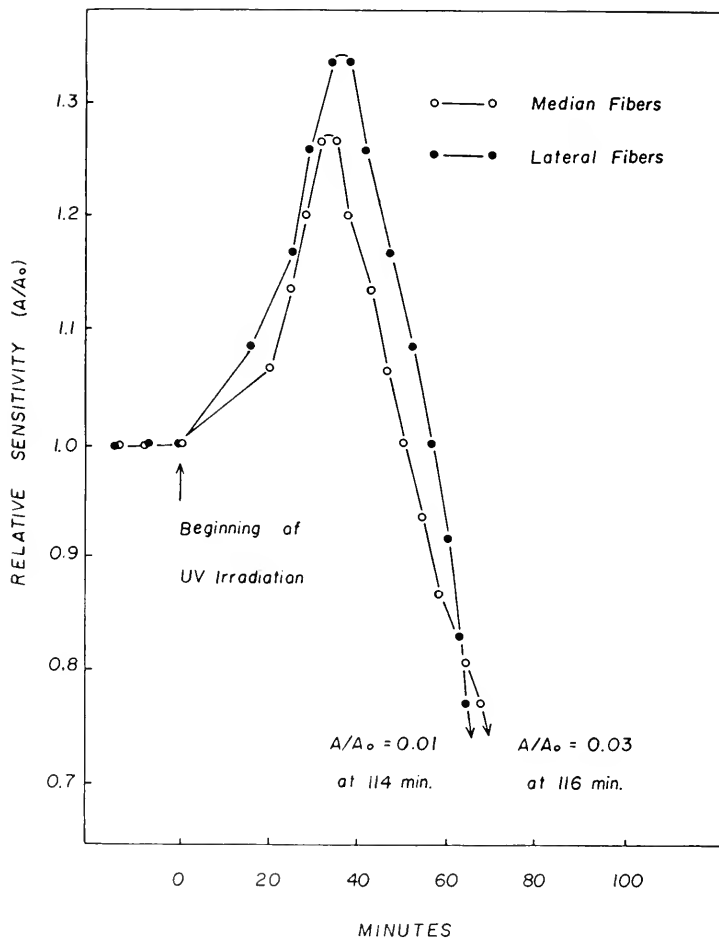


FIG. 12. Relative sensitivity to electrical stimulation during UV irradiation of giant nerve fibers of earthworm, plotted as a function of time of irradiation. Dose rate: 7,200 ergs per cm^2 per sec.

tive amplitude of the spike and the corresponding relative rate of spike rise are shown in Fig. 15. From the beginning of irradiation there was an obvious downward drift in the rate of spike rise, indicated by the dotted line, on which was superimposed a secondary rise corresponding for the most part to the increase in amplitude. This analysis of the rate of spike rise as a function of amplitude of action potential shows that the highest spikes tended to accompany the highest rates of voltage change.

Figure 16 shows the results of UV irradiation on refractory period in three sets of experiments. Only a part of the preirradiation period is shown

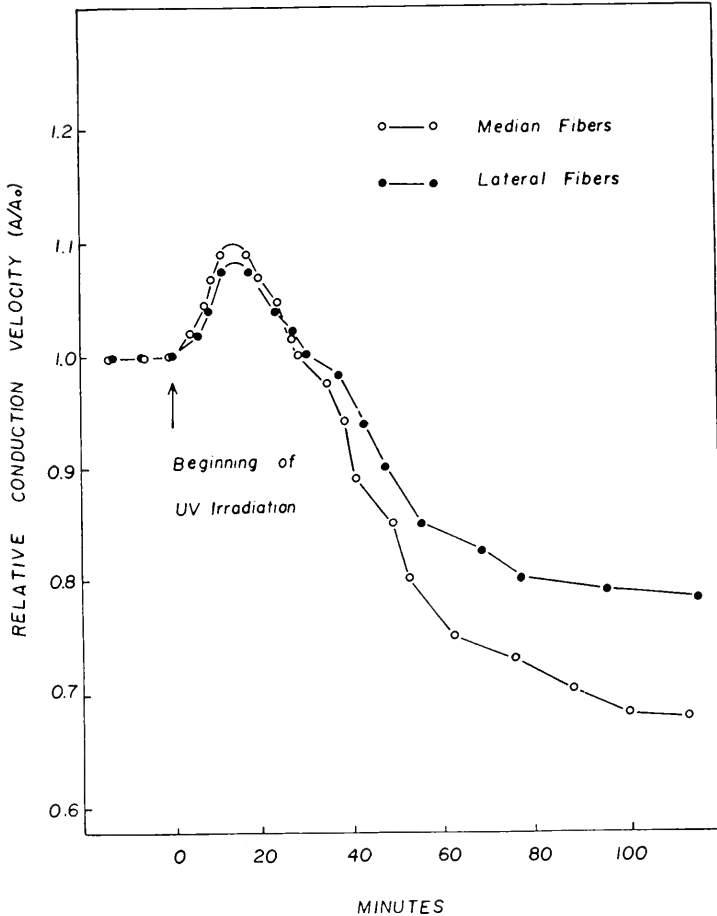


FIG. 13. Relative conduction velocity of giant nerve fiber impulse under UV irradiation, plotted as a function of time of irradiation, as in Fig. 12.

on the graph, since for stable nerves the value did not deviate significantly, once the nerve had settled on the electrodes and the initial period of instability had passed. The relative activity represents the ratio of the refractory period in milliseconds at the beginning of irradiation to the refractory period in milliseconds at any designated time. This inverse relationship is used because a decrease in refractory period connotes an increase in responsiveness of the nerve. Hence, a rise in the curve indicates an enhancement of activity of the nerve. Figure 16 shows the same twofold effect observed previously—an almost immediate enhancement of activity, followed by an

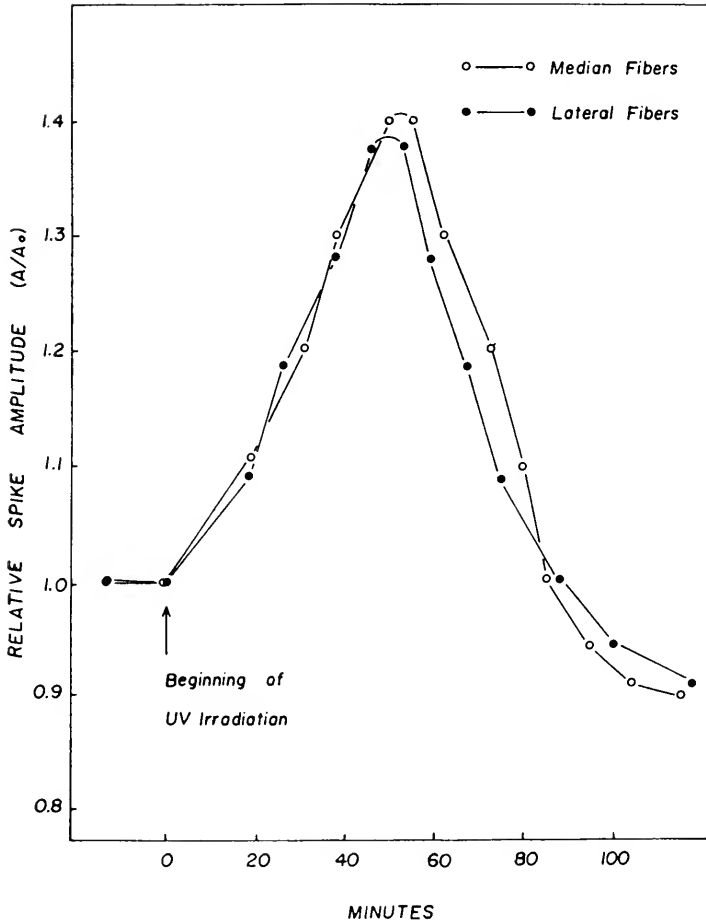


FIG. 14. Relative spike amplitude of impulses of giant nerve fibers under UV irradiation, plotted as a function of time of irradiation, as in Fig. 12.

impairment of activity, leading to ultimate block. The lowest curve depicts the effect on refractory period of UV irradiation at a relatively high dose rate and is contrasted with the center curve, which represents a lower dose rate. The enhancement of activity at the lower dose rate was considerably greater than that observed at the higher dose rate. At even higher dose rates, the results of which are not shown, the enhancement was proportionately less. It was possible to increase the dose rate to the point where no enhancement of activity was observed, only a decrease in activity. This observation suggested a second type of experiment, the results of which are shown in

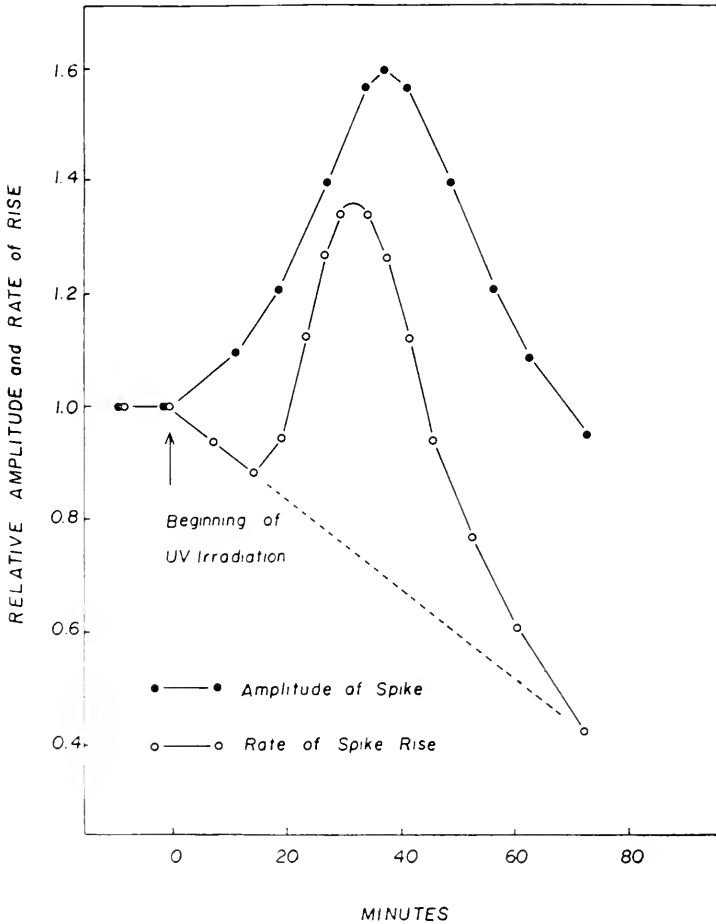


FIG. 15. Relative spike amplitude and rate of spike rise of impulses of giant nerve fibers under UV irradiation, plotted as a function of time of irradiation, as in Fig. 12.

the top curve, in which the nerve was irradiated until enhancement manifested itself, at which time irradiation was terminated, but recording continued. The results show not only that the energy supplied by concomitant bombardment with UV is unnecessary for enhanced activity, but, once the nerve fiber has been altered by UV, that the enhancement continues to increase to even greater values than are possible with nerves subjected to the concomitant destructive action of UV (Bachofer, 1960b).

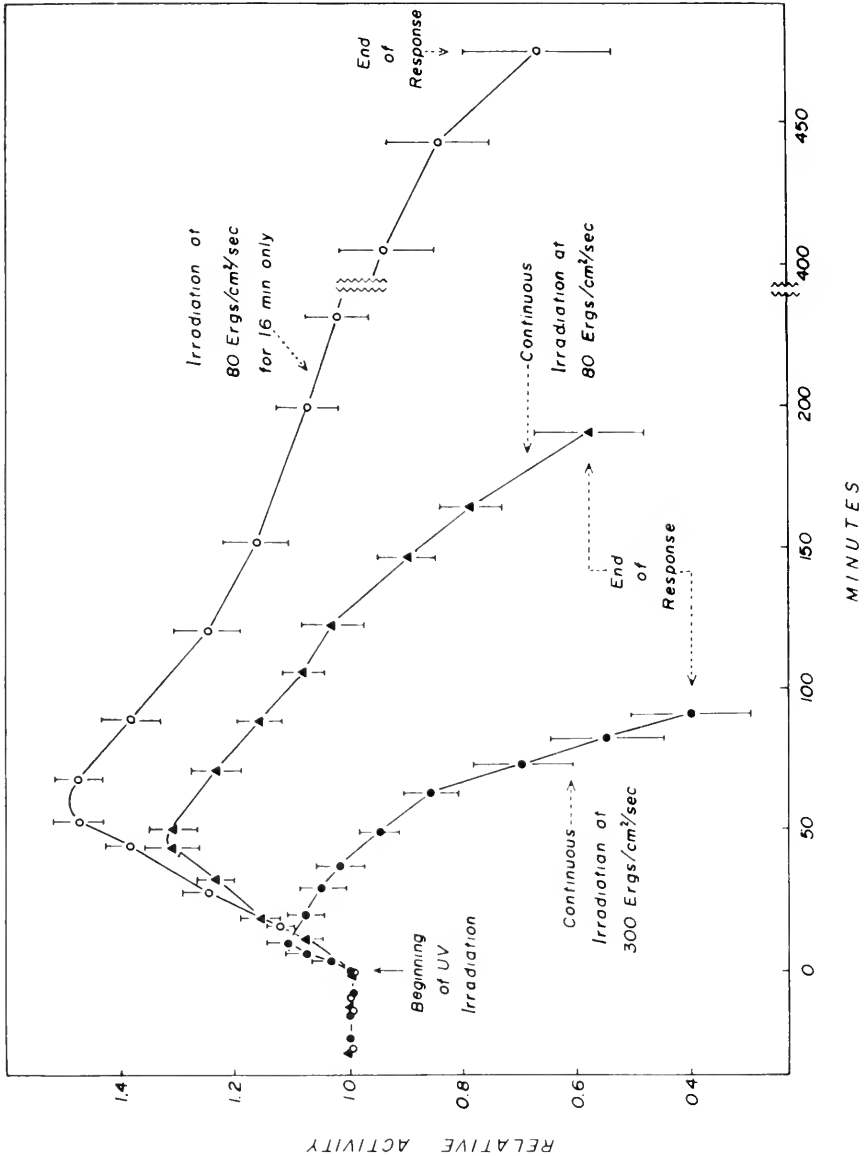


FIG. 16. Effect of UV irradiation on refractory period of single median giant nerve fibers of earthworm. Relative activity is the ratio of the refractory period in milliseconds at the beginning of irradiation to the refractory period in milliseconds at any designated time. This ratio is plotted as a function of time after the beginning of irradiation. Enhancement of activity precedes deterioration and ultimate block. Mean values are plotted together with standard deviations.

Discussion

The enhancement of activity of nerves during irradiation is not due to the additional energy supplied by radiation at the time the nerve responds to stimulation. The possibility of such synergistic action between the energy supplied by radiation and the nerve fiber itself can be ruled out by discontinuing irradiation when the nerve is responding in an enhanced manner; the nerve continues to respond in an enhanced manner even without concomitant irradiation. There is, moreover, the phenomenon of continuation of enhancement even after the cessation of irradiation (Figs. 5 and 16).

The experiments summarized in this paper were not designed to test the theory of excitation proposed by Hodgkin and Huxley, but the results can be shown to be compatible with the theory. According to this theory, an increase in spike amplitude is the result of an increase in the flow of sodium into the cell. Since, in the present experiments, the external concentration of sodium was kept constant, it appears that irradiation altered the properties of the biologic system in such a way that the membrane became more selectively permeable to sodium ions, the result being an increase in amplitude of the action potential. If, according to the theory, the propagation of the impulse depends on sufficient amplitude of the action potential, then the increase in sensitivity due to irradiation would also result from the same increased permeability to sodium. The rate of rise of the action potential has also been shown, according to the ionic theory of excitation, to be related to the magnitude of the action potential; this relationship was also observed as a result of irradiation. Since the velocity of propagation is a function of the resistance of the outside and the inside media of the fiber, as well as the amplitude of the action potential, the situation is somewhat more complex when one deals with the effect of radiation on conduction velocity. It appears that radiation affects the conduction velocity chiefly by altering the resistance of the internal media of the fiber.

The above speculations on sodium permeability appear to be substantiated by the work of Rothenberg (1950), who showed that x-irradiation of squid axons produced increased permeability to sodium. On the other hand, Mullins (1939) concluded that beta irradiation of *Nitella* reduced the rate of sodium transport across the cell membrane. The apparent inconsistency can be reconciled, perhaps, if one recognizes that Rothenberg (1950) gave fairly low doses which allowed the normal excitatory processes to take place. Under these circumstances, he found an increase in permeability. These results would correspond to the period of enhancement observed in the present experiments. Mullins (1939) recorded the rate of sodium transport in cells damaged by irradiation, and his results would correspond to the fall in response reported in the present experiments.

Lest the results of x-irradiation reported here be attributed to any particular or specific derangement requiring ionization as a cause, it should be noted that, in general, all of the effects produced by ionizing radiation can be duplicated with nonionizing radiation, provided only that the biologic preparation be such that nonionizing radiation can penetrate to vital sites. Just where these vital sites are, or what vital components are involved, is not known. Current research in our laboratory indicates that certain components of the metabolic mill may constitute vulnerable targets of radiation. Such components may indeed be the affected entities, and their alteration may be the basic seat of the altered function of the nerve. Observations on membrane permeability, for example, may be valuable indicators of the direction in which we should look in seeking for more basic effects. To speak of changes in permeability of the nerve membrane as the alteration responsible for the altered function of the nerve (as we have done) may amount to singling out one measurable manifestation which has its foundation in more basic aberrations; it is, no doubt, the end result of a complex chain of reactions.

In conclusion, to leave the molecular level of interpretation for a possible application at the systemic level, we might note in the present results that there is a steady fall in conduction velocity at the same time that the spike amplitude is rising. Such results suggest a neuronal basis for the immediate lack of coordination and loss of equilibrium observed in animals subjected to intense irradiation. Even delayed effects of irradiation, manifested in various organs and systems, and of uncertain cause, may have their basis in such neuronal aberrations as reported here.

Summary

The ventral caudal nerves of the rat, the median and lateral giant nerve fibers of the earthworm, and the median giant axons of the lobster were subjected to x-irradiation *in vitro*. The ventral caudal nerves of the rat were also subjected to x-irradiation *in situ*. The giant fibers of the earthworm were, in addition, subjected to UV irradiation. Various electrophysiologic responses were observed before, during, and, in some cases, after irradiation. Electrical stimulation was used to evoke propagated impulses, and measurements were made of amplitude and rate of rise of the action potential, conduction velocity of the propagated impulse, sensitivity of the nerve to stimulation, and relative refractory period. Photographic records of oscillographic traces were projected for measurement and analysis.

The pattern of response for all nerves was essentially the same during irradiation, whether the nerve was irradiated with x-rays or with UV. In response to external stimulation during irradiation, all nerves showed an

increase in activity initially, a period of enhanced activity, a fall in activity, and, ultimately, cessation of activity. The degree of enhancement observed was not the same for all activities, nor did the time of maximum enhancement coincide for all activities. Spike amplitude, for example, consistently showed great enhancement, whereas conduction velocity inconsistently showed slight enhancement.

Enhancement of activity, attributable to bombardment with x-rays or with UV, rarely observed in biologic investigations of this sort, was followed by deterioration of activity and eventual blocking of activity, representative of the lethal action of irradiation commonly observed. The time at which maximum activity of one factor occurred did not coincide with the time at which the maxima of other activities occurred; spike amplitude, for example, was observed to increase, while conduction velocity had already reached its maximum and was rapidly declining.

The energy supplied by bombardment with x-rays or with UV did not act synergistically during the enhanced response; that is, concomitant irradiation was not necessary to produce the enhanced response, once the nerve had been altered by irradiation. Once the nerve was responding in an enhanced manner, cessation of irradiation for short periods had no effect on the response. In certain types of experiments, the response would continue to rise if it was in the rising phase when irradiation was stopped, and continue to fall if it was in the falling phase. The rise or fall proceeded at slower rates when irradiation was stopped.

Stimulation at higher frequencies considerably reduced the enhancement of spike amplitude attributable to x-rays, showed a somewhat lesser effect on enhancement of sensitivity, and showed no effect on enhancement of conduction velocity. Nerves under both stimulation and irradiation failed to respond as long as nerves under irradiation alone. Stimulation apparently imposed an additional stress on the nerves.

The magnitude of enhancement of activity was dependent on dose rate. At low dose rates the enhancement was greater than at high dose rates. It was possible to increase the dose rate to the point that no enhancement was observed.

Acknowledgments

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Alteration of Mammalian Nerve Compound Action Potentials by Beta Irradiation

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Introduction

In an earlier work (Gasteiger, 1959) the radioresistivity to beta irradiation of fast A fibers of cutaneous nerves was reported, with a review of other closely related research. In nearly all studies the assays have been made on large A fibers. Aside from an earlier summary (Gasteiger, 1951) of the work reported here, only Gerstner (1956) has published observations on the comparative sensitivity of other fiber types to ionizing radiations.

Although it is important to accumulate information on the response of various nerve types to radiations, the interpretation of the relative sensitivities is, to a large degree, lost in our limited knowledge of mechanism and structure of the smaller fibers. Since, in a few cases, irradiation of patients reaches very high levels in a small field, and irradiation neuritis does result, investigation of differential sensitivity may be of definite practical importance. Also, with the increasing number of irradiation studies of the central nervous system, a knowledge of the relative sensitivity of fiber types becomes essential for interpretation of experimental observations.

Methods

A total of 43 experiments were performed on sural, saphenous, and vagal nerves removed from 18 Dial-anesthetized cats and placed in a commercial Simms solution at room temperature until needed. Before transferring a

nerve to a lucite recording chamber, extraneous connective tissue was removed, and the nerve ending was crushed to provide an electrical, inactive point for the distal recording electrode.

Across the recording chamber, 11 silver wires of 20 mils diameter were fixed to support the nerve and serve as stimulating and recording electrodes. By means of a switching system, the stimulus, a thyratron-controlled condenser discharge, could be applied to the nerve at positions of choice. All stimuli were supramaximal. Throughout the experiment, the point of stimulation was alternated from between the sites of irradiation and recording to a point on the opposite side of the irradiation site. In this way, both experimental and control recordings could be obtained from the same nerves. In nerves not long enough for recording such "running" controls, a test of the nerve viability could frequently be made after irradiation block by moving the nerve sufficiently to stimulate it just beyond the block or by turning the nerve end-for-end on the electrodes to stimulate an unirradiated portion of the nerve. A third type of control consisted of placing a nerve in the chamber without a radiation source and observing the stability of the action potentials over several hours.

The monophasic action potentials were amplified by an AC differential amplifier with flat frequency response between 10 and 2,000 cycles. They were then photographed from a sweep synchronized oscilloscope. Usually an electrode between stimulating and recording sites was grounded to reduce electrical artifacts.

Drying of preparations was prevented by flooding the floor of the chamber with Simms solution and by keeping the chamber covered after carefully wetting the nerve on the electrodes. When experiments were performed at higher temperatures (31–38°C) as contrasted to room temperatures (24–29°C), the chamber was placed in an incubator and allowed to equilibrate before beginning irradiation.

The beta source used in these experiments consisted of small glass bulbs of radon gas, 3 to 4 mm in diameter, with walls thick enough to absorb all alpha particles emitted by the radon decay products. Such bulbs have been commonly used as beta sources for the treatment of eye disease. Their intensity was measured in terms of their gamma emission, which has a maximum value of 9% of the total ionization—a negligible amount in these experiments, since the biologic effectiveness of the gammas is relatively very low when contrasted to the betas. The intensity of the beta ionization from these radon sources was compared with that of a strontium standard by means of a scintillation counter, and an average value of 10,000 \pm 2,000 rep per 100 mc-min was determined.¹ In practice both radon-filled bulbs

¹ This calibration was made by Mr. J. C. Carlson of the University of Minnesota Hospitals.

and empty bulbs for controls were placed in contact with the nerves. Because of the great irradiation hazard, it was necessary to carefully shield the chamber with lead and maintain a working distance of more than 5 feet during most of the experiment. At no time did a film badge worn on the wrist indicate an above-tolerance exposure of the experimenter.

An analysis of the action potentials was made by projecting them onto squared paper and measuring the amplitude or area of the potential components as being proportional to their activity. Considerable uncertainty was involved in determining the beginning and end of a potential component, but criteria of choice were maintained throughout an experiment, and the relative changes are a valid measure of the irradiation effect.

Results

Potential components were identified and labeled on the basis of their relative conduction velocities and amplitudes. For the sural and saphenous nerves the beta and delta components were readily seen, while the gamma component was far more variable, and the C fiber potentials could usually be viewed only at stimulus intensities damaging to the beta fibers. To view the C fiber potentials, the stimulus strength was increased at infrequent intervals during an experiment, or else a spot check was made at the conclusion of an experiment to determine whether C activity was still present. In a few cases, second beta and gamma fractions could be seen; the second beta peak of the compound action potential usually appeared after the beta peak had been depressed somewhat by irradiation.

The components of the vagal potentials were even more difficult to separate and identify. The delta and B fiber potentials in all but three cases appeared together and were called the "slow" group, while the fastest component was called "A" group, after Middleton *et al.* (1950).

Control recordings under these experimental conditions showed large variation in survival times and order of loss of potential components. However, the control potentials always persisted much longer than the experimental, and the components were lost in random order in contrast to the fast and systematic loss of potential components conducted through the irradiated segment. In some experiments, the shorter conduction distance made it difficult to identify the subpotentials in the control recordings. The three types of controls described earlier were consistent in giving a picture of long term viability for nonirradiated nerves. One must remember, however, that the irradiation effects reported here are a summation of a fast response to the beta radiations, plus a much slower deterioration which occurs without irradiation. The latter effect was enhanced in the higher temperature experiments.

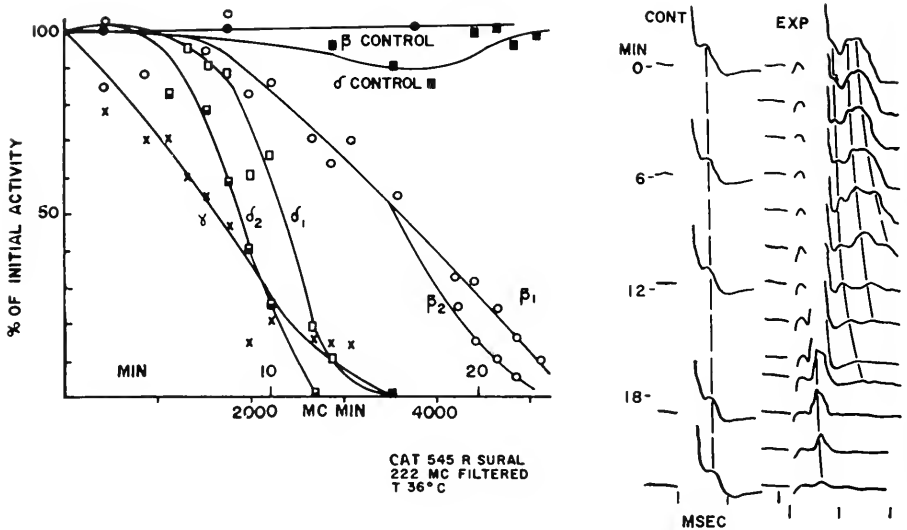


FIG. 1. The potentials to the right illustrate control and experimental responses from the sural nerve during irradiation with a 222 mc Rn source filtered with a 0.1 mm brass foil. Vertical spacing of potentials is proportional to the irradiation time. Decreasing conduction time as irradiation progresses is emphasized by the curving of the lines which connect the potential peaks. To the left, per cent change in activity as measured from amplitude or area of the potentials (see text) is plotted as a function of irradiation time in minutes and dose in millicurie-minutes. In general, these curves tend to be sigmoid, and the smaller fibers of the sural and saphenous nerves were blocked first.

Illustrations of changes in control and experimental action potentials of the sural and vagal nerves are seen in Figs. 1 and 2, respectively. The potentials have been traced one below the other on a time scale which is proportional to the period of irradiation. By connecting their peaks, one obtains a picture of the change in conduction velocity as a function of irradiation time and fiber type. The slower A fibers show a greater sensitivity to the radiations since their conduction velocities are more markedly reduced. Exploration of irradiated nerves revealed that slowing of conduction occurred in less than 1 cm of the nerve, due to the attenuation of beta ray intensity according to the inverse square law.

To appreciate the change in nerve activity as an experiment progressed, the potential components were plotted as a function of irradiation time in minutes and of dose in millicurie-minutes. The plots in Figs. 1 and 2 graphically illustrate changes of the corresponding potentials. An illustration of a similar response of the sural nerve at 27°C is found in Gasteiger's 1959

paper where the differential sensitivity was not discussed. Basically the survival curves are sigmoid—the radiation having little or no effect in the early stage, followed by a fast fall as most of the activity is suppressed, and ending in a more gradual approach to zero activity. These experiments represent the mean results determined from all experiments. The gamma curve of Fig. 1 is an exception, since the gamma fiber usually showed a sensitivity lying between beta and delta fibers. The gamma fibers were the most difficult of the A group to assay and showed a large variability which is evident in the summary of all experiments (Table I).

In the experiment on cat 545 (Fig. 1), a 0.1 mm brass foil filtered the strong beta source. Although such a filter was seldom used, this experiment is particularly interesting, since two components of the delta potential are seen, and a second component of the beta potential is revealed after 16 minutes of irradiation. The "walking out" of such a hidden component was frequently seen, an effect which is difficult to explain, since each potential component results from a population of fibers.

A check for C fibers is illustrated in Fig. 2 at the 16 minute recording. At this time the stimulus strength was increased, and the oscilloscope time

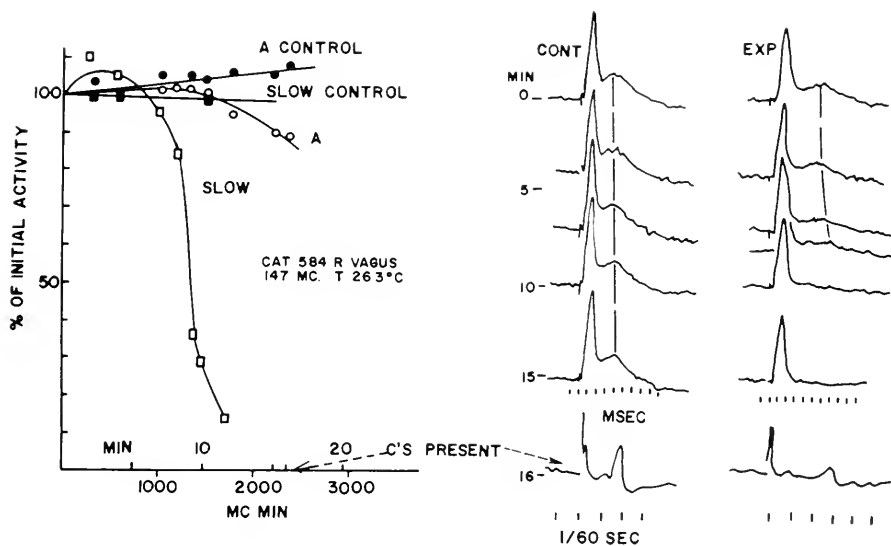


FIG. 2. Response of vagus nerve activity to a 147 mc Rn source. Changes are illustrated as in Fig. 1. The vagal potential was divided into two components: "A" group, which was of high amplitude and velocity, and "slow" group, which is made up of a mixture of B and delta fibers. This latter group was more radiosensitive. After 16 min of irradiation (bottom of figure) the stimulus strength was increased to reveal many conducting C fibers.

TABLE I

SUMMARY OF RELATIVE SENSITIVITIES

<i>Number</i>	<i>Sural and saphenous</i>	<i>Number</i>	<i>Vagus</i>
13	$\delta > \beta$	2	slow $>$ A
4	$\delta > \gamma > \beta$	2	slow $>$ A $>$ C
1	$\delta > \gamma = \beta$	2	B $>$ $\delta >$ A
2	$\delta_2 > \delta_1 > \beta$	1	A and slow $>$ C
1	$\delta > \beta > \gamma$	1	A $>$ slow $>$ C
1	$\delta >$ C $>$ $\beta >$ γ	1	A $>$ C
1	$\gamma > \delta_2 > \delta_1 > \beta$	1	C $>$ A
1	C $>$ $\delta >$ β	1	slow $>$ C
—		—	
24		11	
<i>Spot checks for C fibers</i>			
3	C $>$ B	2	slow $>$ C
1	C $>$ $\gamma >$ β	1	B $>$ $\delta >$ C
1	$\delta >$ C	—	
—		3	
5			

axis was decreased to record the presence of C fibers after the vagal slow fibers had been blocked and the fast A fibers were still conducting.

The scope and range of responses in all experiments are presented in Table I for the sural, saphenous and vagal nerves. The position of a fiber group within a category of observations was based on the irradiation dose required for 37% depression of nerve activity, and the number of experiments which fitted each category is listed to the right of the category. All experiments in which spot checks for C potentials were made are included in the bottom section of the table.

From these experiments, it is concluded that the A fibers of sural and saphenous nerves show a decreasing sensitivity to beta irradiation in the following order: delta, gamma, and beta. Although inconclusive, the observations indicate that C fibers are less than or equal to the deltas in sensitivity, but greater than the betas.

Results from the vagal experiments show the slow fibers (delta and B) to be more sensitive than the fast A fibers. In 3 experiments in which the B fiber potential could be separated from the delta potential, the B fibers displayed greater sensitivity. The vagal C fibers were studied in 7 experiments by continuous recording and in 3 experiments by spot checks at the end of the irradiation periods. In 8 cases C and slow fibers could be contrasted; in 7 of them the C fibers proved to be less sensitive than the slow fibers, and in 1 they were equal. In 6 cases where comparisons could be

made between the C and fast A fibers, 5 showed the C's were less sensitive, and 1 showed the converse. For the vagus, it was concluded that the C fibers are less sensitive to beta particles than the A and B fibers.

Higher temperatures did not affect the quality of the responses, but simply speeded the changes in the potentials. At 24–29°C, the mean 37% survival dose for irradiation of beta A fibers with sources ranging in strength from 48–136 mc was 255,000 rep, while at 31–38°C the survival dose for sources ranging from 84–173 mc was 60,000 rep.

Discussion

The current convention of considering alpha A fibers as being confined to nerves of muscles has been adopted in this work. Consequently, the fastest component of the sural and saphenous compound action potentials has been labeled "beta", since these nerves are cutaneous in distribution. This absence of alpha fibers in our preparations, unfortunately, permits only a partial comparison of our results to those of Gerstner (1956). Our findings that gamma fibers are more sensitive than betas is in agreement with his report for gammas and betas of the frog sciatic nerve irradiated with x-rays. Our observations extend to the delta fibers and suggest a continuum of increasing sensitivity for A fibers as fiber diameter decreases. Gerstner's surprising finding that alpha A fibers are more sensitive than betas mitigates against this generalization. In view of these results, an explanation of the relative sensitivities must indeed be complex.

The sigmoid shape of the survival curve can frequently be interpreted as resulting from an all-or-none destruction of a nonhomogeneous population. There is no doubt that the fibers in the nerve trunks represent a nonhomogeneous population, but at the same time, they are not destroyed here in an all-or-none manner. The reduction of the potential components results from blocking of individual fibers following progressive depolarization of these fibers. That such is the case is attested to by the reduction of the conduction velocity which has often been reported (Audiat *et al.*, 1934; Bachofer and Gautereaux, 1960; Gerstner, 1956) and by the report (Audiat, 1932) of irradiation injury currents in nerve. Progressive reduction of the action potential in the beta irradiation experiments on toad single fiber by Yamashita and Miyasaka (1952) and similar responses in the single fiber experiments of Bachofer and Gautereaux (1959) also substantiate this interpretation.

Since the time of block is a difficult end point to determine for a nerve trunk, the 37% survival dose was used as a superior measure (Lea, 1947). This method makes comparison of our results with those of other investigators somewhat difficult, but it permits more precise comparison of fiber

types and should be helpful in future investigations. The 255,000 rep dose to produce 37% survival of mammalian beta fibers at room temperature compares well with the 300,000 r dose reported for frog sciatic nerve (Audiat *et al.*, 1934; Gerstner, 1956). Gerstner *et al.* (1955) reported a considerably lower blocking dose for x-irradiation of rabbit sciatic. However, in their experiments the block occurred up to 1 hour after the x-ray dose had been delivered, while here the irradiation was continued until block. Increased temperature markedly reduced the 37% survival dose to 60,000 rep. Although a similar effect has been reported for the frog sciatic nerve in response to x-ray (Audiat and Piffault, 1934), the effect was not nearly as profound as seen here for mammalian nerve.

Summary

The compound action potentials of cat sural, saphenous, and vagus nerves were recorded during irradiation of a short length of the nerves with beta rays from a small bulb filled with radon.

For sural and saphenous nerve fibers, the order of decreasing sensitivity to irradiation was delta, gamma, and beta. Less conclusive results show the C fibers to be less than or equal to the deltas in sensitivity, but greater than the betas.

Slow fibers (delta and B) of the vagus nerve were more sensitive than the fast A fibers. The vagal C fibers were less sensitive than the A and B fibers.

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Action of Gamma Radiation on Electrical Brain Activity*

MARCEL MONNIER AND P. KRUPP

University of Basel, Switzerland

In the rabbit, the head was irradiated with a cobalt source producing chiefly gamma radiations. Various doses were applied: weak doses of 400 r. in 7 animals; medium doses of 600 r. in 7 other animals; and strong doses of 900 r. in 6 animals. The irradiation was effected under the following conditions: intensity: 1.3325 and 1.1728 Mv; surface: 29.16 and 23.76 cm²; focal distance: 30 and 20 cm; brass filter: 0.5 mm.

We observed and filmed the behavior of the animal before and after irradiation. The effects of gamma radiation on the electrical brain activity were analyzed using the stereotaxic method we developed with Dr. Gangloff for research on the conscious rabbit (Monnier and Gangloff, 1961).

A stereotaxic coordination system (Fig. 1) helps to locate precisely cortical and subcortical structures, in which electrodes are introduced for stimulation or recording purposes (Fig. 2). The electrical activities of the cortex are derived by means of epidural silver electrodes screwed into the skull, whereas subcortical structures are explored by means of platinum needle electrodes implanted in the caudate nucleus, hippocampus dorsalis, thalamus medialis and lateralis, and midbrain reticular formation. Attention is given not only to the spontaneous brain activity, but also to potentials evoked by electrical stimulation of the midbrain reticular formation, medial and lateral thalamus, and dorsal hippocampus. These structures are stimulated with needle electrodes of the Hess type. The technique allows recording of the electrical brain activity in the awakened restrained animal, as well as in the free moving animal (Fig. 3).

All derived electrical activities were recorded with a 16 channel Schwarzer electroencephalograph and, for closer analysis, with a cathode ray oscilloscope. We analyzed only the acute effects of irradiation, the earliest and latest observations being made at 30 minutes and at 6 hours after radiation.

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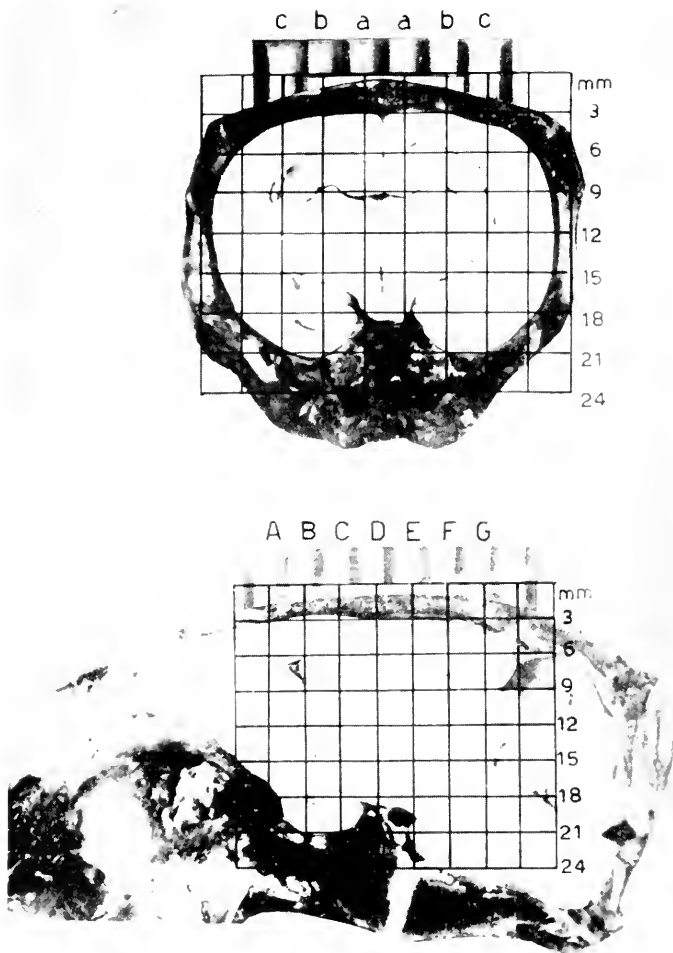


FIG. 1. Top: frontal section through skull and brain with stereotaxic coordinates. Socket screwed onto the skull. Bottom: sagittal section through skull and brain with stereotaxic coordinates. Socket screwed onto the skull (Monnier and Gangloff, 1961).

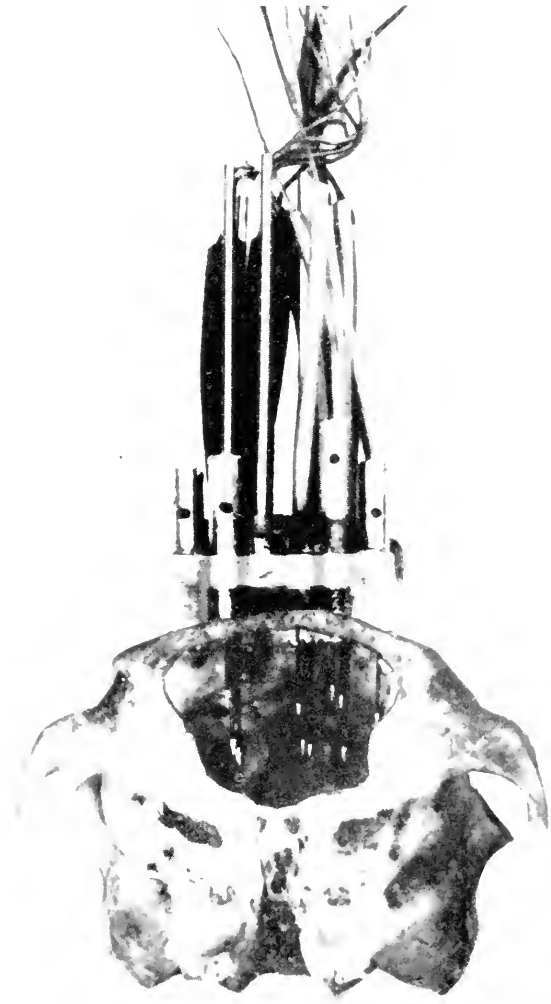


FIG. 2. Frontal view of the skull with stereotaxic socket and final placement of electrodes.

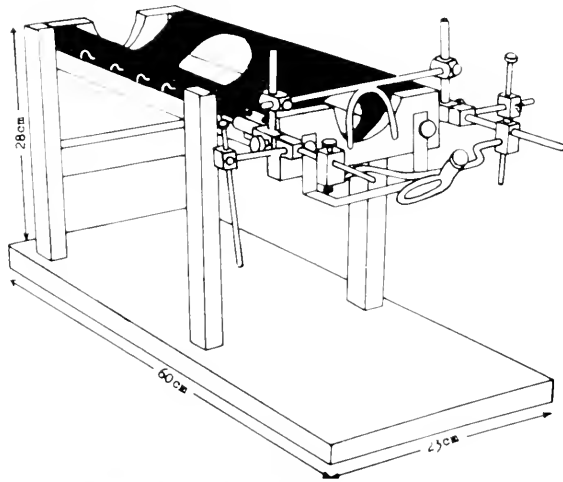


FIG. 3. Top: hammock for the restrained animal. Both forelegs and hindlegs can move freely. Bottom: recording from the brain of unrestrained animal.

respectively. In some animals the brain was removed after the experiment, for histologic control.

Results

ACTION OF GAMMA RADIATION ON THE SPONTANEOUS ELECTRICAL BRAIN ACTIVITY

Gamma radiation slightly, but definitely, alters the spontaneous electrical brain activity in the acute experiment (Fig. 4).

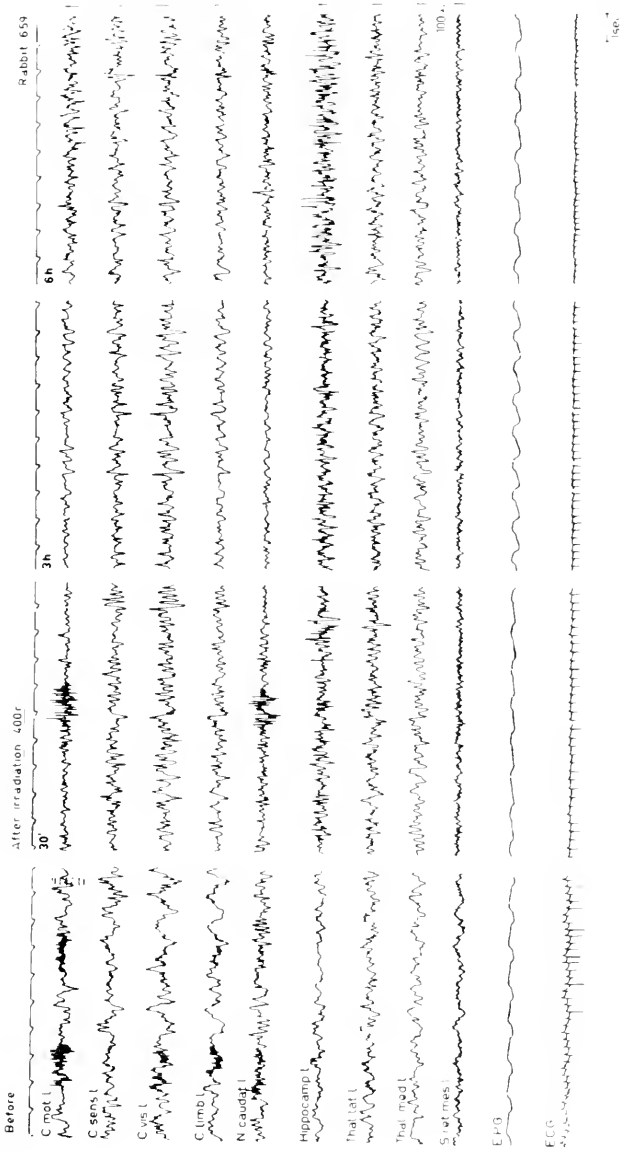


Fig. 4. Action of gamma irradiation (400 r) on the spontaneous electrical brain activity.

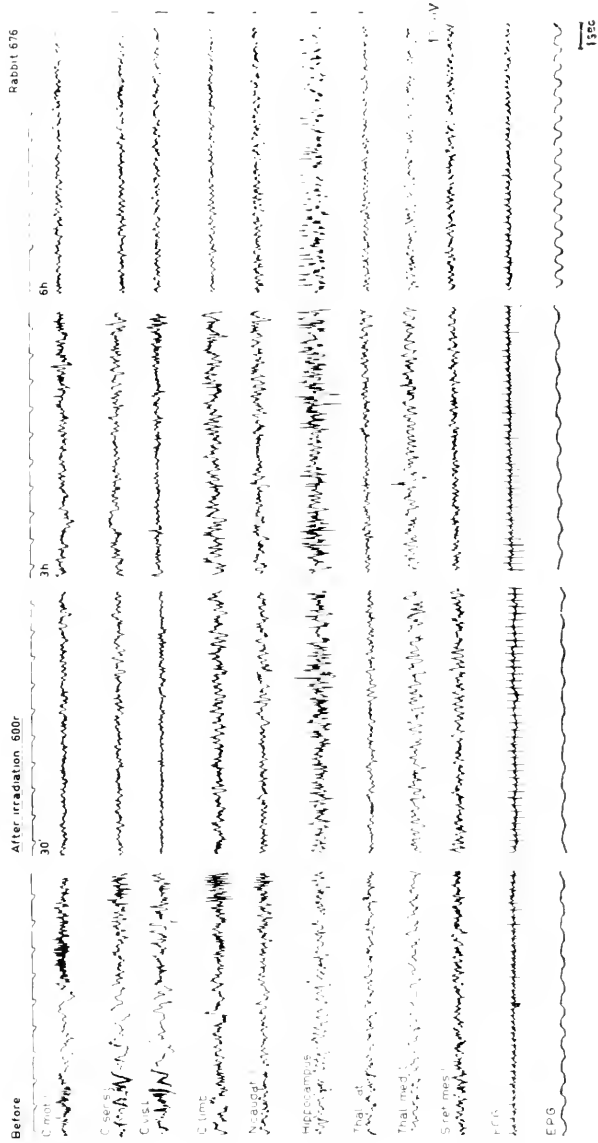


FIG. 5. Action of gamma irradiation (600 r) on the spontaneous electrical brain activity.

Weak irradiation with 400 r produces a marked activation of the archicortex (hippocampus) after only 30 minutes with faster rhythms, spike potentials, and some sharp waves. These symptoms increase steadily, especially the spike potentials, during the experiment, 3–4 hours after irradiation. Simultaneously, the neocortex shows early alterations. The high voltage slow activities (3 cps) of the previous resting state are replaced by low voltage faster activities with numerous sharp waves in the whole neocortex and even in the subcortex (thalamus). These symptoms suggest some early excitatory action of the gamma radiations (Fig. 4).

Stronger irradiation with 600 r confirms the early hyperactivity of the hippocampus with spike potentials of high amplitude and frequency. In the neocortex and subcortex, there is a progressive tendency towards desynchronization. The previous "high voltage slow waves and spindles" activities are replaced more and more by low voltage fast rhythms with sporadic sharp waves (Fig. 5).

Strong irradiation with 900 r shows a different electrographic picture: after 30 minutes a "high voltage slow wave syndrome" develops with numerous spindles. Five per sec rhythms and sharp waves are also present in the neocortex and thalamus. In the archicortex, there is strong hippocampal hyperactivity with series of spike potentials, sharp waves, and high voltage slow waves. All these symptoms reach a maximum after 3 and 4 hours (Fig. 6).

From these observations, we may conclude that weak gamma irradiations (400 and 600 r) tend to desynchronize the spontaneous electrical activity of the neocortex. Low voltage fast rhythms and sharp waves of irritative character replace the previous high voltage slow waves and spindles syndrome of the resting brain. In the archicortex, the hippocampus shows a marked hyperactivity, with series of high and frequent spike potentials and sharp waves. These alterations begin 30 minutes after irradiation and reach a maximum after 3–4 hours, concomitant with a hyperactive motor behavior involving increased oral patterns. Strong gamma irradiation (900 r) tends, on the contrary, to synchronize the whole brain activity at a low frequency (1–3 cps). Irritative symptoms, such as faster rhythms (5 cps), sharp waves in the neocortex, and precritical hyperactivity in the hippocampus, persist. These alterations reach a maximum after 3–4 hours; simultaneously, an abnormal passivity of behavior occurs.

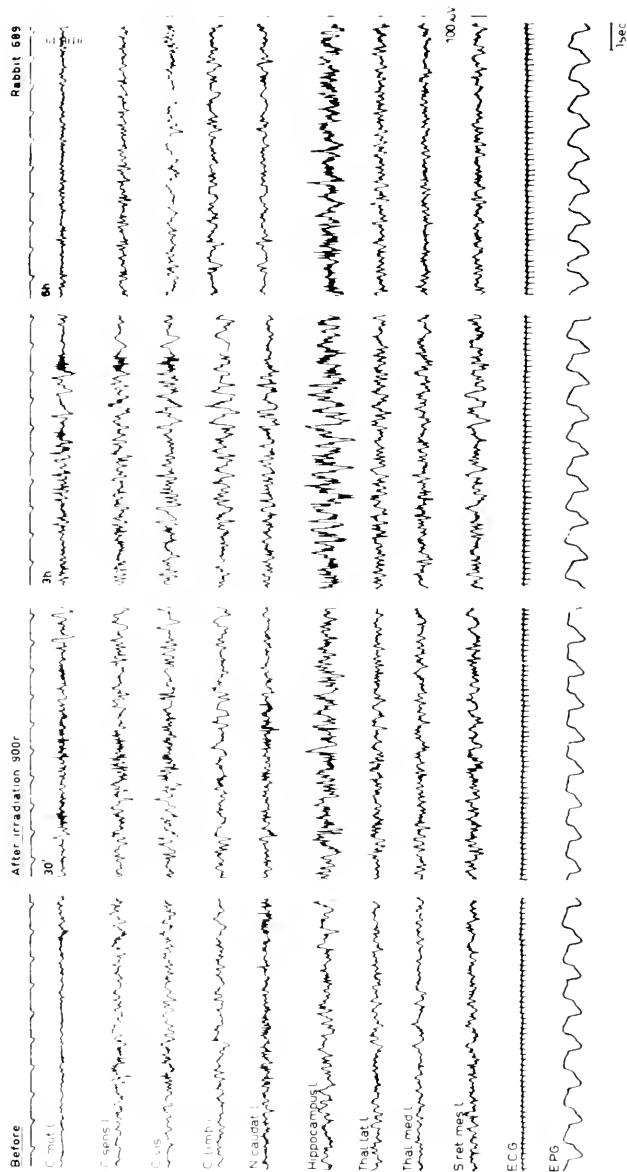


FIG. 6. Action of gamma irradiation (900 r) on the spontaneous electrical brain activity.

ACTION OF GAMMA RADIATION ON ELECTRICALLY EVOKED ACTIVITIES

Electrographic arousal reactions induced by an acoustic stimulus (click), or by electrical stimulation of the midbrain reticular system or posteroventral hypothalamus at high frequency (150 cps) are slightly enhanced by weak irradiations (400 r and 600 r), but slightly depressed by stronger doses (900 r) (Fig. 7).

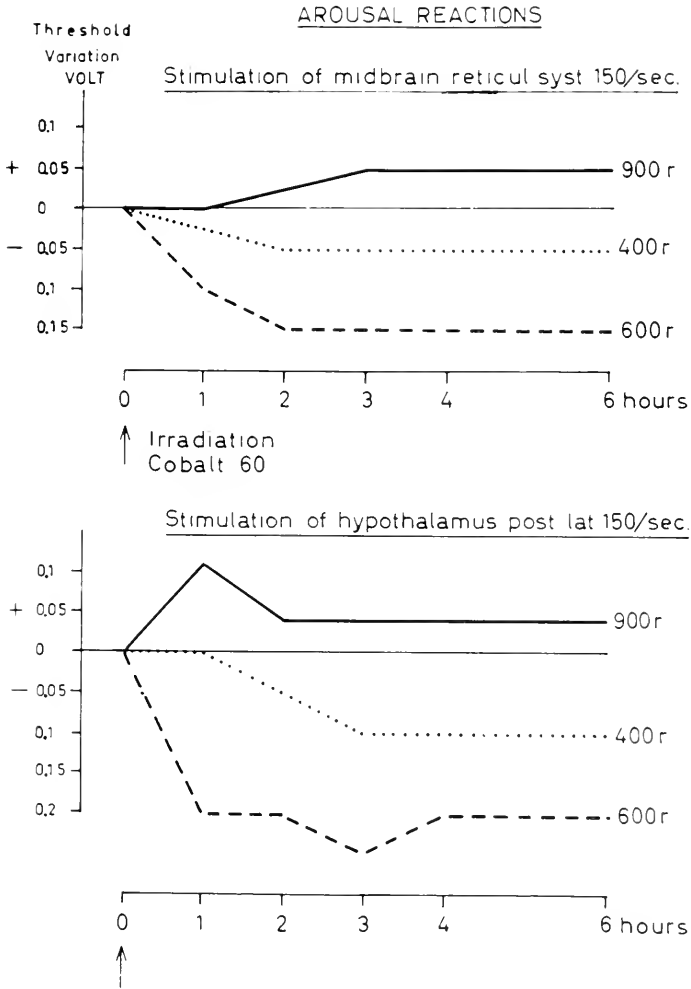


FIG. 7. Action of various gamma irradiations on the threshold of the arousal reactions evoked by stimulation (150 cps) of the midbrain reticular system (above) and of the posteroventral hypothalamus (below).

On the other hand, potentials evoked in the neocortex by stimulation of the midbrain reticular formation at low frequency tend to show increased amplitude and lowered threshold during the first hours after weak irradiation (600 r). This is no longer the case after strong irradiation (900 r) (Fig. 8).

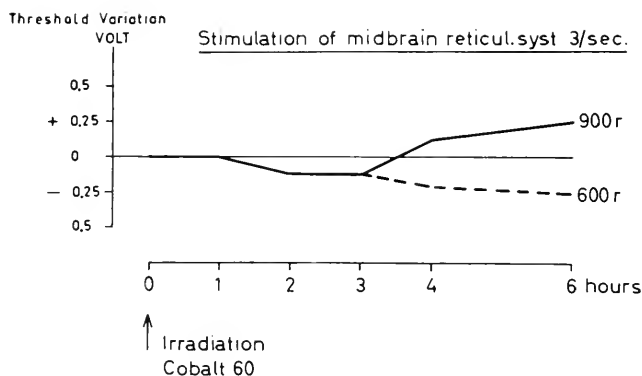


FIG. 8. Action of various gamma irradiations on the threshold of the cortical potentials evoked by stimulation of the midbrain reticular system.

These observations prove that weak gamma radiations slightly and transiently activate the reticular arousal system, but that stronger radiations diminish the effects of reticulo-thalamo-cortical projections, probably by a depressing action at the cortical level.

Potentials evoked in the hippocampus by low frequency stimulation of the contralateral hippocampus show a marked increase in amplitude after weak (400 r) and strong (900 r) gamma irradiation. There is no doubt that gamma radiations activate the archicortex (Fig. 9).

On the other hand, potentials evoked in the cortex by stimulation of the ipsilateral hippocampus at low frequency (3 cps) increase slightly in amplitude after weak doses (400 r), but decrease after strong doses (900 r).

Similarly, the threshold of these evoked cortical potentials shows a slight decrease after weak irradiation (400 r, 600 r) (Fig. 10); this facilitation disappears after strong irradiation.

This suggests again that weak gamma radiations activate the hippocampus and the hippocampocortical projection activity. Strong irradiation, on the contrary, moderates the effect of these projections, probably by depression at the cortical level.

The action of gamma radiations on the specific thalamocortical projection systems is less consistent. Weak doses (400 r) slightly enhance the specific evoked potentials, whereas stronger doses reduce their amplitude and raise

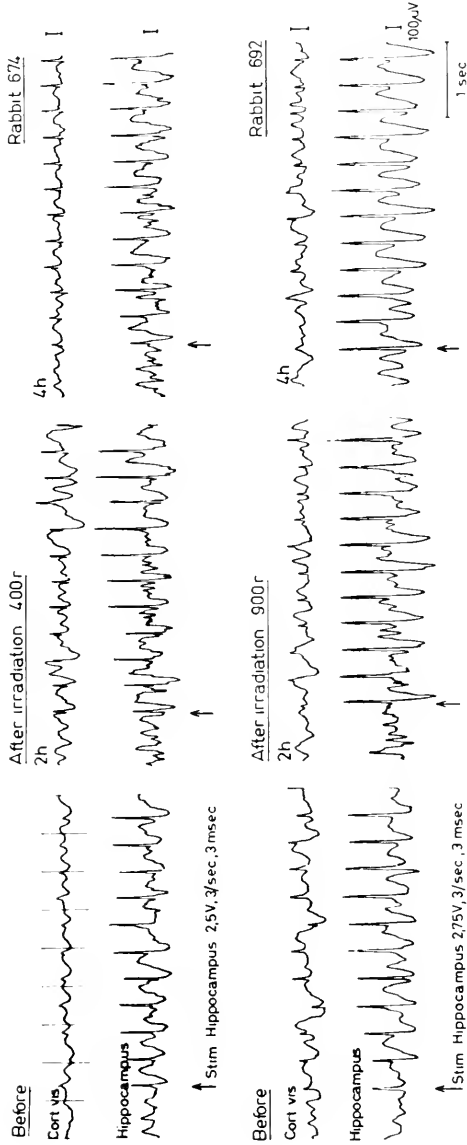


FIG. 9. Action of weak and strong gamma irradiation on the potentials evoked in the cortex (upper record) and in the hippocampus (lower record) by stimulation of the dorsal hippocampus.

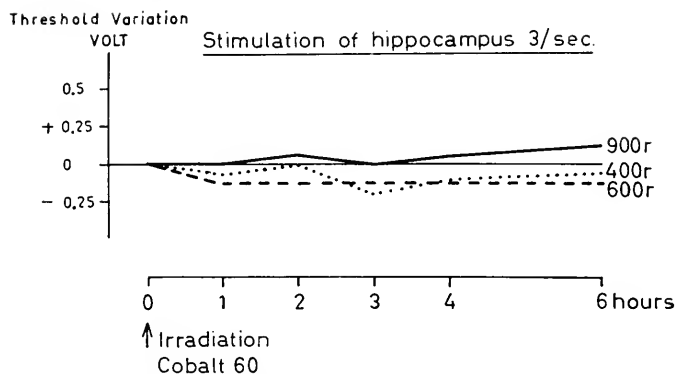


FIG. 10. Action of various gamma irradiations on the threshold variations of potentials evoked in the cortex by stimulation of the dorsal hippocampus.

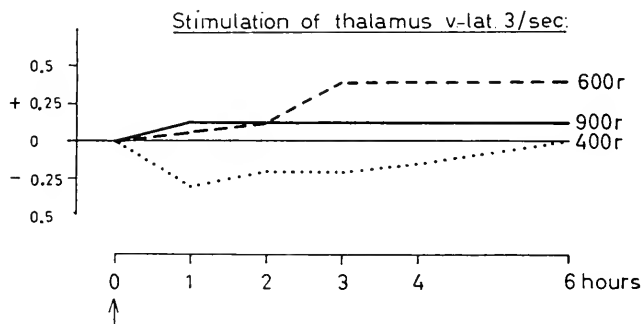


FIG. 11. Action of gamma irradiations on the threshold variations of potentials evoked in the somesthetic cortex (3 cps) by stimulation of the ventrolateral thalamus (specific thalamocortical projection).

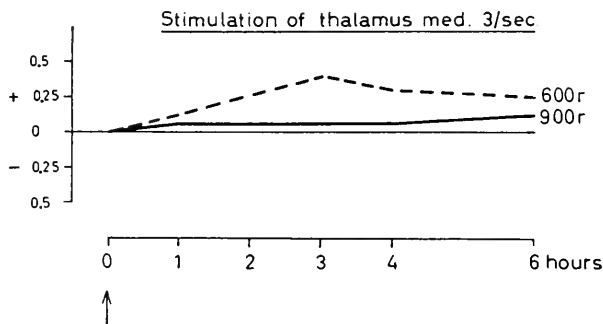


FIG. 12. Action of gamma irradiation on the threshold variations of the recruiting responses evoked in cortex by 3 cps stimulation of the medial thalamus (unspecific thalamocortical projection).

their threshold (Fig. 11). This is also the case with the unspecific thalamo-cortical projections. The threshold of the recruiting responses evoked in the cortex by stimulation of the medial thalamus increases slightly after stronger irradiations (Fig. 12).

The fact that all potentials evoked in the cortex by stimulation of reticular, hippocampal, or thalamic systems are reduced by stronger gamma irradiation (900 r) indicates that the cortical neurons and synapses responsible for the evoked cortical potentials are depressed by such higher doses.

Discussion and Conclusion

In opposition to the previous conception that the brain shows a marked resistance to ionizing radiations, the present results prove that weak gamma irradiation produces transitory, reversible alterations in this organ. Weak irradiation of the head in the rabbit (400 r, 600 r) is followed by hyperactive motor behavior with oral patterns, activated EEG with desynchronization in the neocortex, activation of the reticular ascending system, and activation of the archicortex with precritical discharge of spike potentials in the hippocampus. These observations agree with results of whole body irradiation (400 r) in the cat: transitory discharge of spike potentials in the hippocampus and amygdaloid nucleus, increased arousal rhythms in the cortex (Gangloff and Haley, 1960).

On the contrary, a stronger gamma irradiation (900 r) of the rabbits head moderates the cortical activity (high voltage slow wave syndrome), whereas a precritical hyperactivity persists in the hippocampal archicortex. Similarly, x-irradiation of the head (1,500 r) in the monkey is followed by slow brain activity (Ross *et al.*, 1954).

The reversibility of the electroencephalographic symptoms, namely of thalamocortical effects, observed after weak irradiation suggests an early action of gamma radiations on neuronal and synaptic activities at cortical level.

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GENERAL DISCUSSION

THOMAS J. HALEY (*University of California, Los Angeles, California*): There are four papers I would like to discuss. I was impressed with Dr. Brown's work, and would like to point out that the great sensitivity of the nervous system in the burro is to be contrasted with the high resistance of the blood platelet in the same species. So we must be very careful in all evaluations as to whether we are discussing different species or different reactions within the same species. With regard to Dr. Gasteiger's work, I was happy to hear that it was the preganglionic cholinergic fiber which was involved. We have just completed some work on the postganglionic endings of the vagus in the intestine. We took out the small intestine in guinea pigs receiving 500 r, and over the 1st week postirradiation, including the day of irradiation, we studied acetylcholine synthesis. There was no statistical difference between the control and the irradiated intestine. Thinking this might be a fluke, we then tested the gut under the same radiation conditions and used the Trendelenburg preparation, which sets off the gastric reflex by increasing the interluminal pressure. There was no difference in the response of the gut, pre-irradiation or postirradiation, to hexamethonium or other ganglionic blockers or to atropine. This would seem to indicate that neither the ganglion cells, which synthesize acetylcholine in the gut, or the terminal endings were affected at a dose of irradiation which would kill 100% of the guinea pigs exposed. I was very happy to hear about the results of Dr. Monnier's paper, inasmuch as one of his former students, Dr. Gangloff, and I worked together for a couple of years and came up with similar results in the cat. I might point out, however, that we are in some disagreement with our Russian colleagues inasmuch as when we shielded the head and irradiated the body of the animals, 3-4 r sneaked through, and we did not obtain any changes whatsoever in the EEG. In all other instances, we got the same type of results that Dr. Monnier presented so beautifully this morning.

W. O. CASTER (*University of Minnesota, Minneapolis, Minnesota*): After the review of the Soviet literature, it is most interesting that we can also find in this country changes in the milliroentgen range. I would like to know a little more about the neurology and neuroanatomy of Dr. Miller's audiogenic seizure effect. Does it have a human counterpart? The general characterization of some of the effects was that we were seeing more of these things that tended to be like psychotic tendencies in the human. Secondly, in line with some of Dr. Monnier's good descriptions as a biochemist I would appreciate having someone hook this together. Can you now put your finger on the centers and systems which might perhaps be related to the dolorogenic seizure phenomenon described? The dose levels certainly are the most interesting part of this paper. What was the lowest total dose found to produce an effect? Your ability to detect variations in background presumably due to fallout is particularly intriguing. Do you have any further information on this particular matter?

DOROTHEA STARBUCK MILLER (*University of Chicago*): The fallout background level was rising progressively to a peak in early 1959 and then dropped off. Within three groups of mice, we got three different reactions that were rather revealing. The F_1 group is intermediate in susceptibility and there was an astonishingly close parallel. On the first trial, which is the most delicate indicator, as the fallout level went up, the incidence of fatal seizures also went up. At the highest level we scrubbed the mouse quarters thoroughly, removing all the accumulation of dust, and this certainly reduced the local fallout level.

O. J. ANDY (*University of Mississippi, Jackson, Mississippi*): The implication of Dr. Monnier's fine paper and the other studies on behavior was that the limbic system was primarily involved in the low dosage irradiations. My question to Dr. Monnier involves an interpretation of his results with regard to the cortex being primarily involved in little higher dosages, and second higher dosages depressing the animal and making it passive. Simultaneously, the hippocampus showed increased activity above what it was at the lower dosage, I believe. I think, there was not a depressed cortex, that with higher dosage you could still get the same type of response from the animal with such hippocampal activity. In other words, you may have varying behavior or changes from hippocampal stimulation, the first type displayed, which may result in activation, and the limbic system will cause these tremors in the animals, as Dr. Brown described in burros. If you go above, it is possible to get a discharge in the system, during which time the animal becomes passive and appears depressed. Do you think that the hippocampus could be primarily involved in this instance, and that the cortex and the thalamus might not be as importantly depressed as you made us believe?

JOSEPH SHARP (*University of Utah*): I would like to know if you used different stimulating parameters in the mesencephalic reticulum to get your EEG changes. The reason I ask this is that in our laboratory we have shown that in using central stimulation for conditioned learning experiments, frequency is important. When we monitor the course of learning on the EEG, we create all kinds of havoc when we vary frequency of stimulation to the mesencephalic reticulum.

M. MONNIER (*Basel, Switzerland*): It is always encouraging to have converging research results as in the case with Dr. Haley's and Dr. Gangloff's observations. Dr. Caster asked two questions: The first one concerns the correlation of our electroencephalograph findings with biochemical changes. I cannot contribute personally to this question, but I learned yesterday that increased enzymatic changes were found in the hippocampus. This would correspond beautifully with the hyperactivity of the hippocampus in our experiments. Another impressing correlation was the increased fluorescence shown yesterday in the hypothalamus. This corresponds again with the threshold alterations we found in the posterior-ventral hypothalamus. Concerning the second question about audiogenic seizures, these seizures may be correlated with the increased reactivity of all thalamocortical projection systems and of the reticular arousal observed after weak irradiation in our experiments. If you apply stronger doses, the contrary will occur, because the thalamocortical projections are interrupted at cortical levels. I would expect that higher doses suppress the audiogenic seizures. Dr. Andy raised the question that passivity observed in our animals could not be related to hippocampal discharges into the medial thalamus. Such a mechanism is certainly worth considering. Strong

hippocampus activation produced a stuporous state in our animals resembling psychomotor stupor. The rabbit does not react any more during the critical discharge. Concerning Dr. Sharp's question, we use two stimulation techniques. One—high frequency of 150 per second—is used just for producing the classical arousing reaction. But threshold measurements of evoked potentials are done with single shock or low frequency stimulation (three per second). This is the low frequency stimulation with which we analyze the responses of all thalamocortical projections—specific and unspecific, hippocampocortical and reticulocortical projections. They all were analyzed at this low frequency stimulation.

HERBERT LOCKSLEY (*State University of Iowa, Iowa City, Iowa*): Dr. Brown made the interesting observation of postirradiation increase in spinal pressure in burros. Were these Nevada or Godiva irradiated burros? What was the average dose level? What was the time relationship of his measurements to irradiation?

DANIEL G. BROWN (*University of Tennessee, Oak Ridge, Tennessee*): These cerebrospinal fluid pressure measurements were made on burros that received 485 r to the head. We made no spinal fluid pressure measurements on any of the other burros. This was from the head irradiation experiment. The animals were in a morbid state, that is, they were in a state of coma and were near death. We measured the cerebrospinal fluid pressure at about 28 hours after irradiation and they died at about 30 hours.

EDGAR L. GASTEIGER (*University of Rochester, Rochester, New York*): I would like to ask Dr. Monnier, or anyone else using his implanted electrode technique, whether the effects might result from secondary emissions at the electrodes? When you irradiate a preparation with implanted metallic electrodes might you have localized effects due to secondary emissions from the electrodes?

M. MONNIER: The histologic controls showed a slight increase in the trace of the electrodes. However, it is not possible that the chief effects reported by us result from secondary emissions at the electrode. We have about 20 electrodes in the brain, and only some of them showed the specific changes described. That is why we are not too pessimistic about the role of the implanted electrodes.

C. D. CLEMENTE (*University of California, Los Angeles, California*): Professor Grashchenkov, there are a number of investigators in the United States who, too, believe vegetative centers in the central nervous system may be more radiosensitive than in other areas. Do the Russian investigators believe that the effects that they have shown with extremely low doses on brain areas are a result of humoral agents; and, if so, is this humoral agent epinephrine?

N. I. GRASCHENKOV (*Moscow, U. S. S. R.*): We have many contradictory publications about the part of the brain which is most sensitive to irradiation. I believe the hypothalamic area is more sensitive because I dealt with this area for many years with my collaborators and find much evidence to indicate that this part of the brain plays a very important role in many processes and is sensitive to ionizing radiation. But some Russian investigators, as I review the literature, believe that the cortex plays a very important role, and some show that even the cerebellum plays a very important role in ionizing radiation. Of course, we have different opinions about it, but most publications are concerned with hypothalamic areas or encephalitic areas which are more sensitive to ionizing radiations as most people show it with EEG methods and neurohumoral methods.

C. S. BACHOFER (*University of Notre Dame, South Bend, Indiana*): As one who has been radiating B and C fibers for some time I know that the work is not finished, but I would like to ask Sister Mary Albert if she would make a comment as to the relative sensitivity of B and C fibers to radiation.

SISTER MARY ALBERT (*Notre Dame*): The B and C fibers which I have been irradiating are the sympathetic fibers, and I get just about the same results that Father Bachofer reported as far as enhancement is concerned. I also find that C fibers are more resistant as Dr. Gasteiger pointed out with the vagus. It would seem that the parasympathetic and sympathetic have something in common.

C. T. GAFFEY (*University of California, Berkeley, California*): Dr. Noell, I was wondering what the minimal dose was that knocked out the ERG?

WERNER K. NOELL (*University of Buffalo, Buffalo, New York*): That must depend on the quality of the irradiation, but 2,000 kvp, 4,000 rad . . .

C. S. BACHOFER: If you wait, it goes down, you say, but how long after 4,000 rads does it go out? It does not go out immediately, does it?

WERNER K. NOELL: It drops out somewhere within 2 hours after radiation.

C. S. BACHOFER: A lower dose would knock it out if you waited a longer period of time, would it not?

WERNER K. NOELL: No. These are all acute effects.

C. S. BACHOFER: A higher dose would be required if you tried to knock it out sooner than 2 hours, is that correct?

WERNER K. NOELL: Yes, up to a certain time level.

C. S. BACHOFER: Would you care to say what time?

WERNER K. NOELL: Five minutes after the minimal dose is given.

C. S. BACHOFER: And the minimal dose you would set at what?

WERNER K. NOELL: The minimal dose for a knock out of it is 4,000 rads.

C. S. BACHOFER: Would you qualify it and say at 2 hours time?

WERNER K. NOELL: Yes.

C. S. BACHOFER: I presume it is going down up to 2 hours time, is that correct?

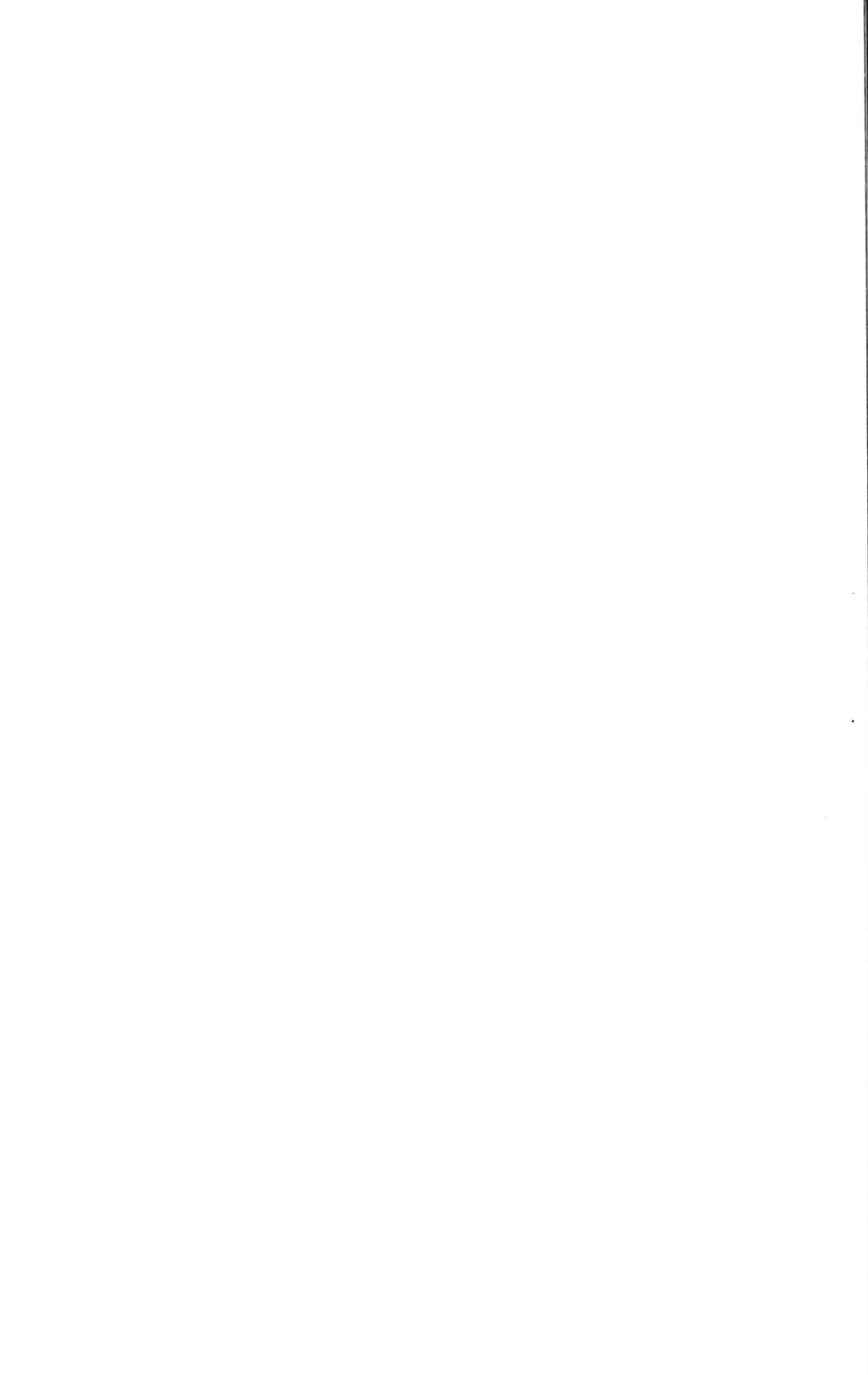
WERNER K. NOELL: No. The ERG effect is a thing which occurs in minutes. It is a sharp decline. Then it continues down for another 1½ hours. These are acute effects, and if these acute effects do not occur, then we have not reached the minimal dose.

C. S. BACHOFER: We have been watching the ERG response after irradiation for a couple of years, and there are two things we found. A heavy dose will knock out the ERG momentarily, but it will usually come back. The other aspect is the ERG after a dose of radiation will continue to decline over a long time and it will go down faster with a high dose and slower with a low dose. So the time and dosage together are important, I think.

LEO E. LIPETZ (*Ohio State University, Columbus, Ohio*): In regard to the paper by Dr. Sato, Dr. Stahl, and Dr. Austin, I would like to say that I had hoped the question of whether the cell body of the neuron is more sensitive than the axon would be settled, but after seeing the slides I have great reservations that this question has been answered at all.

PART V

**Psychological Effects of
Ionizing Radiation**



Effects of Radiation on the Central Nervous System and on Behavior—General Survey*

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*University of Wisconsin,
Madison, Wisconsin*

It has long been known that the adult nervous system, both peripheral and central, is resistant to radiation, and considerable information has been assembled to show that this is true regardless of the criterion taken—atomic, physiologic, or psychologic.

Lyman *et al.* (1933) reviewed the German and Russian literature and concluded that in the adult animal, irradiation produces little or no direct effect on the nerve cells or fibers. Subsequently, Warren (1944) concluded that "interruption of the vascular bed, rather than direct injury of the nerve elements, is probably the cause of the rare nervous symptoms following irradiation of the brain or cord," and others have made the same assumption. Desjardins (1931) reported that the neural portions of the eye are not easily damaged by x-rays and that "no evidence of injury to the auditory apparatus of human beings has ever been reported." Snider (1948) reported no changes in the nervous system of mice or chicks given Si^{89} or radium producing a dose below the LD/50/30, even though the animals were tested 1 year after treatment and even though autoradiographs demonstrated that the radioactive material had reached the central nervous system (CNS).

Gerstner (1958) presented evidence that showed the x-ray dose that would produce cerebral death in man was in the area of 3,500 r and above. Allen *et al.* (1960) demonstrated that high doses from a Co^{60} source, 9,000–40,000 r, produce a pattern of severe and progressive dysfunction of the CNS in macaque monkeys, resulting in so-called CNS death, as contrasted with hematopoietic death or gastrointestinal death. These investigators point out that there was also widespread injury throughout the body which undoubtedly contributed, directly or indirectly, to the short survival time. Allen *et al.* (1960) cited classical CNS symptomatology in some ani-

* Wisconsin researches reported were supported in part by the National Institutes of Health and in part by the University of Wisconsin.

mals at 5,000 r and noted that much smaller doses have produced severe changes in the CNS as reported by Haymaker *et al.* (1954).

Arnold *et al.* (1954), using a 23 Mev betatron source, reported that doses between 4,200 and 8,000 r destroyed all neural components, but left larger blood vessels intact, whereas doses between 1,800 and 3,000 r produced only slight neural or glial cell damage.

In general accordance with these results are data given by Settlage and Bogumil (1955). They implanted needles holding Co⁶⁰ seeds at the surface of the cortex of cats and monkeys. The needles were maintained in place for such a time as to deliver a dose of 4,000 r 5 mm from the radiation source, producing a hemispheric lesion of 8–10 mm in diameter. The transition from totally necrosed cells to apparently normal cells was abrupt, taking place within approximately a millimeter. These investigators also noted resistance of at least the larger blood vessels to damage, and the same phenomenon in man has been reported by O'Connell and Brunschwig (1937) and Wachowski and Chenault (1945). Thus, there is reason to question earlier interpretations that neurologic changes following brain irradiation were the indirect result of vascular damage. It would appear that radiation doses must attain a level of 3,500 r or more to produce widespread or total destruction of the cerebral neurons.

As might be expected, physiologic and biochemical measures of CNS function indicate radiation effects at doses lower than those necessary to produce cell degeneration. Brooks (1956) has shown that the threshold radiation dose necessary to produce prompt depression in the EEG approximated 1,000 r in a group of 12 monkeys subjected to 1,000 r per min from a Ba¹⁴⁰ and La¹⁴⁰ gamma radiation source. Caster *et al.* (1958) demonstrated an acute depression of low frequency EEG 12 hours after irradiation, and subsequently throughout test days 4 through 12. This change and some other changes in EEG pattern occurred after a single 700 r dose and were correlated with depressed concentration of deoxyribonucleic acid.

Arnold *et al.* (1954) reported abnormal EEG records 1 week after a dose of 1,800–3,000 r and 3 months after doses of 900–1,800 r; even doses as low as 375 r produced spiking in the EEG after delay. Recently, Gangloff and Haley (1960) have reported changes in spontaneous and evoked potentials from the hippocampus following 200 and 400 r doses of whole body and head irradiation. They point out that electrical changes from hippocampus, brain stem, and the diffuse thalamic nuclei are more susceptible to low radiation doses than are such changes recorded from the cortex, caudate nuclei, and posterior hypothalamus.

There is other evidence to indicate that the cortex may be more radio-resistant than some of the other cerebral centers. Arnold *et al.* (1954) found damage to the brain stem and moderate damage to the hypothalamus at

doses of 1,800 r and less—approximately half the dose required to produce cortical damage. Similar data have been presented by Clemente and Holst (1954), who reported localized damage in the hypothalamic and medullary regions in 4 monkeys receiving 2,000–3,000 r. They emphasize the susceptibility of the hypothalamus and medulla to radiation effects, in contrast with the cerebral cortex and cerebellum, and point out that this is true not only in terms of cell degeneration, but also in terms of alteration of the blood-brain barrier as measured by penetration of trypan blue.

Since learning, particularly complex learning, is largely dependent on the integrity of the cerebral cortex in mammals, and since the cortex is radio-resistant, one should not be surprised to find that learned behaviors are not adversely affected by whole body radiation. For the monkey the LD/50/30 is approximately 600 r, and the LD/100/30 is probably 700 r or less. Thus, the lethal whole body dose is about 25% of that required to produce serious damage to the cerebral cortex, and it is entirely possible that death from medullary or hypothalamic damage and destruction would supervene before cortical damage took place, even if higher doses could be given through the use of protective agents.

It is possible to demonstrate changes in cortical electrical activity and in some cortical enzyme systems at whole body doses below the LD/50/30, but it is extremely doubtful if changes of the magnitude demonstrated would be associated with any behavioral disturbance. There is now extensive literature on the effects of whole body radiation on a wide range of learning tasks in a fair sample of animal species, and improved performance after irradiation has been reported as often as performance decrement. Thus, improved performance has been found for monkeys by Harlow and Moon (1956), Riopelle *et al.* (1956), and McDowell and Brown (1961) and for rats by Blair and Arnold (1956) and by Blair (1958). The surprising fact is that individual animals have actually shown essentially perfect learned performance a few hours before death in studies using either food or electric shock as incentives. It is possible that some member of the symposium will disclose new data incompatible with my comments, but I believe that the picture is perfectly clear.

There is evidence that the CNS, including the cortical neurons, may be adversely affected by delayed radiation effects induced by sublethal doses. Arnold *et al.* (1954) have demonstrated that monkeys given localized cerebral doses of 3,000–5,000 r from a 23 Mev betatron source showed a virtually complete recovery during an intermediate stage, and then 6 to 8 months later, they developed a fulminating course of delayed radionecrosis which was strikingly selective for the white matter. These investigators emphasized the fact that the brain stem and hypothalamus were particularly sensitive to delayed radiation damage even with doses as low as 1,500 r.

Harlow and Settlage noticed that monkeys subjected to multiple cortical lesions produced by Co^{60} implantations frequently died from an indeterminate cause some months after the last implantation, without additional CNS damage apparent at autopsy. Settlage also observed that monkeys, following amygdaloid and periamygdaloid lesions produced by a Co^{60} source, frequently showed an exaggeration of symptoms some months after implantation, and frequently this terminated in death. The delayed appearance of both petit mal and grand mal seizures is common in monkeys after localized cerebral Co^{60} irradiation.

At Wisconsin we are interested in long term effects of radiation produced by both single doses and fractionated doses. We are building groups of macaques that have been given 500, 600, and 700 r, if they survive it, and will study biochemical, physiologic, and behavioral effects. We are tracing the effects of cumulative doses of 25, 50, 100, and 200 r given to groups of 15 monkeys every 2 weeks. All animals are being carried to lethality with the exception of the 25 r group, to which we plan to give a dose within 80-90% of lethality, as determined by hematologic and physiologic measures (and also behavioral measures if we can obtain adequately sensitive ones). Data obtained from our 100 r group indicate that hematocrit is a sensitive predictor of lethality (Fig. 1).

The 25 r group has at present received only 550 r, and we predict that the lethal radiation dose given at this rate will lie between 2,000 r and 4,000 r, and that this will be the approximate range for individual monkeys within the group.

Of the 15 monkeys forming the 50 r group, we have at present 10 survivors. The first subject died at 900 r, and at present the surviving monkeys have received 1,550 r. Twelve have been tested for retention of discrimination learning sets and acquisition of an oddity learning set. Six started train-

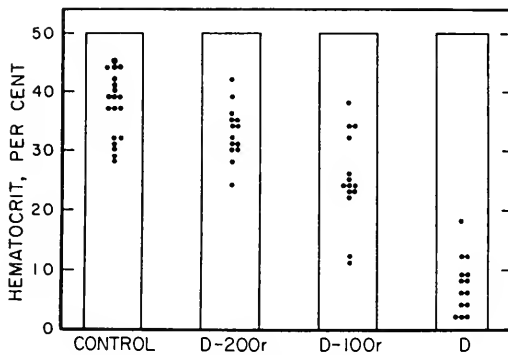


Fig. 1. Hematocrit as lethal radiation dose is approached. D=death.

ing after receiving 650 r and 6 after receiving 1,000 r. They have been given two 6-trial discrimination problems each day and four 6-trial oddity problems. We were interested in the outside possibility that the 3.5 month delay would reveal differences between the two groups, although data such as those of Davis *et al.* (1958) indicated that this interval was probably too short, even if the phenomenon of delayed CNS necrosis following x-irradiation exists. We also wanted to explore the feasibility and practicality of using a skeletonized test program.

Figure 2 shows comparable data for oddity learning for a group of normal monkeys tested only on eight oddity problems a day, and for our radiated monkeys trained on the skeletonized test program. The performance of the normal monkeys, is, of course, superior, since they were not being run simultaneously on interfering problems. However, the important point is the efficient, orderly, and reliable learning of the irradiated monkeys. We are pleased with the consistency of the data obtained by this abbreviated test program and plan to develop and extend the test techniques in future researches; for if one wishes to trace behavior and behavioral changes over long periods, the practical problems are overwhelming unless some efficient, skeletonized behavioral test program can be established.

Oddity learning curves for the two groups of irradiated monkeys, the group trained after receiving 650 r and the group trained after 1,000 r, are presented in Fig. 3. No significant differences were found, and it appears obvious that neither the additional 350 r nor the 3.5 month delay was of any importance. The learning set data are given in Fig. 4, and there is no reason to believe that any significant difference between the groups will appear. The females showed a significant early superiority on the oddity test, whereas the differences for learning set favor the males (Figs. 5 and 6).

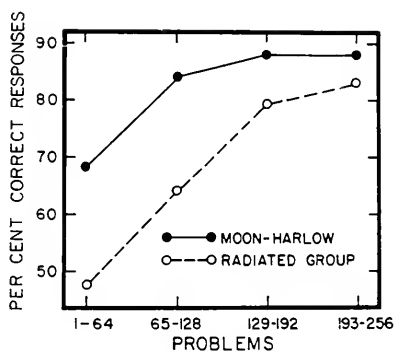


FIG. 2. Oddity performance by normal monkeys and irradiated monkeys on skeletonized test program.

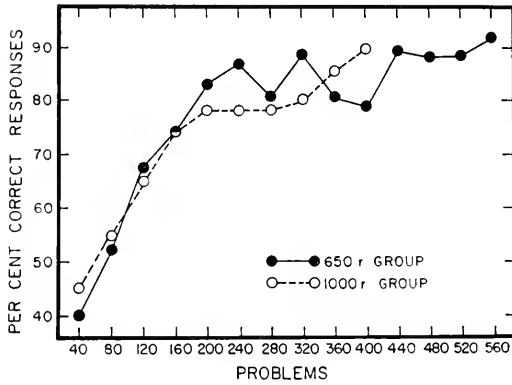


FIG. 3. Oddity learning curves for 650 r monkeys and 1,000 r monkeys.

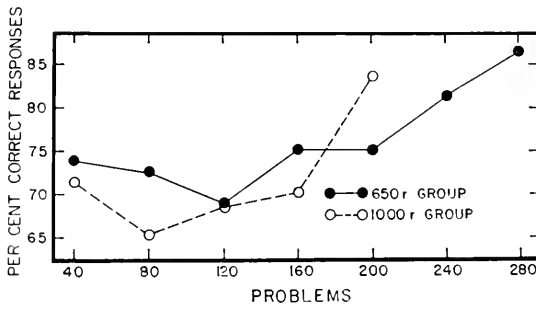


FIG. 4. Learning set curves for 650 r monkeys and 1,000 r monkeys.

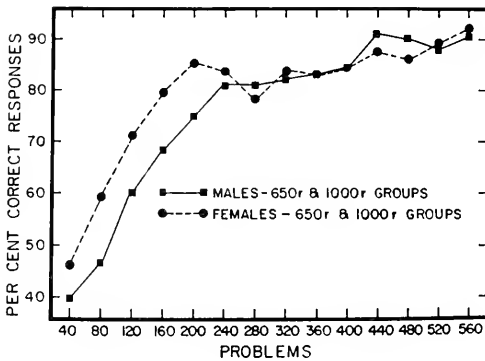


FIG. 5. Male and female monkey performance on oddity tests.

Although whole body radiation does not radically change the mammal's capability for learning, there is a wealth of evidence demonstrating effects on nonintellectual behavior. Depression of total body activity has been reported for monkeys by McDowell *et al.* (1956), and depression of pedometer manipulation in monkeys by Leary and Ruch (1955). Depression of running-wheel activity by rats following irradiation has been described by Jones *et al.* (1954) and by McDowell and Brown (1959).

On the basis of carefully controlled, long term experiments, Davis *et al.* (1958) demonstrated systematic differences in the home-cage behavior of irradiated and nonirradiated monkeys. The irradiated monkeys showed significantly fewer rapid, energy-expending activities and more cage-directed responses. In the test room, irradiated monkeys attended better and were less distractible. Decreased mating activity in male swine and rats following fetal gamma irradiation has been reported by Furchtgott *et al.* (1959).

During the last year, Mr. Sponholz, project supervisor at the Primate

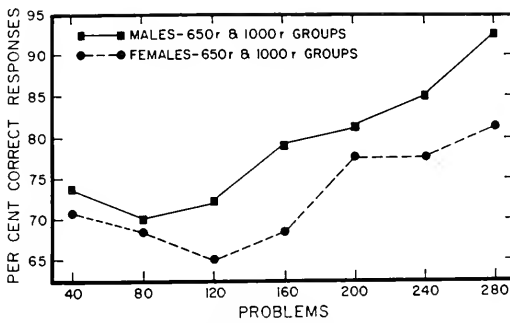


FIG. 6. Male and female monkey performance on learning set tests.

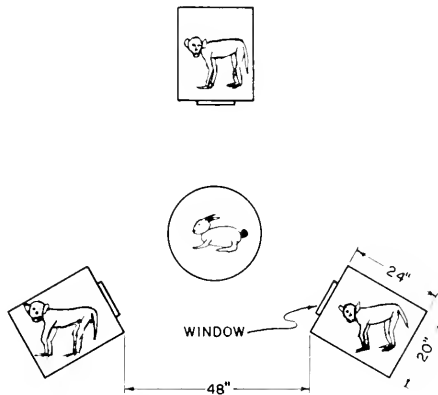


FIG. 7. Visual curiosity test apparatus.

Laboratory, and I have designed and used several "nonlearning" tests on our 50 r and 100 r groups. We established a visual curiosity test by placing three modified Butler boxes (Fig. 7) in such position that the monkeys could view each other when they opened the windows. Figure 8 presents data from our 100 r group that show a lower amount of visual exploration during the last 5 weeks preceding death in irradiated monkeys, as compared with a control group, and a pronounced and obvious drop in exploratory activity in the last week of life. A progressive reduction in visual exploration from 600 r onward is apparent from Fig. 9, and the differences between irradiated and control subjects are significant, even though both show a similar downward trend.

However, we are not satisfied with statistically significant group differences, since we hope to achieve tests capable of predicting death for individual monkeys. Therefore, in an effort to gain better control and to improve our apparatus, we built the boxes into a compact, enclosed unit and had the monkeys of our 50 r group view a rabbit rather than other monkeys. We

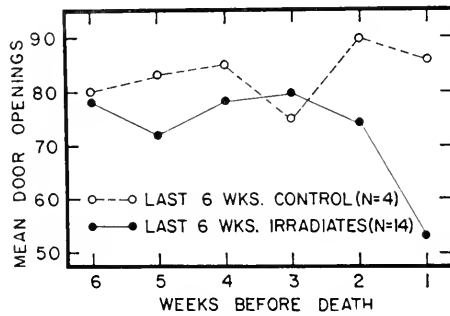


FIG. 8. Visual curiosity performance as lethal radiation dose is approached.

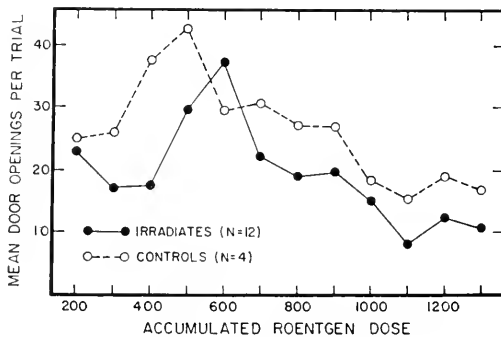


FIG. 9. Behavior of irradiated and control monkeys on visual curiosity test.

failed to pull any rabbits out of hats with this design, and we succeeded in controlling all variables so effectively that the monkeys seldom looked out the windows. There were no data, and consequently no differences. Our next step will be to test visual exploratory behavior when the modified Butler box is attached to the animal's home cage and the testing conducted after an assigned number of hours of visual deprivation.

An attempted stress test without electric shock is shown in Fig. 10. This is a smooth-walled Plexiglas and metal chamber with a steel pole in the center and 4 in. of cold water in the pan at the base. The monkey was introduced to the test through a door at the back of the chamber, and each session lasted 15 minutes.

The data on the 50 r group are somewhat less than spectacular. The normal monkeys spent less time out of the water than those irradiated (Fig. 11). Some monkeys learned ways to brace themselves between the pole and the wall of the chamber, thus avoiding the water, and two control animals made little or no attempt to keep out of the water. We plan to modify the apparatus by making walls with no interior projections and by making the water so deep that the monkeys must either hold to the pole, swim, or drown.

Actually, even the unmodified situation may give us effective predictors of approaching radiation death, since the cold water appears to be a more noxious stimulus for irradiated animals than for the controls, and the single one of the 12 irradiated monkeys that has died evinced a sharp decrease in time on the pole as death approached.

Figure 12 depicts a general activity test in which movements in the home

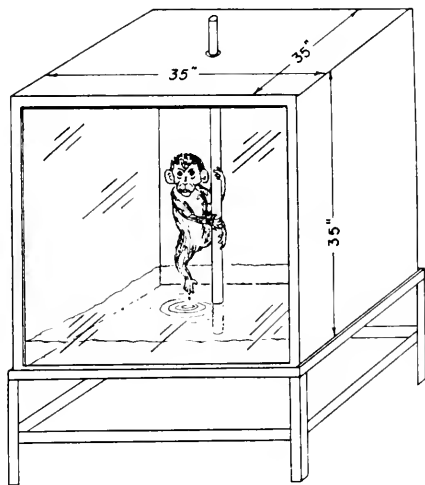


FIG. 10. Stress test apparatus.

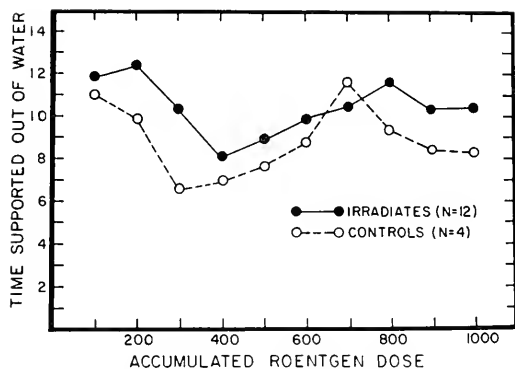


FIG. 11. Performance of normal and irradiated monkeys on stress test.

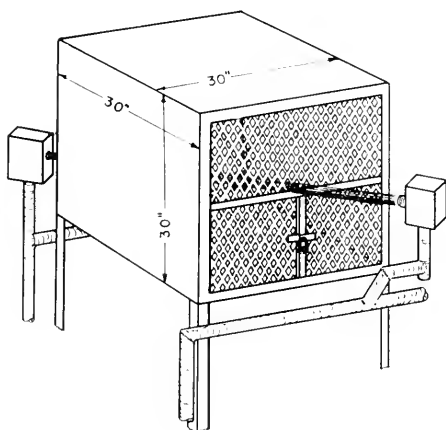


FIG. 12. Activity apparatus.

cage between 8 P.M. and 8 A.M. were measured by interruptions of an electric-eye beam. Difficulty was encountered in obtaining consistent records; there were large individual differences, frequent apparatus breakdowns, and vibration of cages. We now believe that activity may best be measured during testing in a Wisconsin General Test Apparatus (WGTA) or an isolation chamber which we have previously used successfully in determining effects of various cortical and subcortical lesions on total body activity.

Important areas for assessing the behavioral effects of whole body radiation relate to irradiation of the mammal at various stages *in utero* and soon after birth. The anatomic and embryologic basis for these researches is given by Rugh and Grupp (1959), Hicks (1958) and Hicks *et al.* (1959). Hicks

et al. (1959) have shown that specific cerebral structures of rats are differentially affected by radiation on successive days after conception. He suggested that it should be possible to relate behavioral measures to specific malformations of cerebral cortical structure.

The behavior studies on the effects of irradiation early in life give data in keeping with the known radiosensitivity of the developing nervous system. Levinson (1952) fetally x-irradiated rats with 300–600 r on the 11th, 13th, 15th, 17th, and 19th postconception days. When the animals were 50 days old, they were tested on a Lashley Type III maze. Deficits were found to be directly related to the radiation dose and to be most severe for groups irradiated on the 13th day. Tait *et al.* (1952) irradiated rats during the final week of pregnancy using 30, 90, 180, and 360 r. The offspring of the animals receiving 90 r or more were significantly poorer maze learners than control animals.

Subsequently, Levinson and Zeigler (1959) subjected rats 2, 4, 6, 8, 14, 18, and 24 days of age to doses of 0, 150, 250, and 350 r. Beginning at 45 days of age, they measured learning performance on a Lashley Type III maze and on the Hebb-Williams closed-field test. The 350 r group was essentially an LD/50/30 group and so was combined with the 250 r group for analysis of variance. No learning deficits appeared in the surviving infants irradiated at 18 or 24 days, but there were significant differences in all other groups, with maximum deficits in the 2 day and 4 day groups. The severity of the learning deficits increased as a negatively accelerated function of dose level. Furchtgott (1951) had previously irradiated rats at 3 weeks of age and obtained similar negative results.

Recently Furchtgott *et al.* (1958) in part replicated and extended Levinson's (1952) and Furchtgott's (1951) studies by giving rats 100, 200, and 300 r on days 14, 16, or 18 of the gestation period, or neonatally, and testing their performance at 45–50 days of age on the Lashley Type III maze. Few 300 r subjects of any age, or 14 day irradiated subjects at any dose level, met the criterion of two out of three errorless trials in less than 20 sec each. Degree of learning deficit was clearly a function of dose level and age at irradiation. Maximum deficit resulted from irradiation at 14 days after conception, in accord with Hicks' anatomic timetable and with Levinson's prior findings. At postconception day 14, a 100 r dose was effective in producing significant deficit, and on day 18, a 200 r dose. Only neonatal subjects receiving 300 r showed learning deficit.

Although the number of experiments is small and the range of learning tests limited, the obtained data are highly significant and consistent and in excellent agreement with known radiation-anatomic correlations. There can be little question but that fetal and neonatal irradiation at dose levels below

LD/50/30 produce a relatively persistent learning loss and that amount of loss is related to particular developmental state and irradiation dose.

The effects of irradiation at varying times *in utero* or in early neonatal periods have not been measured for the monkey. However, techniques for determining dated pregnancies have been established, and methods for large scale breeding have been developed. Next year we should be in a position at Wisconsin to initiate limited investigations in these areas.

One of the most intriguing discoveries concerning the effects of radiation on behavior is the finding by Garcia *et al.* (1955) that rats developed a conditioned avoidance to saccharin if they were induced to drink it in solution while being irradiated. Recently, they reported such behavioral changes following fractionated radiation doses totaling less than 20 r. Garcia *et al.* (1957) also demonstrated that conditioned spatial avoidance could be obtained, in that after irradiation rats would spend less time in a chamber in which they had been confined during a series of radiations than in a chamber in which they had not been previously exposed to radiation. These general results on radiation-conditioned spatial avoidance have been confirmed by Overall *et al.* (1959) using a different apparatus and comparing the time spent by the subjects in a chamber exposed to radiation with the time spent in a lead-shielded chamber.

Recently Garcia and Kimeldorf (1960), using localized x-radiation, demonstrated that the abdomen is a region of special sensitivity in producing saccharin aversion. Exposures of 54 r and 108 r delivered to the abdomen produced a decrement in saccharin consumption, whereas radiation of the head, thorax, or pelvis required a dose of 252 r to produce the same effect. Garcia and Kimeldorf suggest that sensations triggered by gastric dysfunction may represent the stimuli through which radiation acts to condition behavior in animals, but they recognize that this may not be the sole receptive mechanism, since saccharin avoidance can be produced by direct head irradiation.

During the last year, Sponholz, Bowman, project associate, Primate Laboratory and I have investigated conditioned saccharin avoidance in rats and monkeys. In 33 white male albino rats 40 to 50 days of age, Holtzman strain, changes in preference between a saccharin solution and tap water were tested after association of saccharin drinking with exposure to several levels of x-irradiation. Sham radiation was carried out on 12 controls.

The rats were given a choice of 0.5% saccharin solution or tap water from bottles available in the home cages 24 hours a day except during the 24 hours before and 16 hours after irradiation. Preference levels for saccharin and water were recorded 4 days preceding deprivation and 4 days following

the postexposure deprivation. Only saccharin solution was provided during the 80 minute radiation with either 50 r, 100 r, or 200 r.

The results for the rats (Fig. 13) show unequivocal depression of saccharin consumption and preference reversal for the 200 r group. However, we observed no changes, or minimal changes, after 50 r doses. Thus, our results are generally in accord with Garcia-Kimeldorf findings; the differences in the minimal effective dose level between their data and ours may be a function of strain of rats or amount of preradiation preference testing.

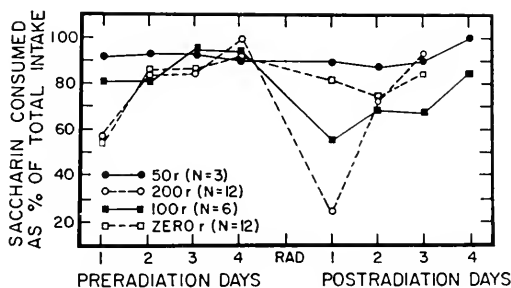


Fig. 13. Radiation-induced saccharin avoidance in rats.

We have conducted a few exploratory studies on conditioned taste aversion with mature rhesus monkeys. Three males were tested on sweetened cherry Kool-Aid vs. tap water, and the data for one that developed a relatively stable preference for the Kool-Aid are presented in Fig. 14. These preradiation preference measures were obtained while the monkey was being rotated in a small cage in the radiation chamber for 45 minutes. During these times, both Kool-Aid and tap water were available to the subject, and

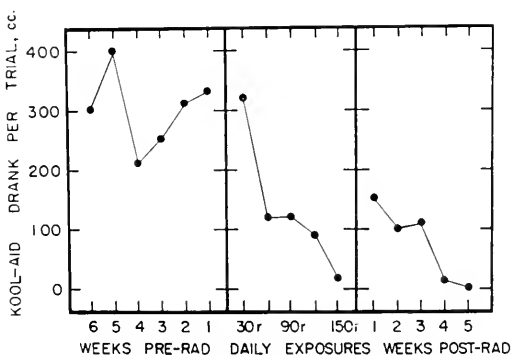


Fig. 14. Radiation-induced Kool-Aid avoidance by monkeys.

preference was determined at the end of each trial by amounts consumed. The subject received two tests per week for 6 weeks.

After the preradiation preference testing, the monkey was exposed to 30 r per day, for 45 minutes, for 5 consecutive days. During the exposures, the animal was restrained in the small rotating cage used during the preradiation tests, with only the Kool-Aid available.

After the total dose of 150 r had been administered during the five day conditioning period, preference tests were repeated at the rate of two per week for 5 weeks. Kool-Aid ingestion was extinguished by the end of the radiation exposure period and was greatly depressed during the 5 post-radiation weeks.

Similar, though less striking, data were obtained with another monkey (Fig. 15) using a preferred and a nonpreferred Kool-Aid solution.

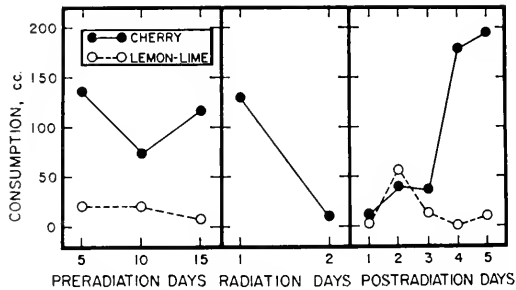


FIG. 15. Radiation-reversed Kool-Aid preference by a monkey.

Since we were not satisfied with Kool-Aid as a stable and commonly accepted high preference fluid, we tested the same monkey for preference of apple juice, grape juice, or tap water by presenting him with a choice between pairs of these three fluids for 18 days. Each possible pair of fluids was presented in random order, one pair per day, for 1 hour periods in the home cage. The cage was moved into the radiation chamber for half the trials and left in the home room for the remaining tests. After 18 days of preradiation preference testing, he was irradiated at the rate of 50 r in 55 minutes each day for 3 consecutive days. Only the preferred apple juice was available to the animal during these exposures. Following the three daily irradiations, the preference test procedure which had preceded the irradiation was repeated for an additional 18 days. The fluids ingested during preference testing were the only liquids the monkeys received during these 39 days.

A second control monkey was given the same program of preference testing except he was subjected to sham radiation. Consumption of apple juice was greatly depressed for the experimental subject during the 3 post-

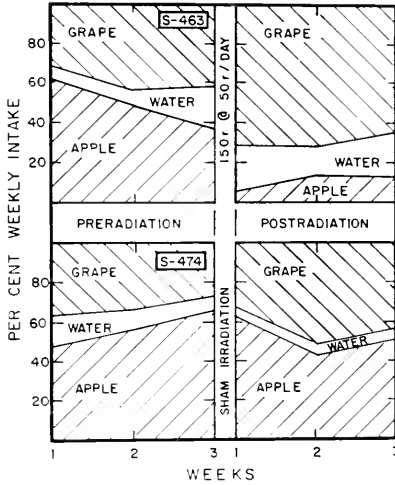


FIG. 16. Depressed Kool-Aid ingestion during irradiation of monkeys.

radiation weeks and was unaffected for the control subject (Fig. 16). These monkeys were the only subjects to give unequivocal preference suppression effects after radiation, but supplementary data showing Kool-Aid and apple juice avoidance during the radiation sessions were obtained on additional subjects (Fig. 17).

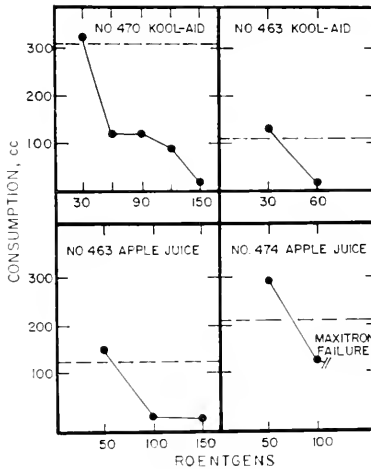


FIG. 17. Radiation-induced depression of cider ingestion by a monkey.

The data from the total group demonstrate that conditioned fluid aversion can be produced in the monkey, even if there are considerable individual differences. Our results and our difficulties are similar to those reported by Kimeldorf *et al.* (1960) for the cat. However, it is clear that after technical difficulties are overcome the Garcia-Kimeldorf phenomenon can be demonstrated to hold for various species.

Although the Garcia-Kimeldorf phenomenon is an established fact, the most recent studies by these investigators suggest that the primary mechanisms involved do not relate to radiation-induced CNS changes. If this is true, the total body of radiation researches in neurology and psychology present data closely correlated and integrated.

In the future we may expect a shift of interest in assessing behavioral effects, particularly those relating to learning, from whole body radiation studies of the mature subject to the effects of radiation on the animal *in utero* and on the neonatal and infantile animal. Direct radiation of specific cerebral centers and areas, including the cortex, remains a worthwhile field of investigation. The effects of long term, chronic radiation from various sources on various species is relatively unexplored. Regardless of subject or dose, additional attention will doubtlessly be given to "nonlearning" tests covering a wide variety of behaviors.

Radiation research in the past has given primary emphasis to the effect of varying doses on groups of subjects. In the future more attention probably will be given to the study and prediction of individual differences. Such problems become more and more important as one progresses from *drosophila* to mouse, rat, monkey, and man.

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Learning Behavior of Rats Given Low Level X-Irradiation in Utero on Various Gestation Days*

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These experiments were done to determine manifestations of behavioral aberration as related to age of subject at time of irradiation, age of irradiated subject at time of testing, magnitude of dose, and type of instrument capable of measuring the observed phenomena.

Since much literature exists confirming the fact that x-radiation delivered *in utero* has an adverse effect on subsequent learning ability of the exposed rat (Furchtgott *et al.*, 1958; Levinson, 1952; Tait *et al.*, 1960), this study is concentrated on portions of the gestation period and levels of radiation dosages not reported nor extensively employed in animal learning studies.

Two disparate, but related, experiments will be presented, one on age at time of maze learning and another on age at time of conditioning and extinction.

Subjects

Of 114 albino rats, bred in and shipped from Columbia University department of radiology laboratory, 43 were approximately 400 days old and 71 were approximately 90 days old at the beginning of experiment one. At completion of the maze experiment, 59 rats to be used in the conditioning-extinction experiment were randomly selected from the respective groups. They were 540 and 120 days old, respectively, by the start of experiment two. Numbers of subjects per subgroup, experimental task, and radiation condition for each are indicated in Table I. All subjects were naive to psychologic experimentation at the start of the study.

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† Roberts Rugh, Columbia University, conducted the irradiation of all subjects; his work was aided in part by a U.S. Public Health grant. The following colleagues and assistants worked in the project obtaining data: Barbara Brodigan, Shirley Clark, Gerald Garrett, Robert Graham, Robert Hall, Allen Holmes, Gale Howell, Vincent Luchsinger, W. H. Melching, and Paul Thomas.

TABLE I
SUBJECTS AND CONDITIONS

Group ^a	Radiation dosage (r)	Time of radiation (days in utero)	Maze experiment		Conditioning experiment	
			Age at beginning (days)	N	Age at beginning (days)	N
A	0	0	400	17	540	6
B	25	2.5	400	3	540	2
C	50	6.5	400	7	540	6
D	50	11.5	400	4	540	4
E	50	12.5	400	6	540	4
F	100	8.5	400	6	540	5
a	0	0	90	13	120	6
b	10	8.5	90	5	120	4
c	25	8.5	90	13	120	4
d	50	7.5	90	7	120	4
e	50	8.5	90	15	120	4
f	100	8.5	90	3	120	3
g	150	8.5	90	12	120	4
h	200	1.5	90	3	120	3

^a Groups identified with capital letters are 400-day-old rats; groups identified with lower case letters; 90-day-old rats.

Radiation and Breeding Procedures

The x-ray facilities consisted of a standard x-ray with parallel tubes run at 180 kvp, 30 ma; filtration was 0.28 mm Cu and 0.50 mm Al. Both upper and lower tubes were used with a half value layer of 0.6 Cu, 67 cm target distance at 50 r per min for 200 r, 150 r, 100 r, and 50 r. For rats receiving 25 r, only the upper tube was used with 69 cm target distance at 25 r per min. For subjects exposed to 10 r, only the upper tube was used, with filtration of 0.5 mm Cu, 0.5 mm Al, half value layer of 0.8 mm Cu, 91 cm target distance at 10 r per min.

To determine the gestation period, females were placed with males at 5 PM and examined the next morning at 9 AM for presence of vaginal plugs. Those found with such signs of successful copulation were recorded as pregnant and in the 0.5 day of gestation. All irradiation for successive days based day of exposure *in utero* on this method of calculation.

Experiment One

Because cortical ablation studies have shown the Lashley III maze (Lashley, 1929) to differentiate between learning ability of animals with cor-

tical lesions and normal controls, investigators studying maze learning have selected this instrument for examining irradiation effects on learning. Their success in establishing differences in Lashley III maze performance in the prenatally irradiated rat, exposed to 90-600 r, has been reported (Furchtgott *et al.*, 1958; Levinson, 1952; Tait *et al.*, 1952). All these studies, however, have utilized young animals in the learning experiments and have devoted their attention to subjects irradiated on or beyond the 11th gestation day. Therefore, no information exists to establish the nature of the learning behavior resulting from these and smaller dose levels given prior to gestation day 11.

Since Hicks (1953), Rugh (1959a, b), and Rugh and Grupp (1959a, b) have called attention to the radiosensitivity of embryonic tissue prior to this day in rat and mouse gestation, it was considered important to investigate these factors as they might be revealed in aberrant learning behavior.

APPARATUS AND PROCEDURE

Subjects were given preliminary training in a six-ft-alley runway to establish a goal-directed running tendency. Each rat was placed on a 23½ hour food deprivation schedule 2 days prior to commencement of this training. During this period they were handled so that they would become accustomed to the experimenter. Subjects then were given two runs daily for 5 days, being allowed 30 sec at the end of each run to eat a wet mash food placed at the end of the runway. Then they were returned to their cages and given three Nutrena nuggets. This diet preserved the subjects' weights and maintained a level of hunger drive to insure adequate motivation throughout the experiment.

One day after completion of preliminary training, all subjects were given one trial daily in the Lashley III Maze (Fig. 1). They were scored for time (opening of the starting box door to entry into the goal box) and for full or partial forward going errors and retrace errors.

RESULTS

Each group was tested for a significant difference from every other group by a *t*-test for mean number of trials required to reach the trial which preceded the criterion of mastery (4 out of 5 consecutive errorless trials). The Mann-Whitney *U* test (Siegal, 1956) was used to test significance between groups for mean number of errors and mean time for trials until the criterion of mastery was achieved. Computations for trials and errors to achievement of criterion excluded the scores for criterion trials, but included mean time scores.

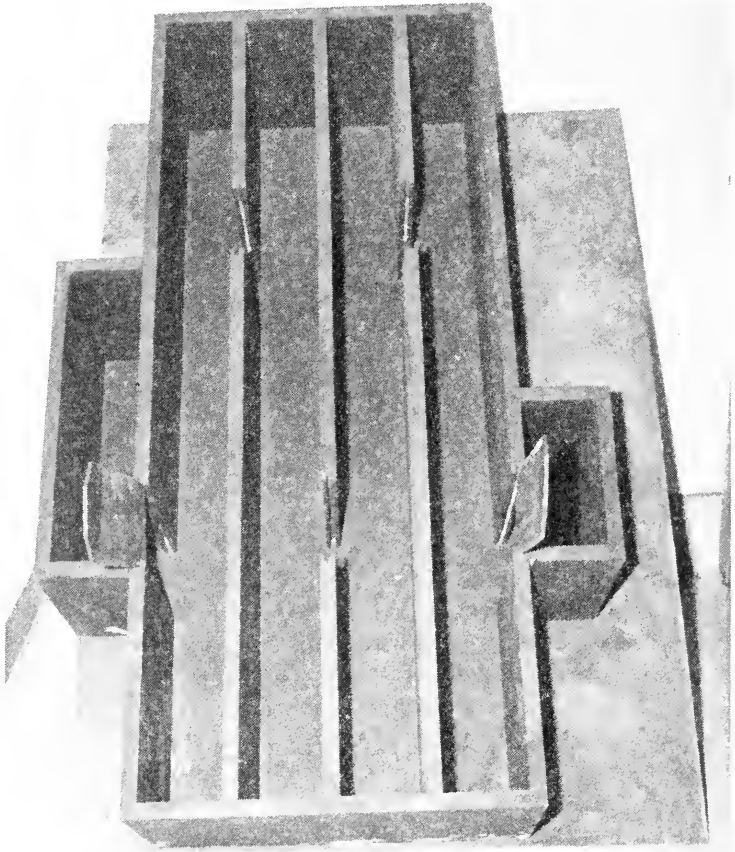


FIG. 1. The Lashley III Maze.

Table II reflects the significance of difference between groups and the direction of the differences. The groups identified with capital letters are 400-day-old rats, while those labeled in small letters are 90 days old.

1. No significant differences on any measures were found between the control groups.

2. The older irradiated groups were found to be significantly inferior to the controls on all measures.

3. The 150 r/8.5 day and the 50 r/7.5 day younger groups were significantly inferior to the control groups on all measures. Aside from these findings and the fact that the 100 r/8.5 day younger group was inferior in terms

TABLE II

SIGNIFICANCE OF DIFFERENCES BETWEEN GROUPS

Group	Measure	<i>h</i> 25/2.5d	<i>C</i> 50/6.5d	<i>D</i> 50/11.5d	<i>E</i> 50/12.5d	<i>F</i> 100/8.5d	<i>a</i> Ch	<i>b</i> 10/8.5d	<i>c</i> 25/8.5d	<i>d</i> 50/7.5d	<i>e</i> 50/8.5d	<i>f</i> 100/8.5d	<i>g</i> 150/8.5d	<i>h</i> 200/1.5d
A (Ch)	Time	.002	.002	.002	.002	.002	—	—	—	.02	—	—	—	—
	Errors	.05	.002	.002	.002	.002	—	—	—	.02	—	—	—	—
	Trials	.02	.001	.001	.001	.001	—	—	—	.02	—	—	—	—
B (25/2.5d)	Time	—	—	—	.01	.02	.002*	.05	.02*	.02	.002*	—	—	—
	Errors	—	—	—	.02	.05	.05*	.02*	.02*	—	.002*	—	—	—
	Trials	—	.001	—	.001	.05	.05*	.02*	.02*	—	.002*	—	—	—
C (50/6.5d)	Time	—	.02	—	—	—	.002*	.001*	.002*	.05*	.002*	.01*	.002*	.02*
	Errors	—	—	—	—	—	.002*	.002*	.002*	.05*	.002*	.01*	.002*	.02*
	Trials	—	—	—	—	—	.001*	.01*	.001*	.001*	.001*	.01*	.001*	.01*
D (50/11.5d)	Time	—	—	—	—	—	.02	.05	.02*	—	.02	—	—	.05*
	Errors	—	—	—	—	—	.02	.05	.02*	—	.02	—	—	.05*
	Trials	—	—	—	—	—	.001*	.02*	.001*	.02*	.001*	—	—	.05*
E (50/12.5d)	Time	—	—	—	—	—	.001*	.02*	.001*	.02*	.002*	.002*	.05*	.05*
	Errors	—	—	—	—	—	.02	.02*	.002*	.02*	.002*	.002*	.002*	.02*
	Trials	—	—	—	—	—	.02	.004*	.002*	.002*	.002*	.002*	.002*	.05*
F (100/8.5d)	Time	—	—	—	—	—	.01	.01	.001*	.002*	.001*	.01	.001*	.02*
	Errors	—	—	—	—	—	.002*	.002*	.002*	.002*	.002*	.02*	.02*	.02*
	Trials	—	—	—	—	—	.005*	.005*	.001*	.02*	.005*	.02*	.05*	.05*
a (Ch)	Time	—	—	—	—	—	.001*	.01*	.001*	.002	.001*	.02	.001*	.05*
	Errors	—	—	—	—	—	—	—	—	.002	—	—	.002	
	Trials	—	—	—	—	—	—	—	—	.02	—	—	.001	
b (10/8.5d)	Time	—	—	—	—	—	—	—	—	.02	—	—	.001	
	Errors	—	—	—	—	—	—	—	—	.02	—	—	.001	
	Trials	—	—	—	—	—	—	—	—	.006	—	—	.002	
c (25/8.5d)	Time	—	—	—	—	—	—	—	—	—	—	—	.02	
	Errors	—	—	—	—	—	—	—	—	—	—	—	.05	
	Trials	—	—	—	—	—	—	—	—	—	—	—	.02	
d (50/7.5d)	Time	—	—	—	—	—	—	—	—	.002	—	—	—	
	Errors	—	—	—	—	—	—	—	—	—	—	—	.02	
	Trials	—	—	—	—	—	—	—	—	—	—	—	.02	
e (50/8.5d)	Time	—	—	—	—	—	—	—	—	—	—	—	—	
	Errors	—	—	—	—	—	—	—	—	—	—	—	.05	
	Trials	—	—	—	—	—	—	—	—	—	—	—	.01	
f (100/8.5d)	Time	—	—	—	—	—	—	—	—	—	—	—	—	
	Errors	—	—	—	—	—	—	—	—	—	—	—	.05	
	Trials	—	—	—	—	—	—	—	—	—	—	—	.01	
g (150/8.5d)	Time	—	—	—	—	—	—	—	—	—	—	—	—	
	Errors	—	—	—	—	—	—	—	—	—	—	—	.05	
	Trials	—	—	—	—	—	—	—	—	—	—	—	.05	
h (200/1.5d)	Time	—	—	—	—	—	—	—	—	—	—	—	—	
	Errors	—	—	—	—	—	—	—	—	—	—	—	.05	
	Trials	—	—	—	—	—	—	—	—	—	—	—	.05	

KEY: * Difference in favor of upper group; difference in favor of group at left when no asterisk is shown; —, no significant difference.

of trials to achievement of criterion, no other significant differences between the younger groups and control groups were recorded.

4. In most cases, measures showing significance of differences favored the younger groups over the older ones. Exceptions should be noted:

(a) The 25 r/2.5 day older group did not differ significantly in errors and trials to criterial achievement from the 10 r/8.5 day, 50 r/8.5 day, 50 r/7.5 day, 100 r/8.5 day, 150 r/8.5 day, or 200 r/1.5 day younger groups. Neither did this older group differ from the 50 r/7.5 day, 100 r/8.5 day, or 200 r/1.5 day groups in running time. Compared with the 25 r/8.5 day younger group, they were inferior in running time and total errors, but no reliable difference was reflected in the mean number of trials to criterial achievement.

(b) The older control group was significantly superior to the 10 r/8.5 day, 25 r/8.5 day, 50 r/7.5 day, and 50 r/8.5 day younger groups in mean number of trials until the criterion was reached. It was superior in time and error scores compared with the 150 r/8.5 day and the 50 r/7.5 day younger groups. No other significant differences were apparent.

5. When age and dose level were constant in 400-day-old rats given 50 r, the 6.5 day group had significantly larger mean time scores than the 11.5 day group, and no differences between 6.5, 11.5, and 12.5 day groups were found other than those stated before. In 90-day-old rats given 50 r, the 7.5 day group was significantly inferior to the 8.5 day group on all measures.

6. When age was held constant and various dose levels and days of *in utero* irradiations were compared in 400-day-old rats with 100 r vs 50 r and 25 r, the 100 r/8.5 day animals (group F) did not differ from the groups given 50 r (C,D,E) on any measures, nor from the group given 25 r (B) in mean time scores. This group was inferior to the 25 r/2.5 day older subjects in trials and errors to achievement of criterion.

In 90-day-old rats with 200 r vs 150 r, 100 r, 50 r, 25 r, and 10 r, group h (200 r/1.5 days) was superior to groups b (10 r/8.5) for time, c (25 r/8.5) for time, d (50 r/7.5) for time and errors, and g (150 r/8.5) for time and errors. It showed no other differences between its control group or other younger groups. In 90-day-old rats with 150 r vs 50 r, no differences were found between group d (50 r/7.5 day) and group g (150 r/8.5 day).

All animals in all groups failed to meet the criterion of mastery in 50 trials (Fig. 2). Only 29, 25, 66 and 50% of the subjects of groups C, D, E, and F, respectively, were able to achieve this criterion. The bar graphs reflect the means for each of the groups for only those subjects which did attain mastery. These data magnify the differences already described. They also reflect the variability of performance in the old animals, particularly those in group C, the 50 r/6.5 day group.

Three animals in the 50 r/12.5 group showed locomotor difficulty in the

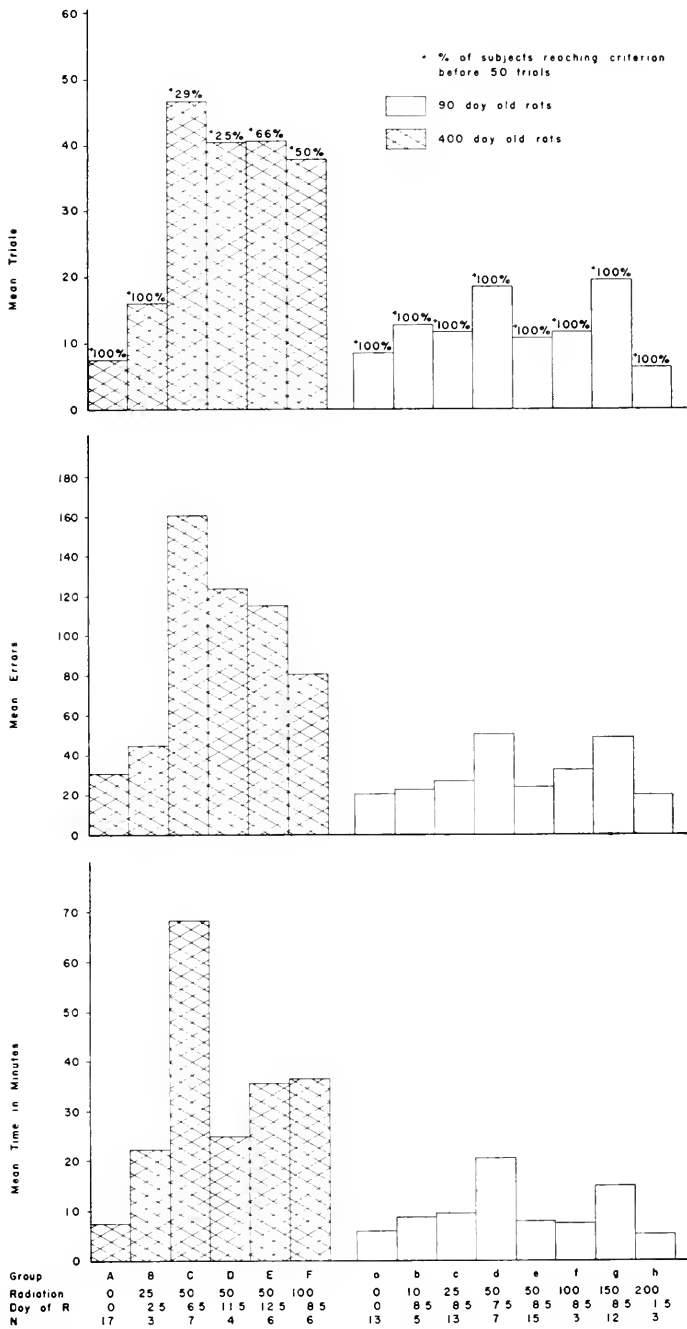


Fig. 2. Mean trials, errors, and time to criterion of mastery.

hind legs. All older animals that were run beyond 25 trials failed to eat in the goal box on completion of the trial. No such behavior was seen in any of the rats in the 90 day groups.

Experiment Two

To ascertain if a behavioral deficit may be determined by a learning instrument other than the maze, an operant conditioning task was made a part of the test battery given the subjects. Melching (1956-1957) previously found in a pilot study an indication that prenatally irradiated subjects show some impairment in capacity to condition and extinguish a bar pressing habit. Since this represents perhaps a simpler and certainly a different type of learning requirement than that of maze learning, it was felt that the differences noted in experiment one might be confirmed and elaborated.

PROCEDURE

The apparatus used was the Model DB-103 demonstration Skinner box (Davis Scientific Instruments, Studio City, California) with Model BM-105 bar mechanism, Model PD-104 pellet dispenser, and Model CB-106 control box and power supply. Automatic recording of the animals' responses was accomplished by the Davis Model CRRC-133 cumulative recorder, the Model 1704 counter, and Model 1721 print-out counter (Foringer, Rockville, Maryland). Food pellets weighed 45 mg (P. J. Noyes Co., Lancaster, New Hampshire).

Testing was conducted on 3 consecutive days. On day 1, each subject was placed in the apparatus to precondition for 30 minutes, and its pre-operant level of responding was determined. During this time, the pellet dispenser was allowed to operate with each bar pressing response, but no food was delivered into the apparatus for reinforcement of responses. On day 2, the subject was placed in the apparatus for 60 minutes of conditioning, and each response was reinforced by one pellet of food. On day 3, the subject was returned to the apparatus and allowed to remain until extinction of the bar pressing responses had occurred. As on day 1, the pellet dispenser operated with each response, but the pellets were withheld from the animal by being allowed to fall outside the test box. Extinction was defined as occurring when the number of responses during any 30 minute period fell to or below the number of responses recorded during the 30 minute preconditioning on day 1.

Each subject was placed on a total food deprivation schedule 24 hours before starting the tests. No food, other than that obtained by the bar pressing on day 2, was furnished during the experiment. Thus, each had

been deprived of food for 24 hours at the start of the preconditioning period, for 48 hours at the start of the conditioning period, and again for 24 hours at the start of the extinction period.

RESULTS

Each group was tested for a significant difference from every other group by the Mann-Whitney *U* test on the following measures: number of responses during the 30 minute preconditioning; number of responses during the 60 minute conditioning; number of responses to extinction; extinction defined as a return to or below the preoperant level of responding over a 30 minute period; number of responses to extinction, extinction defined as 5 minutes without responding; time required for extinction, extinction defined as a return to or below the preoperant level of responding over a 30 minute period; time required for extinction, extinction defined, as 5 minutes without responding; number of responses during the first 5 minutes of extinction.

The entire group of 540-day-old animals was compared with the entire group of 120-day-old animals for each of these measures; no significant differences were found. Of 637 tests, 18 reflected significant differences between the 200 r/1.5 day subjects and all but one of the other groups, for numbers of preconditioned responses and numbers of responses during the first 5 minutes of the extinction period. This consistent direction of difference for the one group and the fact that only 23 other differences were found between the various groups with no pattern of consistency, led to an examination of the probability that all differences noted were due to chance or artifact. Further tests provide evidence that 36 of the 41 differences noted could have been due to chance alone.

Discussion

THE LEARNING TASKS

Several inferences can be drawn from the data of the two experiments. It is apparent that while the maze provided a learning task sufficiently sensitive to detect behavioral aberrations, the bar pressing instrument did not. This does not imply that the operant conditioning technique cannot produce evidence of behavioral anomalies resulting from prenatal irradiation, but it suggests that the schedule undertaken in establishing the conditioned response and studying its extinction was inappropriate to discriminate between prevailing deficiencies in the learning capabilities of the subjects. Now the problem is to explore intensively sequences of schedules; after the Skinner technique (Ferster and Skinner, 1957), to establish appropriate

procedures which will obtain positive differences in groups treated with conditions given in the present experiments.

The maze did provide evidence of differences in the learning ability of the various groups. It is important to establish the validity of this instrument as a measure of sensory, motor, and central nervous system functions. This has not been done to any degree of exactness. Lashley (1929) has shown that this maze will detect cortical and subcortical insult in terms of deficient rates of learning, and evidence is available to establish that it is also sensitive to central and peripheral visual defects. Much work is left to be done to answer the question of precisely what this maze does test.

The maze data provide the principal information for the present discussion. They must be viewed with some caution, since they represent the performance of very few subjects per experimental group. Likewise, generalizations regarding the precise physiologic and anatomic correlates of the behavior observed can be made only with extreme conservatism. The generalizations regarding indication of radiosensitivity of the embryonic tissue at the various periods of gestation and to various dose levels of irradiation can be stated with some confidence. It would seem fairly safe to presume that deficit in learning ability is highly correlated with the amount of intact tissue responsible for effective learning procedure.

FETAL AGE AT TIME OF EXPOSURE AND DOSE MAGNITUDE

When a relatively large dose (e.g., 200 r) was given on the 1.5 day of gestation, it did not seem to impair tissues necessary for the cognitive processes required for maze learning. The group receiving these conditions did not differ significantly from the normal controls. This finding is consistent with the statement of Hicks (1953) that the nervous system at this period of gestation is in its most primitive stage and is relatively insensitive to irradiation.

On the other hand, relatively low doses (25 r, 50 r) were associated with deficient maze learning at 2.5, 6.5, and 7.5 days during gestation. This information is inconsistent with the suggestion by Hicks that irradiation insensitivity prevails throughout the 1st week of embryonic life and supports the findings by Rugh *et al.* (Rugh, 1959a, b; Rugh and Grupp, 1959a, b) that neural tissue, at least in the embryonic mouse is, extremely reactive even to very low doses from the 1st day of gestation on.

This conflict of data can be clarified with some assumptions based on further examination of the current findings. Hicks and Rugh have reported that the period around the 9th day of gestation produces dramatic head malformations (anencephaly). These led to the emphasis on a study of the 8.5 day irradiations in the present work. Yet, with the pronounced indi-

cation of radiosensitivity of tissue at this time *in utero*, the current results failed to yield evidence for learning deficit in rats given 10, 25, 50, or 100 r at this critical day, while they did indicate that rats given 150 r at 8.5 days were deficient in maze learning.

These findings can imply two conclusions: (1) A rat is not as radiosensitive at 8.5 days as it is at 7.5 days, and (2) a rat is not sensitive to damage at 8.5 days (damage detectable by maze learning), unless it received more than 100 r. The first conclusion is based on the fact that group d, the 50 r/7.5 day group, was found to be significantly inferior on all measures of learning, not only to group e, the 50 r/8.5 day group, but also to group f, the 100 r/8.5 day group.

AGE AT TIME OF TESTING

Group F (100 r/8.5 days) was markedly inferior to the normal controls, as was group g (150 r/8.5 days). Moreover, group F was inferior in learning to group g. Age at time of testing apparently accounts for this reversal of expected results. The animal irradiated on the 8.5 day of gestation apparently can receive sufficient insult to reveal learning impairment provided the dose is great enough or the animal is old enough.

Dose magnitude, per se, has been clearly shown to be a factor in producing behavioral deficits in learning when there is marked radiosensitivity of tissues requisite for effective cognition. Tait *et al.* (1952), Levinson (1952) and Furchtgott *et al.* (1958) have supported Hicks' (1953) findings in this regard with doses of 90-600 r between the 11th and the 19th days of gestation. On these days it is apparent that the damaged tissues are requisite for efficient learning. But in all of these behavioral studies the rats were between 45 and 120 days of age at the time of testing, an age which could best be compared with 90-day-old rats of groups d (50 r/7.5 days) and g (150 r/8.5 days). Their findings lend support to a further hypothesis that the 7.5 day represents a period *in utero* of marked radiosensitivity which reveals itself in behavior at a relatively early age.

The results of the present study imply that age at time of testing is extremely critical for detection of tissue damage. One might postulate that any dose given at any time during the gestation period could possibly have an adverse effect on learning at some later period in the life of the organism.

It is apparent that 25 r given on 2.5 days is related to observed learning impairment in the 400-day-old rat. Since the reliability of differences for this group is not high (.05, .02 for errors and trials, respectively) and the N is small (3), perhaps this conclusion bears limited support. The reliability of the results with groups C, D, and E (50 r), with significant differences of magnitudes of .002 and greater, reported between each and their control group, requires that serious consideration be given to the above hypothesis.

Summary and Conclusions

In two learning experiments, 114 normal control and prenatally irradiated albino rats were divided into two age groups (90 and 400 days) at the beginning of psychological testing.

All subjects were trained to a criterion of learning of 4 out of 5 consecutive errorless trials on a Lashley III maze. Then 59 were randomly selected from their respective radiation and control groups and trained on a bar pressing instrument to measure experimental conditioning and extinction ability.

The results indicated that while the data obtained from the maze study yielded differences between groups receiving various doses of irradiation at differing periods *in utero*, data obtained from the conditioning study did not.

No differences were found in the two groups of normal controls, but the older irradiated animals exposed to 25–100 r were found to be significantly inferior to normal control groups in maze learning ability. Learning deficit was prominent in the 90-day-old groups only where conditions included 50 r at 7.5 days and 150 r at 8.5 days. A 90-day-old group given 200 r at 1.5 days was not adversely affected in learning in comparison to controls.

The results lend credence to the hypothesis that magnitude of dose has a functional relationship to *relative* radiosensitivity of tissue at all periods throughout gestation, the function being revealed on the dimension of the age at time of testing.

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Effects of Neonatal Irradiation on Learning In Rats*

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In the radiation literature, the term "protective agent" has been applied to an agent that fulfills one or more of the following criteria: (a) increases survival rate on exposure to a lethal irradiation dose, (b) increases the median survival time, (c) enables reduction of dose, (d) develops radio-resistant subjects, or (e) permits recovery from radiation damage. The last two criteria imply the prevention of generalized or indirect radiation effects and stem from reports of drug protection administered immediately (Künkel and Schubert, 1959) or up to 3 weeks after radiation (Doull and Dubois, 1953; Künkel *et al.*, 1957; Patt and Swift, 1948). Several recent investigators (Latarjet and Gray, 1954; Meier, 1960; Mole, 1959; Pihl and Eldjarn, 1958; Rugh, 1959) have sharply criticized the blanket application of the term "protective agent", and Doherty *et al.* (1958) have pointed out that although chemicals can increase survival rate or median survival time, they do not afford equal protection to every system in the animal.

Although reduction in mortality is an important criterion of the effectiveness of chemoprotection, it is equally important to determine whether the disruptive behavioral effects of radiation can also be decreased. As Rugh has noted (1959) levels of exposure below those producing histologic damage may nevertheless elicit changes in behavior. For these reasons, it is particularly important to determine whether the irradiated, but protected, animal is as adaptable to his environment as he was before radiation. In this experiment, the problem is approached by comparing maze behavior of protected and unprotected irradiated animals.

Inasmuch as the adult nervous system has been considered relatively resistant to ionizing radiation (Prosser, 1947), and since its effects on the fetal

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nervous system are confounded by possible maternal fetal interactions (Meier, 1960), the developing neonatal nervous system (Hicks, 1954) would appear to be the subject of choice for evaluating chemoprotection.

It has been demonstrated (Levinson and Zeigler, 1959) that total body irradiation of neonatal rats results in significant decrements in adult learning performance directly proportional to radiation dose. Animals exposed to a single dose of radiation during the early neonatal period show the greatest learning deficits as measured by maze performance; those irradiated at 18 days or older do not differ significantly from control animals.

The present study was designed to confirm and extend the observations on behavioral effects of radiation administered during the first 6 days of life and to explore the effectiveness of a radioprotective agent, AET [S,-(2-aminoethyl) isothiuronium bromide hydrobromide], in preventing the decrements in maze performance typical of the irradiated animal.

Method

Subjects were 236 offspring born to 20 Wistar-strain mothers and exposed to x-radiation 2, 4, or 6 days after parturition. They were divided into a 0 r (control) group and two groups of 225 r and 275 r. These three main groups were subdivided so that approximately half received radiation following saline injections and half following AET injections. The 0 r non-irradiated group was sham irradiated and contained a saline subclass, an AET subclass, and also some normal subjects who received no injection.

Table I shows the experimental design (excluding the 22 normal subjects). To complete this design, successive groups of neonates were irradiated until each experimental group contained ten 45-day-old surviving animals.

The subjects were weighed at 40 days of age and were prepared for behavioral testing by being handled. The mortality data were collected 45 and 75 days before the maze tests.

IRRADIATION

The radiation source was a G.E. Model OX-250 industrial x-ray unit with 250 kvp, 10 ma, and 0.55 mm Cu added filtration. The target-specimen distance was 70 cm and the dose rate was 26 r per minute, measured in air by a Victoreen condenser r meter. For exposure to the beam, the rat pups were placed in individual shallow lucite chambers arranged on a slowly revolving turntable to ensure equivalent radiation. Each animal received a single dose of total body radiation.

The radiation levels were selected on the basis of data from an earlier experiment (Levinson and Zeigler, 1959) so as to include a moderate radiation level (275 r), a minimal level (225 r), and a control dose (0 r).

TABLE I
EXPERIMENTAL DESIGN^a

Dose (r)	Age at radiation (days)						S.D.
	2		4		6		
	Saline	AET	Saline	AET	Saline	AET	
0	15:60%	—	11:100%	—	14:86%	—	2.5
	—	18:89%	—	15:80%	79%	12:92%	2.1
225	—	83%	—	—	—	—	—
	24:46%	—	24:62%	—	12:100%	—	3.5
275	42%	—	58%	—	—	—	—
	—	47:34%	—	15:100%	—	24:62%	4.5
Mean % surviving 45 days	—	26%	—	93%	—	29%	—
	50:24%	—	36:36%	—	32:31%	—	4.9
S.D.	20%	—	28%	—	25%	—	—
	—	44:20%	—	11:91%	—	11:100%	4.1
	—	16%	—	—	—	91%	—
	36	38	55	90	60	79	
	4.5	5.0	4.2	1.9	3.7	2.8	

^a KEY: Each cell contains the number of rats irradiated and the per cent which survived at 45 days. A second percentage is included for those which survived at 75 days, if this was different.

DRUG

AET was selected as the chemoprotective agent because of its high degree of effectiveness and its relatively low toxicity (DiStefano *et al.*, 1956). It has been shown that pretreatment of adult rats (Preston *et al.*, 1959) and mice (Doherty and Burnett, 1955; Urso *et al.*, 1958) with AET reduces the lethal effects of radiation, almost doubling the LD/50 from 700 r to 1,400 r.

AET, 250 mg per kg of body weight, was administered intraperitoneally 10 minutes prior to irradiation. Adult rats tolerate a maximal dose of 294 mg per kg with no toxic effect (DiStephano, 1959; Doherty, 1959). A somewhat lesser dose was selected because of considerations of increased drug toxicity, later confirmed in fetal rats (Graham, 1960).

The AET, dissolved in sterile saline just before injection, was prepared in a concentration of 1.25% so that an 8 gm infant received 0.16 ml. Oozing of the drug was prevented by inserting a 25 gauge needle approximately 0.5 cm subcutaneously before turning to enter the peritoneal cavity. If damage to abdominal viscera was suspected, the animal was discarded.

BEHAVIORAL TESTING

At 45 days of age, after 3 days of preliminary adaptation in a single unit straight-alley Lashley I maze, the rats were introduced into the Lashley III maze (Lashley, 1929). They were then given five trials per day until they reached a learning criterion of two out of three consecutive errorless trials completed within 10 seconds. An error was defined as entrance into a blind alley by a full body length or as the retracing of one maze unit. The time per trial was measured from the time the animal left the "start" box until it reached the food.

Thirty days after reaching the criterion in the acquisition series, the surviving animals were again placed in the Lashley III maze and given five trials per day until they regained the criterion. These scores constituted the retention scores.

Throughout the testing, the rats were maintained on a 23 hour deprivation schedule. A 24 hour feeding rhythm was established two days before each testing period. The food incentive was Purina laboratory chow, moistened and made into pellets.

Results

MORTALITY

The mortality data appear in Table I, including the number of rats which received each neonatal treatment and the percentage of 45 day and 75 day

survivors. As some irradiated animals died, the behavioral testing was completed on the selected population which survived.

Mortality was directly proportional to amount of radiation, for both the AET and unprotected groups. The protective effect of AET was most noticeable in the 275 r group, where 118 unprotected rats were radiated to obtain 35 rats surviving 45 days, whereas only 66 chemoprotected animals were required to yield 30 survivors (or 30% and 46%, respectively).

The mean survival rate appeared greatest for animals radiated at 4 and 6 days, and it is in these groups that the protective effect of AET was most clearly demonstrated. Mortality was highest in the 2 day group, and for this group no protective effect was observed.

WEIGHT

Separate comparisons of group mean weights were made for males and females. The nonirradiated group averaged 135.4 gm (males) and 106.5 gm (females). The irradiated group averaged 97.5 gm (males) and 82.6 gm (females). A comparison of the unprotected (saline) groups for the three radiation levels indicated that weight decrements in both sexes increased as radiation dose was increased. The protective effect of AET on weight gain was most noticeable at the 275 r level.

Considering age at radiation, the greatest weight decrements appeared in the 2 and 6 day groups.

ORIGINAL LEARNING

Three performance measures—trials, errors, and time in the maze—were used for analysis of the original learning scores in the Lashley III maze. The function relating dose level to performance (Fig. 1) indicated that performance deficits on *all* measures increased with increasing radiation dose.

Substantial decrements in maze performance appeared at the highest radiation level. The performance of 275 r rats differed significantly from that of nonirradiated animals ($p < .01$ for errors and time and $< .05$ for trials). A similar comparison for the 225 r and nonirradiated groups revealed no significant differences and indicated that the effects of 225 r on performance were near threshold and were not very different from the 0 r group.

A trend is visible in the curves of Fig. 1, and the ordering appears consistent in all cases. Superimposed in the graph of trials are data taken from the previous neonatal study (Levinson and Zeigler, 1959); there is considerable agreement between the results of the two studies.

As in the previous study, time and error scores were more sensitive indicators of radiation effects than trials.

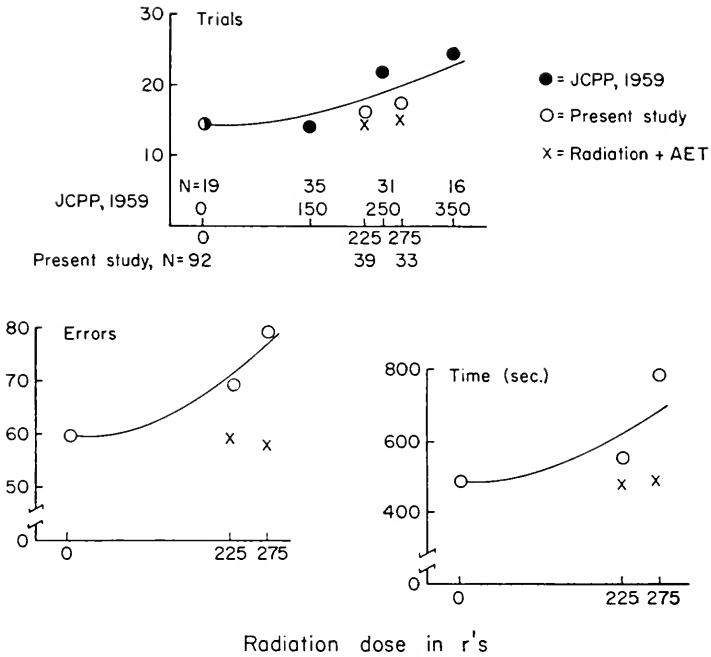


FIG. 1. Maze scores as a function of radiation dose.

The relationship between age at irradiation and performance deficit is indicated in Fig. 2. Radiation in the first 6 days produced significant deficits in adult learning. However, age at radiation was not an ordered variable, since radiation interferes with developing cells which differ with neonatal age.

Figure 2 suggests there may be significant differences due to radiation at 2, 4, and 6 days, but these differences are not consistent with those of the previous study. Also, in the earlier work (Levinson and Zeigler, 1959) the greatest adult learning decrement appeared at day 4; while, in the present data, the least effect occurred at this age. It is possible that these discrepancies between the two studies are attributable to differences in the species and radiation levels used, but further research is necessary to clarify the point.

Groups receiving AET and those without chemoprotection differed significantly for all three performance measures. In fact (Fig. 2), the performance of the AET groups in every instance approximated that of the nonirradiated group, the most striking difference between protected and unprotected groups appearing at the highest radiation level. There were no significant differences between the 275 r AET and the nonirradiated groups in trial,

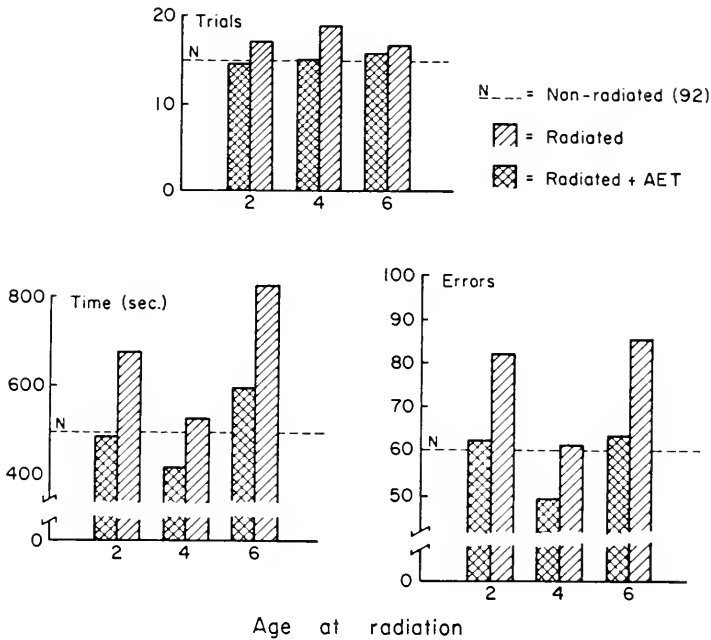


FIG. 2. Maze scores as a function of age at radiation. Number of subjects in radiated group: 25, 25, and 22 for Or, 225r, and 275r, respectively. Corresponding numbers in radiated + AET group are 23, 23, and 26.

error, or time scores ($t = .357, .550, \text{ and } .229$ at 119 degrees of freedom, respectively). Finally, the scores of the AET protected animals were close to the 0 r level for all performance measures.

RETENTION

In the 30 day interval between the acquisition of the maze habit and the relearning test, 29 animals died.

With increases in radiation, dose decrements in all relearning measures were greater. An F test showed that radiation dose had a significant effect on all retention measures ($p < .01$). Relearning was less affected by radiation than was original learning.

As with the original learning data, age at radiation was not a significant variable. But a considerable protective effect was exerted by the AET in the 275 r group. Saline 275 r mean scores were 20.0, 22.1 and 323.7 for trials,

errors, and time, respectively; the equivalent protected group scores were 13.8, 11.4, and 171.1.

The savings percentages¹ for the various groups decreased as radiation dose increased. Indeed, for the 275 r group the savings score for trials was a minus number.

Discussion and Conclusions

Neonatal rats were radiated at three levels of radiation at either 2, 4, or 6 days of age. Half of these animals received pretreatment with AET, a chemoprotective agent. Measures of maze learning behavior, mortality, and body weight were obtained.

Acquisition and retention of the maze habit were inversely related to radiation dose. However, the group receiving AET pretreatment performed significantly better than the unprotected groups. Indeed, the original maze performance of the protected group was hardly distinguishable from that of nonirradiated animals. The protective effect of AET on both original learning and retention measures was most striking at the highest radiation level (275 r).

In addition to evaluation of the chemoprotective effects, the study served to extend the data of a previous experiment on the effects of neonatal irradiation. The present data were consistent with the previously reported relationship between dose level and performance deficit and with the finding that time and error measures are more sensitive indicants of radiation effects. It was again found that radiation in the first 6 days of life produces significant decrements in adult learning.

The mortality was directly proportional to radiation dose; however, rats receiving AET had a higher survival rate. Weight at 45 days of age decreased as radiation dose increased, and the chemoprotective effect on mortality and weight was observed only for the highest radiation dose.

Recent studies have measured chemoprotection in terms of reduction of radiation lethality (Benson *et al.*, 1957; Crouch and Overman, 1957), survival time (Preston *et al.*, 1959), depression of blood forming organs (Cronkite *et al.*, 1951), number of cataracts (von Sallman *et al.*, 1951), splenic atrophy (Patt *et al.*, 1953), epilation (Condit *et al.*, 1958; Forsberg, 1950), and graying of hair (Kulwin, 1953).

The present study demonstrated the importance of measuring the effectiveness of chemoprotection in yet another way—by means of behavioral testing.

¹ The savings percent or savings score is obtained by dividing the retention score by the original learning score and converting to a percentage for each subject.

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Behavioral Effects of Cranial Irradiation of Rats*

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This paper summarizes the results of a series of experiments, carried out over a period of several years, dealing with the effects of irradiation on a variety of behavioral processes, including emotionality, maze learning and retention, instrumental learning, discrimination learning, formation of a learning set, motivation, visual exploration, and general (locomotor) activity. Details of the irradiation techniques used in these experiments have been published elsewhere (Arnold, 1952; Blair and Arnold, 1956; Blair, 1958) and need not be repeated here. Briefly, each rat was individually irradiated in a lead exposure chamber. For some experiments, the whole head of the rat was exposed to 300, 800, 2,000 or 2,500 r, while, for other experiments, only an oval area (11×13 mm) directly over the brain was exposed to 5,000 r.

Gross Effects, Lethality, and Tooth Deformities

The results of some of the behavioral tests can best be understood against a background of the gross effects of cranial irradiation. Preliminary work showed that, for 90-day-old rats, 2,500 r whole head irradiation and 5,000 r brain area irradiation were near the maximum practical doses. Beyond these, the mortality rate was prohibitive. All animals developed symptoms of radiation sickness similar to those reported for much lower doses of total body irradiation. They stopped eating and lost weight, reaching a minimum weight 10 to 20 days after irradiation. Weight loss and lethality were positively related to dosage and inversely related to age when irradiated. A dose of 5,000 r to the brain area of 90-day-old rats shortened their life span by about one third.

Tooth deformities developed after irradiation. They were most severe in

* Most of the research reported in this paper was supported by the Atomic Energy Commission.

the head irradiated animals. In one experiment, all 20 irradiated animals developed tooth overgrowth, supernumerary lower teeth, abnormal breaking off of teeth, and growth of new ones. None of the control animals had tooth defects. The teeth of one of the head-irradiated animals are shown in Fig. 1, photographed approximately 10 months after a dose of 2,500 r. The upper left tooth has grown spirally through more than 360 degrees, penetrating the roof of the mouth and emerging at the side of the nose. The lower right tooth penetrates the roof of the mouth. Later work, in which control animals were starved to match the weight loss of irradiated rats, indicates that these deformities were a primary result of irradiation rather than a secondary effect of not eating.

Emotional Behavior

In one experiment, 40 pied rats were divided into split-litter control and experimental groups of 20 each with 10 males and 10 females in each group. In the second experiment, 16 Sprague-Dawley male rats were randomly assigned to experimental and control groups of 8 each. The apparatus consisted of an "open field" constructed by surrounding a circular area 10 ft. in diameter with a sheet metal barrier 24 in. high. This area was brightly illuminated by a light suspended above its center.

Test trials were given 5 days prior to irradiation and at 5 day intervals after irradiation for 30 days. In the first experiment, a final test trial was

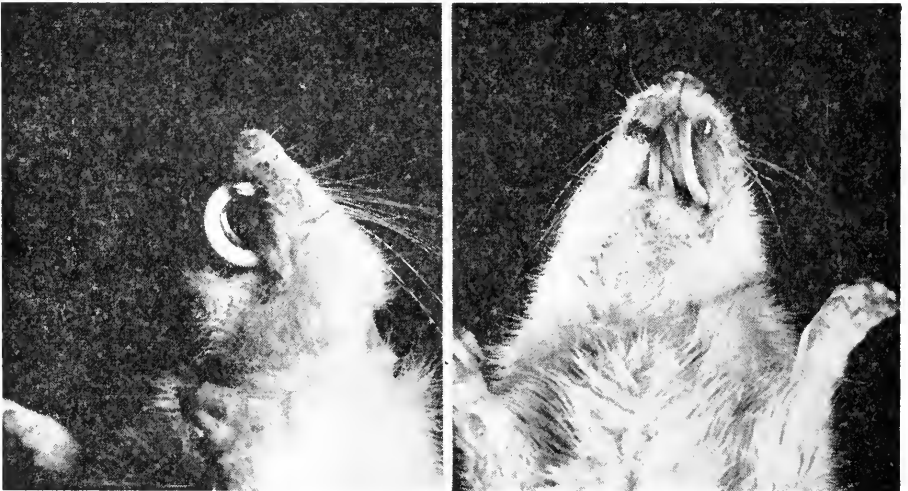


FIG. 1. Tooth deformities developed during 10 months after 2,500 r head irradiation.

given 107 days after irradiation. On each test trial the rats were placed, one at a time, in the center of the field, and the number of defecations and urinations during a two minute interval was recorded. A dose of 2,000 r was given in the first experiment; 2,500 r, in the second.

RESULTS

In the first experiment, the irradiated male rats tended to be more emotionally reactive than the controls, but no such tendency was found for the females. The second experiment on males revealed a significant increase in number of defecations after irradiation. The urination index did not yield consistent results. These results indicate that in terms of the defecation index, male rats show increased emotional reactivity during the first 30 days after head irradiation. This was not true for females.

Instrumental Learning

The first experiment tested acquisition of an instrumental response after 2,000 r head irradiation administered 38 days prior to the beginning of the experiment. In the second experiment, a dose of 2,500 r was given. In order to have a battery of tests on the same animals, the same pied rats that had been previously tested for emotionality were used in the first experiment. In the second experiment, 40 Sprague-Dawley male albino rats were randomly assigned to experimental and control groups of 20 rats each.

In both experiments, a modified Skinner box with a horizontal bar extending through a hole in one side was used. When the rat pressed the bar, an automatic feeding mechanism dispensed a small pellet of food into a food pan below the bar. A shutter, which could be raised and lowered by the experimenter, was located in front of the bar.

The procedure was the same in both experiments except that, in the first experiment, irradiation preceded acquisition, while in the second experiment, irradiation occurred between acquisition and extinction. The dosage in the first experiment was 2,000 r, and in the second it was 2,500 r. All animals were run on a 22 hour hunger drive. In each trial, the animal was placed in the box with the shutter lowered. The experimenter then raised the bar and allowed the animal to make four bar-pressing responses, each of which was reinforced by a pellet of food; the shutter was then lowered. The latency of each response was automatically recorded by means of electrical time markers and a Phipps-Bird polygraph. Training continued for 12 days (48 reinforced responses), and then extinction was begun. Each extinction trial was the same as a training trial except that the feeding mechanism was inoperative. The criterion of extinction was a period of 3 minutes in the box

with no response. After reaching the criterion, each rat was tested for spontaneous recovery.

RESULTS

The measures of acquisition and retention did not show significant differences between experimental and control groups. Neither the dose of head irradiation nor the postirradiation time interval affected instrumental learning with relatively short postirradiation time intervals. However, later experiments suggested that with longer postirradiation intervals time is an important parameter.

Maze Learning and Retention

Of the series of experiments dealing with maze learning, all used the same apparatus and essentially the same training procedure. They differed only in radiation dosage. The apparatus was a 14-unit Tolman-Honzik T-maze. The rats were run under a 23½ hour hunger drive. They were given 1, 3, or 5 trials a day, until they reached the criterion of 3 successive trials with a total of no more than 3 errors.

In two experiments (Arnold, 1952), acquisition and retention were tested after 300 r and 800 r to the whole head. There were no significant differences between irradiated and control animals. Also, no symptoms of radiation sickness were observed, although within 60 to 90 days after irradiation, the dark hair on the heads of the pied animals turned gray.

A third, unpublished experiment tested the effects of 2,000 r whole-head irradiation on maze acquisition. The performance of the irradiated animals was consistently better than that of the controls, but the differences were not statistically significant.

In a fourth experiment (Blair and Arnold, 1956), the effects of 2,500 r whole-head irradiation on retention of maze learning were tested. The rats learned the maze, were irradiated, and then were tested on postirradiation days 3, 12, 25, 40, 60, and 80. The control rats tended to perform better than the irradiated rats on the day 3 test; but, by the day 25 test, there was a reversal, and the irradiated rats were superior to the controls. This superiority was maintained on subsequent tests. These unexpected results were accounted for in terms of motivational factors rather than in terms of direct effects of irradiation on brain tissue. The hypothesized factors were increased hunger and reduced exploration resulting from sickness following irradiation.

A fifth experiment (Blair, 1958) tested the effects of 5,000 r brain irradiation on maze acquisition with training beginning 1, 30, and 60 days after

irradiation. All irradiated groups learned the maze in fewer trials, had faster running times, and were less variable in performance than control groups. In trials to learn, the differences between experimental and control groups became smaller as a function of delay in beginning training. Although histologic examination revealed that brain tissue in some of the irradiated rats had been damaged, it was suggested that a combination of increased hunger motivation and reduced exploratory drive produced the differences in performance.

The last experiment was designed to test (1) whether increased hunger following radiation sickness adequately accounted for facilitation of maze performance, (2) whether possible detrimental effects of brain irradiation might be masked by increased hunger motivation following radiation sickness, and (3) whether radiation sickness and/or facilitation of maze performance might be produced by irradiating a body area other than the head with a dose equivalent to that applied to the brain area. This experiment compared the performance in a 14-unit multiple T-maze of 110 rats divided into the following four matched weight groups: a brain-irradiated group, a group that received an equivalent dose of irradiation to the right hind leg, a group starved so as to lose the same amount of weight lost by the brain-irradiated group, and a control group. The animals were weighed each day and those in the starvation group were given the appropriate amount of food to maintain their weight at the same level as that of the brain-irradiated group.

RESULTS

As in previous experiments, the brain-irradiated animals lost weight rapidly and reached a minimum 12–15 days after irradiation. Weight loss of the starved animals matched that of the brain-irradiated animals; however, the leg-irradiated animals did not lose weight. In each experiment, the average weight of the leg-irradiated group closely matched that of the control group. These findings, along with daily observations of behavior, clearly indicated that the radiation sickness syndrome observed in the brain-irradiated animals was not produced by an equivalent dose of irradiation applied to another part of the body, specifically, to the hind leg.

Both starvation and brain-irradiated animals performed better than the controls. The starvation animals performed better than the brain-irradiated, but the difference fell short of statistical significance. The performance of the leg-irradiated animals was erratic. In the first experiment, it approximated that of the starvation animals and was significantly better than that of the controls while, in the third experiment, it approximated that of the controls.

Considering these results in the light of our previous maze learning test

results, it seems clear that brain-irradiated rats and rats starved to match the weight loss of brain-irradiated rats perform significantly better than control rats. The leg-irradiated rats tend to perform better than the controls, but the difference is not reliable. Finally, the starved rats tend to perform better than the brain-irradiated rats, but the difference is not statistically reliable. Unfortunately, no definite conclusion can be drawn as to whether there were detrimental effects from brain irradiation which were masked by increased hunger motivation, but the possibility of such detrimental effects remains.

Maze Learning as a Function of Age When Irradiated

Subjects were 90 albino rats grouped according to age at the time of irradiation into 60-, 90-, and 120-day-old groups. Each group contained 15 males and 15 females. Dosage was 5,000 r to the brain. Sixty days after irradiation and 7 days prior to the preliminary maze training, the rats were placed on a 24-hour feeding schedule. After the preliminary training, they were given one training trial per day on a 14-unit multiple T-maze until they reached a criterion of 3 consecutive trials with a total of 3 errors or less. On the 60th and 90th days after reaching the criterion, the rats were given one retention test trial. After the 90-day retention test, the rats were retrained on the maze, one trial per day until they again reached the criterion.

RESULTS

In the acquisition phase of the experiment, the irradiated animals in the 60 and 90 day groups tended to learn the maze faster than their controls. The differences, however, were not statistically significant. The direction of the differences was reversed for the 120 day group, where the control animals tended to learn faster than the experimentals. Again, this difference was not significant. None of the differences on the 60 and 90 day retention tests or on the relearning trials was significant. These findings indicate that, within the time limits involved in this experiment, irradiation at different ages does not produce significant decrements in maze performance. This is noteworthy in view of the fact that we have shown that the same dosage produces close to 100% mortality in rats 30 days of age.

Maze Learning As a Function of Higher Dosages

This experiment was designed to test the effects of 6,000, 7,000, 8,000, 9,000, and 10,000 r. Unfortunately, a breakdown of the x-ray machine and

a high mortality rate (particularly at the higher dosage levels) made it impossible to obtain definitive data. The data that were obtained for animals having doses up through 8,000 r were consistent with the previous findings that irradiated rats tend to learn more rapidly than corresponding controls.

Discrimination Learning and Formation of a Learning Set

The effects of 5,000 r brain irradiation on discrimination and on learning set were investigated in two experiments: 20 rats were subjects in the first; and 24, in the second. The apparatus was a 5-choice discrimination box, described fully in a previous publication (Koronakos and Arnold, 1957). The task involved learning to discriminate visually which of 5 doors gave entry from the choice to the reward compartment. Stimulus cards with different geometric figures were attached to each door and the correct door was signified by the "odd," i.e., non-matching, pattern. A series of 9 such visual discriminations were given, with 30 trials on each problem.

RESULTS

The irradiated animals showed a slight superiority on the first two problems. However, on the remaining seven problems, their performance was consistently inferior to that of the controls. Acquisition of the oddity learning set was acquired more slowly in the experimental group. At the same time, the latencies of the experimental group were consistently lower than those of the control group. This suggests that the experimental animals were more strongly motivated than the controls; and, as a result, they tended to respond too quickly to allow time for making a correct discrimination.

Discrimination Learning—Long Term Effects

The work reported thus far involved relatively short postirradiation time intervals, but, because of histologic findings of damage after 9–12 months, we became increasingly interested in the long-term effects of irradiation; and it was decided to test discrimination learning 300 or more days after exposure.

Subjects were 24 albino rats divided into an experimental and a control group; there were 6 males and 6 females in each group. These animals had previously learned the 14-unit T-maze. The apparatus was a two-choice discrimination box. By pushing open either of two doors, the rat gained access to a food reward. Black or white stimulus cards were attached to the doors. The animals were irradiated with 5,000 r to the brain 323 days prior to the beginning of the experiment. After preliminary adaptation to the apparatus, the animals were given 5 trials per day on a black-white discrimina-

tion problem. Training continued until each animal reached a criterion of 8 consecutive trials without an error or until a maximum of 50 trials was reached.

RESULTS

Only one irradiated rat reached criterion, by comparison with 8 rats in the control group. Significantly more errors were made by the irradiated group than by the control group. These findings indicate that the long term effects of irradiation are deleterious.

Effects of Brain Irradiation as a Function of the Interval after Irradiation

The finding that radiation has long-term deleterious effects on discrimination learning led to a more extensive investigation. An experiment was designed to determine the effects of 5,000 r brain irradiation on maze learning, discrimination learning, and concept formation at intervals of 1, 3, 6, 9, and 12 months after irradiation. Two hundred male and 200 female Sprague-Dawley albino rats, 90 days old, served as subjects. Maze learning was evaluated by means of a 14-unit T-maze, and a brightness discrimination was established in a five choice discrimination box. In order to test learning set formation, the previously described series of oddity problems were used. An increasingly rapid solution of the successive problems was taken as evidence of the formation of a learning set.

Within each sex, rats were randomly assigned to experimental and control groups for each of the test intervals (1, 3, 6, 9, and 12 months). All experimental groups were exposed to 5,000 r brain irradiation by our standardized technique, and controls were sham irradiated. At the various postirradiation time intervals, the groups were tested for maze learning. Then they were tested for discrimination learning, and then, concept formation. To keep the postirradiation time interval the same in each group, each of the tests was begun only after all the animals of a group had finished the preceding test.

RESULTS

Because of a high mortality, due in part to an outbreak of respiratory infection and in part to the effects of radiation, maze learning data were obtained only for the 1, 3, and 6 month groups, discrimination learning data only for the 1 and 3 month groups, and learning set data only for the 1 month group.

The irradiated animals were consistently superior in maze learning, and

this was true even of the 6 month group. With respect to discrimination learning, no significant deterioration occurred within 221 days after irradiation. It will be recalled, however, that, in a previous experiment, we tested rats 323 days after irradiation and found the irradiated rats to be inferior to their corresponding controls in discrimination learning. This suggests that there is a critical period between 200 and 300 days after irradiation.

The scanty data on learning set formation allow no definitive conclusions. Of the data obtained, there were no consistent differences between experimental and control animals tested 92 days after irradiation.

Motivation—General Level of Activity

One experiment tested the effects of 2,500 r to the whole head, and three others tested the effects of 5,000 r to the brain area. Fifty days prior to irradiation, the rats were placed in automatically recording activity wheels with attached living cages in order to establish a base-line activity level. Activity of the experimental animals exposed to 2,500 r whole head irradiation was recorded for 120 days. Of the three 5,000 r experimental groups, activity in one was recorded for 120 days; in another, for 300 days; and in the third, until death.

RESULTS

In all four experiments, irradiated rats were less active except for a brief interval between the 10th and 15th days after irradiation. At the peak of this activity burst, experimental rats made three times as many revolutions per day as the controls (2,300 vs 700). Thereafter, the activity of the experimental animals diminished to a level well below that of the controls. In both the experimental and control groups, there was a gradual decline in activity with time, a tendency which continued until death.

Motivation—Hunger and Thirst

In the first experiment on this topic, the effects of 2,500 r to the whole head on hunger motivation were tested; in the second and third, the effects of 5,000 r to the brain area on hunger and thirst motivation were tested. Measurements were made in a Jenkins-Warden obstruction apparatus before and after irradiation. A 48 hour deprivation period was used in the hunger tests and a 23 hour period in the thirst tests.

RESULTS

The 2,500 r dose to the whole head produced a significant increase in hunger drive between the 20th and 100th days following irradiation. Thereafter, the difference between experimental and control groups became smaller, until it virtually disappeared.

The 5,000 r dose to the brain area did not produce a corresponding increase in hunger drive. Except for a period 20–30 days after irradiation, when it was slightly higher, the performance of the experimental group was consistently lower than that of the control group.

The 5,000 r dose to the brain produced a significant decrease in the strength of the thirst drive, a decrease which reached a maximum 30 days after irradiation. At this point, the difference between experimental and control groups was significant. Thereafter, the strength of the drive tended to increase on successive tests, until, at about 75 days after irradiation, there was virtually no difference between irradiated and control groups.

Motivation—Manipulative Activity

Subjects were 20 male albino rats exposed to 5,000 r brain area irradiation.

Mounted on the walls of a hexagonal wooden box 12 in. high and approximately 15 in. in diameter were a horizontal brass bar, a $\frac{3}{4} \times \frac{3}{4} \times 1\frac{1}{2}$ in. wood block, a wire mesh ladder with three large wooden rungs, a chain attached to a piece of wood $\frac{3}{8} \times \frac{3}{8} \times 2$ in., a wheel, and a vertical metal pushing panel with a $\frac{3}{8} \times \frac{3}{8} \times 2$ in. piece of wood mounted near the bottom. A seventh manipulandum, a vertical wooden dowel $\frac{5}{8} \times 2\frac{3}{4}$ in., was mounted in the middle of the floor of the box. A constant speed polygraph was used to record automatically any manipulation.

Fourteen days after irradiation, experimental and control rats were placed one at a time in the box at 5 PM and were removed at 9 AM the next day. During these hours, manipulative activity was recorded. This process continued until each rat had had two such periods in the box. Beginning 110 days after irradiation, the procedure was repeated.

RESULTS

The rats engaged in much manipulative activity, but there were no significant differences between irradiated and control animals in total manipulations or in preferential order for the manipulanda during each of the test periods.

Motivation—Visual Exploration

Subjects were 20 pied rats 90 days old. The experimental group contained 6 males and 5 females, and the control group contained 5 males and 4 females. The apparatus consisted of a plywood box, $10 \times 10 \times 10$ in. with a wire mesh floor and a translucent plastic top. In one wall, a 2×2 in. window, located 1 in. above the floor, was covered by a door hinged so that an easy push on the inside would cause it to fall and to open the window. A guillotine door inside the box could be lowered to deny the rat access to the window.

Throughout the experiment, the rats lived in home cages plentifully supplied with food and water. On each experimental trial, the latency of opening the window was recorded. When it opened the window, the rat was allowed to look out for 30 seconds. If the rat did not respond within 5 minutes, the trial was recorded as a failure. Ten trials per day were given on the 7 days prior to irradiation. After irradiation with 5,000 r to the brain, 10 trials per day were given for the first 30 days. Testing then continued every 5th day for the next 50 days. Next, massed satiation trials were given, i.e., each rat was given continuous trials until it failed to respond on three successive trials. Finally, long term tests were made 190–200 days after irradiation.

RESULTS

During the first 5 days after irradiation, the average latency of the experimental group was virtually the same as that of the control group. Then, beginning with the 6th postirradiation day, there was a sharp increase until, by the 11th postirradiation day, the average latency for the experimental group was 10 times that of the control group. Thereafter, there was a sharp decrease in the latency of the irradiated group to a level about 2.5 times greater than that of the control group, and this differential continued through the remainder of the test trials.

The results of the massed satiation trials are intriguing. Two of the male rats (one irradiated and one control) were still responding after 5 hours of testing—at which time the experimenter extinguished. During this time, the control rat made 212 responses and the experimental rat made 192 responses. All the other rats stopped responding. The difference in trials to satiation for experimental and control groups was not significant. On the long term tests, there was no significant difference between irradiated and control animals.

Discrimination Learning Based on Visual Exploratory Drive

This experiment was designed to test discrimination learning motivated by visual exploratory drive. It was thought that discrimination learning might be a more sensitive indicant of differential drive strength than the latency measure.

Of 14 rats 280 days old and previously tested for maze learning, 7 were irradiated and 7 served as controls. Irradiation was carried out 190 days before the start of the experiment. The apparatus was essentially the same as that used in the visual exploration experiments. It consisted of a box with two windows covered by doors which the rat could push open. Black or white cards were attached to these doors and, in order to respond correctly, the rat was required to discriminate between these cards. Errors (pushes on the incorrect or locked door) were electrically recorded.

After preliminary training, the animals were given 50 discrimination training trials at the rate of 10 trials per day. The criterion of learning was 32 correct responses out of 50. Animals achieving this criterion can be said to have learned the discrimination at the 5% level of significance.

RESULTS

One experimental and one control animal failed to adapt to the situation and were discarded during preliminary training. One of the irradiated rats died during discrimination training. Four of the remaining six control animals and three of the remaining five irradiated animals learned the discrimination. This difference is not statistically significant. Thus, it appears that irradiation did not significantly affect discrimination learning motivated by visual exploration.

Summary and Conclusions

These investigations show that, in rats, sufficiently large doses of cranial irradiation produce symptoms of radiation sickness similar to those for total body irradiation. These symptoms are not produced by equivalent amounts of irradiation to another part of the body, such as the hind leg.

Either directly or indirectly (via the production of radiation sickness), cranial irradiation seems to affect some behavioral processes but not others. At least two parameters are important in testing any of the behavioral processes: dose size and the time interval after irradiation. Most of the work reported here involved doses of 2,500 r to the whole head and 5,000 r to

the brain area. With higher doses, the mortality rate was so great that post-irradiation testing was precluded, especially long-term testing.

Both short-term effects (less than about 150 days after irradiation) and long-term effects (more than about 250 days after irradiation) were tested. Thus far, the results of the latter are inconclusive, and the following summary applies primarily to the short-term effects of irradiation.

Whole head irradiation with 2,500 r increased emotionality in male rats but not in females.

Instrumental learning and retention were not significantly affected by 2,500 r whole head irradiation.

A 2,500 r dose to the whole head and a 5,000 r dose to the brain area produced better maze learning and retention in irradiated animals than in controls. It appears likely that this improvement in performance was due primarily to increased hunger motivation and to reduce exploratory activity following radiation sickness. Rats starved to match the weight loss of the 5,000 r brain-irradiated animals performed consistently better than the irradiated animals. This finding suggests that brain irradiation may have detrimental effects on maze learning which are masked by the facilitative effects of increased hunger motivation and reduced exploration.

In learning set formation, the performance of 5,000 r irradiated animals was inferior to that of their controls. Again, this finding may be interpreted in terms of increased hunger motivation. The irradiated animals may have been so strongly motivated that they responded too quickly, with the result that their accuracy was impaired.

The effects of irradiation on discrimination seem to be a function of the postirradiation time interval. The relatively short postirradiation time intervals revealed no significant differences. Even a 221-day-postirradiation time interval had no effect. However, a 323-day-postirradiation interval revealed detrimental effects. These findings suggest that there may be a critical period between 200 and 300 days after irradiation.

General (locomotor) activity was depressed both by 2,500 r and by 5,000 r except during an interval between the 10th and 15th days after irradiation.

Hunger motivation was increased between 20 and 100 days after 2,500 r whole head irradiation but not after 5,000 r brain irradiation.

Thirst motivation was depressed for about 75 days after 5,000 r brain irradiation. Maximum depression occurred 30 days after irradiation.

Visual exploration was depressed during at least the first 50 days after irradiation. Later tests at 190 to 200 days after irradiation showed no significant difference between irradiated and control animals.

It should be pointed out that, in our recent work, a new irradiation technique is being used. Until now, a vertical beam was applied to the top of the animal's head. As a result, radiation that was not absorbed in brain tissue

may have affected mouth and throat tissue, possibly complicating radiation sickness and its sequelae. The new technique utilizes a horizontal beam which minimizes the dosage to mouth and throat tissues. With the new technique, it is planned to investigate the effects of dosages of 5,000 r or less after longer periods.

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Radiation-Conditioned Behavior*

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Radiation-conditioned behavior was detected initially in studies of physiologic responses during exposure to low dose rate gamma radiation (Table I). The consumption of food and water by rats decreased progressively during successive weekly periods of irradiation, while consumption between exposures was not reduced (Garcia *et al.*, 1956a). This puzzling observation was not consistent with accepted concepts concerning cumulative effects of radiation exposure. However, the behavior did suggest a learning curve in which the animal was conditioned to avoid food and water during irradiation. The experimental research necessary to test the hypothesis that radiation is a stimulus for conditioning behavior has occupied the attention of John Garcia, E. L. Hunt, and myself for the past few years.

It was reasoned that if the progressive reduction in food and water consumption during exposure was a learned behavior, then the manipulations involved in a sham irradiation test should likewise evoke the conditioned (learned) response in previously irradiated animals (Garcia *et al.*, 1956b; Kimeldorf *et al.*, 1955). Table II illustrates the previous exposure history

TABLE I

FOOD AND WATER CONSUMPTION DURING EXPOSURE TO GAMMA RADIATION ^a

<i>Exposure conditions</i>	<i>Consumption during successive exposures</i>	<i>Consumption for 10 hrs. after each exposure</i>
Radiation + food and water	declines	normal
Sham irradiation + food and water	normal	normal
Radiation only	—	normal

^a Exposure pattern: 1-8 weekly exposures, 75 r per exposure at 9.4 r per hr.

* The opinions and assertions contained herein are the private ones of the author and are not to be construed as official views of the Navy Department. This study was supported through funds provided by the Bureau of Medicine and Surgery, USN.

TABLE II

MANIPULATION AND CONFINEMENT STIMULI COMPLEX^a

<i>Previous exposure history</i>	<i>Postirradiation sham exposure test food and water consumption</i>
Radiation + food and water	decline
Radiation + food only	decline
Radiation only	normal
Sham irradiation + food and water	normal

^a Exposure pattern: gamma radiation, 1-9 exposures with 75 r per exposure at 9.4 r per hr.

and the direction of consumption in a postirradiation sham exposure test. Food and water consumption in the sham exposure test was decreased in the groups which had previously experienced food and water consumption during radiation exposure. When animals were given only food during irradiation, the consumption of both food and water was decreased in the subsequent sham exposure test. In contrast, animals without food and water during periodic irradiation exhibited no inhibition of consumption during the sham exposure test. Littermate controls which had been periodically sham irradiated prior to the sham exposure test maintained or increased food and water consumption during the test.

It was concluded that manipulation and confinement to a radiation chamber can serve as a conditioned stimulus complex to produce a reduction in consummatory behavior, provided the animals had previously consumed food or water, or both, during radiation exposure in the same situation. The approach, however, was awkward, principally because of the difficulties in specifying the important variables involved in the conditioned stimuli of manipulation and confinement.

To facilitate further study, a distinctive taste cue was utilized in conjunction with radiation exposure, and the animal was subsequently tested for the presence of a conditioned response towards the taste cue. Saccharin flavored water was selected as the discriminable taste stimulus, since it is normally preferred to tap water by the rat and could be manipulated in a convenient manner.

Preirradiation and postirradiation tests of saccharin preference were made on animals which received either saccharin or water during radiation exposure, with saccharin flavored water and tap water available simultaneously. In terms of preference, the consumption of saccharin flavored water normally constitutes more than 75% of the total fluid intake. Table III describes several conditions investigated with respect to radiation exposure and saccharin preference. It was found (Garcia *et al.*, 1955) that animals

that tasted saccharin during a single 30 r exposure to gamma radiation at 5 r per hour displayed a distinct aversion to this fluid in the postirradiation saccharin preference tests. Animals that tasted saccharin during a 6 hour exposure to 57 r exhibited a nearly complete aversion to saccharin in the postirradiation tests. The effect on saccharine preference persisted for more than 30 days following the conditioning event. Animals with water available during exposure and control animals with saccharin available during sham irradiation continued to exhibit a high postexposure preference for saccharin. In further investigations, it was found that exposure to 10 r of

TABLE III

SACCHARIN AS A CONDITIONED STIMULUS ^a

<i>Exposure conditions</i>	<i>Postirradiation saccharin preference</i>
Radiation + saccharin	avoidance
Radiation + water	normal
Sham irradiation + saccharin	normal
Irradiated rat only	normal
Irradiated saccharin only	normal
Saccharin immediately prior to irradiation (trace conditioning)	avoidance

^a Exposure pattern: gamma radiation in a single exposure of 30-57 r at 5-9 r per hr.

gamma radiation was sufficient to reduce saccharine preference, if the test for saccharine preference were made in a sham exposure situation (Hunt *et al.*, 1961). It was also found that conditioning could be elicited if saccharine consumption occurred immediately prior to exposure (trace conditioning) (Garcia and Kimeldorf, 1957). Trace conditioning made it possible to study the stimulus potential of a 4 minute radiation exposure, by preceding the exposure with a 20 minute presentation of saccharin (Garcia and Kimeldorf, 1960a).

As illustrated in Table IV, the conditioned saccharin aversion has been observed with x-rays (Garcia and Kimeldorf, 1960b; Kimeldorf *et al.*, 1960), gamma rays (Garcia *et al.*, 1955; Hunt *et al.*, 1961), and fast neutrons (Garcia and Kimeldorf, 1960a) over a broad spectrum of exposure conditions and dose rates. Chocolate flavored milk and Kool-Aid have been used with other species. Radiation-conditioned consummatory behavior has been reported in the mouse, rat, and cat by us (Kimeldorf *et al.*, 1960) and in the monkey by Harlow (1960).

Thus far our description of radiation-conditioned behavior has concerned conditioned taste stimuli or has involved consummatory behavior in the test

TABLE IV

EFFECTIVE EXPOSURE CONDITIONS FOR INDUCTANCE OF CONDITIONED SACCHARIN AVERSION IN MALE SPRAGUE-DAWLEY RATS

<i>Exposure factors</i>	<i>Dose rate</i>	<i>Total dose</i>
X-rays, 250 kvp, half-value layer of 2.7 mm Cu	18 r per min	54 r ^a
γ rays, Co ⁶⁰ , 1.2 Mev	5 r per hr	30 ^b
Fast neutrons, Be(p,n)B, Fission energy spectrum	2 rad per min	7.5 rad ^c

^a Garcia and Kimeldorf, 1960b.^b Garcia *et al.*, 1955.^c Garcia and Kimeldorf, 1960a.

situation. The question arose as to whether the radiation stimulus could elicit learning in situations not dependent on consummatory behavior. It was decided to determine whether a rat could discriminate as to the place of radiation exposure and whether radiation has motivational qualities sufficient to elicit a learned avoidance of the place of exposure. Exposure to gamma or x-rays was associated with one of two distinctive compartments of a straight 30-in.-long alley in a forced trial learning procedure (Fig. 1). One compartment of the alley was painted black and had a grid floor, while the other compartment was painted white and had a mesh floor. Prior to the conditioning phase, each animal was tested for choice of residence in the alley by removing the barrier between compartments. The cumulative time spent in one compartment was used to separate the animals into groups having a comparable residence preference. During the conditioning phase, rats were exposed to radiation while confined to one of the two compartments. On the day following each exposure, the animals were confined to the opposite compartment for an equivalent time as a sham irradiation procedure. After several such cycles, the partition between the two compartments was again removed, and the animals were given a free choice of residence in the alley.

Four conditioning sessions with 50 r of gamma radiation at 10 r per hour were sufficient to alter the residence preference in the postirradiation test (Garcia *et al.*, 1957). In contrast to sham irradiated controls, the irradiated animals exhibited a decreased preference for the compartment in which they were exposed, demonstrating that radiation exposure can produce a conditioned spatial avoidance behavior.

Arbit (1959a) has published confirmation of radiation-induced spatial avoidance behavior in the rat and has found that autonomic blocking drugs (tetraethylammonium and hexamethonium) did not interfere with acquisition of the conditioned behavior (Arbit, 1959b). Logie *et al.* (1959) reported that, in the absence of differential cues, intact male rats will spend less time

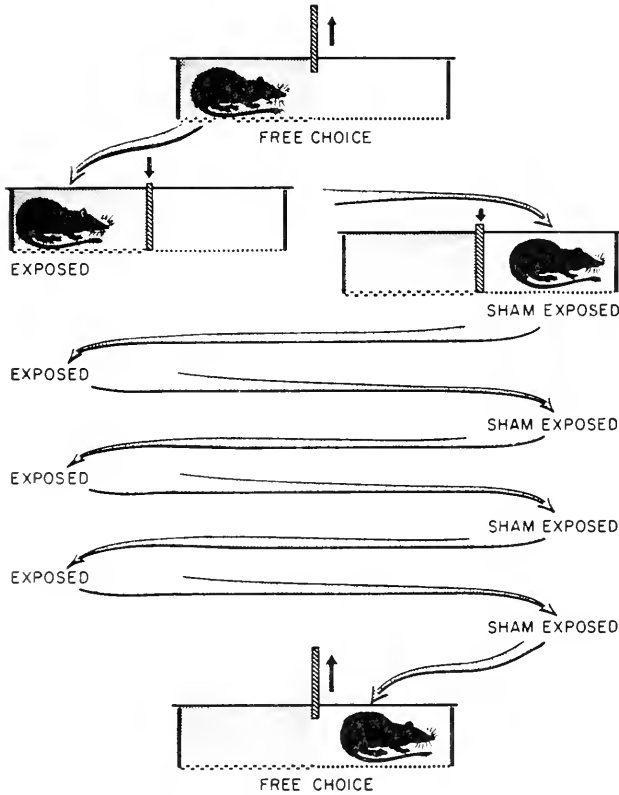


FIG. 1. Schematic design for spatial avoidance conditioning.

in that portion of an alley which is exposed to radiation than in the shielded end. Andrews and Cameron (1960) demonstrated similar avoidance behavior in the mouse.

The search for sensory avenues through which radiation might operate as a stimulus has been undertaken (Table V). It is known that under certain conditions ionizing radiation can act as a visual stimulus through a phosphene effect (Lipetz, 1955); however, we found that a radiation-conditioned aversion to saccharin can be established in bilaterally ophthalmectomized rats (Garcia and Kimeldorf, 1958). It is doubtful that olfactory stimuli associated with radiation exposure lead to conditioned aversive behavior, since the control groups in several saccharin conditioning studies were sham irradiated in the exposure field behind lead shields. Arbit (1959a) was unable to demonstrate spatial avoidance conditioning in sham exposed animals subjected to possible odor cues accompanying radiation exposure.

TABLE V
EXPERIMENTAL CONDITIONS AND SACCHARIN PREFERENCE BEHAVIOR

<i>Test subject</i>	<i>Postirradiation conditioned saccharin aversion</i>	<i>Minimum effective exposure dose used</i>
Intact	present	gamma rays, 10 r
Ophthalmectomized	present	gamma rays, 30 r
Hypophysectomized	present	x-rays, 60 r
Adrenalectomized	present	x-rays, 60 r
Head exposed ^a	present	x-rays, 252 r
Thorax exposed ^a	present	x-rays, 252 r
Abdomen exposed ^a	present	x-rays, 54 r
Pelvis exposed ^a	present	x-rays, 252 r

^a Collimated beam with $\frac{3}{4}$ in. diameter.

Andrews and Cameron (1960), on the basis of controlled ventilation experiments, also concluded that it was unlikely that ozone or oxides of nitrogen provided adequate olfactory cues for radiation avoidance in the mouse, and they emphasized the relative importance of the dose rather than the duration of exposure. Evidence that ionizing radiation causes sensations referable to cutaneous receptor stimulation in mammals does not exist at the dose levels employed with conditioning, although this possibility cannot be dismissed. Saccharin aversion has been produced in hypophysectomized animals and in adrenalectomized animals, indicating that drastic alterations in endocrine state do not interfere with the conditioning process (Garcia and Kimeldorf, 1960b). In an attempt to define a critical site of action for radiation exposure in the conditioning framework, a collimated x-ray beam was used to study saccharin avoidance conditioning in rats with localized radiation exposure of the head, thorax, abdomen, or pelvis. While the abdomen proved to be the most sensitive exposure region, conditioning could be instigated by exposure of other regions with higher doses (Garcia and Kimeldorf, 1960b).

The failure to detect a critical locus for exposure or a direct sensory avenue essential to the behavior has led to a consideration of systemic responses to radiation which may serve as internal stimuli to motivate avoidance behavior in the rat.

Very few functional responses have been reported to occur at comparable dose levels in mammals. Relevant information concerns only events which occur during the conditioning phase, i.e., during a brief radiation exposure.

Several criteria, including heart rate, respiration frequency, deep body temperature, and aortic blood pressure, have been investigated in the conscious rat during exposure (Hunt and Kimeldorf, 1960). The changes observed (Kimeldorf and Hunt, 1958) were not coincident with ex-

posure and, therefore, were not involved in motivating behavior during the conditioning phase. One functional disturbance in the conscious rat was found to fulfill the criteria for a response coincident with exposure and sensitive to small doses of radiation. In a study of gastrointestinal function, radiation exposure throughout a transit period of 2.5–20 minutes decreased the gastric transit rate by a factor of at least three with no latent period or minimum effective dose observable (Jones and Kimeldorf, 1959). Conard (1951) has reported an intestinal disturbance during exposure, namely an increase in motility with exposure doses of 100 r and more after a latency of approximately 1 minute. However, the relationship between gastrointestinal dysfunction during low dose exposure and conditioning remains obscure since prior adrenalectomy profoundly reduced the gastric retention effect during radiation exposure, but the same operation did not inhibit saccharin conditioning.

In summary, it has been possible to demonstrate that radiation exposure can instigate learning and that stimuli associated with exposure can become determinants of postirradiation behavior in several species of mammals. Thus far, it has not been possible to delineate the perceptual properties of the stimulus in terms of a specific receptor mechanism, endocrine state, or anatomic region whose exposure to radiation is critical for the event. The motivational strength of the radiation stimulus is indicated by the relatively small dose which is sufficient to condition a taste aversion in the rat and by the persistence of the conditioned response.

With the demonstration of radiation-conditioned behavior, there is implied at least a transient alteration in neural function contingent upon the radiation. It has not been established whether neural involvement is the result of direct action of radiation on a component of the nervous system or is the result of neural mediation of radiation effects in other systems. The use of radiation as a stimulus in the conditioning situation provides a tool for eliciting neural activity at radiation dose levels well below those available with other methods and should contribute to the analysis of the role of the nervous system in the radiation syndrome.

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Behavioral and Correlated Hematologic Effects of Sublethal Whole Body Irradiation*

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This study is the second of two sets of experiments dealing with the effects of radiation on primates. The need for radiation studies on animals closer to man than mice, rats, or dogs is obvious and was recognized by the National Institutes of Health when they established a committee on radiation studies to encourage such work.

Both sets of experiments involved single sublethal doses of ionizing radiation, 50–400 r in the first set and 300, 400, and 500 r in the second. At the time the study was planned, much radiation research was directed towards lethal whole-body exposure to x-rays and to higher energy radiations. It seemed to us that low level radiation in single doses had considerable importance for civil defense. For example, in an atomic bomb explosion, the number of persons subjected to low level radiation should equal those exposed to lethal and to severe sublethal doses. While the medical problems of low level irradiation are not grave, for psychologic reasons they might be magnified by fear, panic, and neurosis. Subsequent international concern over atomic bomb testing has shifted interest further towards the effects of low level irradiation.

We deliberately chose relatively simple, lightly motivated types of behavior to measure radiation stress or damage. The reflex approach to assaying stress damage is to seek out or devise tests that call for the highest level of cerebral activity and to insure the highest level of performance by employing strong motivation. Common experience teaches us that man is quite capable of extraordinary feats of strength and endurance in crises. Since strong motivation is required to produce these levels of activity, we may actually be testing

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the effect of radiation on motivation rather than on capacity. This distinction is particularly obvious when food is the motivation.

Many military and civil problems relating to performance under stress depend upon what men do when subjected to long-continued low level stress while engaged in critical, but relatively undemanding, tasks. In both war and peace, man performs for brief periods of excitement and intense demand interspersed with long stretches of monotony and low level demand. We believe that there is a wide gap between what men *can* do and what men *will* do; the latter may be more sensitive to stress and may be as important to study as what man can do.

In choosing low levels of radiation and lightly motivated behavior, we expected to encounter and did encounter difficulty from individual variation. A weak effect is obviously harder to establish than a strong effect. In the first series, the radiation levels were even lower than we had intended. This occurred because the available studies designed to determine the lethality curve for the macaque monkey included tubercular monkeys and gave an LD/50/30, which was too low. Our monkeys, free of tuberculosis and well nourished, were considerably more resistant than the normative data led us to predict.

Method

Tests used in the first series of experiments were described previously (Leary, 1955; Leary and Ruch, 1955). The psychologic and physiologic functions studied were: (1) lightly motivated performance tests, such as the puzzle manipulation of Harlow *et al.* (1950) and the plastic pedometer sandwich devised by Leary and Ruch (1955); (2) routine cage activity, spontaneous activity, and food and water intake; and (3) hematologic values.

Sixteen immature male *Macaca mulatta* were divided into 4 groups of 4 each. In each group, 3 monkeys received radiation doses of 300, 400, and 500 r, respectively; a control monkey was not irradiated but was subjected to all other experimental procedures, including rotation before x-ray machine. The monkeys were tuberculin tested before and after a 30 day isolation period, and, by these tests and by general observation, they appeared to be in good health when the experiment began. No illness attributable to intercurrent infection occurred during the experimental period, and, excepting one monkey which died of generalized septicemia during the 11th postirradiation week, the animals remained in good health for months after the close of the experiment.

The monkeys lived and were tested in expanded metal cages, 18 × 24 × 22 in., with double screen bottoms. A false floor of large mesh was inserted

3 in. above the regular cage floor at feeding time. Uneaten food fell through this coarse mesh to the regular cage floor, from which it was collected for weighing. A fine copper screen inserted between the floor and the bottom of the cage insured collection of all rejected food. A Kennard-Ruch-Fulton watering device was attached to each cage, with graduated reservoirs so that water use could be accurately determined.

The monkeys were placed in these cages at least 4 weeks before preirradiation data were recorded. All measurement procedures except blood sampling were carried out during this conditioning period. Data were gathered for 1 week before and 6 weeks after irradiation.

FOOD AND WATER INTAKE

Every morning the monkeys received one-fourth of an apple, one-fourth of an orange, and half of a carrot, and these were usually entirely consumed. In the afternoon, more Purina dog chow than the monkey would eat was left in the cage for 1½ hours. This diet was supplemented periodically with cod liver oil on a lump of sugar. To remove any moisture from the chow, it was placed in a drying oven at 68° C for 24 hours. The remains of the daily ration recovered from the food cup and the fine screen were also dried for 24 hours. The difference in weight between the dried daily rations and the dried remains was considered the animal's food intake for the day.

Since playful splashing can obscure actual intake, water was available to the monkeys only during three 15-minute periods—at the beginning of the feeding period (after 22½ hours of deprivation), in the middle of the feeding period, and at its end. Since satiation was apparently approached during the first period and splashing occurred during the last two periods, the water intake of only the first periods was considered.

ACTIVITY AND MANIPULATION

The method of activity measurement was selected after studying four methods with respect to validity and reliability (Fig. 1, see also Isaac and Ruch, 1956). The electric eye method was chosen on the basis of convenience. Briefly, an infrared light beam bisected the length of the living cage. When this beam was broken, a photoelectric cell was activated to drive a digital counter located in another room. The counters were photographed every 20 minutes throughout the day and night with a Grass kymograph camera.

Pedometers encased in clear plastic were left in the cages except during the afternoon feeding period. When the monkey handled the pedometer in such a way that it was jiggled along its vertical axis, the counting dials were activated. The pedometer scores were recorded once a day and are an

ITEM	RATING	WORK ADDERS	ELECTRIC EYE	OSCIL- LATION
Rating	.976	.968	.946	.849
Work adders		.932	.928	.838
Electric eye			.993	.789
Oscillation				.993

FIG. 1. The coefficients of correlation ($N = 19$) on the diagonal measure the reliability (odd versus even minutes). The top row of coefficients can be construed as measures of validity, if the ratings by humans are taken as the validating measure. The electric eye method yields a reliability coefficient of .948 for odd versus even days over a 44 day period.

index of the monkey's interest in manipulating the objects of his environment.

IRRADIATION

For exposure to radiation, a monkey was placed in a Plexiglas cylinder whose walls were $\frac{1}{4}$ in. thick and were perforated at about 3 in. intervals by $\frac{1}{2}$ in. ventilation holes. The tube was 16 in. long, and, depending on the size of the animal, was 6 or 8 in. in diameter. The bottom of the cylinder was fixed, but the top could be adjusted to accommodate monkeys of different sizes. The size of the cylinder greatly restricted the monkey's movements, as did the darkening of the room during irradiation.

The tube was suspended by strings from a metal shaft that was rotated at 1 rpm by a synchronous motor. To reduce "back scatter," the metal shaft was made narrow and was never closer than 8 in. to the monkey. Air dosage was calibrated with a Victoreen meter before and at the end of each day's run. The Victoreen meter had been recently calibrated and, on several days, was checked against a second meter. At the point corresponding to the center of the animal's body, the rate of irradiation varied between 8.7 and 8.9 r per minute. (For the source distance of 4 ft. used in these experiments, a 5% fall-off at the upper and lower extremes of the body is expected.)

The x-rays were generated by a Picker pulsating therapy generator using a Machlett FCX tube with a tungsten target. A constant kilovoltage of 200 was used at a constant current of 20 ma. The effective filtration was about 0.50 mm Cu and 2.0 mm Al.

ANALYSIS OF THE DATA

In an attempt to counter individual variability, the weekly postirradiation scores of each animal were subtracted from its preirradiation score. The

differences between the 3 experimental groups were determined by analysis of variance with respect to the preirradiation and postirradiation differences. If the F-ratio obtained was not statistically significant, the data from all experimental animals were combined and were compared with the scores of the control group by means of a t-test. When a significant F-ratio was obtained, the means of the separate groups were compared with the control group by means of t-tests.

Results

FOOD AND WATER INTAKE

Determined in somewhat different ways, the results of our two series are essentially similar. Figure 2 shows the results for 300 and 400 r in the first series. This study indicated that the threshold for anorexia lies somewhere below 300 r and perhaps above 100 r. The second finding was unexpected, because food intake was lowest on irradiation day and lower in the 1st week than in subsequent weeks.

In the second series, (Fig. 3), although the curves fall in a logical order, statistically significant changes in food intake related to dosage level could not be established with 4 animals in each dosage group. However, the 3 experimental groups collectively ate less than the control groups during the 1st postirradiation week and this difference was significant at the 1% level of confidence.

The early onset of anorexia noted in the first study is thus confirmed and again the data suggest that the threshold for anorexia is below 300 r.

The early changes are maximal. In fact, in the following 5 weekly periods, food consumption of the combined experimental groups did not differ significantly from that of the control group. There is, however, a second, though less severe, reduction in food intake during the 3rd postirradiation week that occurs in the groups exposed to 400 r and to 500 r.

Water intake roughly paralleled food intake, being maximally reduced in the 1st postirradiation week. The difference between the mean for the combined experimental group and the control mean was significant at the 5% level of confidence during the 1st week. The difference for the subsequent weekly periods was not significant, but the decrease over the entire postirradiation period was significant at the 1% level of confidence.

ACTIVITY

In our first series of experiments, activity was recorded objectively by a strain-gauge penwriter and subjectively by human raters. The objective method indicated some reduction of activity in the first postirradiation day

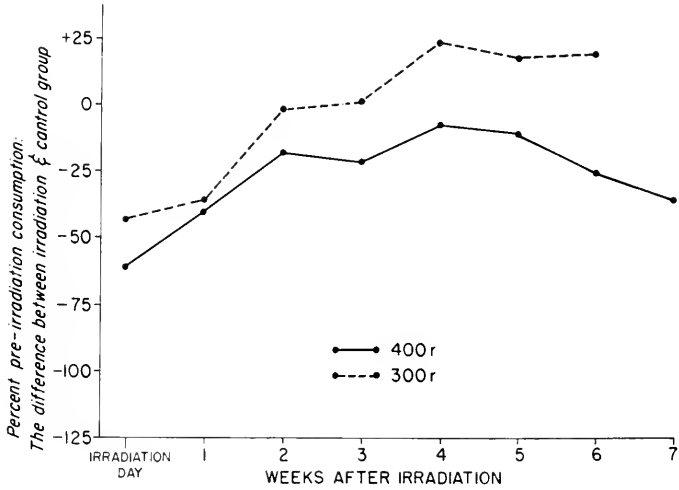


FIG. 2. Food consumption of the two most heavily radiated groups in the first series of experiments.

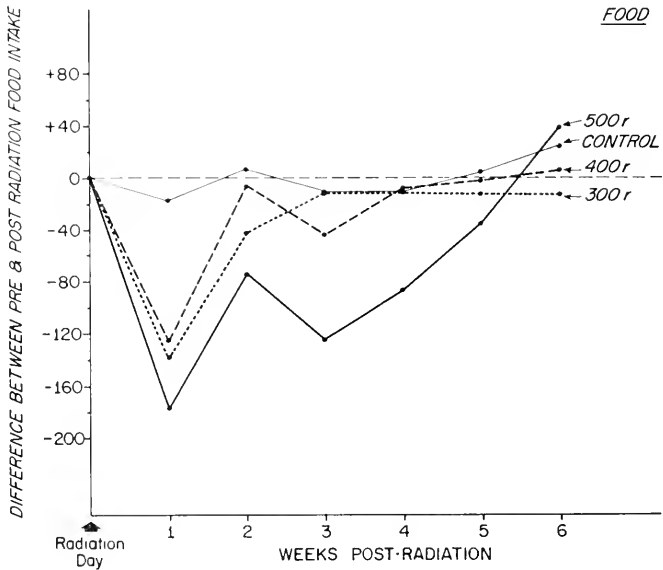


FIG. 3. Food consumption in the second series of experiments. Ordinate is in difference in grams from preirradiation level for the group. On the first postirradiation day, the average intake for a monkey decreased from 83 to 38.5 gm of chow.

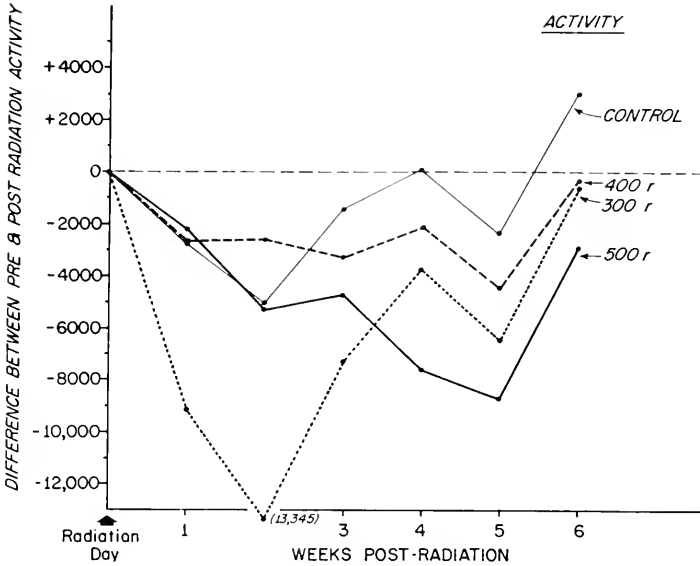


FIG. 4. Spontaneous cage activity. The ordinate is in difference of counts per week from preirradiation level for the group.

and week, but the differences were not statistically significant. By the subjective method, the activity of the 400 r group was significantly lowered on the 1st postirradiation day.

With the electric eye method of recording cage activity used in the second series of experiments (Fig. 4), we were unable to confirm the early post-irradiation change suggested by the first series. There was no immediate decrease in the cage activity of the experimental animals significantly greater than that shown by the controls. However, during the 3rd and 4th post-irradiation weeks, the experimental animals as a group were less active than the control group (5% level of confidence). The activity for the total post-irradiation period did not differ significantly from that of the control animals.

It is pertinent that the electric eye method, after a number of small changes designed to reduce noise and social interaction (and to give greater automation by the use of print-out counters), has proved quite sensitive to such moderate environmental parameters as light and sound level, and ambient warmth and cold (Devito and Smith, 1959; Isaac and Devito, 1958).

OBJECT MANIPULATION

The data obtained from the pedometers yielded no significant difference between the experimental groups in 5 of the 6 postirradiation weeks, largely

because manipulation by the most heavily radiated animals increased greatly. This increase does not seem reasonable in the light of the scores of the other experimental groups, nor does it agree with the data gathered by Leary and Ruch (1955). While this difference may be real, it is regarded with suspicion.

BLOOD CELLS

Anticipating more striking changes in behavior and wishing to make a start at analyzing their cause, we observed the blood picture of the animals intercurrently with behavior testing. Comparatively little was then known of the hematologic effects of sublethal radiation in monkeys (see French *et al.*, 1955). Blood samples were taken 3 or 4 days before the radiation day, on that day, 3 days later, and thereafter at weekly intervals for 9 weeks.

The white blood cell count dropped 24 hours after radiation (Fig. 5), but the difference between irradiated and control animals was not statistically significant. By the 3rd day after irradiation, the mean for all radiated animals was significantly lower than for the control group. This difference was maintained until 28 days after radiation. At that time, there was a statistically significant differential recovery rate among the irradiated groups. The animals that had received the largest dosage of radiation were fastest in recovering, and those that had received the smallest dosage were slowest. Leucophilia was apparent during the next 3 weeks. At 42 days, the mean of the combined experimental groups was significantly higher than that of

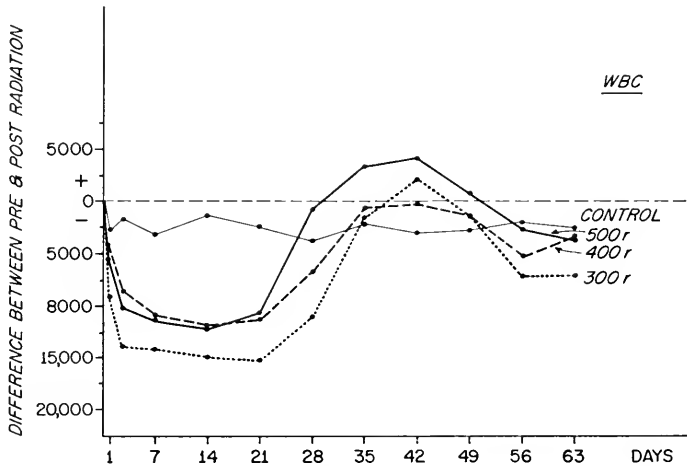


FIG. 5. White blood cells before and after irradiation.

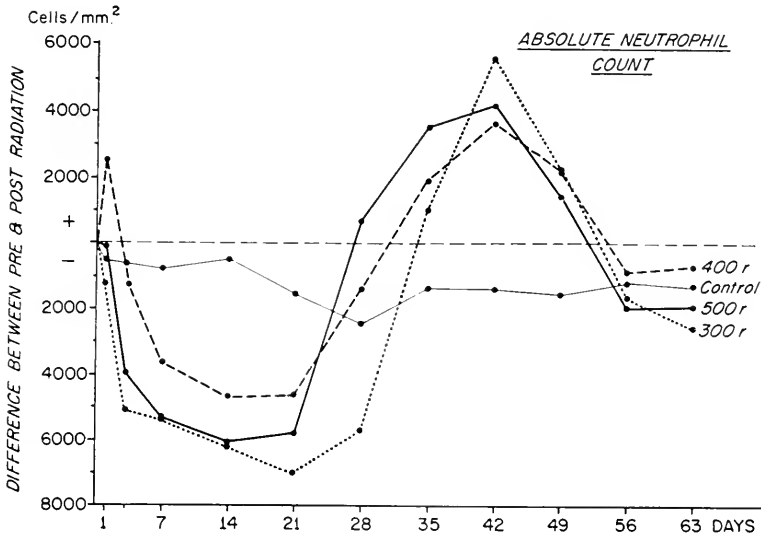


FIG. 6. Neutrophils before and after irradiation. The preirradiation values in cells per 100 ml of blood were: control group, 6,389; 300 r, 7,623; 400 r, 5,280; and 500 r, 6,751.

the control group (1% level of confidence). This phase was followed by a return to the normal range.

The number of circulating neutrophils (Fig. 6) decreased on the 1st irradiation day, the value undoubtedly representing the sum of a rise and a fall shown by French and his co-workers (1955) to occur in the first 24 hours. The mean of the combined experimental groups was significantly below that of the control group at the end of the 1st week and remained so during the 2nd and 3rd postirradiation weeks. Recovery then commenced. A differential rate of recovery among the groups was apparent but was not statistically significant. Again the most heavily irradiated group were fastest in recovering, and the least irradiated group were slowest. By the 5th post-irradiation week, a neutrophilia was apparent. The mean of the combined irradiated groups was significantly above the control group at 5, 6, and 7 weeks after radiation. Values for the irradiated animals fell within the normal range during the 8th and 9th postirradiation weeks.

Twenty-four hours after irradiation, the number of circulating lymphocytes had decreased, and the mean of each experimental group was significantly lower than that for the control group (Fig. 7). The variance among the means of the experimental groups was also significant. The findings at 3, 7, and 21 days after radiation were similar. At 14 days after radiation, the variance among the means of the radiated groups was significant, but

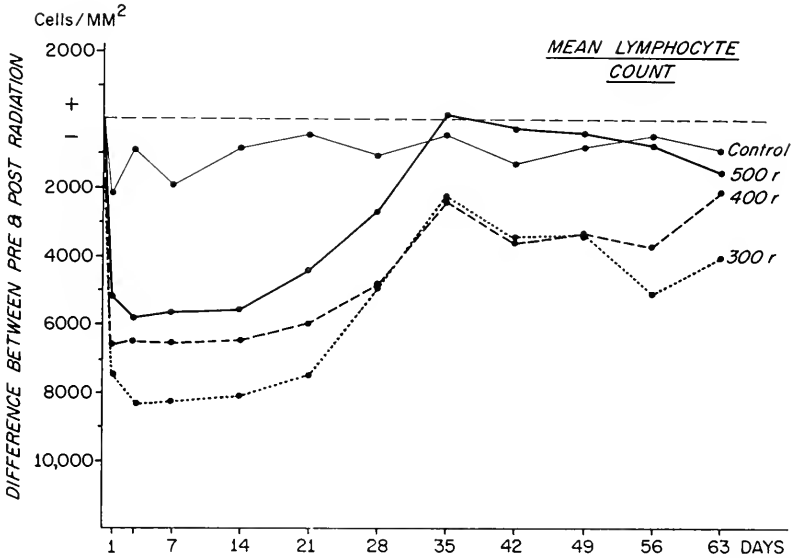


FIG. 7. Lymphocyte counts before and after irradiation. The preirradiation values were: control group, 8,370; 300 r, 9,266; 400 r, 7,328; and 500 r, 6,380.

the mean of the 500 r group did not differ significantly from that of the control group. At 28 days after irradiation, the variance among the group means was not significant, but the combined experimental group mean differed significantly from the mean of the control group. Thereafter, the experimental groups were in the normal range.

The response of the red blood cells (Fig. 8) was prompt, their number decreasing significantly in 24 hours, and some decrease was sustained throughout most of the study. Even at 28 days, the mean for the 300 r group was significantly different from the control means. After 5 weeks, the 400 and 500 r groups still differed significantly from the control group, and in the 500 r group a significant difference persisted after 6 weeks. The decrease in hemoglobin content was somewhat more gradual than the decrease in the red blood cells but followed a similar course (Fig. 9), reaching a nadir at 21 days. The mean of the combined group differed significantly from the mean of the control group 2, 3, and 4 weeks after radiation.

Discussion

Whereas in our first series, the radiation levels (50, 100, 200, and 300 r) were too low, the 300, 400, 500 r series seem to bracket the threshold for changes in activity and food and water intake. However, although 16

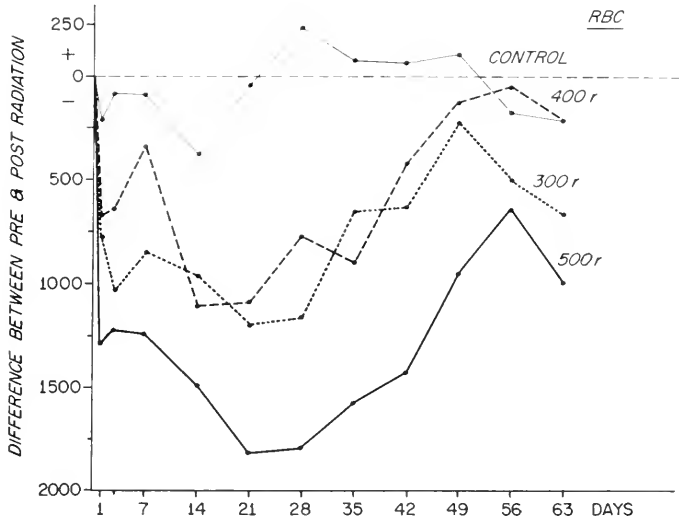


FIG. 8. Red blood cells before and after radiation. The preirradiation count for the control group was 5,602,000; for the 300 r group, 5,980,000; for the 400 r group, 5,560,000; and for the 500 r group 6,250,000.

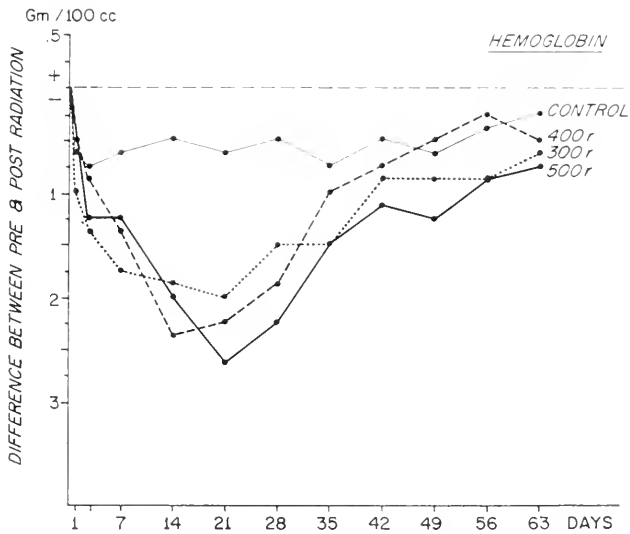


FIG. 9. Hemoglobin. The preirradiation levels for the four groups ranged between 14.25 and 14.60 gm per ml of blood.

disease-free animals were used in the second series of experiments, this number was not sufficient to establish statistically significant differences in the weekly means for individual dosage levels for many of the responses tested. Combined data for all groups seems to provide clear evidence for a decrease in food and water intake in the early postirradiation period and a decrease in activity during the 2nd and 3rd weeks. The early decrease in food intake was not expected from the work of other investigators. For example, Eldred and Trowbridge (1954), in referring to dosages in the lethal range, speak of a "symptom-free period following irradiation, in which almost all animals had undiminished appetite."

These monkeys, unlike several groups which have been used in radiation studies (including that on which the available LD/50 is based), were free of tuberculosis and well nourished. There were no visible signs that would enable an observer to pick out the irradiated animals. In 9 animals at 400 r and in all 4 monkeys at 500 r, there were no deaths and no visible pathologic alterations within the experimental period.

While there is still no published statistically reliable figure for the LD/50/30 for the *Macaca mulatta* monkey, the early estimate of Eldred and Trowbridge—600 r—is probably reasonably correct. The 400–500 r level must therefore be considered a just sublethal dosage.

The observed changes in activity and food and water intake are definite, but are not striking relative to individual variability. The number of red blood cells and the amount of hemoglobin reach their lowest point at about the time that hypoactivity is most pronounced. The reduction in red blood cells by one third is somewhat less than that producing subjective signs in man, but the subjective human and objective simian data do not warrant close comparison, and the rate of onset is a factor. Our data suggest that hematologic changes need to be ruled out as an indirect cause of behavioral alterations before the nervous system is implicated as a primary cause. Whether the blood changes are in the right direction and sufficiently rapid and pronounced to explain the food and water intake data, is doubtful. It is interesting to note that the observed anorexia does not fall in the period of pronounced gastrointestinal pathologic alteration. This fact suggests that anorexia may result from a primary effect of radiation on the nervous system.

Summary and Conclusions

Behavioral and hematologic effects of single dose, whole body irradiation in the sublethal range (300–500 r) were studied. No deaths or visible pathologic lesions were produced within the experimental period (63 days).

- Food and water intake was depressed in the immediate postirradiation period.
- Random cage activity was depressed 2 or 3 weeks after irradiation.
- The results of a test of object manipulation were equivocal.
- The number of red blood cells and the amount of hemoglobin dropped rather precipitously and remained depressed, to some degree, for weeks.
- Total leucocytes, neutrophils, and lymphocytes were markedly altered at this level of radiation damage. A distinctive sequence of increase and decrease for each cellular component occurred. A late neutrophilia was manifested 6 to 7 weeks after irradiation. Its degree appeared to be inversely proportional to the decrease in the preceding weeks and directly proportional to the radiation dosage.

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Performance of Monkeys before and after Irradiation to the Head with X-Rays*

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This investigation sought to determine the effects of large focal doses of x-irradiation to the heads of monkeys and is similar in design to three prior investigations on the effects of whole body irradiation on behavior (Davis *et al.*, 1956, 1958; McDowell *et al.*, 1956). The studies explored a wide spectrum of performance to sort out relevant variables.

Harlow (1953) described two distinct syndromes that result from damaging the cerebral cortex of monkeys: the frontal lobe syndrome, which results from bilateral lesions of the frontal lobes, and the posterior association area syndrome, which follows bilateral lesions of the inferior parietal lobule and the lateral surface of the temporal lobe. The former syndrome is characterized by a permanent deficit in performance on delayed response problems and a significant, but not permanent, deficit in performance on patterned string, oddity, and double alternation problems. The subjects with frontal lobe lesions frequently refused to work and were inattentive and hyperactive.

The performance of monkeys with the posterior association area syndrome was permanently impaired on discriminations of form, color, and size of patterns, but temporarily impaired on discriminations between objects. These monkeys were able to solve delayed response problems nearly as well as subjects that did not have brain operations, and monkeys with bilateral lesions of the frontal lobes were nearly as proficient with discrimination problems as controls.

* These experiments were conducted at the Radiobiological Laboratory of the University of Texas and the U. S. Air Force, Austin, Texas, and were supported with funds provided by the U. S. Air Force and the School of Aviation Medicine. The authors were aided in describing technical details by Lorin Logie, Capt., USAF, and Dr. Sidney Kent, University of Alabama Medical School.

The syndrome that results from whole body radiation of monkeys is totally dissimilar to those produced by surgical lesions of the brain. Immediate and delayed effects of whole body radiation include changes in relative amounts of self care, unrestricted manipulation and social behavior (McDowell *et al.*, 1956), lowered distractibility, lessening of visual acuity, change in posture (Davis *et al.*, 1958), focalization of attention (McDowell, 1958), and changes in preferences for foods (Leary, 1955; Davis, 1958a).

There appears to be great dissimilarity between results obtained in the Soviet Union and in this country in regard to radiation sensitivity of the central nervous system. Several Soviet investigators have reported changes in conditioned responses of laboratory animals following radiation to the head with relatively low doses (Stahl, 1959). In this country Blair and Arnold (1956) found differences in maze running between nonirradiated rats and those irradiated in the head with high doses of x-rays. Harlow (1958) obtained transient, but marked, deficits in the performance of monkeys on complex learning tasks by implanting needles of Co⁶⁰ in the cerebral cortexes of monkeys. The needles impart a high local dose.

Methods

Subjects were 27 male rhesus monkeys given preliminary training, then randomly sorted into three head-irradiated groups and one nonirradiated group. The first experimental group, seven monkeys designated *A*, was given two 3,000 r doses of x-radiation to the frontal lobes; the second group, six monkeys designated *P*, received a comparable dose of radiation to an area of the head lying over the inferior parietal lobule and the posterior aspect of the temporal lobe; the third group, designated *AP*, was treated with the same doses over both the anterior and posterior foci.

Three patterns of radiation were employed. The first pattern, used with group *A*, was designed to irradiate the anterior portion of the frontal lobes. The pattern, 2.2 cm wide, extended inferiorly to a line horizontal to the corner of the eye. The intersection of the anterior line of the pattern with the superior midline lay 2.1 cm dorsal to the supraorbital protuberance.

The second pattern of irradiation, used with the *P* group, was designed to irradiate the posterior association areas. This focus was 0.9 cm wide at the apex and 2.0 cm wide at the base. The angle of the sloping inferior line was determined by extending a line from the corner of the eye parallel to the zygomatic arch. The posterior line of this focus was fixed by measuring 2.5 cm rostral to the anterior aspect of the occipital protuberance.

The *AP* group received both patterns of irradiation.

Dimensions of the patterns and their relationships to external landmarks

were determined by halving the head of one monkey midsagittally. The areas of radiation were identified, and holes that marked the corners of the patterns were bored from the interior surface of the skull, through the skull and through the skin. The external boundaries were drawn by connecting the points made by the drilled holes. Each subject was prepared for irradiation individually, and slight compensations were made for structural variations of individual skulls.

The radiation source was a self-rectifying half-wave 260 kvp, 18 ma Picker therapeutic x-ray machine with an inherent filtration of 0.25 mm Cu plus 2 mm Al. The following radiation factors were employed: 1 mm Al and 0.25 mm Cu filtration added, voltage of 250 kvp, and current of 18 ma. Prior to irradiation, each monkey was intravenously given 14 mg of sodium phenobarbital per kg of body weight, and almost instantaneously went into surgical anesthesia. The monkey was placed on its back at right angles to and 100 cm from the x-ray beam. Its body was shielded by two 0.25 in. lead sheets placed at target-to-object distances of 80 to 87 cm. The x-ray beam was collimated by directing it through rectangular holes in the lead sheets. The size of the collimated beam at the exposure distance (100 cm) was checked several times with film and was found to correspond to the size of the desired pattern. The head was propped so that the beam completely covered the area of the desired drawn pattern. The boundaries of the pattern were further limited by 0.25 in. thick caps fashioned from lead sheeting, cut to the particular pattern, and fitted over the head. The dose rate measured with an NBS calibrated 25 r Victoreen Chamber was 20.5 r per min. The dose rate measured with an Air Force water-equivalent chemical dosimeter was 21.8 r per min. The dose rate measured by the Air Force chemical dosimeters was chosen, since this dosimeter was standard for the Radiobiological Laboratory.

Two periods of irradiation were preceded and separated by training. During 6 months of preliminary training, the animals became proficient in doing eight selected laboratory tasks, six given in the Wisconsin General Test Apparatus, (WGTA) (Harlow and Settlage, 1948), requiring the selection of objects.

After 12 days of massed practice on all eight tasks, each subject was anesthetized and restrained in the radiation chamber. The experimental animals were irradiated with 3,000 r x-radiation. Controls were sham irradiated. On the day following treatment, all were returned to the laboratory and trained during each of the following 27 days. Those in the experimental groups were irradiated with a second similar 3,000 r dose 28 to 30 days after the first irradiation. Following the second irradiation, they were trained during 27 days spaced over 38 calendar days.

Measurement of Performance

OBJECT-QUALITY DISCRIMINATION

Preliminary training

Animals were trained 60 days on 4-trial object-quality discrimination problems in the WGTA. Twelve problems were given to each animal on each day. Discrimination stimuli consisted of pairs of commonly used, randomly assorted dissimilar objects. Each problem required the selection of one of the two objects which shifted in left and right positions in a predetermined random order. One object was arbitrarily correct and was placed over a foodwell containing a raisin; the other object had no reward. A new pair of objects was employed on every four trials.

The learning during preliminary training was comparable to that previously reported for subjects given training on 4-trial object-quality discriminations.

Radiation schedule training

Each subject received six 4-trial discrimination problems a day on each of the 12 days prior to the first irradiation and during each of the 27 days of training following the first and second periods of irradiation. The discrimination problems given after irradiation differed in one important respect from those presented prior to irradiation. Half of the problems given each day consisted of planometric patterns composed of three pairs of pictures selected at random from magazines and pasted on 2 × 2 in. fiberboard squares. The other three pairs were similar to those used daily prior to irradiation.

A detailed analysis of the data, including a breakdown of the various categories of errors outlined by Harlow (1950), indicated no significant trends between groups. One significant trend within a group was a decrease in differential-cue errors made by the *P* group on trial 4 of problems presented following the first radiation. Three parallel analyses, each involving 36 comparisons, were made between the performance of subjects in a particular group on trials 2, 3, and 4 in the preradiation and two postradiation periods, and the occurrence of one significant difference is less than would be expected by chance.

BENT WIRE DETOUR PROBLEM

Bent wire problems occur in various patterns, from a simple straight segment of 0.187 in. welding rod 3.0 in. long to four segments separated by 90°

bends in the wire. The patterns are constructed in pairs and grasped in the center by a vise. A reward, a candy Life Saver or a paper poker chip punched through the center, is threaded onto the pattern, and the subject solves the problem by removing the reward.

Preliminary training

Subjects were trained for 16 days to remove poker chips and Life Savers from simple one segment patterns and for 19 additional days on variations of the one segment problem (Davis, 1958b, experiment III).

Radiation schedule training

Each subject was trained with five bent wire problems during each of the 66 days of training before, between, and after irradiation. Pairs of two segment bent-wire problems were employed in each trial, with two candy Life Savers as rewards, one on either side of the jaws of the vise. Patterns with two segments, one paralleling the bars of the restraining cage, the other extending toward the subject, were used during the 12 days of training prior to irradiation. During the period between the first and second irradiation and following the second irradiation, the second segment extended away from the subject rather than toward it (Davis, 1958a; Davis *et al.*, 1958).

Subjects in the four groups were less well matched on detour problems than on any other problems. During the 12 day preirradiation period, the *P* group failed significantly more often than the control group, $p < .05$ using the U-test. For this reason, we regarded comparisons between groups as invalid and only compared performance within particular groups between periods of training.

The change in difficulty of patterns after the first irradiation produced significantly more failures in the control group. This trend was not significant in the irradiated groups. Performance of the control group became significantly worse after the second irradiation than during the intrainradiation period. The irradiated subjects followed a similar, but not significant, trend.

PATTERNED STRING TESTS

Patterned string tests are among the oldest and most frequently employed tests of "animal intelligence" (Harlow, 1934). They consist of two or more strings or chains arranged in patterns. Subjects must select the arbitrarily correct string and draw it in to obtain the reward attached to the end.

Preliminary training was not given prior to the 12 days preceding irradiation.

Radiation schedule training

Cross strings, box pattern, and angle cross patterns were presented twice a day to each subject on all 12 days preceding irradiation. Thereafter, each was trained once a day on parallel strings, double cross, and pseudocross patterns, in addition to the three preirradiation patterns.

No significant changes were attributable to radiation effects.

THE ELEVATOR DETOUR PROBLEM

The elevator detour problem of McDowell and Nissen (1959) differs from many previously reported in that it requires subjects to sustain and coordinate the use of both hands during problem solution. The apparatus consisted of an elevator and a ladder. The elevator was a wooden box covered with Plexiglas, with the interior forming a shaft 18 in. high and 2 in. wide. A small plastic elevator was mounted on two steel vertical rods and traveled freely up and down the shaft. A stylus was attached to the front of the elevator. The Plexiglas front was slotted so that subjects could raise the elevator in the shaft to get a piece of candy resting on the top of the elevator. A vertical ladder was mounted on the table of the WGTA 5.0 in. in front of the restraining cage and 2 in. in front of the elevator. The monkey had to reach through the rungs of the ladder to move the stylus. It could view the candy through the Plexiglas as it raised the elevator, and, when the elevator reached the top of the shaft, could procure the candy. Since the elevator dropped if released, both hands had to be coordinated to pass the stylus by each of the rungs of the ladder.

Preliminary training

The shaft without the ladder was presented to the subject with the elevator in three starting positions, 4, 8, and 12 in. from the top of the shaft, until the subject could procure the candy five successive times at each position. The ladder was then placed between the subject and the elevator shaft during 10 days of training. Nine trials were given each day, three in each starting position. The dependent variable was the latency of approach between presentation of the problem and subjects' response. If the subject failed to approach the problem within 60 sec after it was presented, the trial was called a failure-without-trying. If it tried to procure the candy within 60 sec, but failed, the trial was called a failure-with-trying.

Radiation schedule training

The subjects received training with the ladder in place during the 12 days before the first irradiation or sham irradiation. Training continued during

the 27 day intrainradiation period and the 27 days following the second exposure. At the end of the training that followed the second exposure, the problem was presented in a more difficult form. Additional rungs were inserted an equal distance between each of the four rungs of the ladder, and subjects were given two 10 day periods of practice separated by 10 days of rest.

Figure 1 shows the efficiency of performance relative to performance during the 12 days preceding irradiation.

The results indicated a significant progressive drop in performance of the *AP* group after irradiation. None of the other trends were significant. Analysis of the approach latency data indicated that the *P* group improved signifi-

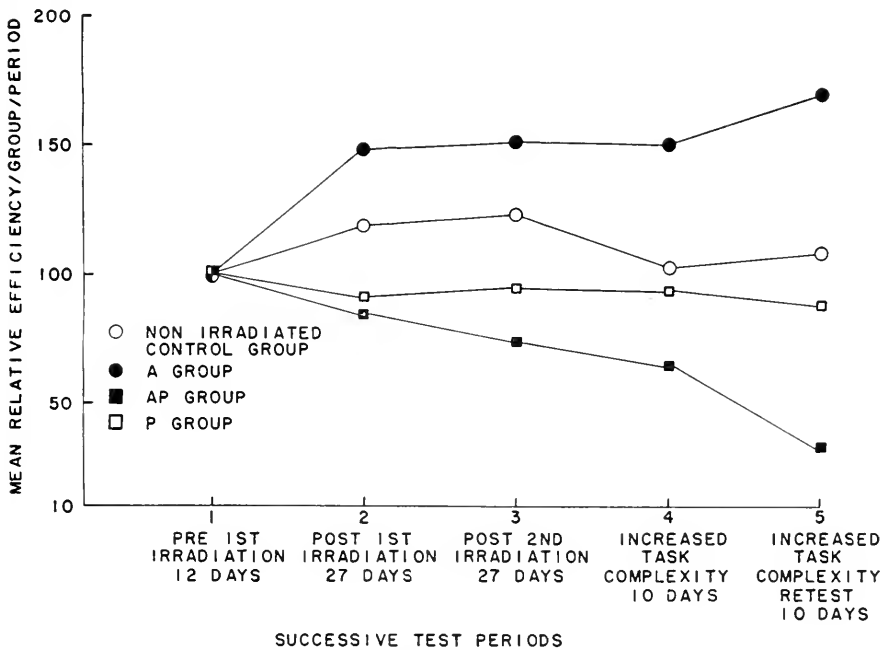


FIG. 1. Efficiency of performance on elevator problem.

cantly, and that the control group approached the problem significantly more slowly following radiation than prior to radiation.

FOOD PREFERENCES

Harlow and Meyer (1952) showed that the preferences of monkeys for foods are stable and can be scaled. Leary (1955) found significant changes

in preferences immediately after whole body radiation, and Davis (1958a) reported significant differences between food preferences of nonirradiated monkeys and those who had received whole body radiation with a mixed source and had survived 14 months.

The free choice method was used in this experiment (Davis, 1958a) in the WGTA. A board, 28 × 9 in., containing 125 food wells, was placed in the stimulus tray carrier. The wells were arranged in 5 rows of 25 columns and held small pieces of raisins, apples, bread, potatoes, and celery, assigned in predetermined random order.

No training preceded that given during the 12 days prior to irradiation.

Radiation schedule training

Each subject was given one trial on each of the 12 days preceding irradiation and on each of the remaining 54 days of the experiment. The tray was loaded with 125 pieces of food; the opaque screen of the WGTA was raised and the subject was allowed to select 40 pieces of food.

The preferences before irradiation were grossly comparable to those obtained by other workers, although direct comparisons are impossible since subjects were allowed only 40 instead of 60 pieces of food on each trial. The order of preference was raisins, apples, bread, potatoes, and celery. Differences between preferences for food were significant, except between raisins and apples.

No significant changes in preferences could be attributed to irradiation.

SYSTEMATIC OBSERVATIONS

McDowell *et al.* (1956) studied the behavior of monkeys in their living cages before and after whole body radiation with 400 r x-rays and contrasted their performance with that of sham irradiated monkeys. Their technique was notably different from that used in naturalistic observations, because they maintained continuous recording and included all behavior that was directed toward objects or represented movement. They reported that whole body radiation produced an over all decline in activity and general malaise, with a significant decrease in the *relative* frequency of self care and a significant decrease in the *relative* instance of initiation of aggression.

More recently, Hammack (1960) described the syndrome resulting from nitrogen mustard poisoning. In eight categories of behavior (McDowell *et al.*, 1956), the syndromes resulting from whole body radiation and nitrogen mustard poisoning were similar, except in time of onset and duration.

No observations were made before the experiment began.

Radiation Schedule

Subjects were placed in a $3 \times 3 \times 3$ ft cage of steel bars located in the center of an octagonal room. Four sides of the octagon were permanent walls, and four contained one-way vision windows.

Each subject was observed simultaneously by four investigators during 5 days prior to irradiation and by two investigators during 5 days following radiation, and selected subjects were observed clinically during subsequent days.

One observer made verbal recordings similar to those made by McDowell *et al.* (1956). Another recorded preselected activities by punching keys of a specially constructed console similar to that of Hammack (1960). The third and fourth observers recorded selected single aspects of behavior when they occurred.

The key-punched activities were manipulatory (manipulation of the cage, puzzle, and self); visually directed behavior (general visual survey, visual regard of the cage, puzzle, and self, and visual regard to sounds); and postural behavior (cage shaking, bouncing, pacing, shifts in location, resting, and body orientation to uncontrolled noise from the laboratory and to controlled hammering on the wall in a predetermined random rhythm).

The subjects engaged in approximately four activities each minute. They spent most of the time surveying the sterile and unfamiliar environment, picking at and inspecting the cage, and pacing. Table I compares our data with data obtained by McDowell *et al.* (1956), who observed pairs of monkeys in their living cages. The most prominent category in their study

TABLE I

A COMPARISON OF THE PERFORMANCE OF MONKEYS IN
TWO STUDIES WHICH EMPLOYED SYSTEMATIC OBSERVATIONS

<i>Category</i>	<i>Percentages from earlier study (McDowell et al., 1956)</i>	<i>Percentages from present study</i>
Self involved	13.7 ^a	4.6
Rapid energy expenditure	6.6	18.8
Visual survey	15.2	42.8
Inanimate object	26.4	33.8
Other animal	38.0	—

^a Values in both studies are computed without including shifts in location. Inanimate object includes looking at as well as manipulation of particular objects.

was other-animal-directed behavior. Visual survey and rapid-energy-expenditure were more frequent in ours. The manipulation and visual regard of inanimate objects were slightly more frequent in our study, but it dropped to 24% of the total behavior during the second 5 days of the experiment, a level almost identical to that attained in their study.

Frequencies of activities are presented in Table II. The most frequently occurring behaviors were highly reliable, and all correlations were significant at or beyond the 5% confidence level. Looking at self, looking toward sounds, orienting to sounds, and resting were so infrequent, they could not be analyzed.

Two changes were attributable to the effects of radiation. The *A* group engaged in significantly more rapid energy expenditure after radiation than before, $t = 3.03$, $p < .05$, and the *AP* group showed significantly more visual survey after radiation than before, $t = 3.90$, $p < .01$.

Two differences were characteristic of all four groups and were probably effects of adaptation to the strange environment. There was a significant decline in the number of times a cage was manipulated and the number of times it was examined visually, $p < .05$ in both comparisons using the sign test.

CHRONIC EFFECTS OF HEAD RADIATION

Table III indicates the subjects that died during the year following irradiation and identifies the focus of irradiation. Three monkeys in the *A* group died between the first and second radiation. Approximately 2 weeks after the experiment, one in the *AP* group died. During the next 150 days the rest of the *AP* group, two in the *A* group, and two in the *P* group died. None in the control group died during the year following the experiment. The clinical pattern was severe, acute motor ataxia occurring approximately in the 48 hours before death. This pattern was followed without variation by the *AP* group.

Although the data are limited, they suggest an immediate cause of death manifest during the 1st week following radiation and a delayed cause evident at an average duration of 144 days after the second radiation.

McDowell and Brown have followed up the original study with investigations of the behavior of subjects that survived. They studied discrimination along a peripheral cue gradient (1960), visual acuity (1960a), oddity reversal and delayed response (McDowell *et al.*, 1961), effects of repetitious work (1960b), and response latency (1960b). Their findings are generally similar to results obtained with surgically brain-damaged monkeys and contrary to the results obtained with large doses of whole body radiation.

TABLE II

THE RELIABILITY OF THE CATEGORIES OBSERVED IN THE PRESENT STUDY AND THE EFFECTS OF RADIATION TO THE HEAD ON PERFORMANCE

Motor component	Categories		Before radiation		After radiation			
	Object involvement	N	Items per min. for each S	rho	Gr. A	Gr. P	Gr. AP	Gr. C
1. Manipulation	a. cage	23	.614	.95	.291	.640	.240	.400
	b. puzzle	26	.310	.92	.177	.260	.220	.529
	c. self	18	.118	.83	.091	.327	.247	.040
2. Looking	a. nonspecific							
	visual survey	28	1.661	.86	1.783	1.613	2.613	1.542
	b. cage	26	.291	.52	.274	.267	.087	.111
	c. puzzle	21	.094	.79	.057	.100	.033	.058
	d. self	12	.056	—	.017	.060	.053	.027
e. sounds	3	.006	—	.000	.006	.040	.000	
3. Body orientation	a. rapid energy expenditure	28	.731	.92	1.411	.653	.367	1.200
	b. shifts in location	22	.108	.48	.086	.060	.070	.062
	c. orientation to sounds	5	.010	—	.017	.020	.000	.000
	d. resting	0	.000	—	.011	.007	.027	.000

TABLE III
MORTALITY DURING FIRST YEAR
AFTER RADIATION

<i>Monkey and group</i>	<i>Survival time (days after radiation)</i>
A-1	radiation 1 + 7
A-2	radiation 1 + 7
A-3	radiation 1 + 26
A-4	radiation 2 + 7
A-5	radiation 2 + 178
A-6	radiation 2 + 198
A-7	Surviving
P-1	radiation 2 + 134
P-2	radiation 2 + 160
P-3-6	Surviving
AP-1	radiation 2 + 53
AP-2	radiation 2 + 95
AP-3	radiation 2 + 120
AP-4	radiation 2 + 145
AP-5	radiation 2 + 146
AP-6	radiation 2 + 210
C-1-9	Surviving

Discussion and Summary

The findings broadly indicate the course of damage to the central nervous system by ionizing radiations, suggest relevant areas for further research, and aid in the understanding of the syndrome that results from whole body radiation.

It is clear that x-ray radiation to parts of the heads of monkeys, in two 3,000 r doses spaced a month apart, does not have the same effect on performance as surgical insult to comparable areas of the brain. Effects similar to those found in brain-injured monkeys do appear, however, among monkeys that survive a year or more. This is in accord with the report by Arnold *et al.* (1954) of long latencies between irradiation and histologic changes in the brain.

The present study indicates that damage from irradiation of the central nervous system is manifest earliest as disturbances of movement, activity, and visual survey. The elevator task requires subjects to make delicate, two-handed, coordinated movements and is the only task to differentiate the performance of subjects in the experimental and control groups.

Subjects given radiation to the frontal lobes showed hyperactivity within

a few days after irradiation. Stahl (1959) indicates that Soviet investigators noted hyperactivity in animals irradiated in the head. Subjects in the AP group have more visual survey after irradiation than before. The present study also provides evidence showing that visual survey is greatly augmented by an unfamiliar environment—our monkeys appeared to be constantly monitoring the setting that contained no monkeys, many investigators, and nothing to hide behind or under.

Reviewing the syndrome that results from whole body radiation with 300–500 r suggests that some of the changes may be due to radiation effects on the central nervous system. The salient features of the syndrome include lowered distractibility, changes in posture (Davis *et al.*, 1958), focalization of attention (McDowell, 1958), relative decrease in initiation of aggression, and relative decrease in manipulation of inanimate objects (McDowell *et al.*, 1956). The authors submit that these changes and the changes which follow radiation to the head in the present study are the type of variation that would be expected if central nervous system integrative functions were impaired. Other facets of the whole body radiation syndrome, such as relative increase in self care (McDowell *et al.*, 1956) and changes in preferences for foods (Leary, 1955; Davis *et al.*, 1958), are probably related to damage of non-nervous tissue.

The present study, together with a recent paper by Biryukov (1957), suggests that differences between the results obtained by Western and Soviet investigators may be less than heretofore supposed. Both studies showed that after irradiation of the central nervous system, impairment of movement is more prominent than impairment of complex learned responses. The design and statistical treatment of data by Soviet physiologists is vague, but possibly the conditioned response is a more sensitive tool for the detection of integrative disturbances of the central nervous system than complex learned behavior.

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Some Behavioral Effects of Ionizing Radiation on Primates*

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For the past eight years, Yerkes Laboratories have been engaged in a survey of the behavioral effects of ionizing radiation in chimpanzees. These studies were begun by Henry W. Nissen and continued under his direction until his death in 1958. They are now being continued in collaboration with C. M. Rogers. Only a small portion of the results are as yet published, and a review of the findings seems appropriate at this time.

The chimpanzees were given 375 or 400 r whole body gamma radiation. Although this dose is less than that usually believed to cause early pathologic changes in the central nervous system, there have been reports of functional changes following irradiation with this amount (Livshits, 1953, reported by Stahl, 1959).

In another investigation, the heads of cynomolgus monkeys (*M. irus*) were irradiated with 2,000 r, a dose which, if given to the whole body, would have killed the animals quickly.

This latter investigation was begun in the hope that it would contribute toward the development of techniques to study the role played by various parts of the brain in the elaboration of certain behavioral patterns, without the artifacts of more conventional methods. Numerous studies of brain functions and behavior made use of the methods of surgical ablation, electrical stimulation, or administration of drugs, most of which reported definite behavioral changes. By eliminating the need for entry into the cranium, the effects of infection, displacement and distortion of tissue, and electrical damage are avoided. Side effects are doubtlessly minimal with irradiation, and the vascular system is left uninterrupted.

In general, the more techniques employed to study the problem, the more surely can we isolate the real from the artifacts, especially where indirect effects are significant. Should behavioral changes be manifested, the sensi-

* This work was performed under contract with the U. S. Atomic Energy Commission.

tivity of the brain to the direct and indirect effects of radiation would be established, thereby permitting quantitative studies of sensitivity.

Chimpanzees

METHOD

Chimpanzees to be irradiated were driven by truck from Orange Park, Florida, to the University of Tennessee Agricultural Experiment Station at Oak Ridge, Tennessee, where they were given 375–400 r of gamma radiation from the Co⁶⁰ facility. Approximately 12 hours were required for irradiation. The animals were then returned to their home cages. During the 51 hours away from the laboratories, they lived in transport cages approximately 2½ ft high, 2 ft wide, and 3 ft long.

Table I shows pertinent facts about the animals and the irradiation. Because of the sequence of irradiation, the animals were not all given identical tests. However, in April, 1959, the irradiated animals were assembled in a single group to compare their performances with appropriate controls. All have been tested continuously since then.

TABLE I
SUMMARY OF IRRADIATION

<i>Name</i>	<i>Sex</i>	<i>Age</i>	<i>Dose</i> (<i>r</i>)	<i>Irradiation date</i>	<i>Remarks</i>
Dolly	F	15 (?)	400	November, 1954	
Pitipie	F	15 (?)	400	November, 1954	Died September, 1959 —Anesthesia during impossible delivery.
Chow	M	5	375	April, 1955	Died August, 1957— Result of experimental treatment.
Lad	M	6	375	April, 1955	
Debi	F	7½	375	April, 1955	
Art	M	17	375	January, 1957	
Bard	M	16	375	January, 1957	Died February, 1957— Myocardiosis, adrenal hemorrhage pneumonia.
Hank	M	11	375	May, 1957	
Dehn	M	11	375	May, 1957	

Observations of behavior were made at frequent intervals by various investigators, including H. W. Nissen, A. A. McDowell, C. S. Ferster, L. J. Peacock, C. M. Rogers, and myself. Tests were selected to cover a wide range of performances and to challenge a variety of skills and capacities at different levels of complexity.

In some tests the animal was required to move quickly; in others, it had to make an appropriate repetitive response. Tests involved food and other rewards. Some tests required the detection of small or obscure stimulus differences; in others, spatial memory was challenged.

Many of the tests were presented in the apparatus shown in Fig. 1. A retractable gray tray bearing test stimuli is propelled against the wire mesh of the cage. The stimuli, in this case two different wood objects, cover food wells. If the animal displaces the correct stimulus, he obtains food. Usually the animal's task is to discover (learn) which object consistently covers the food. An opaque screen immediately in front of the animal can be lowered while the stimuli and rewards are being arranged for the next trial.

RESULTS

The adults, especially the females, appeared to show the effects of radiation sickness more than younger animals. Onset of the acute deterioration



FIG. 1. Chimpanzee displacing stimulus object from food well to obtain reward.

TABLE II

SUMMARY OF TEST PERFORMANCE

<i>Tests showing no decrement in irradiated chimpanzees</i>	
<i>Test</i>	<i>Measure</i>
Consecutive Discrimination	% correct
2-Choice Delayed Response	% correct
5-Choice Delayed Response	% correct
Sameness-Difference	% correct
Matching	% correct
Matching Similarities	% correct
Size Transposition	% correct
Serial Discrimination	% correct
Bent-Wire Detours	% correct
Patterned Strings	% correct
Pendulum	Synchrony of response
Finger Dexterity	Speed of response
Strength of Pull	Intensity of response
<i>Tests showing decrement in irradiated chimpanzees</i>	
<i>Test</i>	<i>Measure</i>
4-Choice Oddity	% correct
Visual Acuity	% correct
Size Discrimination	% correct
5-Choice Delayed Response	Trials attempted
Bent-Wire Detours	Trials attempted
6 Unit Puzzle	Trials attempted
9 Unit Pegboard	Trials attempted
Poker Chip Manipulation	Trials attempted
Wood Block	Frequency of response
Elevator	Latency of response
Social Behavior	Interactions
Operant Conditioning	Rate of response

of test performance occurred about 21 days after treatment. The usual signs of anorexia and lassitude occurred with additional indications of abdominal discomfort. An adult male showed no significant behavioral signs until the 25th day. He deteriorated rapidly, and on the 28th day, he died of pneumonia aggravated by experimental undernourishment.

Table II shows two groups of tests. Above are those tests on which performance remained at preirradiation levels or at the level of a control group. Below are those on which a behavioral change took place. Fourteen tests required the displacement of a particular one of two or more stimulus objects to obtain food reward. Accuracy of performance was assessed

by the percentage of correct responses. Out of the 14 tests, 11 engendered no loss in accuracy on the part of the irradiated animals; 3 tests (4-choice oddity, visual acuity, and size discrimination) produced significant loss.

By way of illustrating tests which produced no differences between normal and irradiated subjects, we may consider the discrimination of pairs of stimulus objects presented in consecutive and in serial order. Under the former, the two stimuli of a given problem are presented 10 times in succession. Then they are exchanged for new ones, which are also presented for 10 trials. Ten such problems are given each day, making a total of 100 trials.

When the problems are presented in serial order, the stimulus pairs are changed on every trial. Each animal, therefore, goes through a list of problems 10 times. Thus, there is maximal opportunity for the different problems to serve as distractions for the other problems, and we anticipate poorer performance by the normal animals. The performance of the irradiated animals should remain fairly high, since irradiation has long been thought to make animals resistant to distraction (Nemenov and Yakovleva, 1944; Harlow and Moon, 1956; McDowell, 1958).

The data of Fig. 2 are based on averages of 3 weeks of testing at the end

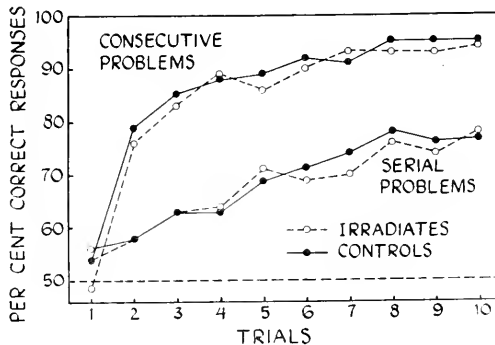


FIG. 2. Performance of normal and irradiated chimpanzees on visual discrimination learning problems presented in serial and in consecutive order.

of training. The performances of the two groups, now consisting of all irradiated animals living until 1960 and their controls, are virtually identical.

As an example of a test involving a loss of accuracy of response, consider the data on 4-choice oddity. In this test, 4 square plaques are suspended by their upper edges in such a way that pushing back a plaque uncovers a food well. On each plaque is pasted a complex design cut from wallpaper. Three of the plaques are identical and the 4th is different. The animal can obtain food only by depressing the unique stimulus. Each combination of stimulus

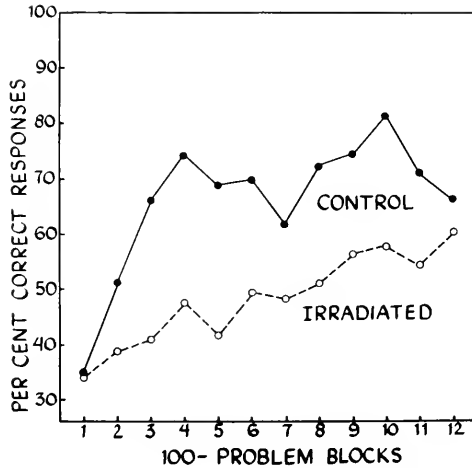


FIG. 3. Performance of normal and irradiated chimpanzees on a task in which they had to select the unique stimulus from three identical stimuli.

patterns appeared only once. New stimulus combinations were constructed each day by re-pairing the supply of 200 triplicate sets of plaques. Figure 3 provides no room for doubt as to the inferiority of the irradiated animals.

The two particular tests described were administered from 3 to 5 years after irradiation, long after the animals had recovered from the acute effects of irradiation. As is well known, radiation sickness produces anorexia, lassitude, and inactivity. When the animals of the present study were afflicted with radiation sickness, they frequently refused to attempt the tests. On other days their responses slowed considerably. Measures based on speed of responding, if they deteriorated, did so during radiation sickness (Table II). After they recovered from this phase, the animals' motivation was restored, and they never failed to respond in the test situation. Also, their activity returned to preirradiation levels.

In summary, irradiation of chimpanzees with 375-400 r of gamma radiation over 12 hours, produced few permanent behavioral losses. On a few tests, conducted several years after treatment, the irradiated animals were significantly inferior to the normals in accuracy of response. Whether this should be attributed to the irradiation, to characteristics of this particular small sample, or to pure chance, is not answerable. Despite the obvious importance of any signs of deficit for treatment of humans, we would wish to be cautious in concluding that 375-400 r will produce permanent incapacitation of an intellectual or perceptual nature. One reason for remaining cautious is that some of the tests which produced loss in this study did not do so in other laboratories or in monkeys (Riopelle *et al.*, 1956; Harlow and

Moon, 1956b). On the other hand, we may have some confidence in the findings of loss in learning of fine visual discrimination (acuity), since it has been found by Davis *et al.* (1958) and by McDowell and Brown (1958).

Monkeys

If there are uncertainties about the statistical interpretation of a few positive differences among many zero differences in the data for chimpanzees getting 375–400 r whole body radiation, there are none whatsoever for the data on the cynomolgus monkeys given 2,000 r to the head.

METHOD

Four animals were given gamma radiation from the Emory University Co⁶⁰ teletherapy unit, and four were given x-radiation from a 250 kvp therapy unit operated on 30 ma with 0.5 mm of Cu and 1.0 mm of Al filtration. Treatment was given to the sides of the head in a single session which was interrupted at the midpoint to rotate the animal 180 degrees. Total treatment required 37 minutes. Doses of 2,000 r were calculated to be midline values, which was approximately 80% of the skin dose.

RESULTS

During the first 24–48 hours, no visible reactions were noted, although appetite and activity waned. In the early part of the 3rd week, the animals developed a diffuse moist reaction of the skin of the head. This reaction required 4 to 5 weeks to completely regress. Towards the end of the healing phase, there was beginning edema, most marked about the eyes, mouth, and inframandibular region (Fig. 4). Death occurred 13–35 weeks after irradiation. Shortly before death the animals developed incoordination of gait, loss of placing and hopping reactions, and an exaggerated response to spatial displacement of their body.

Electroencephalographs taken at various times before and after irradiation were variable. However, two effects could be noted. A "glissando" effect started as a moderately regular 25–30 cps wave and decreased in frequency to about 10 cps in 5–15 sec. It appeared only during the first few days after irradiation. The second effect was a homolateral hypersynchrony in which the leads on one side of the head became synchronized. The synchrony sometimes occurred on one side; at other times the two sides became separately synchronized. The effect appeared only between 3 and 30 days after irradiation.

Within 2 weeks after irradiation, the monkeys were tested on a conditioned



FIG. 4. Cynomolgus monkey (*M. irus*) during edematous phase following 2,000 r to the head.

avoidance task on which they had been given extensive preirradiation training. They had to jump over a low barrier within 4 sec after the onset of a light to avoid shock. Tests were conducted on normal animals and on those drugged with reserpine. No deficit could be detected in the results, nor was there altered sensitivity to reserpine, as had been reported by Ross *et al.* (1954) for pentobarbital.

Post irradiation discrimination training was begun between 2 and 3½ months after treatment. By this time, we could be sure of vision unimpeded by swollen eyelids. The monkeys were given one visual discrimination problem (Fig. 1) every day for 25 days. The stimuli differed in form, color, and size.

The controls significantly surpassed the irradiated animals on this test. The controls averaged 97% on the last six trials of the problem; the x-irradiates, 93%; and the gamma irradiates, 85%.

Two of the x-irradiated and one of the gamma-irradiated monkeys lived

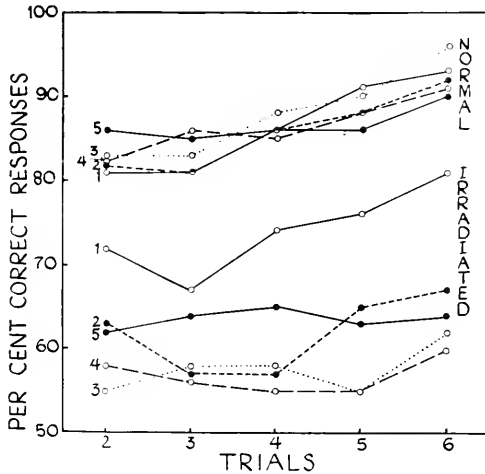


FIG. 5. Performance of normal and head-irradiated monkeys on a series of discrimination learning problems presented consecutively.

to undergo testing on a series of consecutively presented discrimination problems. The learning curves of the normal and the combined irradiated groups for successive blocks of 50 problems are shown in Fig. 5. Although both groups had received identical preirradiation training, their capabilities after treatment were markedly dissimilar. The normal monkeys retained the ability to perform the test during inactivity. The irradiated monkeys showed loss on the first block of problems and further loss on the second and third blocks of problems. There was no overlap of scores between the two groups.

Both of these tests were conducted after the edema had subsided somewhat, and the animals were not tested if there was any doubt as to clarity of vision. At no time was the behavior of the subjects indicative of impeded vision, nor was it ever inappropriate. Food was always eaten when offered, and responses, although somewhat slower than those of the normal subjects, were suggestive of high motivation.

In contrast to the chimpanzee data, the monkey data clearly indicate that 2,000 r administered within 40 minutes produces profound behavioral changes which are probably not transient. The tests described are of proven usefulness for the detection of experimental brain damage in macaques and may be of assistance in identifying those organ systems within the brain which are the more sensitive to irradiation. Although we regret that our macaques did not live long enough to undergo a more extensive battery of tests, the results obtained do suggest that some functional derangement has occurred at least in the so-called "posterior association" areas. Probably the temporal

lobes are involved, for removal of these areas produces deficits of the sort produced here. Whether damage to other parts of the brain occurred, needs further testing.

One cannot help but be reminded that Clemente and Holst (1954) observed that lesions occurring after a long latent period showed a predilection for the white matter. We strongly believe that when delivered in a short time, 2,000 r of gamma radiation produces severe changes in the functioning of the central nervous system, changes which affect the cognitive and integrative perceptual spheres of behavior.

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Some Effects of Radiation on Psychologic Processes in Rhesus Monkeys

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The main radiation research efforts in psychology during the past few years at the radiobiological laboratory of the University of Texas and the U. S. Air Force in Austin, Texas, have been concerned with the relations of radiation-induced changes in distractibility to performance on selected laboratory tasks. It has been found, in general, that such radiation-induced changes are related both to performance facilitation and to performance decrement, depending on the nature of the task employed.

One group of male rhesus monkeys was given chronic whole body exposures to varying mixed doses from a neutron-gamma source in the laboratory. A second group of male and female monkeys was placed at varying distances from "ground zero" at the Nevada Test Site for exposure to nuclear radiations.

The chronic laboratory exposure of the first group was conducted from April 12, 1954, to December 5, 1954 (Table I). Because of the confounding of the radiation variables in the design (a design for which the authors are in no way responsible), most studies with this group have followed the

TABLE I
DOSIMETRY FOR LABORATORY EXPOSED SUBJECTS

Group	Interval (days)	No. of exposures	Duration (hours)	Gamma dose (rep)	n_f Dose (rep)	Total dose (rep)
A	12	20	16	0	0	0
E	4	40	16	0	0	0
B	12	20	16	70	7	77
F	4	40	16	140	14	154
C	12	20	16	140	14	154
G	4	40	16	280	28	308
D	12	20	16	280	28	308
H	4	40	16	560	56	616

precedent established by Davis *et al.* (1957) and have treated each set of experimental results in terms of comparisons of subjects within untreated control, low dose, and high dose subgroups. Treatment groups A and E comprise the untreated control subgroup; treatment groups B, C, and F, the low dose subgroup; and treatment groups D, G, and H, the high dose subgroup. Most of the studies have dealt with a relative radiation dosage variable, rather than with an absolute dosage variable.

The initial suggestion of a radiation-induced change in distractibility came from systematic observations of the free cage behavior of the subjects in the three subgroups described (McDowell, 1958).

These observations were made approximately 1 year after the cessation of exposure of the treated animals. It was found that the frequency of responses to cage parts as manipulanda, the prepotent stimulus class in this behavioral setting, was significantly greater for the irradiated than for the control subjects. The frequency of responses to uncontrolled auditory stimuli occurring outside the test room, however, was significantly greater for control than for irradiated subjects.

These two findings suggested the hypothesis that the chronic irradiated monkey is less distractible than is the normal monkey, i.e., that radiation exposure reduces the probability of response to the weaker stimuli in the environment, with the consequence that the relative effectiveness of the strongest stimulus is increased. Subsequent tests supported the tenability of this hypothesis. In these tests it was found that all experimentally induced stimulus conditions which significantly affected the performance latencies of the irradiated subjects on a simple repetitive task also significantly affected the performance latencies of the controls. Some stimulus conditions, however, which did not significantly affect the performance latencies of the irradiated subjects, did significantly affect the performance latencies of controls. Visual and auditory stimulus conditions were used.

On the basis of the findings on distractibility of the irradiated and control subjects, it was predicted that the learning performance of the irradiated subjects would be superior to that of the controls on tasks such as spatial delayed response and discrimination problems with reduced stimulus cues—tasks which are considered by many researchers to place a premium on attentiveness to the locus of food reward during the trial-setting phase of testing.

A study conducted by McDowell and Brown (1960b) with normal subjects clearly shows that the nature of sensory stimulation during the period of delay, as well as learning during the trial-setting phase, is a factor in spatial delayed response performance. Figure 1 shows the per cent errors per day over successive days of testing for two groups of normals tested with identical stimulus cues on spatial delayed response. One group had darkness

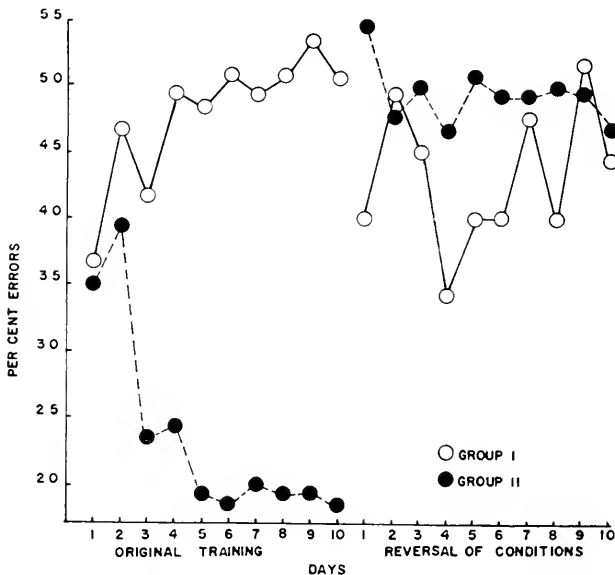


FIG. 1. Per cent errors per day on 10 sec spatial delayed response problems involving identical stimulus cues by *Ss* (Group 1) tested for 10 days with darkness during the delay and, then, for 10 days under regular room illumination and by *Ss* (Group 2) tested on the same problems with the conditions of illumination reversed.

during the delay and manifested no improvement with practice. The second group had regular room illumination during the delay and showed the usual improvement with practice to an asymptote at about 80% correct responses. The right side of Fig. 1 shows the performance of the same two groups with conditions of illumination reversed. Figure 2 shows the per cent errors per day over successive days of testing for two groups of normals tested with discriminative, but ambivalent, stimulus cues on spatial delayed response. Again, one group had darkness during the period of delay and the other group had regular room illumination. In this case, the group with darkness during the delay not only improved significantly faster than the second group, but also improved to errorless performance, in contrast to the usual delayed response asymptote at between 80 and 90% correct. It seems readily apparent from this study that if the trial setting phase of delayed response is adequate to learning, then elimination of sources of sensory stimulation during the delay facilitates learning performance.

Figure 3 presents the results of a study conducted by McDowell and Brown (1959b) to test the prediction of facilitated delayed response performance by the irradiated subjects. Analysis of the data on which this figure was based yielded a significant groups \times practice interaction. The normal

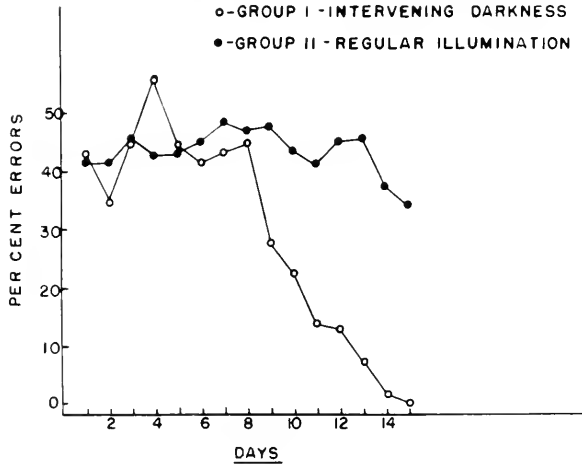


FIG. 2. Facilitated spatial delayed response performance of monkeys tested on discriminative, but ambivalent, cue delay problems with darkness during the delay.

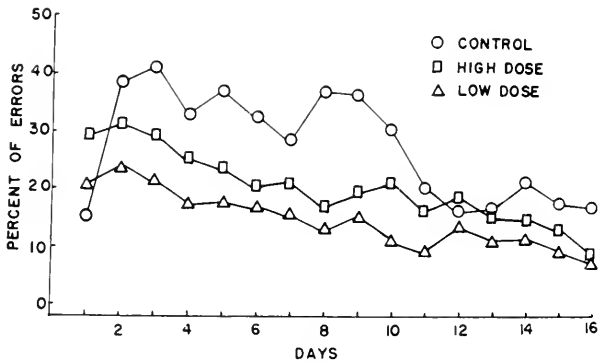


FIG. 3. Per cent errors per group per day on the spatial delayed response problem.

subjects could and did perform as efficiently at some points in time as the chronic irradiated subjects, but they appeared to suffer interference from extraneous stimuli.

Figure 4 shows the results of a study conducted by McDowell and Brown (1958) to test the prediction of facilitated performance by the same irradiated subjects on discrimination problems with reduced stimulus cues. This task, developed by Cowles and Nissen (1937), combines aspects of object discrimination and delayed response. In the Wisconsin General Test Apparatus (WGTA), two identical cues are placed over the food wells. During the learning trial of each problem, a discriminable cue rests on top of that

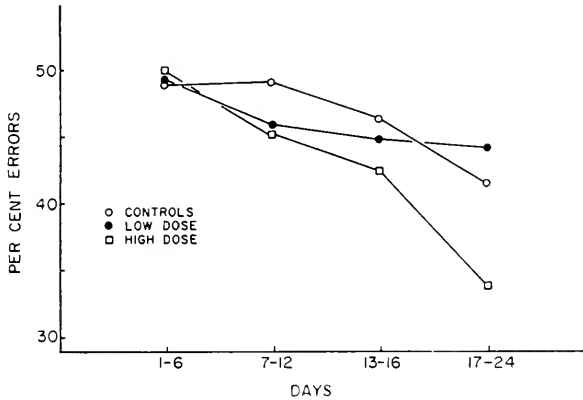


FIG. 4. Facilitated reduced cue discrimination performance of high dose irradiated monkeys.

cue placed over the food well containing the food reward. During the test trial, the discriminable cue is removed and correct response is to that position rewarded during the training trial. Analysis of the data on which Fig. 4 is based, again yielded a significant groups \times practice interaction. The high dose irradiated subjects showed significantly greater improvement with practice than did the low dose or control subjects.

The same subjects then were tested by McDowell and Brown (1959a) on an oddity-reversal problem which required the utilization of the same stimulus cues in antagonistic response patterns for correct solution. In the original training, each subject was tested on 24 trials a day to the criterion of 2 successive days with two or less errors per day on response to the object which was odd in color. During reversal training, each was tested to the same criterion on response to the object which was odd in form. No consistent differences were observed in the number of errors recorded by control, low dose irradiated, and high dose irradiated subjects in reaching either the prereversal or postreversal criterion. In achieving the criterion, all groups showed a statistically significant increase in errors on reversal learning over errors on original learning. The groups did show a statistically significant difference in negative savings scores with the controls manifesting the least savings, and the high dose irradiated subjects showing the greatest savings. These results were interpreted as indicating a superiority of the irradiated subjects over the controls, with respect to reversal problems of this type.

In another study reported by Overall and Brown (1958), each day subjects were given 42 training trials on simple black-white discrimination. Eight test trials presenting two black (positive) stimuli were interspersed among each day's training trials. During the test trials, the position most

recently occupied by the positive training stimulus was chosen by the control and the low dose irradiated subjects to a significant degree. The high dose irradiated subjects manifested no preference for that position associated with reward on the most recent training trial. The results were interpreted as indicative of a narrowed scope of attention in the high dose irradiated subjects.

Brown *et al.* (1958a) hypothesized that, if irradiation results in decreased distractibility with a consequent narrowing of attention, the irradiated subjects should prove less efficient than normal subjects in association of peripheral cues. In the test of their hypothesis, stimuli which were peripheral on the initial problem became focal on the second problem. The results supported the tenability of their hypothesis. The controls evidenced association of peripheral cues, while irradiated subjects did not.

Gentry *et al.* (1958) trained the same subjects on intermediate-size discrimination problems. A test of transposition was then employed to determine the extent to which subjects of the different subgroups utilized relationships between stimuli as a basis for problem solution. Relational learning was found to decrease as a linear function of radiation dosage. The investigators did not interpret the results as suggesting a decrement for the irradiated animal with respect to relational learning. They, rather, interpreted the results as suggesting that, if given a choice, the irradiated animal will utilize learning in terms of absolute stimulus properties, rather than learning in terms of relations.

McDowell and Brown (1960a) studied these same subjects under conditions of repetitious work. Each subject was given 50 trials (or until balking occurred) each day for 44 days on a single form-discrimination problem. If any subject refused to respond within 3 minutes at any point in the daily testing, he was accorded a balk and testing was discontinued. A significantly larger proportion of controls than of irradiated subjects manifested balks, and the balking controls showed significantly more balks. The number of trials worked during successive 4-day periods varied significantly over time for the balking controls.

These findings were considered by the authors to support an hypothesis of greater work decrement for normal than for previously irradiated monkeys under conditions of repetitious work. The results suggest response competition for the normal subjects between the relevant stimuli of the test situation and extraneous stimuli.

Another study was conducted by McDowell and Brown (1960d) to compare the response perseveration of some of the same irradiated subjects with that of normal subjects when tested according to the proactive inhibition paradigm. The study specifically involved the effects of initial training on a peripheral cue discrimination problem, during which no learning was

manifest, on the subsequent transfer of a single learned discrimination along a peripheral cue gradient. The results of the study indicated that the chronic irradiated male monkey, at the dosage used, is less susceptible to proactive inhibition than is the normal male monkey. Initial training on a peripheral cue discrimination, during which no learning was manifest, interfered less with the subsequent transfer of a single learned discrimination along a peripheral cue gradient by the chronic irradiated subjects than by the normal subjects.

The proactive effect appears to have been due to failure to learn the initial problem. The consequence of the failure to learn the initial discrimination was apparently an association of the stimulus events of peripheral cue testing with a 50% reinforcement schedule. An hypothesis of decreased distractibility and narrowed scope of attention for the irradiated animal would predict that fewer such stimulus events would be associated by the irradiated animal than by the normal animal. Any change, then, in one of the stimuli associated by both normal and irradiated monkeys would constitute a proportionately greater alteration of the situation for the irradiated animal. Thus, the irradiated animal should more easily discriminate a change in the situation and redefine the problem, as appeared to be the case in the present study.

While the results of all of the aforementioned studies could be accounted for in terms of decreased distractibility, another study conducted by McDowell (1960) with some of the same subjects suggests that decreased distractibility alone cannot account for all of the learning performance differences between controls and irradiated subjects. In that study, subjects were tested for transfer of a single learned discrimination along a peripheral cue gradient. When the subgroups were compared with respect to efficiency of transfer performance, they were found to differ significantly as a nonlinear function of radiation dosage. The author interpreted the results as reflecting differential increments in incentive motivation for the subgroups during the initial focal cue discrimination training. To explain these differential increments, he hypothesized that ratio of response rate to range of effective stimuli defines incentive motivation when the other determiners of incentive motivation, as given by Spence (1956), are held constant.

These same subjects were tested by Brown and McDowell (1960) on each of eight visual acuity problems presented in order of increasing difficulty. Each problem required the subject to choose between circles and circles with breaks in order to procure a food reward. Previous visual acuity testing (Davis *et al.*, 1957) had shown a deficit only in the high dose irradiated subjects (616 rep) during the 1st year following the radiation exposure given 3 years prior to the initiation of the present study (Fig. 5). The visual acuity deficit noted during the 1st year after exposure in the high

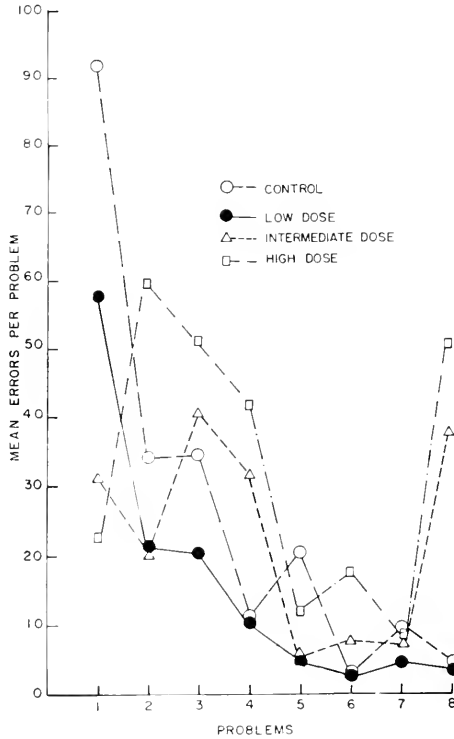


FIG. 5. Mean errors to achievement of the criterion for each group on each of the eight successive problems.

dose irradiated subjects was still manifested 3 years after exposure. Intermediate dose subjects (308 rep) that had shown no visual acuity deficit during the 1st year after exposure, manifested such a deficit 3 years after exposure. An interpretation of the results in terms of a basic deficit in learning ability for these two groups was contraindicated by their superiority over normal and low dose irradiated subjects on the initial and easiest problem.

Before proceeding to the research results on the second group of monkeys, the field-exposed males and females, it would seem in order to show why it was deemed necessary to analyze the test results in terms of the sex factor as well as the radiation factor.

Recent findings by McDowell *et al.* (1960), with respect to sex as an inherent difference between subjects are strikingly comparable to those with respect to radiation as an experimentally induced difference. These researchers conducted a study to determine if male and female monkeys of com-

parable ages differ in distractibility or in concentration of attention on the prepotent stimulus in both a test situation involving the presence of food and a free cage environment with no food. The study proposed further to test the prediction of facilitated delayed response performance by that sex showing the greater concentration of attention if, in fact, evidence for sex differences in attention were manifest.

One experiment concerned a comparison of 21 female and 20 male monkeys on preliminary WGTA training involving food as the prepotent stimulus. Figure 6 shows the per cent of each sex having completed preliminary WGTA training on successive days of testing. Females required significantly fewer days to complete this training than males. A second experiment compared the sexes on object-directed responses in the free cage environment. Figure 7 shows the proportion of total object responses directed by each sex to each of the four stimulus classes. Each of the sexes ordered its responses significantly and in the same manner with respect to these stimulus classes. Females directed a significantly larger proportion of their object responses to the prepotent stimulus class than males. Because of these findings, facilitated spatial delayed response performance was predicted for females. Figure 8 shows the per cent errors on successive days of spatial

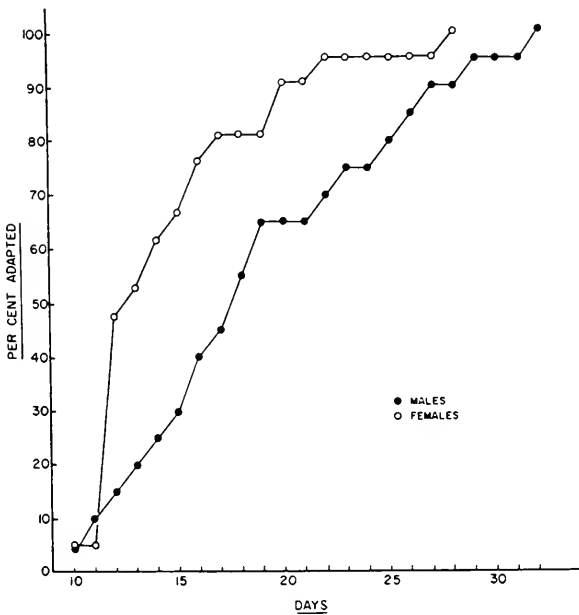


FIG. 6. Per cent of *Ss* of each sex having completed preliminary WGTA training on successive days of testing.

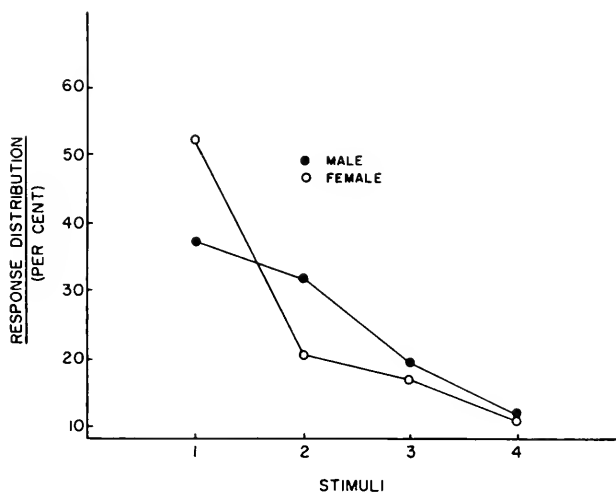


FIG. 7. Proportions of total object responses directed by the Ss of each sex to each of the four stimulus classes in the free cage environment.

delayed response testing for the two sexes. The prediction proved valid. The females improved significantly faster than males.

In a second group of 64 rhesus monkeys, 56 had been exposed to nuclear radiations at the Nevada Test Site. The number of each sex in each subgroup and the radiation dosages are shown in Table II. For statistical analysis, the control group and radiation subgroups I and J constituted the

TABLE II

DOSE LEVELS FOR AND NUMBER OF SUBJECTS OF EACH SEX IN EACH SUBGROUP

Subgroup	Gamma (r)	Neutron (rep)	Estimated total dosage (rem)	No. of males	No. of females
C	252	209	670	5	1
D	242	183	608	4	1
E	204	154	512	5	3
F	187	126	439	3	4
G	169	114	397	5	3
H	151	102	355	3	4
I	129	85	299	6	2
J	119	77	273	5	2
Control	0	0	0	3	5
				—	—
				39	25

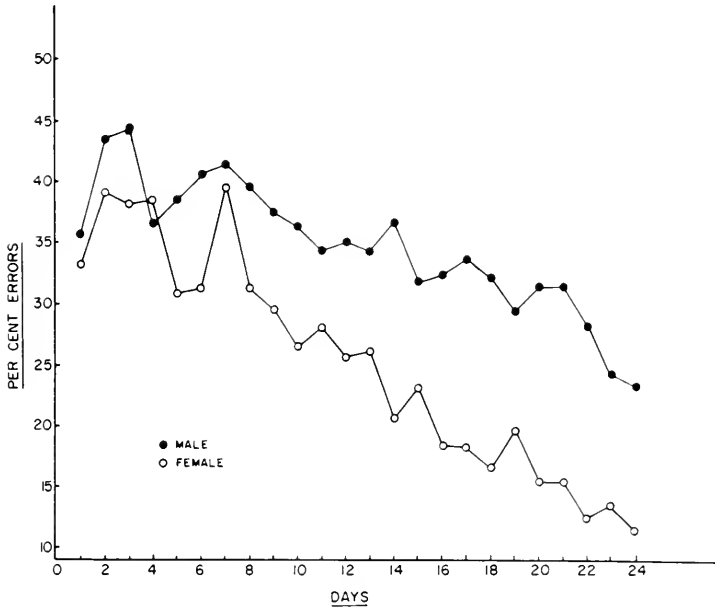


FIG. 8. Per cent errors on successive days of testing on spatial delayed response for the 20 male Ss and for the 21 female Ss.

low dose group, radiation subgroups F, G, and H constituted the medium dose group, and radiation subgroups C, D, and E constituted the high dose group.

The initial study conducted by McDowell *et al.* (1959) was concerned with progression through a series of training stages designed to prepare the subjects for object discrimination testing on the WGTA. The three relative radiation dosage groups were also compared on their initial object quality discrimination learning. The radiation exposure of the irradiated subjects predated the present study by 11 months. In this study, the higher the relative radiation dosage, the faster the response to food, the slower the response to a wooden object block, and the faster the discrimination of a food-rewarded object block after object blocks had acquired the stimulus value of food.

The same subjects then were tested by McDowell *et al.* (1961a) on discrimination problems with reduced stimulus cues and on spatial delayed response problems. Figure 9 shows the per cent errors on successive 4 day periods of testing on reduced cue problems for these 40 males and 24 females. The females learned significantly faster on this problem than males. Figure 10 shows the per cent errors on successive 4 day periods of testing

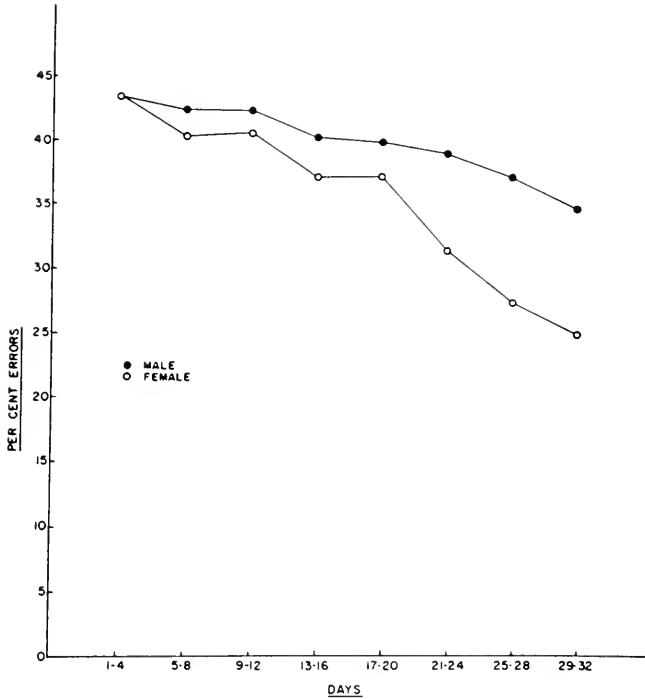


FIG. 9. Per cent errors for each sex on successive 4 day periods of reduced cue discrimination training.

on spatial delayed responses for the same groups. The females were significantly superior to the males throughout the testing.

The same subjects were tested by McDowell and Brown (1961b) on a graded series of dot discrimination problems. Analysis of the error data yielded a significant sex \times problems interaction (Fig. 11). As the discriminations became increasingly harder, the females became increasingly superior to the males. Figure 12 shows that a similar phenomenon was manifest with respect to the relative radiation dosage groups. The higher the relative radiation dosage, the more efficient was the performance as the discriminations became increasingly more difficult.

Another study by McDowell and Brown (1961c) involving the same 64 subjects concerned peripheral cue discrimination learning. The essential apparatus is shown diagrammatically in Fig. 13. Each subject, to obtain consistently the food reward, was required to respond to one of two identical pieces of plumber's chain, on the basis of how near it was to a discriminable cue. The same two discriminable stimulus blocks were used throughout 48

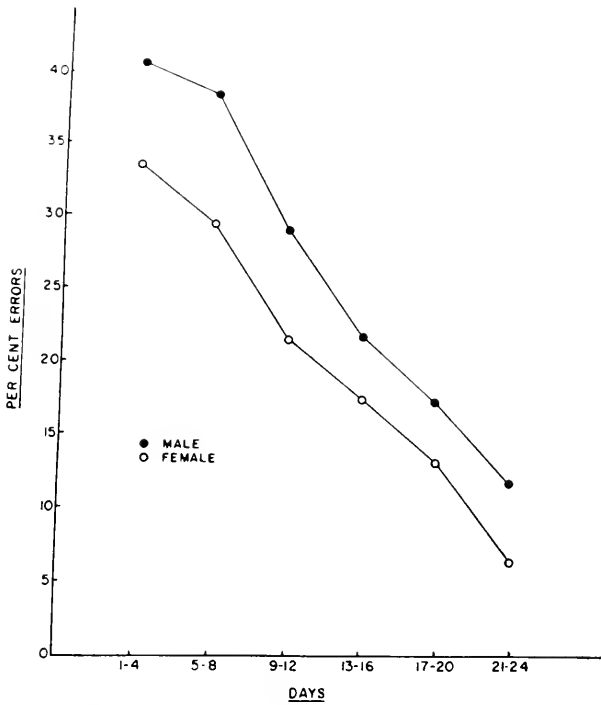


FIG. 10. Per cent errors for each sex on successive 4 day periods of delayed response training.

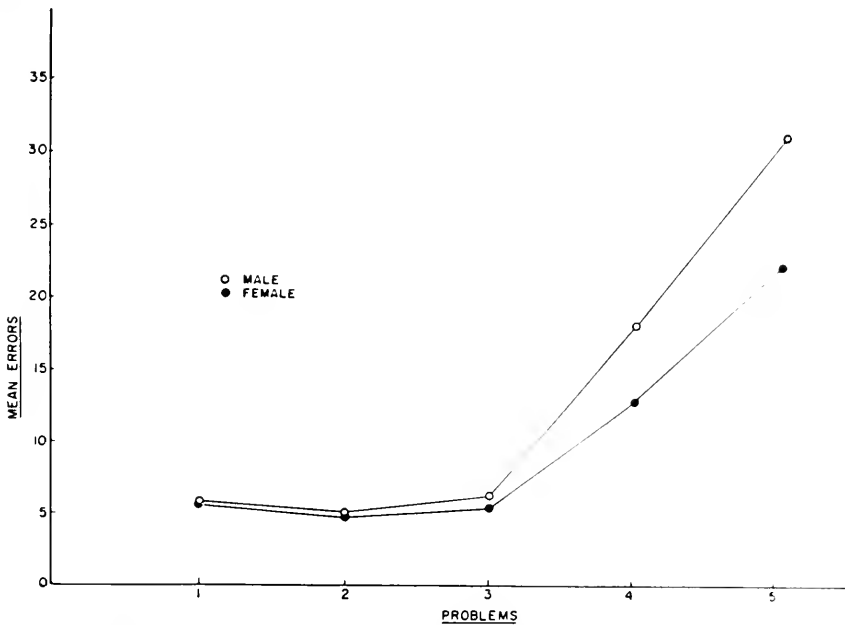


FIG. 11. Mean errors for each sex on each of the five dot-discrimination problems.

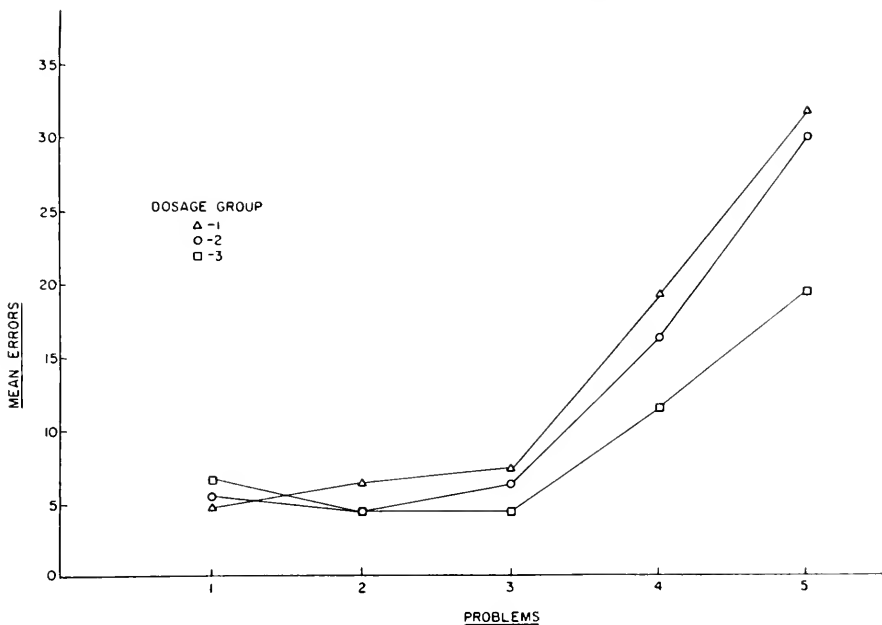


FIG. 12. Mean errors for each radiation dosage group on each of the five dot-discrimination problems.

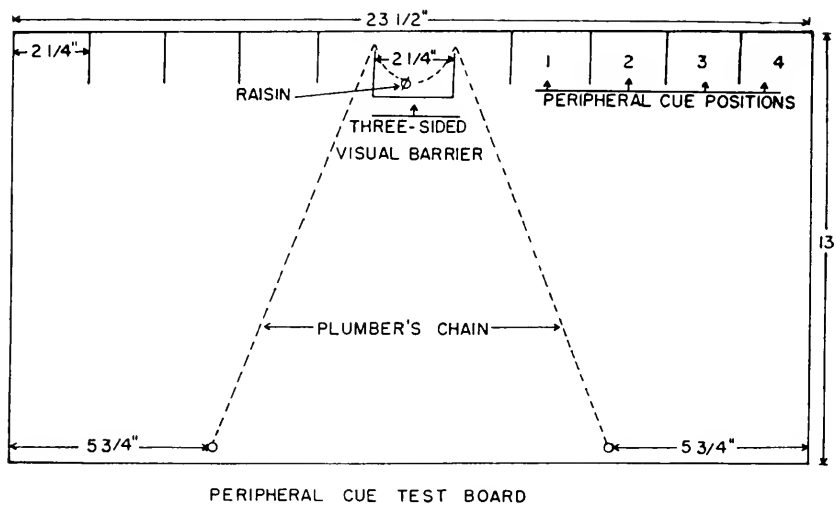


FIG. 13. The modified two-string patterned string board used for the peripheral cue testing.

days of testing. Figure 14 shows the mean errors for each sex in each radiation dosage group on successive 8 day periods of testing. Statistical analysis of the error data yielded a significant radiation \times sex \times practice interaction. The appropriate interpretation of the interaction would appear to be that as the relative radiation dosage is increased, the males show increasingly more rapid rates of improvement and the females show increasingly less rapid rates of improvement. One might speculate that radiation exposure increases attentiveness for males and females alike; but because of the inherent sex differences, the result for the males on this problem is the exclusion of irrelevant stimuli, and the result for the females is the exclusion of the relevant stimulus.

A recent experiment conducted by McDowell *et al.* (1961b) provides additional evidence that radiation exposure reduces the number of stimuli responded to in the discrimination problem situation. The same low, medium, and high dose subjects were tested in the WGTA on response

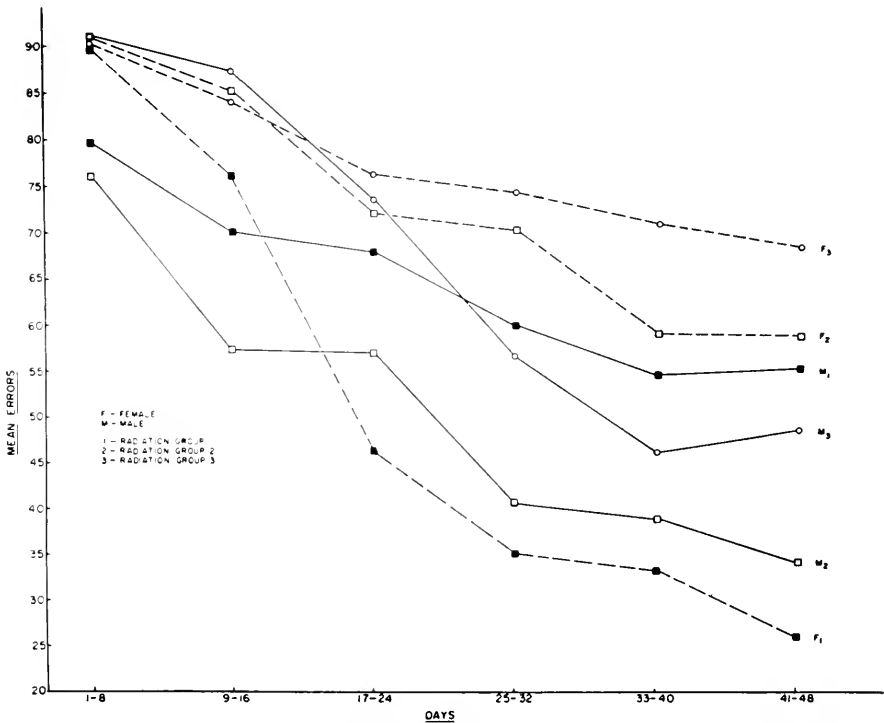


FIG. 14. Mean errors during successive 8 day periods for each sex of each radiation dosage group. F=female; M=male; subscripts 1, 2, 3 indicate respectively radiation groups 1, 2, 3.

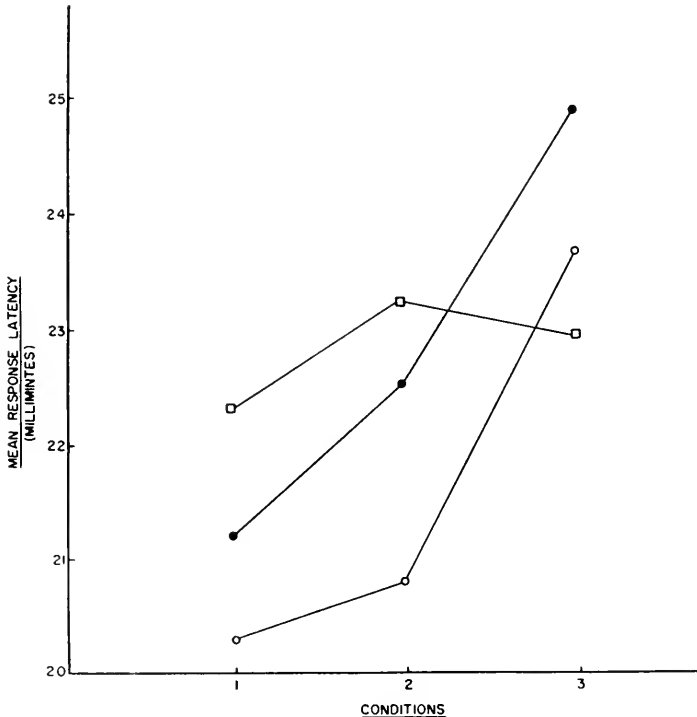


FIG. 15. Mean median latencies for low dose (Group 1), intermediate dose (Group 2), and high dose (Group 3) irradiated Ss on response to (1) a single food-rewarded object block in a constant position, (2) the same object block with left-right position "randomized," and (3) either the same food-rewarded object block or a novel non-food-rewarded object block presented simultaneously.

latency to a single food-rewarded object block in a constant position, on response latency to the same object block with left-right position "randomized," and on response latency to either the same food-rewarded object block or a novel nonfood-rewarded object block presented simultaneously. Figure 15 shows the mean median latencies for each radiation group under each condition of testing. Statistical analysis of the data yielded a significant condition effect and a significant groups \times conditions interaction. With the single block conditions, the higher the relative radiation dosage, the higher the response latency. When the novel unrewarded stimulus block was introduced, the higher the relative radiation dosage, the less the disruption of response latencies. The high dose subjects continued to respond just as they had in the single object "randomized" position condition.

While it is recognized that the null hypothesis is never accepted, the consistent negative findings on concept formation problems with both of

the major groups discussed is worthy of note. Studies by Brown *et al.* (1958b) on conceptual discrimination, by Brown *et al.* (1959) on novelty learning set, by McDowell and Brown (1960c) on peripheral cue learning set, and by McDowell and Brown (1961a) on approach-avoidance learning set have yielded negative results with respect to the radiation variable. One might speculate that the frequent introduction of novel test stimuli in problems of this type keeps the attention of the controls focused on the problem and, in consequence, militates against radiation differences.

The procedure in the conceptual discrimination research is illustrative of the recurrent novelty factor. In one experiment, object-quality discrimination problems were presented in a series in which the positive (rewarded) stimulus object on any given problem (after the first) was the negative (unrewarded) stimulus of the preceding problem. In a second experiment, the negative (unrewarded) stimulus object of each successive problem was the positive (rewarded) object of the preceding problem. Since each problem was presented for only four trials, a novel stimulus object was presented on every fifth trial. It was also the case on each of the other concept formation studies that novel stimuli were introduced on every fifth trial.

In summary, radiation for both field and laboratory exposed rhesus monkeys produces decreased distractibility or, in other words, increased concentration of attention. The general resultant for learning is facilitation of performance on tasks placing a premium on attention to the site of food reward and decrement in performance on tasks requiring attention to peripherally placed stimuli. Whether test results of this nature reflect direct or indirect effects of radiation exposure on the central nervous system remains to be solved.

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GENERAL DISCUSSION

T. C. RUCH (*University of Washington, Seattle, Washington*): I want to ask Dr. Brown what he means by "high, medium, and low" doses in terms of lethality? As you know we have no reliable published data on the lethality of radiation in monkeys and this might give us objective definitions. The other thing I would like to know is whether members representative of the high, medium, and low groups are trained simultaneously by the same personnel, the same time of year, on the same apparatus, and so on?

W. LYNN BROWN (*University of Texas, Austin, Texas*): First of all, the groups were divided before the act so that we could reach certain statistical requirements in the handling of the data. As to high, medium, and low, we are using descriptive terms. Group C was the highest group. All the animals in Group A were lost. Of 8 animals in Group B, 7 were lost. Consequently, that group or the single animal was not included. In the high dose group, we lost 5 of 24 animals. In the other groups, we have few deaths. We had two deaths in the medium group and one in the low dose group. So, we were using these terms rather than stating each time what the dosage was, and we are dealing with a variable dose of radiations—mixed gamma-neutron radiation. The problem is an Air Force design problem in radiation. We psychologists did not design this problem, but the Air Force has certain problems with reference to radiations that they wanted solved, and these animals were assigned to these dose levels. Psychology had a somewhat minor role, compared to hematology and bone marrow. Despite the designs, we have been able to test out a number of relations which are consistent between the laboratory exposed animals to gamma-neutrons and the Nevada field tests.

T. C. RUCH: Have you an LD/50 for 30 days?

GEORGE M. KRIZE (*Texas A & M College, College Station, Texas*): Mackey has reported it in the same report, 555 plus or minus 25.

HARRY R. HARLOW (*University of Wisconsin, Madison, Wisconsin*): I am sure we are gradually accumulating data on the LD/50/30. We have had no animals go out on 500 r. We have had better than 50% survival after a single dose of 600 r. We are accumulating a large amount of data on fractionated doses, and when these are extrapolated, these factors on the LD/50/30 for monkeys will be somewhere between 600 and 650 units.

W. LYNN BROWN: In the range of 650 units would be a guess, for a while then, for a healthy animal.

C. D. CLEMENTE (*University of California, Los Angeles, California*): I would like to ask a question about the onset of generalized radiation sickness in animals that have sustained head radiation alone. Dr. Arnold made the statement that head radiation in rats was able to produce a generalized radiation sickness. In our

previous studies (Clemente and Holst, 1954), it was found that, even though we shielded the body of monkeys sustaining head radiation with a $\frac{1}{8}$ in. lead shield, 0.5% of our head radiation dose was scattered and absorbed by the bodies of the animals. These were doses that were calculated for us by the physicists and seemed reliable, and I wonder whether or not Dr. Arnold took the trouble to measure the scatter to the bodies in his rats and whether this is the reason that a generalized radiation sickness was observed. In this regard I would also like to ask Dr. Riopelle whether the monkeys which sustained brain radiation in his series showed symptoms of diarrhea, blood cellular changes, or gastrointestinal pathology, such as intestinal hemorrhages, usually described as indicative of the generalized radiation sickness syndrome.

ARTHUR J. RIOPELLE (*Yerkes Laboratories, Orange Park, Florida*): No. Our head irradiated monkeys die from a variety of things. Temperature control went out in the laboratory one night, and we lost a few animals a week or two after that. Autopsy showed a variety of ailments scattered throughout the body, as one would anticipate, from infections. Chins were necrotic. They sloughed off. There was also sloughing of the lips. Infection via that route certainly appeared. But we did not see the usual radiation sickness syndrome.

WILLIAM J. ARNOLD (*University of Nebraska, Lincoln, Nebraska*): I did not say that I thought head irradiation alone would produce a total pattern of radiation sickness exactly comparable to that of total body irradiation. I feel sure that if someone checked carefully, there would be differences. With respect to the back scatter, we did not check the amount which seeped through or scattered from the radiation site. I believe it would be small. We did radiate the hind legs of animals using a comparable area and dosage with all conditions the same as for the brain area irradiation. We noted no evidence of radiation sickness from our general observations or from careful measurement of weight loss or food intake. We observed no evidence of radiation sickness in those animals.

T. J. HALEY (*University of California, Los Angeles, California*): In confirmation of what Dr. Harlow and Dr. Riopelle stated about conditioned avoidance, we have studied rats under this situation using sound as our conditional stimulus and in doses of 300, 600, and 900 r we could detect no statistically significant difference between the control period prior to irradiation and the results obtained afterwards. In fact, with animals at the 900 r level, where they live only 6 or 7 days, we actually saw animals on the grid perk up their ears at the sound, make one last lunge for the pole, and drop dead. I would also like to comment on this controversy on the LD/50 in the monkeys. It seems as though we need a definition of terms. As I understand it, Dr. Harlow is working with pure x-rays or gamma rays. Dr. Lynn Brown is working with gamma rays and neutrons. The LD/50 will not be the same under those two conditions. We should always define the conditions of our experiments implicitly so that no one will get the wrong impression.

MARCEL MONNIER (*Basel, Switzerland*): I would like to ask Dr. Ruch if he thinks it is possible to discriminate two mechanisms for the high percentage of errors in monkeys, one type related to hyperdistractibility with motor hyperactivity, and the other type related to an abnormal passivity. Our observations point on one

hand to hyperexcitability of the reticular ascending system; on the other hand, to a passivity depending on extensive cortical depression.

T. C. RUCH: I can not encourage the idea from our own data because the direction of the effect on spontaneous activity, which would be the only data we have, might reflect a general level of cortical excitability or something of that sort. There was a reduction in both low level and sublethal level.

BILLEY LEVINSON (*University of Buffalo, Buffalo, New York*): Dr. Monnier's idea is a fine one. It is something that has been bothering me for a long time. Rats make mistakes in the maze because they are plain stupid or because the building is not soundproof, and there are a lot of distractions. Particularly in the field, we have observed that these rats are hyperexcited and hyperdistractable. I believe it would be very easy to design an experiment that would separate these two different kinds of errors.

JASBIR SINGH (*Stritch School of Medicine, Loyola University, Chicago, Illinois*): Dr. Levinson has mentioned that radiation is a protective and non-protective agent to certain systems of the body, and I would like to know which of those systems particularly can we classify as protective or non-protective agents. Dr. Harlow has mentioned that radiation reduced the lemon water consumption of animals if they are exposed to radiation as compared to the normal consumption of water. Is it due to the acidity of the lemon water or could it be due to some other factors involved in the whole process?

BILLEY LEVINSON: This is a rather difficult question to answer. This has been referred to as the "radiation chimera" or "radiation mosaic". I think it is worth pointing out that there are many different systems in the body. Some of this is having differential effects. By AET some of these systems have been worked out, e.g., cataracts, some changing in spleen efficiency, epilation, graying of the hair. AET has also been worked with the hemopoietic systems and mean survival time. There is a bibliography of some 30 or 40 items listing various systems affected by AET. The main thing is that it does not act on any of these systems in the same way, and there are probably interaction effects between systems.

HARRY F. HARLOW: I would like to express my gratitude to Dr. Haley. He could not be more right in terms of LD_{50/30} or anything else. In the case of chemical composition of any of the substances which we used in conditioned aversion, I am frank to state that we have no data. Everything we have done has been a preliminary struggle to find conditions in which the phenomenon can be demonstrated. We just chose substances for which the monkeys had a high preference.

ERNEST FURCHTOTT (*University of Tennessee, Knoxville, Tennessee*): Having worked on the problems of irradiation on behavior longer than any other American psychologist, since about 1948, and having reviewed the field in 1955, I would like to make some general comments about the state of the problem at present. In my 1956 review, I commented about some of the problems which need investigation in this field. I am glad to see that some of these problems have been investigated. I also pointed out there are several problems which I think would profit from a behavioral investigation, namely, an analysis of certain perceptual or sensory changes. A number of your open investigators have pointed out that

there are definite sensory changes associated with radiation, for example, adaption and changes in taste. To the best of my knowledge, nobody has worked on this problem. On the other hand, I also said at that time that there are some problems, like learning, in the adult animal that seem to me like a dead-end; and all the work since that time simply tends to confirm this. I have one question for Dr. Levinson. In many behavior studies it is necessary to run a conversion operation, that is to study this problem from several viewpoints. We need to have several different tests to get at the basis of the behavioral phenomenon. Dr. Levinson compared the effects of AET on learning and maze retention with animals which did not receive AET and were irradiated. Dr. Levinson, did you use control animals which received AET? It is possible that AET affected, not the radiation mechanism, but rather motivation and other variables.

BILLEY LEVINSON: I think Dr. Furchtgott is wrong in thinking there are no effects of radiation on the adult animal. I believe there is evidence on this from Russian and American sources. In my paper under the zero radiation group were three subject groups: one received only sham irradiation; one received sham irradiation and a saline injection; one received an AET injection and sham irradiation. On the acquisition learning task there were no significant differences, the means being almost identical for all three groups. There were some slight differences in the retention test, probably a function of age such as Dr. Kaplan demonstrated of changes over time. I suspect that if an animal is injected at age 2, 4, or 6 days with saline or AET, there is bound to be some effect. The retention test data suggests there was a difference between those receiving AET and those receiving saline. It is well known that AET is toxic. Mr. Thomas Graham, of Western Biological Laboratories, found in preliminary results that AET is greatly toxic in fetal animals. I selected a much lower AET dose than recommended, and if studies had been done with higher doses, we probably could have found some toxicity.

JACK ARBIT (*Northwestern University Medical School, Chicago, Illinois*): I would like to discuss Dr. Harlow's comments about the marked individual differences in obtaining avoidance conditioning, and also pertaining somewhat to the controversy regarding the reproducibility of the avoidance conditioning phenomenon using irradiation as the unconditioned stimulus. We recently have completed a study using Dr. Kimeldorf's spatial avoidance paradigm and x-radiation as the unconditioned stimulus. It was an analysis of variance design, varying the number of cues available as conditioned stimuli, and emotionality of the animals as measured in an open field test by the number of fecal boluses deposited before the conditioning series took place. Those animals who would be called emotional in terms of their open field behavior showed the avoidance conditioning phenomenon with x-irradiation as the unconditioned stimulus in the spatial avoidance design. Those animals who were in the unemotional group in terms of previous behavior in the open field test did not show avoidance conditioning. This may have some bearing on individual differences in animals or on differences in this phenomenon from one laboratory to another.

JOHN L. FALK (*Harvard School of Public Health, Boston, Massachusetts*): I would like to comment on a series of experiments done by Dr. Kimeldorf on radiation-conditioned taste aversion and radiation-conditioned spacial avoidance. It

might be possible to use radiation as a discriminative stimulus. I am not aware that any work like this has been done. One could determine the time required to show such a discrimination; and if a situation were set up where the responding and behavior were measured from moment to moment, it might throw some light on whether conditioned avoidance and the conditioned taste aversion were due to a direct sensory perception of the irradiation, or whether the effect was due to the animal's perceiving the autonomic or other secondary effect resulting from the radiation. I think such an experiment could be set up using differential reinforcement of low rad versus a variable ratio, and having the radiation turned on during one of the components, the radiation, therefore, being the only possible way in which the animal could tell what the reinforcement schedule would be. I wonder if Dr. Kimeldorf has done any work along this line, using radiation as a discriminative stimulus. If he has not, I would like to know whether he would hazard a guess as to whether the aversions and avoidances he found are due to direct sensory perception of the radiation source or due to the autonomic or other effects of the radiation.

DONALD J. KIMELDORF (*U. S. Naval Radiological Defense Laboratory, San Francisco, California*): Dr. Falk has been reading our secret protocols. We are in the process of attempting to use radiation as a conditioned stimulus, rather than as the unconditioned stimulus. Other than what we have said in our formalized presentation, which was carefully considered with regard to perception and motivation of radiation, I hesitate to make any judgments yet. What appears to be indications and perhaps proof of late effects of alteration in behavior has been of interest to me in another regard. We know that radiation exposure does shorten life span. This has been shown in several species. We do not know if this is a simulation of or stimulation of the normal aging processes. It may be that the same system that responds to radiation is the one that reacts to radiation processes which show up in aging. Those people who find these late effects hold their controls for a longer time to determine whether there is any relationship between the alteration behavior which occurs early in the irradiated animal. It might not occur in the same time sequence with regard to life span. If someone has not tested controls at a later date, it might be valuable to do so.

DONALD B. LINDSLEY (*University of California, Los Angeles, California*): It seems to me that during the past 3 days we have heard some very interesting papers which have brought definite questions before us. One relates to the dosage; one relates to a question that Dr. Furchtgott brought out. We have heard that in those cases where doses were approaching sublethal doses, or even considerably less, there often was a decrement in performance or a depressant effect. Where the dosage was considerably less, say under 200 r or 75 r, we noticed in several instances, in Dr. Harlow's survey and several others, that there was for these lower dosages a definite sensitizing influence with increasing performance on the various tests. Above that, the decrement began to set in. In the papers from other sessions of the symposium, we noticed something of the same sort where a peripheral nerve or receptor system was radiated. There was indication on Gaffey's paper, Noell's, Lipetz's, and others, that there was at first an increased responsiveness even of peripheral structures. In C. A. Tobias' paper, pointing out the nature of

the fluorescent effect in the brain stem, the lower brain stem, hippocampus or parts of the limbic system, one might wonder whether certain things might not be happening here. Our receptor system and peripheral nerves, perhaps at some level of radiation, sensitize temporarily to give some of these results. Where the brain itself is radiated or perhaps the whole body, the influence of this might suggest that the reticular activating system and the associated limbic system might be somehow sensitized. This may not be as absurd as it first sounds, because in Dr. Arnold's results a number of his tests were essentially negative; but emotionality was one of the things in the open field tests which was not measured. In another paper by Davis, he pointed out particularly negative results on some tests but noted that non-visual survey and restlessness might be coupled with a slight sensitization to the reticular activating mechanism to incoming sensory modes. I wonder whether others, Dr. Monnier particularly, since he has been concerned not only with reticular activating systems, but also with the diffuse thalamic nuclei, would see in this a possibility. Certainly, as Dr. Furchtgott mentioned, the sensory or perceptual modalities may be sensitized or desensitized, and this may be a determining factor in learning tests or discrimination tests. So we have to look carefully at the kind of measurements and observations we are making and attempt to determine what particular system or element in the system may be responsible for some of the results.

ROGER T. DAVIS (*State University of South Dakota, Vermillion, South Dakota*): Dr. Lindsley, I should like to say that in looking at some of the things which have been reported in the literature, we find lower distractability, changes in posture, narrowing of attention, relative decrease in initiation of aggression and manipulation of inanimate objects. These things suggested to me what you have said, but I did not feel I was enough of an anatomist to make that statement. I am glad you did.

DONALD B. LINDSLEY: I do not think I am enough of an anatomist to make it, either, but I have been at least concerned with it. I would like to make a final statement, since this is the last session of our meeting. I think we are all agreed that this has been a fine meeting, and I think we would also like to extend our thanks to our two hard-working co-chairmen, Dr. Tom Haley and Dr. Ray Snider, to the Public Health Service, and the Atomic Energy Commission for having made this conference possible. We can all now not take AET or TEA, but TWA. The meeting is adjourned.

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