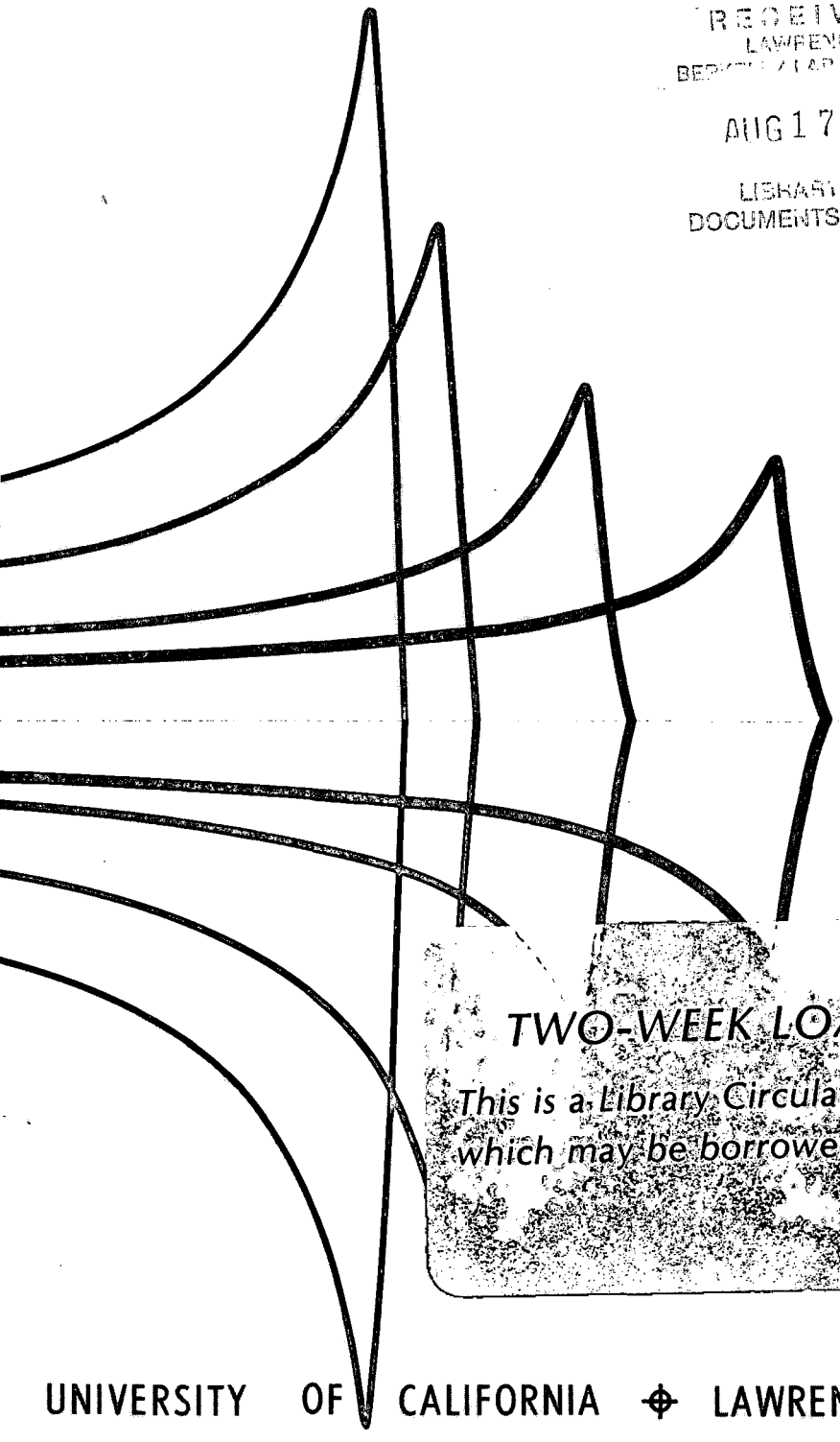
BIOMEDICAL STUDIES WITH HEAVY-ION BEAMS



Meeting of the Advisory Committee for Biology and Medicine

United States Atomic Energy Commission

March 9-11, 1967

UNIVERSITY OF CALIFORNIA ⊕ LAWRENCE RADIATION LABORATORY

DISCLAIMER

UCRL-17357

BIOMEDICAL STUDIES WITH HEAVY ION BEAMS

Donner Laboratory and Donner Pavilion

LAWRENCE RADIATION LABORATORY
University of California
Berkeley, California

March 1967

Research work related to space radiobiology has received support from the National Aeronautics and Space Administration through the Lawrence Radiation Laboratory.

Contents

Historical Survey

C. A. Tobias and J. H. Lawrence 1

Stopping-Power and Range-Energy Data of Heavy Ions in Nongaseous Media

P. G. Steward 8

Penetration of High Energy Heavy Ion Beams into Matter

G. M. Litton, R. W. Wallace, and C. A. Tobias 18

Heavy Particles in Space: Physical Aspects

S. B. Curtis 31

Fluctuations of Ionization by Heavy Ions

H. D. Maccabee 38

Distribution of Ionization in Heavy Charged-Particle Tracks

J. T. Lyman 42

Measurements of Heavy Charged Particles with Semiconductor Detectors

M. R. Raju 47

Effects of Heavy Ions upon the Production of Free Radicals in Organic Compounds

W. B. G. Jones and T. Henriksen 58

Inactivation of Enzymes in Dilute Aqueous Solutions by X-Rays and Heavy Ions

T. Brustad 68

Radiobiology and Radiochemistry of Lipoproteins

A. V. Nichols, N. K. Freeman, and F. T. Lindgren 79

Some Effects of Accelerated Charged Particles in Bacterial Spores

E. L. Powers, J. T. Lyman, and C. A. Tobias 81

Cellular Radiobiology of Heavy Ions: Cell Recovery and DNA Repair

R. H. Haynes, W. R. Inch, and J. T. Lyman 86

Cellular Radiobiology of Heavy Ions: Mammalian Cells

P. W. Todd 91

Proposed Omnitron Experiments by Members of the Laboratory of Chemical Biodynamics
M. Calvin, R. M. Lemmon, and M. P. Klein 101
Radiation Genetics with Densely Ionizing Radiations
R. K. Mortimer 105
Heavy Ion Radiography and Microscopy
C. A. Tobias, T. L. Hayes, H. D. Maccabee and R. M. Glaeser 108
Proton and Alpha Radiation Effects in Mice
J. K. Ashikawa 121
Heavy Ion Beams in Experimental Population Genetics
J. L. King 124
Morphological Studies of Laminar Lesions
W. Haymaker 126
Anatomical Studies with Laminar Lesions
L. Kruger 136
Possible Usefulness of Accelerated High Energy Nuclei in Studies on Regeneration in the Spinal Cord
D. L. Singman, D. C. Van Dyke, and G. P. Welch 146
Differential Sensitivity of Components of the Visual System to Alpha Particle Irradiation
C. T. Gaffey 155
Studies of the Vestibular Organs with Heavy Particles
L. W. McDonald 159
Studies on Brain Activity and Learning Behavior Using Heavy Particle Irradiation
R. L. Schoenbrun 164
Space Flight and Mammalian Radiobiology
H. Aceto 175
Radiation Stimulation of Nerve Action
C. A. Tobias, J. T. Lyman, and J. R. Luce 189
Pituitary and Hypothalamic Irradiation
C. A. Tobias and D. C. Van Dyke 197

Heavy-Particle Pituitary-Suppressive Therapy in Diabetic Retinopathy
J. A. Linfoot, J. L. Born, J. F. Garcia, C. A. Tobias, R. P. Kling, E. Manougian, C. Y. Cheung, R. A. Fink, and J. H. Lawrence 200
Growth Hormone Studies in Acromegaly in Patients Receiving Pituitary Irradiation
J. F. Garcia 213
Heavy Particles in Acromegaly
E. Manougian 219
Heavy Particles in Radiosurgery of the Pituitary in Cushing's Disease
J. A. Linfoot, J. L. Born, C. A. Tobias, E. Manougian, C. Y. Cheung, and J. H. Lawrence 223
Preliminary Observations on an Untreated Control Series in Diabetic Retinopathy
R. P. Kling and J. A. Linfoot 230
Long-Term Follow-Up on 45 Patients with Diabetic Retinopathy Treated with Heavy-Particle Pituitary-Suppressive Therapy Between 1958-1961
J. L. Born, J. H. Lawrence, J. A. Linfoot, E. Manougian, and R. P. Kling 232
Radiation Studies in Parkinson's Disease
R. Tym and R. Weyand 241
Application of the Omnitron to Research in Neurological Surgery
R. A. Fink 244
Heavy Ion Effects on Ascites Tumor Cells
J. M. Feola 246
Therapeutic Possibilities with Heavy Particles
G. J. D'Angio 258
Physical Studies with Negative Pions
C. Richman, M. R. Raju, and S. B. Curtis 264
The RBE of Negative Pion Beams
W. D. Loughman 266

An Integrated Low-Background Counting Facility for the Omnitron
W. E. Siri, D. Van Dyke, and T. W. Sargent 273
In Vivo Visualization of an Accelerated Particle Beam Using the Positron Scintillation Camera
D. C. Van Dyke 278
In Vivo Activation Analysis with the Omnitron
T. W. Sargent 281
Biomedical Applications of Short-Lived Radionuclides
W. E. Siri 287
The Therapy of Neoplasms and the Oxygen Effect
C. A. Tobias and J. H. Lawrence 290
Educational Activities at the Donner Laboratory and Possible Future International Cooperation
J. H. Lawrence and C. A. Tobias 297

HISTORICAL SURVEY

C. A. Tobias and J. H. Lawrence

For many years there has been close cooperation between the Biomedical and the Physics and Chemistry groups of the Lawrence Radiation Laboratory, and many of the biomedical discoveries and techniques were made possible by basic developments in the physical sciences. Since the acceleration of particles is a difficult and complex speciality, developments in this field often took place in several stages. Ideas for developments in the field often appear several years before they can be technologically executed; whenever new particles have been accelerated successfully, this was followed by a wave of applications and discoveries, and the results achieved often could not have been fully predicted initially. Generally speaking, heavy particles — that is, protons, alpha particles and fast-moving heavy ions — have interest in science because of four distinctly different aspects:

First, these particles are a natural phenomenon; that is, they occur in nature. Their presence in interstellar space is indicative of the nuclear processes occurring in stars, and the fact that they impinge on the outer surface of planets leads us to assume that they may have influenced the evolution, physiochemical properties, and ecology of our planet.

Second, heavy particles can be used to probe the properties of inert or living matter. In this respect, as an information-gathering tool they may be regarded as similar to light. We use light and vision for discovery of the structure of the macroscopic and microscopic world. At the submicroscopic level, we must resort to the use of penetrating radiations or to accelerated particles to understand living and nonliving matter and the structure of atoms and nuclei. The heavy ions have particularly desirable properties for such purposes.

The third aspect of interest is the modification of matter by heavy ions. A very wide range of phenomena is encountered here. The nuclear interest began with the production and discovery of radioisotopes. Such

studies not only led to the identification and production of new elements, but also to our understanding of nuclear structure. Radioisotopes became essential tools in biology and medicine. Radiation studies are continuing as attempts are made to better understand how crystal lattices, biomolecules, and living cells are modified by heavy particles.

Finally, applications of accelerated particles include diagnostic and biophysical applications for disease-controlling processes, and in this field some unique approaches are available. The extent of the involvement of the Laboratory with accelerators can be perhaps most easily understood in a historical review.

Table I lists chronologically some of the dates of interest in the development of the Laboratory. The last thirty years may be divided into three periods. The first is that of nuclear discovery and development of neutron and isotope science (1931-1945). The second period concerns initial biomedical use of protons, and biophysical elaborations of isotope techniques yielding basic solutions to the problems of synthesis and turnover of biological systems (1945-1957). The third period began with the availability of mesons and heavy ions (1957 to present) and is continuing with elaboration of biomedical applications.

Biomedical interest began shortly after Ernest O. Lawrence and associates built the first cyclotron in 1931. Among the many medically important isotopes discovered at Berkeley, we might mention sodium-24, carbon-14, and hydrogen-3. The first demonstration of selective uptake of a radioisotope by the human body was made in 1936 by the use of iodine-131, demonstrating its uptake in vivo in the thyroid gland. This experiment as much as any other event has stimulated the development of tracer biology.

Carbon-14 was discovered in 1939 after initial attempts for following the pathway of carbon in biosynthesis had already been made in photosynthetic organisms by the use of the short-lived isotope, carbon-11. The discovery of ^{3}H at Berkeley followed that of ^{14}C by only a few months. Another milestone concerned the initial therapeutic application of radioisotopes, first attempted in 1936 by use of radioactive phosphorus-32 for the treatment of leukemia and related blood diseases. Many other medical applications of radioisotopes were made since that time.

Table I. Dates of interest in the development of the Laboratory.

1931	First cyclotron.
1933	Discovery of ^{24}Na.
1935	First biological experiment with neutrons (on mice).
1936	Selective uptake of an isotope *in vivo* (^{131}I) in the thyroid of a child.
1936	First isotope therapy of leukemia with ^{32}P.
1938	Carbon-11 used in photosynthesis research.
1939	Discovery of ^{14}C.
1939	Acceleration of C^{6+}.
1940	Discovery of ^{3}H.
1941	Acceleration of Ne^{10+} in cyclotron.
1942	Proton microscope proposed.
1943	Radioactive inert gases used in respiratory study.
1944	Synchrotron principle.
1946	Description of Bragg-peak effects.
1947	First large cyclotron completed.
1947	Neutron activation analysis for biology.
1948	First biological experiments with protons.
1948	Discovery of heavy primaries in cosmic rays.
1952	Radiation hypophysectomy in animals.
1955	First proton therapy — radiation hypophysectomy for mammary cancer.
1956	First large solar flare observed.
1957	Heavy ion linear accelerator completed.
1957	First heavy ion exposures of unicellular organisms.
1957	Discovery of proton radiation belt around Earth.
1958	Laminar cerebral lesions by protons.
1959	Treatment of acromegaly and diabetic retinopathy.
1961	Gamma-ray camera completed.
1961	Radiation induction of nerve reflex at Hilac.
1963	Carbon-11 activation by helium beam observed in humans.
1964	Corpus callosum cut by helium ion beam in animals.
1964	Omnitron principle.
1965	First exposure of biological specimens to pi mesons.
1967	Studies begin on heavy ion microscope.
1967	Heavy ion radioactivation of humans proposed for diagnostic studies.

In the initial period of the use of radioisotopes, many technical problems had to be overcome in the production of sufficient quantities of radioisotopes for biomedical use through grappling with the problems of measurement and quantitation. Techniques such as the radioautographic technique, special methods of measurements for carbon-14 and hydrogen-3 and other soft beta ray-emitting isotopes, had their origins in the early 1940's. Parallel with accelerator development, inquiries were made into the biological effects of the various particles, too, with the discovery that neutrons produced by the cyclotron appear to be more effective in producing biological effects than the X-rays. Beginning with 1935 neutron biological studies were initiated, and by 1940 some therapeutic approaches with neutrons were attempted.

It was realized early that in the cyclotron it is possible to accelerate not only hydrogen or helium nuclei, but heavier ions as well. The first few accelerated ions of carbon were produced in 1939 and of neon in 1941. It was in 1946 that the advantageous properties of protons for medical therapy due to the Bragg ionization property was described at Berkeley. Prior to this, due to the early neutron work and the early cellular studies, it was known that heavy ionizing particles had many interesting radiobiological properties. Biological studies with high energy protons and deuterons followed almost immediately after the first large cyclotron, the Berkeley 184-inch cyclotron, was completed. It was then realized that the heavy particles were much more suitable than other types of external radiation for delivery of well-localized depth dose inside of the body, with the regions of entry or exit receiving only minor doses. Since at this time it was well known that therapeutic attempts for tumor irradiation were limited by one's ability to concentrate most of the dose in the tumor and much less in the surrounding and intervening tissues, thoughts of improving radiation therapy spurred the investigations with heavy ions.

The radiobiological properties of the protons were so suitable for localized irradiation that, following detailed animal investigations, medical use of protons commenced in 1955. The first patient with widespread mammary cancer received pituitary irradiation with 340 MeV protons. We have twelve years' experience with therapeutic irradiation in several special

types of disease, particularly for the control by irradiation of the pituitary gland of a small percentage of patients with widespread metastatic breast neoplasms, and we have excellent results after irradiation of the pituitary in acromegaly and Cushings disease, and beneficial results in ameliorating the retinopathy in some patients with diabetes mellitus leading to blindness.

Certain procedures involve heavy particle exposure of brain, particularly for Parkinson's disease and brain tumors. All these techniques have been greatly augmented by research in other laboratories, particularly at Uppsala and at Harvard University.

In 1948 it was discovered (at the University of Minnesota in cosmic ray studies with high altitude balloons) that particles of high atomic number bombard the upper atmosphere. Some of these particles with considerable kinetic energy arrive from the sun and others are components of the galactic cosmic rays. The fact that these particles existed in nature with high velocities, and the potential application in biology of heavy charged particles, spurred scientists at Berkeley to consider accelerating multiply-charge ions with high intensity. Ideas were being formulated about 1950 and several methods were suggested then for a technique of accelerating multiply-charged ions to high velocities. Some of these methods are coming into full application in current design of new accelerators. In 1957 two heavy ion linear accelerators (Hilac) were completed at Berkeley and Yale University, and immediately thereafter a stream of data began to flow concerning the effects of heavy ions on cells and molecules of biological interest. Today heavy ion radiobiology is an established field.

In 1957, the same year that the Berkeley Hilac was completed, American scientists predicted and then discovered the space radiation belt around the earth. This is a region of high particle flux density mainly composed of fast protons and electrons. Recognition of solar flares and of the solar wind, both consisting predominantly of protons, followed shortly thereafter, particularly under the interest generated in 1956 by the appearance of a very large solar flare, which caused easily demonstrable changes in cosmic ray intensity in many places, even at ground level. Today we know that solar flares contain not only protons but alpha particles and heavier ions as well, and that if man should venture into space far enough and for long enough, he will be exposed to a significant dose from a stream of heavy ions of solar flare origin.

The application of proton and heavy ion beams to neurophysiology has become of particular interest during the last decade since neurological lesions may be made in well-defined locations in the brain without the hazard of bleeding. The lesion can resemble in effect bloodless cutting by the surgical knife, or in fact can be made in a variety of shapes. Radiation lesions are becoming of significant importance in studies of brain anatomy, physiology, and function. In 1961 it was demonstrated in the laboratory that pulsed beams of heavy ions may cause action potentials in excitable tissue. This finding has raised a number of questions with respect to the mechanism involved in initiation of the nerve impulse.

About 1958 we realized that the particles and energies available in current accelerators would not completely satisfy biomedical interests and that production of parallel beams of particles of high atomic number would be of great interest in many research fields.

Studies were then initiated in the Donner Laboratory with the cooperation of the Lawrence Radiation Laboratory toward finding adequate schemes for acceleration of the various ions of interest. Four years ago a study was completed on a heavy ion synchrotron which used a linear preaccelerator and which could accelerate ions up to krypton with reasonable intensity. This machine, however, had not sufficiently advantageous properties to warrant the large effort and expenditure of building it. The Laboratory has since then continued accelerator studies. Spiral ridge cyclotrons, monoenergetic high energy cyclotrons, linear accelerators, and synchrotrons with weak and strong focusing were studied in attempts to solve the need. A breakthrough in the design ideas came in 1964 when the principles involved in the Omnitron were first utilized. These are described in detail in the Omnitron report.[1] The Omnitron is probably the most versatile accelerator ever designed. It completely fulfills research needs for biology and medicine at the Laboratory for many years ahead. It is based on the synchrotron principle: The synchrotron becomes its own preaccelerator because it can be used in several successive acceleration cycles of partially or fully stripped ions. The storage ring enables one to store particles while the synchrotron ring is being reset for further acceleration. The Omnitron promises to accelerate beams of any of the nuclei in the periodic table, including nuclei of the heaviest elements with energies of approximately 500 million volts per

nucleon and protons up to 1.4 BeV. The beam is focused into parallel bundles of highly monoenergetic particles with considerable intensities.

The variety of beams produced by the Omnitron is of definite interest in several fields including nuclear chemistry, low energy nuclear physics, meson physics, and astrophysics, and of course in many branches of biology and medicine.

A chief mission of the Donner Laboratory is to provide a place for fundamental investigations into the biological effects of radiations from the molecular level through the cellular and organic level, and to serve progress in nuclear medicine and in related biophysical fields. For many of these studies we regard the Omnitron as a unique tool yet not available anywhere else in the world.

Reference

1. The Omnitron, a Multipurpose Accelerator, Lawrence Radiation Laboratory Report UCRL-16828, July 1966.

STOPPING-POWER AND RANGE-ENERGY DATA OF HEAVY IONS IN NONGASEOUS MEDIA

P. G. Steward

Introduction

The Omnitron will open to experimental study heavy ions that are presently unavailable. The behavior in a stopping material of ions with energies of greater than 10 MeV/amu and with atomic number Z greater than 18 have not been thoroughly investigated, either theoretically or experimentally, in the past, because no means of attaining these ions has been available. This accelerator's making such ions available for use in nuclear medicine and in radiobiology has stimulated a need to generate range-energy and stopping-power data in any arbitrary medium for ion energies up to 500 MeV/amu and Z numbers through 92.

We have developed a method for obtaining the stopping power of these particles by extending presently available experimental and theoretical studies of charged particle stopping power. Results of this method, which is described in some detail elsewhere,[1] are presented here. These results contain some degree of uncertainty, and thus adjustments in the data presented is inevitable as experimental data from the Omnitron becomes available.

Method

Emphasis has been placed, in the development of this method, upon being able to extrapolate the results of available stopping-power studies to any ion in any stopping medium at any kinetic energy ϵ for which $0.01 \leq \epsilon \leq 500$ MeV/amu. For some small subset of these ions, stopping media, and energy range, perhaps more accurate data can be derived at the expense of generality.

The method for obtaining this range-energy and stopping-power data is designed to be conveniently incorporated into a computer program. This program may be used as a subroutine for a main program in which this data is required, or it may be used alone to produce range-energy and stopping-power tables.

The present state of the experimental and theoretical studies of heavy ion stopping power makes it convenient to treat the problem separately for $Z \leq 10$ and for $Z > 10$. This division is due to the fact that experimental data are available for some ions with $Z \leq 10$ for most energies at which the atom is not completely stripped of electrons. Experimental stopping-power data for this energy region, which is very difficult to treat theoretically, is quite incomplete for ions of $Z > 10$.

Method for Z Less than Ten

For ions with $Z \leq 10$ we divide the calculation into two energy regions. For $\epsilon \leq 10$ MeV/amu we use a modification of a method proposed by Northcliffe[2] for smoothing experimental data. Our results calculated by this method differ from Northcliffe's best guess of the correct stopping power by less than the uncertainty we attribute to his results.

For energies above 10 MeV/amu we assume the ions with $Z \leq 10$ are completely stripped of their orbital electrons. Thus we can use the known proton stopping power with the relative stopping-power formula

$$-\frac{dE}{dX} = Z^2 \left(-\frac{dE}{dX}\right)_p, \qquad (1)$$

where $-(dE/dX)$ and $-(dE/dX)_p$ are the stopping power of the ion and the proton at the same velocity and in the same medium, Z is the atomic number of the ion. The proton stopping power is taken from the results of Barkas and Berger.[3] They have essentially solved Bethe's formula incorporating shell corrections.

Method for Z Greater than Ten

For ions with $Z > 10$ it is convenient to study the stopping power separately in each of four energy regions. The first is the low energy region defined by $137\,\beta \leq Z^{1/3}$. Here $\beta = v/c$, the ratio of ion velocity to the velocity of light. The reason for expressing the region boundary by this means is apparent if it is realized that $137\,\beta/Z$ is the ratio of the ion velocity to the average orbital velocity of its K-shell electron if screening effects are ignored. Thus the energy region boundary is defined in terms of the degree of ionization of the ion. In this low energy region, theory developed by

Lindhard et al.[4,5] for fission product stopping power has been normalized to some experimental fission product range data and adapted for computer programming. The theory includes both the effect of ionization energy loss and nuclear coulomb interactions.

A second energy region is the high energy region in which the ions can be treated as completely stripped of orbital electrons. This region is defined by $137\beta > 3Z$. Here we use the relative stopping power formula, Eq. 1, together with the Barkas and Berger proton stopping power as discusses above.

The third energy region is the medium-high energy region defined by $9 < 137\beta \leq 3Z$. This region is bounded on the low energy side by 2 MeV/amu and on the high side by three times the velocity of the ion's K-shell electron. The stopping power in this region is calculated from Eq. 1, again using the Barkas and Berger data for the proton stopping power. However, in this energy region the atomic number Z that appears in Eq. 1 must be replaced by an effective charge $Z_{eff} \leq Z$, which takes into consideration the fact that the ion is not completely stripped of its orbital electrons. To obtain Z_{eff}, charge state data for argon is derived from range-energy data taken from Northcliffe.[6] Extreme care is then taken in extrapolating this argon data to all other ions for which $Z > 10$. The extrapolating method is discussed in detail in Ref. 1.

The remaining region is the medium-low energy region. Here $Z^{1/3} < 137\beta \leq 9$. This is a very narrow energy region. Thus a cubic polynomial matching the slope and magnitude of the stopping power at both boundaries yield satisfactory values for the stopping power throughout the region.

Method for Obtaining Ion Range

We obtain the range by a simple numerical integration of the inverse of the stopping power from zero energy through the energy for which the range is desired. Minor exceptions to this statement are discussed in Ref. 1.

Results

This method for calculating heavy ion stopping power and range has been incorporated into a computer program. This program has been used to obtain data to be presented here for several selected ions and stopping

media. The ions chosen were hydrogen, helium-4, carbon-12, neon-20, argon-40, krypton-84, xenon-131, and uranium-238. Data is presented for each of these ions incident upon water, aluminum, copper, silver, lead, and uranium.

The stopping power of water is, of course, quite nearly the same as the stopping power of tissue. We can, therefore, use Fig. 1 as an approximation to the stopping power of particles in tissue. This quantity equals the total linear energy transfer (LET) when secondary particles are also included. The latter term is better known in radiobiology. The curves in Fig. 1 are also proportional to the Bragg ionization curve of individual particles. When many particles form a beam, statistical fluctuations and nuclear interactions will broaden the shape of the Bragg curve—a circumstance that is discussed in the following report by Litton et al. The shaded area in Fig. 1 corresponds to the stopping power of accelerated ion beams that are now available in the Hilac and in various cyclotrons. Thus this figure gives an estimate of the extended radiobiological potential of the beams of the Omnitron. The following points may be worthy of note:

1. Heavy particles above helium will be available with a range about 50 times greater than at present, allowing penetration of these ions of considerable depth of tissue; in fact, all the ions up to krypton will penetrate to 1/2 depth of the human head or more. This will allow research on multicellular organisms and on animals for the first time with heavy ions.

2. We do not have appreciable beams of particles heavier than argon available in any accelerator at the present with appreciable range; the Omnitron will be able to deliver beams of all the particles up to uranium and even uranium will have several g/cm^2 range penetration in tissue. It is also apparent from the graph that much higher stopping powers will become available for experimental investigation than heretofore. The maximum LET will be about 10 times the highest available now, reaching to nearly 20 000 keV per micron in the case of accelerated uranium.

3. The high-LET porton of the particle tracks will be much greater than that of particles available at present. For example, the argon range beam will have linear energy transfer above 100 keV/micron for about 10 cm of its range in tissue. This is an important point since laboratory data indicate that the oxygen effect in radiobiology becomes small only above 100 keV per

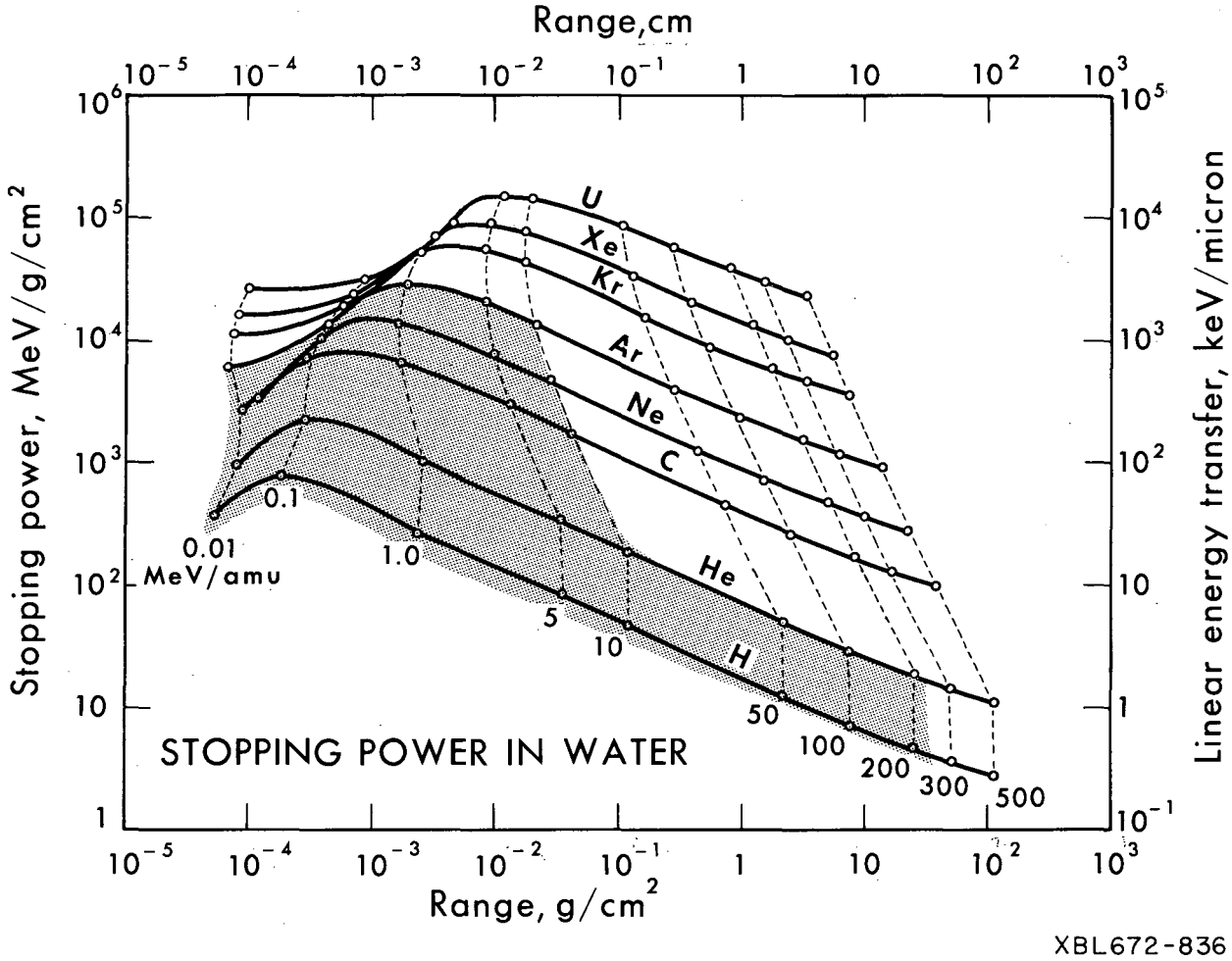


Fig. 1. Stopping-power curves as a function of range for various ions in water, as calculated by the computer program. Various ion energies in units of MeV/amu are designated on each curve. The Ne-C and Xe-U crossovers at low energy, although possibly a physical reality, occur in regions of low confidence.

micron. Should tumor therapy be attempted with heavy ions for elimination of the oxygen effect, the particles with intermediate atomic numbers might prove to be most suitable. (See in this volume, "Cancer Therapy and the Oxygen Effect" by J. H. Lawrence and C. A. Tobias and "Therapeutic Possibilities with Heavy Particles" by G. J. D'Angio.)

4. Attention is drawn to the lower energy portion of the very heavy ion tracks where an anomaly appears to occur in the stopping power. This is the domain where nuclear collisions predominate in stopping, and the energy loss process here is comparable with very intense heating of an extremely localized region at the core of the track. Ionization effects of this type appear to cause biological effects best explained by the so-called thermal spike model. Some of these effects have already been explored. For example, scientists at the General Electric Company have produced extremely localized melting of the absorber with fission recoils which led to the formation of extremely small holes of a few angstroms in diameter.

Figure 2 gives stopping-power curves in aluminum and Fig. 3 in lead. The curves will have to be verified of course by experimental investigations after the accelerator is available.

Figure 4 is a graphic representation of the integrated data showing the range-energy relationship for various particles in water. The data are plotted to 500 MeV/amu, the design energy of the Omnitron.

References

1. P. G. Steward, Calculation of Stopping Power and Range-Energy Data for Any Heavy Ion in Nongaseous Media, Lawrence Radiation Laboratory Report UCRL-17314 (1967).
2. L. C. Northcliffe, Passage of Heavy Ions Through Matter, Ann. Rev. Nucl. Sci. 13, 67 (1963).
3. W. H. Barkas and M. J. Berger, Tables of Energy Losses and Ranges of Heavy Charged Particles, in Studies in Penetration of Charged Particles in Matter (National Academy of Sciences—National Research Council, Washington, D. C., 1964), p. 103.
4. J. Lindhard and M. Scharff, Energy Dissipation by Ions in the keV Region, Phys. Rev. 124, 128 (1961).

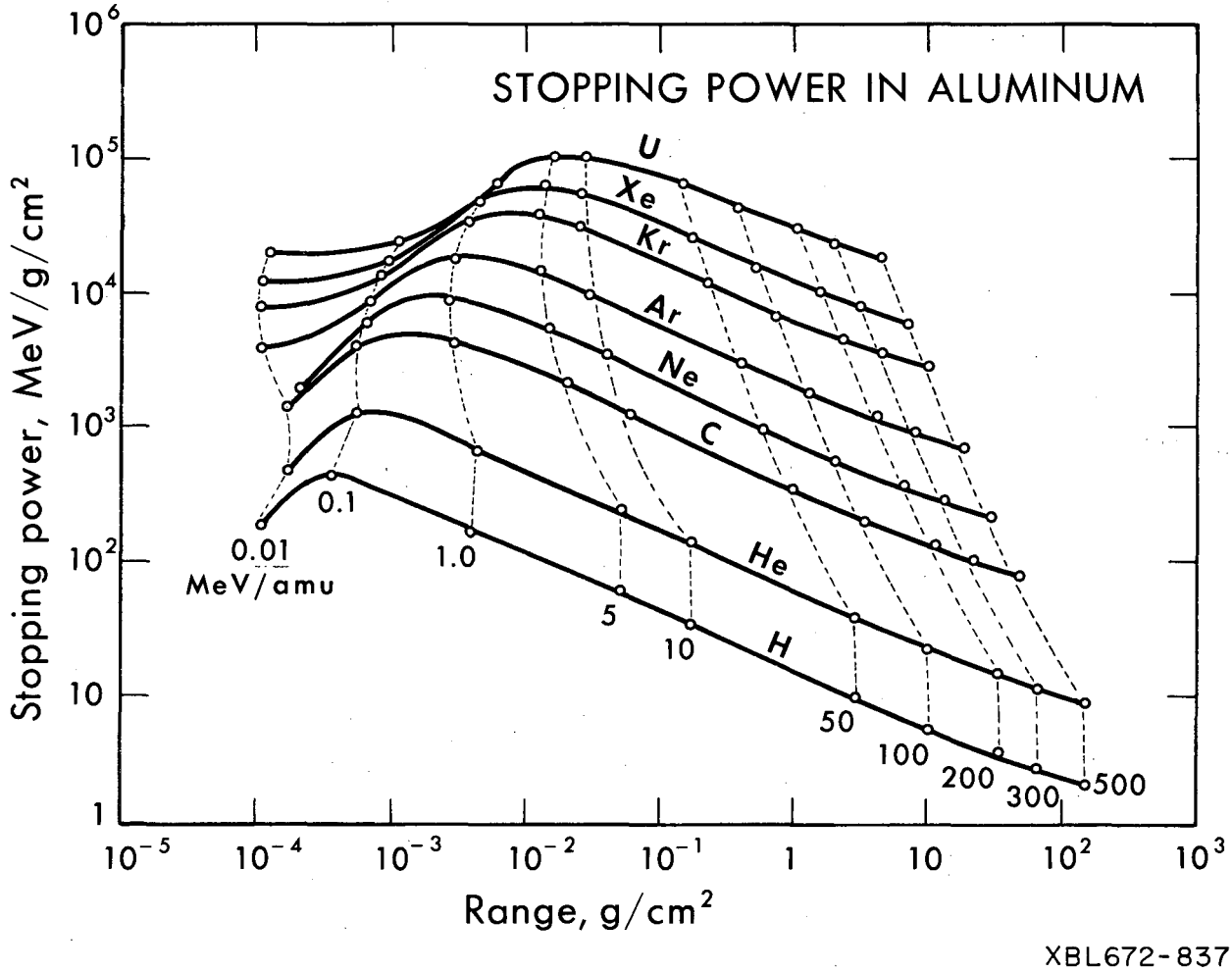


Fig. 2. Stopping-power curves as a function of range for various ions in aluminum, as calculated by the computer program. Various ion energies in units of MeV/amu are designated on each curve.

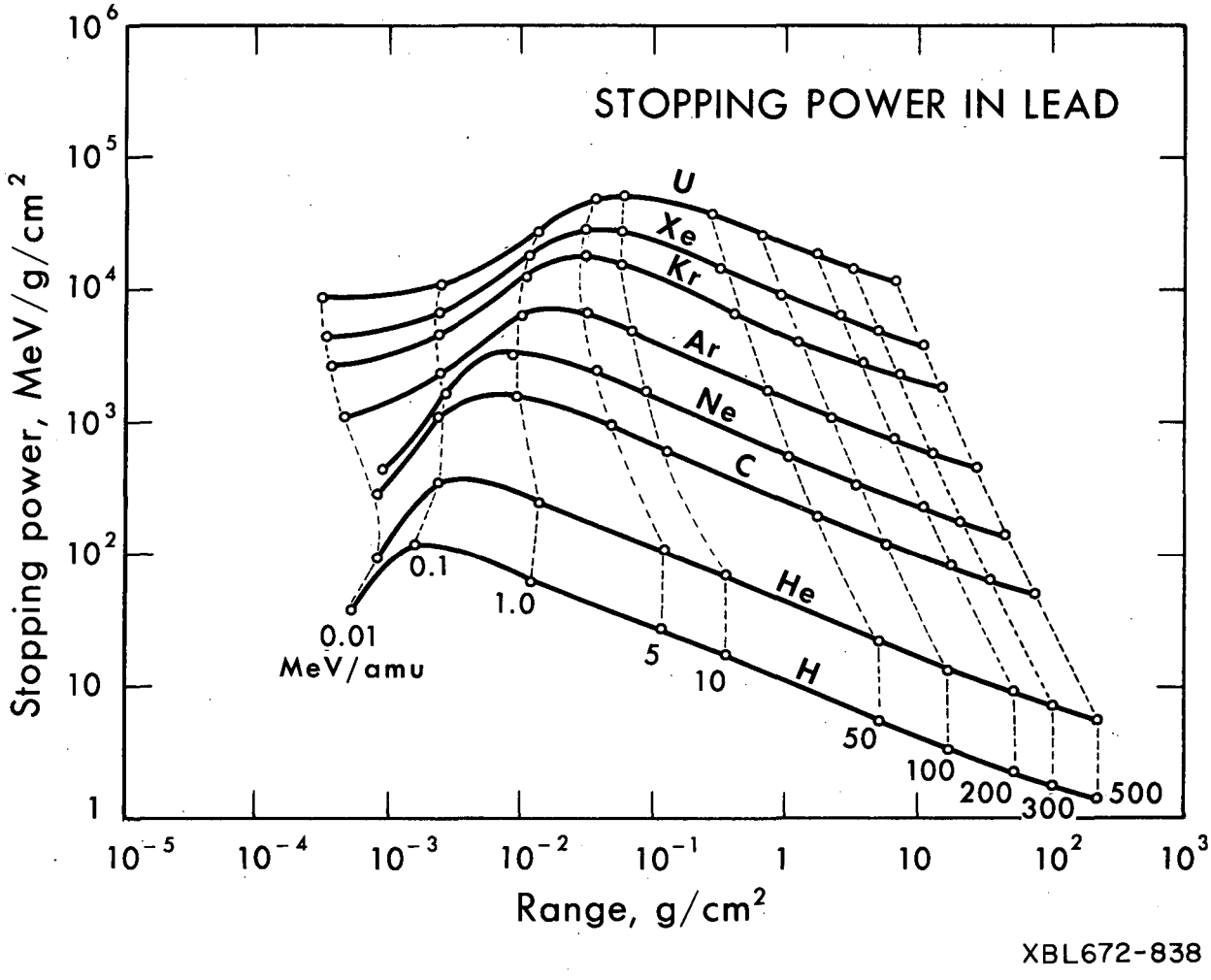


Fig. 3. Stopping-power curves as a function of range for various ions in lead, as calculated by the computer program. Various ion energies in units of MeV/amu are designated on each curve.

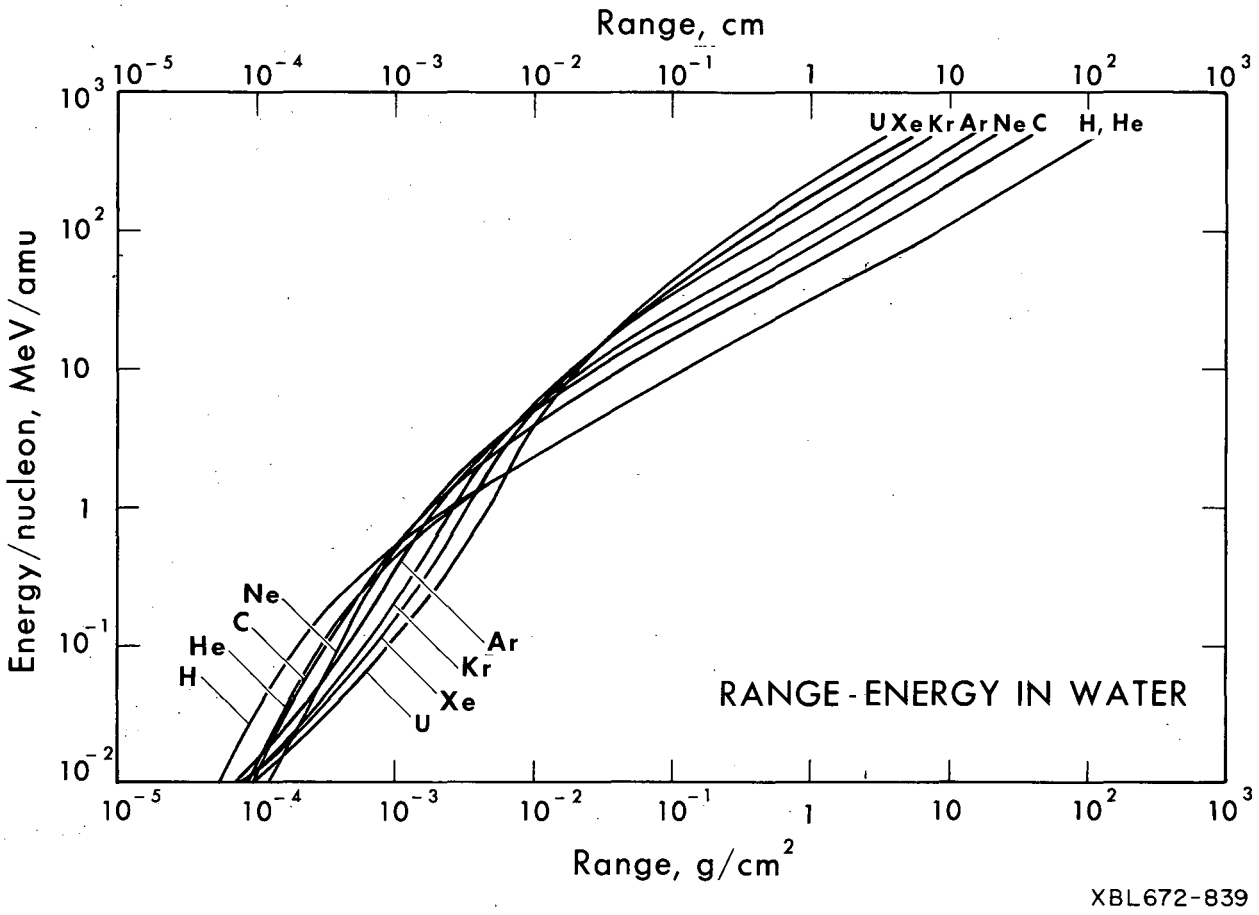


Fig. 4. Range-energy curves for various ions in water, as calculated by the computer program. For any energy on the ordinate, the residual range is read from the abscissa.

5. J. Lindhard, M. Scharff, and Schiott, Range Concepts and Heavy Ion Ranges (Notes on Atomic Collisions, II), Kgl. Danske Videnskab. Selskab, Mat.-Fys. Medd. 33, 14 (1963).

6. L. C. Northcliffe, Passage of Heavy Ions Through Matter II: Range-Energy Curves, in Studies in Penetration of Charged Particles in Matter (National Academy of Sciences—National Research Council, Washington, D.C., 1964), p. 173.

PENETRATION OF HIGH ENERGY HEAVY ION BEAMS INTO MATTER

G. M. Litton, R. W. Wallace, and C. A. Tobias

Abstract

A method for calculating Bragg ionization curves and number-distance curves in various materials has been developed for high energy, very heavy particles.

Previous attempts for calculating Bragg curves did not adequately take into account the various nuclear interaction processes. At the energies of interest, these processes can play an important role in determining the shape of the Bragg curves. In the present method, a mathematical model is developed which couples together the processes of ionization energy loss and nuclear interactions.

Another feature of the present study is the manner in which multiple-scattering corrections are applied. Previous work has been generally limited to relatively light ions. We have made the extension to arbitrarily heavy ions.

The calculated results agree very well with experimental data for those cases in which secondary-particle production is of minor importance. It is further demonstrated that even when secondaries are a large contributing factor, the method yields valuable information regarding the variation in energy deposition with the atomic number of the penetrating particles.

Introduction and Method of Analysis

In the experimental use of accelerated heavy ions for producing lesions in living tissue, important considerations with respect to depth-dose distributions relate to the efficiency by which the various types of lesions can be produced. For example, for knife-edge cutting lesions one would wish to have a sharp thin sheet of beam with relatively little lateral scattering. For producing laminar lesions deep in tissue one would wish to have the width of the Bragg peak to be as small as possible compared to the depth penetration of the beam, and for producing deep lesions it is of advantage to have the so-called Bragg ratio (the ratio of the peak ionization to the initial ionization at the point of entry of tissue) as high as possible. The calculated data presented

in this summary paper indicate that heavy ions with atomic number between 10 and 30 appear to have a distinct advantage for the production of deep lesions compared to electrons, mesons, protons, or alpha particles. It will be shown that the Bragg ratio is higher for atomic numbers between 5 and 10 for range penetrations of interest in biology, that the width of the Bragg peak is smallest for the same range penetration when heavy ions are used but particularly between atomic numbers of 10 and 20, and that the lateral scattering of the beam is also drastically reduced, particularly for atomic numbers greater than 10.

The geometry considered is that of a parallel beam of monoenergetic particles incident on a slab of material whose transverse dimensions are large compared to both the beam dimensions and the depth of penetration of the beam.

Ionization Energy Loss

If the particles are neutral atoms and if their energy is sufficiently high, then as they traverse the medium, some or all of their electrons will be stripped off. The ionization energy loss results from many inelastic collisions with the electrons of the medium, causing a net transfer of energy to the medium. The ionization energy loss rapidly increases as the particles are slowed down, until their speed is so low that they begin to pick up atomic electrons.

Although there are many facets to the highly complex process of charge exchange and ionization energy loss, a wealth of experimental evidence exists which backs up a great deal of theoretical work initiated by Bohr and Bethe. Consequently, for protons very good estimates are available for the rate of ionization energy loss of many different materials.

The calculation of the ionization energy loss used in this study is based on the work of Steward for heavy nuclei (described in this volume).

Multiple Scattering

The process of small-angle multiple scattering leads to a divergence of the beams and also to a decrease in the mean range of the particles. Compared to this process, the effect due to large-angle Rutherford scattering is of second order. Correction factors due to multiple-scattering are applied to the basic results.

Straggling

The process of ionization energy loss occurs in discreet steps, rather than as a continuous process. This fact leads to the general concept of straggling, whereby a given particle energy does not correspond to a unique position in the medium. Instead, at a given position, there is a distribution of energies, or conversely, at any given energy, there is a distribution in distances.

In calculating the Bragg curves, the analysis is performed in such a way that the processes of ionization energy loss, nuclear attenuation, and straggling are all taken into account simultaneously.

Nuclear Interactions

At very high particle energies, nuclear reactions play an important role in the energy loss processes. A great many different reactions occur, and the total process is exceedingly complex. In such an inelastic collision, many different secondary particles can be produced, including recoil nuclei, protons, neutrons, and gamma rays of medium particle energies and mesons at higher particle energies. The secondary particles will exchange energy with the medium by the processes of ionization loss (charged particles only) and nuclear interaction (all particles).

A complicating factor is that the angular and energy spread of the secondary particles, as functions of the incident particle atomic number and energy, and the composition of the absorber, are not accurately known.

Fortunately, at relatively low incident energies, some experimental and theoretical data are available from which we have drawn the basis for many simplifying assumptions with regard to the production of secondaries and the mode of energy loss of these particles. It will be necessary to perform additional theoretical and experimental studies on these matters.

Other Processes

There are some other processes which influence the passage of charged particles through matter. Most of them are not very significant from the standpoint of energy deposition. They include Rutherford scattering, energy transport via fast secondary electrons, and Cerenkov radiation.

Calculations and Results

A computer program (Bragg) was written for calculating Bragg curves and number-distance curves for various atomic numbers. This program will be made available shortly. In making comparisons with experimental results, two calculation parameters were allowed to vary so as to obtain the best possible agreement. The first of these parameters related to the total cross section for nuclear interactions and the second parameter to the degree of straggling.

Figures 1 and 2 show typical Bragg curves. For these two cases, the experimental data were available as a check; these data are also plotted on the figures. The shapes of the curves are quite typical, although the full width at half maximum and the peak-to-plateau ratio are strongly dependent on the charge and initial energy of the beam. Figure 3 shows a typical number-range penetration curve. Again, experimental data are shown for comparison. The discrepancies are due to secondary particle production, multiple-scattering processes, and energy spread and imperfect collimation of the beam.

Comparisons of the calculations with available data indicate the following:

a. The nuclear radius r_0 that gives good approximation for the ionization data is 1.1×10^{-13} cm.

b. The straggling parameter which appears to fit existing measurements with alpha particles is closely similar to that calculated by Bethe for straggling in protons.

To demonstrate the effect of the incoming particle charge on the shape of the Bragg curve, Figs. 4 through 6 are shown. These give Bragg curves for various ions in water, with the initial energies being chosen such that the Bragg peaks are all at 10 cm.

Using these and other similar data, the results shown in Fig. 7 are obtained. This figure shows the peak-to-plateau dose ratio for Bragg peaks at 5 and 10 cm. The following conclusions may readily be drawn. The full width at half maximum falls off extremely rapidly with increasing atomic number, up to a value of approximately 10. For greater values of the atomic number, the width increases very slowly. The peak-to-plateau ratio appears to have a maximum in the vicinity of an atomic number of approximately 10,

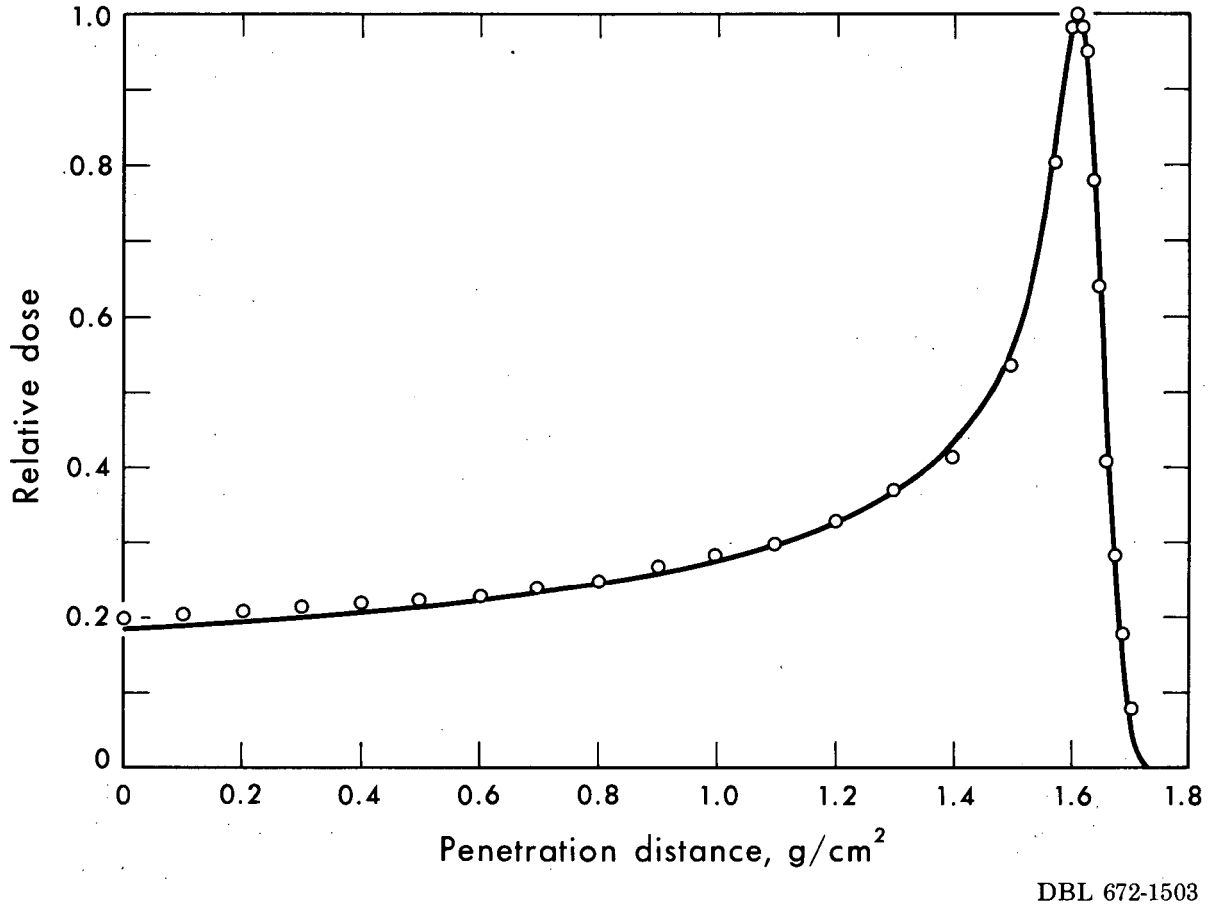


Fig. 1. Bragg curve for 43 MeV protons in water. The enclosed points are the experimental data.

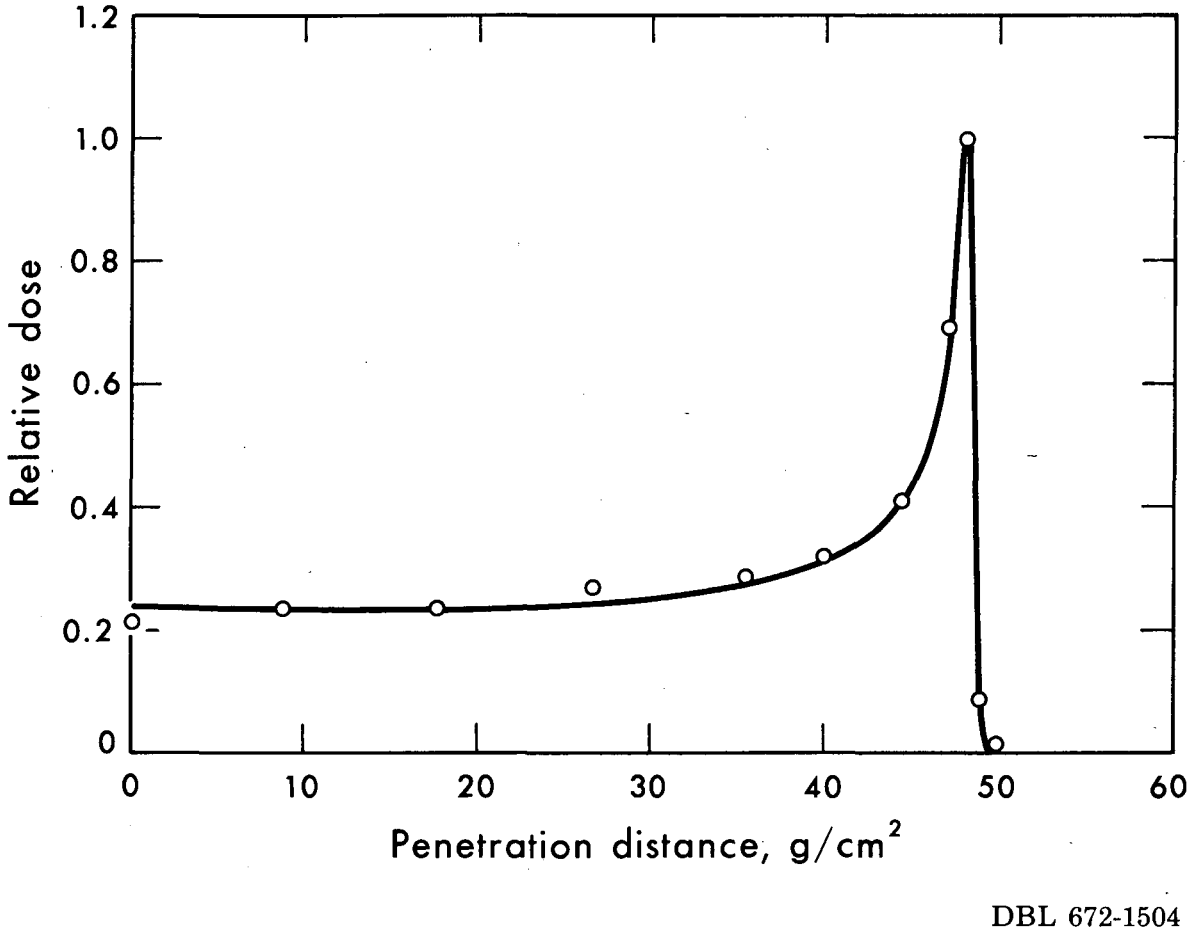


Fig. 2. Bragg curve for 910 MeV helium ions in copper.

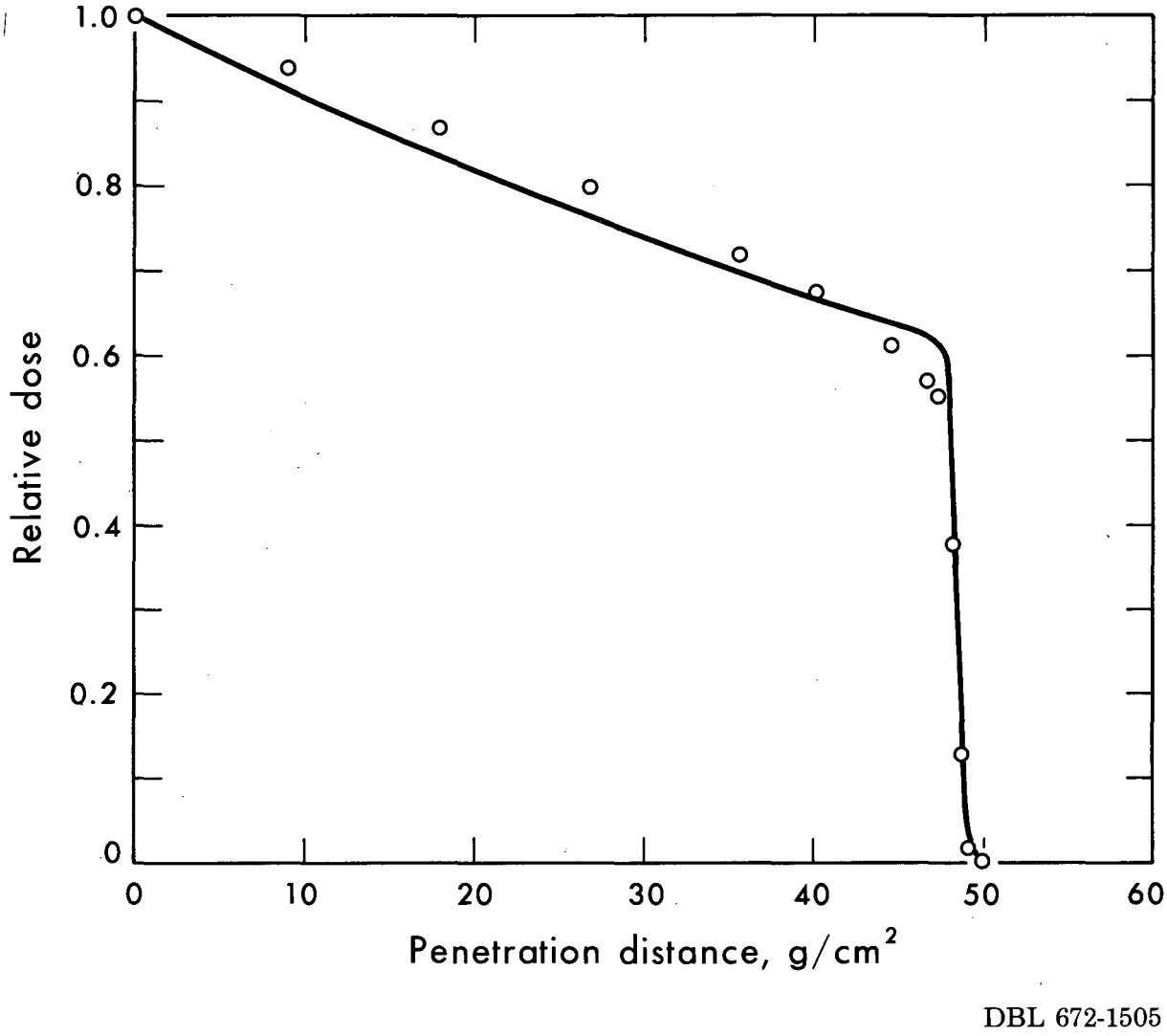


Fig. 3. Number-distance curve for 910 MeV helium ions in copper.

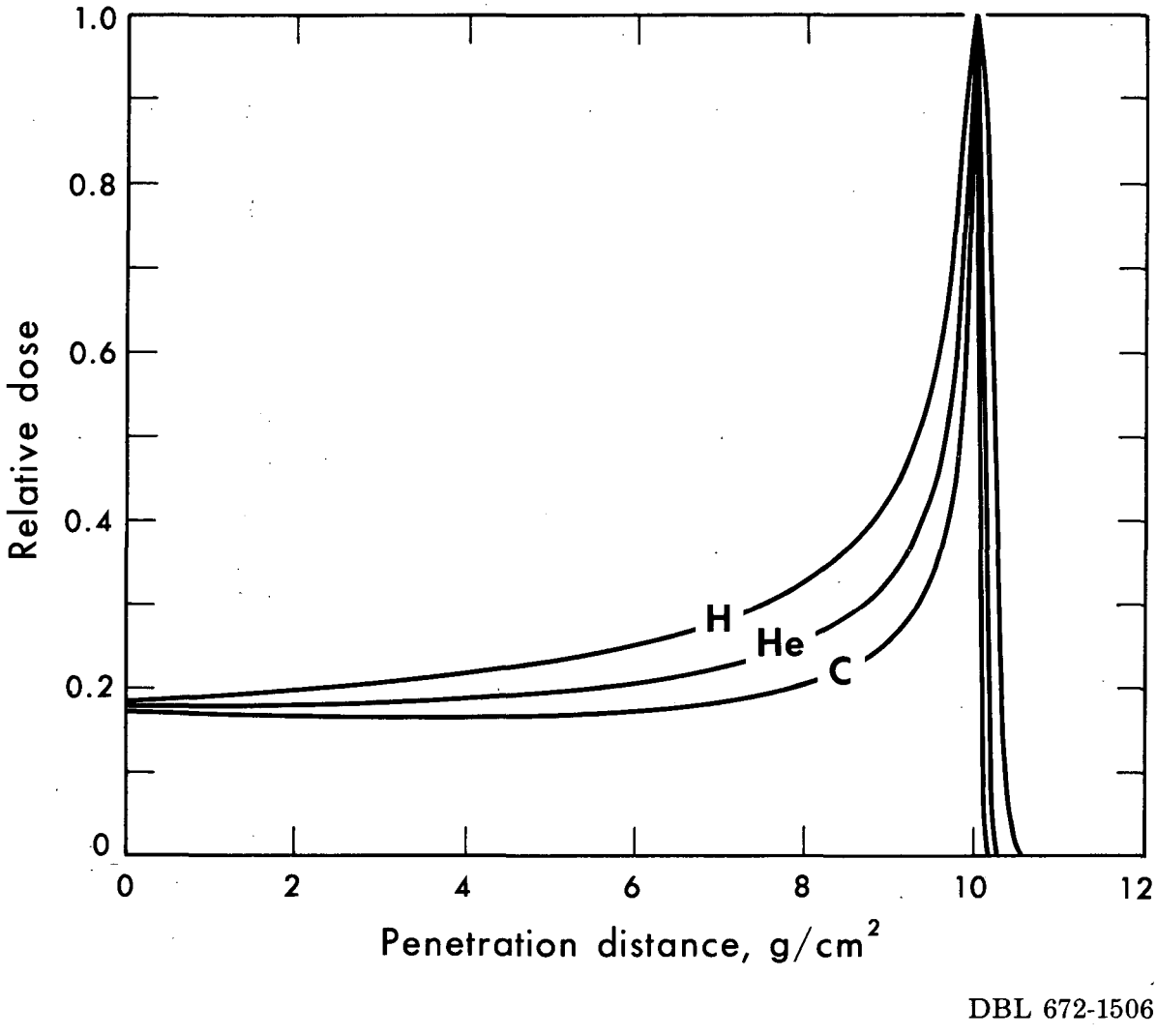


Fig. 4. Bragg curves in water for H, He, and C ions, with the Bragg peak at 10 cm.

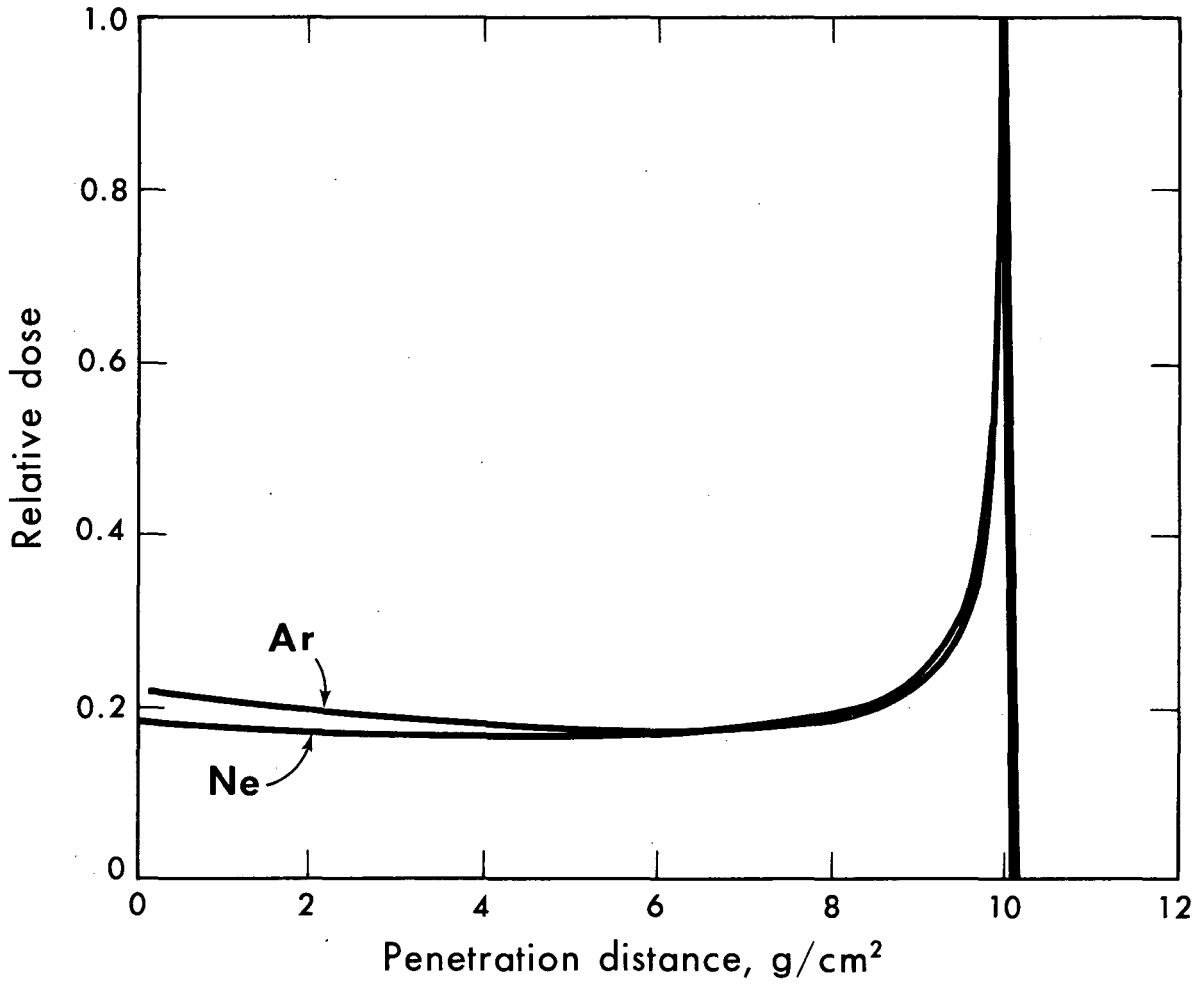


Fig. 5. Bragg curves in water for Ar and Ne ions, with the Bragg peak at 10 cm.

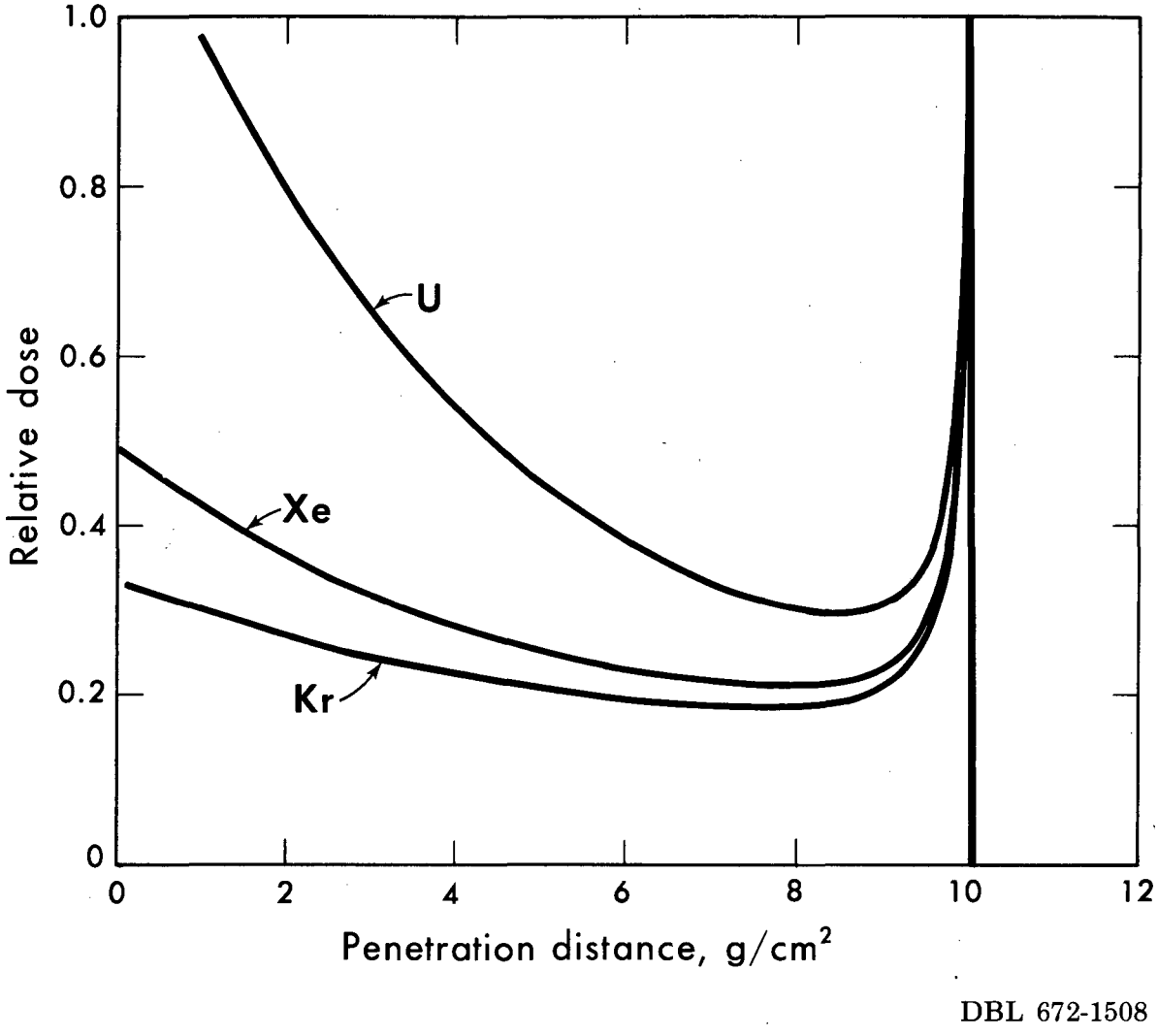
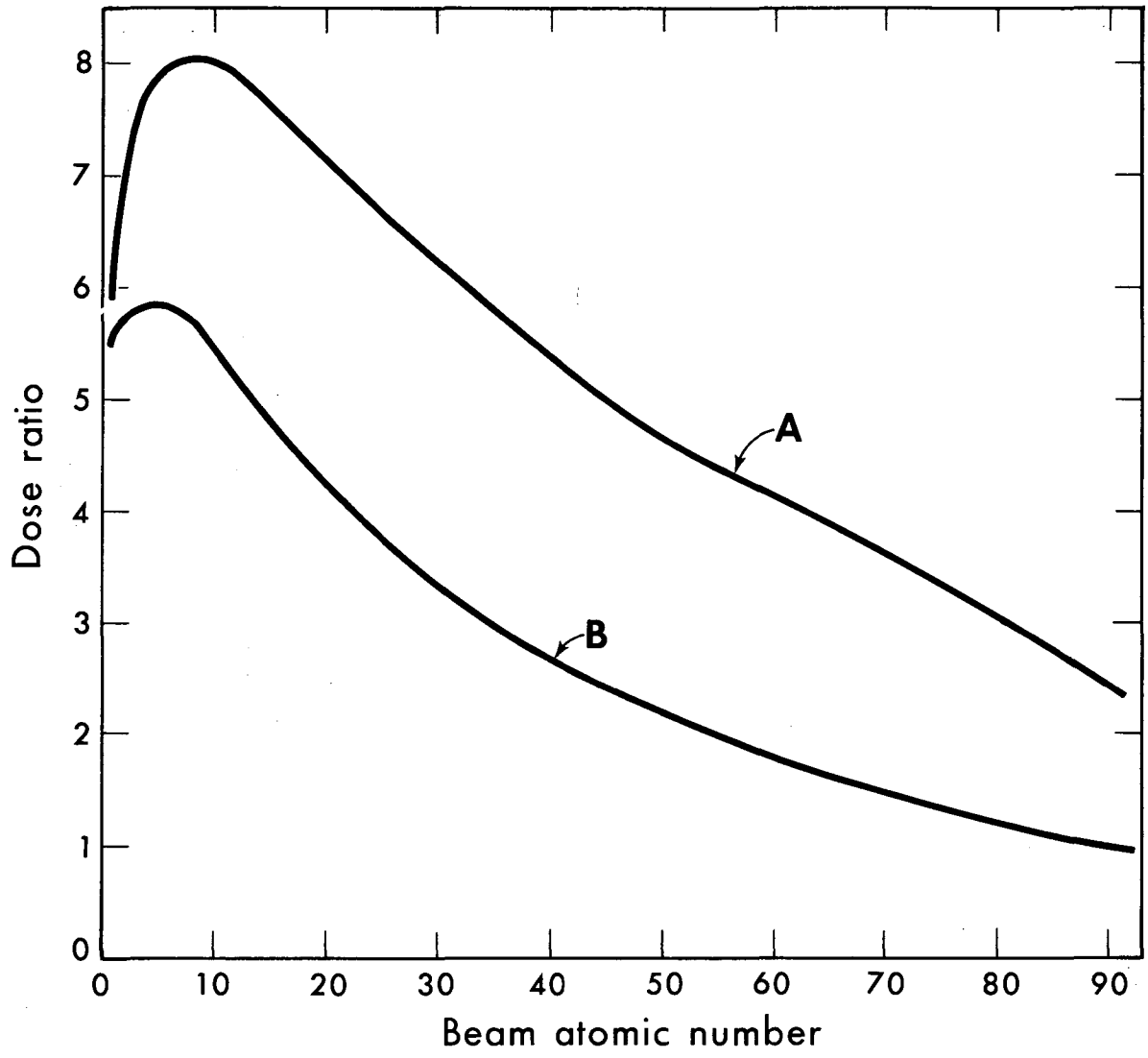


Fig. 6. Bragg curves in water for U, Xe, and Kr ions, with the Bragg peak at 10 cm.



DBL 672-1509

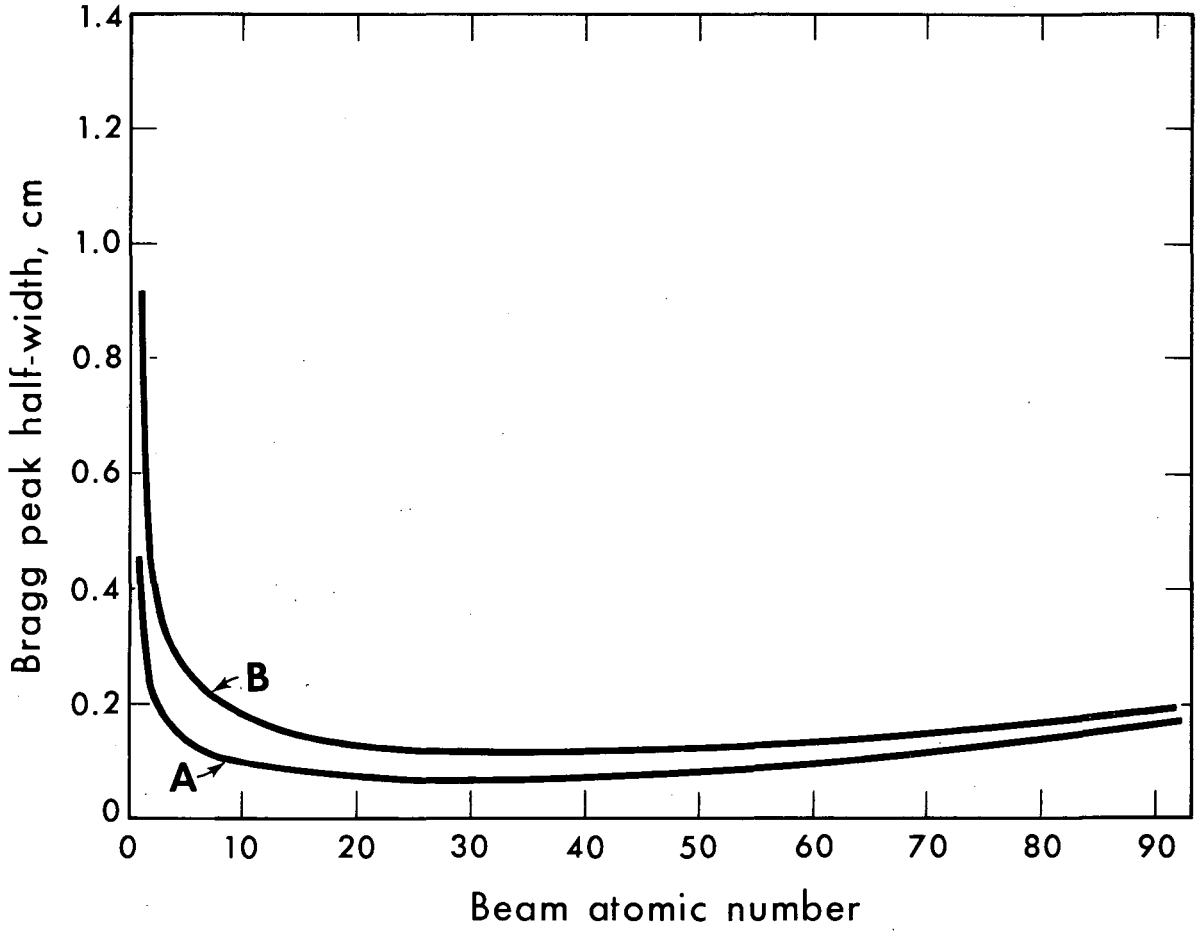
Fig. 7. Peak-to-plateau dose ratio as a function of beam atomic number for ions in water.
Curve A: Bragg peak at 5.0 cm
Curve B: Bragg peak at 10.0 cm

after which the ratio decreases fairly slowly with increasing atomic number. At the 184-inch cyclotron, alpha particles used in therapy have a Bragg ratio of about 3. Thus, considerable improvement can be produced with the Omnitron.

A point of interest is the width of the Bragg peak at 1/2 maximum since this determines the depth of the lesion that may be obtained in certain biological-biomedical experiments; for example, in the production of laminar lesions in the brain it is essential to keep the depth of the lesion minimum. The data plotted on Fig. 8 summarize our conclusion for range penetration of 5 and 10 cm to the fact that the half-width of the Bragg peak can be minimized at the given range penetration by applying an ionizing particle of high atomic number. The optimum appears to be atomic number of about 25, but already neon with an atomic number of 10 gives satisfactory results. For example, at 5 cm penetration into tissue, the width of the Bragg peak might be as small as 1 mm.

Our preliminary calculations also indicate that multiple scattering can play an important role in the energy loss process. By going to very heavy ions, the multiple scattering effect is greatly reduced—by as much as a factor of 5. The importance of this cannot be overemphasized, inasmuch as multiple scattering effectively places a lower limit on the size of a beam. In other words, the use of heavy ions will allow beams of significantly small size to be utilized, and such beams promise to obtain sharper cutting lesions than obtainable at present.

The calculations made to date have been concerned only with energy loss from primary beam particles. For those energies at which secondary particle production is significant, one would expect the results to be at least somewhat different from those obtained here. At several hundred MeV/amu, we predict that the contribution of secondaries can be a significant part of the ionization process for heavy ions (e.g., up to 30% of ionization may come from this source). Due to the paucity of data concerning secondary particle production, it is, however, difficult to perform such calculations with precision. Consequently, reliance must be placed on experimental measurements, which cannot be made at present.



DBL 672-1510

Fig. 8. Bragg peak full width at half maximum as a function of beam atomic number for ions in water.
Curve A: Bragg peak at 5.0 cm
Curve B: Bragg peak at 10.0 cm

HEAVY PARTICLES IN SPACE: PHYSICAL ASPECTS*

S. B. Curtis

Ever since the heavy component of the galactic cosmic rays was discovered,[1] some concern has been expressed on the possible danger that such ions might present to humans in high-flying aircraft and in spacecraft on extended missions.[2-5] Analyses have been hampered by lack of information not only on the biological effects of these ions but also on the physical parameters involved. For instance, the identity and energy spectra of the various heavy components were poorly known until recently, and even now we lack information in certain regions of the periodic table. Secondly, the penetration of heavy ions through matter is poorly understood, so that doses deep in absorbers can only be crudely estimated.

In the past year or so, information on the identities and spectra of ions in the galactic cosmic rays has accumulated quite rapidly from well-instrumented satellite and balloon flights. However, due to a lack of a laboratory source, progress in the area of the penetration of high energy heavy ions has been considerably slower.

Heavy Ions in Galactic Cosmic Rays

Recent experiments on the OGO-I satellite,[6,7] the IMP III satellite,[7] and balloon flights,[7,8] have yielded extensive new information on the fluxes of heavy ions in the galactic cosmic radiation during the minimum of the solar activity cycle, when these fluxes reach their maximum values. A recently published compilation of data from the OGO-I satellite[6] is shown in Fig. 1. It is interesting to note that the spectra are all close to their maximum values in the several hundred MeV/nucleon range, and ions of these energies can penetrate shielding of a few g/cm^2. At 200 MeV/nucleon, an iron ion, one of the ions of highest Z observed and therefore of shortest range, will travel 2.8 g/cm^2 (1.04 cm) of aluminum if it does not undergo a nuclear interaction. The Bragg curve in aluminum for a monoenergetic beam of iron ions of energy 200 MeV/nucleon is shown in Fig. 2. It is seen

*A portion of this study was performed under NASA contract NASw-1362.

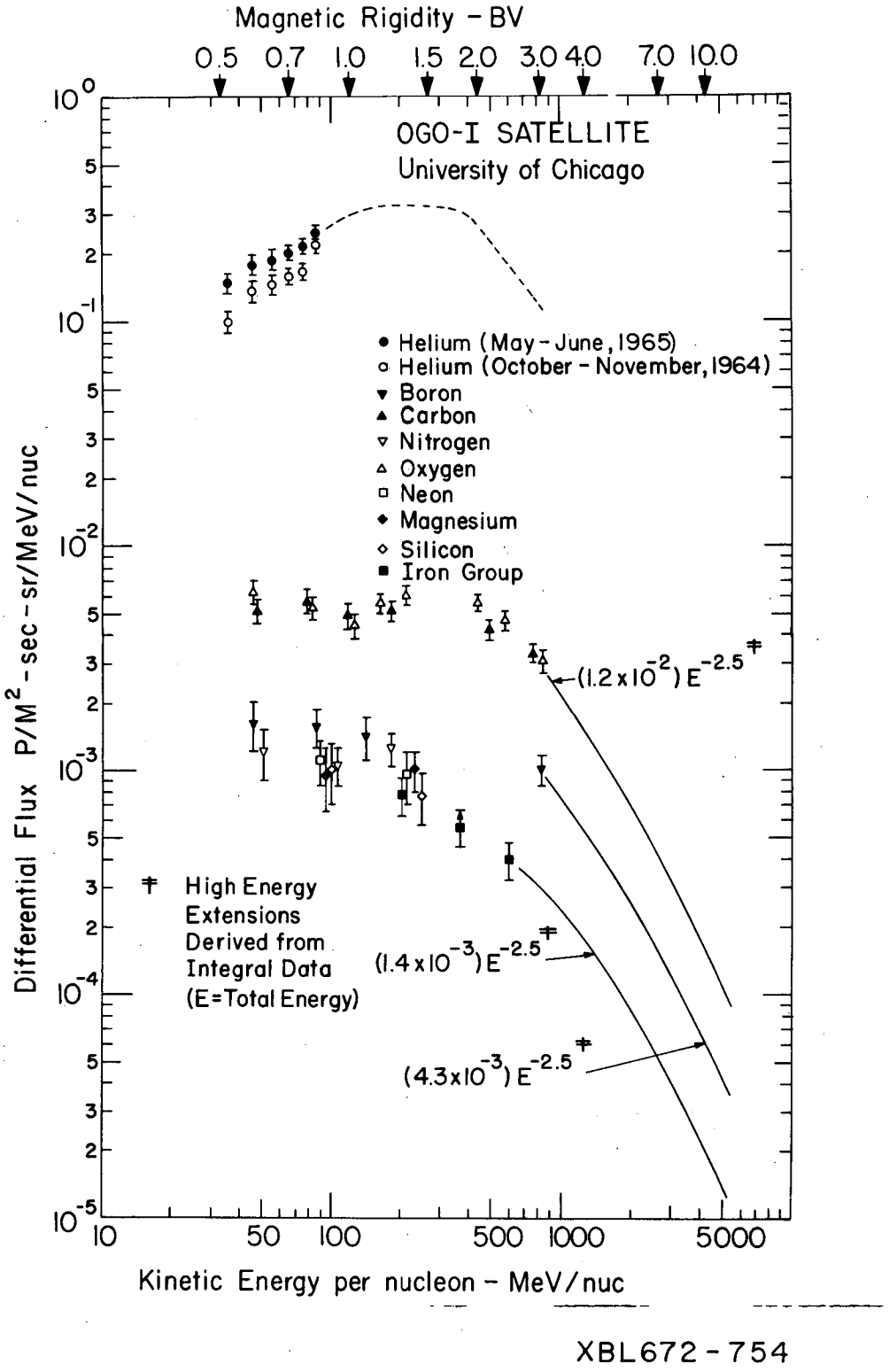


Fig. 1
(from reference 6)

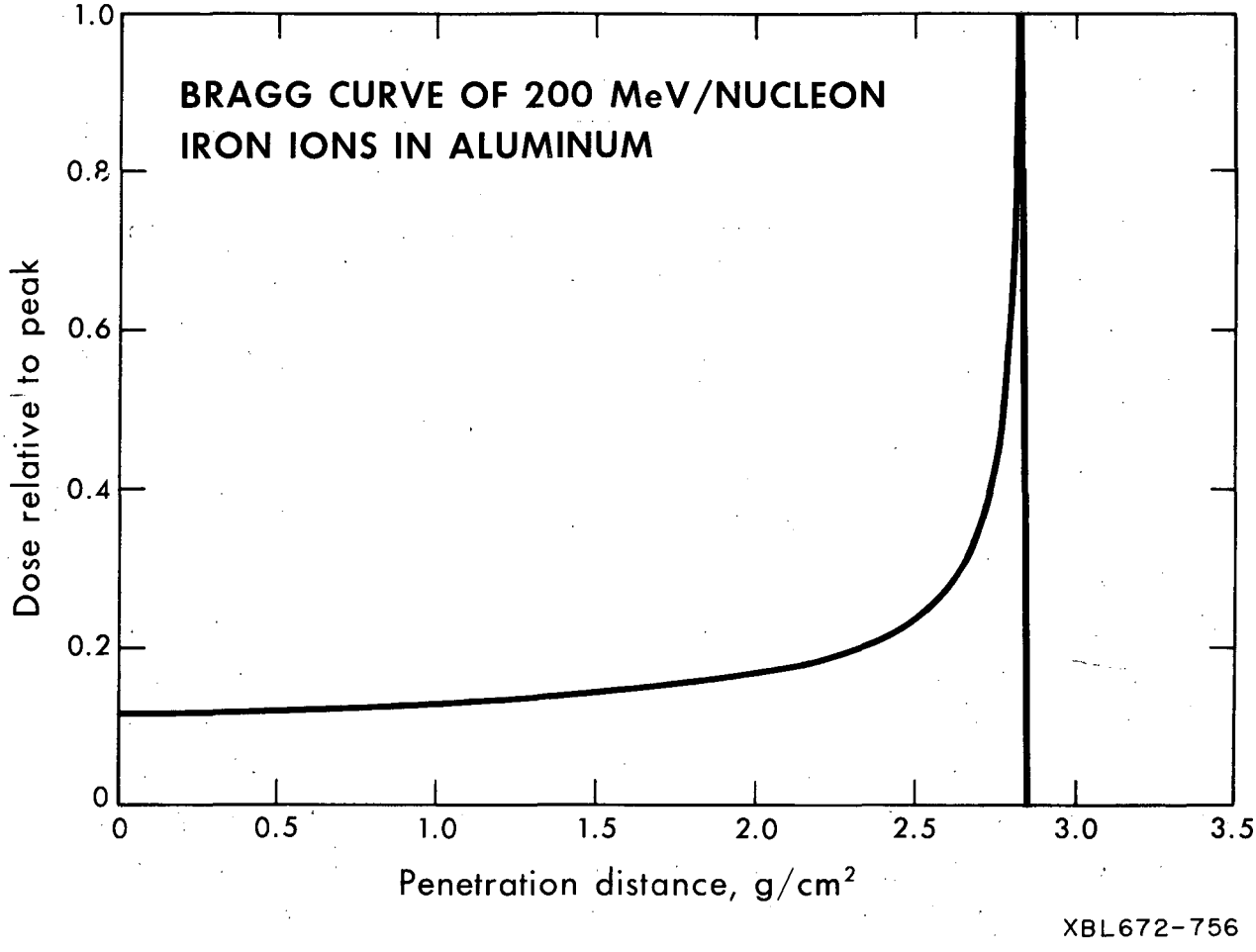

BRAGG CURVE OF 200 MeV/NUCLEON
IRON IONS IN ALUMINUM
Dose relative to peak
1.0
0.8
0.6
0.4
0.2
0
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Penetration distance, g/cm²
XBL672-756

Fig. 2

that thicknesses greater than 2.8 g/cm^2 are needed to stop such ions. The secondary products of nuclear interactions of the ions with the aluminum nuclei can be expected to penetrate more deeply than this. Other ions of the same energy/nucleon but with a lower Z will travel farther. We also note that although the flux of iron-group ions ($26 \leq Z \leq 28$) is down from the helium flux by two orders of magnitude, as shown in Fig. 1, the dose deposited is multiplied by the square of the charge on the ion, and since this ratio between iron and helium is 170, the doses in free space from each ion should be of the same order of magnitude. The following maximum yearly free-space doses have been calculated from recent spectra of the various components of the galactic cosmic radiation during solar minimum:

Protons:	4.8 rads/yr	From Pioneer 6 data[9]
Helium ions:	3.1 rads/yr	From IMP III data[10]
M-particles:	1.9 rads/yr	($6 \leq Z \leq 9$)
LH-particles:	1.3 rads/yr	($10 \leq Z \leq 14$)
VH-particles:	1.3 rads/yr	($26 \leq Z \leq 28$)
Total:	12.4 rads/yr	

We note that over one-third of the dose is from components heavier than helium, and the protons alone account for less than one-half. From the standpoint of radiation within a spacecraft, the dose from the heavier components, after an initial small build-up, will decrease in the shielding of the spacecraft walls more rapidly than will the proton component. Because of their high initial energy, however, the number of stopping ions (sometimes called thin-down hits) falls off rather slowly as a function of absorber depth. An accurate estimation of this fall-off is difficult because of the complexity introduced by the fragmentation of heavier ions into lighter ones within the absorber. There is no experimental information on interaction cross sections and secondary particle emission probabilities in interactions of high-energy heavy ions with light nuclei. Lower limits to the number of thin-down hits per cm^3-day can be obtained, however, by assuming a nuclear mean free path, but neglecting entirely the heavy secondary ions emerging from the nuclear interactions. This is not a bad approximation for the heaviest ions, the VH-particles or iron group ($26 \leq Z \leq 28$), but becomes increasingly worse for ions of lower mass. To give a rough idea of the dependence of

thin-down hits on absorber, such lower limits are shown as a function of absorber thickness in Fig. 3 for slab water shielding. It is interesting to notice that from 1 to 15 cm, the number of M-particle ($6 \leq Z \leq 9$) thin-downs decreases by only a factor of 3 and the number of LH-particle ($10 \leq Z \leq 14$) thin-downs decreases by only a factor of 4. Proper account of the heavy secondaries, of course, would cause this decrease to be even less severe. In this calculation, average values for A and Z^2 were chosen for each group. Also, since no data are available on interaction mean free paths of the various heavy ions in water in this energy region, the values for hydrogen and oxygen taken from Waddington[11] were used to calculate the mean free path in water, thus increasing somewhat the uncertainty of the calculation.

Heavy Ions from Solar-Particle Events

M-particles have been detected in several large solar-particle events.[12] Fluxes appear to be less than 2% of the flux of helium ions in the same energy/nucleon range, and this ratio seems to remain relatively constant from event to event. The energy spectrum of these particles peaks at very low energies, and the largest total dose has been estimated[13] for the giant event on November 12, 1960, at 2 rads behind 2 cm of water and 0.2 rads behind 5 cm. There is some evidence that low fluxes of particles with $Z \geq 10$ are also present in these events, with neon ions being the most prevalent in this group.[14]

In summary, the following points can be made concerning the physical aspects of heavy ions in space:

(a) Fairly good experimental data are now available on heavy galactic cosmic-ray fluxes in space, up to Z = 28, as measured during the maximum flux period of the last solar cycle (1964-65). Ions heavier than helium contribute over 35% of the dose in free space.

(b) The energy spectra of all components seem to peak around 200 MeV/nucleon. Because of this large initial energy, the number of thin-down hits per cm^3-day in 15 cm of water is still at least 25 to 35% of its value at 1 cm of water for all ions except those in the iron group.

(c) Physical parameters, such as the cross sections for heavy secondary formation and interaction mean free paths, have not been measured in water at these energies. The lack of this information hampers an accurate

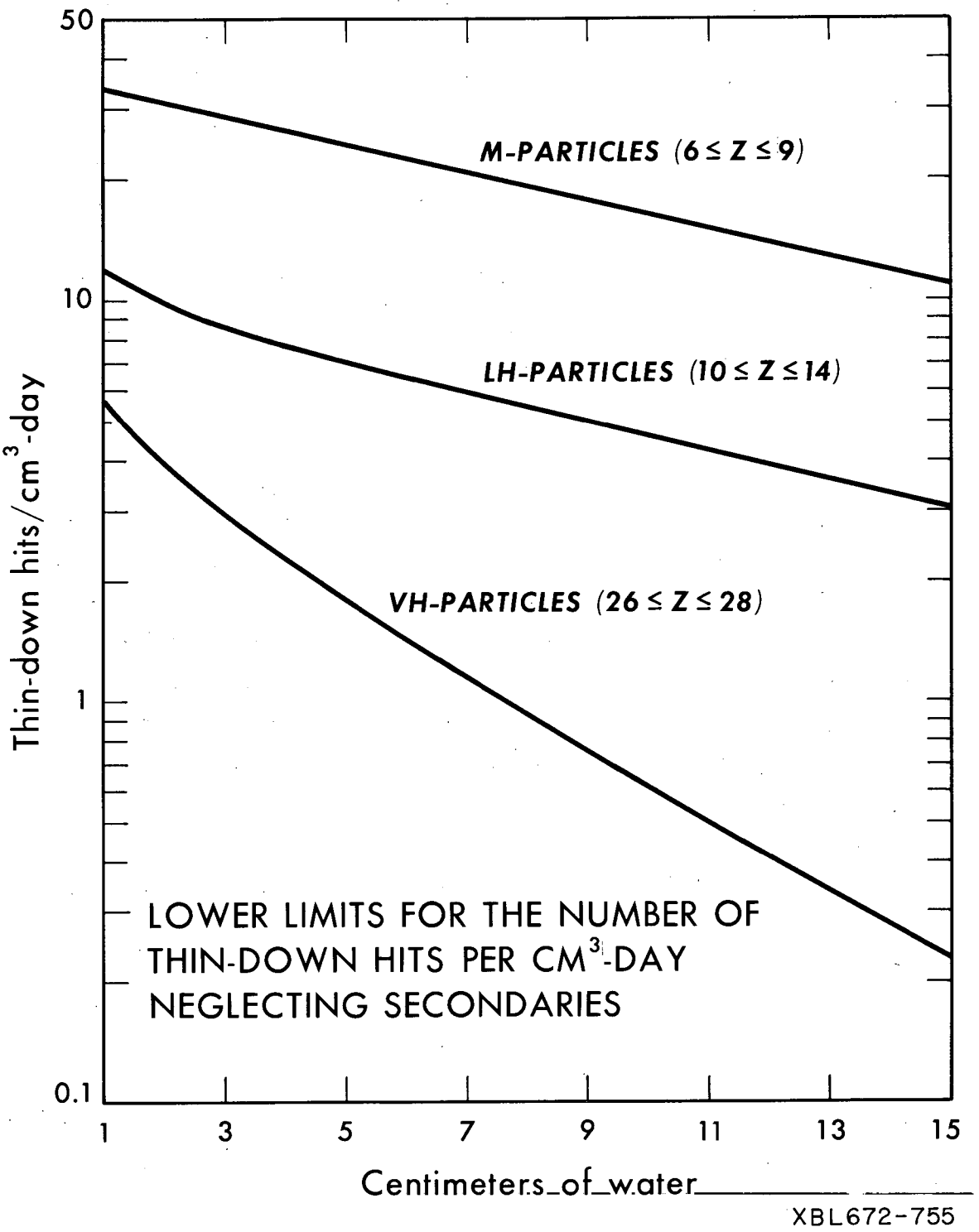

50
10
1
0.1
M-PARTICLES (6 ≤ Z ≤ 9)
LH-PARTICLES (10 ≤ Z ≤ 14)
VH-PARTICLES (26 ≤ Z ≤ 28)
LOWER LIMITS FOR THE NUMBER OF
THIN-DOWN HITS PER CM3-DAY
NEGLECTING SECONDARIES
Thin-down hits/cm3-day
1
3
5
7
9
11
13
15
Centimeters of water
XBL672-755

Fig. 3

evaluation of the radiation caused by these ions deep inside an absorber.

(d) Fluxes of ions in the carbon, nitrogen, and oxygen group plus a few heavier ions have been observed in large solar-particle events.

References

1. P. Freier, E. J. Lofgren, E. P. Ney, F. Oppenheimer, H. L. Bradt, and B. Peters, Phys. Rev. 74, 213 (1948).
2. H. J. Schaefer, J. Aviation Med. 23, 334 (1952).
3. C. A. Tobias, J. Aviation Med. 23, 345 (1952).
4. P. Todd, in Second Symposium on Protection Against Radiations in Space, NASA SP-71, 105 (1965).
5. S. B. Curtis, Energy Loss Distributions and Fraction Cell Lethality, Lawrence Radiation Laboratory Report UCRL-17283 (1966).
6. G. M. Comstock, C. Y. Fan, and J. A. Simpson, Astrophys. J. 146, 51 (1966).
7. V. K. Balasubrahmanyan, D. E. Hagge, G. H. Ludwig, and F. B. McDonald, Goddard Space Flight Center preprint X-611-65-480 (1965).
8. W. R. Webber, private communication, 1966.
9. C. Y. Fan, J. E. Lamport, J. A. Simpson, and D. R. Smith, J. Geophys. Res. 71, 3289 (1966).
10. G. Gloeckler and J. R. Jokipii, Phys. Rev. Letters 17, 203 (1966).
11. C. J. Waddington, in Progress in Nuclear Physics, O. R. Frisch, ed., Vol. 8, 1 (1960).
12. S. Biswas, C. E. Fichtel, and D. E. Guss, J. Geophys. Res. 71, 4071 (1966) (and references therein).
13. W. R. Sheldon and S. B. Curtis, Bull. Am. Phys. Soc. 9, 141 (1964).
14. S. Biswas and C. E. Fichtel, Astrophys. J. 139, 941 (1964).

FLUCTUATIONS OF IONIZATION BY HEAVY IONS

H. D. Maccabee

When an energetic heavy ion passes through matter it loses energy by several competing processes. The predominant mode of energy loss in the ion velocity range considered here is that involving inelastic collisions with the electrons of the stopping material, resulting in ionization and excitation of the atoms of the material. Because the collisions are discrete and random, statistical fluctuations are expected in the number of collisions.

In first approximation, the probability of energy loss ϵ in a single electronic collision is proportional to ϵ^{-2}. Thus collisions resulting in a large energy transfer to an electron are relatively infrequent compared with small-energy-transfer collisions. Although they are relatively infrequent, the large-energy-transfer collisions account for a significant proportion of the total energy loss. In a "thin" absorber (one in which the energy loss is small compared with the kinetic energy of the particle), the probable number of large-energy-transfer collisions may be so small that the random statistical fluctuations in this number are relatively large, and result in significant fluctuations in the energy lost in this mode and thus fluctuations in the total energy loss occur.

This phenomenon has been investigated theoretically by Landau,[1] Symon,[2] Vavilov,[3] and others, and is often called the Landau effect. There have been several experimental investigations, and most recently the work of Maccabee[4] and Raju[5] has provided a comprehensive verification of the Vavilov theory.

In Vavilov's exact treatment, the dimensionless parameter κ is introduced, and it is shown that for $\kappa << 1$, the fluctuations are large, while for $\kappa >> 1$, the fluctuations are negligible and the normal Gaussian shape of the total energy loss distribution is valid.

$$\kappa \equiv 0.150 \frac{SZ}{A} z^2 \left(\frac{1-\beta^2}{\beta^4} \right),$$

S = thickness of absorber in g/cm^2

Z = atomic number of absorbing material

A = atomic weight of absorbing material

z = effective charge on incident ion

β = speed of incident ion ÷ speed of light.

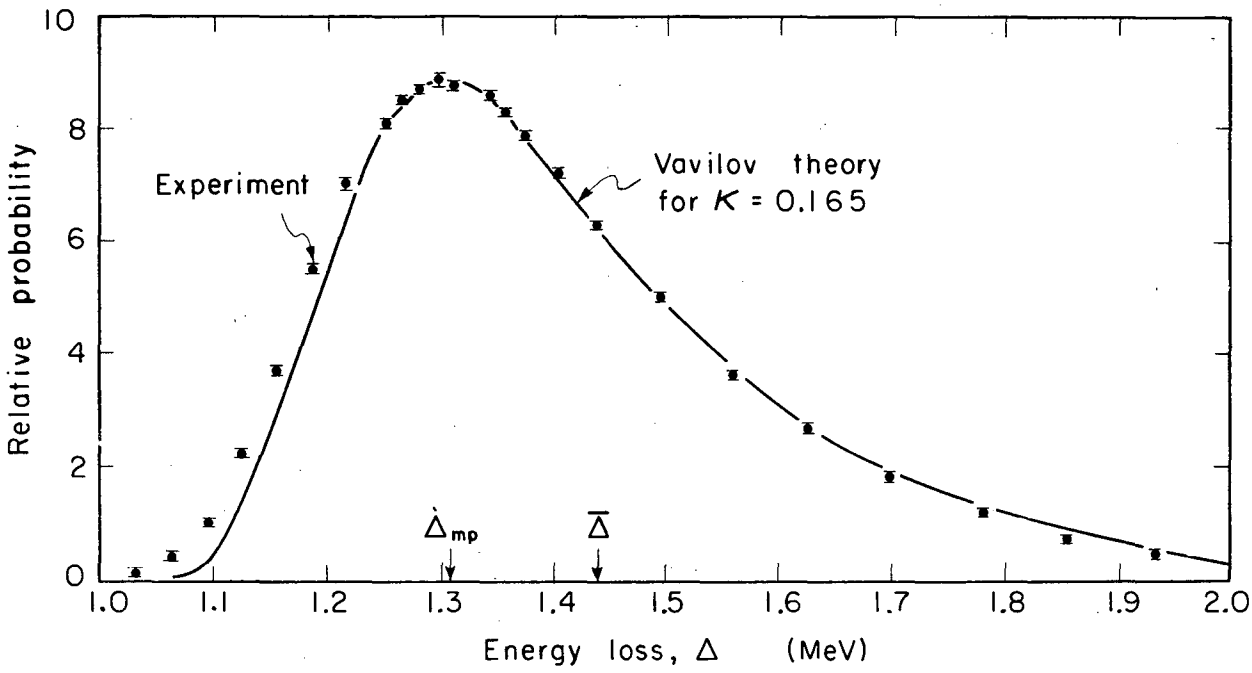
κ may be thought of as a measure of the ratio of the total energy loss to the maximum possible energy loss in a single collision, i.e., a measure of the number of large-energy-loss collisions suffered by the ion in passage.

For example, consider the case of 910 MeV He^{2+} ions passing through 0.046 cm of silicon (0.107 g/cm^2). In this case the value of κ is 0.165 and large fluctuations in energy loss are expected. Figure 1 shows a comparison of the theoretical energy loss distribution with the experimental distribution measured with a lithium-drifted silicon detector at the Berkeley 184-inch synchrocyclotron. Note the good agreement between theory and experiment, the significant difference between $\bar{\Delta}$, the average energy loss, and Δ_{mp}, the most probable energy loss, and the long high-energy-loss "tail."

Of course, many biological targets are "thin" with respect to energetic heavy ion beams, and thus fluctuations are expected in the amount of energy transferred to the target. For example, consider a 4 BeV Ar^{18+} ion (100 MeV/nucleon) in a cell of 4 microns thickness; the value of parameter κ in this case is 0.17 and the shape of the spectrum of energy transfer to the cell is expected to be similar to that of Fig. 1, with an average energy transfer of about 1 MeV.

Consider also a 132 MeV Ne^{10+} ion (6.6 MeV/nucleon) traversing a DNA strand of 20 Å thickness and density about 2; the value of κ in this case is 0.015, and the fluctuations about the average energy transfer (about 1 keV) will be even broader than those of Fig. 1.

The concept of linear energy transfer (LET) is of fundamental importance in the interpretation of biological experiments with radiation. Indeed, the need for penetrating beams of very-high-LET particles is one of the primary arguments for the desirability of the Omnitron for biomedical research. The previous examples underscore the importance of knowing not only the average energy transfer to a biological target, but the complete LET distribution, including the effects of fluctuations. Before continuing, we note that the ion energy loss is only identical with the linear energy transfer if none of the secondary electrons (delta rays) escape the target.



MUB 11436

Fig. 1. Energy loss distribution of 910 MeV He^{2+} in 0.107 g/cm^2 silicon; $\kappa = 0.165$.

By now it should be clear that the cumulative effect of the fluctuations of energy loss along "thin" segments of the ion track will be fluctuations of the total range of the ion in the medium, or "range straggling." In principle, it should be possible to relate the range straggling distribution to statistical fluctuations in the collisions along the whole track in a similar way as the energy loss distribution is related to the fluctuations of collisions in a track segment.

Of course the theory of ionization on which our understanding of fluctuations is based assumes a simple z^2 dependence of the rate of energy loss by ionization. As is well known, charge-exchange effects becomes important in the lower range of ion velocity ($\beta \lesssim z/137$), when the ion speed becomes comparable to that of its own orbital electrons. Since there are no accelerators in existence which accelerate ions heavier than helium to greater than 10 MeV/nucleon, there are no experimental data on energy-loss, fluctuations, charge exchange, range, or straggling in this very significant range of charge and energy. Our best hint at the validity of the extended theory of energy loss and fluctuations comes from the good agreement between theory and experiment for 910 MeV helium ions. Although there are several experimental difficulties associated with such measurements at the Hilac, studies of this type should be undertaken. The best source of data on these questions, however, will be the Omnitron itself.

References

1. L. Landau, J. Phys. U.S.S.R. 8, 201 (1944).
2. K. R. Symon, Ph.D. Thesis, Harvard University, 1948; summary in B. Rossi, High Energy Particles (Prentice-Hall, New York, 1952), p. 10.
3. P. V. Vavilov, Soviet Phys. JETP 5, 749 (1957).
4. H. D. Maccabee, Ph.D. Thesis, University of California, 1966; Lawrence Radiation Laboratory Report UCRL-16931.
5. H. D. Maccabee, M. R. Raju, and C. A. Tobias, IEEE Trans. Nucl. Sci. V. NS-13 No. 3, 176 (1966).

DISTRIBUTION OF IONIZATION IN HEAVY CHARGED-PARTICLE TRACKS

J. T. Lyman

It is well known that the biological effects of heavily ionizing particles depends on the dose and also on the linear energy transfer of these particles. In addition to these factors, however, there is also some evidence to show that the transverse distribution of energy deposition away from the particle track is also of significance. Qualitatively this distribution is understood, but we do not have an exact theory to show its distribution. Experimentally there have been only a few recent studies; however, techniques and methods are developing that will enable scientists to study the detailed structure of particle tracks in the near future. The presentation below concerns the demonstration that the energy distribution of secondary electrons is the same for particles of light or heavy ionization and that the number of such secondary electrons depends primarily upon the square of the charge carried by the particle. Experimentally the structure of heavy ion tracks has not been measured in detail. A brief account of the experimental work done to date is given; much more remains to be done.

The main interaction by which a heavy charged particle loses energy in matter is by inelastic collisions with the atomic electrons. The atomic electrons receive energy sufficient to leave them in an excited state (excitation) or in an unbound state (ionization). The specific energy loss to the atomic electrons per unit path traversed by a heavy charged particle is given by the following expression:[1]

$$\frac{-dE}{dx} = \frac{4\pi z^2 e^4}{mv^2} NZ \left[\ln \frac{2mv^2}{I} - \ln (1 - \beta^2) - \beta^2\right], \qquad (1)$$

where z is the units of charge of the incident particle, e and m are the charge and the mass of the electron, v is the velocity of the incident particle, N is the number of atoms per cubic centimeter of the stopping material, Z is the number of electrons per atom of the material, I is the adjusted ionization potential of the material, and β is the velocity of the particle in units of the velocity of light ($\beta = v/c$).

In every primary ionization collision between a charged particle and an atom, one or more electrons are ejected. The more energetic of these electrons are responsible for secondary ionizations. If we restrict the consideration to these so-called delta rays or knock-on electrons (i.e., electrons which have been ejected with an energy which is large compared with the ionization potential), then the binding of these electrons can be neglected and the interaction can be treated as if it occurred with a free electron. By use of the Rutherford scattering formula, it can be shown that the cross section $d\Phi$ for the ejection of an electron with an energy between W and dW is

$$d\Phi = \frac{2\pi z^2 e^4}{mv^2} \frac{dW}{W^2} . \tag{2}$$

The relationship between the energy of the electron and the angle, θ, the ejected electron makes with the incident particle is,

$$W = \frac{2mc^2\beta^2}{1-\beta^2} \cos^2\theta . \tag{3}$$

The electrons which have been ejected in the forward direction are the most energetic and have an energy nearly equal to[3]

$$W_{max} = \frac{2mc^2\beta^2}{1-\beta^2} . \tag{4}$$

To find the number of electrons dN_δ of a given energy emitted per unit path length, the cross section (Eq. 2) must be multiplied by the number of electrons per unit volume of the stopping material, n,

$$n = \frac{N_0 Z}{A} \rho , \tag{5}$$

where N_0 is Avogadro's number, A is the atomic mass of an atom of the stopping material, and ρ is the density of the material.

$$dN_\delta = \frac{2\pi z^2 e^4}{mv^2} \frac{N_0 Z}{A} \rho \frac{dW}{W^2} . \tag{6}$$

The energy loss to these electrons is the energy of the electron times the number of such electrons

$$W dN_\delta = \frac{2\pi z^2 e^4}{mv^2} \frac{N_0 Z}{A} \rho \frac{dW}{W}. \qquad (7)$$

The total energy per unit path given to all such electrons by the incident particle is calculated by integration of W dN from W_{min} to W_{max}.

$$T_{>W_{min}} = \int_{W_{min}}^{W_{max}} W\, dN_\delta$$

$$= \frac{2\pi z^2 e^4}{mv^2} \frac{N_0 A}{A} \rho \ln \frac{W_{max}}{W_{min}}. \qquad (8)$$

The fraction of energy given to these electrons can be calculated from Eqs. 1 and 8. Since $N = (N_0/A)\rho$, we see that

$$\frac{T_{>W_{min}}}{dE/dx} = \frac{\ln \frac{W_{max}}{W_{min}}}{2\left[\ln \frac{2mv^2}{I} - \ln(1-\beta^2) - \beta^2\right]}$$

$$= \frac{\ln \frac{2mv^2}{W_{min}} - \ln(1-\beta^2)}{2\left[\ln \frac{2mv^2}{I} - \ln(1-\beta^2) - \beta^2\right]}. \qquad (9)$$

If W_{min} is taken to be the same value as I, then nearly half of the energy is transferred to electrons which are capable of further ionization. The fraction of the energy and the energy spectrum of these electrons are independent of the charge and mass of the incident heavy particle, but are dependent upon the velocity of the incident particle.

Although there is no adequate theory with which to do a similar calculation for the low energy electrons and the bound electrons in excited states, to a first approximation, the relative numbers of such electrons probably follow the same functional dependence as the high energy electrons. Oda and Lyman[4] have measured the yields of the low energy electrons (< 50 eV) emitted from the surfaces of metallic foils (aluminum and nickel), by bombarding the foils with various heavy-ion beams. Curves obtained from

plotting the yields measured with different retarding potentials applied to the foils were very similar in shape for each heavy-ion beam used (a range from helium to neon). This indicates that the spectra of emitted electrons are also very similar. Of course, the total yield of electrons is much higher for the more heavily charged ions. The yield of the emitted electrons can be expressed as

$$\delta = \frac{dE/dx}{\epsilon} \Delta x, \qquad (10)$$

where δ is the number of emitted secondary electrons per incident primary, ϵ is the average energy required to produce one emergent secondary electron, and Δx is the thickness of the region in which the escaping electrons are produced. Both ϵ and Δx are unknown and cannot be determined in this experiment, although their ratio can be found. The ratio $\epsilon/\Delta x$ was not constant when the velocity of the incident beam was changed. The ratio got larger when lower velocity heavy ions were used. One possible explanation is that with the slower particles, the energy of an electron ejected in any given direction is lower, and consequently the range of the electron is shorter; and therefore the thickness of the region in which the escaping electrons are produced is reduced. The results of these experiments can be used to derive the number and energy of the electrons which pass through a given point within the medium.

A second experiment has been initiated which will yield data on the secondary-electron energy soon after ejection from the atom, that is, before the electrons have been appreciably slowed down in passing through matter. The secondary electrons will be produced by the various heavy ions in a gaseous medium at low pressure. By electrostatic analysis of the secondary-electron momenta, it may be possible to obtain the initial energy distribution of the secondary electrons.

References

1. R. D. Evans, The Atomic Nucleus, (McGraw-Hill, New York, 1955), Ch. 18.
2. H. A. Bethe and J. Ashkin, Passage of Radiations through Matter, in Experimental Nuclear Physics, Ed. E. Segre (John Wiley & Sons, New York 1959), Vol. I.

3. W. H. Barkas and M. J. Berger, Tables of Energy Losses and Ranges of Heavy Charged Particles, NASA SP-3013, 1964.
4. N. Oda and J. T. Lyman, Secondary-Electron Distribution for Heavy Ions, Radiation Res. Suppl. (in press).

MEASUREMENTS OF HEAVY CHARGED PARTICLES WITH SEMICONDUCTOR DETECTORS

M. R. Raju

For biological and medical research with accelerated heavy ions it is important to know the dose in energy units and also the distribution of linear energy transfers of the individual particles at any given point within the tissue studied. The measurement of the quantities with absolute accuracy is difficult because the usual types of radiation detectors have some difficulties in measuring the high-LET portion of the radiation with accuracy.

The semiconductor detector has revolutionized radiation detection. The use of a solid as a detector is very attractive because of its high stopping power. Another advantage is the low energy required to produce a hole-electron pair (3.66 eV for silicon): nearly ten times as much charge is liberated for a given energy loss in silicon as in gas, which leads to small statistical fluctuations in the number of charge pairs and to improved energy resolution over gas-filled counters. The energy resolution for germanium detectors is one or two orders of magnitude better than scintillation counters for gamma radiation. The efficiency of germanium detectors is an order lower than scintillation counters because the atomic number of germanium is lower than that of the scintillators used for gamma detection. The energy resolution of scintillators for gamma detection is limited to about 6% at best and they are inseparable from the photomultiplier tube. In most of the biomedical applications where energy resolution is not particularly important, silicon detectors are more useful. The intrinsically high speed of the semiconductor detectors is due to the high mobility of the carriers in the electric field coupled with the short distance between the electrodes. Besides having an excellent energy resolution and fast response, another attractive feature of these detectors is that their response is linear with energy deposited in the detector, regardless of the type of particle. This linear response is of paramount importance for their use with heavy ions.

It must be noted, however, that there is a small departure from linearity for heavy ions at low energies. This departure was at first

attributed to incomplete charge collection from the densely ionized tracks. But the nonlinearity is still present to a slightly lesser degree even when the results are extrapolated for very high collecting field in the detector. This deviation from linearity is now known to be due to the inherent nature of interaction of heavy ions at low energies. At higher energies most of the energy of the heavy ion is dissipated through electronic collisions. However, at very low energies (i.e., 94 keV for carbon ions, 180 keV for oxygen ions) the energy losses due to nuclear collisions are more important than electronic collisions. Radiation detectors convert only the energy lost in the detector through electronic collisions into a proportional electrical signal, and hence for the energy that is lost in a nuclear collision, there is no signal from the detector. This effect, however, is very small and can usually be neglected.

The optimum performance of these detectors can be realized only if the detector and preamplifier and the other associated electronics are designed to match each other. In this respect the semiconductor detector and associated electronics development group headed by Fred S. Goulding at the Lawrence Radiation Laboratory, Berkeley, has been of great help for our heavy-charged-particle measurements with semiconductor detectors. A short summary of our results will be presented here.

The relative pulse height from a surface barrier detector as a function of energy for various heavy ions that have ranges less than the depletion thickness of the detector are shown in the Fig. 1.[1] Further measurements by Lyman, using a 1 mm thick lithium-drifted silicon detector, gave linear response up to the investigated energy of 400 MeV ^{40}Ar ion. Semiconductor detectors are being used at the heavy ion linear accelerator (Hilac) routinely by the Biomedical group to measure the energy of the heavy ion beam.

Depending on the energy of the particles and the thickness of the detector, a given detector may be used to measure either their specific energy-loss distribution or their energy distribution. If the detector is thin, so that the energy deposited by the particle in it is very small compared with the energy of the particles, the detector can be used to measure the specific energy-loss distribution. On the other hand, if the detector is thick enough to stop all the particles in the beam, then it can be used to measure the energy distribution of the particles.

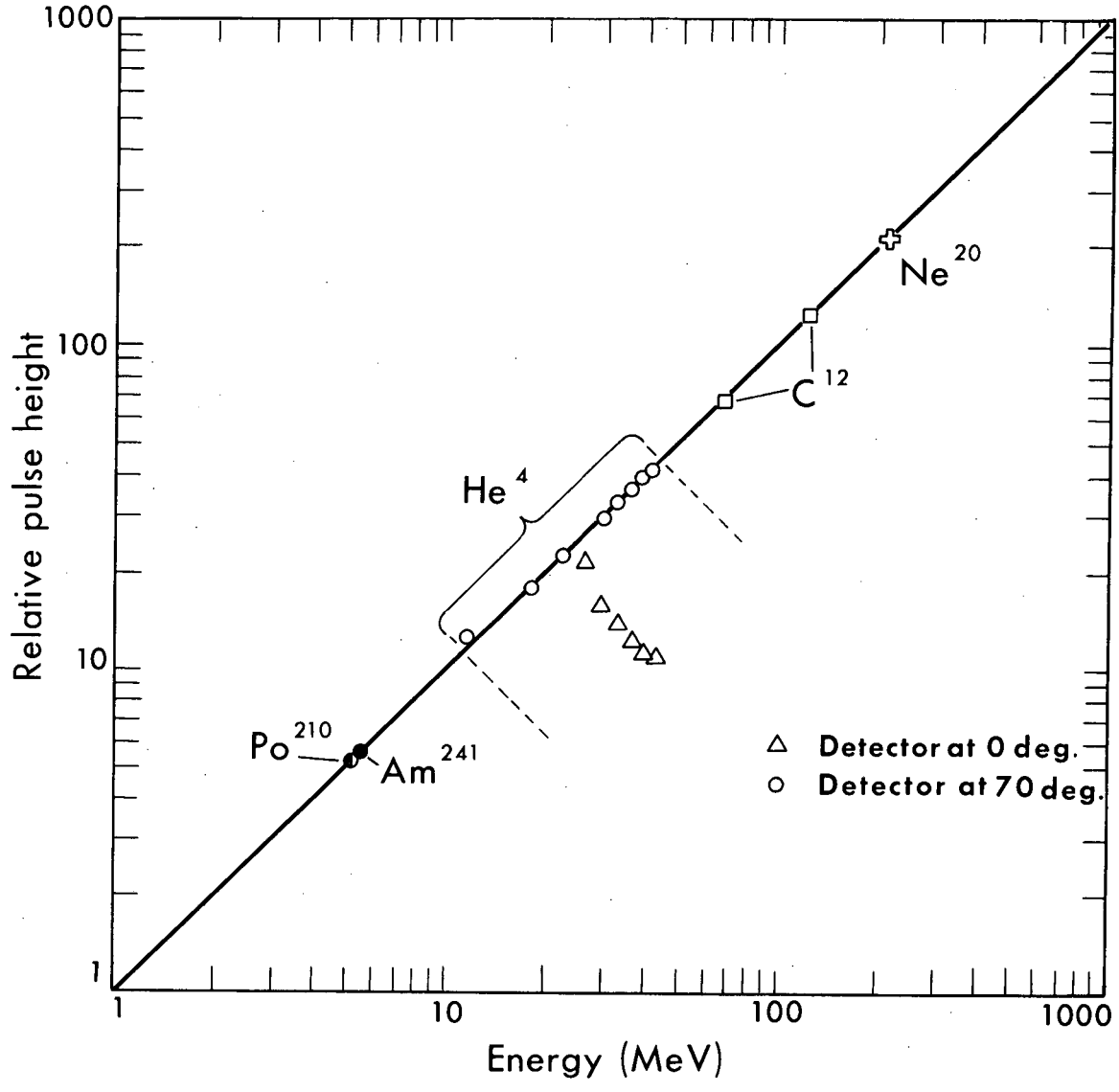


Fig. 1. Linearity of pulse height with particle energy for heavy ions. ^{4}He ions with energy greater than 22 MeV will not be stopped within the depletion layer when the ions are incident normal to the surface of the detector, as demonstrated by points (normal incidence) with detector position at 0 degrees.

The most probable energy-loss measurements of protons of energies up to a maximum of 730 MeV and α-particles up to a maximum of 910 MeV, using lithium-drifted silicon detectors of different thicknesses up to 5 mm, agreed well with the values predicted by theory.[2] Theories of fluctuations of energy loss for high- and intermediate-energy protons and α-particles have been thoroughly investigated by using semiconductor detectors of various thicknesses and the results are found to be in good agreement with theory.[3,4,5] A detailed report on fluctuations of energy loss has been included in this volume ("Fluctuations of Ionization by Heavy Ions," by H. D. Maccabee).

The most probable energy loss of pions of energies extending from 50 to 365 MeV has also been measured by using lithium drifted silicon detectors, and the results again are found to be in good agreement with theory.[6] The behavior of the pion beam with its muon and electron contamination as it passes through various thicknesses of absorbing material has also been displayed by using lithium drifted silicon detectors. Also, for the first time, the energy distribution of negative pion stars in silicon has been measured and is found to be a constantly decreasing function with increasing energy, with a high energy tail extending beyond 60 MeV.[6]

The Bragg peak of heavy charged particles is used for therapy and for radiobiological investigations. Besides knowing the dose at the Bragg-peak position, one needs also to know the LET distribution at this position. In order to evaluate the LET distribution of heavy particles at the Bragg-peak position, the energy distribution of these particles was measured by using lithium drifted silicon detectors thick enough to stop the beam, and was found to be much higher than one would normally expect.[2] Figure 2 shows the energy distribution of α-particles at the Bragg-peak position measured with a 5 mm thick lithium drifted silicon detector. It can be seen from the figure that the modal energy of the α-particles at the Bragg peak was 85 MeV. This data is translated into LET distribution in water and is also shown in the figure; the modal LET was around 10 keV/μ. Figure 3 shows the energy distribution at the Bragg-peak position of a 50 MeV proton beam. It can be seen from the figure that the modal energy of the protons at the Bragg peak is around 5 MeV and the corresponding LET value is around 8 keV/μ. From the data it seems that the modal energy of the heavy charged particles at the

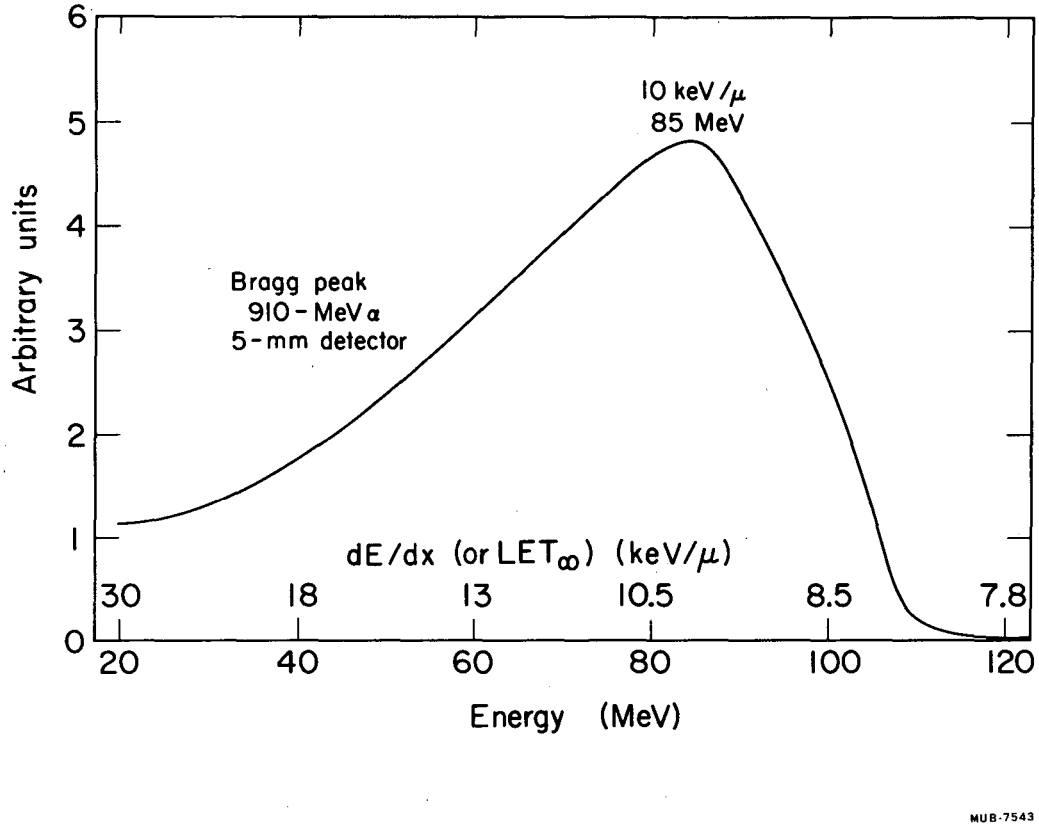


Fig. 2. Distribution of α-particle energy at the Bragg-peak position 910 MeV α beam.

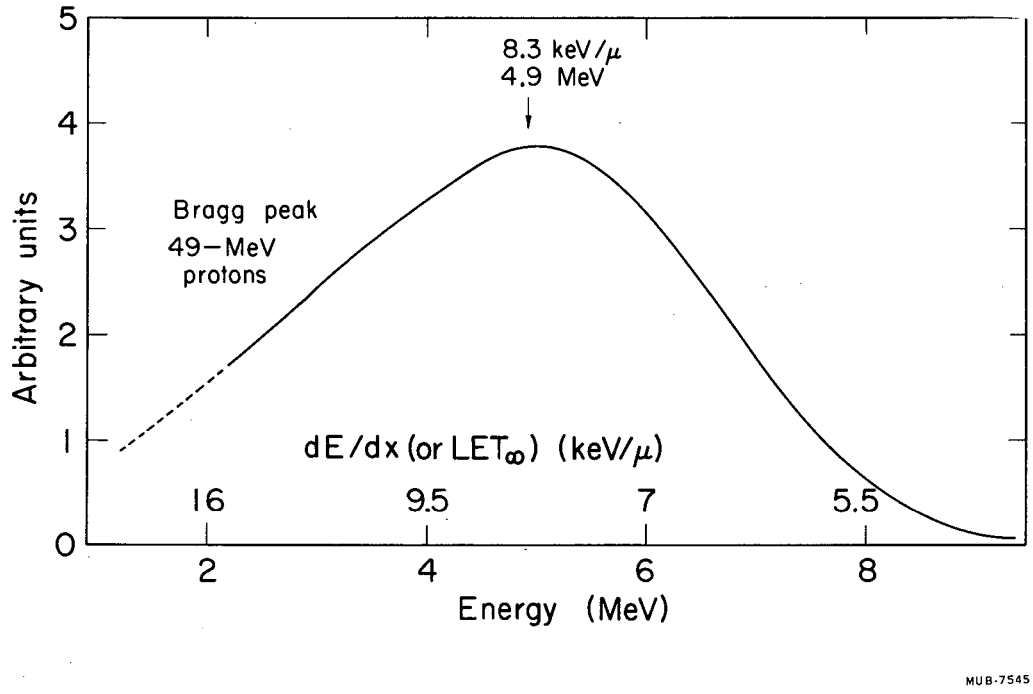


Fig. 3. Energy distribution at the Bragg-peak position of 49 MeV proton beams.

Bragg-peak position is roughly 10% of the primary beam energy and this rough rule is also found to apply to the 150 MeV proton beam of the Harvard cyclotron.

The lithium drifted silicon detector is also being used as a pulse dosimeter in some special dosimetric problems associated with negative pion dosimetry. The method consists of feeding the signal from the lithium drifted silicon detector to a charge-sensitive preamplifier that yields a voltage pulse proportional to the energy deposited in the detector. The voltage pulse is further amplified by a linear amplifier. The detector leakage current is blocked by ac amplification used in the system. The amplifier output is thus fed to a polarity-clipping circuit to eliminate the overshoot contribution, and the resulting pulses are then integrated.[7] The depth-dose distribution of both positive and negative pions in water measured with this dosimetric system is shown in Fig. 4. As can be seen from the figure, the negative pion beam gives rise to a much higher dose than that of the positive pion beam near the end of the range, because of considerable contribution of dose from the negative pion stars.

This system measures the dose due to each particle, thereby making it possible to measure the dose fraction due to particles of interest. Measurements have been made of the fractional dose due to particles depositing energy above a particular energy in the detector for a pion beam, using a threshold discriminator in the system. Such measurements yield information on the distribution of ionization density. The results of these measurements for positions designated 1, 2, 3, and 4 in Fig. 4 are shown in Fig. 5. For comparison purposes, the integrated output of the detector at zero threshold setting of the discriminator at the above-mentioned position is normalized to unity. Note that the two curves for the negative pion beam at positions 1 and 2—corresponding to the peak and halfway down the falling portion of the depth-dose distribution curve of negative pion beams—are similar. This suggests that the ionization density distributions at and beyond the peak of the negative pion depth-dose curve may be similar. On the other hand, the curve corresponding to position 3 halfway up the rising slope of the depth-dose curve falls below the curves 1 and 2 with increasing threshold setting. This suggests that the ionization distribution at that point is considerably less than at the other two points. As expected, the fractional dose for

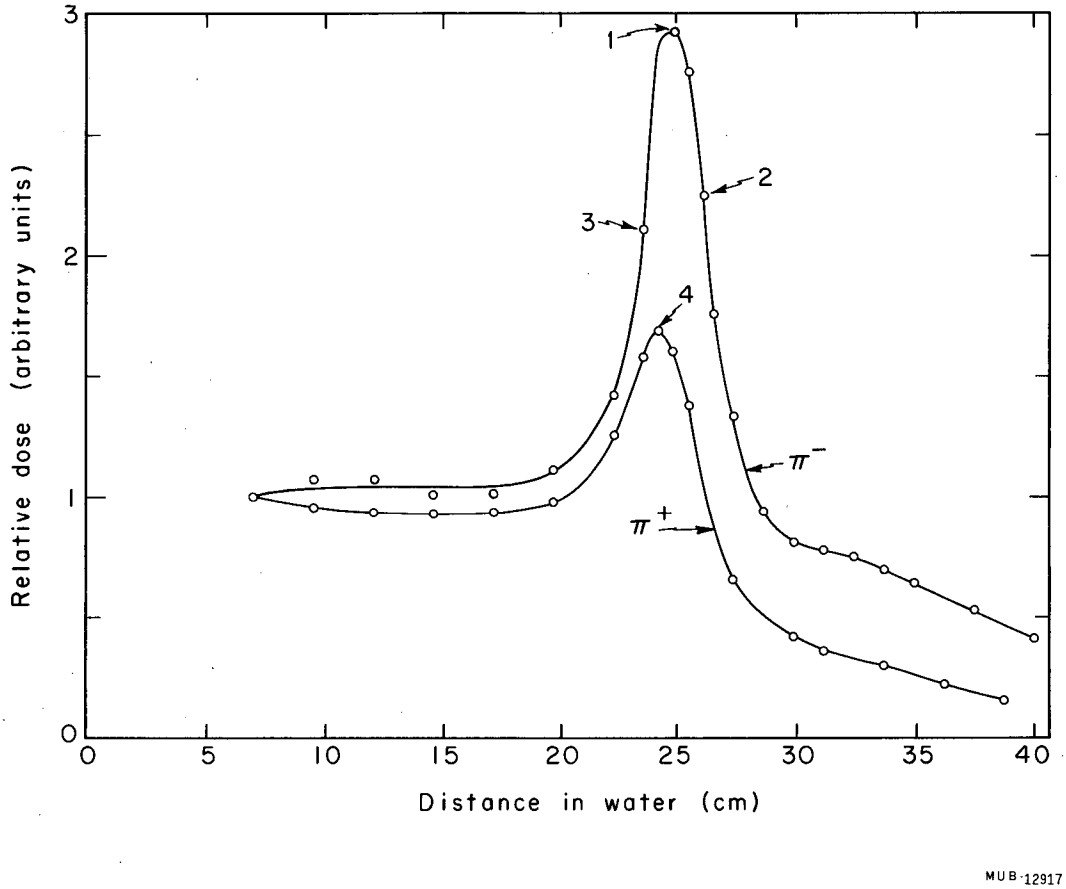


Fig. 4. Depth-dose distribution of 190 MeV/c π^+ and π^-.

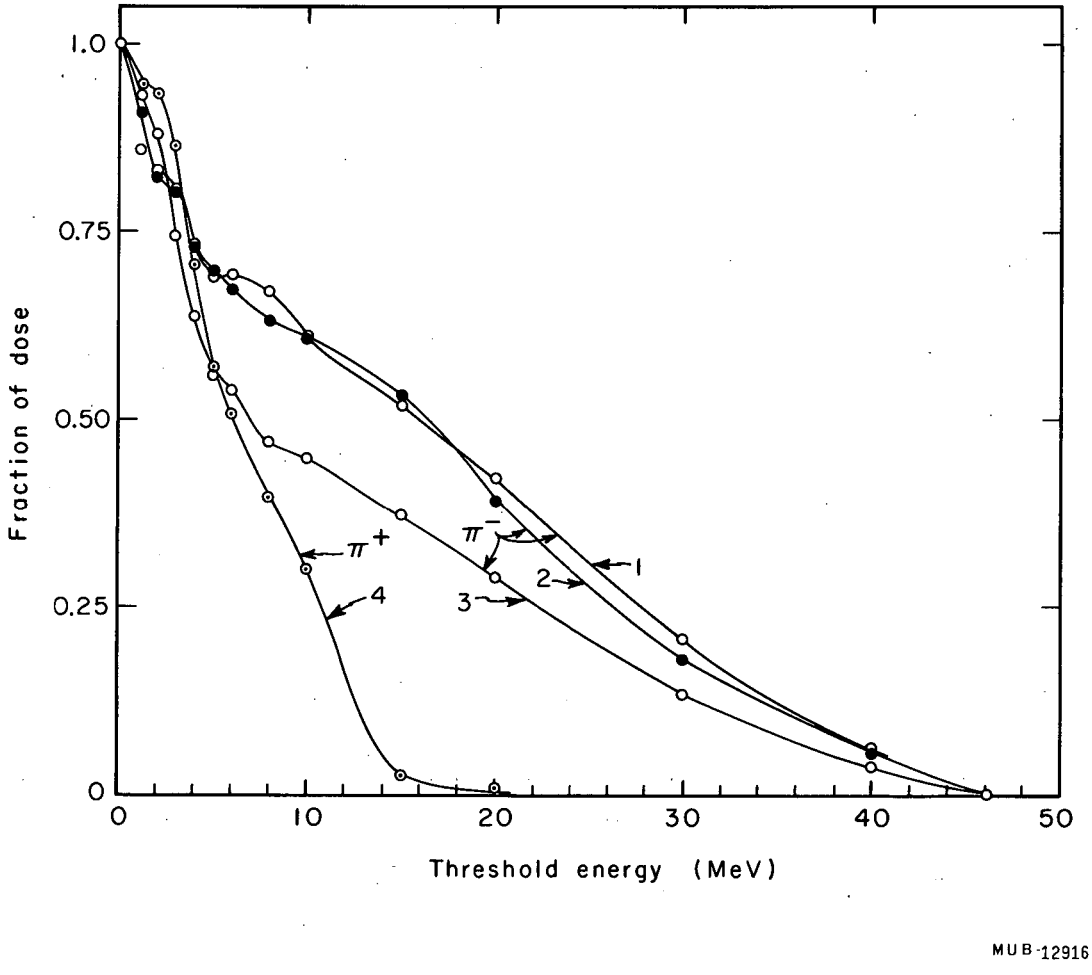


Fig. 5. Fraction of dose due to particles depositing energies higher than the threshold-energy setting, as a function of threshold energy.

threshold setting greater than 10 MeV at position 4, corresponding to the positive pion depth-dose distribution peak, falls to zero quickly as there are no stars contributing to the dose here. It should be mentioned that for the detector thickness used (3 mm), pulses greater than around 9 MeV cannot be due to the passage of pions through the detector. In the case of negative pions, pulses greater than around 9 MeV are definitely due to star formation. It can be seen from Fig. 5 that the dose contribution from the negative pion stars at the Bragg peak is roughly 60% for the negative pion beam, which is contaminated with roughly 25% electrons and 10% muons. This dosimetric system is currently being used to measure the depth-dose distribution of negative pions alone, when the pion beam is associated with muon and electron contamination, by using a threshold Cerenkov counter connected in anti-coincidence with the dosimetric system.

The useful life of the semiconductor detector is limited by the onset of radiation damage, which first deteriorates its energy resolution, then causes a decrease in response and, finally, a complete breakdown. Because of thicker depletion regions, lithium drifted silicon detectors are more sensitive to radiation damage than diffused junction or surface barrier detectors. Typical allowable exposures are tabulated below.[8, 9]

Radiation	Surface barrier surface junction detectors	Diffused junction detectors	Lithium drifted silicon detectors
5-50 MeV α	$\approx 10^{10}$	$\approx 10^{13}$	$\approx 10^{9}$
5-10 MeV protons	$\approx 10^{11}$	$\approx 10^{13}$	$\approx 10^{9}$-10^{10}

Semiconductor detectors have been extremely useful for energy and energy-loss measurements of heavy charged particle beams as well as for measuring doses of low intensity beams. The use of these detectors to measure doses of intense beams is rather limited at the present state of technology.

However, some of the commercial signal silicon diodes made for general circuit applications can profitably be used for measuring doses of intense heavy charged particle beams. The radiation damage need not be a limiting factor for their use as radiation dosimeters.[10] If the diode is

preexposed to radiation doses of the order of 10^6 rads, the sensitivity of the diode is reduced by a factor of the order of 3, but the sensitivity does not change significantly thereafter with further radiation exposures involved in dose measurement. The short-circuit current of such a preexposed diode is found to be proportional to the dose. These diodes can be used to measure doses in the range from 25 rads/min up to a few thousand rads/min.[11] Because of their small physical size (of the order of a cubic millimeter), these diodes are ideal for measuring beam profiles of small collimated beams. Such diodes are being used routinely at the biomedical facilities here and at the Harvard cyclotron.

References

1. J. T. Lyman, Ph. D. thesis, Lawrence Radiation Laboratory Report UCRL-16030 (1965).
2. M. R. Raju, Lawrence Radiation Laboratory Report UCRL-16354, August 1965; also in Proceedings of the Workshop Conference on Space Radiation Biology, Berkeley, Calif., Sept. 1965 (to be published in Radiation Research).
3. H. Maccabee and M. R. Raju, Nucl. Instr. Methods 37, 176-178 (1965).
4. H. D. Maccabee, M. R. Raju, and C. A. Tobias, IEEE Trans. Nucl. Sci. Vol. NS-13, 176-179 (1966).
5. H. D. Maccabee, Ph. D. thesis, Lawrence Radiation Laboratory Report UCRL-16931 (1966).
6. M. R. Raju, H. Aceto, and C. Richman, Nucl. Instr. Methods 37, 152-158 (1965).
7. M. R. Raju, E. J. Lampo, S. B. Curtis, J. M. Sperinde, and C. Richman, Lawrence Radiation Laboratory Report UCRL-16924 (June 1966); also in Proceedings of the 13th Nuclear Science Symposium, Boston, Oct. 1966 (to be published by IEEE).
8. G. Dearnaley, J. Sci. Inst. 43, 869-877 (1966).
9. F. S. Goulding, private communication, 1966.
10. A. M. Koehler, in Proceedings of the Workshop Conference on Space Radiation Biology, Berkeley, Calif., Sept. 1965 (to be published in Radiation Research).
11. M. R. Raju, Phys. Med. Biol. 11, 371-376 (1966).

EFFECTS OF HEAVY IONS UPON THE PRODUCTION OF FREE RADICALS IN ORGANIC COMPOUNDS

W. B. G. Jones* and T. Henriksen†

The study of the production of free radicals (i.e., an unpaired electron the magnetic moment of which can be detected through electron spin resonance analysis) in organic compounds of biological importance occupies an important position in the elucidation of the molecular mechanisms of radiation damage. It has been argued by workers in the field that free radicals are intermediates in the production of biological damage by ionizing radiation. The body of evidence which supports this contention has undoubtedly provided much of the incentive for further investigation into the factors which affect the formation of free radicals. Furthermore, it is well known from low temperature studies that the free radicals which are observed at room temperature are the end result of a sequence of events which was initiated by the absorption of radiation energy in the primary ionizing process. It would therefore seem reasonable to assume that both the type and yield of these "secondary radicals" can be influenced by the local distribution of the energy absorption as well as by the absolute temperature at which the secondary reactions take place. The general trend of the experiments which have borne out this assumption will be the basis of the brief discussion which follows. Except where otherwise noted, the source of the heavy ions was the Berkeley heavy-ion linear accelerator (Hilac). ESR centers are the secondary type rather than initial centers.

If polycrystalline samples of amino acids, peptides, enzymes, and nucleic acid components are irradiated with the heavy ions helium, lithium, boron, carbon, oxygen, fluorine, neon, silicon, and argon and the types of free radicals and their relative yields are measured, it appears that the types of secondary radicals which are formed are independent of the stopping power of the radiation, while the yields of the different types of radicals do vary with the stopping power.

---

*AEC postdoctoral fellow.

†Present address: Norsk Hydros Institute for Cancer Research, Oslo, Norway.

The compound which showed the greatest spectral changes when irradiated with heavy ions of different stopping power was reduced glutathione, GSH (Fig. 1). It should be noted that in the middle of the spectrum a resonance appears which becomes more pronounced at high LET. The samples in Fig. 1 had been kept at room temperature for about 20 minutes after irradiation before the spectra were recorded. While there are reasons to believe that there are several different types of ESR centers formed initially in reduced glutathione,[1] slow secondary reactions will lead to the formation of sulphur radicals, which will completely dominate the spectrum after the samples have been at room temperature for several hours. The spectra of Fig. 1 are therefore an intermediate case between these two extremes, and seem to show that the relative yields of the primary species depend upon the stopping power. When the GSH samples of Fig. 1 were kept at room temperature, the spectra gradually become equal to each other and quite similar to the cysteine sulphur pattern.[1]

For Fig. 2 the variation of radical yield with stopping power is clearly seen for alanine, cytosine, cytidine, and reduced glutathione. Slight changes (if any) are observed in the radical yields for these compounds in going from sparsely ionizing radiation up to 40 MeV helium ions with a stopping power of approximately 190 MeV gm^{-1} cm^2. As the stopping power of the radiation increases above this level, the radical yield shows a continuous decrease. It appears that the shape of the yield curves is the same for both aliphatic and aromatic compounds.

A similar graph of radical yield vs. stopping power is shown for glycine in Fig. 3, but the results are given for three different temperatures. The variation of radical yield with stopping power becomes smaller with decreasing temperature. This seems to be a general observation, for similar results have been seen for the radical yield in the enzymes trypsin and lysozyme,[2] and Brustad has reported a similar effect of the irradiation temperature on the loss of enzymatic activity of trypsin.[3]

While it is not our purpose here to discuss the loss of enzymatic activity due to heavy ion irradiation, since Dr. Brustad will cover that topic elsewhere in this volume, it still might be worth mentioning in passing that not only does the stopping power-vs.-inactivation curve for trypsin follow

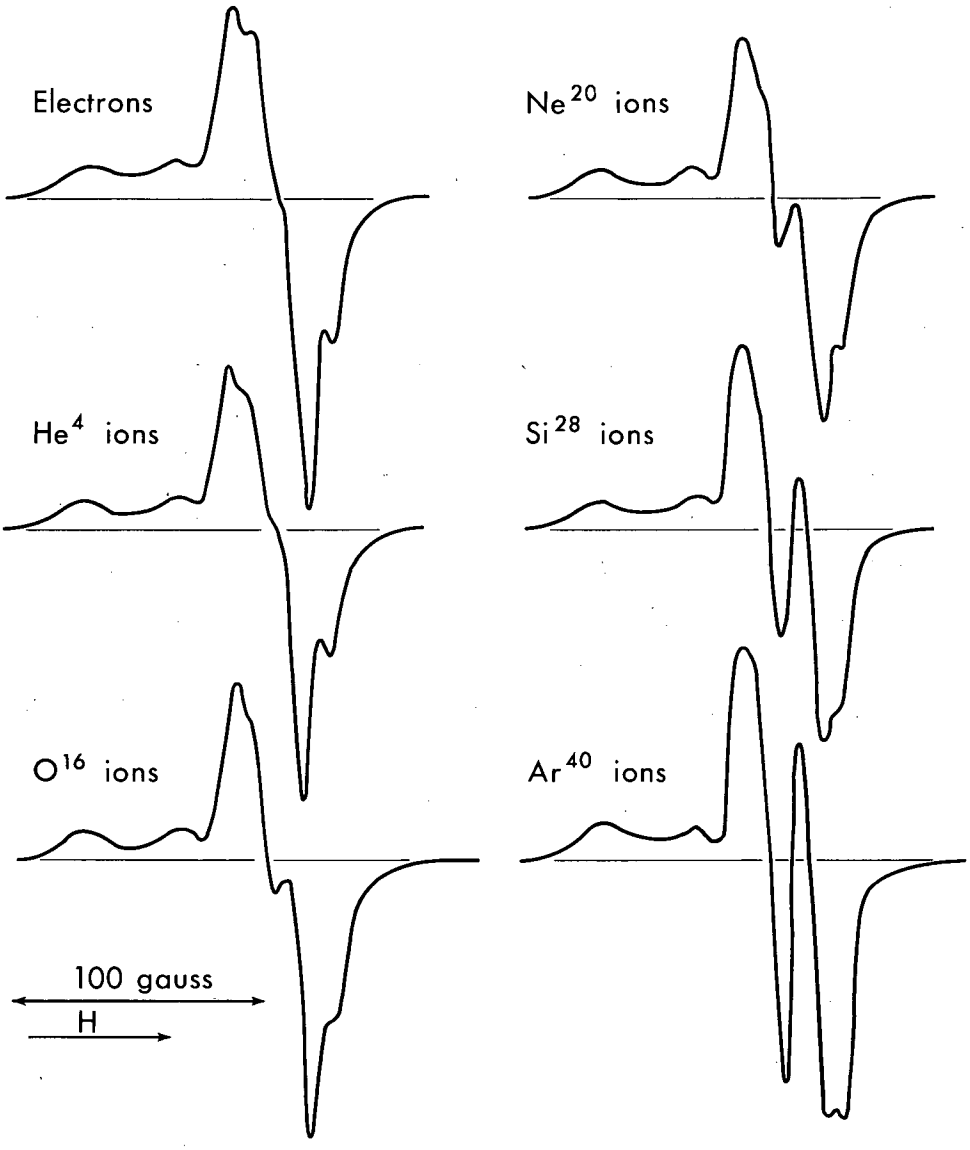


Fig. 1. The qualitative spectra of reduced glutathione irradiated with different types of heavy ions. Both the irradiations and the measurements were carried out at room temperature. Dose: 1.5×10^6 rads.

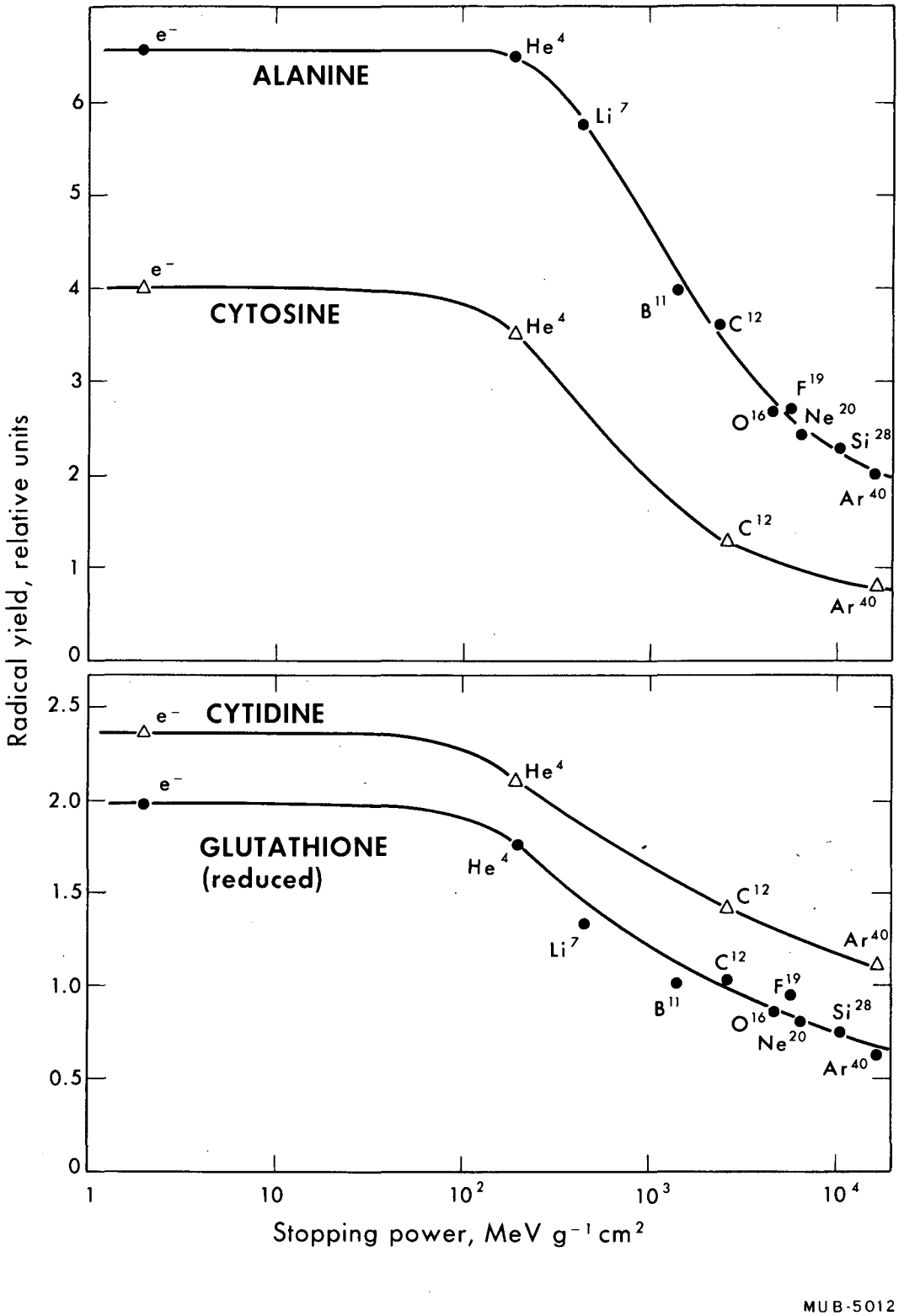


Fig. 2. The yield of secondary radicals as a function of the stopping power for several compounds.

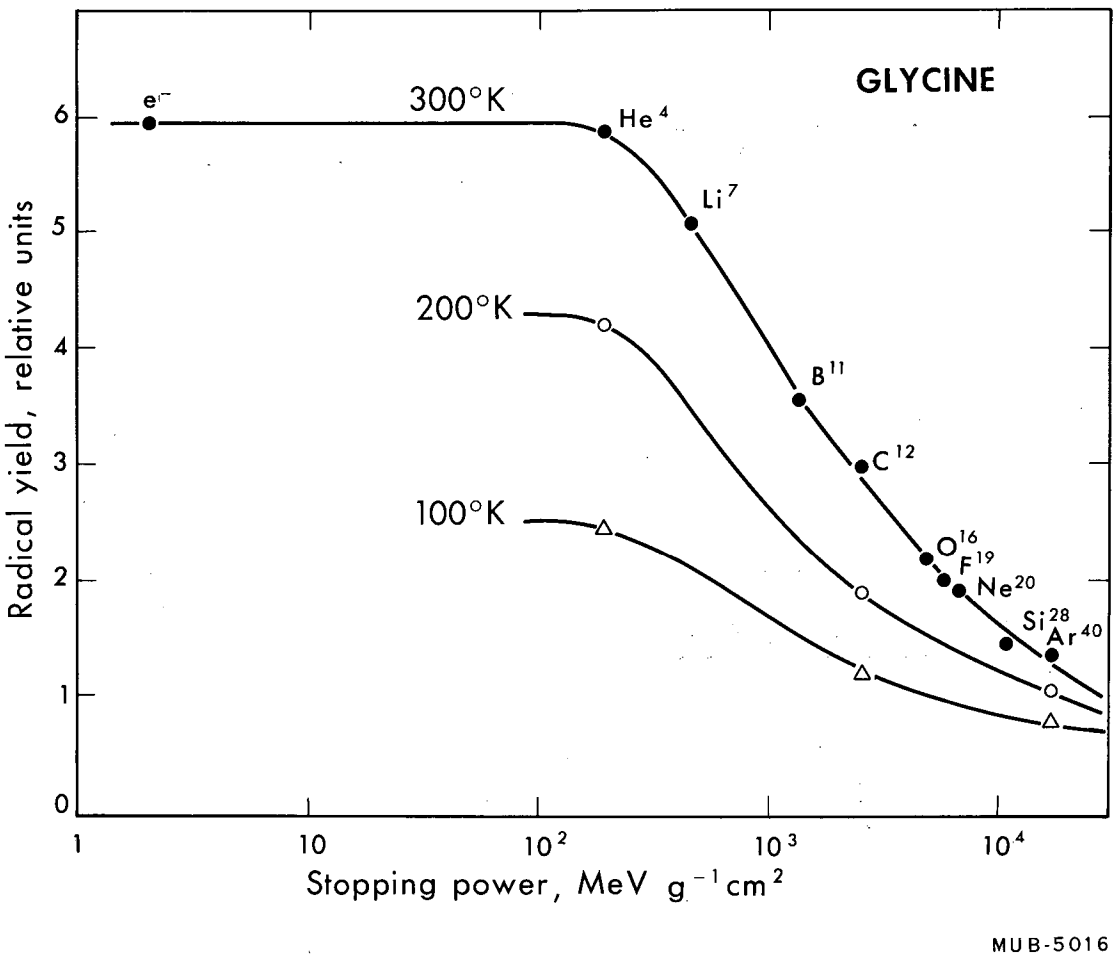


Fig. 3. The yield of secondary radicals as a function of the stopping power for glycine. The different curves indicate different irradiation temperatures.

the same pattern as the stopping power-vs.-radical yield curve for this enzyme (Fig. 4), but that the pattern of the curves shown in Figs. 2, 3, and 4 is the same as that found in the stopping power-vs.-inactivation curve for T1 bacteriophage.[4, 5] This correlation strongly suggests that the secondary radicals somehow are involved in the sequence of reactions which lead to the loss of biological activity.

An indication of the variation in radiosensitivity as a function of stopping power can be derived from the stopping power-vs.-yield (inactivation) curves presented above, by taking the ratio of the yield (inactivation) for the flat portion of the curves to the values observed for argon ions.[6] The results of such calculations indicate that radiation with a low stopping power is from two to five times as efficient as the densely ionizing argon ions. Thus it appears that molecular damage, whether measured by the production of secondary radicals or by the inactivation of enzymes and phages, is largely influenced by the local distribution of the initial energy absorption.

It is also of interest to study the yield of radicals as a function of dose. In Fig. 5 are shown the dose-effect curves for radical production in solid glycine by the ions helium, carbon, and argon. While the curves are straight lines over a large range of dose up to about 10^7 rads, it should be noted that for all curves of Fig. 5 the slope is less than 1.0. Two observations should be made about the dose-effect curves:

(1) Different ions produce different saturation levels. The maximum number of free radicals that can be trapped in solid glycine appears to decrease as the stopping power of the radiation increases.

(2) The dose-effect curves actually decrease at higher doses after passing through a maximum at a dose of about $(6 \text{ to } 8) \times 10^7$ rads. Similar effects have been observed with 15 MeV electrons,[7] and it was suggested that in this case the decrease was due to thermal annealing from the high dose rate used. While the decay of radicals in solids does increase at elevated temperature, the average sample temperature for the glycine samples of Fig. 5 was never allowed to reach such high values that thermal annealing alone could explain the decrease in radical concentration, which amounts to about a factor of 3. An alternative explanation is to assume that the number of trapping sites is limited and that the incident radiation not only produces free radicals which

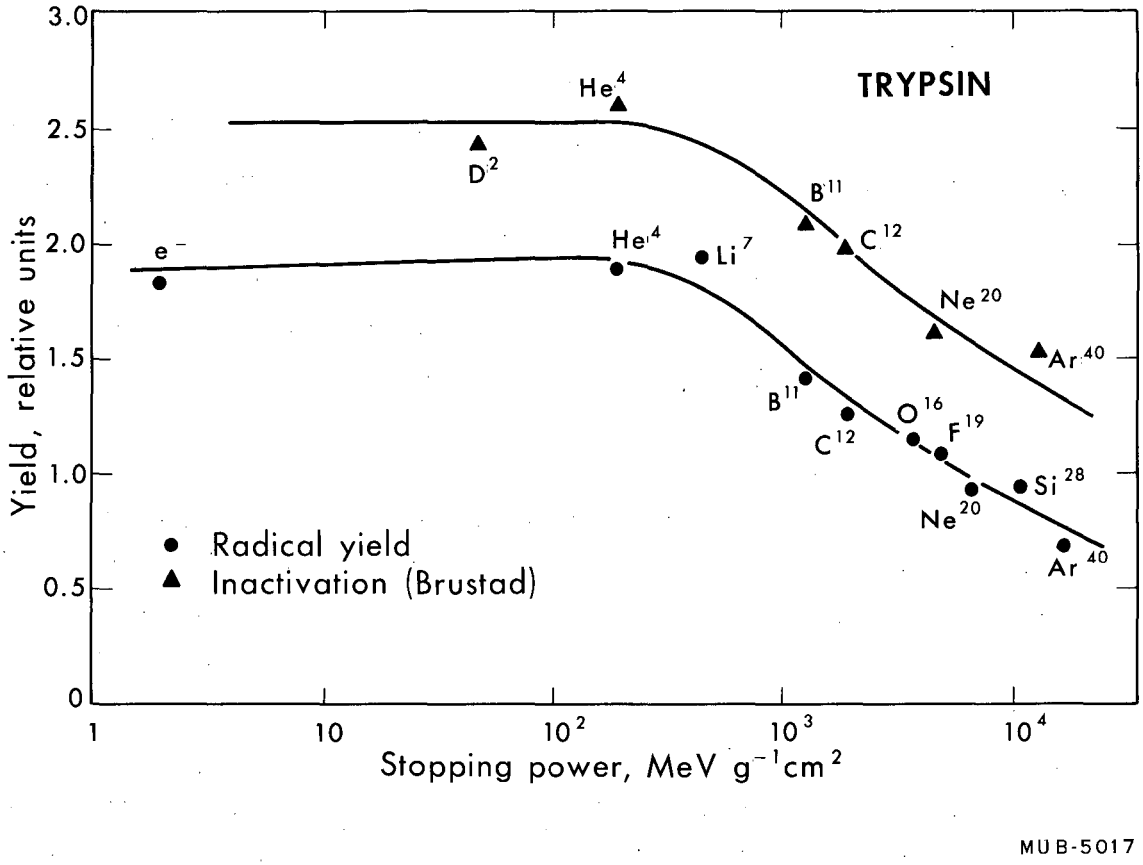


Fig. 4. The radiation effect in solid trypsin, as measured by (1) the loss of enzyme activity (these data are taken from Brustad,[3] and (2) by the production of secondary radicals as a function of the stopping power.

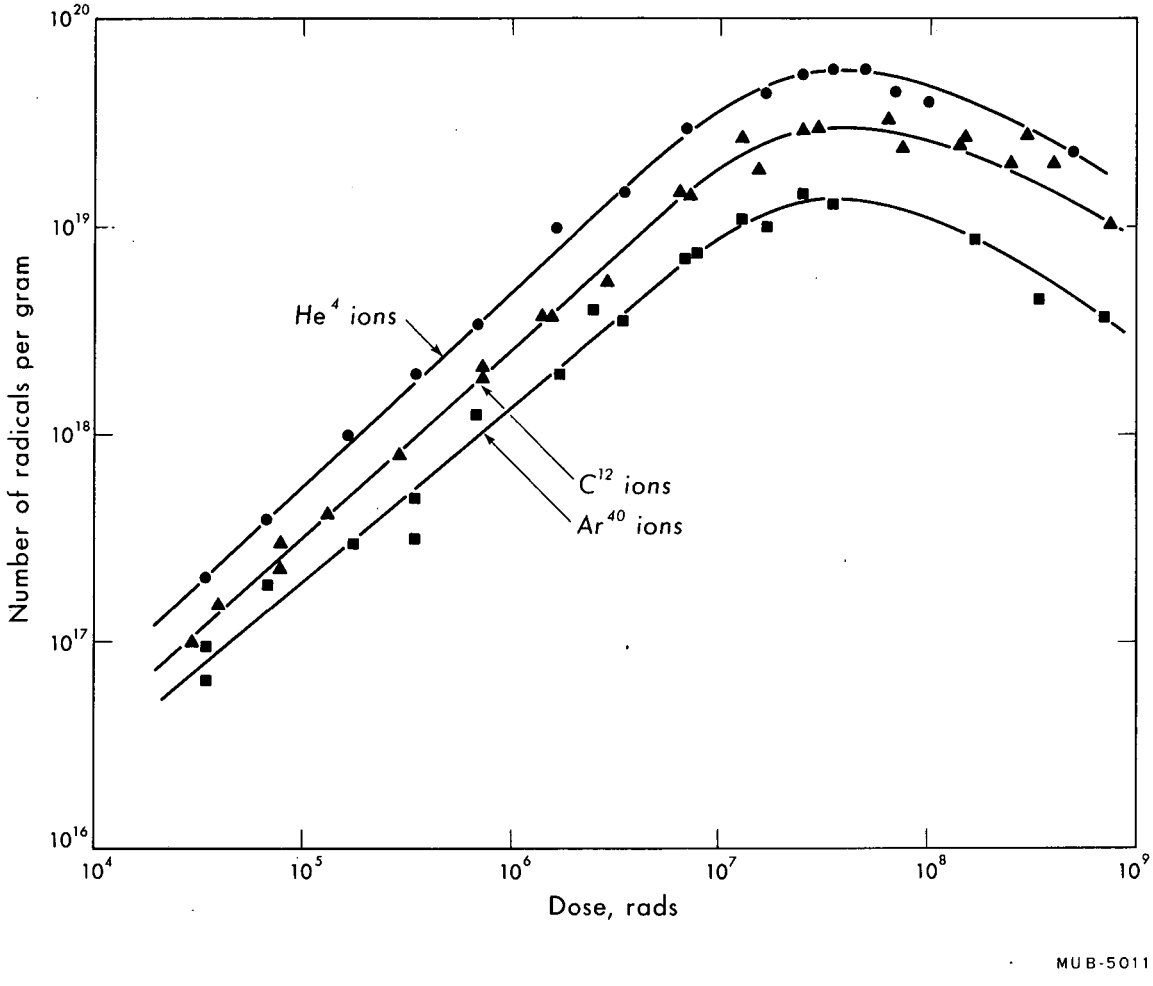


Fig. 5. Production of secondary radicals in solid glycine as a function of the radiation dose of helium, carbon, and argon ions.

may then be trapped in these sites, but also destroys trapping sites. If the action of this destructive process is dependent on the stopping power of the radiation, this would explain the decrease in saturation level with increasing stopping power seen in Fig. 5.

A simple model can be constructed for the production of free radicals by densely ionizing particles.[6] The number of secondary radicals trapped along the track of a bombarding ion is assumed to be the sum of those which are produced by the energy which is deposited in the track core and those which are produced by the δ rays outside the core. As the stopping power of the bombarding particle increases, the radical density increases and eventually the saturation level is reached, with the result that the curve for yield vs. stopping power starts to fall (Figs. 2, 3 and 4). The saturation level is first reached in the track core and then gradually outside the core. Thus an increasing region around the center of the track is saturated with radicals and hence only that portion of the δ-ray energy which is dissipated outside this saturated region is available for the production of additional radicals. To a first approximation, the extra energy deposited in the saturated region may be assumed to be used to sustain a steady-state concentration of radicals. However, as previously mentioned, there is the possibility that because of the increase in the "temperature" of the track core, the radiation also will destroy trapping sites, with the result that the radical density in the track core decreases with increasing stopping power.

It can be seen from the brief outline presented here that we have need to continue our studies in two major directions. First, by working at liquid helium temperatures, we should be able to obtain spectra for the initial ESR centers formed by heavy-ion irradiation. The engineering difficulties have been largely overcome so that irradiations and measurements of ESR spectra can be carried out at 4° K for electron irradiation, and it is felt that the design lessons learned here can be successfully applied to the heavy-ion work. Second, we need to extend our studies of the influence of the stopping power of the radiation upon the type and yield of the free radicals formed. Further studies of the saturation levels and the "track temperatures" are also warranted in order to learn more about the molecular mechanisms of radiation damage. In order to follow this second avenue of research, we have need of

a more powerful and versatile accelerator which is capable of giving us ions of far greater stopping power. We need the Omnitron.

References

1. T. Henriksen, J. Chem. Phys. 36, 1258-1262 (1962).
2. T. Henriksen, Rad. Res. 27, 694-709 (1966).
3. T. Brustad, in Biol. Effects of Neutrons (IAEA, Vienna, 1964).
4. T. Brustad, Advan. Biol. Med. Phys. 8, 161-224 (1962).
5. P. E. Schambra and I. Hutchinson, Rad. Res. 24, 514-526 (1964).
6. T. Henriksen, Rad. Res. 27, 676-693 (1966).
7. J. Rotblat and J. A. Simmons, Phys. Med. Biol. 7, 489-498 (1963).

INACTIVATION OF ENZYMES IN DILUTE AQUEOUS SOLUTIONS BY X-RAYS AND HEAVY IONS

T. Brustad*

Introduction

Because of the short range of heavy particles available from present heavy ion accelerators, few studies of the effect of heavy ions on enzymes in dilute aqueous solutions have been performed. On the other hand, a great many investigations have been performed with X-rays, which have led to the conclusion that the radiation injury from sparsely ionizing radiations is caused predominantly by the reaction of radical species resulting from radiolysis of the aqueous medium. Thus, the species OH, H, and e^-_{aq} are assumed to be of particular importance for the injury resulting from radiation of low LET. However, our knowledge about the exact amount of enzyme injury caused by any of these radiolysis products is rather scanty for radiations of low LET[1] and almost nonexistent for radiations of high LET.

The purpose of the present work was to compare the radiosensitivity of the enzyme lysozyme in dilute solution when exposed to 120 MeV stripped C-nuclei with that after exposure to X-rays.

Materials and Methods

Twice-crystallized and lyophilized hen egg lysozyme was dissolved in a concentration of 0.1 mg/ml in buffers of various pH values, made up from citric acid and sodium phosphate. Details of the experimental procedures including the method used to determine the lysozyme activity are described elsewhere.[2, 3]

For X-ray exposure, the enzyme solution was contained in a sintered glass filter funnel with 0.25 mm Lucite cover. Aliquots of enzyme solution were here withdrawn through a small hole in the Lucite cover-by-means-of a syringe, without interruption of the gas flow through the solution. The dose

*Norsk Hydro's Institute for Cancer Research, Montebello, Norway, and Donner Laboratory, University of California, Berkeley.

rate in the radiation vessel was 2.5 krad/min, determined with a victoreen ionization chamber.

C^{6+}-ions of energy 120 MeV were accelerated in the Berkeley heavy ion linear accelerator (Hilac). An irradiation chamber as shown in Fig. 1 was designed. The beam enters the chamber through a 0.25-mil Mylar window over which area the particle fluence was constant. The thickness of the chamber in the direction of the beam is about 4 mm. The carbon ions (which have a range in water less than 1 mm) are therefore stopped in the solution in the chamber. The enzyme solution was bubbled with either O_2 or N_2 during the exposure, by flushing the gas through the inlet tube and the sintered glass filter, shown in the figure. The bubbling action was insufficient, however, to ensure homogeneous irradiation of the entire enzyme solution in the chamber. Therefore, a small magnet stirrer was used, as shown in the figure.

The dose delivered to a sample by exposure to C^{6+}-particles was calculated from the number of C-ions stopped in the sample, the energy of the C-ions when entering the solution, and the thickness of the chamber holding the enzyme solution. The average dose rate was about 85 krad/min. Details of the C^{6+} dosimetry and exposure procedures are given elsewhere.[3]

Results

Shape of Dose-Inactivation Curves

The dose-inactivation curves of lysozyme irradiated with X-rays under oxygen bubbling were usually exponential. When exposed in nitrogen atmosphere, on the other hand, they were always found to be of the type shown in Fig. 2a, e.g., a low efficiency for inactivation in the low-dose region, followed by an exponential or nearly exponential portion with higher inactivation efficiency as the dose increased. This type of curve shape was never observed after exposure to C^{6+}-ions. When the heavy ions were used, the dose-inactivation curves were always exponential. Sometimes the exponential portion extended over the entire dose range studied, as shown in Fig. 2b, whereas under other conditions the dose-inactivation curves showed a "resistant tail" in the high-dose region. We have not studied the conditions under which this "tail" appears, but it may at least in part be ascribed to insufficient mixing of the enzyme solution during the irradiation. The present

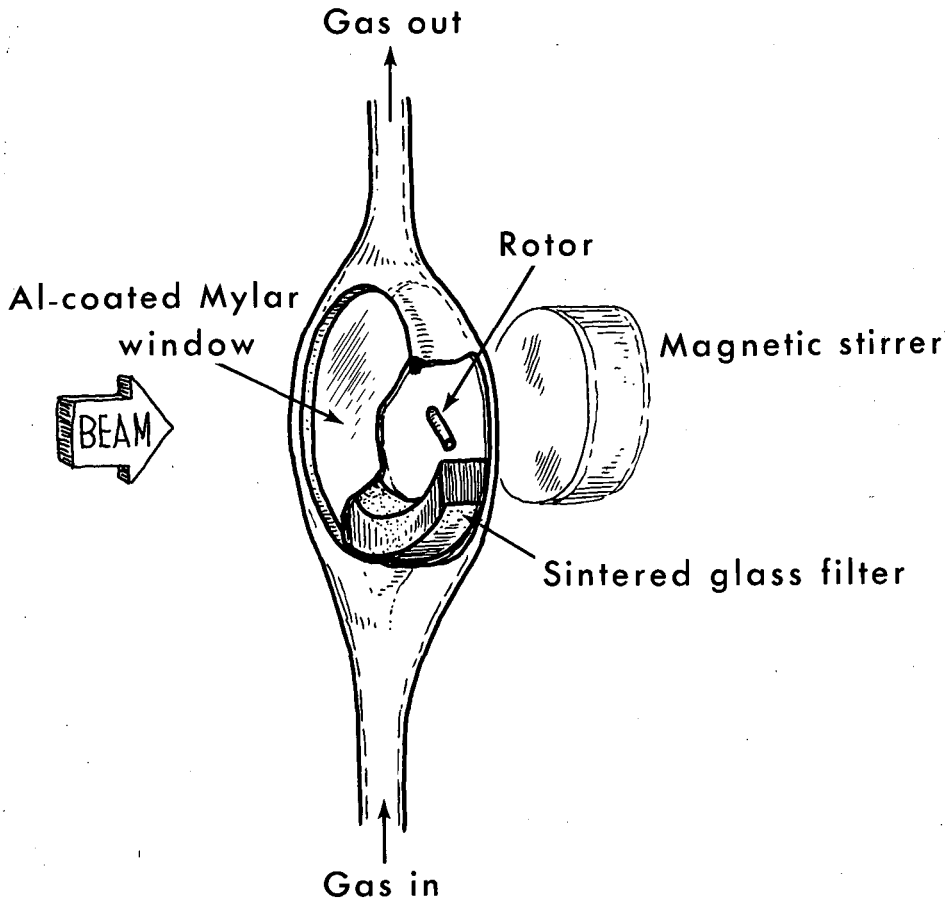


Fig. 1. Drawing of the exposure chamber used when irradiating enzyme solutions with heavy ions. The heavy ions enter the chamber through the thin Mylar window, which is Al-coated on one side, facing away from the solution.

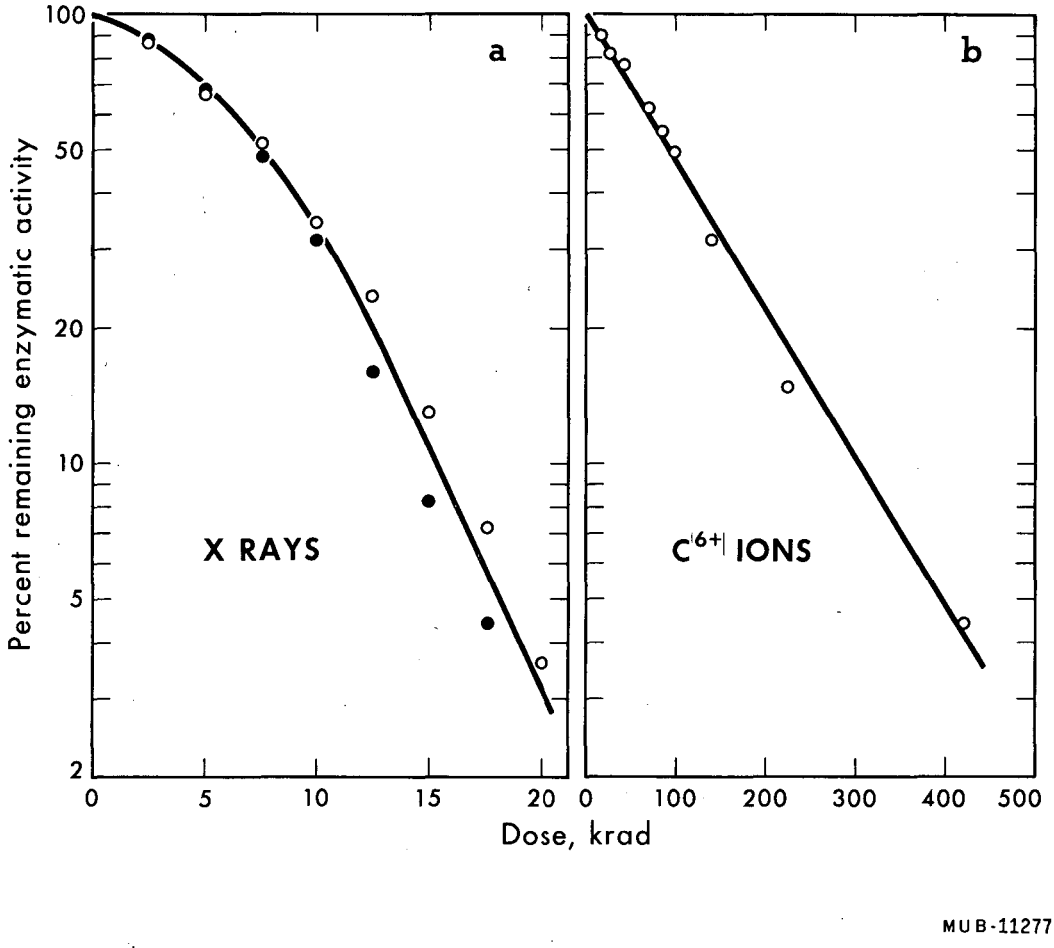


Fig. 2. Dose-inactivation curves of lysozyme in N_2 saturated aqueous solution of pH 3.5, exposed to (a) 150 kV X-rays, (b) 120 MeV C^{6+}-ions.

finding that densely ionizing radiation gives rise to exponential dose-inactivation curves under conditions that cause sparsely ionizing radiation to produce "multiple hit" curves, parallels results obtained on the cellular level, for inactivation of the hatching ability of Archemia eggs[4] and for inactivation of the reproductive capacity of human kidney "T1" cells.[5]

Radiosensitivity of Lysozyme Exposed to C^{6+}-ions and X-rays

The heavy ion inactivation data presented in the following are very incomplete and based on experiments which were usually carried out just once. Because of these serious shortcomings of the present data, we will here only point out some of the most striking differences between the effects of heavy ions and of X-rays. Figure 3 shows the radiosensitivity of lysozyme given as the reciprocal value of the 63% inactivation dose in rads, plotted as a function of the pH of the irradiated enzyme solution. The results are given for the densely ionizing C^{6+}-ions as well as for the sparsely ionizing X-rays. For comparison, the radiosensitivity of lysozyme exposed inthe dry state to C^{6+}-ions is also given, to show the contribution to the inactivation from the so-called "direct" effect. The samples were here dried from the same enzyme solution as used in the other experiments reported in this paper. Thus, the pH refers here to the solution from which these samples were dried. Two conclusions can readily be drawn from the data presented in Fig. 3:

(a) The sensitivity in nitrogen atmosphere is about 10 times as great in the case of X-rays as in the case of C^{6+}-ions.

(b) Although oxygen for both radiations provides a radioprotection over the entire pH range studied, the magnitude of this protection is smaller for C^{6+}-ions than for X-rays.

Protection Against Injury from Densely Ionizing Radiation

Because of the large differences observed between the radiosensitivity of lysozyme irradiated with X-rays and that after irradiation with C^{6+}-ions, it was of interest to compare the effectiveness of various chemical radioprotectors in reducing the degree of enzyme inactivation caused by the two types of radiations. So far we have studied the effect of ethanol, glycerol, histidine, and cysteine on the radiation injury of lysozyme irradiated in O_2-free solution.

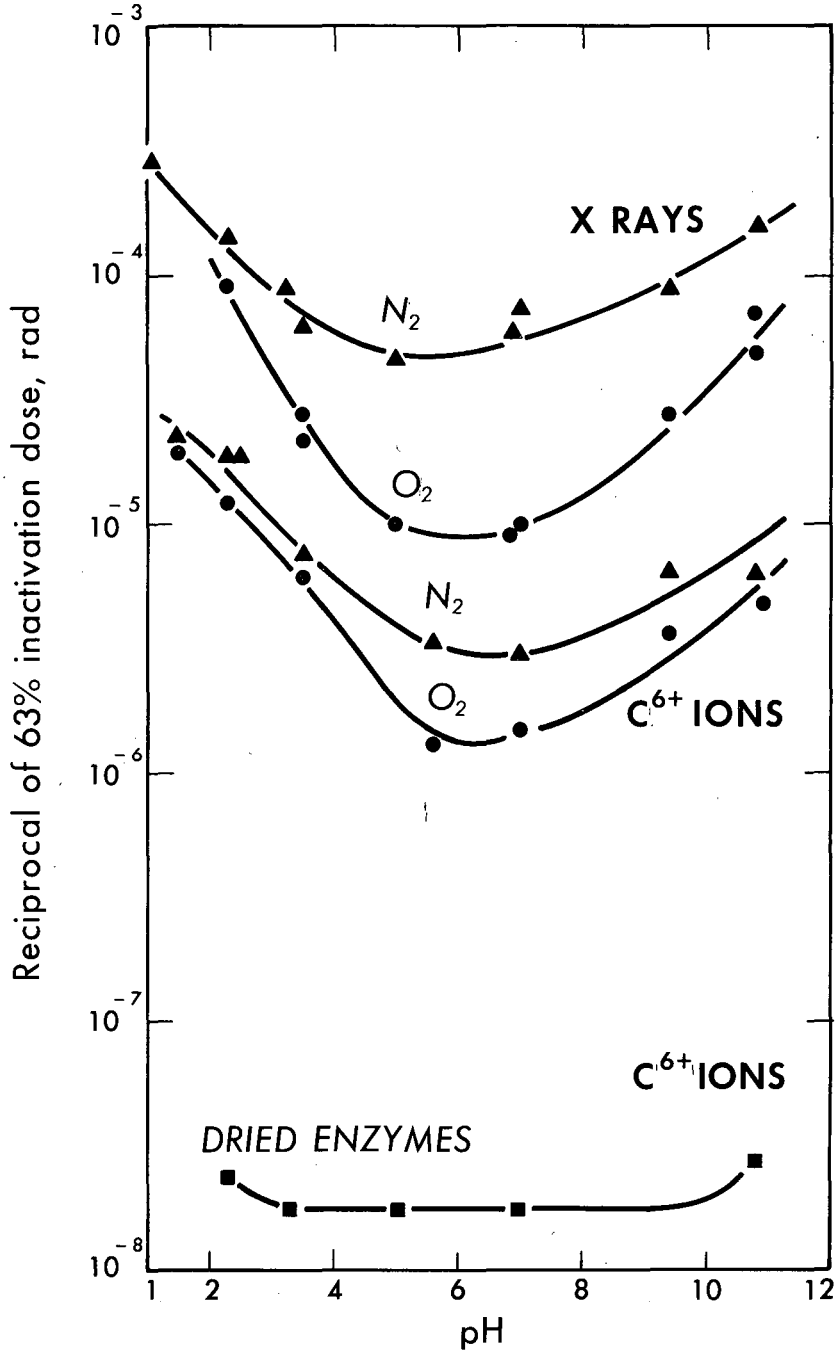


Fig. 3. The radiosensitivity of lysozyme in N_2 and in O_2 saturated solutions of various pH, for 150 kV X-rays and 120 MeV C^{6+}-ions. The radiosensitivity of lysozyme, dried from solutions of various pH and irradiated in high vacuum with C^{6+}-ions is shown for comparison.

The first two compounds have high rate constants for reactions with OH radicals ($\approx 3 \times 10^8$ M^{-1} sec^{-1}), an order of magnitude smaller for reactions with H radicals, and both are quite inefficient as scavengers of hydrated electrons.[6] Histidine[7] was used because of its high rate constants for reactions with hydrated electrons ($\approx 3 \times 10^9$ M^{-1} sec^{-1}). Cysteine-HCl was incorporated in this comparison because of its extensive use as a radioprotector in numerous radiobiological investigations. Complete dose-effect curves were determined for three or more concentrations of each of the radioprotectors under investigation. From such curves plots of the radiosensitivity as a function of the protector, concentrations were obtained. Figure 4 shows an example of how the radiosensitivity of lysozyme depends on the concentration of glycerol during the exposure to C^{6+}-ions. It is seen that the radiosensitivity decreases considerably even for rather moderate concentrations of glycerol and levels off as the protector concentration is increased. Thus, at the highest concentration tested the sensitivity is reduced to about 5% of that of an unprotected enzyme solution. It is of interest to note that the radiosensitivity observed in the presence of such a high concentration of glycerol is still about 35 times as high as that observed for dry samples. In Table I are shown for each of the four protectors tested, the concentrations required to reduce the radiosensitivity of lysozyme to 50% of that value characteristic of the unprotected enzyme solution. From this table two conclusions can be drawn:

(a) All four compounds protect lysozyme against radiation injury resulting from X-rays as well as from C^{6+}-ions. Surprisingly, less than 2 times higher concentrations of the protectors appear to be required to provide the same degree of protection against injury from C^{6+}-ions as from X-rays.

(b) Ethanol and glycerol are about equally effective radioprotectors, whereas cysteine is more effective by almost two orders of magnitude.

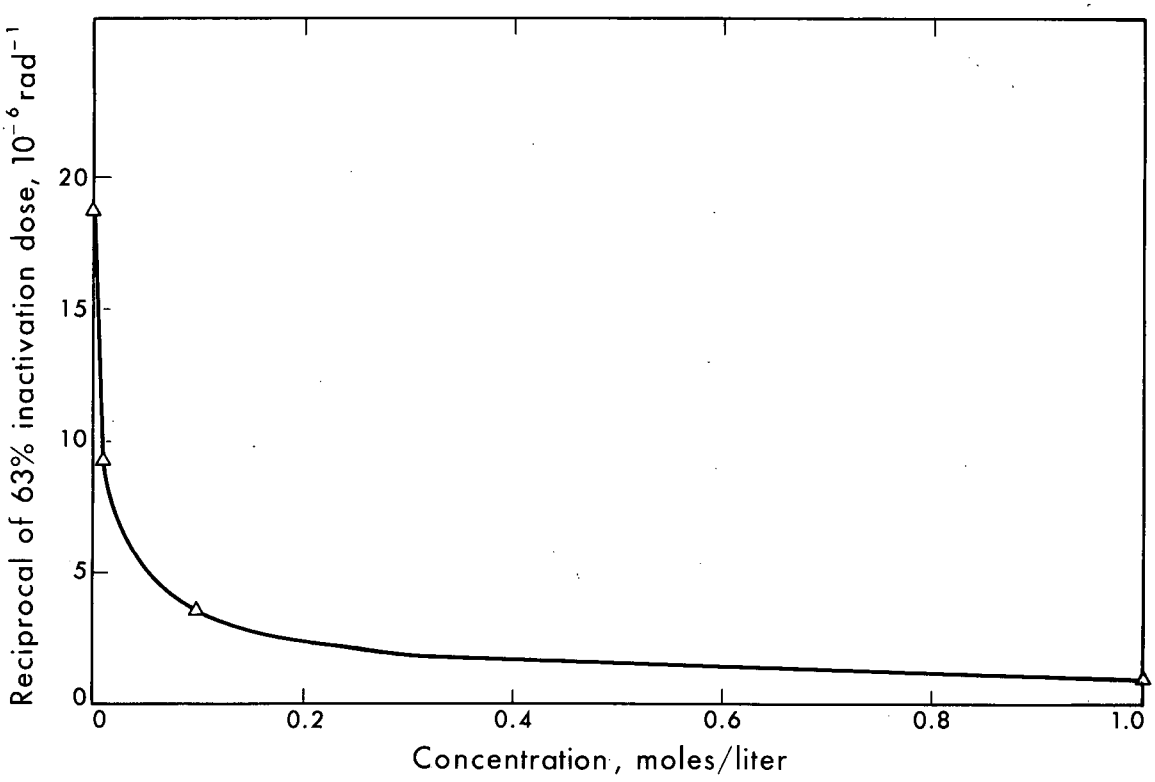


Fig. 4. The reciprocal of the 63% inactivation dose of lysozyme, irradiated with C^{6+}-ions in a N_2-saturated solution of pH 2.5, as a function of the concentration of glycerol in the solution.

Table I. Concentrations of protectors (10^{-4}M) which reduce sensitivity to 50%.

Protector	pH = 2.5		pH = 5.0		pH = 9.4
	X-rays	C^{6+}	X-rays	C^{6+}	C^{6+}
Ethanol	60	75	90	135	--
Glycerol	60	100	90	125	--
Histidine	--	21	7	12	4
Cysteine	1	1	1	2	1

Discussion

When radiation by "indirect" action through water radicals (H, OH, or e^-_{aq}) act on saturated organic molecules, such as enzymes, the major reaction is presumed to involve removal of a hydrogen atom from the molecule, forming a free radical in which the free bond lies on a carbon or, perhaps, a nitrogen atom. When radiations act "directly" upon the organic molecule, similar radicals are presumably also formed. It is well known from studies of simple solutions such as ferrous sulphate, exposed to polonium alpha radiation (or other radiations of high LET from disintegrating nuclei) that very few water radicals escape the tracks of such radiations.[8] Since the stopping power of water for C^{6+}-ions used in the present investigation is greater than that of polonium alpha particles, one may suspect that very few water radicals escape from the "core" of the C^{6+}-tracks also. When enzyme solutions are irradiated by X-rays the enzyme radicals will be formed mainly as a result of indirect action, whereas when C^{6+}-ions are used the enzyme radicals will be formed to a much greater extent by direct action. The observation that the radiation injury in nitrogen atmosphere is about 10 times higher for X-rays than for C^{6+}-ions is in line with this reasoning: The heavy ion beam is expected to be able to affect only a small fraction of the solution, while the free radicals resulting from the X-rays are able to pervade the entire solution. Under these latter conditions it is expected that oxygen or other radical scavengers can exert their scavenging action quite efficiently. The fact that oxygen provides a strong protection against the X-ray-induced injury of lysozyme, as seen in Fig. 3, has been

interpreted to indicate that lysozyme is sensitive to attack by diffusible reducing radical species, in particular, H and e^-_{aq} (Ref. 2). Since the injury to the enzymes after C^{6+} exposure to a higher extent than after X-ray irradiation presumably stems from "direct" action, one expects here less protection by the presence of specific radical scavengers, which also is seen from Fig. 3. This suggest that if the observed protection by oxygen, of the enzyme injury resulting from C^{6+} irradiation, is caused by scavenging of water radicals, the latter are presumably created by high energy δ-rays of low LET, outside the core of the tracks of the C^{6+}-particles. Another possibility here is that enzyme radicals which are formed by the direct action of the C^{6+}-ions may be able to diffuse in time out of the tracks where they are formed and to react with oxygen or other solute molecules which may be present only in low concentration. This is possible because of the longer lifetime of these enzyme radicals as compared with the highly reactive water radicals. Oxygen molecules are, however, known to react very rapidly with carbon radicals to form peroxy radicals. Subsequent reaction of such peroxy radicals may be such that the activity of the enzyme is not destroyed, while in the absence of oxygen the enzyme radical may undergo some spontaneous rearrangements that have a certain probability of destroying the enzymatic activity of the molecule. The present data are too incomplete to allow us to distinguish between these two different explanations of the protection of enzymes in dilute solution against heavy-ion-induced injury. Further experiments are, however, being planned to elucidate this question.

A pulse radiolysis technique has proved to be very useful in elucidation of the mechanisms of injury induced by radiations of low LET on biological macromolecules in aqueous solutions.[9] Pulse radiolysis studies of heavy-ion-induced effects have, however, not been performed so far because of unsuitable beam characteristics of the heavy ion accelerators available.[9] If the Omnitron plans materialize, however, heavy ion beams of sufficient energy, intensity, and pulse length can be obtained to make heavy ion pulse radiolysis studies possible and very promising. Combined with general chemical approaches as presently used, considerable progress in our understanding of mechanisms behind observed LET effects in molecular radiobiology is then to be expected.

Acknowledgments

I am very grateful to Professor C. A. Tobias for the opportunity to work in his group and for his interest in this work, to Dr. John Lyman for discussions of heavy ion dosimetry, and to Mr. David Love, Mr. Jerry Howard, and Mrs. Jean Luce for their unfailing help and support during the arduous Hilac experiments. The work was jointly supported by the U. S. Atomic Energy Commission, the National Aeronautics and Space Administration, Norsk Hydro's Institute for Cancer Research, and The Royal Norwegian Council for Scientific and Industrial Research, Norway.

References

1. R. A. Luse, Basic Mechanisms in the Radiation Chemistry of Proteins and the Nucleic Acids, Radiation Res., Suppl. 4, 192-212 (1964).
2. T. Brustad, The Effect of Radical Scavengers on the Radiosensitivity of Lysozyme in Dilute Aqueous Solutions of Varying pH, Radiation Res. 27, 456-473 (1966).
3. T. Brustad, On the Mechanisms of Radiation Inactivation of Enzymes in Dilute Solutions, in Third International Congress of Radiation Research, Cortina, 1966 (to be published).
4. F. Hutchinson and S. S. Easter, Jr., A Difference Between Biological Effects of Gamma Rays and Heavy Ions, Science 132, 1311-1312 (1960).
5. P. Todd, Reversible and Irreversible Effects of Densely Ionizing Radiation upon the Reproductive Capacity of Cultured Human Cells, Med. Coll. Virginia Quart. 1, (4), 1-14 (1966).
6. M. Anbar and P. Neta, Tables of Bimolecular Rate Constants of Hydrated Electrons, Hydrogen Atoms and Hydroxyl Radicals with Inorganic and Organic Compounds, Intern. J. Appl. Radiation Isotopes 16, 227-242 (1965).
7. R. Braams, Rate Constants of Hydrated Electron Reactions with Amino Acids, Radiation Res. 27, 319-329 (1966).
8. A. O. Allen, The Radiation Chemistry of Water and Aqueous Solutions (D. Van Nostrand Company, Inc., New York, 1962).
9. Pulse Radiolysis, ed. by M. Ebert, J. P. Keene, A. J. Swallow, and J. H. Baxendale (Academic Press Inc., New York, 1965).

RADIOBIOLOGY AND RADIOCHEMISTRY OF LIPOPROTEINS

A. V. Nichols, N. K. Freeman, and F. T. Lindgren

The radiobiology and radiochemistry of lipoproteins have not been adequately explored, and further studies in these areas are needed. An approach to the study of the possible chemical sensitization of lipoprotein and membrane structures to disruption by radiation is proposed. It is planned to evaluate the physical and chemical effects of low and high LET radiations on lipoprotein structures containing specific organic lipid-soluble materials. Initially, human serum lipoproteins will serve as the lipid-protein structures, and changes in their physical and chemical properties will be investigated. Among the various kinds of radiation and radiation sources that will be used for these investigations, the unique characteristics of the Omnitron should prove to be of unusual value. Our rationale for the above radiobiologic studies is discussed below.

Recent studies on cellular and subcellular membranes clearly show a strong dependence of many integrated metabolic functions of a cell on the molecular organization of the membrane.[1] The complex lipoprotein surfaces of cellular membranes provide highly specific loci for organized enzyme activity. The integrity of the membrane, hence, is crucial not only for maintaining a barrier between the cell or organelle and its environment, but also is a critical factor in maintaining the organization required for sequential metabolic processes. Specific examples of such membranes are the endoplasmic reticulum and the mitochondrial membrane in cellular cytoplasm.

Release of enzymes from their physiological sites of activity has been observed following exposure of subcellular particles to agents such as radiation, auto-oxidizable compounds, and peroxides.[2] These agents have all been shown to produce, presumably via free radical mechanisms, substantial modification of the chemical and physical properties of lipids and proteins, the principal components of biological membranes.[3] Therefore, the underlying basis for the disruption of many of the functions associated with membranes by such agents is believed to consist of structural changes in lipids and proteins induced by free radical attack.

An agent capable of producing dramatic alterations in subcellular membrane (endoplasmic reticulum) structure and function is the hepatotoxin, carbon tetrachloride. Extremely minute amounts of this agent administered in vivo disrupt protein synthesis, lipoprotein formation and excretion, and other enzymatic activity associated with an intact endoplasmic reticulum.[4] Several kinds of evidence suggest that the action of carbon tetrachloride is due, in part, to a cleavage of this compound into a trichlormethyl free radical and monatomicchlorine at or near the endoplasmic reticulum. Once formed, the free radicals are believed to spark an autocatalytic, peroxidative degradation of the unsaturated lipids present in the membrane. It is suggested that such alterations in the lipids could drastically change their physical and chemical properties and could lead, in turn, to extensive changes in membrane structure and function. The metabolic processes responsible for the potential formation of the free radicals from carbon tetrachloride have not as yet been established. It is interesting to note, however, that the high solubility of carbon tetrachloride in lipoprotein structures probably ensures significant exposure of this material to the enzymatically active surfaces of the endoplasmic reticulum.

The uptake of lipid-soluble organic materials into lipoproteins and cellular membranes by living systems may be significantly increasing by virtue of the continuing contamination of the environment by industrial wastes, pesticides, cigarette smoke, auto exhaust, etc. Various processes may initiate free radical formation in these materials and hence lead to localized disruption of normal structure and function by the oxidative changes already discussed. It is hoped that the proposed study will provide some further information on the bases for the correlations being developed between environmental agents and biological dysfunction and disease.

References

1. D. E. Green and A. Tzagoloff, J. Lipid Res. 7, 587 (1966).
2. I. D. Desai, P. L. Sawant, and A. L. Tappel, Biochim. Biophys. Acta 86, 277 (1964).
3. W. T. Roubal and A. L. Tappel, Arch. Biochim. Biophys. 113, 5 (1966).
4. R. O. Recknagel and A. K. Ghoshal, Lab. Invest. 15, 132 (1966).

SOME EFFECTS OF ACCELERATED CHARGED PARTICLES IN BACTERIAL SPORES

E. L. Powers,* J. T. Lyman, and C. A. Tobias

Bacillus megaterium is a convenient organism for the analysis of biological damage produced by various radiations, as a number of aspects of X-ray damage have been described. We have used dried spores of this organism to test the kinetics of survival after bombardment by a number of heavy ions at the Berkeley heavy ion linear accelerator (Hilac). Each of the ions—He, B, C, N, O, Ne, and A—was used at a kinetic energy of 8.3 MeV/nucleon, so that the distribution of the secondary ionization was the same for each particle but the linear energy transfer (LET) changed from one particle to the other as the ratio to the squares of the charges on the particles. Here we summarize these results; a detailed paper is in press.[1]

Prior to this work Powers[2] found that radiation damage to the survival of bacterial spores may be classified into three different types: I. Oxygen-independent damage. This is observed if oxygen is kept from the spores during and after irradiation. II. Immediate oxygen effect. This type of damage necessitates the presence of oxygen during exposure to irradiation. III. Delayed oxygen effect. This appears when organisms are irradiated in nitrogen and later exposed to oxygen. By exposing the bacterial spores to the different accelerated particles in the so-called "track segment method" and by varying the environmental conditions for exposure, we were able to study each type of damage induced by heavy ions.

The organisms were exposed to heavy particles in the presence of air, in nitrogen, and in nitrogen followed by H_2S prior to exposure to air. These three types of exposure allowed differentiation between the three classes of biological effects.

Survival curves of this particular bacterial spore are exponential to all the radiations tested. However, it was found that the heavier particles proved to be more effective in eliminating self-proliferation than the light particles. The surviving fraction of spores N/N_0 can be expressed by a

*Present address: Dept. of Zoology, University of Texas, Austin, Texas.

simple formula as:

$$\frac{N}{N_0} = e^{-\sigma f}$$

where σ is the interaction cross section and f is the flux of particles. Here σ is a function of the linear energy transfer ϵ. The cross section as a function of LET is plotted in Fig. 1. The cross section is essentially proportional to the efficiency by which a given particle is able to cause lethal effect.

Because the data points were quite accurate, we were able to subject them to mathematical analysis and to compare them with various models of the biological effects of radiation. In order to do this, a least-squares fit was made to a number of models by optimizing the fit for the parameters for each model. The least-square sum for each model was compared.* The best fit was obtained when the cross section had the following analytical form:

$$\sigma = \sigma_{\alpha\infty} (1-e^{-\alpha\epsilon}) + \sigma_{\beta\infty} (1-e^{-\beta\epsilon^2}) , \qquad (1)$$

where $\sigma_{\alpha\infty}$ and $\sigma_{\beta\infty}$ are limiting values σ for $\epsilon \to \infty$ and α and β are constants. It is clear from the model that the mode of radiation damage is different at low LET and at high LET. Most of the oxygen-dependent inactivation with required immediate oxygen or late effect of oxygen is due to the mechanism which is most effective at low LET values. The cross section for this process is expressed in the first term of Eq. 1 as a simple saturation curve. At high LET the second term predominates; over a considerable range of values, this predicts a rapidly rising cross section, proportional to the square of LET, and at very high LET there is little demonstrable difference for inactivation in the presence or absence of oxygen. A simple interpretation for the finding that at high LET the radiation effect can be proportional to the square of LET is the explanation that two ionization events along a single track of an ionized particle interact to produce radiation inactivation.

The presence of the two types of effects explains the high relative biological effectiveness of heavily ionizing radiations. The values of the limiting cross sections and inactivation constants are presented in Table I.

---

*We are indebted to Merlin Dipert of Argonne, and to Eric R. Beals and Mark W. Horovitz at Berkeley for these calculations.

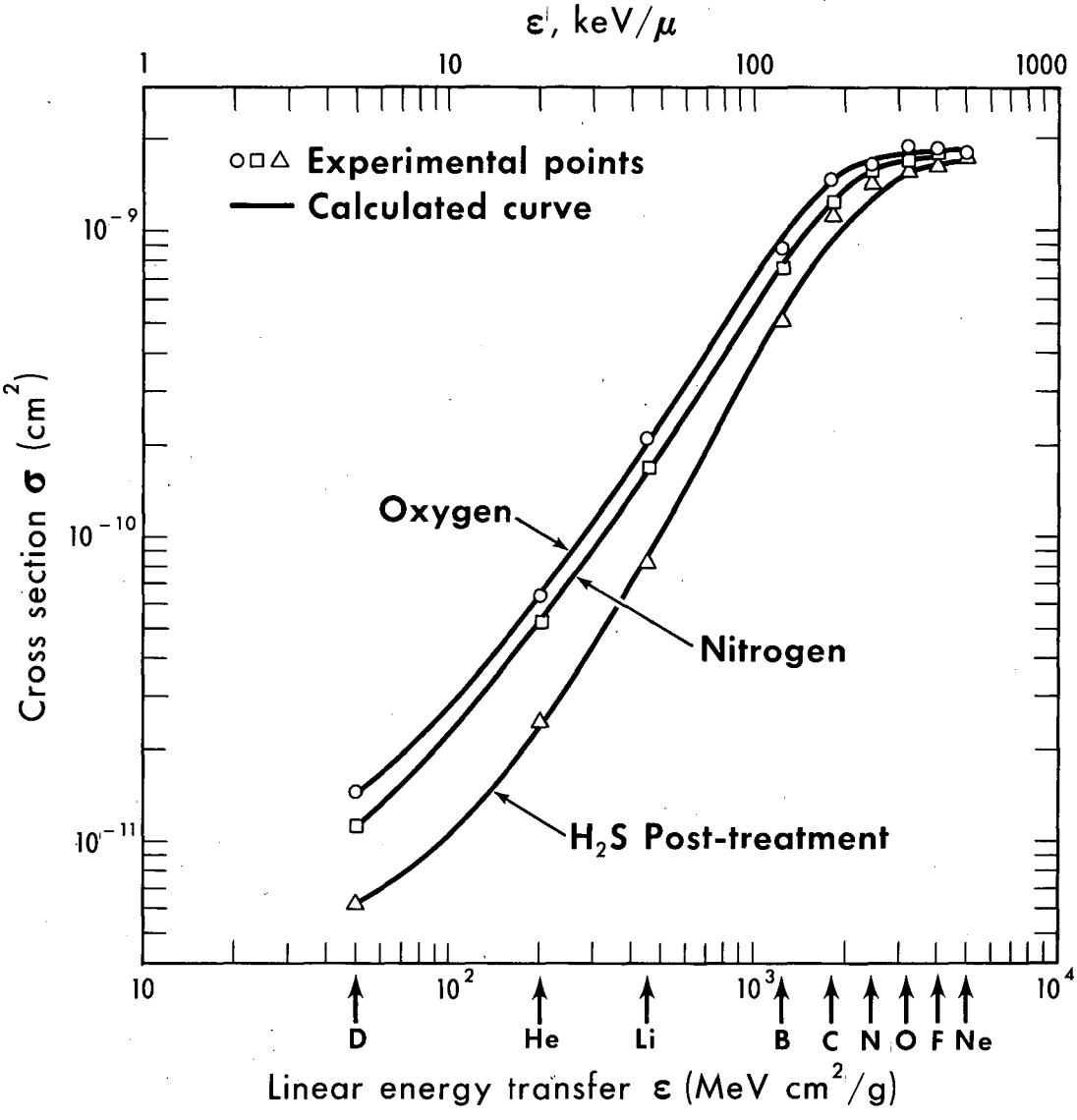

ε', keV/μ
1
10
100
1000
○□△ Experimental points
Calculated curve
Oxygen
Nitrogen
H2S Post-treatment
Cross section σ (cm²)
10⁻⁹
10⁻¹⁰
10⁻¹¹
10
10²
10³
10⁴
D
He
Li
B
C
N
O
F
Ne
Linear energy transfer ε (MeV cm²/g)
MUB-6985

Fig. 1

Table I. Best values of cross sections and constants.

	$\sigma_{\alpha\infty} + \sigma_{\beta\infty}$ (cm^2)	$\sigma_{\alpha\infty}$ (cm^2)	α (cm^{-1} MeV)	$\sigma_{\beta\infty}$ (cm^2)	β ($cm^{-2}MeV^2$)
Exposure in air	1.84×10^{-9}	0.045×10^{-9}	6.35×10^{-3}	1.795×10^{-9}	4.56×10^{-7}
Exposure in nitrogen	1.77×10^{-9}	0.046×10^{-9}	4.6×10^{-3}	1.624×10^{-9}	3.66×10^{-7}
Exposure in N_2 with H_2S post treatment	1.72×10^{-9}	0.894×10^{-11}	1.74×10^{-2}	1.712×10^{-9}	2.33×10^{-7}

By applying a similar model to other experiments with unicellular organisms, for example to yeast cells and to mammalian cells, it is found that lethal effects can be well explained by the presence of the two types of inactivation components. For cells which have large nuclei and large chromosomes, the second term, depending on LET square, is of greater importance than the first term. A number of current experimental studies have indicated that under certain conditions bacteriophage are likely to suffer lethal effect if radiation produces a double chain scission in their DNA. It is known, however, that most low LET radiations do not often produce double chain scission, but rather they can produce damage to only a single nucleic acid chain. It will be of interest to make an experimental test of the types of molecular alternations that high LET radiations produce in DNA.

Some preliminary studies with free radicals induced in the dried spores have indicated that there is a parallelism between the amount and type of free radical induced in the final biological effect. High LET radiation appears to produce free radicals in the bacterial spores that are not annealable, whereas the concentration produced by free radicals in the low LET region is modified by post-temperature exposure to H_2S or to high temperature.

The results presented in this summary point to a way of classifying and quantitatively accounting for the effects of high LET radiation. Most of these effects are accounted for by assuming that the cell nucleus is affected; the detailed physicochemical events and the relationship of biological effects to the free radicals induced remains to be elucidated.

References

1. E. L. Powers, J. T. Lyman and C. A. Tobias, Some Effects of Accelerated Charged Particles on Bacterial Spores (submitted to Rad. Res.).
2. E. L. Powers, Some Physicochemical Basis of Radiation Sensitivity in Cells, In Proc. 18th Annual Symposium on Fundamental Cancer Research, "Cellular Radiation Biology," M. D. Anderson Hospital, Houston, Texas (1964) (Williams & Wilkins Co., Baltimore)

CELLULAR RADIOBIOLOGY OF HEAVY IONS: CELL RECOVERY AND DNA REPAIR

R. H. Haynes, W. R. Inch,* and J. T. Lyman

When any agent such as X-rays, ultraviolet light (UV), or nitrogen mustard (HN_2) is applied to cells it produces a "spectrum" of chemically distinct structural defects in the genetic material (DNA) and other cell components. Inactivation and mutation are perhaps the biological effects of most general interest, and they result from those defects that are formed in DNA. The type and number of defects depends on the agent used and its dose. If the observed biological effects were determined solely by the initial spectrum of DNA defects, our major task would be to carry out appropriate radiochemical studies to identify and enumerate these defects for various doses of the agents of interest. However, cells also possess enzymic mechanisms which repair certain of the initially produced defects before they express themselves biologically. Clearly then, the fate of the cell depends jointly on the probability of formation and repair of each type of defect. Therefore, in addition to radiochemical studies, the relative repairability of all the various defects induced in DNA must be determined if we are to understand fully the molecular basis of action of any such agent.

Strand breaks, pyrimidine nimers, and guanine cross-links figure prominently among the various DNA defects produced by X-rays, UV, and HN_2 respectively. The enzymic repair of each of these defects and the associated recovery of cell viability has been demonstrated in both bacteria and yeast.[1-6] Beams of heavy ions can be used to change the initial distribution of the DNA defects produced by ionizing radiation through an increase in linear energy transfer (LET). A number of workers have suggested that this change consists primarily in an increase in the ratio of double- to single-strand breaks. However, this suggestion has not so far been verified quantitatively, nor has the relative repairability of strand breaks and other types of defects produced by ionizing radiation been determined for the various known repair systems. Appropriate techniques have been designed to measure strand breakage in bacterial DNA following exposure of the cells to

*Ontario Cancer Foundation, London, Ontario, Canada.

X-rays.[1] Similar studies should be carried out for radiations of higher LET. However, the limited penetration of the currently available Hilac (heavy ion linear accelerator) beams restricts the amount of material that can be conveniently irradiated. With the Omnitron beams, it will be a simple matter to irradiate the size of bacterial sample required to extend this analysis into the high-LET region.

The colony-forming ability of a cell is a convenient means for assessing damage to its genetic material, and the analysis of dose-survival curves is useful in constructing a general picture of the fundamental processes involved. In the bacterium E. coli, DNA repair manifests itself in the marked difference in radiosensitivity between the reactivating strain B/r and the non-reactivating mutant B_{s-1}. It would appear that the principal known DNA lesions produced X-rays, UV, and HN_2 can be reparied by the same enzymic mechanism in B/r. In view of this versatility of the coli repair mechanism it is natural to ask if there are any lesions that are not repairable.

Studies with mammalian cells indicate that high-LET radiations produce a greater proportion of irreversible damage than do X-rays, and it is not implausible to imagine that unrepairable DNA damage might be produced within the columns of dense ionization formed along the tracks of such particles. In a series of experiments with Hilac beams, we found that the differential sensitivity of B/r and B_{s-1} declines as the LET of the particles increases, and that the two strains are almost equally sensitive to partially stripped argon nuclei (Fig. 1). The simplest interpretation of these results is that the fraction of repairable defects declines with increasing LET. This could be attributed to an increasing probability of producing unrepairable DNA double-strand breaks by densely ionizing particles. However attractive this interpretation, it remains open to the criticism that DNA may be a relatively less important target for these high-LET particles, and that lethality might be due to membrane damage or other processes.

Further experiments are necessary to determine if an increase in double-strand breaks and declining repair are in fact correlated with an increase in LET.

Recovery after irradiation has been observed in organisms of wide phylogenetic distribution, and in many instances DNA repair might be involved. However, it is by no means certain that the enzymic steps are the

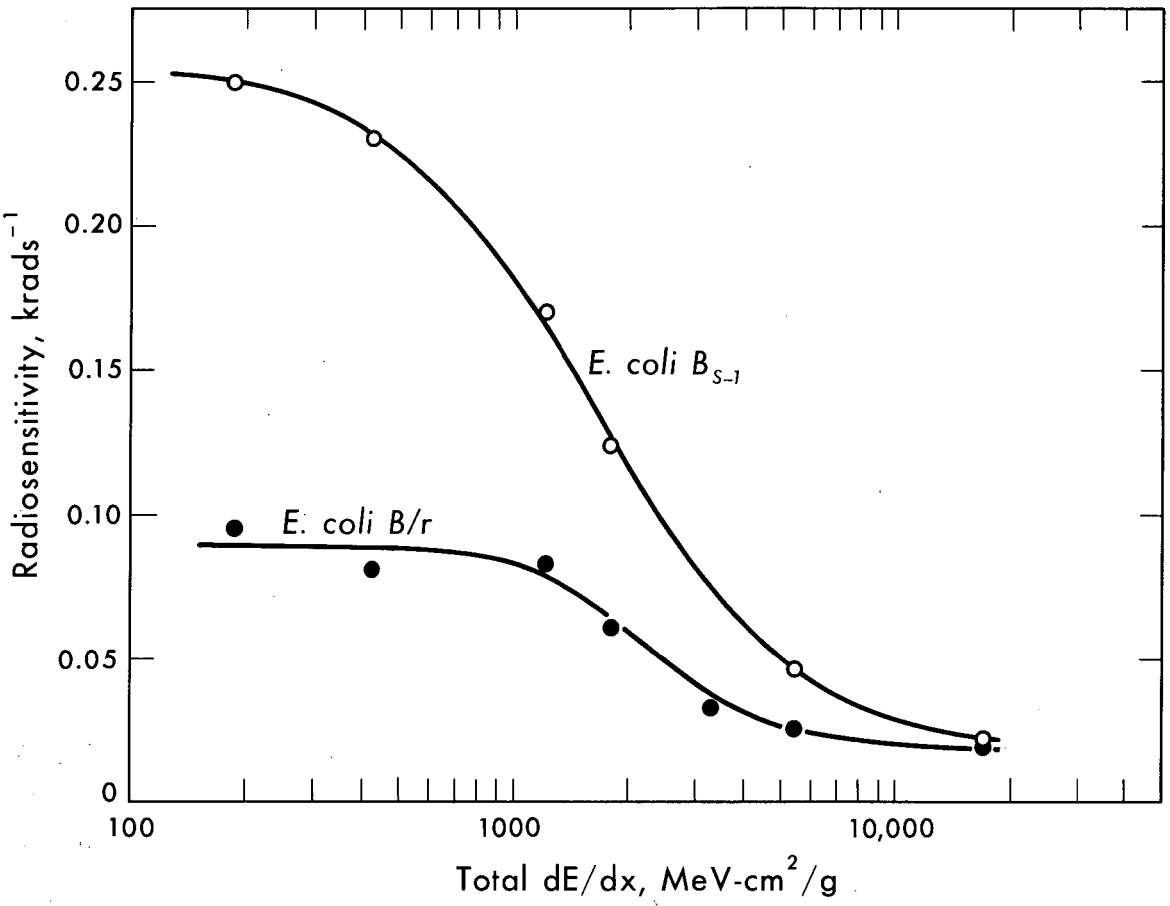


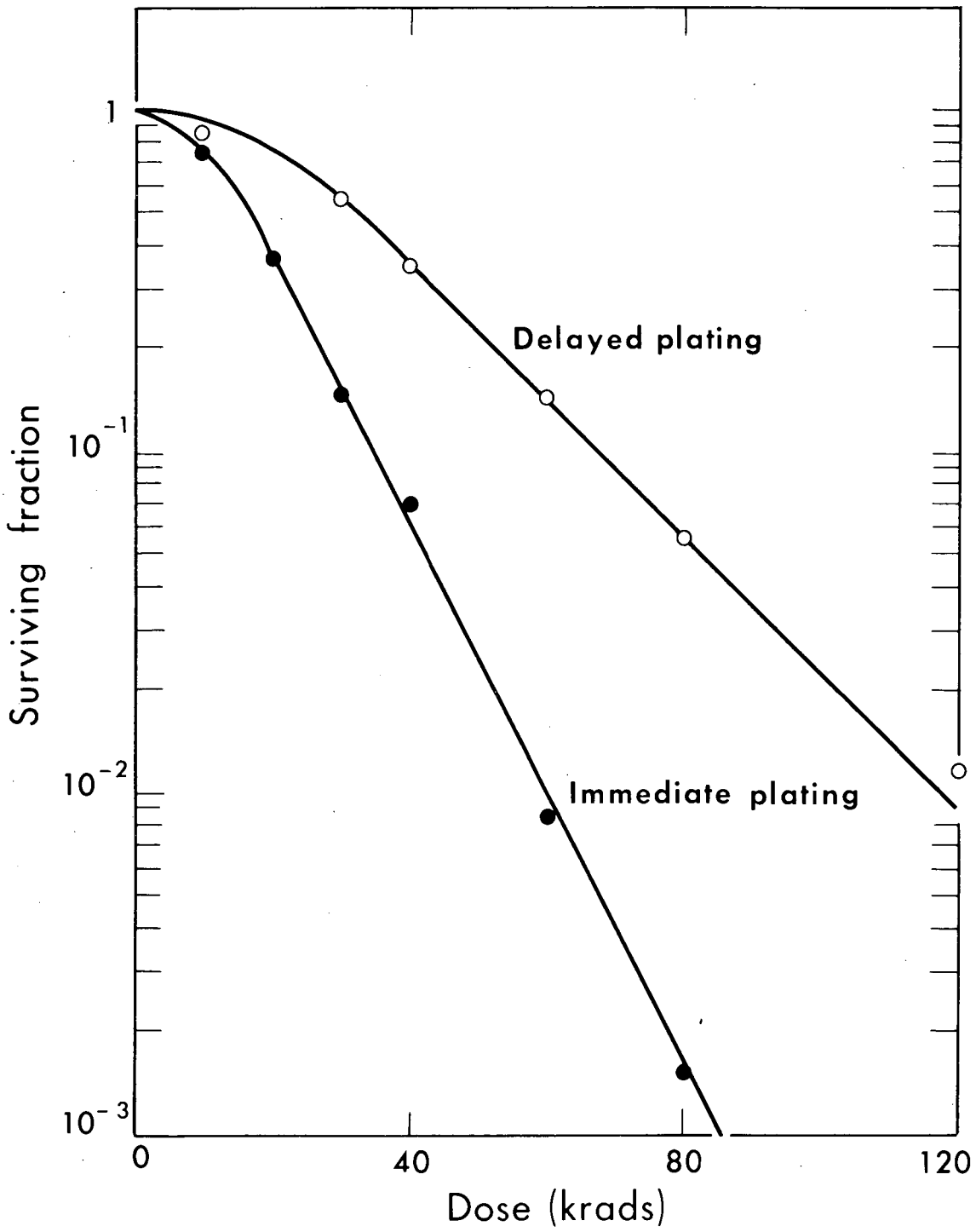
Fig. 1. Radiosensitivity (LD_{90}^{-1}) of E. coli B/r and B_{s-1}, irradiated under fully oxygenated conditions, as a function of the total mass stopping power of the various heavy-ion beams (9 MeV/amu) produced by the Berkeley Hilac. All collisional losses (including δ-electrons) were included in the calculation of dE/dx, which is used as an approximate mean LET.

same in each case. Liquid-holding recovery in diploid yeast and dark reactivation in E. coli are similar in that both appear to be enzymic, energy-requiring processes capable of reversing damage caused by X-rays, UV, or HN_2, but incapable of dealing with damage caused by heat or acridine-sensitized photodynamic action. However, they differ markedly in their response to densely ionizing radiation. We have shown that it is unlikely that high-LET damage can be repaired in E. coli; however, both the survival-curve shoulder and the magnitude of recovery in diploid yeast are independent of LET (Fig. 2). The macromolecular basis of recovery in yeast is unknown but these results strongly suggest that it differs in some fundamental way from excision-repair in E. coli.

It would therefore be desirable to extend the study to still higher-LET values to see if the effect declines in yeast for sufficiently high values of LET as it does in E. coli.

References

1. R. A. McGrath, R. W. Williams, and C. S. Swartzendruber, Biophys. J. 6, 113-122 (1966).
2. R. B. Setlow and W. L. Carrier, Proc. Natl. Acad. Sci. U. S. 51, 226-231 (1964).
3. D. Pettijohn and P. C. Hanawalt, J. Mol. Biol. 9, 395-410 (1964).
4. P. C. Hanawalt and R. H. Haynes, Biochem. Biophys. Res. Comm. 19, 462-467 (1965).
5. M. H. Patrick, R. H. Haynes, and R. B. Uretz, Radiation Res. 21, 144-163 (1964).
6. R. H. Haynes, Radiation Res., Suppl. 6, 1-33 (1966).



MU-35521

Fig. 2. Recovery of diploid yeast (Saccharomyces cerevisiae, strain X841) elicited by 48-hr storage (delayed plating) after irradiation with stripped neon nuclei (9 MeV/amu) under fully oxygenated conditions. The magnitude of recovery is the same as that observed for X-ray or 40 MeV alpha-particle irradiation despite the 30-fold increase in LET.

CELLULAR RADIOBIOLOGY OF HEAVY IONS: MAMMALIAN CELLS

P. W. Todd*

Since the discovery by Puck[1] in 1956 that human cells can be caused to multiply and form colonies in culture in much the same way as bacteria, cellular bases have been discovered for many physiological phenomena known to occur in mammals. The widespread use of ionizing radiation in medicine exploits its cytotoxic effects, and these effects have been subjected to extensive study in isolated cells in culture during the past decade. It occurred to Puck and co-workers[2] that the ability of an isolated human neoplastic cell to multiply into a colony of cells might be a legitimate end-point with which to investigate the effects of ionizing radiation, in much the same way as described in the preceding paper (Cellular Radiobiology of Heavy Ions: Cell Recovery and DNA Repair). With the use of this colony-formation assay, it was discovered that less than 500 rads of X-rays was adequate to inhibit 90% of most types of mammalian cells from forming colonies in culture. This extraordinary sensitivity of mammalian cells in culture has also been confirmed in intact organisms, either by implanting single tumor cells into receptive hosts[3] or by injecting normal stem cells into immunologically suppressed hosts and assaying for the ability of the implanted cells to form "colonies."[4]

Since beams of heavy ions are incapable of penetrating the tissues of intact organisms, we chose the isolated cell culture technique for assessing the potential efficacy of these radiations in inhibiting the proliferation of mammalian cells. Our general method may be simply described as the exposure of cultures containing known numbers of viable cells to graded doses of X-rays and heavy ions, followed by approximately 2 weeks of proliferation and the counting of the colonies that result from surviving cells. From such experiments, one obtains "survival curves" by plotting the logarithm of the fraction of cells which formed colonies against the dose to which they were

---

*With the assistance of John T. Lyman, Robert Tym, David Love, Jerry Howard, Rollin A. Armer, Catharine L. Boerke, and Willie M. Jackson; author now at Department of Biophysics, Pennsylvania State University.

exposed, as described in the previous section. These experiments were performed with a variety of heavy ions, each of which had a different ionization density, or rate of energy loss, in energy-loss units of MeV-cm^2/g.

For a long-term line of cultured human kidney cells,[5] designated T1, exposed to 50 kVp X-radiation, we find survival curves like those given in Fig. 1. As noted previously, about 500 rads of this radiation inhibits colony formation by 90% of the cells when air is present during the irradiation. For heavy ions, the dose required for this end-point is found to be decreased except for heavy ions having -dE/dx very large. This point is illustrated in Fig. 2, which is a plot of 90% inactivating dose against -dE/dx. The accelerated ion used to obtain each value of -dE/dx is indicated on the plot. Heavy ions (in particular boron and carbon ions) with -dE/dx in the vicinity of 2000 MeV-cm^2/g appear to be maximally effective for this end-point.

Another obvious feature of Fig. 1 is that cells irradiated in the absence of air experience a higher survival rate. The ratio on the abscissa of the upper curve to the lower is referred to as the "oxygen enhancement ratio" or OER. It is a ratio of doses and tends to be about 2.8 for the curves in Fig. 1. It shows that about 3/4 of the effect of X-rays depends upon the presence of oxygen. The denser the ionization, the less stringent is this dependence upon oxygen, and Fig. 3 illustrates this point with a plot of the OER against the -dE/dx, with the corresponding heavy ions indicated on the graph. Apparently the effect of oxygen is abolished when ions with -dE/dx greater than about 2500 MeV-cm^2/g are used.

Cells which have incorporated drugs (for example iododeoxyuridine, IUdR) into their DNA behave as if they were previously irradiated; that is, they are more sensitive to X-ray inactivation than normal cells. Heavy ions abolish this effect also. Figure 4 presents survival curves for cells with and without IUdR in their DNA to X-rays and heavy ions.[6] The cells are clearly sensitized to X-rays and helium ions, but not to carbon ions.

Another feature of the curves of Fig. 4 is that the X-ray and helium ion curves are "sigmoid" curves, whereas the carbon-ion survival curve is a straight line on the semilogarithmic plot. The curved lines have been interpreted to indicate that some radiation injury must be accumulated before lethality is expressed; hence, this injury must be sublethal. It was discovered by Elkind[7] that such injuries could be repaired by sublethally irradiated cells in a matter of a few hours. The straight-line nature of the carbon-

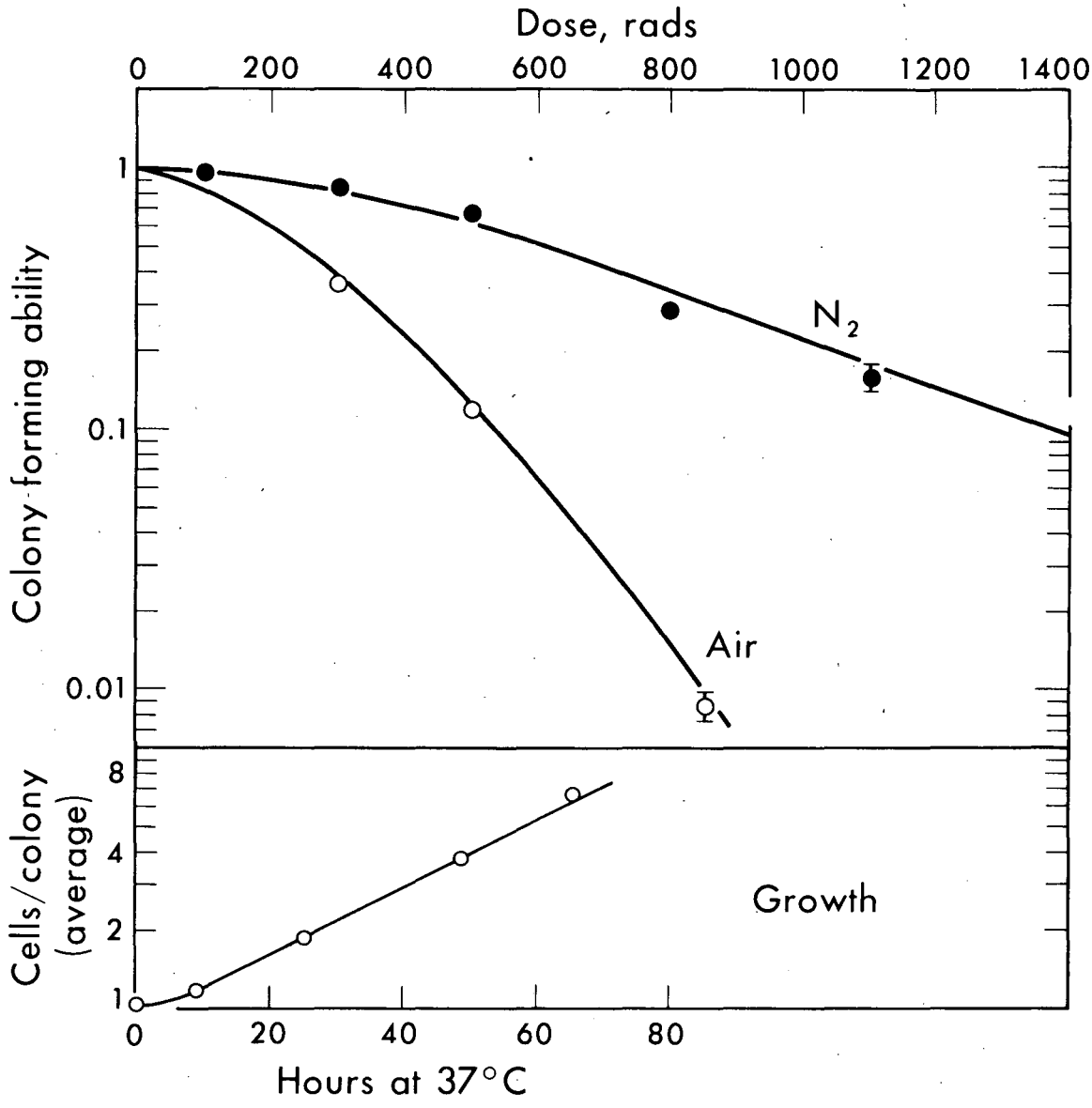
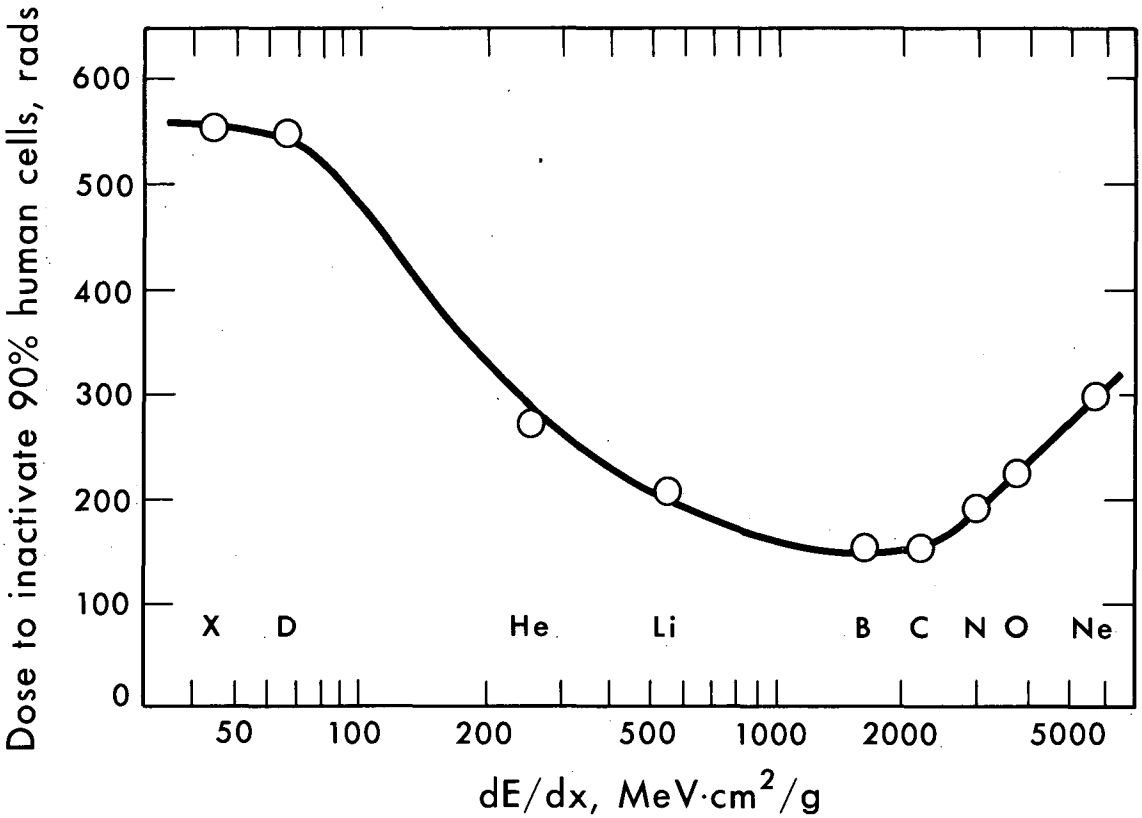


Fig. 1. Survival curves for human kidney T1 cells exposed to 50 kVp X-rays in the presence of air (lower curve) and in pure nitrogen (upper curve). Bottom axes describe growth of control population.



XBL671-664

Fig. 2. Dose in rads required to inhibit the colony-forming ability of 90% of human kidney T1 cells by X-rays and various accelerated heavy ions. The total -dE/dx is shown on the abscissa, and the corresponding ions are shown at the bottom of the plot.

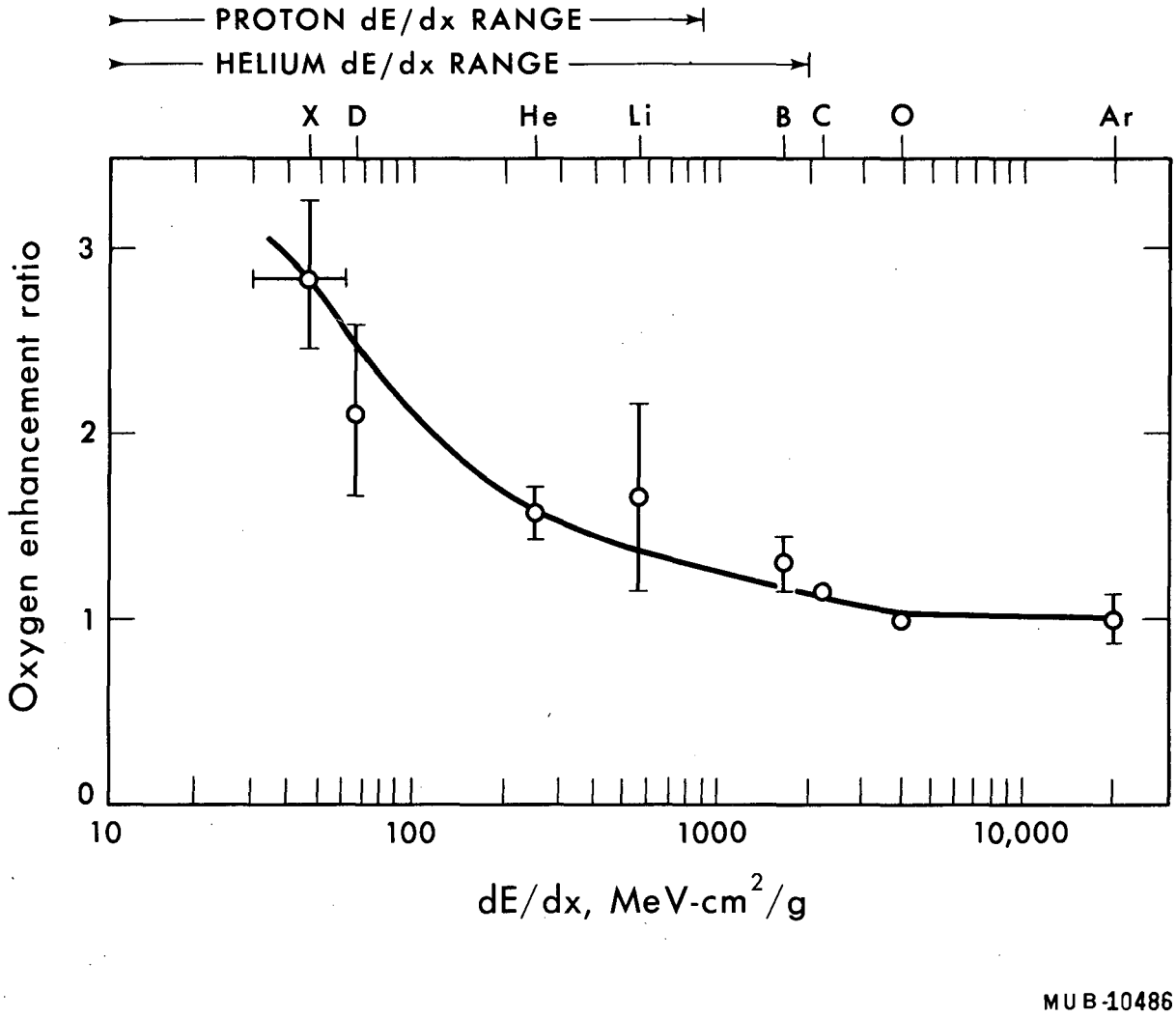


Fig. 3. The dependence of the oxygen enhancement ratio (OER) upon -dE/dx for the inhibition of colony formation of human kidney cells.

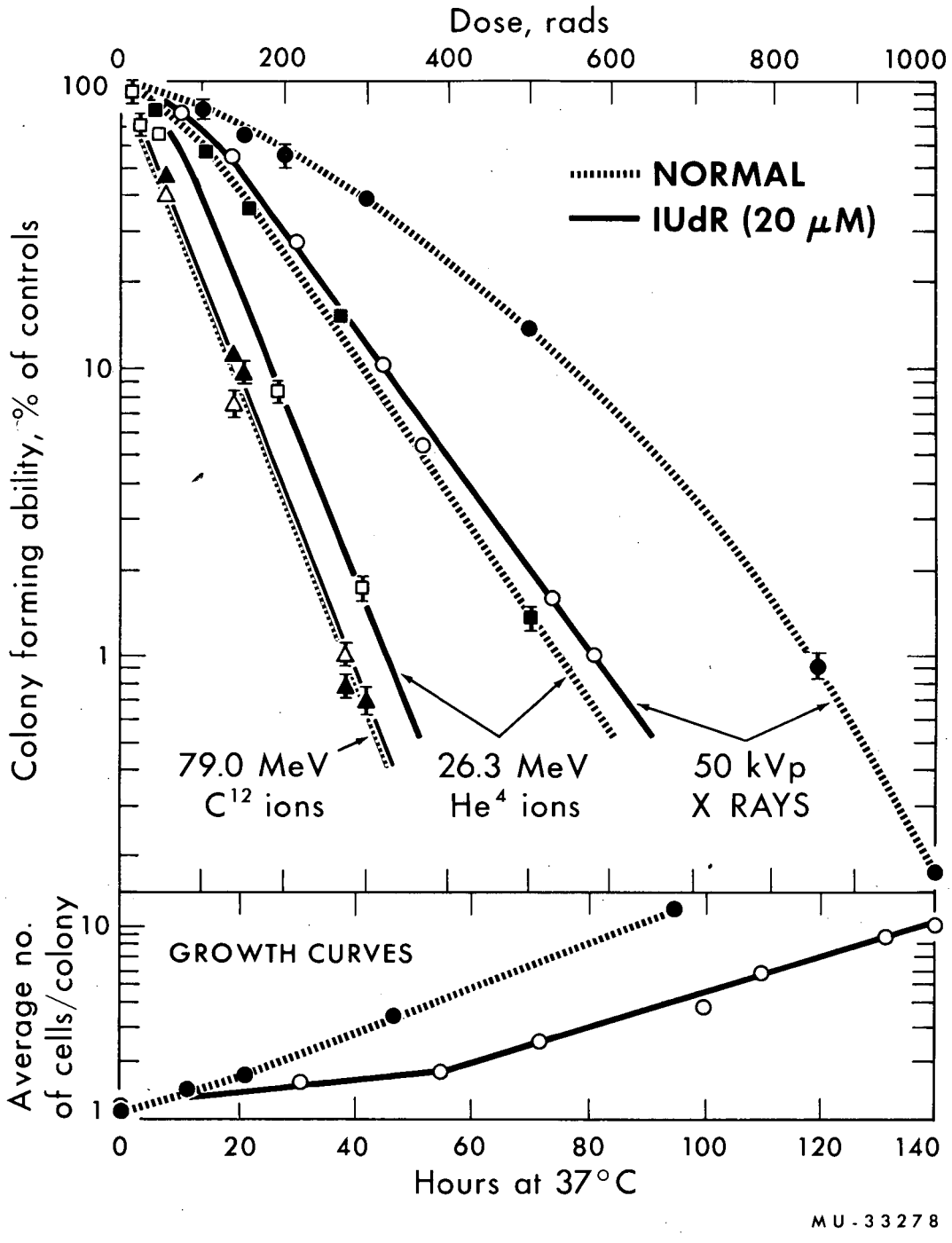


Fig. 4. The effect of incorporation of iododeoxyuridine into DNA upon the inhibition of colony formation of T1 cells by X-rays and heavy ions.[6]

ion survival curve is interpreted to indicate that there is no such thing as sublethal carbon-ion injury. We tested cells exposed to a variety of heavy ions for sublethal injury by allowing them to "recover" between two doses of ionizing radiation separated in time. If sublethal injury of the type described by Elkind exists, then survival of cells should increase as the time between the two doses is increased. We found this to be the case for radiation injury inflicted by all ions that result in sigmoid survival curves, but not by ions that result in straight-line survival curves. This result is pointed out in Figs. 5 and 6. (For further explanation, see figure captions.)

Other interesting effects of heavy ions have been demonstrated in other laboratories. It has been demonstrated that cysteamine, a "radioprotective" drug, is impotent against the lethal effects of alpha particles at the ends of their tracks.[8] It appears that the efficiency of the various heavy ions for the induction of chromosome aberrations closely parallels that for lethality, but the induction of division delay by heavy ions appears to be independent of either of these processes.[9] Cells are found to be more sensitive to X-ray lethality during mitosis and a period of time surrounding mitosis; it appears that the same is true for heavy-ion lethality.[10] This last appears to be the only observation of a radiation-modifying influence that persists for radiations of high $-dE/dx$.

The results obtained in this series of experiments performed at the Berkeley heavy-ion accelerator have been applied to generalized radiobiological theory[11] as well as to more pragmatic end-points for the future, namely the potential usefulness of heavy ions in therapeutic radiology.[12]

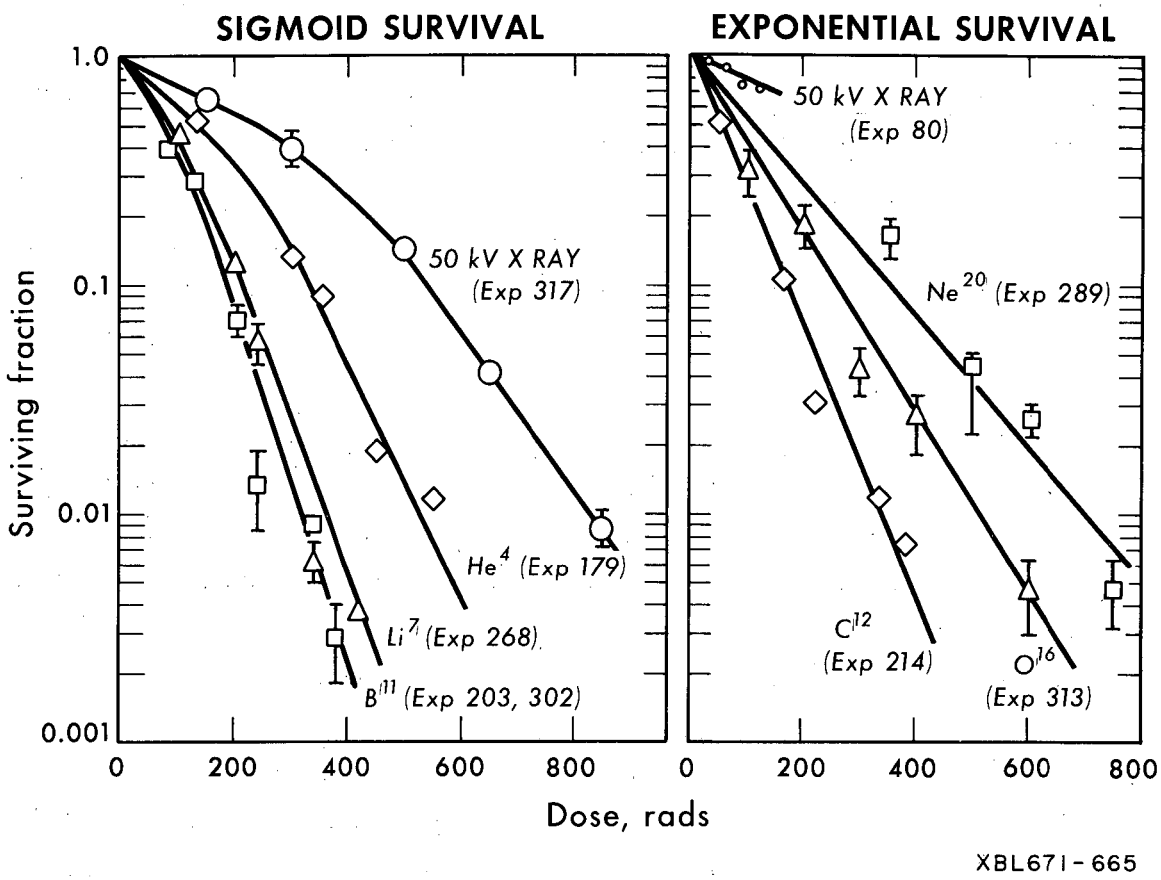


Fig. 5. Sigmoid survival curves for the inhibition of colony formation by X-rays, helium, lithium, and boron ions and exponential survival curves for carbon, oxygen, and neon ions. All ions were accelerated to an energy of about 6.6 MeV/amu.

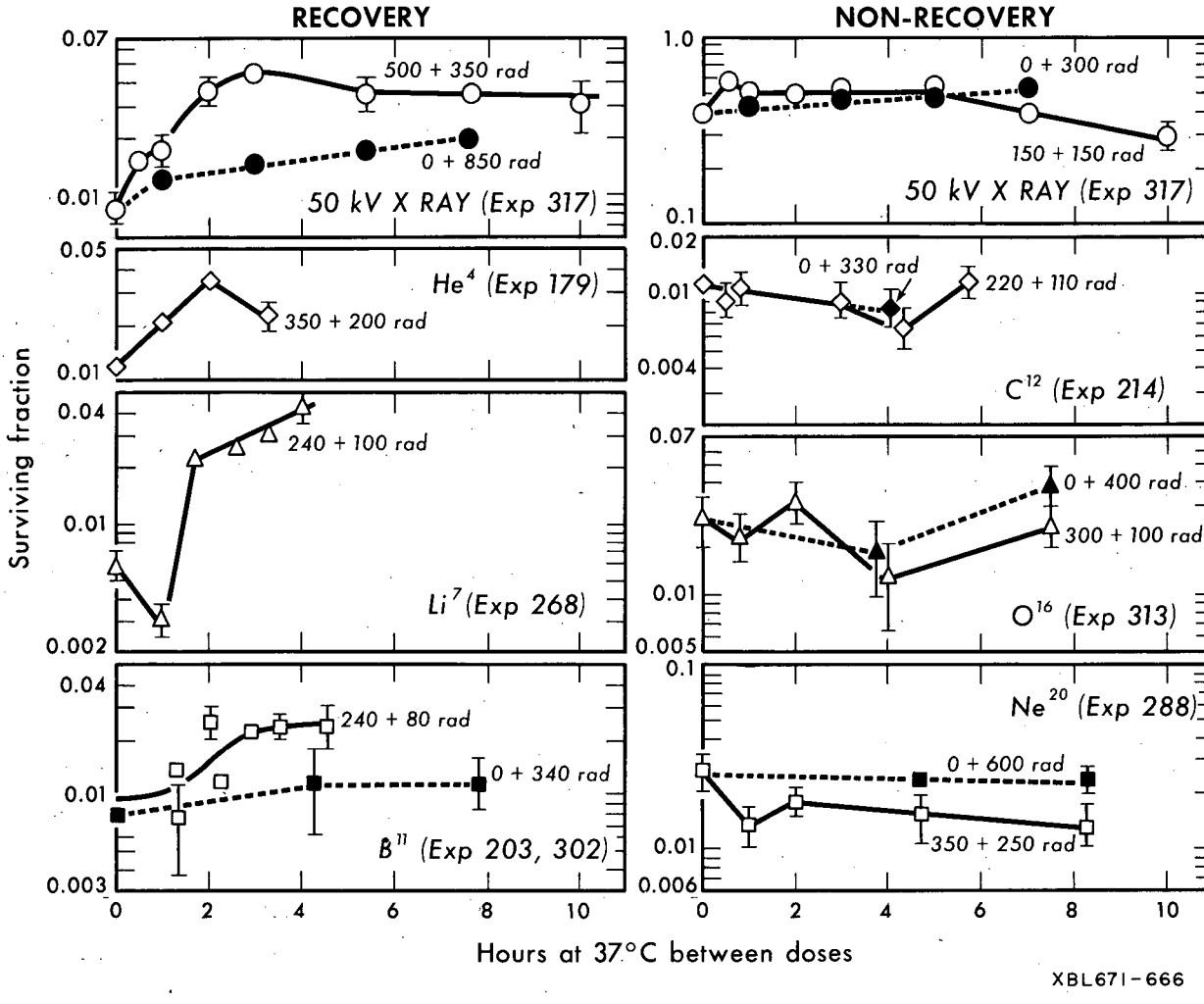


Fig. 6. Survival of T1 cells to fractionated doses of X-rays and heavy ions. Two doses were delivered, the magnitudes of which are indicated in each frame for each type of radiation. The time allowed to elapse (with the cells at 37°C) between the two doses is on the abscissa. Solid plotting symbols indicate that a single dose equivalent to the sum of the two was given at that time for comparison. If fractionation resulted in a persistently higher survival than that due to single doses, then recovery from sublethal injury is said to have occurred. Data were taken from the same experiments as in Fig. 5, and it can be seen that no recovery occurred from radiations that result in exponential survival curves.

References

1. T. T. Puck, P. I. Marcus, and S. J. Cieciura, Clonal Growth of Mammalian Cells in Vitro: Growth Characteristics of HeLa cells with and without a "Feeder Layer," J. Exptl. Med. 103, 273-284 (1956).
2. T. T. Puck and P. I. Marcus, Action of X-Rays on Mammalian Cells, J. Exptl. Med. 103, 653-666 (1956).
3. H. B. Hewitt and C. W. Wilson, A Survival Curve for Mammalian Cells Irradiated in Vivo, Nature 183, 1060 (1959).
4. J. E. Till and E. A. McCulloch, A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells, Radiation Res. 14, 213-222 (1961).
5. G. W. Barendsen, T. L. J. Beusker, A. J. Vergroesen, and L. Budke, Effects of Different Ionizing Radiations on Human Cells in Tissue Culture II. Biological Experiments, Radiation Res. 13, 841-849 (1960).
6. R. Tym and P. W. Todd, The Sensitization by Iododeoxyuridine of Cultured Human Cells to the Lethal Effect of X-Rays and Heavy Ions, Intern. J. Rad. Biol. 8, 589-603 (1964).
7. M. M. Elkind and H. Sutton, Radiation Response of Mammalian Cells Grown in Culture I. Repair of X-Ray Damage in Surviving Chinese Hamster Cells, Radiation Res. 13, 556-593 (1960).
8. G. W. Barendsen and H. D. M. Walter, Effects of Different Ionizing Radiations on Human Cells in Tissue Culture. IV. Modification of Radiation Damage, Radiation Res. 21, 314-329 (1964).
9. L. D. Skarsgard, Comparison of Some of the Biological Effects of Heavy Ions and X-Rays on Mammalian Cells, in Radiation Effects in Physics, Chemistry and Biology, ed. by M. Ebert and A. Howard (Year-Book Publ., Chicago, 1963), p. 5.
10. L. D. Skarsgard, personal communication.
11. P. W. Todd, C. A. Tobias, G. Roy, and L. Lawlor, Radiation Inactivation Cross Sections for Biological Test Objects, Biophysical Society Abstracts, p. 114 Abstract No. FC10 (1966).
12. C. A. Tobias, and P. W. Todd, Heavy Charged Particles in Cancer Therapy, J. Nat. Cancer Inst. (to be published).

PROPOSED OMNITRON EXPERIMENTS BY MEMBERS OF THE LABORATORY OF CHEMICAL BIODYNAMICS

M. Calvin, R. M. Lemmon, and M. P. Klein

Production of Microholes in Membranes

We are beginning to recognize a major limitation in our knowledge of the growth and development of living cells—both individual cells and aggregates of them. This limitation concerns the means by which these cells communicate not only with the outside world—that is, the environment in which they are situated—but also with each other. This problem has gradually focused attention on the fact that one of the defining characteristics of a cell is its limiting membrane, and all communication between the inner workings of the cell and its environment, including neighboring cells, takes place by virtue of the passage of materials through these limiting membranes. The selectivity of this passage and the mechanism by which materials are retained or expelled, pumped in our out, seem to be properties closely associated with the nature of the limiting membrane, both in structure and in dynamics.

So far, the chemistry of these limiting membranes has provided only the crudest kind of data concerning the materials of which they are constructed. The detailed geometry of their construction is now undergoing a complete re-examination. The primitive view of the simple lipid double layer, which has been extant for some twenty years or more, no longer seems tenable. There are a number of evolving notions in which both the passive selectivity and active transport are controlled by molecular organizational changes at specific points in the membrane.

A variety of models (for mathematical purposes) has been devised, but very few have been susceptible of experimental tests. One of these models involves the notion that the membrane is a continuum in which exists a pattern of "holes" of different sizes, and with different types of binding surfaces. These binding surfaces of the holes are generally conceived as being patterns of charges, usually achieved by dipolar lipids or proteins, or combinations of them, which can change their conformation under suitable influence, either electronic or chemical, so as to change their permeability, or activity, in a variety of ways.

One way of constructing such models for experimental purposes involves the punching of a continuous film, either inorganic (mica cleavage sheet) or organic (synthetic or natural organic polymer) with micropores of controlled size. The only feasible method now available for creating such holes is the exposure of the film to fission fragments under proper collimation, followed by etching of the resultant orifice to the desired size. It is clear that this is a very limited operation, both in terms of the number of pores that can be created and the accuracy of their direction. The method is also hampered by the limited kinds of fission fragments available, and the limited associated energies available.

We propose to use the Omnitron, with its capability of producing many kinds of ions of very heavy mass, as a means of providing a variety of membranes with a variety of pore densities and sizes. These pores can then be subjected to the adsorption of a variety of molecules which, in turn, will give the pores their passive selectivity and some active qualities as well. There is reason to suspect that this is already possible even in the porous membranes that are made by the fission fragment method, although the capacity of such membranes for transfer and transport is extremely limited.

A detailed description of the materials that would be used, both as substrates for the membranes themselves and for adsorbent molecules to give specificity to the pores, remains to be determined and would, in fact, be guided by the accumulation of knowledge of real biological membranes.

It is clear that the Omnitron, capable of producing a penetrating particle of about 200 MeV mass, is the ideal instrument for constructing these systems which will undoubtedly have both theoretical and practical significance. The Omnitron's complete control of particle mass and direction, as well as energy, will make possible the construction of model systems free of the randomness of direction and hole size which the present (fission fragment) system imposes. Thus, the theoretical understanding of such porous systems can be approached more closely in a quantitative manner, and the testing of concepts governing membrane permeability, activity, and electrical properties can be achieved on a more explicit basis.

Radiation Chemistry of Crystalline Choline Chloride

Crystalline choline chloride, $[(CH_3)_3NCH_2CH_2OH]^+Cl^-$, is of great interest to radiation chemists because of its extraordinarily high G value

(molecules decomposed per 100 eV of ionizing radiation). It is, in fact, the most radiation-sensitive compound known. Under certain conditions of irradiation, it decomposes by a chain reaction that gives G values as high as 55 000. The anomalous radiation sensitivity is shown only in the crystalline form—in solution, choline chloride exhibits normal radiation stability. The radiolysis products are (almost exclusively) trimethylamine and acetaldehyde.

Recent studies, in particular the X-ray crystallography and ESR studies of the irradiated crystals, have indicated that the chain radiolysis occurs because of some unique (and as yet not understood) property of the choline chloride crystal structure. The radiation sensitivity is not shown by crystals of very close chemical analogs of choline chloride—neither is it shown by at least one other crystalline form (face-centered cubic) of choline chloride. In the radiation-sensitive form (orthorhombic), it is known that the appearance of lattice defects inhibits the chain decomposition.

We anticipate that the Omnitron, with its capacity to produce a great variety of high atomic weight ions at almost any desired energy, will be a powerful tool to aid us in the understanding of choline chloride's radiolysis mechanism. We know, for example, that γ-rays and accelerated electrons produce the same kind of free radicals in choline chloride crystals, and that these free radicals are chain initiators (not propagators) in the radiolysis mechanism. It will be important to know if heavy ions will produce the same free radicals (observable in the ESR spectrometer). If the densely ionizing heavy ions produce different free radicals, which also initiate the chain decomposition, then we will know that the mechanism is not unique, and can be started by different agents.

We also wish to determine whether choline's decomposition may be initiated by the introduction of point defects into the crystal. If, for example, the chloride ion is displaced from its normal position in the choline's crystal lattice, this displacement might initiate the decomposition. More likely, such displacements will inhibit the chain decomposition. The kinetics of such inhibitions (decomposition rates and radical decay rates as functions of temperature, time, and radical concentrations) should do much to reveal the nature of this unique solid-state decomposition mechanism. The selective displacement of a heavy ion, such as chloride, can best be accomplished by heavy-particle irradiation—for this purpose the Omnitron will prove very valuable.

Locations of Photosynthetic Trace Metals

The locations and concentrations, within an algal cell, of the important photosynthetic trace metals (such as Mg, Mn, Fe, Cu, Co, and Zn) are not known well enough. The functions of some of them (for example, Mn) are quite unknown. This knowledge is essential for a better understanding of the processes by which green plants convert sunlight into chemical energy, and then use that energy to operate the numerous biochemical pathways.

The Omnitron has the potential of greatly helping us to locate the trace metals within the photosynthetic apparatus. The diverse ions that it will accelerate may provide a kind of expanded "neutron activation" analysis. By selecting a specific irradiating ion with a high cross section for reaction with a particular trace metal, and then applying autoradiographic techniques on individual algal cells, one may hope for a far better localization of trace metals than is now possible.

At present there are very little data available on the radionuclides to be expected when the above metals are irradiated with a variety of ions. Consequently, one needs first to accumulate such data so that one may pick out radiating ions that will have high enough cross sections, and whose radioactive products will have useful properties (e.g., long-enough half lives and low-enough energies) for the subsequent autoradiography. The high energies of the Omnitron ions will be necessary when one wishes to carry out the activation in a water suspension or ice matrix.

RADIATION GENETICS WITH DENSELY IONIZING RADIATIONS

R. K. Mortimer

The main stimulus for using heavy ions to induce genetic changes lies in the chance that these radiations may cause qualitatively different forms of damage. For example, the importance of the two main classes of lesions leading to mutational change, i.e., base pair substitution and base pair addition or deletion, varies considerably with the mutagen used. X-rays induce considerably more addition-deletion type changes than does ultraviolet or most of the chemical mutagens. It seemed possible that heavy ions might induce this type of damage exclusively.

Work done to date does not allow a complete assessment of the properties of heavy ions as mutagens. In a study of reverse mutation in yeast,[1] the efficiency of ions with LET values in the range 10 to 5×10^4 MeV $g^{-1} cm^2$ approximates that observed for inactivation (Fig. 1). For three different reversion systems, a maximum efficiency was observed in the LET range 1000 to 1200 MeV $g^{-1} cm^2$. Neon ions were quite inefficient and also showed an oxygen effect suggesting that delta rays were causing most of the reversions with these ions. For two of the reversions, HI and TR_{NF}, it is known that mutation of suppressor genes is involved. Work by Magni[2] and us has indicated that such mutations occur preferentially by addition-deletion changes. However, one of the reversions, TR_F, probably involves a base pair substitution change[3] (von Borstel, personal communication). Thus, these studies are not definitive with regard to characterizing the molecular basis of heavy-ion mutagenesis.

DeSerres (Oak Ridge) has induced forward mutations at the ad_3 locus of Neurospora with heavy ions.[4] He finds both a higher efficiency and a higher frequency of irreparable (deletion) changes in the locus compared with those induced by X-rays. Nakai and Mortimer (Rad. Res. Suppl. - 1967) found that carbon ions were not qualitatively different from less densely ionizing radiations for induction of intra- or intergenic recombination.[5]

Future genetic studies with heavy ions should be designed to permit precise characterization of the molecular lesions induced. A forward

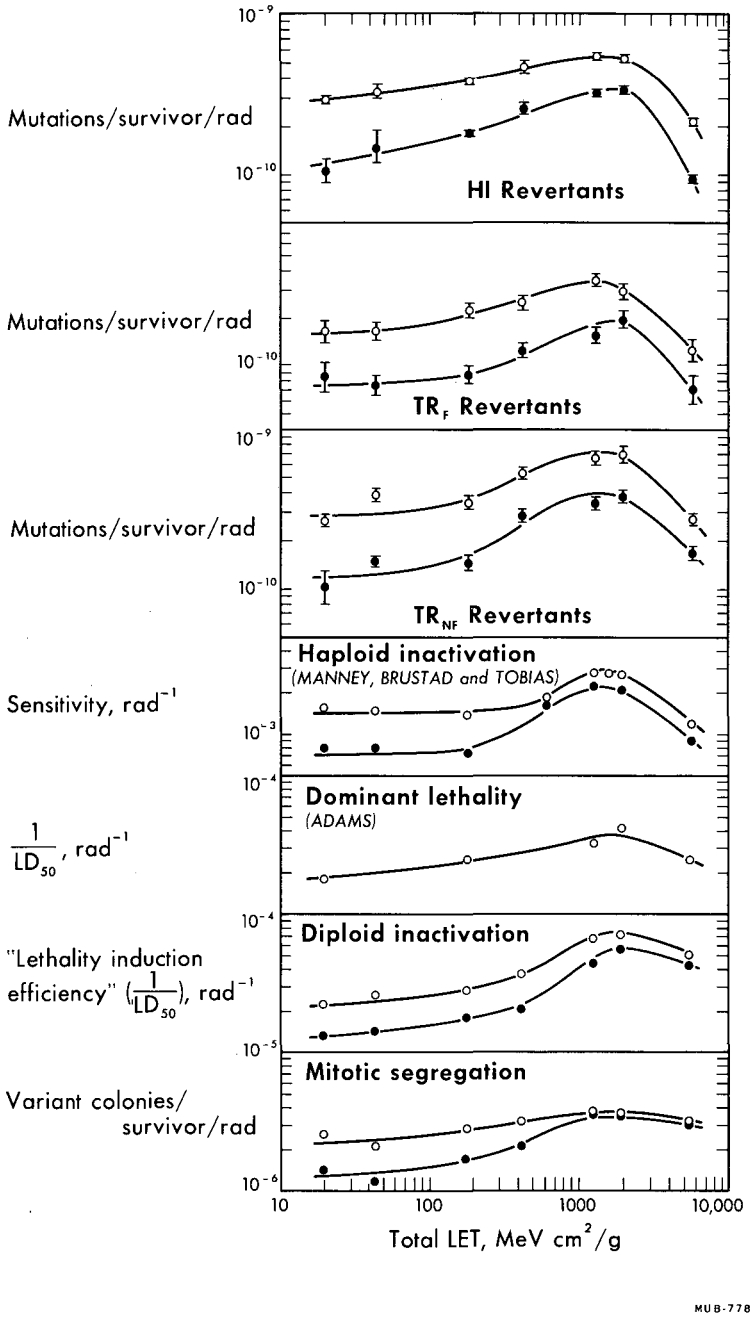


Fig. 1. Mutation induction efficiency as a function of LET$_\infty$ for three classes of reversions with culture X841 (Ref. 1). Open circles, arobic; solid circles, anarobic. For comparison, the corresponding curves for induction of lethality in X841, lethality in a haploid strain (T. R. Manney, T. Brustad, and C. A. Tobias, Rad. Res. 18, 374-389, 1963), dominant lethality in a haploid strain (L. R. Adams, Dominant Lethality in Yeast, LET Studies with the LRL Heavy Ion Linear Accelerator, in Semiannual Report, Biology and Medicine, UCRL-9897, Fall 1961, pp. 147-150), and mitotic segregation in X841, are presented.

mutation system, such as is used by DeSerres, should be employed. The mutants should be characterized by the following tests: complementation, leakiness, temperature and osmotic pressure reversibility, suppressibility, fine structure map location, and reversibility by specific mutagens such as 2-amino purine, hydroxylamine, and ICR-170. These studies would establish whether heavy ions induce specifically frameshift (addition-deletion) mutations or are relatively nonspecific, as is the case with X-rays and uv.

The apparent uncoupling of track core and delta-ray effects observed in the earlier study (Fig. 1) would also be worth following up. It would be expected that efficiency and oxygen effect should remain constant as LET is increased beyond that of neon ions, since the delta-ray component of the dose is constant. Varying the velocity of the beam would also permit a test of this hypothesis.

References

1. R. K. Mortimer, T. Brustad, and D. V. Cormack, Rad. Res. 26, 465-482 (1965).
2. G. E. Magni, R. C. von Borstel, and C. M. Steinberg, Super-Suppressors as Addition-Deletion Mutations, J. Mol. Biol. 16, 568-670 (1966).
3. R. C. von Borstel (Biology Division, Oak Ridge National Laboratory), private communication.
4. F. J. DeSerres, Genetic Analysis of the Structure of the ad-3 region of Neurospora crassa by means of irreparable recessive lethal mutations, Genetics 50, 21-30 (1964).
5. S. Nakai and R. K. Mortimer, Induction of Different Classes of Genetic Effects in Yeast Using Heavy Ions, Rad. Res. Suppl. (in press).

HEAVY ION RADIOGRAPHY AND MICROSCOPY

C. A. Tobias, T. L. Hayes, H. D. Maccabee, and R. M. Glaeser

As heavy ions pass through matter, their energy loss can be correlated with the atomic composition, density, and thickness of the specimen traversed. The range penetration is a very sensitive function of such parameters, and there are quantitative methods for measurement of the energy loss in a given traversal.

A second useful property of heavy ion beams is that as they strike matter, secondaries are formed. These may be of the atomic variety, such as secondary electrons, X-rays, or visible light, or they may be nuclear reaction products. Some of the heavy ion secondaries are similar to those obtained upon electron bombardment; others are different: for example, heavy ions may produce spallation recoils from nuclei of the absorber.

It is well known that a wavelength (λ) may be associated with charged particles of mass M traveling with velocity v:

$$\lambda = \frac{h}{Mv}.$$

The wavelength turns out to be approximately 2000 times smaller for protons than it is for electrons traveling with the same velocity. Protons or heavy ions may thus be considered to undergo diffraction as they pass through homogeneous matter in a similar manner to light or to electrons. Thus, in theory, heavy ions can be used for microscopy. Because of the smaller wavelength, heavy ion microscopy can theoretically yield more than 2000 times greater resolution than electron microscopy. In the course of the design of the Omnitron, we realized that perhaps this is the first high energy accelerator that is designed with sufficiently refined electro-optical techniques so that the emerging beams might be used for such exacting problems as radiography or microscopy. We thus decided to survey some of the possibilities.

Heavy Ion Radiography (Macroscopy)

If monoenergetic heavy particles are allowed to pass through a sample, it is possible to accurately determine their energy loss by analyzing the particles emerging on the other side. It may be that heavy particles can be used for a much more sensitive "thickness gauge" than either X-rays or electrons. The situation is illustrated in Fig. 1. Suppose that a thickness variation of Δt is to be measured at depth t. Then the depth resolution will depend on the fraction $\Delta I/I$ for each type of radiation, where ΔI is the intensity variation. By using the Bragg ionization curve as indicated in the graph, it is then possible to obtain a much larger ratio for particles than for X-rays over a wide range of thickness:

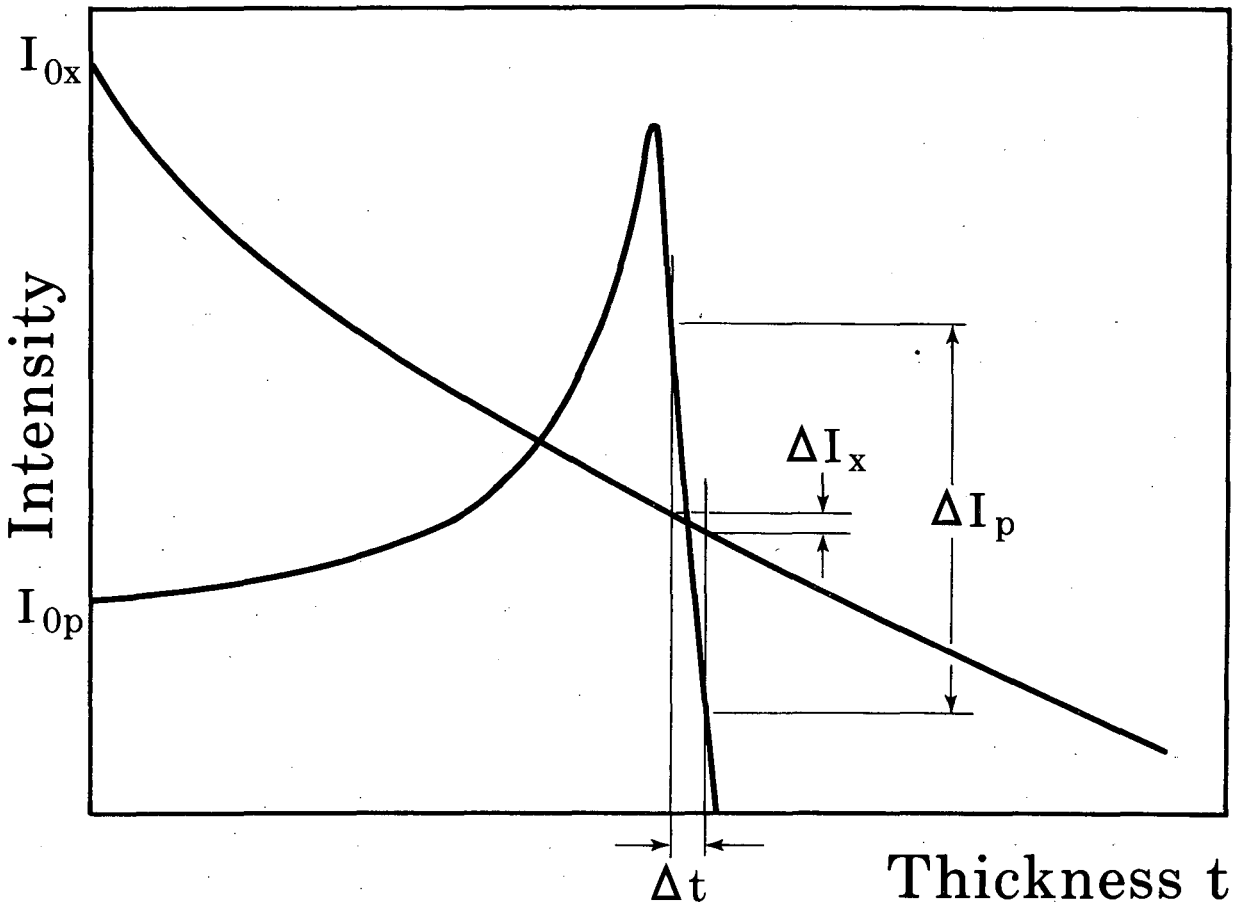
$$\frac{\Delta I_p}{I_p} \gg \frac{\Delta I_x}{I_x}.$$

Potentially, this method then can be used for radiography by obtaining pictures on an X-ray film of the penetration of heavy ions through the specimen or by means of scanning a beam and determining the residual energy of the particles as the scan proceeds. A simple arrangement for this is shown in Fig. 2; the residual energy of the beam is determined by pulse-height analysis as the beam scans through the specimen. Each heavy ion can be used to measure more than one property, namely, the presence of the heavy ion as well as the residual range, and it may well be that thickness scans can be obtained with the use of heavy ions at a much lower dose than would be the case for X-rays.

It may be worthwhile to mention some problems in diagnostic roentgenology that might be solved by the use of heavy particles:

(a) It is well known that radiologists often have trouble resolving the presence of a small metastatic lesion invading bone. The patient may already have subjective indications—e.g., pain—in the absence of objective measurement. The lesion is often detected by X-rays when it has already grown to a rather large size. It is possible that proton scans over a predetermined area of interest would prove to be very useful to improve diagnostic depth resolution.

(b) Another radiological example is the type of problem that usually arises when an air pocket is to be found somewhere in the body. For diagnostic



DBL 672-1512

Fig. 1

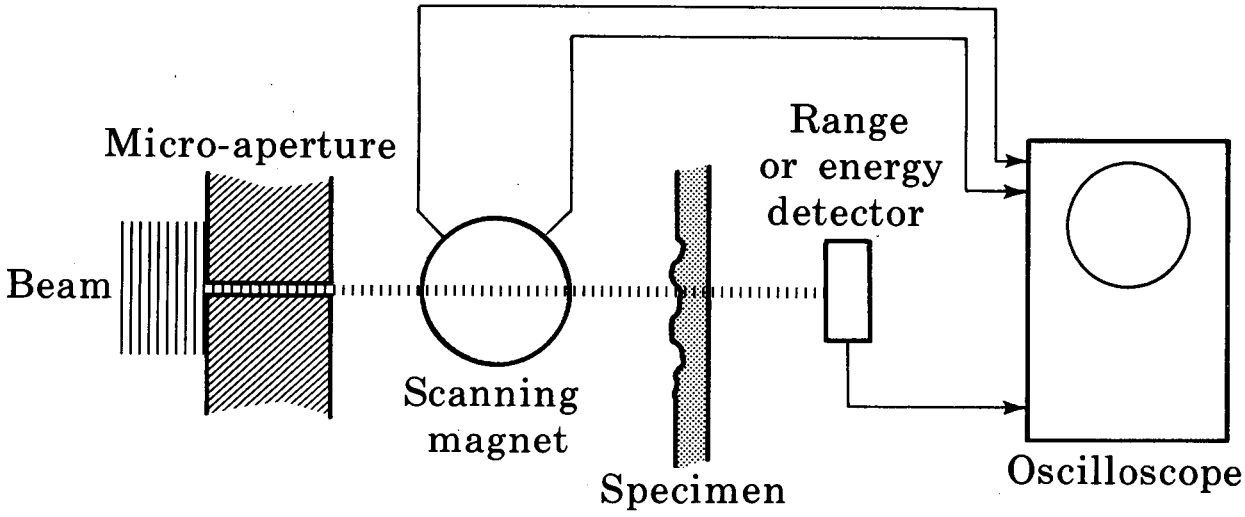

Micro-aperture
Beam
Scanning
magnet
Specimen
Range
or energy
detector
Oscilloscope
DBL 672-1513

Fig. 2

localization of the third ventricle in the brain, air is injected at the base of the spine with hopes of detecting (by diagnostic roentgenography) the resulting air pocket in the brain. It may well be that protons or heavy ions would prove to be more sensitive for the detection and localization of such an air pocket than X-rays.

(c) The detection of adipose tissue or soft tissue tumors by heavy ion techniques is another possibility. It is well known that diagnostic X-rays are relatively insensitive to small changes in tissue composition. If, however, the atomic composition of tumors shows deviations from normal, then it is possible or even likely that such tumors could be detected in a matrix of tissue of normal composition.

(d) The thickness gauge concept is effective either at large thicknesses or at very small thicknesses. One must, however, be able to regulate the energy of the particles over a wide range. This can be easily done in the Omnitron, which generates monoenergetic beams of widely variable penetration. Thus it may be possible to use a thickness gauge at the microscopic level for microradiography of cells. Subcellular particulates, e.g., in the nucleus, are often much denser than the cytoplasm and this may provide sufficient contrast for detection by heavy particles, even in the absence of staining.

Heavy Ion Microscopy

There are two basic parameters to be considered in any form of microscopy. The first is the localization in a small volume of information relevant to the specimen. The second is the kind of information and its significance in biological studies. Several of the heavy ion microscopic techniques (scanning, PRIM) discussed in this report permit a separation of these two parameters that is not found in the standard light microscope or the standard electron microscope. Such separation results in an increase in kinds of information available (stereoscopic, chemical, nuclear). This increase in information is one of the main goals of the heavy ion microscopy proposals and emphasizes our position that high resolution is not the only important factor in microscopy.

It is possible, however, that resolution can also be improved. As early as 1932,[1] it was shown that the resolution of a proton microscope can be much higher than that of an electron microscope, because of the wavelength

limitation. In practice, however, resolution limitations come from a number of other factors, including spherical aberration in magnetic lenses, chromatic aberration due to inconstancy of particle energy, inhomogeneity of the particle source, and vibration occurring during the experiment. There are some reasons to believe that heavy ions could prove to be at least as good as electrons, and in some cases better.

The Problem of a Small Diameter Parallel Beam

The beam of the Omnitron will be about 1 mm in diameter. It may be possible to use quadrupole and octupole lenses to focus this down to a much smaller dimension, and then use a microaperture to produce ultrasmall diameter beams.

Recent advances in the studies of the interaction of heavy particles with matter point to methods for preparing submicroapertures. Fleisher[2] and others have used fission fragments in mica; certain fission fragments can be used to blast holes of extremely small diameter, sometimes only a few angstroms in diameter. By the use of accelerated heavy ions, it may be possible to produce such submicroapertures in multiple layers, using an aperture already completed as a screen for an aperture to be made in a second foil. Apertures made by heavy ions may be used to produce a proton microbeam.

It seems clear that the initial beam must be focused down by a linear factor of 10^2 (10^4 in area) in order to achieve sufficient beam intensity after a submicroaperture. If the initial beam is 10^{12} particles/mm^2 sec, focusing of 10^4 in area yields 10^{16}/mm^2 sec, which results in 10^4 particles/sec coming through an aperture of 10 Å cross section (100 Å^2 = 10^{-12} mm^2).

Microbeam Lesions

Microlesions in tissue were produced by the use of 2 MeV protons by Zirkle and associates[3] and by others,[4] using natural alpha particles. The precision with which the heavy ion beams can be controlled by magnetic deflection, along with the control of the range, should make the Omnitron a versatile microbeam apparatus. At Berkely, attention is being directed toward some specialized applications of microbeams. It has been proposed that the study of fertilized egg cells and of embryonic developing organisms

in early multicellular stages might be fruitful, since it could give clues to some of the processes of normal development and differentiation and the mechanisms of origin of congenital malformations. It would add data to the understanding of the hazards of very heavy particles on embryonic systems, a problem area that is sure to become of practical interest with supersonic transport planes flying at high altitude. Because of limitations on beam quality and range, the initial embryonic studies were carried out with the insect Tribolium confusum; this study has led to interesting results on congenital wing abnormalities and their etiology,[5,6] as well as on the role of magnetic fields in altering the development process.[7]

Another area of current interest is the possible applicability of microbeams to localized radiobiological studies on neuron explants in tissue culture. These may help elucidate specific regions in the neuron susceptible for initiating action potentials, and the chain reactions involved in impulse initiation. Microbeams also might prove to be useful in cytological studies of: protein and nucleic acid synthesis, crystalline systems (e.g., choline chloride), photosynthetic systems (e.g., chloroplasts), and trace-element activation.

Conventional Proton Microscopy

The use of microapertures as a source and quadrupole magnets, with octupole correction, for focusing, might make transmission proton microscopy practical with the Omnitron. Initially one would not expect to improve on the resolution of electron microscopy since similar practical limitations exist for both systems concerning lens aberrations, surface contamination, vibration, etc. However, at low energy, protons have higher relative cross sections for scattering off specimen atoms, and proton images could have more contrast than electron images.

Scanning Heavy Ion Microscopy (SHIM)

The possibility of focusing the beam and passing the accelerated particles through a submicroaperture and the availability of magnetic deflection scanning would make it feasible to build heavy ion scanning microscopes such as have been described for electrons by Knoll and Theile,[8] von Ardenne,[9] McMullan,[10] Crewe[11] and Oatley et al.[12]

Hayes and Pease[13-16] have recently described the applications of the scanning electron microscope to biological studies. The resolution of this instrument is still only about 250 ; however, all of the pictures produced in this scanning microscope indicate that it is not necessary to analyze the particles transmitted through the specimen itself as the beam strikes the target. The basis of analysis can be scattered or secondary electrons, characteristic X-rays, ultraviolet or visible light.

The fact that the information is not carried on the same radiation that allows for localization of this information permits a large increase in the kinds of information available. The same principles could be applied by using heavy ions instead of electrons as the primary scanning beam; in addition, the increased range of the particles could make observation of hydrated and living material possible.

As an alternative to the method of deflecting the beam, it would be possible to scan by mechanically moving the specimen or the beam aperture. It should be possible to achieve the small-amplitude, well-controlled mechanical movements required by coupling the stage or the aperture to electrical-mechanical transducers that are based upon the piezoelectric or electrostriction effects.

Particle Random Impact Microscope (PRIM)

Analysis of the properties of microscopes indicate that several criteria must be satisfied to obtain satisfactory images. One of the criteria is that the radiation used for microscopy should in some way interact with the specimen. In the case of light microscopy and in traditional electron microscopy, the diffracted or scattered primary radiation is the important quality. In other instances (SHIM, PRIM) the secondary radiation emitted from the specimen is used as an indicator of the interaction. Another requirement is to have knowledge of the position of the particles when they strike the specimen. In conventional microscopy this is satisfied by producing an image of the specimen on the target screen by using primary radiation that has been altered by interaction with the specimen. In a scanning microscope, the place of interaction is localized by recording the parameters of magnetic, electrical, or mechanical deflection as a function of time. However, scanning implies the production of a microbeam and this in turn implies a loss

of resolution because of the difficulties of producing a microbeam. One of us (C. T.) has proposed another principle of microscopy, in which the interaction of the particle and specimen is again separated from the problem of localizing the place of interaction, as in the scanning microscope, without the use of a microbeam. One would allow a beam of particles to fall at random on the specimen, the only condition being that they should form part of a parallel beam and that the particles should be closely monoenergetic. Interaction with the specimen would be measured by registering secondary electrons, photons, or recoils. The particles would also be allowed to penetrate an "analyzer" placed near the specimen for the sole purpose of determining where the particle is. The analyzer could be a detector with a known regular submicroscopic structure. Interaction of the heavy particles in the analyzer should produce information leading to location of the particular heavy ion. Information on the location, when it is combined with the information on the secondaries emitted, can be displayed on an oscilloscope. The problem of resolution then depends less on the microaperture or diffraction than on the structure and interactions of the analyzer. At this time it is probably too early to talk about the limit of resolution that may be reached by such techniques, but by combining high resolution detectors with electromagnetic lens magnification, resolution on the order of angstroms might be possible.

Figure 3 is a schematic for the random impact microscope. The location-analysis device shown in this instance is a wedge-shaped lithium-drifted semiconductor detector. It is known that when a particle crosses the detector, an output pulse may be obtained that is proportional to the energy loss produced in the detector by the particle. The pulse heights from monoenergetic parallel particles then can be correlated with the positions of the particles. By applying wedge-shaped solid crystal detectors oriented perpendicularly to one another, it is possible to localize the particle in two dimensions. The principle as described here has some similarity to the principle of the gamma ray camera as described by Anger,[17] whereby imaging can be obtained without the necessity of scanning. Two other semiconductor devices[18, 19] for particle position detection have been constructed: the ribbon detector and the checkerboard detector. These devices are of centimeter dimensions, however, and must be microminiaturized for use

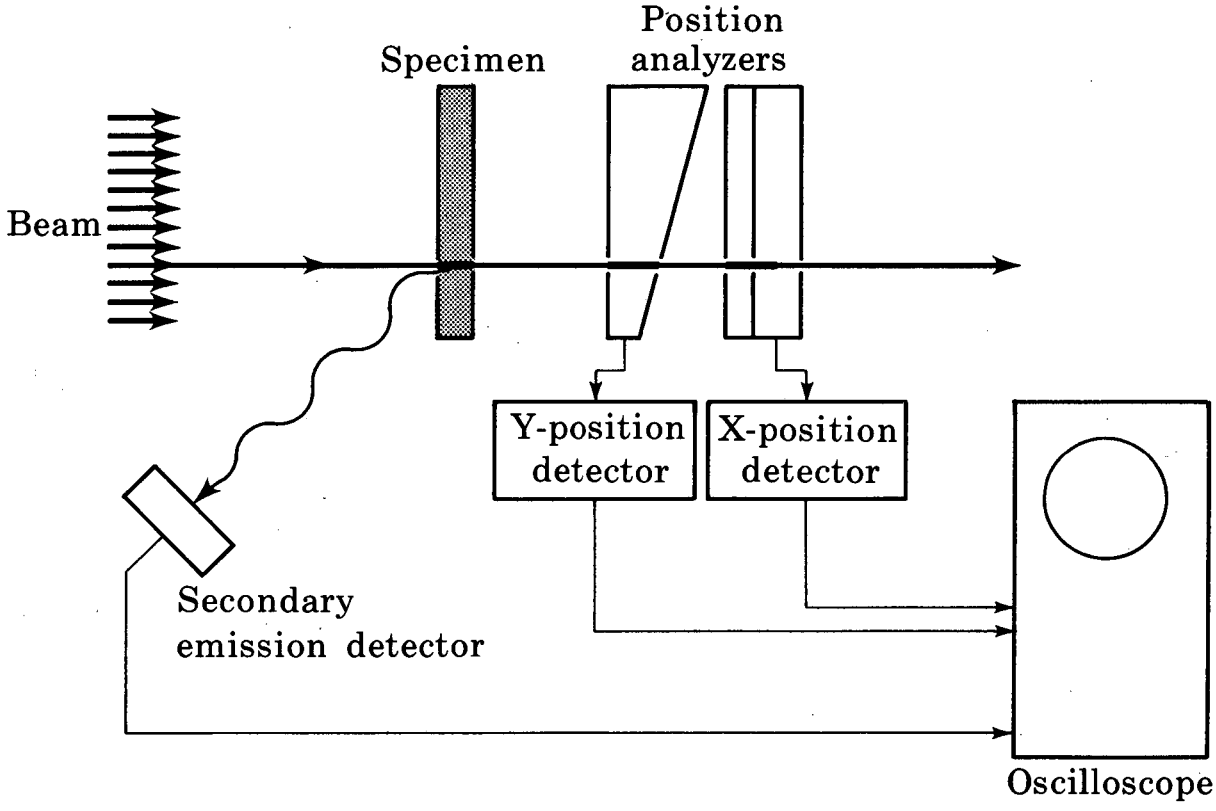

Position
analyzers
Specimen
Beam
Y-position
detector
X-position
detector
Secondary
emission detector
Oscilloscope
DBL 672-1514

Fig. 3

in microscopy. Another device that might be considered for a detector could consist of a fluorescent screen plated onto a "light-sensitive" cathode ray tube and generally similar to the "light pencil" technique used in computer applications. One may be able to interpose a magnetic lens between the specimen and analyzer of Fig. 3, thus magnifying the image and easing the problem of particle location.

The advantages of large information content, increased range, and the possibility of working with hydrated and living specimens would all be associated with the PRIM.

Heavy Ion Micro-Etching

When a beam of short-range particles impinges on the surface of a specimen held in vacuum, evaporation or sublimation of some atoms or molecules may take place if the energy released in the interaction is sufficiently high to heat the local region of the specimen above a critical temperature for a sufficient length of time. Kanaya et al.[20] have already explored the consequences of implanting argon. Heavy ions of a broad distribution of atomic number and energy may be particularly suitable to perform micro-etching, perhaps more so than electrons; they can deliver more concentrated energy packets, and due to relatively less scattering, there is a great deal more uniformity in their depth of penetration, given a monoenergetic impinging beam. Studies of track formation in organic and inorganic media by fission recoils have already demonstrated that local properties of the specimen, e.g., binding energy, thermal conductivity, etc. may have much to do with the efficacy of the etching process. We then visualize rather uneven etching of frozen-dried biological specimens when the proper particle and dose rate is chosen. Subsequent examination of the etched specimens by particle microscopy or other techniques may reveal some of the local properties of the specimen itself.

Recoil Track Microscopy

According to a suggestion of Robert Glaeser of this laboratory, the particle microscope studies of recoil tracks formed in frozen-dried biological specimens (or in suitable replicas mounted adjacent to the specimens) could be of some interest. The surface of a biological organelle could be

labeled with a stable isotope that is incorporated in a suitable organic compound and the specimen and its replica could be exposed to a heavy ion beam from the Omnitron. The subsequent study of spallation track distribution in specimen and/or replica may give information on the location of the specific biomolecule under study.

Discussion

Many of the ideas in this summary paper were developed somewhat incidentally to the main objectives of the Omnitron. It appears that the Omnitron may be suitable for experimental application of some of these ideas. Others may wait for further extension of nuclear and engineering knowledge before they can be fully applied. It has become clear, however, that the problems confronting the accelerator physicist and engineer, as well as the biophysicist, in building the Omnitron itself require similar techniques and solutions as the problems confronting high resolution heavy ion microscopy. We are therefore continuing a small-scale study with the hope that when the Omnitron is finished, some contributions might be made to heavy ion macroscopy and microscopy.

References

1. Claude Magnan, The Proton Microscope, Nucleonics 4, 52-66 (1949); and M. Knoll and E. Ruska, Ann. Physik 12, 607 and 641 (1932) and Z. Physik 78, 381 (1932).
2. R. L. Fleischer, P. B. Price, and R. M. Walker, Method of Forming Fine Holes of Near-Atomic Dimensions, General Electric Research Lab. Reprint 4573.
3. R. E. Zirkle and W. Bloom, Science 117, No. 3045, 487-493 (1953).
4. M. Davis and C. L. Smith, Exp. Cell Res. 12, 15-34 (1957).
5. N. M. Amer, V. V. Slater, and C. A. Tobias, Radiation Res. 16, 574 (1962).
6. V. V. Slater, J. Lyman, C. A. Tobias, N. M. Amer, J. S. Beck, M. Beck, and A. J. Slater, Radiation Res. 21, 541-549 (1964).
7. N. M. Amer, Radiation Res. 19, 215 (1963).
8. M. Knoll and R. Theile, Z. Physik 113, 260 (1939).
9. M. von Ardenne, Z. Physik 109, 553 (1938).
10. D. McMullan, Proc. Inst. Elec. Engrs. (London) B100, 245 (1953).

11. A. V. Crewe, Science 154, No. 3750, 729-738 (1966).
12. C. W. Oatley, W. C. Nixon, and R. F. W. Pease, Scanning Electron Microscopy, in Adv. Elect. Elec. Phys. (Academic Press, New York, 1965), Vol. 21, pp. 181-247.
13. R. F. W. Pease and T. L. Hayes, Nature 210, 1049 (1966).
14. T. L. Hayes, R. F. W. Pease, and L. W. McDonald, Lab. Invest. 15, 1320 (1966).
15. R. F. W. Pease and T. L. Hayes, in Sixth International Congress for Electron Microscopy, Kyoto (Marusen Co., Ltd., Tokyo, 1966), pp. 19-20.
16. R. F. W. Pease, T. L. Hayes, A. S. Camp, and N. M. Amer, Science 154, 1185 (1966).
17. H. O. Anger, Rev. Sci. Instr. 29, 27 (1958).
18. E. J. Ludwig, W. M. Gibson, and J. S. Hood, IEEE Trans. Nucl. Sci., Vol. NS-12, No. 1, 247-254 (1965).
19. W. K. Hofker et al., IEEE Trans. Nucl. Sci., Vol. NS-13, No. 3, 208-213 (1966).
20. K. Kanaya, S. Matsui, K. Shimiza, and H. Yamazaki, in Sixth International Congress for Electron Microscopy, Kyoto (Marusen Co., Ltd., Tokyo, 1966), pp. 255-256.

PROTON AND ALPHA RADIATION EFFECTS IN MICE

J. K. Ashikawa*

One of the hazards of space flight is cosmic radiation. These ionizing radiations of solar and galactic origin not only permeate outer space but constantly bombard earth.

Our present knowledge indicates that in space flight of long duration, astronauts in all probability will be exposed to these dangerous radiations. We further know that in interplanetary flights the greatest radiation hazard will be the protons and alpha particles from solar flares. It has been estimated that had an astronaut been caught in the violent solar storms of 1959-60, he would have received a whole-body dose of about 200 rads. Can man tolerate such high doses in outer space and survive to carry out his mission successfully? At present we cannot definitely answer such a question since we are not yet able to make a generalized statement regarding the biological effectiveness of all components of cosmic radiation and we have not completely explored the possibilities for synergism between radiation and other factors, e.g., weightlessness. But concerted efforts are being made at Berkeley and elsewhere to determine the biological effects of these ionizing radiations in mice and other animals. The problem is not simple, because exposure to solar flares results in a characteristic pattern of depth-dose distribution, with the LET spectrum different at each level.

We at Berkeley have observed that gastrointestinal death predominates in mice that have been lethally irradiated with 730 MeV protons and 910 MeV alpha particles, while hematopoietic death predominates in mice that have been irradiated with X-rays.[1, 2] Thus, at doses which are 98 to 100% lethal in 30 days, the incidence of gut death is 8 to 12-fold greater in the particle-irradiated than in the X-irradiated animals. Since the LET values of 730 MeV protons, 910 MeV alphas, and 250 kVp X-rays (1 mm Cu HVL) are comparable, this difference in mode of death apparently is due to differences in the microscopic dose distribution of these radiations in bone marrow cavities and in soft tissues.

*USC School of Medicine, 1200 No. State Street, Los Angeles, California.

The effectiveness of both high energy proton and alpha radiation relative to 250 kVp X-ray for acute mortality in mouse varies with time as well as with dose, and depends on the time after irradiation when mortality is evaluated. The relative effectiveness of both 730 MeV protons and 910 MeV alpha particles decrease with increasing time post-irradiation. Thus, from a value of about unity at LD_{50}^{4} days, the value decreases to 0.9 at LD_{50}^{6} days, and to 0.75 at LD_{50}^{30} days.

We found that gastrointestinal death is also enhanced by increasing the proton and alpha particle dose rate.[3] For protons, a 10-fold increase in dose rate from 100 to 1000 rads/min was found to increase its relative effectiveness by more than 20%. A converse effect on gastrointestinal death was noted in fractionated dose studies. In these studies we found that single proton dose which normally kills 80 to 90% of the mice in 6 days (gastrointestinal death) when given in two equal doses separated by 3 hours, will kill only 10 to 20% during this time. This is equivalent to a dose reduction of about 1.3. Hence, in these animals the whole-body dose must be increased by about 30%—from 940 rads (air) to 1220 rads—to produce the same gut effect as in the single-dose group. A similar effect was noted in animals irradiated with 910 MeV alpha particles.

Some studies have been conducted on the genetic effects of spermatogonial irradiation with 730 MeV protons and 910 MeV alpha particles. As with X-rays and fast neutrons, mice irradiated with acute whole-body doses of protons and alpha particles show an initial fertile period, followed by sterility for about 6 weeks, then resumed fertility. Based on abnormal embryo:total implantation ratio, the RBE of 730 MeV protons and 910 MeV alpha particles, relative to 250 kVp X-rays, appear to be about unity. In comparison, the RBE of fast neutrons is reported to be five to six times that of X-rays. These results are not unexpected since the LET values of fast neutrons are about 10 times greater than the radiations we have used in our experiments.

References

1. C. C. Wang, J. Lyman, C. A. Tobias, Relative Biologic Effectiveness of 730 MeV Proton Particles for Acute Lethality of Mice, in Lawrence Radiation Laboratory Report UCRL-10211, June 1962, pp. 43-50.

2. J. K. Ashikawa, C. A. Sondhuas, C. A. Tobias, A. G. Greenfield, V. Paschkes, Difference in Injury Mode, Dose-rate Dependence and RBE of 730 MeV Protons, 100 kVp X-Rays, and 250 kVp X-Rays, Vol. 1, pp. 249-260 in Biological Effects of Neutron and Proton Irradiations; also, Lawrence Radiation Laboratory Report UCRL-11014, September 1963.

3. C. A. Sondhuas, J. K. Ashikawa, C. A. Tobias, V. Paschkes, and D. Love, Studies on the Mammalian Radiation Syndrome with High-Energy Particulate Radiation. II. Some Factors Affecting RBE of 730 MeV Protons, in Lawrence Radiation Laboratory Report UCRL-11184, January 1964, p. 128.

HEAVY ION BEAMS IN EXPERIMENTAL POPULATION GENETICS

J. L. King

The branch of experimental population genetics that deals with the effects of induced mutation in animal populations is still in a state that is too primitive and uncertain to benefit from the use of heavy ion mutagenesis now or, probably, in the immediate years ahead. It is possible to foresee even now, however, that questions exist in this field which may be answerable only with the use of such highly specialized sources of mutagenic irradiation.

Population studies are necessary in the evaluation of the genetic aspect of radiation hazard to man, and useful to the understanding of normal mechanisms of gene interdependence and the fate of mutations in populations. Results of studies of genetically irradiated animal populations have been published in any quantity only in the last four years. Unfortunately, the only firm conclusion that can be drawn at present is that the matter is fraught with difficulty and uncertainty: published results have almost universally been negative, contradictory, unreproducible, or not statistically significant. Thus, mice with irradiated ancestors have been reported to show: increased longevity; decreased longevity; no effect on longevity; increased adult body weight; decreased adult body weight; no effect on adult body weight; decreased litter size; no effect on litter size; and so forth. An indication of the complexity of the problem is the quite significant reproducible result of an experiment reported by D. F. Cox:[1] paternal X-irradiation of Hampshire swine reduces the mean litter size by 5%; paternal X-irradiation of the Duroc breed of swine increases the mean litter size by 5%.

A major potential source of ambiguity is that radiation-induced mutations are not of a kind. Qualitatively different types of mutations presumably have different effects on populations, and these effects may frequently cancel or confuse one another. Some population effects are apparently due to the simple killing of germ cells, or to dominant lethals which, for instance, reduce competition within litters. Surprisingly enough, there is extensive evidence that beneficial mutations may be induced with something like the same frequency as detrimental mutations--beneficial and detrimental being defined

in relation to some parameter such as viability or longevity. It is possible that this condition is true for, say, base substitutions but not for chromosome rearrangements.

To untangle this skein of divers effects, it will undoubtedly be helpful to have an array of sources, each with a relatively narrow mutation spectrum. The responses of populations to different types of mutation could then be differentiated. Heavy ion beams are promising in this respect, as there is evidence that high LET mutation spectra have high ratios of chromosomal to intragenic effects. The experiments of Mortimer et al.[2] with the Hilac here indicate, in fact, that the point mutation (revertant) rate among surviving yeast cells begins to fall off markedly near the present upper limit of available LET. The tendency would probably continue with the higher LET values of the Omnitron, and it is possible that at some level virtually all mutation will be of the gross chromosomal type. If this were known to be the case, there are a number of questions in experimental population genetics that could be resolved.

Before the use of the Omnitron in experimental population genetics can be justified, two developments must occur. One is a more extensive understanding of the patterns of response of animal populations to the more conventional mutation sources. A lot of work is being done on this now, including a major project here. The second development must be the characterization of the mutation spectra of the Omnitron as well as of other radiation sources. Most of this must await the operation of the Omnitron itself. In perhaps six years the questions will be much more sharply defined; at present it appears probable that the Omnitron will be useful or necessary for some of the answers.

References

1. D. F. Cox, Genetics 50, 1025-1028 (1964).
2. R. K. Mortimer, T. Brustad, and D. V. Cormack, Rad. Res. 26, 465-482 (1965).

MORPHOLOGICAL STUDIES OF LAMINAR LESIONS

W. Haymaker*

As Jerzy Rose once related, the first effort to produce a laminar lesion had behind it a neurophysiological objective. If one or another of the six laminae of the cerebral cortex could be destroyed, leaving the others intact, then it might be possible to assess the contributions made by the various laminae to cortical electrical activity, as recorded from the cerebral surface. So the first experiment was done to see if a lamina could be selectively destroyed. This pioneer study, reported 10 years ago (1957) by Malis et al.[16] proved that the approach was feasible. In two cats in which a 5 by 5 mm area of the cerebrum was exposed to 10 MeV proton radiation they demonstrated that a longitudinal zone of the cortex 0.8 mm deep to the cerebral surface could be severely damaged without materially injuring the intervening tissue. In stained sections the damaged zone was found to have a sharp lower border and an uneven upper border, and its width was 40 to 100 μ, depending on dosage. Since tissue shrinks about 35% during processing, the actual width of the band would be correspondingly greater. To this "Bragg-peak zone," or "band," they gave the misnomer, "laminar" lesion, a term we shall probably have to live with. In actuality the zone was pseudolaminar, as would be expected from bombardment with a monoenergetic beam. Nonetheless, the lesions were precisely enough situated to serve as a wholly satisfactory model for the neurophysiological purposes intended. Some results along this line are available.[15]

Rose and his associates,[23] in following up that first study in cats, decided that the initial step should be a careful histological study. They used rabbits and concentrated on silver methods that specifically impregnate nerve cells and their processes. As in the cats, the "Bragg-peak band" of damage in the rabbits was peculiar in that only nerve cells were destroyed, leaving intact the rest of the tissue elements, which included glia and fiber processes. From this study came the remarkable observation that new axons

*Ames Research Center, NASA, Moffett Field, California.

grew into the cell-depleted band. That nerve fibers in the brain can "regenerate" had long been known, but here in the laminar preparation was a model in which the phenomenon was open to study. When a peripheral nerve is sectioned, as many as 40 new axons sprout from a single nerve fiber in the proximal stump, and sprouting of much the same magnitude seemed also to be occurring in the laminar preparation. The comparison is, however, not entirely apt, as no proof was provided that the particle radiation amputated a sizable number of the fibers in their course vertically through the band. Be that as it may, the persisting tissue matrix in the band served as a latticework on which the new axons could climb. There was sometimes an orderliness in the regrowth in the band in that a semblance of the previous fiber-architecture was achieved, but usually not.

A subsequent study, by Kruger and Clemente,[15] confirmed all this, and showed that new axons could grow out not only in the cat and rabbit, but also in the rat and monkey. Further, in the rat, Estable-Puig et al.[5] observed that axonal growth occurred not only in the cerebral cortex but also in the cerebellar cortex. Such a heavy growth of axons occurred at both sites that after a few months the axons could easily be seen in stained sections with the unaided eye. Entrance of the new axons into the band was evident on about the 18th day postexposure. Many grew upward into the band from a long distance away, i.e., from a region of the white matter near the corpus callosum. Moreover, it was shown that the new axons in the band were being surrounded by a sheath of myelin, but the mechanism by which this occurred was obscure. Oligodendroglia appear to be requisite to myelin formation, in the sense that the myelin layers encircling axons are an extension of the oligodendroglial cytoplasm. One reason for mentioning all this is to indicate that in vivo models are needed if one is to get at the the heart of the problem of how, for example, remyelination is achieved under some condition of central nervous system (CNS) injury,[3, 13] but not under other conditions, such as multiple sclerosis. The laminar preparation is admirably suited as such a model.

Speaking of possible applications of the laminar model to clinical problems in man, we wonder whether, with the use of the model, nerve fibers could be induced to grow more effectively up and down the spinal cord in paraplegics. It is a wild idea, but could not exposure of the cord to particle

radiation in the acute stage of injury create a latticework on which new axons could more effectively grow?

Studies on laminar preparations were undertaken in the early days without any idea as to where they might lead. We used to come at times from Washington to work with Dr. Tobias and his associates. I would put brain material into all kinds of fixatives, then, on coming home, stain sections in various ways. On one of these trips Igor Klatzo, of the NIH, came along and brought Rossman's fluid, which contains a number of fixing ingredients. He put some of the brain blocks in this fluid. On staining the sections by the PAS method with and without pretreatment with α-amylase he found that the astroglia in the irradiated area (but not in the control areas) contained particles of glycogen.[12] Glycogen is normally present in the brain, but is at a level (45 to 70 mg per 100 g of tissue in the rat) usually not detectable with use of the PAS stain. Only on considerable increase does the glycogen become evident in sections, and then in the form of granules, which are stained red by PAS. It was appropriate that the glycogen increase should occur in the astrocyte, for carbohydrate metabolism in this cell is probably very intense. Through its cytoplasmic processes wrapped around vessels, this cell also has transport functions, carrying, for example, glucose from the blood stream and discharging it into the tissue, for use by such elements as nerve cells.

Klatzo, and shortly thereafter Miquel et al.,[19,21] observed that the glucose coming from the blood stream into the irradiated part of the brain was not being used properly and was being stored as glycogen. This observation was a breakthrough, in that a dependable means was now available for the study of CNS energy metabolism. Essentially the glycogen accumulation represented a "biochemical lesion," the intensity of which reached a peak in 48 hours, then decline over the next week or two, when normality was reached.

The glycogen increase raised all kinds of questions as to its genesis. A favored view was that the radiation directly damaged the tissue such that inhibition of aerobic metabolism occurred, with the result that glucose in the tissue was converted to glycogen--in other words, that the accumulation was the outcome of a profound reduction in oxydative metabolism. This view found support in an observation made by Snezhko,[25] in an experiment

in rabbits in which determinations were made of free O_2 content in cerebral tissue following X-irradiation of the head at 1-3 kR. The O_2 tension in the tissue fell transiently in some of the animals. However, in all the animals the tension soon increased drastically (by 70 to 100%). Subsequently—for at least 7 hours—the O_2 concentration fluctuated in a wave-like manner, and remained above the 70%-increase level. No parallel was found with hemodynamic shifts, and thus it was concluded that the rise in O_2 concentration in the brain was indicative of lowered oxidative processes. There seems no better explanation of acutely developing glycogen increase.

The problem of O_2 tension in the brain following irradiation is, however, not that simple, as shown by Aleksandrovskaya[1] in a histology study in rats. The radiation conditions she used were, however, so different from those in the preceding experiment that comparison is not valid. The rats were totally irradiated in fractional doses of 50 R weekly for a maximum of 250 R, and they were sacrificed at various periods of time up to one month after the last exposure. Pathological changes favoring a hypoxic effect were: laminar lesions in the cerebral cortex, destruction of nerve cells, and multiplication of oligodendroglia. According to the author: "On taking into account the high oxygen requirement of an organism exposed to the action of penetrating radiation it can be assumed that the usual amount of oxygen supplied to the brain is found to be inadequate and a deficit develops in the oxygen supply of the brain, or to put it differently, a secondary anoxia develops." Lack of visible changes in vessels was considered to support this view. While lack of visible vascular change does not necessarily mean that it did not exist, and while vascular change may have occurred in this experiment and been pathogenic, nonetheless the viewpoint is refreshing, and certainly has some substance.

As if the O_2 factor were not enough to cope with in evaluating tissue damage, there is still another factor to be reckoned with, and that is brain edema. Here, a hypoxic state of the tissue is inevitably a complication.

To evaluate the significance of an edematous process in radiation injury, a model was needed. None could be found that had more advantages than the laminar preparation, in which, as stated, only the upper part of the cortex is damaged. In studies on the rat and cat carried out with the use of this model, it was found[20] that very quickly after 48 MeV α-particle

irradiation given in a large dose (24 krad), edema fluid began to permeate the white matter beneath the band, until in a day or two all the white matter throughout the cerebrum was flooded by the fluid. But the interesting point was that glycogen accumulated in astroglia throughout all the cerebral cortex, even in that farthest removed from the site of radiation injury (Miquel, unpublished). Then it was found that an equally far-flung glycogen disturbance occurred after a very small stab wound had been made in the cortex (Miquel and Ibrahim, unpublished). This raised questions which have not as yet been answered. Could some reflexly induced edematous process not visible under the light microscope be held accountable? Increased glycogen content in the cortex must necessarily go hand-in-hand with a reduction in brain function. Indeed, Křivánek[14] found a correlation between duration of glycogen accumulation (following application of strychnine to the cortex, which should have induced an edematous state) and duration of abnormal conditioned reflexes. In this connection, Miquel and Haymaker[19] observed in the totally irradiated brain (of the rat) that the largest accumulation of glycogen was in a structure heavily concerned in emotive functioning, i.e., the hippocampus. The future requires that more consideration be given to these wider implications of laminar and other radiation-induced lesions. A more sensitive means of glycogen detection is needed, as the glycogen response about which we have been talking is not detectable at doses below 1 krad. Perhaps further studies for the detection of glycogen biochemically, by a method already established for laminar preparations,[26] will prove profitable.

This gives something of the story of the investigations that were opened up in our own laboratory by the chance observation that particle irradiation resulted in glycogen accumulation.

This discussion is not supposed to exceed a certain length, and since something needs to be said about the nerve cell, little can be said about other matters. Alpha-particle radiation in a dose of 12 krad (surface dose) invariably results in 2 or 3 days in the appearance of mitotic figures at the sites of neuroglia. However, some say that neuroglia do not divide mitotically! At about 6 krad (surface dose) vascular permeability disturbances occurring in the laminar preparation can persist for as long as 13 days [as shown with the use of fluorescein-labeled albumin (FLA)[8, 11, 12]] as prominent vascular changes, as viewed electron microscopically, can last some 22 days.[17]

Thus, vascular alterations during such time periods can be a source of parenchymal injury. Vessels in irradiated tissue can be functionally defective even though morphologically they appear perfectly normal, or practically so.[4, 24] In this connection, any claim that ionizing radiation primarily destroys nerve cells—say after a latency of some days or weeks—should be considered unfounded unless backed up by vascular permeability studies. The oligodendrocyte has such a refined digestion system that mesenchymal cells may not be called into the laminar preparations to clean up the debris.[18] Lastly, if one is looking for a sensitive system for the detection of CNS radiation damage, the paper by Neumayr and Thurnher[22] should not be neglected.

In regard to nerve cells, some people might think that the brain is composed mostly of these cells. But only about 1/10 the cells are nerve cells. It thus takes an average of 10 cells, such as vascular endothelial cells and astrocytes, to keep a nerve cell going. The nerve cell is thought to be particularly radioresistant because it is not subject to divisional processes which make other cells radiosensitive; the nerve cell uses practically all its energy in communication. The defenses set up for the preservation of the integrity of the nerve cells suffice when the entire head is irradiated, as under these conditions the nerve cell can go uninjured following exposure to thousands of rads, yet oligodendroglia not far away will be destroyed by 200 rads[2, 10] (primarily? secondarily?). However, despite all the defenses available, the nerve cell is the most vulnerable element when the radiation field is very small, such as in the laminar preparation. What is meant by "most vulnerable" in this context is that while vascular cells and glial cells react actively to the radiation injury and persist, the nerve cell dies.

There seems to be a discrepancy in the dosage needed to wipe out a narrow band of nerve cells. In one experiment, Janssen et al.[11] found that a 1.5 krad surface dose of 48 MeV α-particles (7.5 krad, peak dose) was sufficient to destroy a very narrow band of nerve cells—say a band 25 μ thick—in 7 months. Particle flux in this experiment was 1×10^{8} α/cm^{2}-sec, and dose rate 10 krad/min. On the other hand, Zeman et al.,[28] in bombarding the brains of mice with 22.5 MeV deuterons through an aperture 25 μ in diameter, found no discernible damage in 6-1/2 months after exposure to 225 krad (10.7×10^{9} d/cm^{2}-sec; 8 krad/sec), though nerve-cell destruction

did occur in 24 days when the dose was doubled. The need for massive dosage to destroy nerve cells in a 25 μ track was subsequently confirmed.[27] How is one to account for such wide differences in results? People say it must be a matter of geometry, that the greater volume of brain irradiated in the Janssen et al. experiment can account for the difference.

More concrete information is needed. Although much is known in regard to dose-tissue volume factors in pathogenesis when larger areas of the brain are irradiated, much still remains to be learned of the factors involved in damage incurred in tiny brain areas. What we are hoping to do in collaboration with Dr. Tobias and his group in Berkeley is to bombard many 25 μ fields in the same brain with protons or α-particles in an effort to settle this problem. Tolerance doses would be established in relation to spacing of the particle tracks. This would be, as it were, a first approximation to a study of the effects of heavy primaries once the presently planned accelerator becomes available.

In this connection, there is the problem of the RBE of particle radiation. For most endpoints the RBE has been shown to be close to 1. But in regard to the brain it seems likely that the RBE of particle radiation is higher than 1, at least for certain particle energies. In the monkey, 6 krad proton radiation (55 MeV) given at 2 krad/min caused far greater damage in the same period (unpublished) than 30 krad γ rays (1.2 to 1.4 MeV) given at 1 kR/min.[9] The comparison seems to be valid. Also it has been found that 138 MeV protons had less of an effect on the brain than 55 MeV or 32 MeV protons (unpublished). As to the effect of particles of different nuclear charge on the brain, we once ran an experiment with Dr. Tobias in which laminar lesions were made both with protons and α-particles of equal energy per nucleon (12 MeV), and given in the same dose and at the same dose rate. According to the published results, the width of the band was approximately the same in both series.[11] But there remains the hankering suspicion that the experiment was not well enough designed to provide a definitive answer. Repetition is therefore in order. If we are to be ready to do the crucial studies when accelerated larger nuclei become available, and thus to get some idea of the RBE of galactic cosmic rays so far as the brain is concerned, then considerably more work is needed with particles now available.

References

1. M. M. Aleksandrovskaya, The Effect of Small Doses of X-rays on the Morphology of the Central Nervous System of Animals, in works of All-Union Conf. Med. Radiology, pp. 58-61, Moscow, State Publ. House of Med. Literature, 1957 (Russia) (AEC-tr-3661, Bk. 1, pp. 108-112, 1959).
2. R. H. Brownson, D. B. Sutter, and D. D. Diller, Acute Brain Damage Induced by Low-dosage X-irradiation, Neurology 13, 181-191 (1963).
3. R. B. Bunge and P. H. Settlage, Neurological Lesions in Cats Following Cerebrospinal Fluid Manipulation, J. Neuropath. Exp. Neurol. 16, 471-491 (1957).
4. C. D. Clemente and E. A. Holst, Pathological Changes in Neurons, Neuroglia, and Blood-Brain Barrier Induced by X-irradiation of the Heads of Monkeys, A. M. A. Arch. Neurol. Psychiat. 71, 66-79 (1954).
5. J. Estable-Puig, R. F. de Estable, C. Tobias, and W. Haymaker, Degeneration and Regeneration of Myelinated Fibers in the Cerebral and Cerebellar Cortex Following Damage from Ionizing Particle Radiation, Acta Neuropath. 4, 175-190 (1964).
6. T. J. Haley and R. S. Snider (eds.), Response of the Nervous System to Ionizing Radiation (Academic Press, New York, 1962).
7. T. J. Haley and R. S. Snider (eds.), Response of the Nervous System to Ionizing Radiation (Little, Brown and Company, Inc., Boston, 1964).
8. W. Haymaker, Studies of Brains Exposed to Cosmic Rays and to Accelerated Cosmic Particles, in Brain Function; Cortical Excitability and Steady Potentials; Relations of Basic Research to Space Biology, ed. by M. A. B. Brazier (University of California Press, Berkeley, 1963), pp. 347-378.
9. W. Haymaker, G. Laqueur, W. J. H. Nauta, J. E. Pickering, J. D. Sloper, and F. S. Vogel, The Effects of Barium^{140}-Lanthanum^{140} (Gamma) Radiation on the Central Nervous System and Pituitary Gland of Macaque Monkeys (a study of 67 brains and 77 pituitary glands), J. Neuropath. Exp. Neurol. 17, 12-57 (1958).
10. S. P. Hicks and P. O'B. Montgomery, Effects of Acute Radiation on the Adult Mammalian Central Nervous System, Proc. Soc. Exp. Biol. Med. 80, 15-18 (1952).

11. P. Janssen, I. Klatzo, J. Miquel, T. Brustad, A. Behar, W. Haymaker, J. Lyman, J. Henry, and C. Tobias, Pathologic Changes in the Brain From Exposure to Alpha Particles From a 60-Inch Cyclotron, in Haley and Snider, pp. 383-409, 1962.[6]

12. I. Klatzo, J. Miquel, C. Tobias, and W. Haymaker, Effects of Alpha Particle Radiation in the Rat Brain, Including Vascular Permeability and Glycogen Studies, J. Neuropath. Exp. Neurol. 20, 459-483 (1961).

13. H. Koenig, M. B. Bunge, and R. P. Bunge, Nucleic Acid and Protein Metabolism in White Matter, A. M. A. Arch. Neurol. 6, 177-193 (1962).

14. J. Křivánek, Changes of Brain Glycogen in the Spreading EEG-Depression of Leao, J. Neurochem. 2, 337-343 (1958).

15. L. Kruger and C. D. Clemente, Anatomical and Functional Studies of the Cerebral Cortex by Means of Laminar Destruction with Ionizing Radiation, in Haley and Snider, pp. 84-104, 1964.[7]

16. L. I. Malis, R. Loevinger, L. Kruger, and J. E. Rose, Production of Laminar Lesions in the Cerebral Cortex by Heavy Ionizing Particles, Science 126, 302-303 (1957).

17. D. S. Maxwell and L. Kruger, Electron Microscopy of Radiation-Induced Laminar Lesions in the Cerebral Cortex of the Rat, in Haley and Snider, pp. 54-83, 1964.[7]

18. D. S. Maxwell and L. Kruger, The Reactive Oligodendrocyte (an electron microscopic study of cerebral cortex following alpha particle irradiation), Am. J. Anat. 118, 437-459 (1966).

19. J. Miquel and W. Haymaker, Astroglial Reactions to Ionizing Radiation: With Emphasis on Glycogen Accumulation, in Progress in Brain Research, vol. 15, ed. by E. D. P. De Robertis and R. Carrea (Elsevier, Amsterdam, 1965), pp. 89-114.

20. J. Miquel and W. Haymaker, Glycogen Accumulation in Monkey and Cat Brain Exposed to Proton Radiation, in Proc. 5th Internat. Congr. Neuropath. (Exc. Med. Found., Amsterdam, 1966).

21. J. Miquel, I. Klatzo, D. B. Menzel, and W. Haymaker, Glycogen Changes in X-irradiated Rat Brain, Acta Neuropath. 2, 482-493 (1963).

22. A. Neumayr and B. Thurnher, Über den Einfluss lokaler Röntgenbestrahlung auf die Permeabilität menschlicher Kapillaren, Strahlentherapie 84, 297-305 (1951).

23. J. E. Rose, L. I. Malis, L. Kruger, and C. P. Baker, Effects of Heavy, Ionizing, Monoenergetic Particles on the Cerebral Cortex. II. Histological Appearance of Laminar Lesions and Growth of Nerve Fibers After Laminar Destructions, J. Comp. Neurol. 115, 243-296 (1960).

24. W. Scholz, W. Schlote, and W. Hirschberger, Morphological Effect of Repeated Low Dosage and Single High Dosage Application of X-irradiation to the Central Nervous System, in Haley and Snider, pp. 211-232, 1962.[6]

25. A. D. Snezhko, Biofizika 1, 585 (1956); Biofizika 2, 67 (1957); Tr. Vses. Nauch. Tekh. konf. po Primenen. Radioaktiv. i. Stabil. Izotopov i Izluchenii v Narod. Khoz. i Nauke Radiobiol., p. 176, Moscow, 1958. [Cited by N. N. Livshits, Physiological Effects of Nuclear Radiations on the Central Nervous System, in Adv. in Biol. & Med. Physics, ed. by C. A. Tobias and J. H. Lawrence (Academic Press, New York, 1960), pp. 225-226.]

26. L. S. Wolfe, I. Klatzo, J. Miquel, C. Tobias, and W. Haymaker, Effect of Alpha-Particle Irradiation on Brain Glycogen in the Rat, J. Neurochem. 9, 213-218 (1962).

27. W. Zeman, H. J. Curtis, and C. P. Baker, Histopathologic Effect of High-Energy-Particle Microbeams on the Visual Cortex of the Mouse Brain, Radiation Res. 15, 496-514 (1961).

28. W. Zeman, H. J. Curtis, E. L. Gebhard, and W. Haymaker, Tolerance of Mouse-brain Tissue to High-Energy Deuterons, Science 130, 1760-1761 (1959).

ANATOMICAL STUDIES WITH LAMINAR LESIONS

L. Kruger*

The possibilities of employing the sharp peak in energy release at the end of the path of ionization of positively charged particles (the Bragg effect) was suggested as a means of producing precise destruction at a given distance from the surface in a biological system. This original suggestion was first applied to the cornea by Tobias in collaboration with clinical ophthalmologists (notably Von Sallman[1]) and was subsequently employed for destroying a layer of nerve cell bodies at a given distance from the surface in mammalian cerebral cortex.[2] The experiments on cerebral cortex were extended to the cerebellar cortex[3] with essentially similar results, and lesions in nervous tissue have now been studied with high energy alpha particles, protons, and deuterons by using several of the Berkeley accelerators and the 60-inch Brookhaven cyclotron. Interaction and overlap between the Berkeley and Brookhaven groups has been extensive and at present active work is being pursued principally at the Berkeley Hilac. The principle findings have been related to radiation dosimetry, neuropathology, cortical connections (or neuroanatomy), and fiber dynamics within the cortex.

Radiobiology

Although there is a vast literature in radiobiology, few studies have the advantages inherent in employing heavy ionizing particles. With this method, doses of ionizing radiation can be stated in precise physical terms with respect to the number of ion pairs or the energy (in meaningful units such as ergs or calories) in relation to the number of nerve cells or per gram of tissue or per mm^3 of tissue. While these data have not been the primary interest instigating such research, the extensive series of over 2000 animals irradiated by several groups (principally under the guidance of C. A. Tobias at this laboratory) provides the most exact and extensive information on the dose and time relations in mammalian cells. In addition, because of the nature of the relative ionization curve, one can compare surface and peak doses in the same lesion and obtain some index of the range of

*Department of Anatomy, University of California at Los Angeles.

energies that might be effective for destruction. From these data it has been found that doses of approximately 15 000 rads (peak dose) or 10^9 to 10^{10} particles per cm^2 of brain constitute an effective range for producing a laminar lesion with apparently intact overlying layers of neurons (Fig. 1). The above general figures can now be stated more precisely in terms of two relevant variables: dose-rate and survival time (or post-irradiation interval). Although the original data were derived principally from rabbits and cats[2] and rats,[3-8] these findings have now been extended to monkeys.[3]

Neuropathology

A finding of particular interest in laminar lesions is the apparent selectivity of destruction whereby all nerve cell bodies are destroyed but glial cell bodies are not reduced in number, and in fact with relatively higher doses can be seen to proliferate numerically. The first response to radiation can be detected within glial astrocyte cytoplasm and is characterized by a marked increase in glycogen followed by the appearance of lipid droplets (Fig. 2). At later stages, mitoses and a marked increase in filament content is noted in reactive astrocytes (Fig. 3). An extensive series of papers from the Berkeley group has resulted, describing the changes in electron and light microscopy (Maxwell, Kruger, Lkatzo, Kaymaker, Tobias, Estable-Puig, etc.) of the astrocyte reactive response.

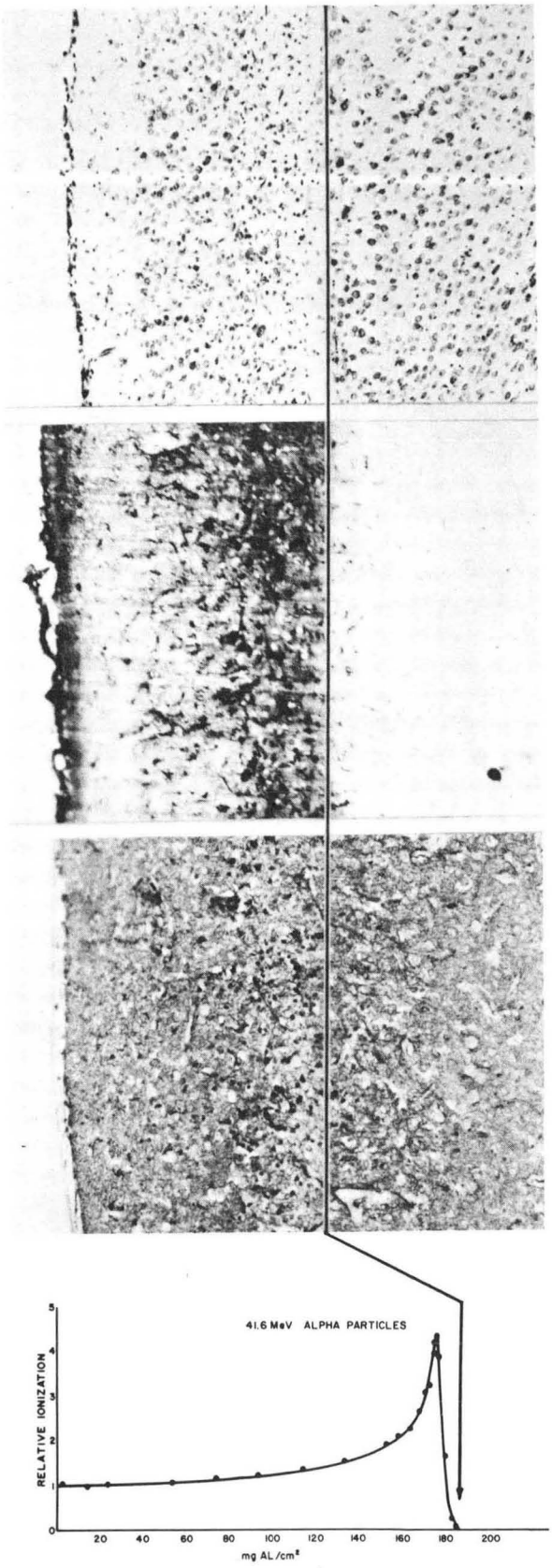
A far more difficult problem has been to ascertain the means by which degenerated material (principally neurons and their processes) are disposed of, and although reactive glial cells acquire numerous osmiophilic bodies which have been interpreted as evidence of phagocytosis,[3] there has been no electron microscopic evidence of either free debris or debris ingestion with low dose laminar lesions.[4] These results have led to a re-examination of the actual existence of freely mobile macrophages of mesodermal origin within brain parenchyma and the identification of "microglia" has been seriously questioned.[5] It is clear that oligodendrogliocytes participate in degenerative processes and that the osmiophilic dense bodies that appear in these cells are not ingested inclusions but are specialized organelles of the "lysosome" variety displaying acid phosphatase positivity (Fig. 4). Evidence is accumulating to demonstrate specialized mechanisms of intracellular digestion are principally responsible for disposal of degenerating tissue in brain[6]

Fig. 1. (a) Laminar lesion in the cerebral cortex, indicated by the absence of neurons and a slight increase in number of glial nuclei. Forty-three days after irradiation. Tissue irradiated with alpha particles, 6000 rads (surface dose). Thionin stain. × 90. A line which indicates the end of range of particles constitutes the lower limit of the lesion a, b, and c, and corresponds to the position of the arrow in d.

(b) Silver impregnation of reactive astrocytes in the irradiated portion of the cortex in the same animal as in (a). Modified Bielschowsky stain. × 90.

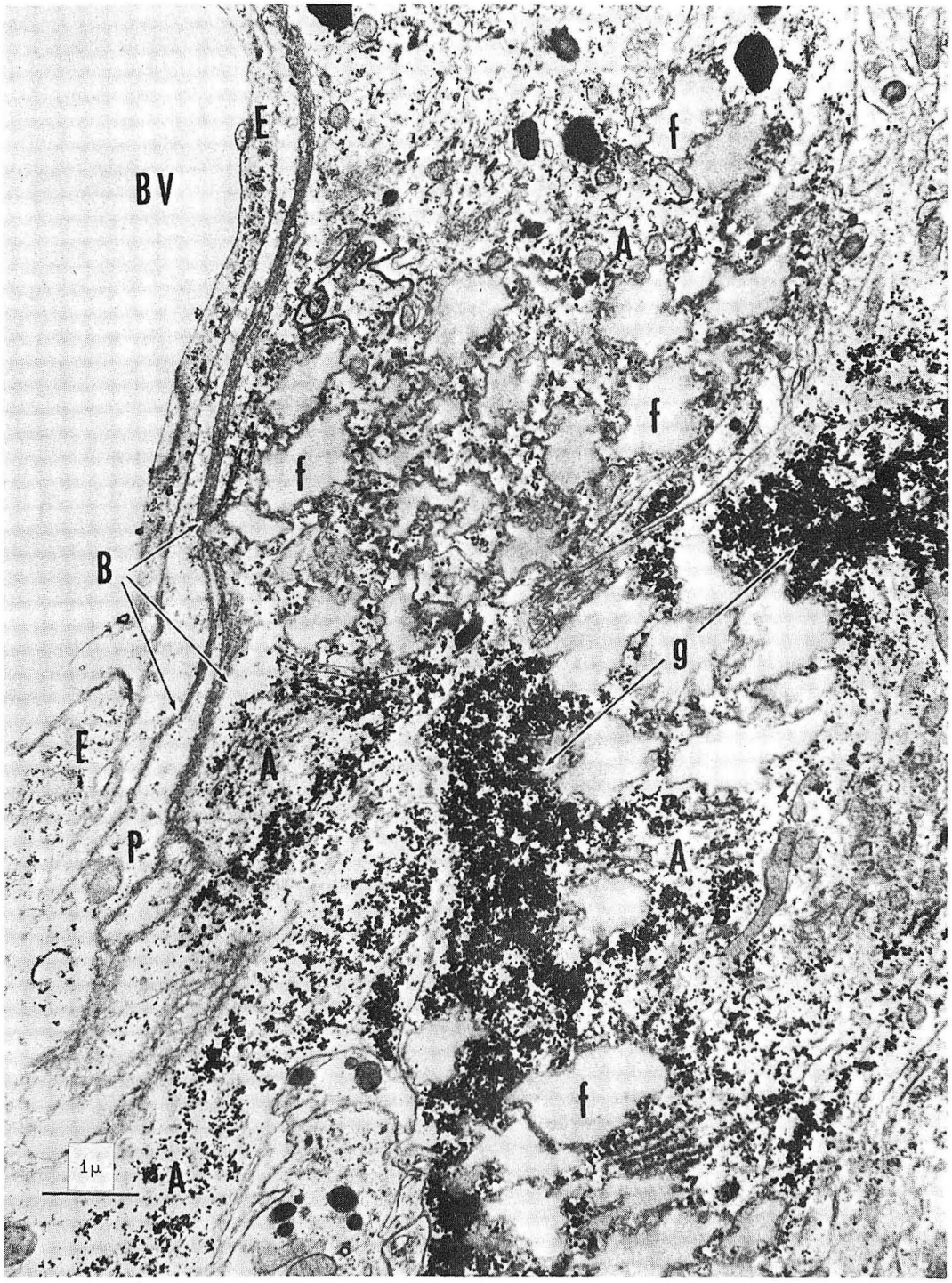
(c) Periodic acid-Schiff (PAS) preparation of a heavy dose laminar lesion (20 000 rads, surface dose) 2 days after irradiation. The rapid accumulation of the PAS-positive material is associated with the zone of astrocytic reaction. × 90.

(d) Energy release curve (Bragg curve) for particles used in this study, showing relative ionization (or energy release) registered in a saturated ionization chamber after passage through aluminum foils of varying thickness. The peak of this curve is responsible for producing the laminar lesion at those doses that do not produce destruction with a surface dose. Only the cortex from the surface to the end of the particle range (arrow) is bombarded by heavy ionizing particles.



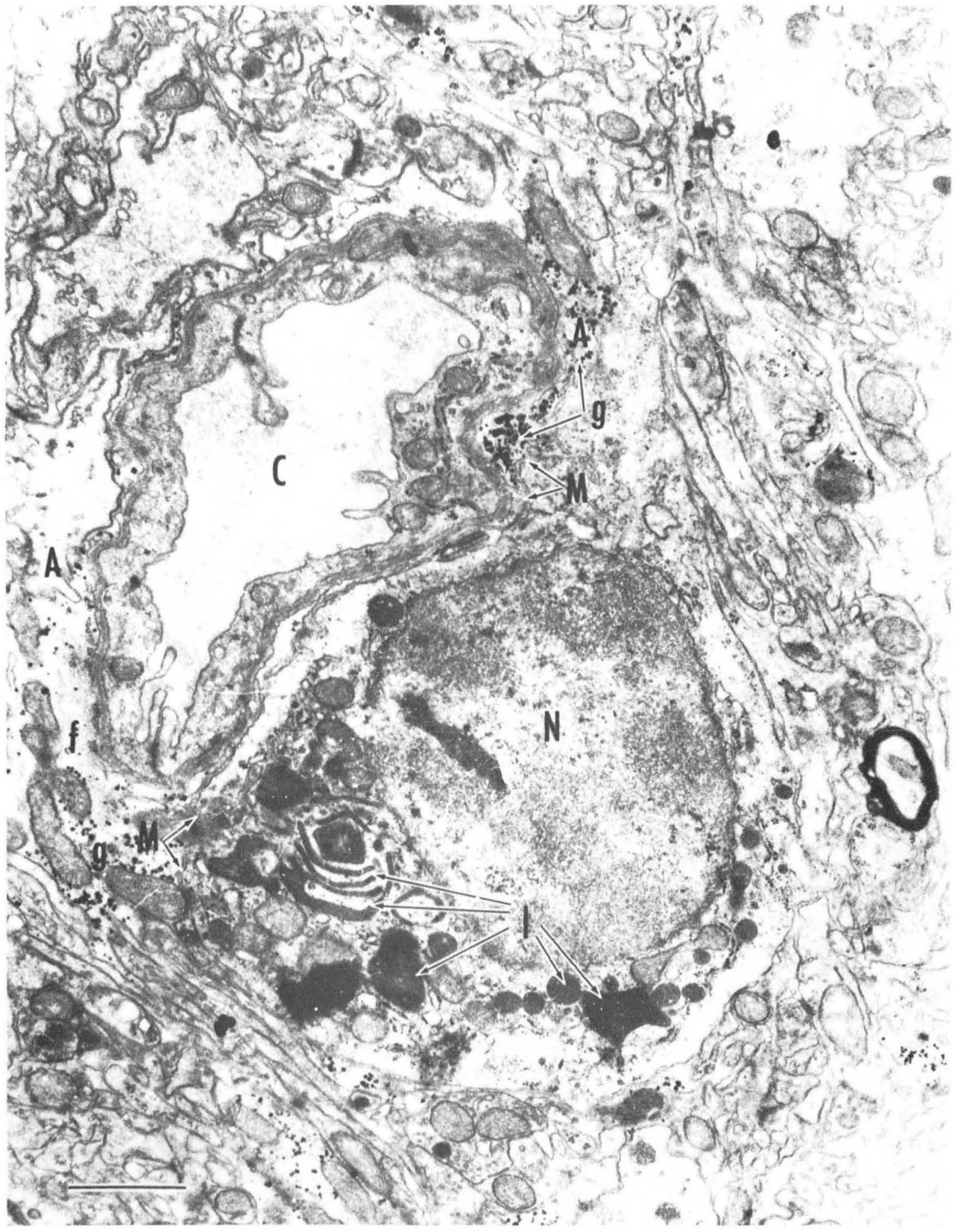
BBH 672-41

Fig. 1



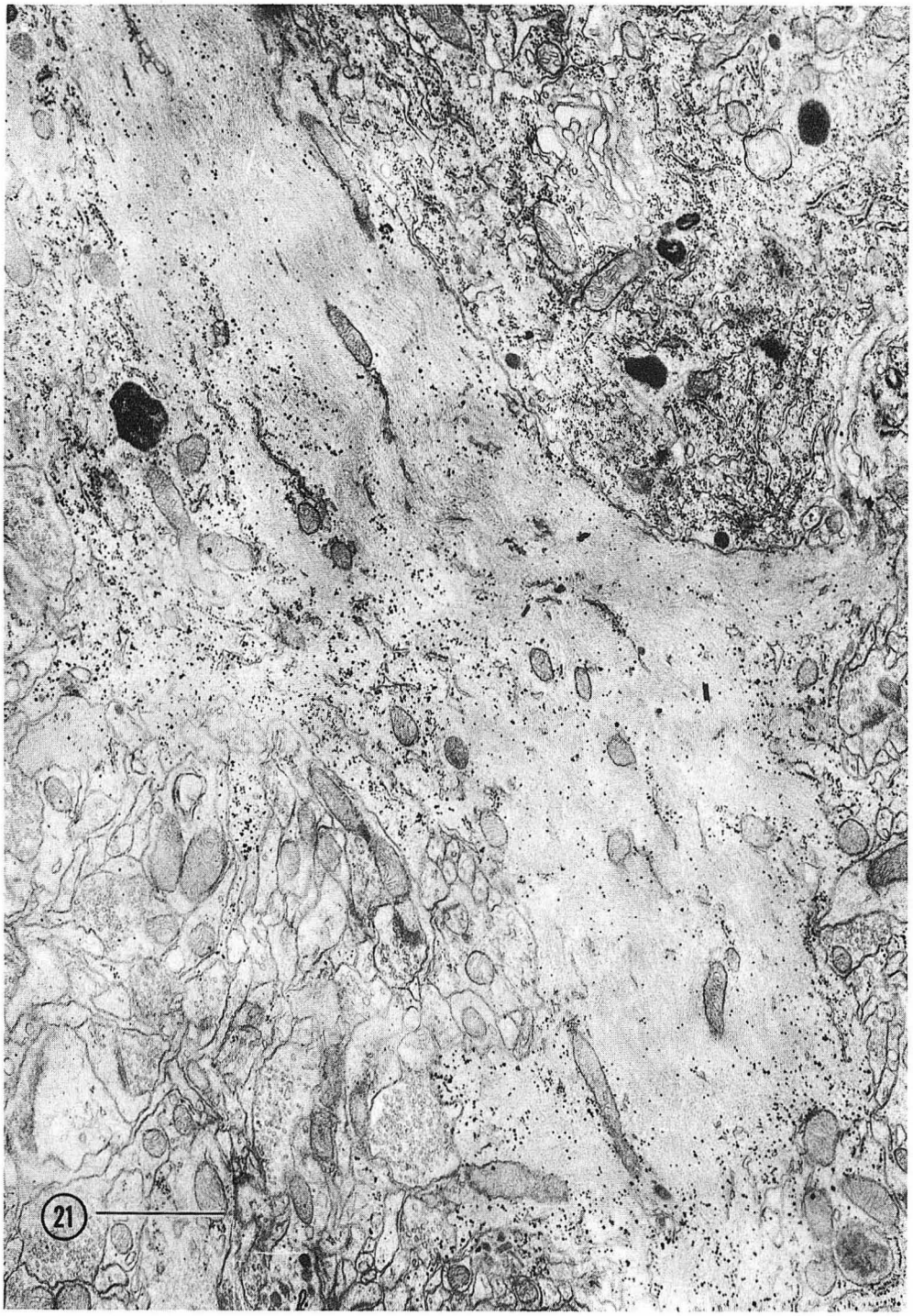
BBH 672-42

Fig. 2. EM of lesion area 11 days after irradiation with 9000 rads. A large blood vessel (BV) occupies the left of the field. Its wall consists of endothelium (E) and several layers of pericytes (P), each surrounded by a basal lamina (B). The vessel wall is invested with astrocyte cytoplasm (A) containing dense accumulations of glycogen (g) and fat droplets (f).



BBH 672-43

Fig. 3. Reactive astrocyte process 22 days after irradiation with 20 000 rads. Note the marked increase in filament content throughout and presence of glycogen granules.



BBH 672-44

Fig. 4. EM of lesion 5 days after irradiation with 6000 rads. A reactive oligodendrocyte with typical nucleus (N) contains lysosome-like bodies (l) some of which appear to be plates or sheets suggestive of Golgi formation. This cell abuts upon a reactive capillary (C), which is also invested with astrocyte processes (A) containing glycogen (g) and filaments (f). These processes are clearly separated from the oligodendrocyte at plasma membranes (M).

and that phagocytosis is a rare phenomenon associated with vascular inflammation. At higher radiation doses, tissue disruption and inflammation is indeed accompanied by macrophage invasion, and these cells are derived from blood leukocytes of pericapillary pericytes, the latter presumably corresponding to the microglia of classical neuropathology. Current work in progress is principally devoted to electron histochemistry of dense bodies in reactive neurons and oligodendrocytes, and mechanisms for auto-digestion in irradiated cells are becoming more clearly understood.

Neuroanatomy

The functional significance of the laminar pattern of cerebral cortex has remained obscure because of unavailability of appropriate methods of study, but the production of precise laminar lesions with clearly defined limits has opened several previously inaccessible features of laminar organization.

Laminar lesions at different depths revealed that although the supragranular layers (laminae I-IV) remain intact regardless of depth of the lesion, the infragranular layers (laminae V and VI) reveal retrograde neuronal degeneration when undercut,[9] thus suggesting that the upper layers are afferent or sensory and the lower layers are essentially efferent or motor. These findings on cellular atrophy were supported by Marchi studies which also revealed a smaller efferent or descending fiber projection from supragranular layers to the thalamus.[7] Retrograde thalamic atrophy was found only if lesions extended into layers V and VI.[7] From reconstruction of an extensive series of lesions placed in different sectors of the striate field, the detailed projection of the lateral geniculate nucleus upon the visual cortex was secured[10] and the severity of thalamic degeneration was related to the depth of lesion, with complete degeneration of a thalamic sector secured only if the lesion reached the bottom of the cortex.

Fiber Dynamics

One of the unexpected findings in earlier studies was the demonstration that nerve fibers (principally axons) were disrupted at the same time as neuron cell bodies were destroyed within the laminar lesion zone. At later sacrifice times, similarly produced lesions revealed a return of fiber architecture and indeed silver-stained preparations revealed more fibers than

were normally present. This was interpreted as a manifestation of fiber growth, an inherent propensity of damaged or interrupted nerve fibers to sprout new branches, rather than a true regeneration of new fibers.[9] The absence of a glial scar in the lesion was interpreted as the principal factor accounting for this previously undemonstrated phenomenon in central axons. Further studies revealed that fiber architecture is not reliably revealed by silver-impregnation methods during the period of intense glial reaction[11] and that it is doubtful that radionecrosis could produce a synchronous destruction of all nerve fibers at doses consistent with laminar lesion production. This finding was further supported by employing other silver methods, and was demonstrated clearly with the electron microscope, which revealed intact as well as degenerating fibers at periods extending from 1 day to 22 months post-irradiation (Maxwell and Kruger). Current studies in progress reveal that although the destruction and reappearance of nerve fibers is not an all-or-none phenomenon, the fiber architecture is nevertheless a reconstituted one rather than merely one of partial and gradual axonal depopulation, and artificial zonal laminae with an unusual array of longitudinal axons can develop after appropriate post-irradiation intervals. These findings support the parsimonious view that all nerve fibers (central as well as peripheral) possess some capacity for growth and that in an appropriate milieu lacking a fibrous barrier of glia, a reconstitution of nervous connections is demonstrable for the first time in the central nervous system.

References

1. L. Von Sallman, C. A. Tobias, H. O. Anger, G. Welch, S. F. Kimura, C. M. Munoz, and A. Drungis, Effects of High-Energy Particles, X-rays, and Aging on Lens Epithelium, Arch. Ophthalmol. 54, 489 (1955).
2. L. I. Malis, C. P. Baker, L. Kruger, and J. E. Rose, Effects of Heavy Ionizing Monoenergetic Particles on the Cerebral Cortex. I. Production of Laminar Lesions and Dosimetric Considerations, J. Comp. Neurol. 115, 219-242 (1960).
3. P. Janssen, I. Klatzo, J. Miguel, T. Brustad, A. Behar, W. Haymaker, J. Lyman, J. Henry, and C. Tobias, Pathologic Changes in the Brain from Exposure to Alpha Particles from a 60-Inch Cyclotron,

in Response of the Nervous System to Ionizing Radiation, ed. by T. J. Haley and R. A. Snider (Academic Press, Inc., New York, 1962), p. 382.

4. D. S. Maxwell and L. Kruger, The Fine Structure of Astrocytes in the Cerebral Cortex and Their Response to Focal Injury Produced by Heavy Ionizing Particles, J. Cell Biol. 25, No. 2, Part 2, pp. 141-157.
5. L. Kruger and D. S. Maxwell, Electron Microscopy of Oligodendrocytes in Normal Rat Cerebrum, Am. J. Anat. 118, No. 2, 411-436 (1966).
6. D. S. Maxwell and L. Kruger, The Reactive Oligodendrocyte: An Electron Microscopic Study of Cerebral Cortex Following Alpha Particle Irradiation, Am. J. Anat. 118, No. 2, 437-460 (1966).
7. L. Kruger and L. I. Malis, Distribution of Afferent and Efferent Fibers in the Cerebral Cortex of the Rabbit Revealed by Laminar Lesions Produced by Heavy Ionizing Particles, Exptl. Neurol. 10, No. 6, 509-524 (1964).
8. D. S. Maxwell and L. Kruger, Small Blood Vessels and the Origin of Phagocytes in the Rat Cerebral Cortex Following Heavy Particle Irradiation, Exptl. Neurol. 12, No. 1 (1965).
9. J. E. Rose, L. Malis, L. Kruger, and C. P. Baker, Effects of Heavy Ionizing Monoenergetic Particles on the Cerebral Cortex. II. Histological Appearance of Laminar Lesions and Growth of Nerve Fibers after Laminar Destruction, J. Comp. Neurol. 115, 243-296 (1960).
10. J. E. Rose and L. I. Malis, 1964. Geniculo-Striate Connections in the Rabbit. 1. Retrograde Changes in the Dorsal Lateral Geniculate after Destruction of Cells in Various Layers of the Striate Region, J. Comp. Neurol. (submitted for publication).
11. L. Kruger and C. D. Clemente, Anatomical and Functional Studies of the Cerebral Cortex by Means of Laminar Destruction with Ionizing Radiation, in Second International Symposium on the Effects of Ionizing Radiation on the Nervous System, ed. by T. Haley (Little, Brown, and Co., Boston, Mass., 1964), p. 84.

POSSIBLE USEFULNESS OF ACCELERATED HIGH ENERGY NUCLEI IN STUDIES ON REGENERATION IN THE SPINAL CORD

D. L. Singman, D. C. Van Dyke, and G. P. Welch

When the spinal cord is severely injured by contusion or laceration, there is permanent loss of function in the injured area. Loss of function appears to be due to three factors: widespread necrosis in the area of injury even when cord manipulation is minimal; limited or abortive regeneration of the axons; and formation of a glial and mesodermal scar tissue barrier to axonal regeneration.[1,2] Even if considerable axonal regeneration should occur, the viable portions of the cord are so widely separated by the extensive necrosis which accompanies even a small atraumatic surgical incision that nothing but extensive and prolonged axonal growth could be expected to bridge the lesion. Proliferation of glial and connective tissue elements may interfere with axonal regeneration, but if scar proliferation could be inhibited[3] and axonal regeneration stimulated, the extensive necrosis makes it such that the axons would have nothing but a mass of necrotic tissue at first and a cyst or cavitation through which to grow later. Thus, assuming that some degree of axonal regeneration is possible, the major deterrent to re-establishment of neuronal connections across an injured segment of the spinal cord would seem to be the extent of the necrosis which accompanies even the most minimal trauma.[4]

This paper presents evidence that a parallel beam of accelerated high energy nuclei, the "atomic knife," can be used to cut the spinal cord without necrosis of adjacent tissue and with minimum formation of either neurological or connective tissue scar.

Materials and Methods

In order to avoid the complications of distended bladder, hemorrhagic cystitis, hydronephrosis, and uremia which follow transection of the cord, rats were parabiosed and one member nephrectomized, the partner serving as the renal excretory organ. For successful parabiosis, highly inbred rats of the Buffalo strain were used.

For surgical exposure of the cord or for removal of the fixed cord after autopsy, a small, double bladed, high speed, electric, circular saw was used. A spacer between the two blades controlled the width and depth of the two cuts. The use of such a saw reduced the operative time and the chance of injury to the cord.

All rats were maintained on terramycin in the drinking water following surgery. All experiments were terminated by perfusing the rat first with saline to remove all blood, and second by 10% neutralized formalin. A segment of the thoracic vertebrae and spinal cord containing the lesion was immersed in formalin, and the cord was dissected free 24 hours later. Histological sections were prepared by standard paraffin imbedding techniques and stained with hematoxylin, eosin, and Luxol blue.

Surgical Incision of the Cord

In order to minimize the possibility of infection, the spinal cord was not exposed through laminectomy, but was severed through a stab wound with a sterile #12 Bard-Parker blade. The cut was made in the lower thoracic region of one member of a parabiotic pair. This procedure did not guarantee complete severance of the cord, and subsequent gross and microscopic examination revealed that the cut involved from 50 to 90% of the cord.

Surgical Incision Followed by Irradiation

In an attempt to inhibit glial proliferation at the site of surgical incision, the area of the incision was exposed to moderate doses of radiation. The lower thoracic segment of the spinal cord of one member of a parabiotic pair was incised through a stab wound under ether anesthesia. The rat was allowed to wake so that we could be certain that the cord had been severed sufficiently to result in paralysis of the hind legs. The incision was then positioned in front of the cyclotron beam, and a dose of approximately 1700 rads of 50 MeV protons was given through a 3.1×10 mm slit aperture oriented transversely across the spinal cord in line with the surgical incision. The incised cord was irradiated within a few minutes after it was cut.

Transection of the Cord with a Beam of Accelerated High Energy Protons

A lightly anesthetized parabiotic pair was fastened to a holder on an optical bench in line with the aperture of the Berkeley 88-inch cyclotron.

The area of the lower thoracic spine of the rat to be irradiated was shaved and aligned to the beam aperture by means of a pointer. The pointer was removed and the animal holder was then moved along the bench so that the back of the rat pressed against the aperture. The aperture was a 2×10 mm slit oriented transversely across the spine.

Results

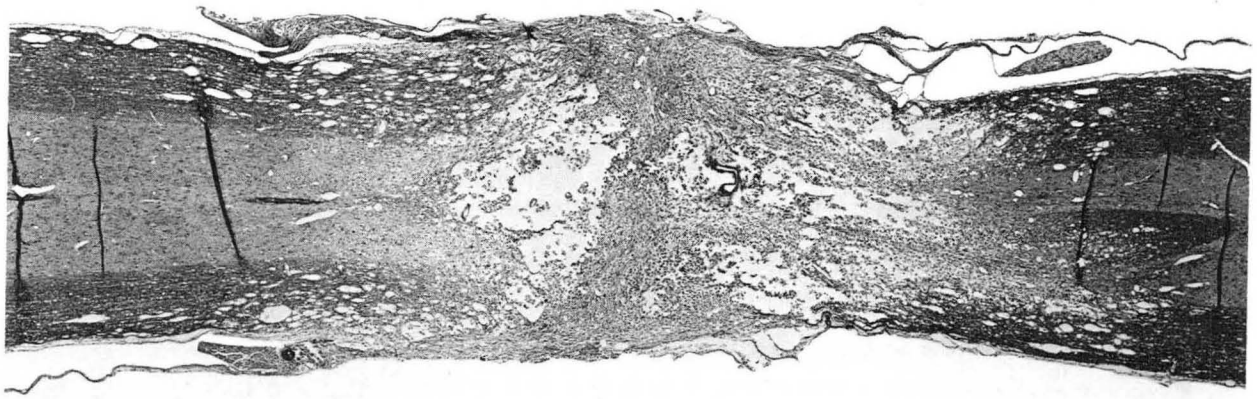
Surgical Incision of the Cord

When approximately 50% of the cord of the rat was cut, leaving a large part of either the right or left half of the cord intact, the rat was usually able to use one leg normally immediately after waking from the anesthesia and to regain some use of the paralyzed leg within 1 week, with completely normal ambulation by 3 weeks post-operation.

When only a very small part of the lateral portion of the cord (cortico-spinal tract) was not cut, the rat had complete paralysis of the hind legs for 1 to 3 weeks, but eventually recovered sufficient motor ability to walk (50-90% normal function as judged by ambulation). This result illustrates the difficulty in judging regeneration by functional recovery unless great care has been taken to ensure the absence of residual intact neurons. This point has been stressed previously.[2]

On gross examination of formalin-fixed cords, 1 to 4 weeks post-operatively, the lesion was evidenced by slight thickening and discoloration at the site of incision.

Microscopic examination revealed loss of normal structure extending in an irregular pattern proximal and distal to the incision. The involved cord was necrotic with invasion of gitter cells and variable amounts of dense glial scar depending on the post-operative interval (Fig. 1). The most striking result of the incision was the extensive, irregular necrosis of the spinal cord extending well above and below the actual incision. This had the appearance of a bland infarct due either to interruption of the nutrient vessels by the incision itself, or to obliteration of the blood supply by edema involving the cord above and below the incision. The final result was that the viable ends of the cord were widely separated by a zone of necrosis. Whether this zone consisted of necrotic tissue, cystic degeneration, or dense glial scar seems of secondary importance. Of primary importance is the fact that the



BBH 672-27

Fig. 1. Appearance of rat spinal cord surgically transected 14 days previously. No irradiation or other treatment. There was complete paralysis of the hind legs. Hematoxylin and eosin × 20.

viable tissue was so widely separated that only extensive axonal regeneration capable of penetrating extensive cystic areas and/or masses of necrotic debris or glial scar could bridge the gap. Thus a major factor in the failure of the mammalian spinal cord to repair even the smallest laceration would seem to be the extent of the adjacent necrosis which invariably accompanies such an injury.

Incision of the Spinal Cord Followed by Irradiation

Because it has been suggested that rapid glial proliferation following injury to the mammalian spinal cord may be an important barrier to axonal regeneration,[1] and since the glial elements are thought to be extremely sensitive to irradiation, an attempt was made to assist regeneration by inhibiting glial proliferation with irradiation.

Gross examination of cut-irradiated spinal cords 1 to 4 weeks after surgery showed some thinning and structural weakness at the operative site. This seemed to be a fairly consistent difference from the cut but not irradiated cord which was usually thicker and did not hinge at the operative site.

Microscopic examination of the cut-irradiated cord revealed essentially the same findings as in the cut but not irradiated cord, i.e., extensive necrosis above and below the site of incision with necrotic tissue, cystic degeneration, and scar tissue interposed between the viable ends of the cord (Fig. 2). Glial scar formation may have been decreased by irradiation, but the extensive necrosis would make bridging of the lesion impossible except by anything but the most energetic and prolonged axonal regeneration. Thus, unless the extensive necrosis can be eliminated, little can be gained by inhibiting glial proliferation.

Transection of Rat Spinal Cord with a Parallel Beam of Accelerated High Energy Nuclei (Protons)

As it has been shown that areas of the mammalian cortex can be "excised" without extensive adjacent necrosis or scar formation,[5] and since necrosis and scar formation appear to be major offenders in preventing repair of injuries to the spinal cord, it was thought worthwhile to determine the response of the spinal cord to the atomic knife. As the object was to produce a "clean" or sharply delineated cut, it was necessary to have a

BBH 672-28

Fig. 2. Spinal cord of rat which had estimated 60% use of its hind limbs 14 days after surgical transection followed by 1.670 rad through a 3.1 mm slit. Perfused on 14th day and sectioned serially. The section illustrated showed the greatest area of continuity of any of the sections. Movies of this rat walking were taken on the previous day. Luxol blue × 20.

beam as parallel as possible. In order to avoid "smearing" of the edge of the cut which would result in a zone of partial injury, minimum movement of the animal during the period of irradiation was desirable. This was accomplished by stabilizing the rat as much as possible and minimizing the time for delivery of the dose.

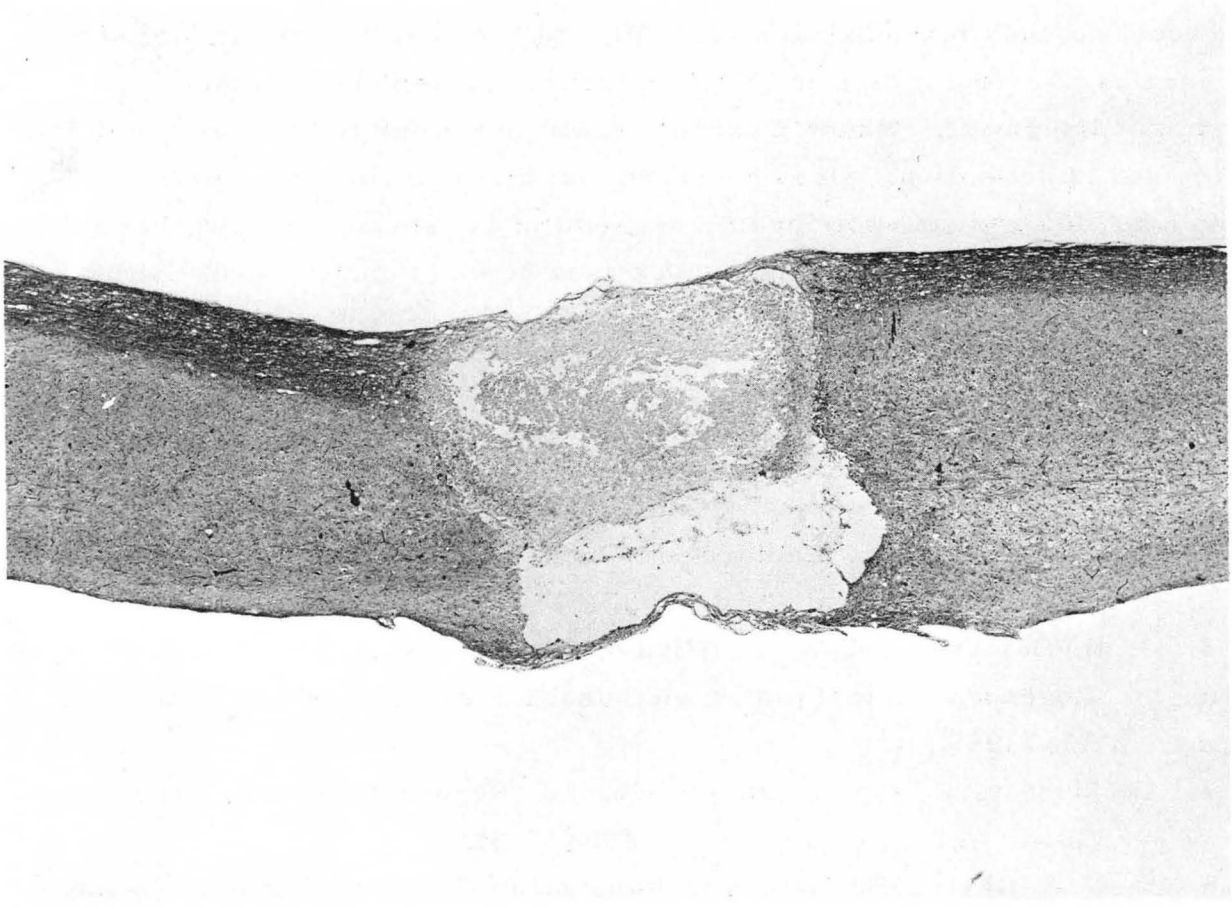
The hind legs of the rat became completely paralyzed one week following delivery of 38,000 rad with a proton beam through a 2-mm aperture. Twenty-eight days post-irradiation the rat was killed and the cord sectioned. (This rat was not parabiosed and was undoubtedly severely uremic during most of this time—could this have an effect on the morphology of the lesion?)

Microscopic examination showed complete necrosis of the irradiated segment without significant glial proliferation within the damaged area and with considerable self-debridement. The resultant lesions was singular not only for the lack of extensive glial scar formation, but for the sharp boundaries of the lesion. The lesion was bounded by viable tissue, and the irregular necrosis characteristically occurring proximal and distal to any surgical incision was entirely absent in the first rat examined, which had been subjected to the atomic knife (Fig. 3). In none of our later studies or studies by others[6] was such a "clean" lesion obtained. More work is needed to determine under what conditions the most sharply defined lesions are obtained.

Discussion

When the spinal cord is compressed or incised, the adjacent tissue undergoes extensive necrosis which results in a wide separation of the viable tissue proximal and distal to the site of injury. It is suggested that this wide separation of functional tissue is probably the major deterrent to repair following injury to the mammalian spinal cord.

The atomic knife may offer a method for cutting the spinal cord without the occurrence of necrosis adjacent to the knife edge. It is assumed that, as in the cortex,[5] necrosis of adjacent tissue is avoided because of the slow obliteration of vessels, allowing time for development of adequate collateral circulation. As extensive necrosis and scar formation are thought to be major deterrents to axonal regeneration in the spinal cord, the atomic knife provides unique advantages in operating on the spinal cord. However, as the "knife" is necessarily wide, the cut ends would have to be approximated



BBH 672-29

Fig. 3. Spinal cord of rat 28 days after 38 000 rad (proton beam from 88-inch cyclotron) through a 2 mm slit aperture. Hematoxylin and eosin × 20.

before any functional healing could possibly occur. Such approximation would not only be technically difficult, but would have to be undertaken months after the initial "cut" to allow time for self-debridement of the excised segment. Whether axonal growth is limited to the early post-injury period is not known.[2] It is proposed that this technique may provide a method for a clean excision of a segment of the spinal cord and that attempts to approximate the clean ends with a view to functional recovery would be profitable.

References

1. P. Lampert and M. Cressman, Axonal Regeneration in the Dorsal Columns of the Spinal Cord of Adult Rats, Lab. Invest. 13, 825 (1964).
2. W. J. O'Callaghan, Factors Influencing Axonal Regeneration in the Mammalian Spinal Cord, Ph.D. Thesis, Univ. of Alberta, 1963.
3. J. B. Campbell and W. F. Windle, Relation of Millipore to Healing and Regeneration in Transected Spinal Cords of Monkeys, Neurology 10, 306 (1960).
4. G. Murray, E. Ugray, and A. Groves, Regeneration in Injured Spinal Cord, Amer. J. Surg. 109, 406 (1965).
5. D. Van Dyke, and P. Janssen, Removal of Cerebral Cortical Tissue with Negligible Scar Formation Using a Beam of High-Energy Particles, J. Neurosurg. 20, 289 (1963).
6. B. Larsson, L. Leksell, B. Rexed, and P. Sourander, Effect of High Energy Protons on the Spinal Cord, Acta Radiol. 51, 52 (1959).

DIFFERENTIAL SENSITIVITY OF COMPONENTS OF THE VISUAL SYSTEM TO ALPHA PARTICLE IRRADIATION

C. T. Gaffey

The relative importance of vision for vertebrate activity is disclosed by the fact that of all the nerve fibers that enter the central nervous system 38% are optic tract fibers.[1] The visual system is normally credited as being the most important sensory receptor channel. For comparative purposes, it is interesting to note that the rate of visual information entering the brain is 10^7 times larger than the rate of auditory information.[2] A signal received by the eye traverses the retina, optic nerve, optic chiasma, optic tract, lateral geniculate nucleus, and its radiations to the visual cortex. Brain centers control pupil size, lens focusing, and tracking eye-movements. Since the visual system is of such value, it is surprising that very little is known concerning the radiologic sensitivity of the components of the visual pathway. It has been our attempt to assay the effects of ionizing radiation on the cat's visual system by using bioelectric techniques as criteria of change of state.

Cats were chronically implanted with electrodes in various components of the visual system (Fig. 1). This permitted electrophysiologic data to be acquired on alert animals before and after irradiation. Cats were exposed to photic stimuli of five different intensities, and the stability of the evoked responses was established in the optic chiasma, optic tract, lateral geniculate nucleus, and visual cortex. Electroretinograms (retinal response to a light flash) were obtained on anesthetized animals. In addition to single evoked responses, 50 light-evoked bioelectric responses were summed by using the Computer of Average Transients (Mnemotron Corporation).

Electrodes were implanted in homologous structures in the left and right hemisphere. The recordings from the visual pathways of the contralateral hemisphere served as an index of the state of the cat. It was important to restrict the volume of tissue irradiated to a minimum, since the effect of radiation on parts of the visual system was to be assayed. It was equally important to be certain that the component of the visual system to be

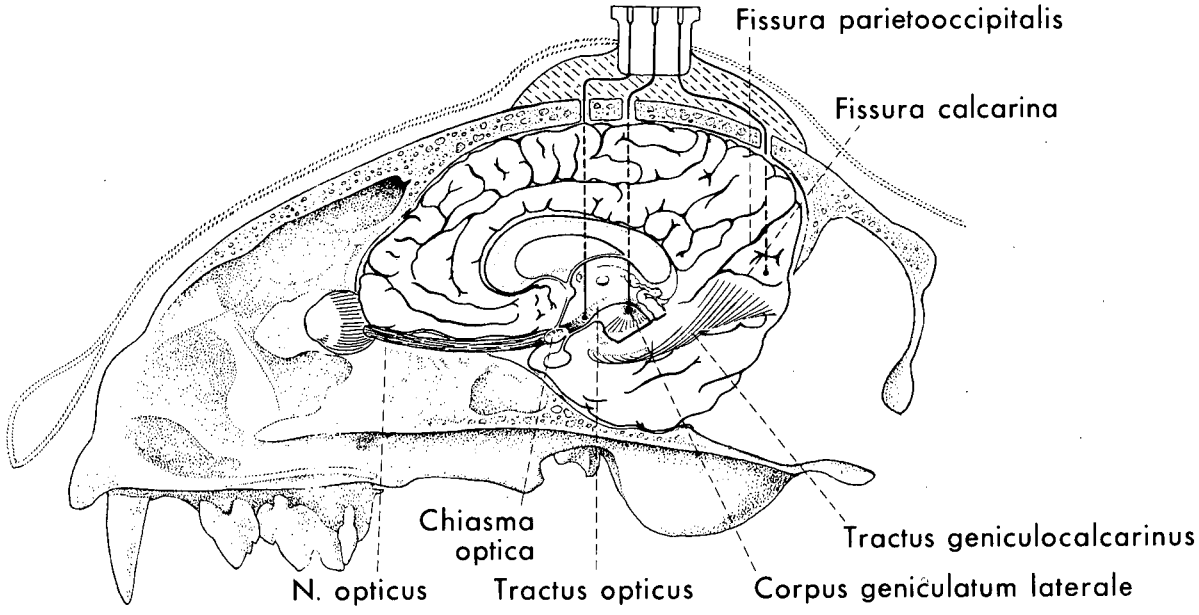

Fissura parietooccipitalis
Fissura calcarina
Chiasma optica
Tractus geniculocalcarinus
N. opticus
Tractus opticus
Corpus geniculatum laterale
MU-26053

Fig. 1

studied was at the site of restricted irradiation. A solution to both these requirements was assured by using the tips of the implanted electrodes as targets at which to aim the 910 MeV alpha particles generated by the 184-inch cyclotron. A cat to be irradiated was immobilized in a stereotaxic instrument and locked into an alignment apparatus at the 184-inch cyclotron. By means of orthogonal roentgenograms, the terminals of one pair of brain electrodes were positioned at the focus of a mechanical rotator that was located in the center of the cyclotron's alpha particle beam. The tip of the electrode in the cat's brain—having been uniquely localized at the rotation focus of the alignment apparatus—remained in the center of the irradiated volume when the mechanical rotator passed the head of the cat through a procession of planes and angles in the path of cyclotron-accelerated alpha particles. By this procedure it was possible to irradiate a discrete volume of brain tissue and avoid damaging the surrounding cells.[3] A dose rate of approximately 1 000 rads of 910 MeV alpha particles per minute was employed. (One rad is equivalent to 1.07 roentgen or 100 ergs absorbed per gram of tissue.)

It was found that the survival time for the photic-evoked response of the optic chiasma, optic tract, and lateral geniculate nucleus was a logarithmic function of the alpha particle dose for doses greater than 10 000 rads. The amplitude of the b-wave of the electroretinogram was attenuated by irradiation but the survival time for the electroretinogram was not a logarithmic function of the dose or a simple linear relationship of the dose. It is significant that the optic tract's response to a photic stimulation required 200 000 rads of alpha particles for immediate blockage. This was one hundred times the dose found to promptly inhibit the electroretinogram. From this is appears that the relative radiosensitivity of the retina to the optic tract (also optic chiasma and lateral geniculate nucleus) is 100:1.

The electrophysiologic techniques for assaying the efficiency of 910 MeV alpha particles has been varied. In retina, optic chiasma, optic tract, and lateral geniculate experiments the criteria for the influence of ionization radiation was the attenuation and suppression of a photic-evoked bioelectric response. Bioelectric current in these experiments was outgoing from the cat to the recording instruments. In research dealing with the sensitivity of the pupillodilation center to 910 MeV alpha particles, the current was ingoing

to the cat's brain from a stimulator (Grass, S-4). Current delivered to the nucleus perifornicalis (pupillodilation of W. R. Hess) induced a maximum pupil size. It was found that the action of electric current on the pupillodilation center could be inhibited immediately and irreversibly by 100 000 rads of 910 MeV alpha particles. Below 8 000 rads there was no detectable effect. The dose-survival relationship for the pupillodilation was nonlinear and nonlogarithmic.

In summary it can be stated that the relative radiosensitivity of the retina : pupillodilation center : optic tract (also optic chiasma and lateral geniculate nucleus) is 100:50:1. Since the retina is the component of the visual system injured most easily by radiation, it is hoped that the mechanism of action of ionizing radiation can be determined in experiments using tissue culture techniques combined with electrophysiology and biochemistry.

References

1. S. R. Bruesch and L. B. Arey, The Number of Myelinated and Unmyelinated Fibers in the Optic Nerve of Vertebrates, J. Comp. Neurol. 77, 631-655 (1942).
2. E. Ackerman, Biophysical Science (Prentice-Hall, Inc., Englewood Cliff, N. J., 1962).
3. C. A. Tobias, H. O. Anger, and J. H. Lawrence, Radiological Use of High-Energy Deuterons and Alpha Particles, Amer. J. Roentgen. 67, 1-27 (1952).

STUDIES OF THE VESTIBULAR ORGANS WITH HEAVY PARTICLES

L. W. McDonald

The vestibular organs are those sensory receptor organs for strength and direction of gravity, linear acceleration, and angular acceleration. These sensory organs are located deep within the bone of the skull, and along with the sensory receptor for hearing—the organ of Corti—constitute the inner ear. As familiar manifestations of functioning of the vestibular organs, one may cite the awareness of motion in an elevator (strength of gravity), the awareness of starting and stopping without visual stimuli even in the smoothest of operating vehicles (linear acceleration), and the dizziness and tendency to fall that one experiences following rapid rotation (angular acceleration).

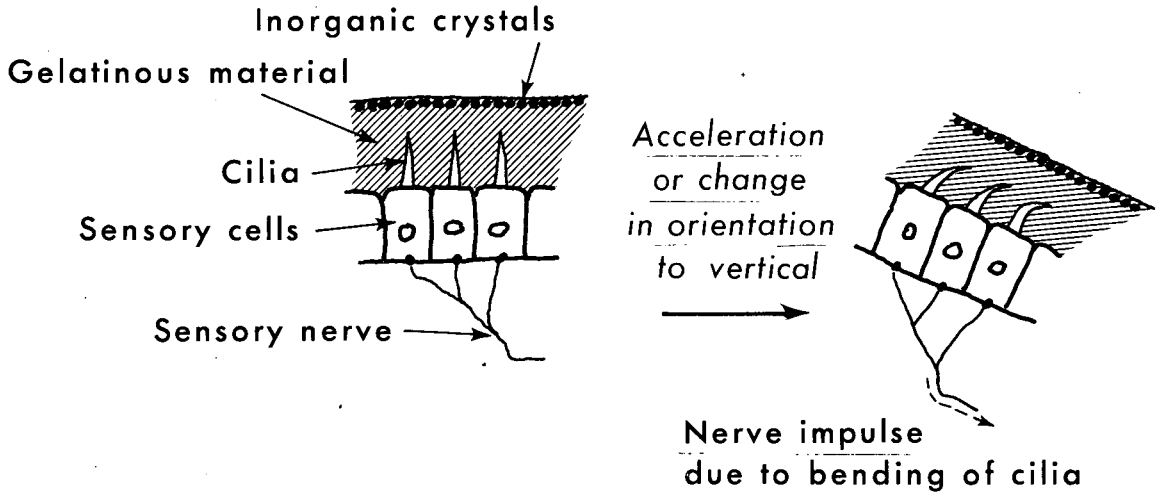
The sensory receptors for gravity and linear acceleration consist of microscopic hairs (cilia) which project into a gelatinous mass containing dense inorganic crystals. When the orientation of the dense inorganic crystals changes with respect to the vertical, these crystals move the gelatinous mass and the cilia. In some as yet unknown way the motion of the cilia causes a sensory impulse to occur in the cells to which the cilia are attached. This sensory receptor impulse is transmitted to the sensory nerve, which in turn transmits the nerve impulse to the central nervous system.

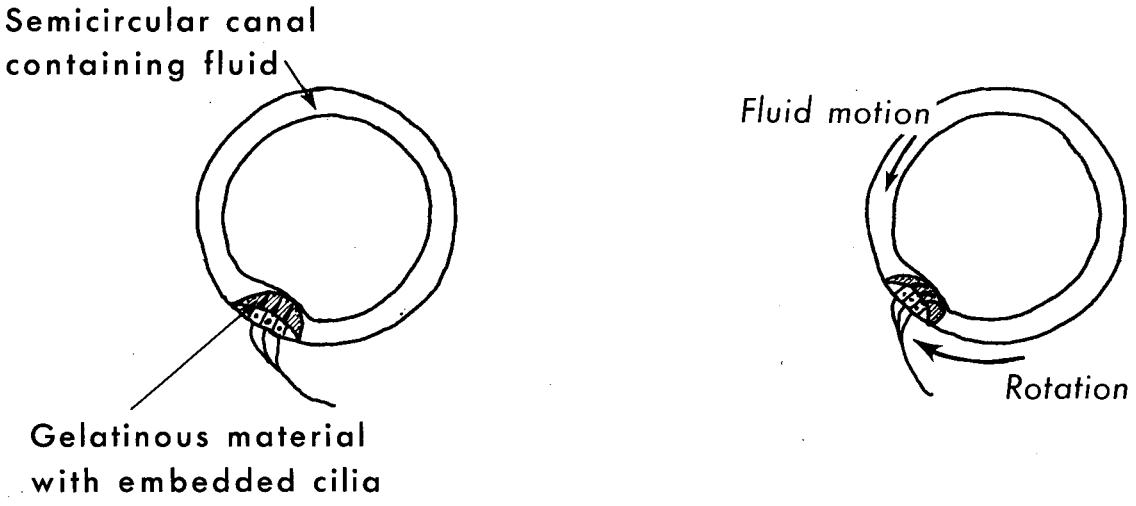
The sensory receptor for angular acceleration has similar cilia embedded in a gelatinous material, but here the gelatinous material does not contain the dense inorganic crystals. Instead, the cilia and the gelatinous material are deflected by the motion of fluid in the semicircular canals (Fig. 1).

Past Experiments

Our interest in radiation effects on the vestibular system began with the known radiation hazards in space under conditions of weightlessness when vestibular mechanisms may already be under stress. Since space radiation is mostly protons and helium ions (except for the Van Allen belts), the effects of this type of radiation are of most importance. The order of questions which

SCHEMATIC DIAGRAM SHOWING FUNCTIONING OF VESTIBULAR ORGAN RECEPTORS





XBL672-789

Fig. 1

we originally set out to answer is as follows:

1. What is the effect of radiation on vestibular mechanisms when man is on the earth's surface? Of particular interest is the minimal radiation dose which will produce an effect on the vestibular mechanisms. If such a dose is less than that which would be immediately lethal, vestibular problems due to radiation may play a role in the failure of a future space mission.

2. Do radiation and weightlessness have a combined effect, i.e., do they act synergistically or antagonistically on vestibular mechanisms?

3. What can be done to reduce the effects of radiation on vestibular mechanisms if such effects are important, or what can be done to ensure the completion of a space mission if an astronaut receives sufficient radiation to cause effects on his vestibular mechanisms?

Previous studies done by others using X-ray relate to the first of these questions. Generally, these studies have shown fairly substantial radiation doses of the order of 5000 to 10 000 rads required to produce a detectable effect on the vestibular mechanisms in the experimental animal. The radiation dose to the vestibular organs was generally uncertain in these studies or there was no attempt to limit the radiation to the vestibular organs.

In whole-body radiation studies using both X-ray and particle radiation, vestibular effects have been reported in the Russian literature with radiation doses as low as 50 rads. We have also done such studies and have failed to show any effects with radiation doses of less than 6000 rads when the radiation is limited to the vestibular organs. Our methods of testing for vestibular function have often been similar to those used in the Russian studies.

We have done whole-body radiation studies using ^{60}Co gamma radiation on rabbits; these studies are preliminary, but with involved statistical analysis some effects on vestibular function can be found with radiation doses of 500 to 700 rads. These functional changes may reflect only the debilitated state as radiation sickness develops in the animal. Repeated studies are planned with X-ray and particle radiation to confirm this effect of whole-body radiation and further explore the nature of the effects utilizing our present methods of vestibular testing.

At present we have two procedures which we have adopted for testing vestibular function of the experimental animal. The first of these is the

measurement of the threshold for nystagmus (the back and forth eye movements which occur as a response to angular acceleration) and the rate of nystagmus eye movements at various angular accelerations. Such testing equipment is not commercially available; it has therefore been necessary to design and construct it. The equipment which we now have in operation gives measured angular accelerations with a precision of ± 2.5% and records eye movements electronically with the animal in the dark so that there is no interference by visual stimuli. This test is a measure of semicircular canal function. The second of these two methods which we have in operation is that of measurement of the rotation of the eye when the animal is turned about a horizontal axis (ocular counter-rolling). We measure this rotation of the eye photographically in a manner which excludes any visual cues to the animal. This test is a measure of the gravity sensing and linear acceleration sensing function of the inner ear.

In preliminary studies prior to the development of our more precise testing methods, we believed that we had found vestibular effects with inner ear particle radiation doses of 500 rads. We have now repeated these studies using our present testing methods, and find that normal animal variations were responsible for effects which we had thought were due to radiation in our preliminary studies. We have been unable to find any measureable alterations in vestibular function with local particle radiation doses of less than 6 rads, although preliminary morphological studies indicate that changes are occurring with radiation doses much less than this.

Up to the present time we have partially answered the first of the three questions which we initially set out to answer. The other two questions—i.e., the combined effect of radiation and weightlessness on vestibular function, and what can be done to prevent the failure of a space mission because of the effects of radiation upon vestibular function—have not yet been answered.

General Plans for the Future

The most difficult problem in evaluating the effect of a drug or a physical agent such as radiation upon the function of the vestibular system is testing for vestibular function. It has been shown by other experimenters that vestibular receptor cell damage due to streptomycin may be very severe

without there being any functional change detectable with common methods of vestibular testing (caloric test and test of general equilibrium). Our preliminary observations indicate a similar situation for radiation. It would appear that the central nervous system adapts to changes in the functional state of the receptor. Methods of reducing or eliminating the central nervous system adaptation are being considered.

If alterations in vestibular function can be demonstrated at the level of 1000 rads, then further studies to answer questions 2 and 3 will be necessary. If radiation doses greater than 2000 rads are required to produce functional effects on vestibular mechanisms, other immediate effects of radiation will outweigh those on vestibular function.

Particle beams offer a great deal to the experimental study of the individual receptor cell groups of the vestibular organs. Thus there are five separate areas of sensory epithelium which have differing functional roles in each ear. The five areas are the three separate cristae of the semicircular canals and the two maculae of the sacculus and utriculus. Particle beams have the advantage over X-ray for such use because of their low scatter, high depth dose, and definite range. Since the vestibular receptors are encased in dense bone, the surgical approach is difficult. The landmarks provided by dense bone in radiographs of the inner ear area of the skull, however, provide a means for localizing the receptor sites in space so that particle beams may be directed to and localized in these sensory epithelia. With the development of such specific destruction of sites of vestibular sensory epithelium, a means will be at hand for the treatment of the more severe cases of Menière's disease in man without any loss in hearing or in those vestibular functions which are not actually involved in the most distressing symptoms of the disease.

STUDIES ON BRAIN ACTIVITY AND LEARNING BEHAVIOR USING HEAVY PARTICLE IRRADIATION

R. L. Schoenbrun

Full exploitation of high energy particulate radiation in the neurosciences is severely hampered by the lack of appropriate accelerator facilities. Although the current literature is meager, there is no lack in enthusiasm for the use of heavy particle radiations to study brain function and animal behavior. Since 1935, when heavy particle effects were first studied in mammalian systems,[1] diverse experimentation and practical applications have firmly established the utility of accelerated particles to biology and medicine. Yet it has been only recently that heavy particles have been employed to study neural mechanisms of behavior.

Orthodox approaches to the problems of learning and cerebral localization originated with the objective behavioral testing methods of E. L. Thorndike (1874-1933) and the regional cerebral ablation techniques of S. I. Franz (1874-1933). These procedures have obviously been refined, and are still widely used in experiments involving the production of specific behavioral changes by means of restricted brain lesions. An elegant application of this method was recently executed by the ethologist Eric Fabricius who studied the neural control of instinctive and learned behavior in pigeons by producing discrete brain radiolesions with 185 MeV protons.[2] Lesions were made in deep brain structures and decrements were subsequently detected in certain innate behavior patterns including, for example, spontaneous feeding and drinking, escape responses, and social behaviors. Learning experiments were also performed in which the birds were initially trained to make a visual discrimination with food reward and later tested for retention. In most cases the ability to acquire and retain new conditioned responses was not impaired by the intervention of radiation.

Based on the pioneering work of Tobias and his co-workers,[3] Malis made a unique neurophysiologic application utilizing monoenergetic particle irradiation to produce discrete laminar lesions in the cerebral cortex.[4] Fundamental to this technique is the Bragg-peak effect for homogenous

particle beams in which all particles have nearly the same range, and ionization reaches a maximum, the so-called Bragg peak, just before the end of range. The depth and thickness of the laminar lesion can be precisely controlled by appropriate selection of particle species and accelerator voltage or by the imposition of absorbers. Physiological studies[5] have dealth primarily with the effects of laminar lesions on evoked electrical activity in the visual cortex of the cat. In these studies, lesions ranging in thickness from 50 to 150 microns were placed at various depths in the visual cortex by using 10 MeV protons or 20 MeV deuterons at Bragg-peak doses of 20 000 to 40 000 rads. Subsequent alterations in patterns of electrical activity in cortical neurons were studied by using the "evoked potential" technique. Here sensory stimuli in the form of brief photic flashes were presented in order to evoke changes in electrical potentials recorded from the visual cortex. Electrical responses evoked by photic stimulation were abnormal during early phases of repair and reorganization after irradiation. Evoked responses were of normal configuration but increased in amplitude when studied after repair was essentially complete some 200 days after exposure. By these means it was possible to analyze structural and functional aspects of information storage, processing, and retrieval in the central nervous system.

In the search for neural mechanisms of learning, attention has recently been directed to deep temporal lobe structures comprising a functionally related unit, the limbic system, and its major component the hippocampus. These structures are part of the phylogenetically ancient rhinencephalon or "olfactory brain" of the vertebrates. Although initially considered in relation to olfaction, the limbic system is now thought to mediate important non-specific functions relating to emotional arousal, attention-focusing, and motivated behavior. Clinical evidence has suggested an important role for the hippocampus in mechanisms of memory. There is an apparently enduring impairment of memory storage function in patients who have undergone bilateral removal of the hippocampus.[6]

Temporal lobe systems have also been extensively studied by Adey and his group who have stressed computer analysis of brain wave recordings in their efforts to detect subtle changes correlated with behavioral performance. For example, certain highly rhythmic hippocampal wave trains in the cat have been correlated with aspects of discriminative motor performance and levels of learning.[7, 8]

The hippocampus has long been known to be highly susceptible to a variety of chemical and physical agents, including ionizing radiation. For example, the earliest signs of cerebral hypoxia or circulatory embarrassment appear as abnormalities in the electrical activity of the hippocampus.[9] Other studies have indicated that low doses of X-rays delivered to the head produced seizure-like activity in the hippocampus at dose levels not affecting other areas.[10] These data coupled with the functional role of the hippocampus in neural mechanisms of learning provided the basis for our study of radiation effects on learning.

The author, in collaboration with professors W. R. Adey and C. A. Tobias, initiated a project to examine the effects of X-rays and high energy alpha particles on brain wave activity recorded from temporal lobe structures during conditioned behavior in the cat.[11, 12] Initial studies utilized 250 kV X-rays in doses ranging from 100 to 1000 R delivered selectively to the hippocampus. Later studies involved 910 MeV alpha particles.

The technique of focal irradiation restricted to the hippocampus utilized a highly collimated narrow beam of X-rays directed through the head of the subject, which was stereotaxically positioned so that the beam intersected the appropriate structure. An improved technique was developed, utilizing high energy alpha particles which could be focused into small volumes of tissue. By centering the radiation site at the tip of an electrode, precise anatomical localization was achieved. The incident 910 MeV alpha particles, aligned by focusing magnets and collimated with a brass tube, were aimed through the head in a 6.36 mm beam. Focal irradiation was accomplished by a "cross-fire" technique using five body positions in the horizontal coupled with rotation through 70° about the longitudinal axis (Fig. 1). The ellipsoidal focus at the center of rotation received about 90% of the total dose (Fig. 2).

Several months prior to irradiation the animals were stereotaxically implanted with electrodes in brain regions known to mediate aspects of learning behavior, such as, for example, the hippocampus and other limbic system structures. After full recovery from the implantation surgery the animals were trained in a two-choice discrimination to approach a cue light for milk reinforcement of correct responses. Brain electival activity was recorded from the implanted electrodes during the behavioral training

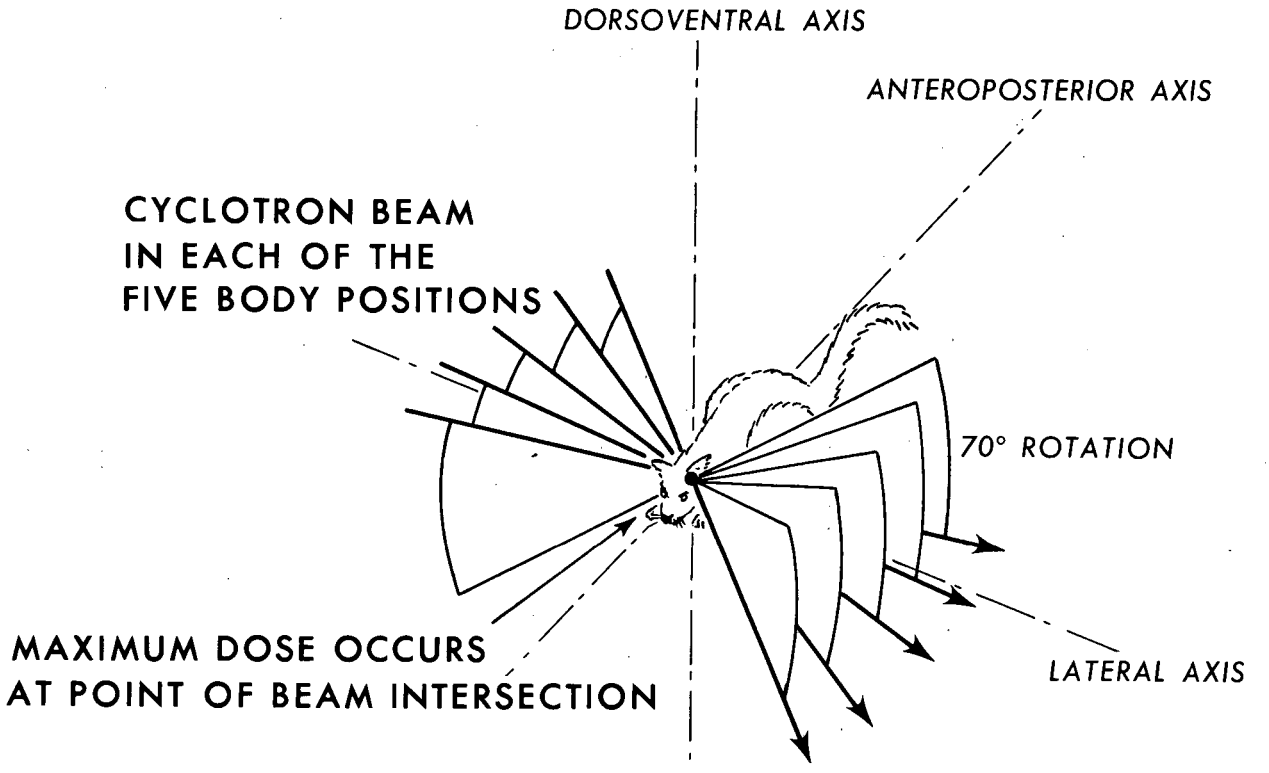
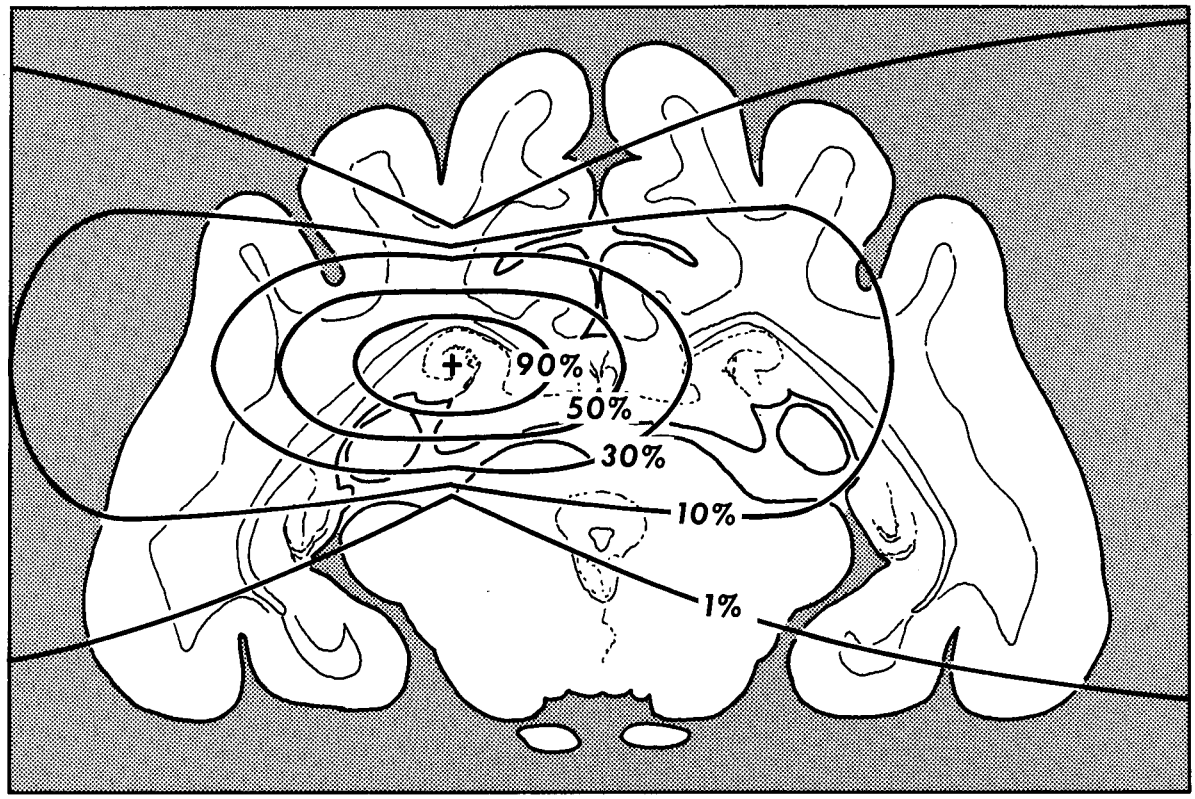

DORSOVENTRAL AXIS
ANTEROPOSTERIOR AXIS
CYCLOTRON BEAM
IN EACH OF THE
FIVE BODY POSITIONS
70° ROTATION
MAXIMUM DOSE OCCURS
AT POINT OF BEAM INTERSECTION
LATERAL AXIS
DBL 672-1515

Fig. 1



ANTERIOR 4.0 mm FRONTAL SECTION DORSAL HIPPOCAMPUS IRRADIATION

DBL 672-1516

Fig. 2

sessions. Focal irradiation of the hippocampus was administered when behavioral performance attained levels of better than 95% correct for each daily test session.

Typical brain wave changes can be seen in a recording made during an approach trial (Fig. 3). At the start of a trial, before the door opens, rhythms in the hippocampus (labeled L. Dorsal Hip., L. Ventral Hip., and R. Dorsal Hip.) are somewhat irregular and unsynchronized in the different recording sites. In contrast to this, during approach for milk reinforcement, the hippocampal rhythms change abruptly to highly synchronous and very regular sinusoidal waves at 6 to 7 cycles per second. With sessation of approach and attainment of milk reward, the rhythms revert to irregular patterns. Synchronous activity again occurs when the animal walks back to the start area. Brain wave correlates of behavioral performance, such as those recorded from the hippocampus during conditioned approach testing, provided the sensitive measure required to detect subtle effects of ionizing radiation on learning.

Ionizing radiation directed to discrete portions of the limbic system in appropriate doses was capable of disrupting on-going electrical activity and associated behavior performance. The effective total dose was usually in excess of 1000 rads and depended on the type of radiation and geometry of the lesion. Following limbic system radiation, conditioned approach performance was greatly slowed in all subjects and completely blocked in some animals. Brain activity concomitant with the decrements in performance was grossly altered by the occurrence of seizure-like waves and sharp "spikes" (Fig. 4; abbreviations: L. D. H., left dorsal hippocampus; DO, door open; L1, approach; L2, milk reinforcement). Pre-irradiation base-line activity is seen on 3/9/64 and 3/10/64. Subsequent to irradiation with 910 MeV alpha particles (20 000 rads), sharp seizure-spikes are seen coincident with failure of approach performance on a "no go" trial, 3/13/64. These studies demonstrated the effectiveness of discrete radiolesions placed in critical structures. By these means it is possible to establish functional relationships between different anatomic regions and specific patterns of behavior.

Another aspect of the problem of learning and information-processing concerns the organization of functional systems at cellular and subcellular

EEG CHANGES DURING APPROACH AND FEEDING IN UNIRRADIATED CAT

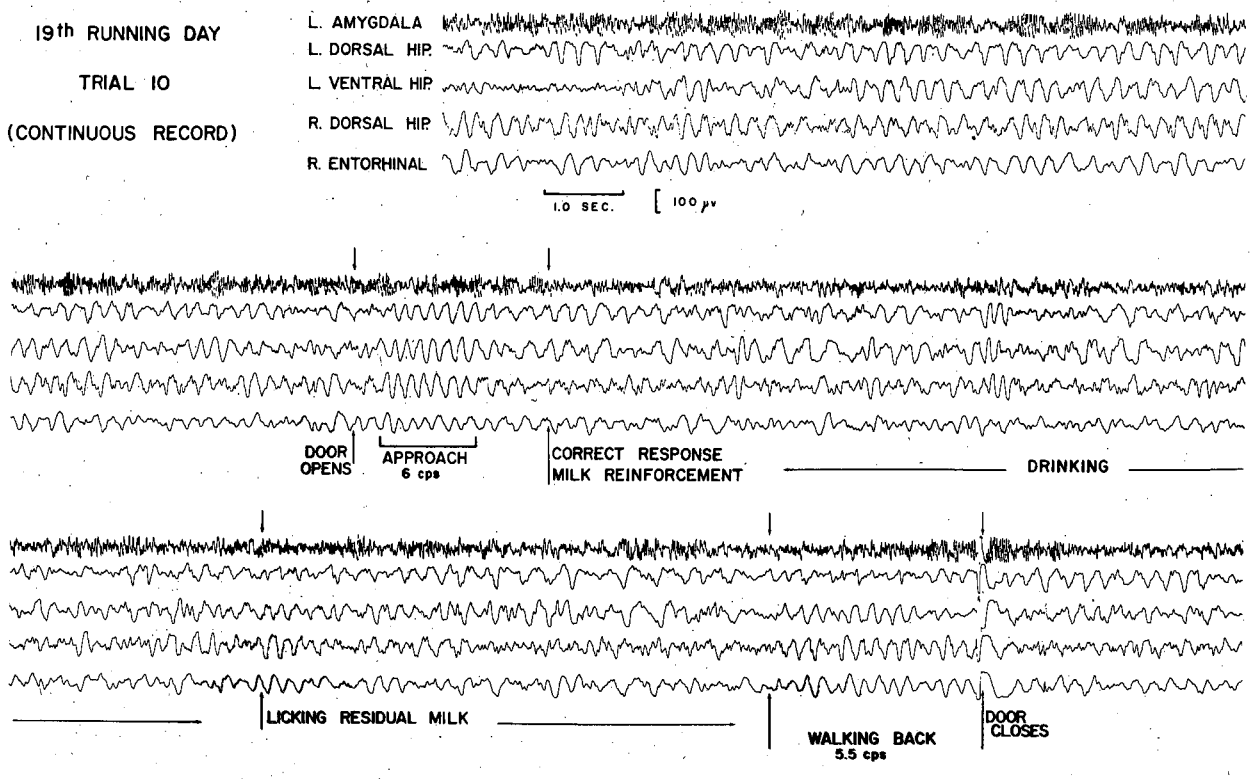


Fig. 3

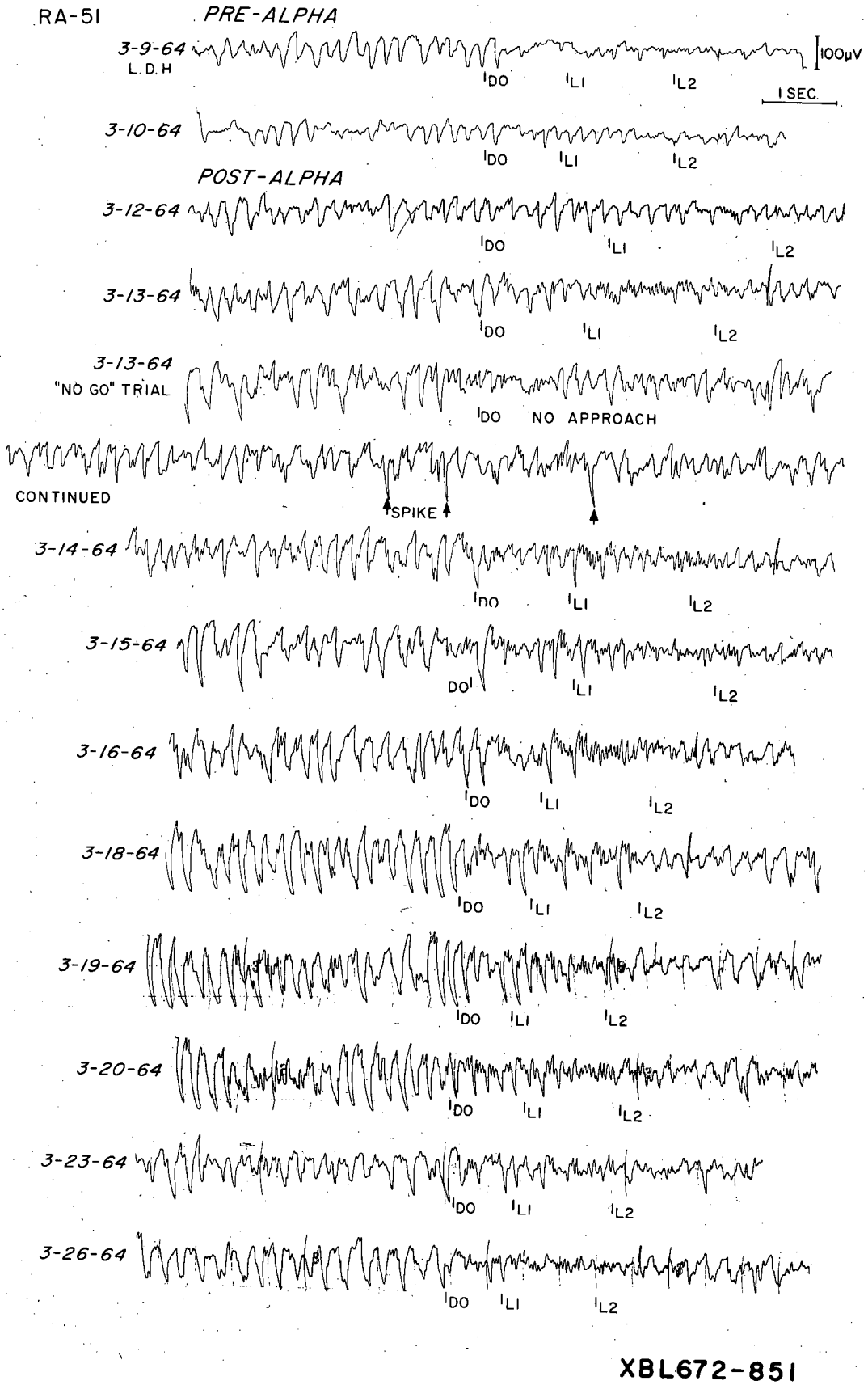

RA-51
PRE-ALPHA
3-9-64
L.D.H
100μV
1 SEC.
3-10-64
POST-ALPHA
3-12-64
3-13-64
3-13-64
"NO GO" TRIAL
NO APPROACH
CONTINUED
SPIKE
3-14-64
3-15-64
3-16-64
3-18-64
3-19-64
3-20-64
3-23-64
3-26-64
DO
L1
L2
XBL672-851

Fig. 4

levels. The hippocampus has many anatomical advantages for the study of fine structural details, including, for example, an uncomplicated cortical structure with a relatively uniform and simple lamination. It is tempting to consider the use of radiation-induced laminar lesions to further study the role of the hippocampus and other cortical structures in learning. A recent paper on the organization of memory functions has stressed the value of selective lesioning of the cortex of the limbic system.[13]

Our current interest is directed to cellular mechanisms involved in the central nervous system response to heavy particle radiation. In addition to conventional brain wave recordings, cerebral impedance is monitored. This method has revealed changes in tissue conductance relating to physiological changes in sleep, wakefulness, arousal, epilepsy, and in relation to storage of information in learning.[14] After brain irradiations there is a transitory drop in impedance followed by a sharp twofold increase above normal levels.

Some aspects of radiation damage may be explained on the basis of a tri-compartmental model of cerebral tissue, with intraneuronal, intraglial, and extracellular compartments. The extracellular compartment is generally considered to be small in cerebral tissue and probably has a high conductivity. The membrane resistance of glial cells, which virtually enclose the neuronal compartment, may be estimated at less than 10 to 30% of that of neurons. It may be assumed that most of the current flow during impedance measurement occurs through extracellular and neuroglial compartments. This emphasizes the possible role of glial tissue in modifying neuronal electrical activity by changes in impedance loading.

Movement of fluid between cerebral compartments may be reflected by changes in impedance values.[15] Increased permeability of cerebral vessels and associated changes in neuroglia have been implicated in radiation damage.[16] In these studies the initial radiation effect was the movement of fluid blood vessels into extravascular channels. We would suggest that this shift in fluid distribution is signaled by the initial small drop in cerebral impedance, which corresponds to increased conductance in the extracellular and possibly intraglial compartments. The subsequent sharp rise in impedance may signal a shift in ions back into the neuronal compartment. There is every expectation that cerebral impedance measurement will reveal changes

in neuronal subsystems that are of fundamental importance to both radiobiology and neurophysiology.

Future experiments with improved heavy particle beams hold exciting possibilities for the neurophysiologist. For example, much current speculation concerns the "molecular basis of memory." Central to most theories of memory storage is the involvement of large proteins such as RNA. By use of appropriate heavy particle beams it may be possible to irradiate the brain and produce selective destruction of individual molecular species resulting in specific "memory lesions." There is evidence that large protein molecules, such as enzymes, are differentially sensitive to radiation.[17] Theoretical speculations on the molecular basis of memory and learning will eventually be transformed to fact with the development of new tools of discovery.

References

1. J. H. Lawrence and E. O. Lawrence, The Biological Action of Neutron Rays, Proc. Natl. Acad. Sci. U. S. 22, 124 (1936).
2. E. Fabricius, Effects of Radiolesions in the Brain of the Pigeon, in Response of the Nervous System to Ionizing Radiation, ed. by T. J. Haley and R. S. Snider (Little, Brown and Co., Boston, 1964), pp. 708-721.
3. C. A. Tobias, H. O. Anger, and J. H. Lawrence, Radiological Use of High-Energy Deuterons and Alpha Particles, Amer. J. Roentgenol. 67, 1-27 (1952).
4. L. I. Malis, R. Loevinger, L. Kruger, and J. E. Rose, Production of Laminar Lesions in the Cerebral Cortex by Heavy Ionizing Particles, Science 126, 302-303 (1957).
5. L. I. Malis, Effect of Laminar Lesions on Evoked Activity in the Visual Cortex, in Response of the Nervous System to Ionizing Radiation, ed. by T. J. Haley and R. S. Snider (Little, Brown and Co., Boston, 1964), pp. 522-553.
6. W. B. Scoville and B. Milner, Loss of Recent Memory After Bilateral Hippocampal Lesions, J. Neurol. Neurosurg. Psychiat. 20, 11-21 (1957).

7. W. R. Adey, C. W. Dunlop, and C. E. Hendrix, Hippocampal Slow Waves, A. M. A. Arch. Neurol. 3, 74-90 (1960).
8. W. R. Adey, Computer Analysis of Hippocampal EEG Activity and Impedance in Approach Learning, in Pharmacology of Conditioning, Learning, and Retention (Pergamon Press, Ltd., Oxford, 1965), pp. 287-317.
9. W. Spielmeyer, Die Pathogenese des epileptischen Kampfes, Ztschr. Neurol. Psychiat. 109, 501-520 (1927).
10. H. Gangloff and T. J. Haley, Effects of X-irradiation on Spontaneous and Evoked Brain Electrical Activity in Cats, Radiation Res. 12, 694-704 (1960).
11. R. L. Schoenbrun, E. Campeau, and W. R. Adey, Electroencephalographic and Behavioral Effects from X-irradiation of the Hippocampal System, in Response of the Nervous System to Ionizing Radiation, ed. by T. J. Haley and R. S. Snider (Little, Brown and Co., Boston, 1964), pp. 591-620.
12. W. R. Adey and R. L. Schoenbrun, Irradiation Effects on Brain Wave Correlates of Conditioned Behavior, CFSTI, Natl. Bureau Standards, Dept. Commerce, Springfield, Va. (1966); see also Nucl. Sci. Abstr. 20, #38602, Nov. 15, 1966.
13. C. M. Fair, The Organization of Memory Functions in the Vertebrate Nervous System, Neurosci. Res. Prog. Bull. 3, 27-62 (1965).
14. W. R. Adey, T. Kado, and D. O. Walter, Impedance Characteristics of Cortical and Sub-cortical Structures, Exp. Neurol. 11, 190-216 (1965).
15. A. van Harreveld and S. Ochs, Cerebral Impedance After Circulatory Arrest, Am. J. Physiol. 187, 180-192 (1956).
16. C. D. Clemente and H. Richardson, Some Observations of Radiation Effects on the Blood-Brain Barrier and Cerebral Blood Vessels, in Response of the Nervous System to Ionizing Radiation, ed. by. T. J. Haley and R. S. Snider (Academic Press, New York, 1962), pp. 411-428.
17. L. G. Augenstein, T. Brustad, and R. Mason, The Relative Roles of Ionization and Excitation Processes in the Radiation Inactivation of Enzymes, in Advances in Radiation Biology (Academic Press, New York, 1964), pp. 227-266.

SPACE FLIGHT AND MAMMALIAN RADIOBIOLOGY

H. Aceto

According to contemporary data, the primary sources of possible radiation hazards to man in space are heavy primary cosmic particles, geomagnetically trapped corpuscular radiations, and solar flare protons. It appears that for the lunar mission, scheduled for 1969, the greatest of these hazards is represented by the large solar flares that eject high fluxes of protons and alpha particles capable of penetrating the wall of a lightly shielded space vehicle. When one considers more prolonged flights into the more distant reaches of the heavens, it is conceivable that the heavy primary cosmic particles may represent a significant increment to the radiation hazard.

Despite the obvious necessity for such information, the physical data that is presently available is not adequate to provide a definitive evaluation of solar flare activity. The only data that is available relating a complete 11 year cycle and its associated solar flare activity comprehensively is that obtained for the last cycle (solar cycle No. 19). Our present predictions of the ranges of radiation dose rates and integral doses to be expected from solar flare events are based on this information alone and therefore can hardly be described as being conclusive. Nonetheless, a general assessment of the relevancy of the existing radiobiological data to this contingent physical data can be made. Such an assessment reveals that information relating the modification of the dose-response relationships for both early and late effects pertinent to space-exposure conditions is indeed meager. The heavy cosmic particles also represent a serious void in our knowledge, in that our understanding of the biological effect of the extremely dense tracks of ionization produced by these particles is incomplete.

On the basis of calculation and experimental data, it has been shown that the diurnal galactic cosmic ray dose in the spacecraft in the region beyond the magnetosphere will not exceed 40 mrads even during periods of peak dose from that source. The maximum dose one would receive during a year's occupancy in space would be about 15 rads. More than 1-1/2 times this dose is now allowed as an emergency dose for workers engaged in atomic industry.

However, as noted above, one should take into account the fact that unlike the conventional radiations encountered in industry, the major fraction of the biological effect caused by galactic cosmic rays is due to the action of nuclei of heavy elements that produce extremely dense tracks of ionization and whose relative biological effectiveness for man or animals is uncertain at this time.

The geomagnetically trapped protons and electrons form circumterrestrial belts. The dosage in the inner belt inside the command module amounts to about 10 rads per hour, mainly resulting from protons with energies of about 100 MeV. During a single traversal of the inner belt the crew can obtain a dose of radiation equal to approximately 2 rads. Radiation of the outer radiation belt consists mainly of electrons with energies from several keV to several MeV, producing surface dose rates on the outside surface of the spacecraft on the order of 10^4 rads per hour. Effective shielding from particles of the outer radiation belt is less difficult than from protons of the inner belts; this results in an extremely small direct contribution to the total dose inside the spacecraft. However, one must still consider bremsstrahlung inside the cabin, which appears when electrons interact with the walls of the spacecraft. Preliminary data based on several of the recent large solar-particle events indicate total doses in the command module (from both protons and alpha particles) of 20 to 50 rem at the skin of the chest, 15 to 30 rem at the eye, and 3 to 9 rem at the blood-forming organs (5 cm depth in the tissue). Perhaps the greatest radiation hazard on a lunar mission will come if the astronauts are caught in the lunar excursion module (LEM) or on the lunar surface during a large solar-particle event. For such a case, the available solar flare information for a single relatively large event suggests doses of 350 to 800 rem at the skin of the chest, 100 to 180 rem at the eye and 3 to 12 rem at the blood-forming organs. [1]

With this rather brief analysis of the space radiation environment in mind, one may now attempt to discuss its radiobiological implications with respect to space travel.

The biological effects of radiation are generally divided into two groups: (1) acute effects, i.e., changes which are manifest from minutes to a few weeks after exposure, and (2) delayed effects, i.e., changes occurring from several weeks to many years after exposure.

From the point of view of space travel, the acute effects are particularly significant. Not only must the risk of death from acute radiation exposure be considered, but even more significant is the possibility that acute sublethal radiation-induced illness may cause abnormal function, thus reducing the performance efficiency of the astronaut.

On the basis of the available space radiation information, it appears that the acute lethal effects in the region of the gastrointestinal and central nervous system death do not represent a significant hazard to space flight. However, it is presently not possible to say unequivocally that this statement applies also to acute radiation effects in the region of bone marrow depression. Radiation lethality in the region of hematopoietic death is measured and visualized in terms of the LD_{50}^{30} dose. Data on radiation effects in this dose region for man are limited to a few observations on persons involved in nuclear accidents and those exposed to nuclear weapons. The lack of accuracy in the estimations of dose delivered and its geometrical distribution presents serious limitations to the usefulness of these data. Opinions as to the LD_{50} of penetrating electromagnetic radiation for man, utilizing these data, range from about 250 to 700 rads of acute whole-body radiation.

Because of the paucity of useful information relating the biological effects of space-type radiation in man, the radiobiologist has had to resort to studies in other mammals and extrapolate these results to man. Existing proton data obtained by using both large and small mammals and relating the biological efficiency of charged-particle beams as compared with X and gamma ray indicate that protons of energies above 50 MeV have an RBE approaching one for 50% mortality at 30 days. This would be expected on the basis of average LET distributions.[2-6] The deviation from unity that is observed for RBE is generally attributed to the increased absorption in bone of 200 kV X-rays compared with protons, and possibly to the pulsed character of the charged-particle beam. Experiments with 910 MeV alpha particles in mice reveal a biological effectiveness that is identical to that of high energy protons. This is not surprising, considering the dose uniformity and LET similarity between these radiations.

It appears that among the acute sublethal effects of radiation, the prodromal response may be one of the limiting factors for an astronaut. Under weightless conditions vomit would behave as any other fluid and could

form an obstructive mass over the mouth and nostrils, leading to suffocation. Thus the most urgent requirement would appear to be to avoid vomiting during flight. Unfortunately, the threshold for vomiting is extremely variable and the mechanism of post-irradiation vomiting remains to be elucidated. Data from irradiated Marshall Islanders indicates that 175 R of gamma rays can cause vomiting in 10% of the exposed individuals.[7] Gerstner has estimated that a uniform whole-body dose of 100 rads would result in a 3% incidence of vomiting.[8] It appears that if nausea is to be completely avoided, a much lower limit than 100 rads would have to be applied. It is interesting to note that delay in gastric emptying in rats (the probable counterpart of nausea and vomiting in these animals) was observed with doses as small as 20 R of X-rays.[9] The time of onset of nausea and vomiting is about 2 hours after exposure, and the climax occurs between about 5 to 8 hours—both being relatively independent of dose. It is important to recognize the fact that this information for man is derived mainly from Japanese atomic-bomb casualties and other cases of accidental irradiation and from therapeutic irradiation of cancer and other diseases with high energy X or gamma rays. It is, however, quite likely that none of these groups react to irradiation in exactly the same way as a select group of astronauts

Both the major fraction of the solar flare radiation dose and the highest average LET will be delivered to the skin. The relatively high intensity surface exposure with little deep tissue dosage may result in widespread erythema and skin blistering. Previous studies indicate that erythema will appear within hours to days following 500 to 800 R. Due to the restrictions and abrasive contacts of the space suit, even a partial body moderate erythema could become extremely uncomfortable and somewhat incapacitating. Furthermore, it is known that both skin injury and psychological stress markedly enhance the adrenocortical output. Corticosterone is a protein synthesis and a mitotic activity inhibitor. Moreover, thermal burns and skin disruptions have already been shown to push a dose of radiation that would normally cause a hematopoietic mode of injury into a gastrointestinal mode (which would—— suggest that there is impairment of repair). Therefore there is a rationale for expecting the normal physiological stress mechanism to enhance some forms of radiation damage. Moreover, periods of long stress may result in the depletion of the adrenal cortex, which ceases to secrete cortisone.

Aldosterone is then secreted with a concomitant retention of sodium and water. We do not know whether this response would be influenced by radiation, but there is reason to expect it might.

A great deal of work remains to be done in this area with respect to both acute and chronic charged-particle irradiation of the skin. The development of an alpha particle beam whose physical characteristics are appropriate for skin irradiations has been initiated at the Lawrence Radiation Laboratory 88-inch cyclotron to answer this need.

Sublethal hematopoietic depression is still another parameter which must be considered when discussing functional decrement factors. It is well known that the circulating lymphocytes are among the most radiosensitive cells in the body. A detectable decrease in lymphocyte count has been observed with penetrating radiation doses of 25 to 50 rads while doses of 100 to 200 rads may result in a 50 to 90% drop, with maximum depression in 2 to 4 days. At doses of about 100 rads and above, lymphocytopenia is accompanied by granulocytopenia, erthyrocytopenia and thrombocytopenia of varying degrees. The possibility of a diminished red blood cell count may have particular significance if one considers the "adaptive" loss in blood volume that was first observed in the early Gemini flights. One GT-5 pilot, for example, showed a 20% drop in red cell mass. If we add to these factors the possibility that when sublethal radiation is given chronically the capillary bed may be damaged, leading ultimately to local tissue hypoxia, a potentially serious problem becomes apparent.

Most radiobiological data available to date have been obtained under carefully controlled conditions of relatively uniform whole-body exposures to highly penetrating radiations. This information may be useful in assessing the hazard from galactic cosmic rays, but it is inadequate in assessing the variable depth-dose characteristic of solar flare radiation. Solar flare particle radiations exhibit a characteristic that exercises a profound effect on their radiobiological response: Absorbed dose declines steeply with tissue depth dose because of the spectral character of the radiation. The radiation hazard should always be evaluated in terms of the spectral composition and type of penetrating radiation acting upon the body. If the penetrating ability of the radiation is great and there is a relatively small dose drop within the body (as is the case with galactic radiation), then the average dose

absorbed by the tissue may be used. However, if the penetrating ability of the radiation is such that the absorbed doses at various points in the body differ greatly, then the amount of the average dose in the tissue (average for the entire body) does not clearly define the mutual relation of various organs and tissue affected by the radiation. Therefore in this case it is necessary to use data relating the spatial distribution of the absorbed dose within the body. This variable depth-dose pattern then makes it necessary to evaluate radiation danger on the basis of damage to the so-called "critical organs" (bone marrow, gastrointestinal mucosa, gonads, crystalline lens, skin).

Since there is no genuine counterpart on earth for the heterogeneous mixture of radiation that composes a solar particle event, biological effects must be deduced from analogous radiobiological experimentation in radiation environments that simulate in some manner the three-dimensional depth-dose and LET spectral patterns given by a typical solar flare. Unfortunately, there are no experiments to date in large animals which simulate, in any realistic sense, both depth-dose and LET spectral profiles that are predicted from solar flares.

Jackson,[10] using a ^{60}Co gamma-ray source and rotational exposures behind properly shaped fields, studied acute lethality in rats exposed to a depth-dose distribution simulating that calculated for a typical solar event and compared the results with those of uniform ^{60}Co irradiation. Under the depth-dose exposure conditions, the midline dose to the animals was 25% of the surface dose. The average LD_{50} surface dose for nonuniform exposure was approximately 3 times that for uniform exposure, and the ratio of integral absorbed dose (nonuniform to uniform) under the two conditions was 1.5. The tissue depth at which the LD_{50} doses were the same was approximately halfway between the surface and the midline, which corresponds roughly to the mean effective depth of the bone marrow of the rat.

Although Jackson has shown that it is certainly possible to duplicate charged-particle LET and depth-dose geometry with gamma radiation, the secondary reactions constitute one of several reasons why studies of this same type should be extended to charged-particle beams. Indeed, such studies are being initiated at the Lawrence Radiation Laboratory, utilizing a simulated solar flare beam at the 184-inch cyclotron.[11] The first simulated flare exposure of primates will be performed at the 184-inch cyclotron in the

Spring of 1967 in collaboration with the group of Dr. Ashton Graybiel at Pensacola Naval Air Station.

Furthermore, the radiation exposure encountered in space will not be delivered at a constant dose rate (as is the case in most radiobiologic investigations), but in a rather complex pattern. For example, there will be brief periods of exposure during transit of the radiation belts with the random occurrence of solar events superimposed on this. Throughout the mission there would also be a continuous low-level exposure due to galactic cosmic radiation. A great deal of work remains to be done to simulate more closely the dose protraction and fractionation patterns anticipated in space travel.

Each of these physical factors—depth-dose distribution and protraction and fractionation of exposure—may modify the dose-response relationships for both early and late effects. Some of these factors may make space exposure conditions less hazardous and others more. Still another modifier that is in dire need of active investigation is the area of the combined effect of radiation with other flight factors. Little consideration has been given to such imponderables as synergisms between weightlessness, acceleration, environmental factors, and psychological stress. On an intuitive basis, many of these factors would be expected to lead to an enhanced probability of personal indisposition, particularly in the case of the prodromal syndrome.

Earlier work has demonstrated that mice as well as rats exhibit a greater mortality response to a given X-ray dose if subjected to hypoxia or cold during the post-irradiation period.[12,13] The effect of prolonged altitude exposure on the irradiated animals was equivalent to a 100 R decrease in the X-ray dose necessary to produce a 50% mortality response at 30 days. Hematologic data suggest that the increased mortality of animals maintained at altitude following lethal radiation exposure is the result of a reduced blood cell volume and an inadequate erythropoietic response during exposure to hypoxia. In contrast, a similar study involving a single sublethal whole-body proton irradiation in beagles indicates that at short post-irradiation times (less than about 1 week) the erythropoietic response of the hypoxic-irradiated animals is comparable to that of the hypoxic-control animals.[14] Figure 1 provides an illustration of this preliminary finding as expressed by the plasma clearance rate (PCR). The response pattern of the irradiated-hypoxic animals suggests that despite severe damage the stem cells and perhaps some of the

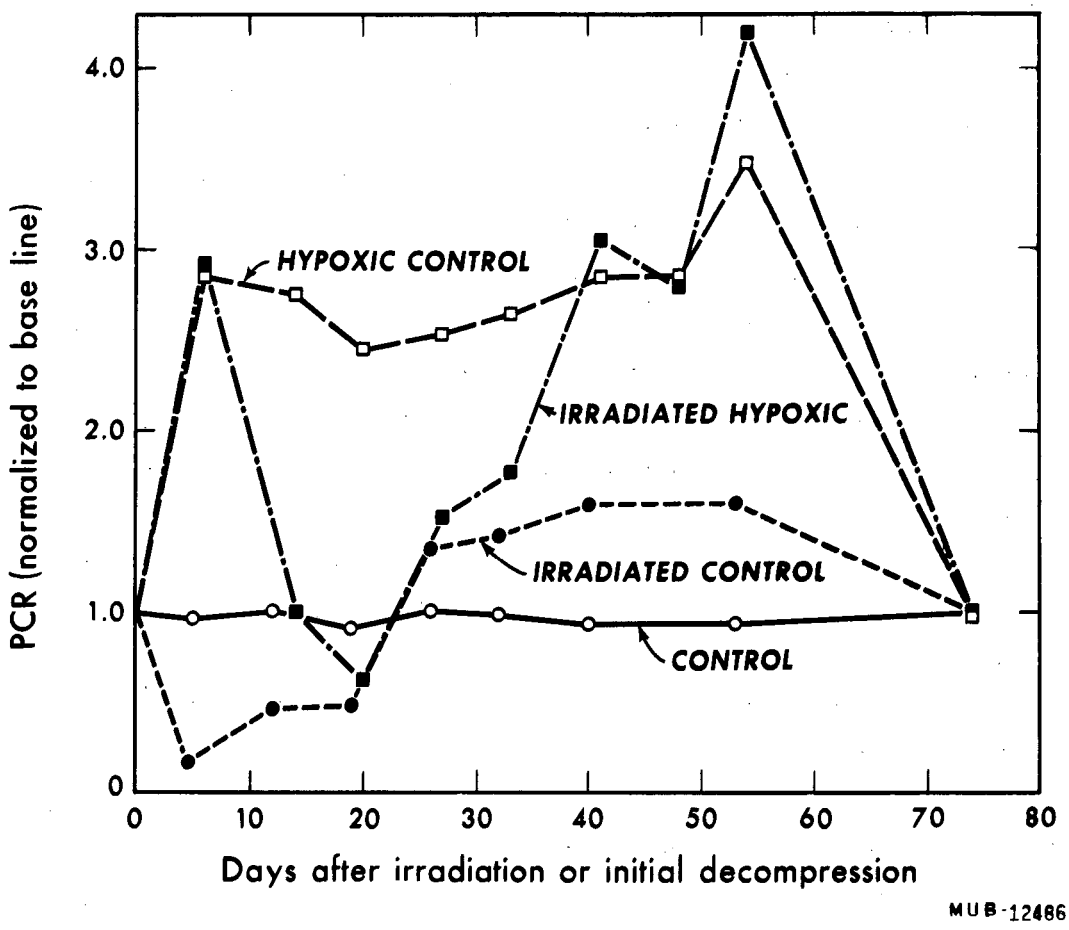

PCR (normalized to base line)
4.0
3.0
2.0
1.0
0
HYPOXIC CONTROL
IRRADIATED HYPOXIC
IRRADIATED CONTROL
CONTROL
0
10
20
30
40
50
60
70
80
Days after irradiation or initial decompression
MUB-12486

Fig. 1

very early pronormablasts could still be stimulated by the hypoxia. However, subsequent to the exhaustion of cells capable of hypoxic stimulation despite radiation damage, the proliferation of red cell precursors rapidly decreased until recovery from radiation damage had occurred.

Until fairly recently it has always been presumed that nervous tissue was very resistant to ionizing radiation. It is still true that nerve cells are not easily killed by radiation and that in order to produce convulsions a dose of several thousand rads is needed. However, high amplitude, low frequency activity associated with sluggish behavior and slow motor performance has been observed after exposures to just 500 rads delivered bilaterally to each hippocampus. Moreover, Russian workers report changes in conditioned reflexes from doses as low as 0.5 to 20 rads, and a distinct change in electric activity of the brain has been observed in rabbits after only 0.05 to 1.3 rads.[15] Work in this country indicates an increased susceptibility to audiogenic seizures in mice following 0.14 rad given at a very low dose rate. It has been demonstrated that rats will avoid drinking the saccharine-flavored water which they earlier had been induced to drink while being irradiated with a single dose as low as 7.5 rads of neutrons; they will also avoid the surroundings in which they were for four sessions of 50 rads of gamma rays.[16, 17]

The response elicited by radiation as the signal in conditioning experiments was abolished when anosmic animals were used, thus suggesting that the conditioning response is not due to a direct effect on neural tissue, but is due to stimulation via the olfactory system, i. e., a peripheral effect. A latent effect of low doses (10 rads), which appears 2 hours post-irradiation, involves a toxin-like reaction whereby rats will avoid gustatory and olfactory stimuli associated with X-ray, but not visual, auditory, or tactile stimuli. This same type of adverse reaction might also function in man. There may well be adverse stimuli occurring in our lives that we are not consciously aware of that influence our behavior and performance. Radiation might turn out to be such a stimulus in the astronaut's environment by making the tasks he must perform less pleasant to do, or even by causing a strain in the social relationships between astronauts.

The heavy primary particles in space may represent a particularly significant hazard to the long-term post-Apollo missions. The acute and

chronic effects of "nonrandom" exposure that would result from the extremely high local doses that will occur in cells and tissues as a result of the passage of these high energy heavy particles are not really understood. The biological effects of such particles have not been adequately investigated because it is impossible at the present time to produce them in the laboratory with an energy high enough to use for mammalian experiments. The heavy ion linear accelerators at Berkeley and Yale produce ions up to and including argon of 10 MeV/nucleon, which are mainly adequate for studies in single-cell systems.

Rather extensive studies have been designed to investigate the lethal effect of heavy ions on human tissue culture.[18] The survival criterion is the ability of single cells to grow into visible colonies. The extrapolation of this data to the total integrated mammalian system raises many serious questions when one considers the fact that in functioning tissue, the interaction between cells may be more important than the viability of single cells. Formation of clones is not the task of most cells found in differentiated tissue, thus "cell lethality" may have an entirely different meaning in vivo.

It is anticipated that the concentrated cellular destruction produced by these particles in highly proliferative tissues like bone marrow, lymph nodes, and intestinal epithelium would hardly result in a significant alteration of function. In the central nervous system (CNS), however, it is conceivable that death of a relatively small number of cells might have far-reaching effects.

Curtis has used microbeams of high energy deuterons to simulate the ionization track of heavy primaries and to study their effects on the visual cortex of the brain and the ocular lens of mice.[19] The dose required to produce histologically observable damage in the brain decreased rapidly with beam diameter until almost one-half million rads were required for beam diameters approximating that of primary cosmic particles. The dose required to produce observable effects in single cells of the lens was not dependent on beam diameter, but the probability of progression to a persistent cataract seemed to require a beam diameter large enough to damage a cluster of cells. Before one yields to the temptation induced by these observations, of minimizing the risk of gross local damage by heavy cosmic ray particles, one should first consider the fact that the ionization pattern and the energy of

secondaries produced by these microbeams is not identical to that expected from the delta rays that are generated by the passage of these particles in tissue. This is an important consideration since it is the delta rays that cause almost all of the dense ionization of the track and are mainly responsible for the biological effect observed. Even more important is the possibility of effects in areas such as the midbrain, where a small number of nerve cells may perform some essential function. Disturbances of hearing might result from tracks of damage in a medical geniculate body; similar damage in a lateral geniculate body might interfere with the fields of vision. If certain regions of the hypothalmus are affected, it is conceivable that a significant alteration of homeostatis may ensue.

Chronic or long-term effects of radiation exposure appear to be of secondary importance in the evaluation of immediate radiobiologic hazards in space. However, they may certainly represent a hazard long after the astronauts' safe return to earth. Chronic injury is cumulative, though subtle and often undetected, and can be the limiting factor for individual exposure histories. At the same time, it appears that our information is most complete for doses that induce acute injury and it becomes progressively more uncertain as dose declines or time to expression of damage increases. The chronic or delayed radiation effects of primary concern are a general life-shortening, increased incidence of leukemia and other malignant changes, cataract production, and genetic damage.

Incidence of cataracts is perhaps one of the more serious sequelae of exposure to space radiation because of the relatively high surface dose and its high LET component. The latent period for cataract production is highly variable and may range, in the adult, from about 2 to many years after exposure.[20] Usually, the higher the dose, the shorter the time interval. The minimum cataractogenic dose for a single acute exposure (200 kVp X-rays) is about 200 rads. A single acute exposure is more effective and produces opacities sooner than the same dose given in divided or continuous exposures spread out over periods ranging from 3 weeks to a few years. The minimum dose for the latter case is between 400 and 1000 rads. Of course one should keep in mind that these figures are for radiations of low ionizing power. On the basis of experiments with experimental animals, recoil protons from fission neutrons (average LET $\approx$ 50 keV/μ) appear to be as much as 5 to 10 times

as cataractogenic as 200 kVp X-rays. This behavior emphasizes the great need that exists for information relating the cataractogenic properties of the charged particles expected in space.

There is no clear-cut evidence on the possible life-shortening effects of ionizing radiation in man. The life-shortening by single doses of X or gamma rays in rodents is on the order of 0.3 day per rad. For fast neutrons, however, the efficiency per rad is essentially independent of fractionation of the dose and so the RBE compared with X or gamma radiation increases from about 2 or 3 for single doses to about 10 for chronic doses.[21] When estimates of life-shortening in man have been made by extrapolation from animal data, it has usually been assumed that the proportional shortening per rad would be independent of species. On this basis, values of 1 to 15 days per rad have been suggested for man, assuming single acute doses of low-LET radiation.

Estimates indicate that leukemogenic response to radiation increases linearly with increasing radiation dose. The threshold, if one exists, appears to be small and the dose that doubles the incidence of the disease may be as low as 30 to 50 rads. It should be noted that leukemia is relatively uncommon and if only a few astronauts were subjected to this doubling dose the likelihood of occurrence of radiation-induced leukemia would be quite small.

In addition to leukemia, other neoplastic diseases are also increased following irradiation. Of particular concern here is the possibility of chronic or long-term skin injury culminating in cancerous changes. A comprehensive assessment of this effect must await further skin carcinogenesis studies, particularly those involving alpha particles and heavier ions.

The genetic hazard is certainly not a major one at the present time. Most of the astronauts may be beyond the median reproductive age. Also, this is a very small group whose genetic impact on the population will be equally small. Despite these practical considerations, the genetic hazard may be of particular interest to one who happens to be a member of that small group. Doses as low as 25 rads to the testicles, either locally or as whole-body exposure, will produce a detectable decrease in sperm count. About 150 rads may induce brief subfertility, and about 250 rads may produce temporary sterility for 1 to 2 years.[22] The general course of events as observed both in man and animals is a pre-sterile period following irradiation,

a sterile period, and a post-sterile period. The importance of distinguishing between pre-sterile and post-sterile period lies in the type and frequency of mutations observed from matings in these periods. Offspring produced in pre-sterile matings largely come from irradiated mature germ cells or spermatazoa. The mutation rate for recessive genes in these cells may be twice that observed in the spermatogonia or stem cells. Moreover, the irradiated mature germ cells carry a high incidence of dominant lethal mutations compared with the immature cells. The neutron RBE for these mutations is 7, with the neutron-dose response data increasing linearly with arithmetic dose while the X-ray data is proportional to dose-squared. Thus, the particulate radiation of space may present an extra threat because of its dense ionizing tracks and greater probability of inducing genetically lethal effects.

References

1. S. B. Curtis, Semiannual Report on Biology and Medicine, Lawrence Radiation Laboratory Report UCRL-16613, Fall 1965, p. 1.
2. C. A. Sondhaus, Effect of High Energy Protons and Alpha Particles on Small Animals, in Second Symposium of Protection Against Radiations in Space, Gatlinburg, Tennessee, NASA SP-71 (1964), p. 97.
3. P. Bonet-Maury, A. Deysine, M. Frilley, and C. Stefan, Relative Effectiveness of 157 MeV Protons, C. R. Acad. Sci. (Paris), No. 251, 3087 (1960).
4. R. W. Zellmer, Aerospace Med. No. 10, 32, 942 (1962).
5. G. Grigoriev, N. G. Darenskaya, M. P. Domshlaf, A. V. Lebandinski, G. Nefedor, and N. I. Ryzhov, Characteristic of the Biological Action and Relative Biological Effectiveness of High Energy Protons, in Proc. Symp. Biol. Effects of Neutrons and Proton Irradiations (IAEA, Vienna, 1964).
6. S. T. Taketa, Biological Effects of Protons and Neutrons in Large Animals, in Second Symposium on Protection Against Radiation in Space, Gatlinburg, Tennessee, NASA SP-71 (1964), p. 73.
7. N. R. Shulman, E. P. Cronkite, V. P. Bond, C. L. Dunham, and R. A. Conrad, Some Effects of Ionizing Radiation on Human Beings, edited by E. P. Cronkite, V. P. Bond, and C. L. Dunham, U.S.A.E.C. (1956), p. 13.

8. H. B. Gerstner, Ann. Rev. Med. 11, 289 (1960).
9. E. V. Hulse, Brit. J. Exp. Path. 38, 498 (1957).
10. K. L. Jackson, The Lethal Effectiveness of Solar-Flare Type Dose Distribution Delivered to the Rat, in Proceedings of the Symposium on the Protection Against Radiation Hazards in Space, Gatlinburg, Tennessee, Nov. 5-7, 1962, TID-7652, Bk. 1, pp. 375-392.
11. J. T. Lyman and H. Aceto, Space Radiation Simulation in the Laboratory, U.S.A.E.C. Bio-Medical Program Director's Meeting, February 1966, Berkeley (Lawrence Radiation Laboratory, Berkeley, 1966), p. 124.
12. B. D. Newsom and D. J. Kimeldorf, Am. J. Physiol. 195, 271 (1958).
13. B. D. Newsom and D. J. Kimeldorf, Am. J. Physiol. 199, 445 (1960).
14. H. Aceto, C. A. Tobias, D. H. McKelvie, R. W. Springsteen, and W. Gee, Combined Stress Study: Effects of Radiation and Hypoxia on Erythropoiesis, 1966 (unpublished).
15. T. J. Haley and R. S. Snider (eds.), Response of the Nervous System to Ionizing Radiation (Academic Press, New York, 1962), p. 297.
16. J. Garcia and D. J. Kimeldorf, Nature 185, 261 (1960).
17. J. Garcia, D. J. Kimeldorf and E. L. Hunt, Brit. J. Radiol. 30, 318 (1957).
18. P. W. Todd, Reversible and Irreversible Effects of Ionizing Radiations on the Reproductive Integrity of Mammalian Cells Cultured in vitro, Lawrence Radiation Laboratory Report UCRL-11614 (1964).
19. H. J. Curtis, Some Specific Considerations of the Potential Hazards of Heavy Primary Cosmic Rays, in Proceedings of the Symposium on the Protection Against Radiation Hazards in Space, Gatlinberg, Tennessee, Nov. 5-7, 1962, TID-7652, Bk. 1, pp. 291-308.
20. G. R. Merriam, Jr., and E. F. Focht, A Clinical Study of Radiations Cataracts and the Relationship to Dose, Amer. J. Roentgenol., Radium Therapy Nucl. Med. 77, 759-85 (1957).
21. G. J. Neary, Nature 187, 10 (1960).
22. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, General Assembly, Seventeenth Session, Suppl. No. 16 (United Nations, New York, 1962), p. 152.

RADIATION STIMULATION OF NERVE ACTION

C. A. Tobias, J. T. Lyman, and J. R. Luce

Persons exposed to ordinarily available sources of penetrating radiations usually do not indicate sensory perception of radiation. Nevertheless it has been known for many years that the retina is very sensitive to X- or gamma-rays.[1, 2] Exposure to 1 roentgen or less of X-rays has been reported to cause an alteration in absolute threshold to light sensation in animals and man.[3] Moderate radiation exposures sometimes result in alteration of EEG patterns.[4] Conditional reflexes are also claimed to be sensitive to low doses of radiation.[5] Hug[6] has observed the reactions of snails, sea urchins, and various sea animals to moderate doses of X-rays.

The use of heavy accelerated ions for neurobiological experiments became of interest when it was realized that by the use of the Bragg ionization property, considerably more dose may be deposited at a predetermined location in depth at any part of the central nervous system than at the surface. Under certain conditions the dimensions of the beam can be made smaller and finer than that of a needle, and very sharply localized irradiations can be obtained. Some years ago several members of the laboratory[7] posed the question as to whether it is possible, generally, to obtain action currents in nerve cells by application of heavy ions and whether immediate reflex action can be elicited. When studying the manner in which electrical currents or other stimuli cause action potentials in nerve cells, it is quite apparent that the current must exceed a certain minimum threshold value in order to produce action potential and that a minimum amount of electric charge must be driven across an axon membrane in time less than about 1 millisecond. The principle for minimum action is true for nonelectric stimuli as well. Applying these principles to radiation, it was decided to attempt to "irritate" nerve tissue by the application of particle pulses of short duration. The heavy ion linear accelerator (Hilac) has a fixed pulse length of about 2 milliseconds. In order to obtain a simple and rapid evaluation of the potentialities of the 40 MeV alpha particle beam at the Hilac as a stimulating or irritating agent it was decided to test the induction of the "corneal blinking reflex" in

rabbits. The geometrical arrangement of the epithelial cells which are in the cornea is one of accurate parallel layering suitable for doing experiments with varying beam penetration. Following the application of a single brief pulse of alpha particles, it was possible to elicit the corneal blinking reflex. In Fig. 1 we have indicated the schematic experiment, and an electrical recording from the orbicularis oculi muscle is shown in Fig. 2. The time delay following the initiating beam pulse and the waveform were typical. The reflex was not obtained due to the action of the secondary light on the retina, since there was no alteration in the response when the optic nerve was cut a few days prior to the application of the beam. Local anesthesia of the surface layers of the cornea abolished the response. It was possible to vary the depth of penetration of the particles and thereby obtain a relative sensitivity of the different layers of the cornea. Maximum sensitivity for initiation of the blinking reflex was obtained for the upper 200 microns; this is a finding of some anatomical interest. Following the application of a number of particle impulses to the cornea, the blinking reflex is abolished, and in some cases late pathological changes occurred in the cornea a few days following irradiation. These were of course restricted to the upper layers of tissue where direct damage was caused by the radiation.

It became of some interest to find methods of lowering the threshold to particle irradiation. Pilot experiments indicated that photosensitizing substances, for example, eosin, caused a significant drop in the threshold dose for the corneal blinking reflex. It is believed that the action of eosin was typical of a series of photodynamic substances.

Additional experiments were carried out in the laboratory to understand some of the basis of the irritative effect of the alpha particles obtained in the laboratory. Gaffey has shown that when the sciatic nerve was irradiated even at high doses no action potential was obtained.[8] When nitella was used as a test object, the particles directed at a segment of a single cell in large doses caused a bending of the cells but no action potential. On the other hand, we exposed the plant mimosa pudica to pulsed irradiation. This plant is known to fold its leaves and bend its stem as a response to mechanical or heat irritation, due to a propagated, nerve-impulse-like action. Single pulses of 1000 to 3000 rads of helium ions reproduced the entire scope of contractions obtained by other methods.

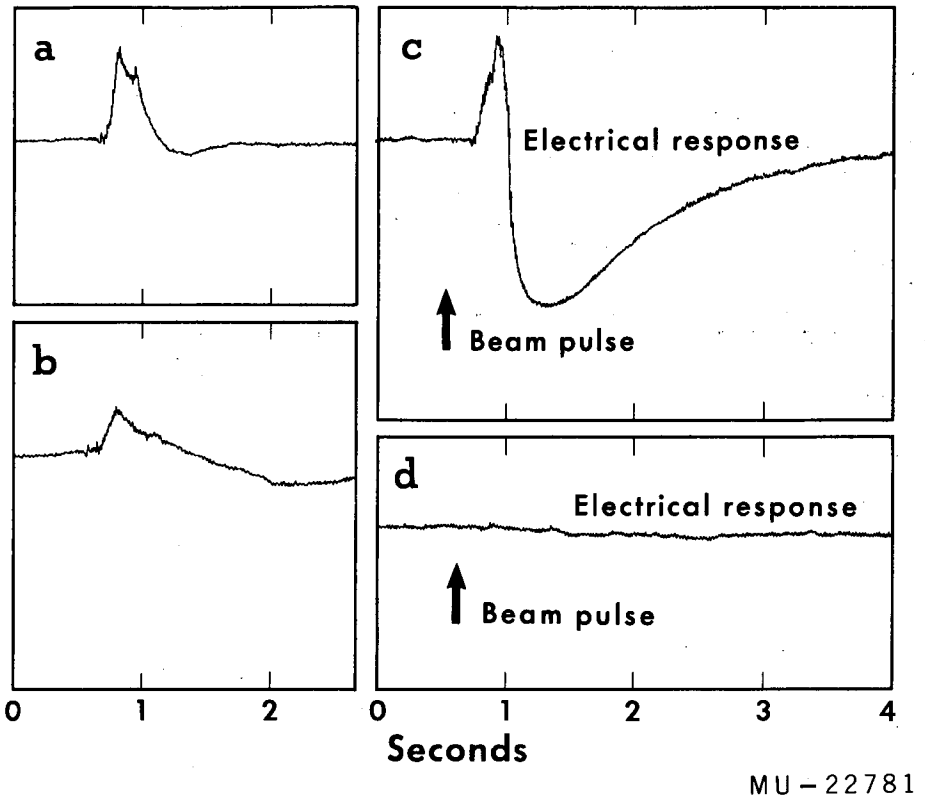


Fig. 1. (a) Response to mechanical stimulation by fine brush. (b) Response to radiant-heat stimulation. (c) Response following 50 000 rads in 2 msec; note delay of about 0.3 sec. (d) No response from sub-threshold stimulation of 10 000 rads in 2 msec. (Figures redrawn from original recorder tracings.)

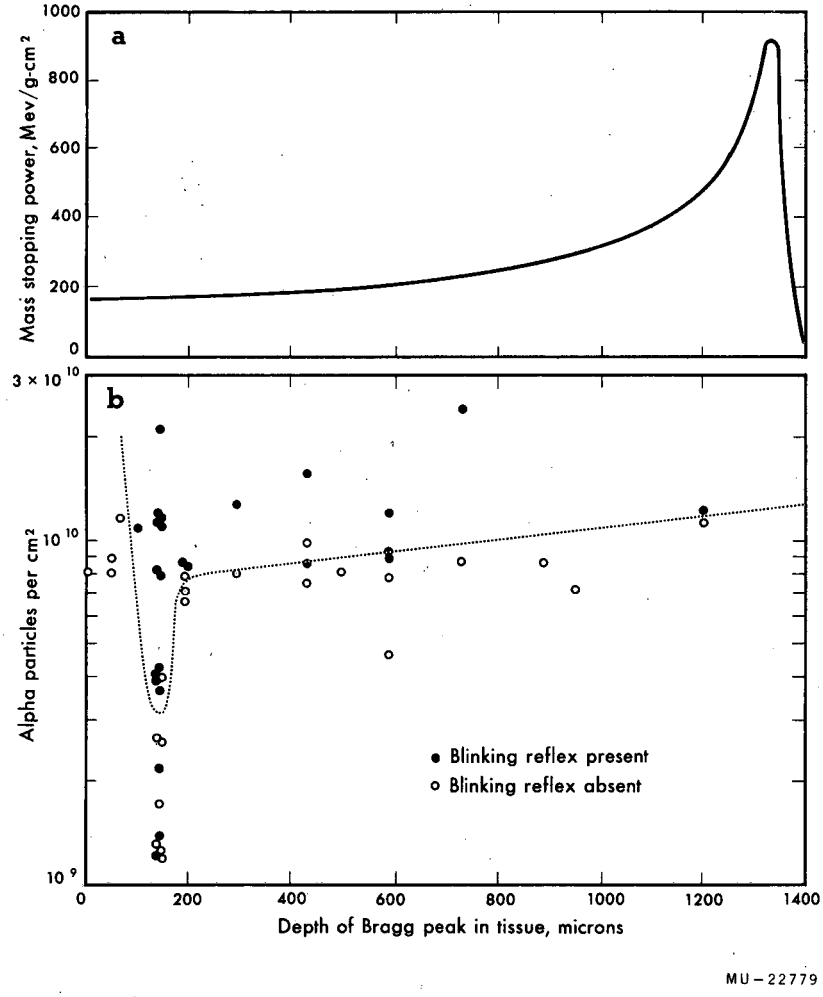


Fig. 2. (a) Bragg ionization curve in tissue-equivalent material of 10.4 ± 0.2 MeV/amu ^{4}He (calculated from unpublished data, Brustad and Lyman). (b) Presence of blinking reflex ●, and absence of reflex O, with the Bragg peak at different depths in tissue. The dose is given in terms of particles per cm^2 in the beam pulse. Data from six normal animals were used in the construction of this graph; the dotted line represents a depth-threshold curve fitted to the data.

Using the motor cortex of rats, and small-diameter beams of 10 MeV/nucleon alpha particles from the Hilac in bursts of 2 millisecond pulses, we attempted stimulation of motion of the extremities. In this type of experiment there are many variables one must control; among these is the depth of anesthesia, the size of the microbeam utilized, the depth of Bragg peak, the locality of irradiation, the rate of scanning over a given area, etc. The current status of experiments may be summarized as follows.

(1) It is possible to obtain motor activity of individual muscles or of groups of muscles by unilateral particle stimulation of the motor cortex of the rat. The stimulation by radiation may be repeated several times in succession.

(2) A single 2 millisecond impulse is usually not sufficient to elicit motor activity. A train of impulses usually of about 1 second duration (15 pulses) is required.

(3) Contrary to expectation, small microbeams of 1/10 mm in diameter appeared to be less effective than a beam of 1/2 mm or 1 mm diameter.

(4) The threshold for eliciting motor action from motor cortex irradiation in anesthetized animals is several thousand rads per second, and there is a very great variability in threshold values. It appears, however, that in wakeful animals the sensitivity is greatly increased. In experiments when animals are electrically stimulated in the motor cortex, anesthesia also decreases the response.

(5) Larger doses of radiation sometimes elicit more generalized response with several muscle groups stimulated.

(6) If radiation stimulation is applied in the same location repeatedly, after a certain number of responses the responses cease. We do not know whether this is due to the deleterious effects of the particles or an intrinsic property of the system itself.

(7) After response has ceased at a given location, more responses may be elicited if the beam is slowly scanned so that the location of impact is slightly changed between each consecutive pulse.

(8) The response although always contralateral from stimulation of a given location was not always the same muscle movement. Stimulation of several different localities clearly can elicit the same muscle response.

In the course of these experiments it has become clear that it would be of advantage if the experiments could be carried out in the unanesthetized animal, if the animal were larger than a rat, and if our ability to control the penetration of the beam, the bean intensity, and the duration of succession of impulses would improve. It is also apparent that this method of studying central nervous system function would become much more practical if radiation-sensitive structures were to be found or if the sensitivity could be increased. Hug has made the suggestion that photosensitive nerve endings are also sensitive to penetrating irradiation, and it appears to be worthwhile to explore the action of photodynamic substances further; these could render nerve endings or synapses more susceptible to radiation stimulation.

The Omnitron will have penetrating heavy ions with exactly controllable range suitable for microbeams. The control on beam intensity and pulse frequency will also be much improved over what is available now (Fig. 3). It is quite possible that very heavy ions, for example, argon or krypton, may be much more effective in initiating nerve action potentials than alpha particles. The use of radiation pulses for irritation or stimulation would have certain advantages over the use of needle electrodes since the trauma and hemorrhage caused by needles would be absent; the location of the region irritated can be chosen precisely and in fact varied continuously.

In certain systems, for example, the retina, very small doses of radiation are able to cause action potential.[1, 2] In other nerves that are more refractory, more energy is required. It appears that in many instances the particle energy transfer required to produce a nerve impulse is less than the electrical energy deposited to the same end. It appears that radiation may initiate in these instances an amplifying process or chain reaction. The origin of such a reaction appears to be either at the sensory nerve endings or in synapses and probably not in nerve trunks. By performing microbeam experiments with heavy particles on neuron explants, we hope to be able to answer some of the fundamental points raised by the above comments. Werner Schlapfer and Abdel Mamoon of the laboratory are actively working on tissue culture experiments.

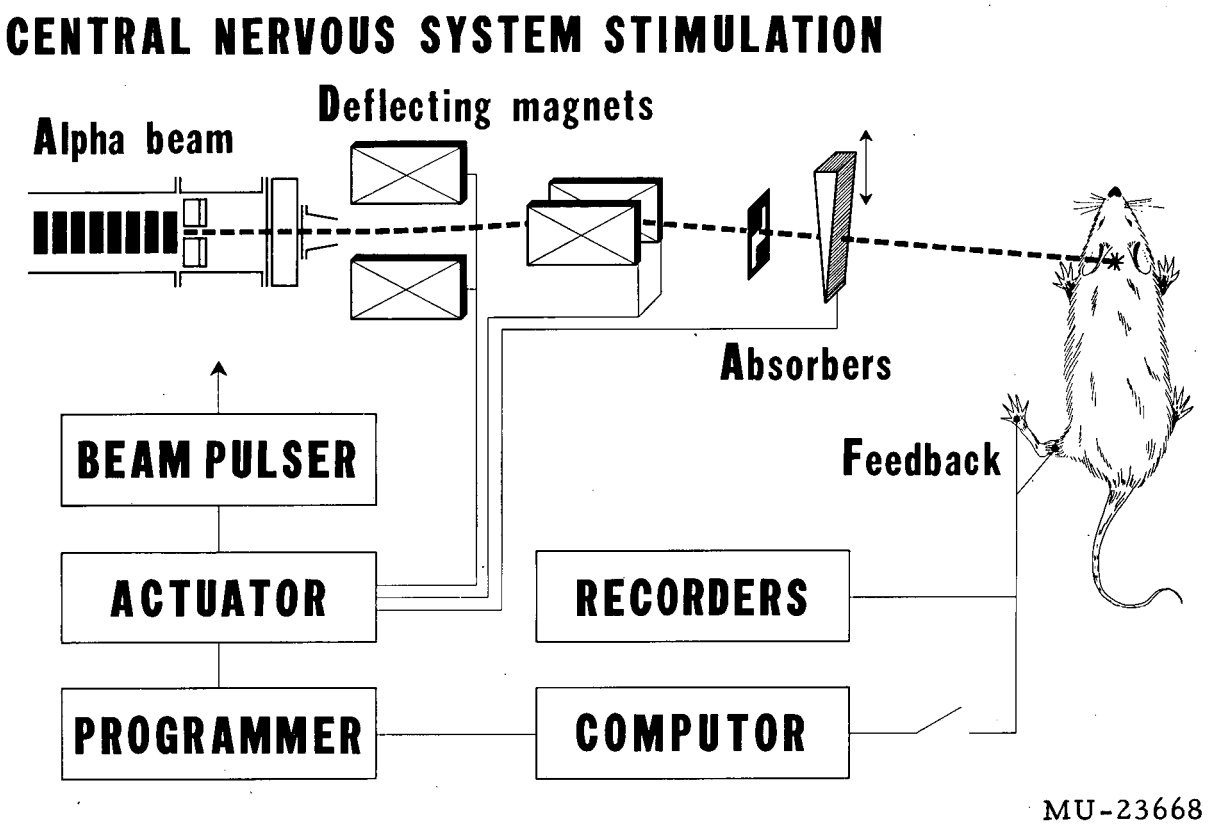


Fig. 3. Schematic concept of future uses of heavy ion pulses for conveying "coded" messages to the brain. The coding may occur by (a) deflection of beam in predetermined sequence, e. g., scanning; (b) passage of beam through absorbers of predetermined profile to position the Bragg ionization peak where stimulation occurs; (c) coding time sequence and intensity of pulses of beam; (d) using "feedback" information from periphery to change coded messages.

References

1. L. R. Lipetz, Electrophysiology of the X-ray Phosphene, Radiation Res. 2, 306 (1955).
2. L. R. Lipetz, The X-ray and Radium Phosphenes, Brit. J. Opthalmol. 39, 577 (1955).
3. K. Motakawa, J. Umetsu, M. Kobayashi, and M. Kanieyama, The Effect of a Small Dose of Roentgen Rays Upon the Human Body as Revealed by the Method of Electric Flicker, Tohoku J. Exp. Med. 64, 151 (1956).
4. H. Gangloff and O. Hug, The Effects of Ionizing Radiation on the Nervous System, Advan. Biol. Med. Phys. 10, 1-90 (1965).
5. N. N. Lifshitz, (for review) Physiological Effects of Nuclear Radiations on the Central Nervous System, Advan. Biol. Med. Phys. 7, 174 (1960).
6. O. Hug, Reflex Like Responses of Lower Animals and Mammalian Organs to Ionizing Radiation, in: Immediate and Low Level Effects of Ionizing Radiations, Suppl. to Intern. J. Radiation Biol. p. 217 (1960).
7. Cornelius Tobias, Jean Luce, Nicholas Yanni, Tor Brustad, John Lyman, and Samuel Kimura, Stimulation of the Corneal Blinking Reflex by Ionizing Radiation, Lawrence Radiation Laboratory Report UCRL-9681, May 4, 1961.
8. C. Gaffey, Response of Sciatic Nerve to High Energy Radiation, in International Symposium on the Response of the Nervous System to Ionizing Radiation, Chicago, 1960 (Snyder & Haley, 1962).

PITUITARY AND HYPOTHALAMIC IRRADIATION

C. A. Tobias and D. C. Van Dyke

Some of the earliest neurophysiology applications of accelerated particle research was directed toward the production of small lesions in pituitary and hypothalamus of the rat. The 190 MeV deuteron beam of the 184-inch cyclotron was suitable for the production of lesions inside of the skull of about 1 mm in diameter and such lesions could be accurately placed by X-ray diagnostic techniques, using bony landmarks of the skull and locating of the lesion. Since the lesions are histologically well delineated and since they do not involve immediated hazard of mortality or excessive hemorrhage they appear to be very suitable for studies of homeostatic control mechanisms, particularly hypothalamic control of pituitary function.

An initial finding was that the hypothalamic region is much more sensitive for acute lethality than the cortex, the brain stem, or the pituitary.[1]

Pituitary irradiation was carried out on a number of groups of rats with various dose levels from 950 rads to 40 000 rads. It was not only established that radiation hypophysectomy can be as effective as surgical, but also that there is some gradation of radiosensitivity for the production of various groups of pituitary hormones. In the rat, as judged by studies of the target organs, the most sensitive to radiation is the rate of production of growth hormone and of thyroid stimulating hormone.

The production of ACTH is in the intermediate radiosensitivity range, whereas the level of gonodatropic hormones is diminished if several thousand rad dose has been delivered to the pituitary.

At the lowest dose levels, 950 and 1500 rads to the pituitary,[2] two interesting findings emerged. First, many of the Lond Evans rats developed a late obesity accompanied by thyroid hypertropy. Secondly, after an interval of many months, practically all of the animals developed pituitary tumors (100% at 1500 rads dose).

Approximately 2 years following irradiation, there were 3.5 tumors per gland (1500 rads) and the tumors included eosinophilic, basophilic, and chromophobe adenomas. This finding has opened possibilities for further

study of the relationship of induction of pituitary tumors to the function of target organs and the role of pituitary tumors in the etiology of other endocrine-originated neoplasms.

By only irradiating part of the rat hypothalamus[3, 4] it was possible to avoid late lethality in many of the rodents. Hypothalamic irradiation not only produced some of the well-known signs of hypothalamic injury such as rage or docility, it was also possible to isolate other known chronic metabolic disorders caused by the radiation lesions. Anterior hypothalamic irradiation led to hypothyroidism, diabetes insipidus, glycosuria. Physiological effects of posterior hypothalamic irradiation included inopthalamus and disturbances in temperature balance.

Working with Blanquet[5] of the University of Bordeaux, it was shown that injury to the median eminence to the hypothalamus by radiation caused abnormalities in thyroid function which could be correlated to changes in the submicroscopic structure of the thyroxin-producing cells in the thyroid gland. The cytomembranes which are associated to production of thyroid appeared to be damaged. Whereas the iodine uptake of the thyroids were normal, the biochemical step leading to the formation of thyroxine was permanently impaired.

At the University of Uppsala, bilateral hypothalamic lesions were produced in goats, particularly in a study of the manner of control of water turnover in these animals. The regions responsible for control of water turnover were mapped in this fashion, and it was found that a more extensive region of the gland was involved than previously believed on the basis of studies with lesions produced by inplanted needles.

The energy of the beams of the Berkeley cyclotron have been changed to higher energy due to the needs of physical research, and the focusing properties of the beam became less favorable for local lesion studies than previously. The beams of the Omnitron are designed, however, for optimum utilization in deep-seated lesions, and can be so utilized even in large animals or man.

References

1. C. A. Tobias, D. C. Van Dyke, M. E. Simpson, H. O. Anger, R. L. Hugg, and A. A. Koneff, Irradiation of the Pituitary of the Rat with High Energy Deuterons, Lawrence Radiation Laboratory Report UCRL-1986 (1953); Am. J. Roentgenol. Rad. Ther. Nuc. Med. 72, 1-21 (1954).
2. D. C. Van Dyke, M. E. Simpson, A. A. Koneff, and C. A. Tobias, Long Term Effects of Deuteron Irradiation of the Rat Pituitary, Endocrinology 64, 240-257 (1959).
3. A. Anderson, J. Garcia, J. Henry, C. Riggs, J. E. Roberts, B. Thorell, and C. A. Tobias, Pituitary and Hypothalamic Lesions Produced by High Energy Deuterons and Protons, Lawrence Radiation Laboratory Report UCRL-3737 (1957); Radiation Res. 7, 299 (1957) abstract.
4. A. Anderson, J. E. Roberts, B. Thorell, and C. A. Tobias, Arrest of Growth in Young Rats Following Hypothalamic Deuteron Irradiation, Lawrence Radiation Laboratory Report UCRL-3738 (1957); Radiation Res. 7, 300 (1957) abstract.
5. P. Blanquet and J. Faure, Hypothalamus and Thyroid, in Advances in Biological and Medical Physics, Vol. VIII, pp. 225-306, 1962.
6. B. Anderson and B. Larsson, Influence of Local Temperature Changes in the Preoptic Area and Rostral Hypothalamus on the Regulation of Food and Water Intake, Acta Physiol. Scand. 52, 75-89 (1961).

HEAVY-PARTICLE PITUITARY-SUPPRESSIVE THERAPY IN DIABETIC RETINOPATHY

J. A. Linfoot, J. L. Born, J. F. Garcia,
C. A. Tobias, R. P. Kling, E. Manougian,
C. Y. Cheung, R. A. Fink, and J. H. Lawrence

Introduction

Prior to the introduction of insulin therapy, diabetic retinopathy was rarely observed because it does not usually develop before the age of forty and is rarely seen in juvenile diabetics. With the marked extension of life that accompanied the therapeutic use of insulin, the incidence of vascular complications has increased and diabetic retinopathy has become one of the major causes of blindness, second only to glaucoma. The frequency of retinopathy increases with the apparent duration of diabetes. Only 4% of diabetics have retinopathy when the duration of diabetes is less than 10 years, whereas over 50% have some degree of retinopathy when diabetes has been present for longer than 15 years.[1] The cause of the retinal changes of long-standing diabetes remains an enigma, and by and large therapy has been disappointing. However, following the report by Poulsen[2] of the amelioration of diabetic retinopathy in a young woman who suffered post-partum pituitary necrosis, hypophysectomy has been performed in a limited number of patients with advancing retinopathy.[3] Although the results in some patients seem to have been quite dramatic, the over-all effectiveness of hypophysectomy is not clear because of a lack of an adequate knowledge of the course of diabetic retinopathy with reference to vision and retinal changes. The absence of a satisfactory control group and the lack of a reasonable baseline to allow proper evaluation has contributed further to this confusion. Although transfrontal and transethmoidal surgical hypophysectomy were the original procedures employed, pituitary-stalk section, implantation of ^{90}Y in the pituitary fossa, and cryo-hypophysectomy employing a nitrogen probe are procedures currently in use. The delivery of heavy particles to the pituitary[4,5] is the only non-surgical technique that has been successfully employed in the treatment of diabetic retinopathy.[6,7]

Description of Diabetic Patient Population

Our first patient with diabetic retinopathy was treated in 1958. Since that time, 161 patients with advanced stages of retinopathy have received heavy-particle pituitary irradiation. The majority of the patients have had symptomatic retinopathy for a significant period of time, and although most are juvenile-type diabetics with the onset of diabetes in their early years, there were several patients in the series who developed diabetes after the age of 50 in whom retinopathy was the first recognized sign of the disease. The duration of follow-up in these patients ranges from 2 months to 96 months, with 24 patients having been followed for 60-96 months, 22 for 48-59 months, 23 for 36-47 months, 29 for 24-35 months, 29 for 12-23 months, and 34 patients have been followed for less than 1 year; in a few cases other therapeutic procedures were undertaken later which eliminated these patients from the heavy-particle series.

The first patients treated in this series were given sufficient amounts of heavy particles to induce complete hypophysectomy. However, difficulties in managing the totally hypophysectomized diabetic patient and concern over the generalized vascular disease, and consequently the greater degree of vascular radiosensitivity in the cerebral blood vessels of the diabetics with its attendant risks, prompted us to use smaller doses to produce partial pituitary ablation, or what we have referred to as pituitary suppression. Significant beneficial effects on retinopathy and an early fall in insulin requirements were observed in some patients in spite of the fact that hormonal replacement therapy was not required for many years after treatment.

Analysis of Results

It is important to emphasize that the problems in evaluating the effects of any treatment on the complications of diabetes are extremely great. Knowledge of the natural history of the microvascular complications is extremely limited. Under ideal circumstances it would be desirable to set up a double-blind study (or simply to treat every other patient), but professional and practical reasons make it extremely difficult to bring oneself to adopt such an approach. Over the years we have accumulated a series of patients whom we have followed for varying periods of time before treatment, and in the past year a series of patients who were refused treatment, and in addition

we are currently expanding another control series and these are now being carefully analyzed. On the basis of our experience and the few reports in the literature,[8, 9, 10] we feel that untreated, progressive diabetic retinopathy advances to blindness at a rapid rate. Although our experience has been longer than 8 years, our analysis must remain in the form of a progress report which will gain greater significance as the period of follow-up is extended, and as our control series for comparison becomes larger and of longer duration.

Initially, no rigid criteria were set up for selection of patients, the only consideration being that they had advancing and serious retinopathy, but sufficient visual acuity to warrant the procedure. As a result we have treated a large number of patients with far-advanced renal and cardiovascular disease, and with retinal pathology that we now realize is irreversible. The patients were selected for preliminary evaluation after a review of the medical and ophthalmological reports of the referring physicians. After a screening evaluation, the patients who were thought to be candidates likely to benefit from pituitary-suppressive therapy were hospitalized in Donner Pavilion for 4 to 5 days. During this time complete medical and ophthalmological evaluations were carried out, including laboratory assessments of renal and hormonal function. On the majority of patients, retinal photographs were taken with a Zeiss fundus camera; 8 to 12 separate views of each visible fundus were obtained. Following treatment, similar metabolic and ophthalmological evaluations were carried out at 1 to 6 month intervals. Renal biopsies were carried out on a small series of patients in order to evaluate the histological patterns in patients with different degrees of retinopathy, and the histological findings were similar to those that have been observed by others. In evaluating our fundus photos we originally selected seven major retinal pathological lesions for evaluation, and this report will present the results of this analysis. More recently we are employing an even more involved analysis; we have now accumulated approximately 7000 fundus photos and when this more detailed analysis has been completed, we shall report our further findings.

Of the 161 treated cases, there were five in whom there were insufficient follow-up data to satisfactorily evaluate the effect of treatment on their retinopathy. All patients who had been followed less than 1 year were

considered as having been treated too recently to be evaluated, and six patients who died within a year after treatment were also excluded. All other patients were grouped by the duration of follow-up (see Table I) and analyzed by groups. Thus patients who expired after 1 year were included with the group of patients who had been followed for a comparable period. Similarly, three patients who subsequently had additional treatment which prohibited evaluation after that time, were evaluated only prior to the subsequent treatment. There were eight patients in this category; three underwent surgical hypophysectomy and five received photocoagulation of the retina in an attempt to stop recurrent hemorrhages.

One hundred twenty-seven patients were analyzed and the pertinent data are presented in Fig. 1. There were 77 males and 50 females in this group. The median age at the onset of retinopathy was 36 and the median duration of diabetes prior to the onset of retinopathy was 18 years. The median duration of retinopathy was 2 years, with the men having slight but significantly shorter duration of retinopathy than the females. Thirty-nine percent of the patients had stable or improved vision and retinal pathology in both eyes, 22 percent remained stable in one eye but progressed in the other, and 39 percent progressed in both eyes.

The causes of progression (see Fig. 2) were as follows: 42% progressed because of vitreous hemorrhage (and in looking back over these particular cases, nearly all had shown progression of their retinopathy within the first year following treatment); 22% progressed because of retinal detachment (and, in contrast to the above, this was usually a later developing event); 21% developed secondary glaucoma; cataracts and macular degeneration accounted for visual deterioration in the remaining 15% of patients.

The question always arises as to what effect the therapy has had on the other diabetic vascular lesions. It is only natural to hope that if one can arrest the progress of the vascular lesions in the eye, then similar effects might be produced in the kidney and other parts of the vascular system, thus retarding the development of renal failure and other cardiovascular complications which are the main causes of death in diabetics. It has been our experience, as well as that of others, that patients who have already developed renal failure, or have coronary artery or other advanced cardiovascular disease, are already in the end stage of the disease, and therapy does little

Table I. Analysis of 127 patients with diabetic retinopathy who had received heavy-particle pituitary irradiation 1 to 8 years prior to January 1, 1967.

Patients treated and followed 1-8 years: 77 males 50 females	127 patients
Median age	38 years
Median duration of diabetes	18 years
Median duration of retinopathy	2 years
Current status of follow-up:	
12-23 months	127 patients
24-35 months	98 patients
36-47 months	69 patients
48-59 months	46 patients
60-96 months	24 patients

to alter their downhill courses. The effects on patients with lesser renal abnormality is more difficult to assess, since it is generally agreed that the natural history of this lesion is significantly longer than that of the major retinal lesions that lead to blindness. Our follow-up studies confirm this clinical observation, and although there is almost invariably a detectable abnormality in renal function in the majority of cases with diabetic retinopathy, we have found little evidence for a correlation between the extensiveness of the retinal and renal lesions in a given patient.

In our series there are 15 patients (12%) who have normal creatinine clearances and no proteinuria; by and large these patients have had little change in their renal function during their follow-up periods. There are 41 patients (32%) who have had clinical azotemia, defined in our series by having a serum creatinine of 1.5 mgm. per 100/ml or higher; half of these patients have shown further deterioration of their renal function, and the other half have not shown a significant change. There are 71 patients (56%) in the series who had no proteinuria, although the majority of these did have significant decreases in the creatinine clearance. Thus 88% of the patients had some

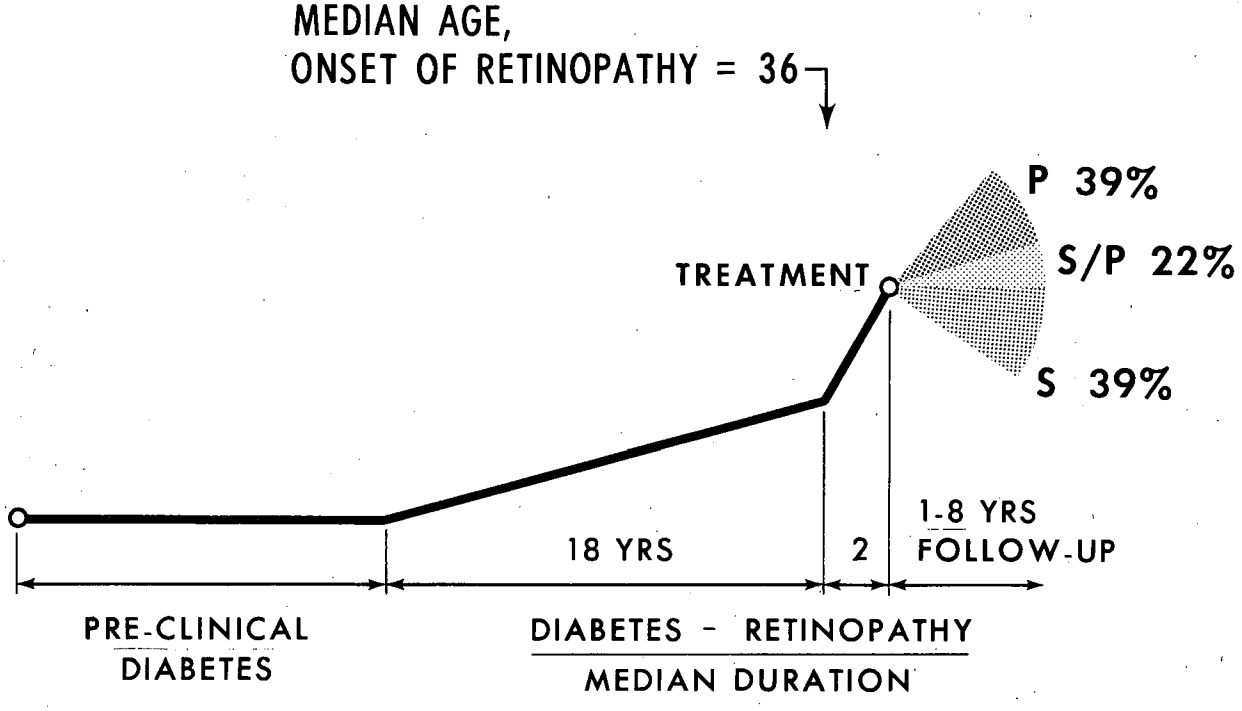
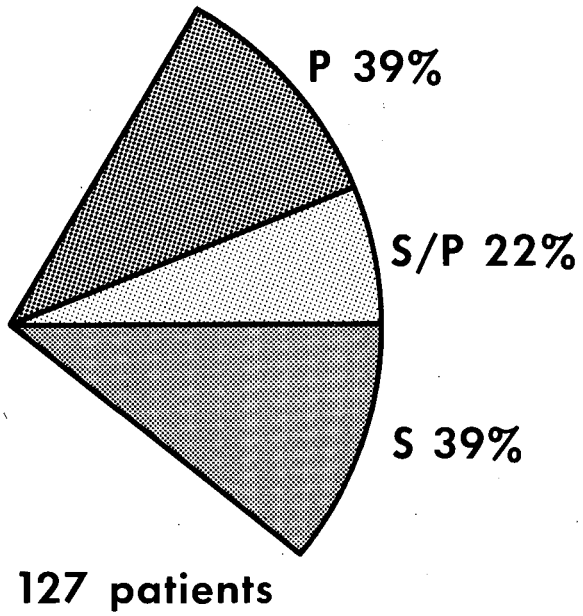


Fig. 1. The course of preclinical diabetes, clinical diabetes, and of diabetic retinopathy in 127 patients is illustrated graphically. The results of treatment are summarized on the right side of the graph. The patients with stabilized or improved vision are shown below, and those with progressive loss of vision above.



CAUSES OF PROGRESSION

1. Vitreous hemorrhage 42%
2. Retinal detachment 22%
3. Glaucoma 21%
4. Miscellaneous 15%

DBL672-1525

Fig. 2. Causes of progression in diabetic retinopathy. In general, vitreous hemorrhages occurred shortly after treatment whereas retinal detachments often did not occur until later.

abnormality of renal function and were thus considered to have detectable diabetic nephropathy. Follow-up of 1 to 8 years in these cases revealed that nearly three-fourths of the patients had either a decrease or no change in their urinary protein excretion, and about one-half have also shown improvement or lack of change in their 24-hour creatinine clearance; approximately one-fourth of the patients had a significant increase in proteinuria, and nearly one-half of these also had some decrease in the creatinine clearance. It is obvious that more prolonged follow-up and additional histological studies of the vascular system will be required to assess significant progression, stability, or regression of these vascular lesions.

The third member of the so-called triopathy of diabetes is diabetic neuropathy. We have employed only clinical examinations in our assessment of this lesion, and therefore we have stuided neuropathy somewhat less critically. But on the basis of these clinical observations, we have failed to find any influence whatsoever on pre-existing diabetic neuropathy, and in fact have observed aggravation of autonomic neuropathy with postural hypotension in patients with panhypopituitarism.

Thirty-one of all the 161 treated patients have expired. The most common causes of death (see Table II) were uremia and myocardial infarction. Two of the patients who received hypophysectomizing doses of radiation died of adrenal insufficiency, and a third patient died of hypoglycemia. The autopsy findings in the pituitaries of these patients showed changes ranging from less than 10% destruction in patients receiving low doses of radiation who expired shortly after treatment, to 99% destruction in those patients receiving ablative doses of radiation. Three patients in the series of 161 cases developed extraocular motor nerve palsies. One additional patient developed symptoms compatible with temporal lobe seizures, which were controlled with anticonvulsant therapy. All of these side effects occurred in the patients receiving large doses of pituitary irradiation, and no clinically apparent complications have been observed in any of the patients treated in the last 5 years. Thus, the morbidity of the procedure remains low, with observable side effects in only 2.5% of all the treated patients.

Table II. Causes of death.

Causes of death		
	Renal	17
	Cardiovascular	10[a]
	Adrenal crisis	2
	Hypoglycemia	1
	Suicide	1
	Total	31
Ages at death		
	22-30	4
	30-40	14
	40-64	13

[a] 1 CVA.

Metabolic Effects

The metabolic effects in these patients are of considerable interest. As indicated previously, the patients treated with ablative amounts of heavy particles developed clinical hypopituitarism within 6 weeks to 6 months after treatment, and replacement therapy with hydrocortisone and thyroid had to be instituted. Many of the patients treated with lesser amounts of heavy particles have not required replacement therapy for 3 to 4 years after treatment. At all treatment levels a significant change of insulin requirements had been observed, with the most striking changes, of course, observed in those groups receiving complete pituitary ablation. In this high-dose group, the median fall in insulin requirement was 56% (see Fig. 3); in those receiving intermediate doses, it was 44%; while in those receiving low doses it was only 29%. It also became apparent that there were individual variations that have to be taken into consideration. In general, in this low-dose group there were a number of patients who displayed no significant fall in insulin requirements, and in addition, they showed less beneficial effect on their retinopathy. We have felt that this is the lowest dose that could be effectively employed in diabetic retinopathy, and that higher doses were probably more desirable. The majority of patients receiving 8000 to 14 000 rads have not required

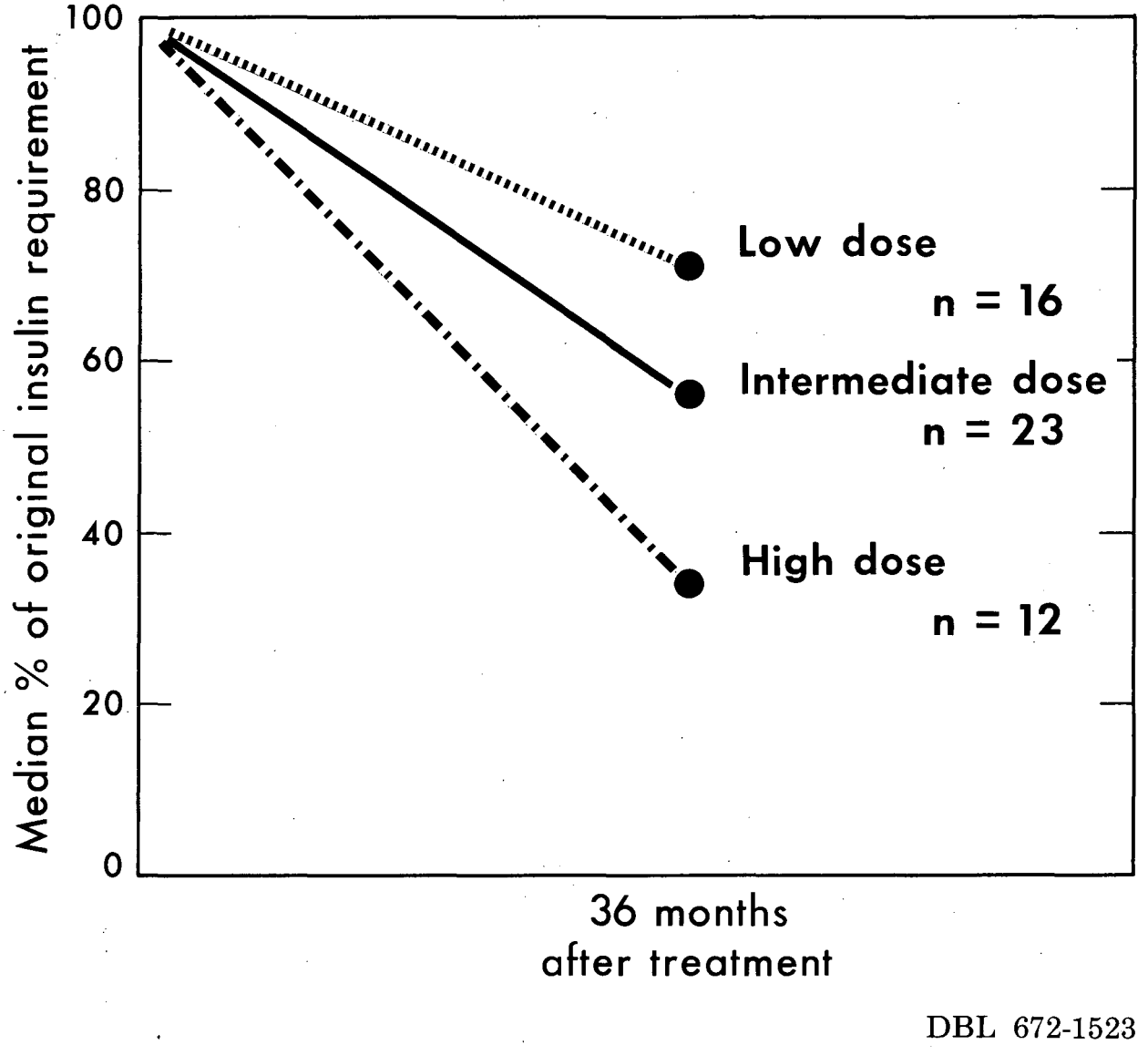


Fig. 3. The median fall in insulin requirement 36 months after treatment, comparing groups of patients treated at three dose levels. The greatest fall was seen in those patients receiving the higher doses, although there is some overlap within these groups, indicating individual factors influencing the susceptibility to pituitary suppression.

replacement therapy even 3 to 4 years after treatment. Shortly after treatment, this group had striking falls in insulin requirements, but maintained normal, although reduced, basal excretion of adrenal corticosteroids and thyroid function. Many of these patients show some reduction in their ACTH reserve, as measured by the Metopirone test, and some patients have had some decrease in the urinary gonadotropin excretion. None of the patients who has had a significant fall in insulin requirement (10 units or more) has had an increase in insulin requirement later, except when replacement therapy with cortisone or thyroid had been instituted.

With the availability of a sensitive radioimmunoassay for growth hormone, we have been able to observe the development of increased insulin sensitivity and loss of the growth hormone response to hypoglycemia in a number of the patients who have normal basal thyroid and adrenal function (see Fig. 4). Furthermore, one can follow the progressive change in growth hormone response to hypoglycemic stimulus when these tests are done serially following heavy-particle treatment. A similar gradual decline in growth hormone levels is observed following heavy particle treatment in acromegaly.

Summary

One hundred and sixty-one patients with progressive retinopathy have received heavy-particle pituitary irradiation and have been followed from 2 to 96 months. Of 127 patients in whom there were sufficient data and who had been followed for 1 to 8 years, 39% had improved or stable vision in both eyes. Twenty-two percent had stable vision in one eye, but showed progressive loss of vision in the other. Thirty-nine percent of the patients developed progressive loss of vision in both eyes. The major causes of progression were recurrent vitreous hemorrhages, retinal detachment, and secondary glaucoma. Stabilization of the retinopathy, observed in patients receiving as little as 8000 rads, does not seem to depend upon complete suppression of pituitary function. A significant drop in insulin requirements was seen with greater frequency in the patients who had a favorable response to therapy. No definite effects on nephropathy or neuropathy have been observed. Many patients were found to retain adequate basal adrenal and thyroid function, but to lose the ability to secrete growth hormone in response to a hypoglycemic stimulus. Thirty-one patients have expired, 27 of the

HEAVY-PARTICLE IRRADIATION
GROWTH-HORMONE RESPONSE TO HYPOGLYCEMIA

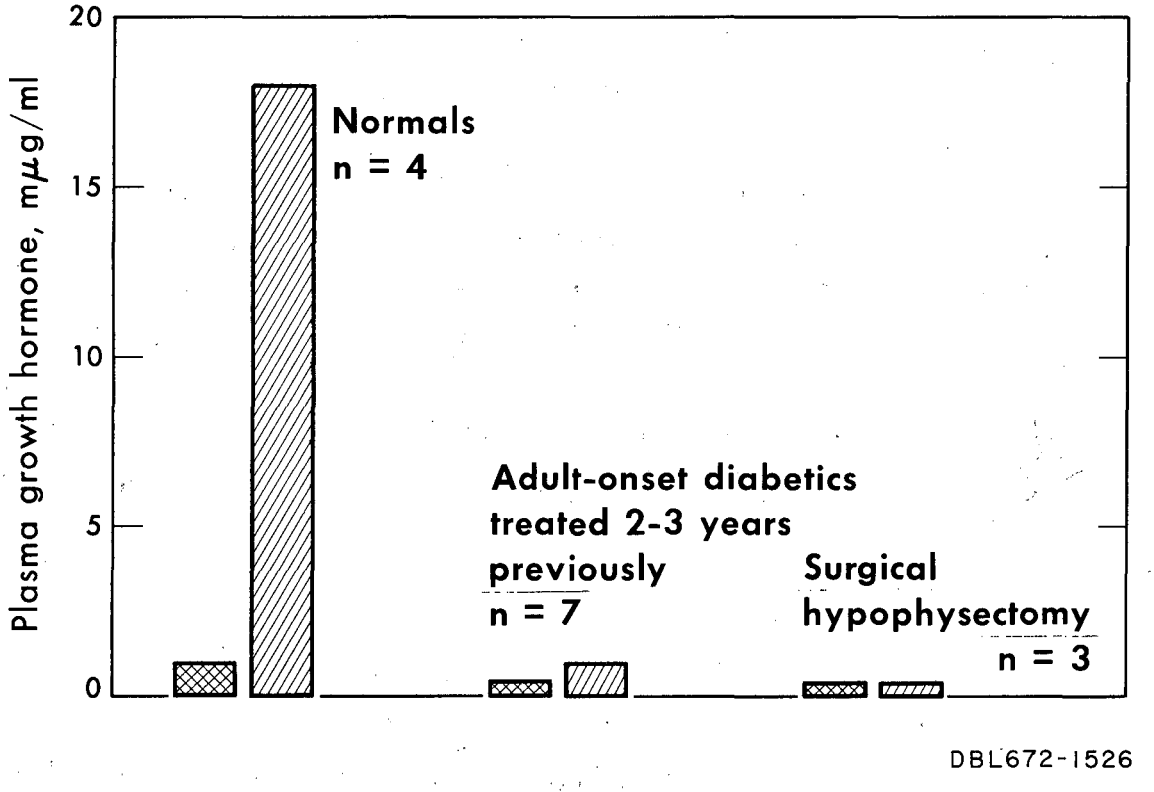


Fig. 4. The growth hormone response to hypoglycemia was reduced in seven adult-onset diabetics who had received heavy particle therapy 2 to 3 years previously. The shaded bars represent the growth hormone level prior to injection of 0.1 u of insulin intravenously, and solid bars the maximum growth hormone rise in response to hypoglycemia. In all cases the blood sugar fell 50% or more after insulin. The response of the diabetics (middle graphs) is compared to four normal subjects on the left, and three surgically hypophysectomized patients on the right.

deaths being due to renal or cardiovascular complications. The procedure, as described, is associated with no mortality, has a very low morbidity, and can be safely utilized in patients with early diabetic retinopathy.

References

1. H. W. Larsen, Diabetic Retinopathy, Acta Ophthalmol., Suppl. 60 (Munksgaard, Copenhagen, 1960).
2. J. E. Poulsen, The Houssay Phenomenon in Man; Recovery from Retinopathy in a Case of Diabetes with Simmond's Disease, Diabetes 2, 7-12 (1953).
3. Symposium on the Influence of Hypophysectomy and Adrenalectomy on Diabetic Retinopathy, Diabetes 11, 46-94 (1962).
4. J. H. Lawrence and C. A. Tobias, Radioactive Isotopes and Nuclear Radiations in Treatment of Cancer, Cancer Res. 16, 185-193 (1956).
5. J. H. Lawrence, J. L. Born, C. A. Tobias, R. Carlson, F. Sangalli, and G. Welch, Clinical and Metabolic Studies in Patients After Alpha-Particle Subtotal or Total Hypophysectomy, Jap. Med. 5, 859-862 (1959).
6. J. H. Lawrence, C. A. Tobias, J. A. Linfoot, J. L. Born, A. Gottschalk, and R. P. Kling, Heavy Particles, the Bragg Curve and Suppression of Pituitary Function in Diabetic Retinopathy, Diabetes 12, 490-501 (1953).
7. J. L. Born, J. H. Lawrence, J. A. Linfoot, C. A. Tobias, E. Manougian, and M. J. Snyder, Hypophyseal Suppression with Heavy Particles in Diabetic Retinopathy, in On the Nature and Treatment of Diabetes, invited papers and related discussions presented at the Fifth Congress of the International Diabetes Federation (Excerpta Medica Foundation, Amsterdam, The Netherlands, 1965), Chap. 33, pp. 475-482.
8. F. C. Cordes, The Diabetic: His Visual Prognosis, A. M. A. Arch. Ophthalmol. 48, 531-556 (Nov. 1952).
9. F. I. Caird and C. J. Garrett, Prognosis for Vision in Diabetic Retinopathy, Diabetes 12, 389-397 (1963).
10. W. P. Beetham, Visual Prognosis of Proliferating Diabetic Retinopathy, Brit. J. Ophthalmol. 47, 611-619 (1963).

GROWTH HORMONE STUDIES IN ACROMEGALY IN PATIENTS RECEIVING PITUITARY IRRADIATION

J. F. Garcia

The study of the physiology of growth hormone secretion in normal and pathological situations has been stimulated in recent years by the development of highly sensitive radioimmunoassay procedures.[1] With the relatively recent application of a variety of methods for the treatment of acromegaly, an objective means of assessing the effect of treatment, other than by clinical signs, has become mandatory. The ability to measure the plasma growth hormone (PGH) level should provide such an objective measure of tumor activity, and thus of the effect of the treatment on the course of the disease. A preliminary study by Linfoot and Greenwood[2] revealed a striking decrease in PGH levels of acromegalic patients after pituitary irradiation with heavy particles. The present study presents our further experience with a plasma growth hormone radioimmunoassay on a large group of acromegalic patients studied before and after heavy particle pituitary irradiation.

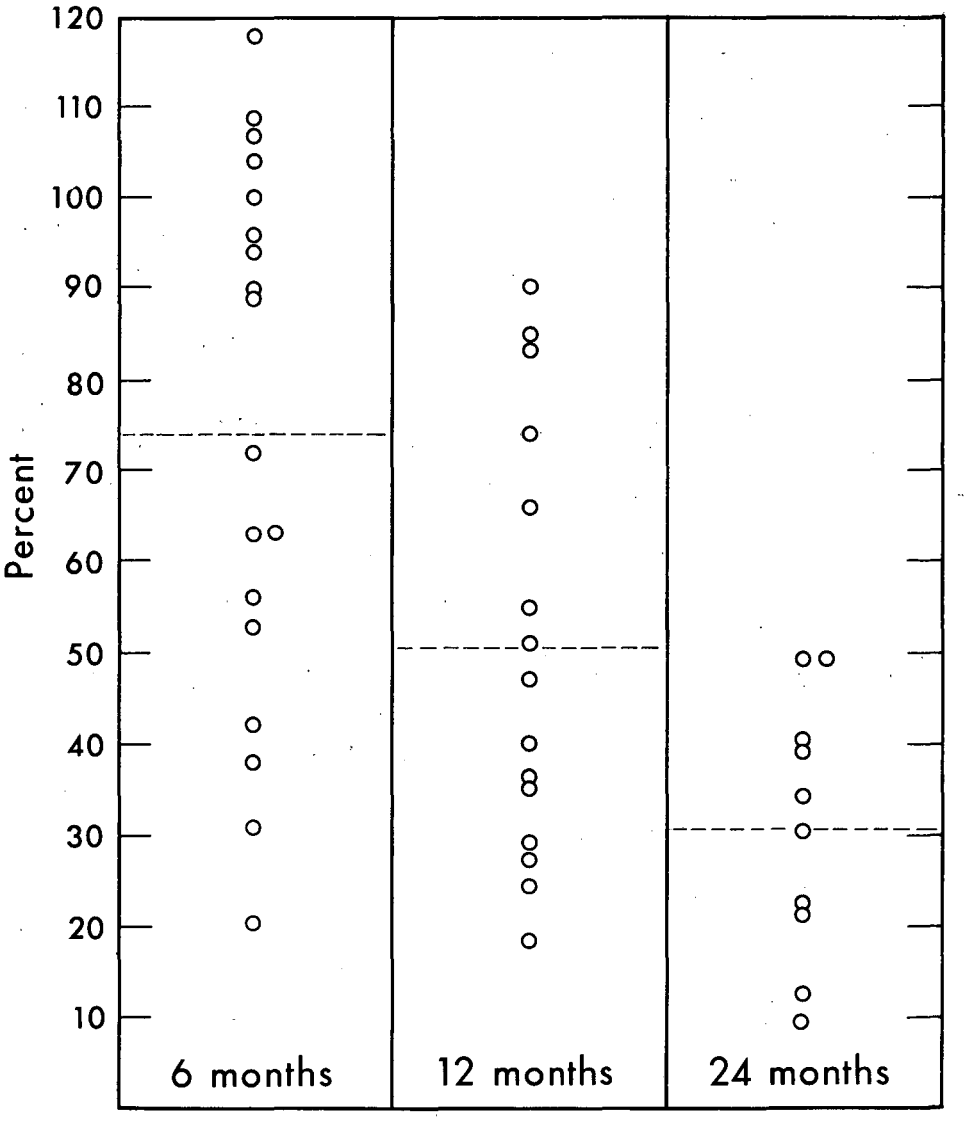
The method used for the radioimmunoassay of growth hormone has been modified from that of Hunter and Greenwood.[3] Human growth hormone (HGH) (kindly supplied by Prof. C. H. Li, University of California, Berkeley) was used to immunize rabbits, and also labeled with iodine-125, using the Chloramine T method of Greenwood et al.[4] The antibody-bound HGH-iodine-125 was precipitated by using a goat antirabbit gamma globulin serum (obtained from Antibodies, Inc., Davis, California). The percentage of the labeled HGH that was bound to antibody was plotted against the logarithms of the concentrations of standard HGH (a gift from Prof. A. E. Wilhelmi, Emory University, Atlanta, Georgia. Lot No. HS503A had a specific activity of 1.7 I.U./mg, standardized in terms of the bovine growth hormone international standard). The concentrations of HGH were read directly off such a curve and are therefore expressed in mμg of the standard HGH preparation used.

Plasma growth hormone studies have been carried out on a total of 56 acromegalic patients thus far. In addition to other studies, the PGH levels have been determined in 23 of these patients throughout insulin tolerance, glucose tolerance, and hydrocortisone-modified glucose tolerance

studies before and periodically after heavy particle pituitary irradiation. Figure 1 summarizes the results in terms of fasting PGH levels thus far obtained in acromegalic patients following treatment. The average fasting value at bed rest in the 23 acromegalic patients before treatment was 50.7 mμg/ml with a range of 5.7 to 212 mμg/ml. Because of the tremendous variation in the initial PGH values, it was necessary to relate the later PGH values in each patient to his or her pretreatment value in an attempt to summarize the overall effectiveness of the treatment. Thus, the points are plotted as the percentage of the pretreatment fasting values. Thirteen patients of 18 studied at 6 months following treatment showed a certain degree of depression in fasting PGH values. In spite of the fact that five patients showed no change or a slight elevation at this time, the overall average was a depression to 74.7% of the pretreatment fasting PGH value. All of the values in 15 acromegalic patients studied at 12 months after treatment showed a depression in fasting PGH to approximately 51% of the pretreatment value. At 24 months following treatment the PGH values were further reduced to 30.5% of the initial values. Typical curves of the actual fasting PGH values observed in six of the acromegalic patients are presented in Fig. 2 as a function of time following alpha-particle irradiation to the pituitary. Unfortunately, no pretreatment data were available on a series of acromegalic patients who had been treated more than 2 years prior to this study. However, in 10 acromegalic patients studied 5 to 7 years after treatment, the mean fasting PGH value was 3.0 mμg/ml with a range from 0.2 to 12.5 mμg/ml. At this point the only comparison that can be made is with the pretreatment average of the other group of acromegalic patients, which was 50.7 mμg/ml, or approximately a 17-fold difference in PGH level 5 to 7 years after treatment.

As a group the acromegalic patients showed very little or no increase in PGH in response to the hypoglycemia produced by the injection of 0.1 unit of insulin per kilogram. Also, all acromegalic patients showed a failure of PGH to suppress to normal levels during oral glucose tolerance tests. However, the majority of the patients showed a slight to moderate fall 2 to 2.5 hours after the 100 gram glucose load. In a group of 29 untreated acromegalic patients the mean fasting PGH level prior to the glucose administration was 45 mμg/ml (range 6.5 to 245 mμg/ml), while the mean values at 90 and 180 minutes were 33 and 32 mμg/ml respectively.

FASTING PLASMA GROWTH HORMONE VALUES IN ACROMEGALIC PATIENTS AT VARIOUS TIMES AFTER TREATMENT AS A PERCENTAGE OF THE PRETREATMENT FASTING VALUE



MUB-12270

Fig. 1

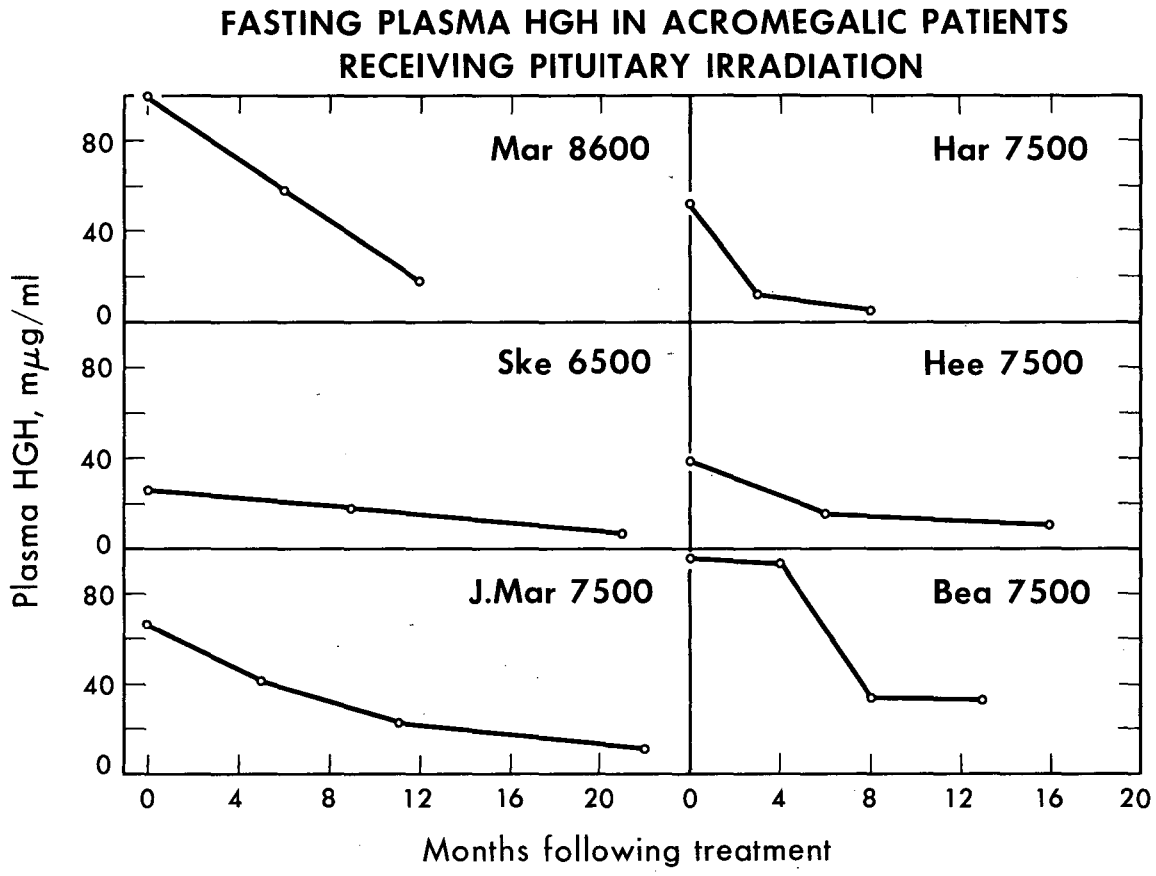

FASTING PLASMA HGH IN ACROMEGALIC PATIENTS
RECEIVING PITUITARY IRRADIATION
Mar 8600
Har 7500
Ske 6500
Hee 7500
J.Mar 7500
Bea 7500
Plasma HGH, mμg/ml
0
40
80
0 4 8 12 16 20
Months following treatment
MUB-9735

Fig. 2

The effect of the administration of hydrocortisone on fasting PGH levels in acromegalic patients was of considerable interest. Nineteen untreated patients received hydrocortisone (40 to 60 mg) 8.5 and 2.0 hours prior to obtaining fasting plasma samples. The fasting PGH values were lower in all patients except one when compared with the usual fasting values in these patients. The mean fall was 30% or approximately that seen following the administration of oral glucose in these patients. A similar suppression by hydrocortisone of PGH levels was observed in acromegalic patients studied at 6, 12, and 24 months following treatment, in spite of the appreciable changes produced by the treatment.

The plasma growth hormone level in the acromegalic patients appears to require considerable time to reach low levels following alpha-particle irradiation to the pituitary; however, the consistency of the depression in PGH levels is rewarding. This gradual fall in PGH observed in the acromegalic patient appears to be unique to this form of therapy and would seem to distinguish this mode of therapy from surgical hypophysectomy[5] or cryohypophysectomy,[6] which result in a relatively sudden depression in PGH levels.

In summary, a radioimmunoassay for HGH which employs iodine-125-labeled HGH and a precipitating antigamma globulin antiserum has been used to measure the plasma growth hormone levels in acromegalic patients before and after treatment with alpha-particle irradiation to the pituitary.

In addition to clinical signs of improvement of acromegaly following treatment, objective evidence of improvement was obtained in all treated patients in terms of their PGH levels. Following treatment of acromegalic patients, the fasting PGH level appears to be the best assessment of the effectiveness of the treatment.

References

1. S. M. Glick, J. Roth, R. S. Yalow, and S. A. Berson, Nature 199, 784 (1963).
2. J. A. Linfoot and F. C. Greenwood, J. Clin. Endocrinol. Metab. 25, 1515 (1965).
3. W. M. Hunter and F. C. Greenwood, Biochem. J. 91, 43 (1964).
4. F. C. Greenwood, W. M. Hunter, and J. S. Glover, Biochem. J. 89, 114 (1963).

5. S. M. Glick, J. Roth, and E. T. Lonergan, J. Clin. Endocrinol. Metab. 24, 501 (1964).
6. L. Lazarus, K. F. Bleasel, T. J. Connelley, and J. D. Young, Lancet 2, 90 (1966).

HEAVY PARTICLES IN ACROMEGALY

E. Manougian

Acromegaly is a disease characterized by overproduction of a growth hormone by a nonmalignant anterior pituitary tumor. The predominant cell type in the tumor usually contains eosinophilic granules, but occasionally these cells contain no granules.

The disease is uncommon but not rare. Indeed, since the changes it causes are often subtle, the disease may be more common than is generally believed.

Signs and symptoms of acromegaly can often be related to the pituitary tumor itself or to the effects of excessive growth hormone. However, certain symptoms, such as headache, , may sometimes be inexplicable. The tumor itself may compress surrounding structures such as the optic nerves, the oculomotor nerves, or the anterior pituitary gland, and thereby produce varying degrees of visual deficit, weakness of the extrinsic eye muscles, or hypopituitarism. The overproduction of growth hormone induces various changes, among which are the enlargement of the soft tissues of the body, including many of the internal organs and glands, as well as growth and cortical thickening of some or all bones (depending upon whether or not the epiphyses of the bones have closed). Changes in carbohydrate tolerance, including a form of resistance to the effects of insulin, also occur.

The causes of death are frequently due to the heart disease which develops more rapidly in this group of patients than in the general population. Less frequently death is due to (1) diabetes mellitus which develops in a large percentage of these individuals, or (2) hypopituitarism with secondary hypoadrenocorticism, or (3) extension of the tumor.

The objectives of treatment are (1) to correct the metabolic error, namely the excessive production of growth hormone, (2) to relieve tumor compression and (3) to prolong life expectancy.

Many forms of treatment have been tried for this disease. The various forms of therapy include surgical excision of the tumor, administration of necrotizing agents to the tumor, and inhibition of the adverse metabolic

effects through the use of medications. The various medications used include the hormones estrogen and testosterone (and, of course, insulin in individuals who developed juvenile-type diabetes). The necrotizing agents used are photons, elementary particles, ionized helium atoms, or various forms of freezing, such as the liquid-nitrogen probe. These necrotizing agents may be administered either externally or internally, this latter requiring surgical implantation of the necrotizing agent. As one of my mentors once remarked, the effectiveness of treatment for any given disease is inversely proportional to the number of forms of treatment for that disease. Nonetheless, it would appear that we are approaching a form of therapy that may become the "ideal" modality for this disease in that it best fulfills the objectives of treatment.

This ideal method is based on the use of external irradiation of the pituitary tumor. It has the least morbidity and no mortality risk. However, with the present forms of administration, we are limited in the dose we can deliver to the pituitary fossa without affecting surrounding tissues. Thus in patients with excessively large tumors, only less than optimal doses may be delivered, and in patients with neurological signs, such as visual deficit due to tumor compression, surgical excision of the tumor is still necessary. Thus, we have not reached a single ideal form of therapy for this disease. To show, however, that when applicable it does fulfill the objectives of the treatment, we present the following data.

Since February 1958, 57 acromegalic patients have been treated at the 184-inch cyclotron, using α-particle irradiation. Only two have died: one, 3-1/2 years after treatment, from a heart attack (following long-standing cardiomegaly); the other patient took his own life 7 months after treatment, before maximum effect of therapy was achieved. As can be seen from Fig. 1, there has not been sufficient post-treatment time to evaluate the effect of treatment on life expectancy.

The metabolic error in this disease is best studied by determining the concentration of circulating plasma growth hormone. This has been possible only recently. Other useful parameters are the glucose tolerance test, and the insulin tolerance test. Table I shows that there was a return to normal in nearly all subjects who had been followed for five to seven years after treatment. It is clear from these data that accelerated particles are effective in the therapy of acromegaly.

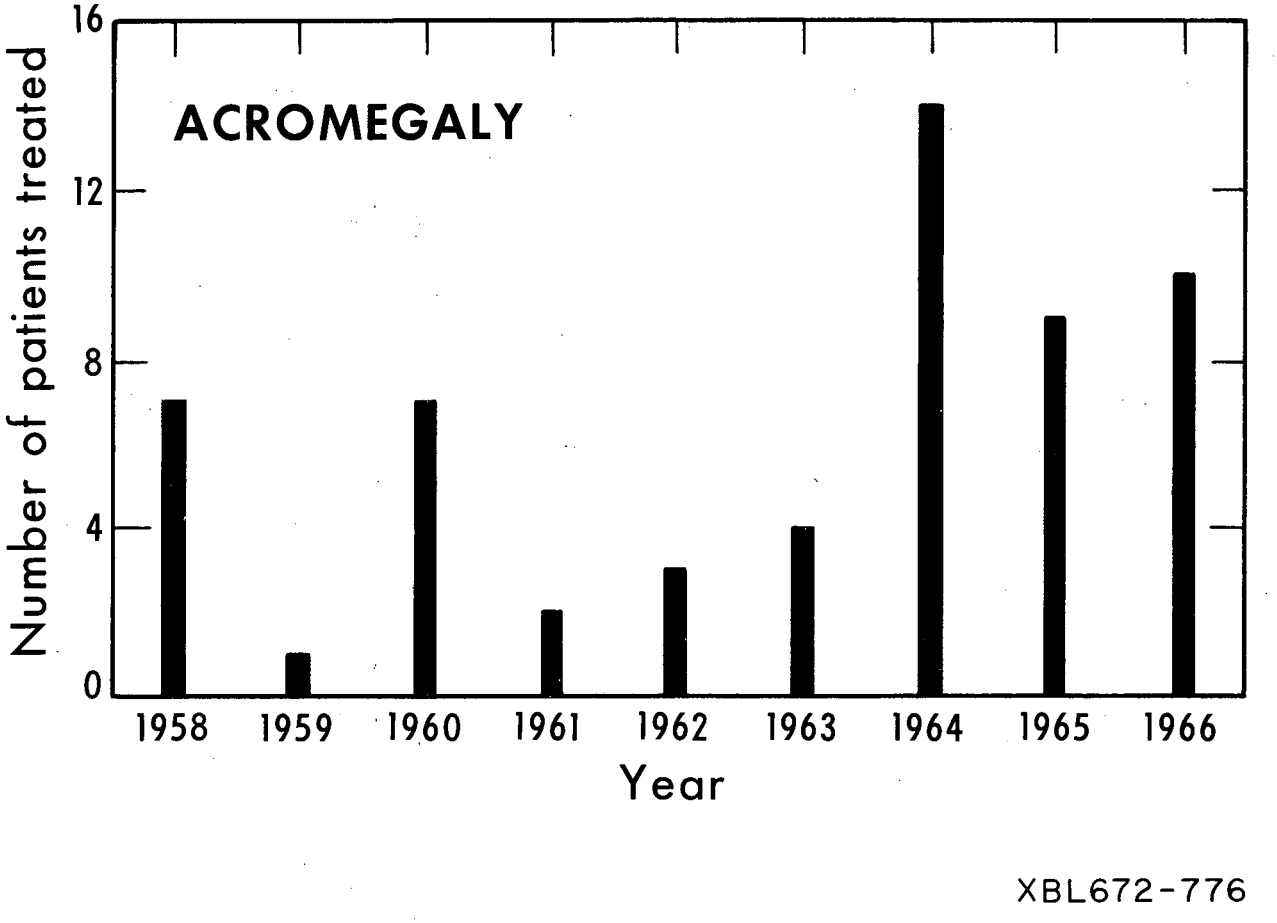


Fig. 1. Number of patients treated from 1958-1965.

Table I. Changes in glucose tolerance, insulin tolerance, and plasma growth hormone up to 7 years after tumor irradiation in acromegalic patients.

Test	Percent Abnormal		
	Pretreatment	2 years after treatment	5-7 years after treatment
Glucose tolerance	52	0	--
Insulin tolerance	86	12	--
Plasma growth hormone	100	73	10

The use of α-particles in the therapy of acromegaly is a step beyond the use of photons in the form of conventional X-ray and cobalt-60 gamma rays in that the α-particles can be delivered safely at larger doses than photons. However, further development is needed along the line of external radiation therapy to the pituitary tumors. A need exists for the study of the effectiveness of various ions as well as the need for techniques to better localize the radiation. It seems quite likely that with such methods we will be able to effectively treat not only acromegalic patients with moderately enlarged pituitary tumors, but also those with markedly enlarged tumors with or without compression effects.

HEAVY PARTICLES IN RADIOSURGERY OF THE PITUITARY IN CUSHING'S DISEASE

J. A. Linfoot, J. L. Born, C. A. Tobias, E. Manougian, C. Y. Cheung, and J. H. Lawrence

Adrenal hypercorticism may result from benign or malignant adrenal tumors or hyperplasia of the adrenal cortex. The former are ordinarily unilateral while adrenal cortical hyperplasia is always bilateral. Congenital adrenal hyperplasia results from an inborn enzyme defect affecting steroid biosynthesis, especially hydrocortisone production. Since hydrocortisone is the most potent naturally produced corticoid-inhibiting adrenocorticotrophic hormone (ACTH) release by the pituitary, it is essential for the proper function of the hypothalamic-pituitary-adrenal axis. Under normal circumstances the "feedback" mechanism operates in such a fashion that high levels of hydrocortisone suppress ACTH and low levels permit a rise in ACTH. In adrenal hyperplasia the hypothalamic-pituitary system is functioning normally but the low levels of circulating hydrocortisone result in increased ACTH secretion, causing a compensatory increase in production of all adrenocortical hormones. Androgens are notably increased, resulting in a masculinizing syndrome which is usually detectable at or shortly after birth. The defect can be detected by the presence of large amounts of hydrocortisone precursors which are not normally present in the urine. Congenital adrenal hyperplasia can be successfully treated by administering hydrocortisone or one of the newer synthetic analogues which suppresses pituitary ACTH secretion and provides adequate amounts of hydrocortisone to sustain life.

Cushing's syndrome is a term reserved for a group of acquired forms of hyperadrenocorticism. While the original description by Cushing in 1932[1] referred to those conditions associated with either hyperplasia or adenomata of the basophilic cells of the anterior pituitary, it was subsequently found that similar clinical pictures were seen with benign or malignant adrenal tumors (they are usually unilateral) and adrenocortical hyperplasia (always bilateral). The latter condition almost always is the result of an altered hypothalamic-pituitary-adrenal axis (i.e., small amounts of ACTH are released in spite of high circulating levels of hydrocortisone). However,

it has recently been found to result occasionally from the secretion of ACTH by malignant extra-pituitary tumors.[2] Between 15 and 25% of the former cases[3,4,5] are associated with clinically detectable pituitary tumors, although the true incidence of these tumors has not been determined. Many of these tumors are not detectable until after bilateral (total) surgical removal of the adrenal glands, which of course, corrects the hyperadrenocorticism but would not be expected to affect the altered hypothalamic or pituitary function. Although total surgical adrenalectomy,[6] made possible by the availability of adrenocortico-steroid therapy, has been a highly successful treatment for acquired adrenocortical hyperplasia, the relatively high incidence of pituitary tumors in these cases plus the abundant physiological data that altered ACTH secretion is the primary disturbance, has returned interest to the pituitary as the initial site for therapy as originally suggested by Cushing. Unfortunately orthovoltage X-ray and more recently high voltage X-ray therapy have only irregularly been associated with adequate and sustained remissions.[7,8] Surgical hypophysectomy is a formidable procedure and will almost invariably necessitate sacrifice of all pituitary trophic hormone function. Highly satisfactory results have been reported following the implantation of ^{90}Y into the pituitary fossa.[9] Results with such techniques are superior to conventional X-ray and gamma ray therapy, which cannot safely deliver adequate depth doses without endangering parasellar structures, e.g., the cranial nerves, hypothalamus, and temporal lobes. Heavy particle therapy is the only form of teletherapy that can accurately and safely deliver large doses of high energy particles to the pituitary and is the only non-surgical technique that has been shown to be effective in the treatment of acquired bilateral adrenocortical hyperplasia.[10] Since 1959, 12 patients with Cushing's syndrome due to bilateral adrenal hyperplasia, which we shall refer to as Cushing's disease, have been treated with heavy particles from the 184-inch synchrocyclotron. All of the patients were extensively studied to rule out the presence of adrenal tumors as well as extrapituitary ACTH-producing tumors.[3] Four of these patients (3F, 1M) had pituitary tumors which developed after total adrenalectomy. All had marked hyperpigmentation which is characteristic of this clinical syndrome. Two patients (JV, JA) had had previous adrenal surgery, but hyperadrenocorticism had recurred due to hypertrophy of a small adrenal remnant in one patient (JV)

and presumably aberrant adrenal tissue in the other. The remaining 7 cases (5F, 2M) had heavy particle therapy as primary treatment. The pretreatment hormonal data is summarized in Table Ia. All of the patients had slight to markedly elevated urinary 17-hydroxycorticosteroids (17 OHCS or hydrocortisone metabolites), but only 3 patients had elevated 17-ketosteroids (androgens). All of the patients had exaggerated responses during metyrapone tests (a procedure that separates hyperplastic from neoplastic adrenals, since the latter fail to respond). This proved to be a valuable means of following the cases after treatment. A marked response to ACTH was also seen in all cases. All but one of the cases had a suppression of urinary 17 OHCS after 3 days of high dose (8 mg/d) dexamethasone, a synthetic hydrocortisone derivative which is even more potent than the latter in suppressing ACTH secretion. These three tests indicate that while the pituitary and adrenals respond in an exaggerated fashion to metyrapone and ACTH and require an inordinately large amount of dexamethasone to reduce urinary steroid output, the direction of response in all instances is normal, suggesting that the threshold at which a given response occurs has been altered in Cushing's disease. Further confirmation of hypothalamic-pituitary abnormality in these cases lies in the loss of the normal diurnal or more correctly circadian variation (rhythm) in plasma steroid levels. Normally the early morning value is significantly higher than the evening value, while in these cases evening values were only slightly lower than the morning levels in all patients in whom it was tested.

The post-treatment data is summarized in Table Ib. All of the patients showed an early effect following heavy particle therapy (6000 to 12 000 rads delivered in 12 days), as evidenced by less exaggerated metyrapone tests and subsequently a gradual fall in basal urinary steroid excretion. After longer periods had elapsed, less exaggerated (normal) responses to ACTH were often observed. Dexamethasone suppressability was only studied in a few patients after treatment and by and large was unchanged even when the basal steroid excretion had returned to normal. The circadian rhythm was restored in only one patient and in fact, the rhythm was observed to be reversed in four patients in spite of a fall in steroid output. The significance of this remains to be determined.

Table Ia. Pre-treatment.

Patient		Urinary 17 OHCS	Urinary 17 KS	Metyrapone test	ACTH test	Dexamethasone test	Diurnal steroids
RK	F	↑	N	↑	↑	↓	A
RS	F	↑	N	↑	↑	↓	A
RC	M	↑	N	↑	↑	↓	A
DM	F	↑	N	↑	↑	↓	A
JV	F	↗	N	↗	↗	→	A
JA	M	↑	N	→	↑	↓	A
JD	F	↗	↑	↑	↑	↓	A
PO	F	↑	↑	↑	↑	↓	A
PD	D	↑	↑	↗	↑	↓	A

Table Ib. Post-treatment.

RK	N	N	↓	↓	--	A
RS[a]	N/↑	N	↓/↑	↓/↑	--	R
RC	N	N	↓	↓	↓	R
DM[b]	N/↑	N	↓/↑	↓/↑	--	R
JV	N	N	→	→	↓	N
JA	N	N	→	→	--	A
JD	→	→	↓	--	--	N
PO	N	N	↓	--	--	R
PD[c]	--	--	--	--	--	--

↑ increased; ↗ slightly increased; → no change; ↓ decreased

N = normal; A = abnormal; R = reversed.

[a] Relapsed at 24 months.

[b] Relapsed at 12 months.

[c] Treated too recently to evaluate.

As mentioned previously, all the patients showed an early effect of treatment on pituitary function and went gradually into partial or complete remission within 3 to 6 months after treatment. Two patients subsequently relapsed, one of whom underwent bilateral adrenalectomy 40 months after treatment. The second patient (D. M., who may have different hypothalamic pathology than the other cases) has had a severe neurological disability (spastic quadriplegia) which had been present for many years and was the result of an attack of encephalomyelitis at 11 months of age. She is currently being cared for in a county institution and is unlikely to tolerate any surgical intervention. Seven of the remaining patients appear to be continuing to show gradual improvement as indicated. The first patient was treated over 7 1/2 years ago and remains in remission. Typical response of these patients who have shown a favorable response to treatment is shown in Fig. 1. While the results are encouraging, further follow-up will be necessary before final conclusions can be reached. The four patients with hyperpigmentation and pituitary tumors have all had arrest of further pigmentation and two have shown gradual regression of pigment. Further sellar enlargement has not developed and none have required surgical decompression. Two of the nine patients have become mildly hypothyroid and required thyroid replacement. Other trophic hormone functions appear to be intact in the remaining patients.

References

1. Harvey Cushing, Bull. Johns Hopkins Hosp. 50, 137 (1932).
2. G. W. Liddle, D. Island, and C. K. Meador, Recent Prog. in Hormone Res. 18, 125 (1962).
3. R. M. Salassa, T. P. Kearns, J. W. Kernohan, R. G. Sprague, and C. S. MacCarthy, J. Clin. Endocrinol. Metab. 19, 1523 (1959).
4. D. H. Nelson, J. W. Meakin, J. B. Dealy, Jr., D. D. Matson, and G. W. Thorn, New Engl. J. Med. 259, 161 (1958).
5. D. A. D. Montgomery, Pituitary Tumors in Cushing's Syndrome, paper presented at the Twelfth Annual Meeting of Congress of Neurological Surgeons, Inc., Houston, Texas, November 1, 1962.
6. R. G. Sprague, R. E. Weeks, J. T. Prisetly, and R. M. Salassa, in Modern Trends in Endocrinology, ed. by H. Gardiner-Hill (Paul B. Hoeber, Inc., New York, 1961), p. 84.

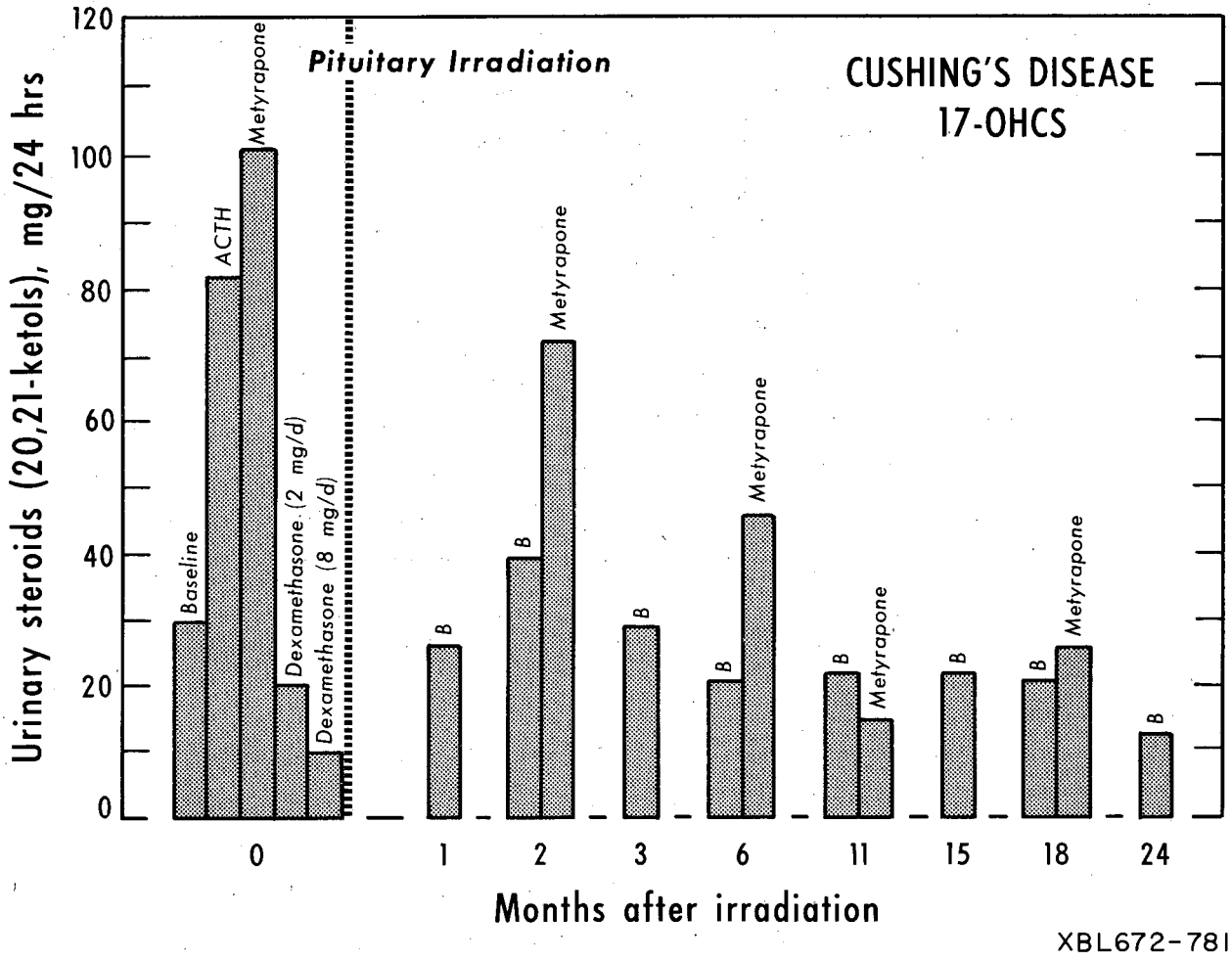


Fig. 1. Changes in urinary adrenal steroid excretion (basal and during metyrapone tests) in patient R. C. before and after heavy particle pituitary irradiation.

7. H. Lisser, J. Nervous Mental Disease 99, 727 (1944).
8. K. W. Thompson and L. Eisenhardt, J. Clin. Endocrinol. Metab. 3, 445 (1943).
9. G. F. Joplin, R. Fraser, R. Steiner, J. Laws, and E. Jones, Lancet 2, 1277 (1961).
10. J. A. Linfoot, J. H. Lawrence, J. L. Born, and C. A. Tobias, New Engl. J. Med. 69, 597 (1963).

PRELIMINARY OBSERVATIONS ON AN UNTREATED CONTROL SERIES IN DIABETIC RETINOPATHY

R. P. Kling* and J. A. Linfoot

A major problem in evaluating the effectiveness of therapy on the vascular complications in diabetes mellitus is the lack of specific information about the natural history of the lesions. Thus the question continually arises: How would the lesions in a given patient behave if left untreated? Several techniques have been employed in solving similar problems in clinical investigations, e.g., the double blind study, sham operations, alternating treatment schedules, etc. Properly done, these techniques are undoubtedly superior to prospective and retrospective case reviews. Unfortunately, certain ethical and practical problems arise when one is dealing with patients with advanced vascular complications and impending blindness. It has been our feeling, as well as that of others, that the visual prognosis is poor enough in the majority of patients treated for rapidly advancing diabetic retinopathy, that a group of untreated patients with similar complications followed in comparable medical facilities could serve as a "control" group. Two such groups have been set up. The first group consists of over 100 patients who have been refused heavy particle therapy for a variety of reasons, mostly, however, because of coexisting advanced renal and cardiovascular complications. Analysis of this group is now in progress. This report deals with a study established at another institution.

Since 1964, 116 patients with diabetic retinopathy have been followed at the District of Columbia General Hospital Eye Clinic. Ophthalmological and medical examinations, fundus photos, and appropriate laboratory studies have been obtained in the majority of the patients. The ages ranged from 17-74 years and the duration for diabetes 2-29 years; about one-half had a family history of diabetes. Diabetic control was considered poor to fair as

*Assitant Professor of Ophthalmology, Georgetown University School of Medicine, Washington, D. C. Consulting Ophthalmologist, Donner Laboratory of the Lawrence Radiation Laboratory, Berkeley, California.

a group and, in view of the economic status of the patients, might be expected to be somewhat poorer than the patient treated at the Donner Laboratory. In general, most of the patients' retinopathies would be considered "mild" or "early" since patients with anything more than minimal retinitis proliferans (irreversible scarring) or retinal detachment were excluded from the group. Thus these patients compare best with the Donner patients who had early retinopathy and were treated in the past several years.

Analysis of the fundus photos in these 116 patients reveals that about 50% of the patients had progressed during the $1\frac{1}{2}$-2 years of follow-up and about 50% have remained stable or progressed little in the interval. The latter group are mostly older patients with later onset of retinopathy.

A more detailed analysis of the specific lesions showed that microaneurysms and retinal and vitreous hemorrhages come and go. Venous and arteriolar changes never improved. Neovascularization never disappeared, although it did hemorrhage and scar. It never stabilized completely, although in some cases the rate of progression was remarkably slow. Hard retinal exudates diminished but were replaced by others, while soft exudates disappeared completely or were followed by hard exudate.

While this "control" series has not been followed a sufficient length of time to draw final conclusions, the changes that have been observed have provided valuable information on the natural behavior of retinal lesions in diabetics who have not received pituitary ablation. Extension of follow-up, of course, will enhance the value of this series and make it possible to make further clinico-pathological comparisons.

LONG-TERM FOLLOW-UP ON 45 PATIENTS WITH DIABETIC RETINOPATHY TREATED WITH HEAVY-PARTICLE PITUITARY-SUPPRESSIVE THERAPY BETWEEN 1958-1961

J. L. Born, J. H. Lawrence, J. A. Linfoot
E. Manougian, and R. P. Kling

The effect of pituitary manipulative procedures on diabetic retinopathy has never been precisely assessed. This is because the natural history of the disease shows a variability in the course of its progression to blindness[1, 2] which almost necessitates the establishment of a control group for each series of patients subjected to any of the treatment modalities. During the last several years we have been building up two such control groups of randomly selected untreated patients with diabetic retinopathy: one here at Donner Laboratory, University of California (100 patients now being analyzed) and another at Georgetown University School of Medicine by one of our collaborators (Kling and Linfoot[3]). However, we can find no control groups at present which exist concurrently from the time of institution of any long-term treatment series. Keeping this problem in mind, we have written the present report, which is a re-evaluation in January 1967 of a group of 45 patients who had received pituitary irradiation with heavy particles prior to December 1961 and who have now been followed for periods of 5 to 8 years. It is hoped that the conclusions reached, despite the absence of an analysis of our long-term control series, will aid in an understanding of the effect of pituitary heavy particle irradiation on diabetic retinopathy, and thus be of help in determining some of the factors important in selecting patients who are likely to benefit from this therapy.

During the period from March 1958 through December 1961, a total of 45 patients with diabetic retinopathy were treated. In this group there were 14 women and 31 men. The patients were almost equally divided between growth-onset diabetics (those whose diabetes was diagnosed before age 20) and maturity-onset diabetics, the distribution being 21 and 24 respectively. The median duration of diabetes among the growth-onset group was 22 years while that of the maturity-onset group was 15 years. The duration

of retinopathy before treatment of the growth-onset group was 2 years while that of the maturity-onset group was 1 year. We have 100% follow-up.

This group of 45 patient were patients whose remaining vision, however slight, was threatened with total blindness from progressive disease. They ranged from those having good vision to those with economic and legal blindness but not total blindness. It was assumed that if these patients had sufficient remaining vision to care for their economic and personal needs, the attempt to preserve any degree of vision was warranted. No patients were excluded from this early group on the basis of cardiovascular or renal disease. This is reflected in the loss of 22 patients from the series by reason of death; in all but 2 cases deaths were from cardiovascular or renal disease, the median survival perdiod being 33 months.

A histogram of the 45 patients comprising this series is shown in Fig. 1. This graph shows cumulatively the number of patients irradiated in the years 1958 through 1961, separating within each year those with stabilized vision in both eyes, those with stabilized vision in one eye, those whose loss of vision had progressed, and those who had died. The graph then passes over the intervening years of follow-up from 1962 through 1966 and re-evaluates the status and composition of this same group of 45 patients on January 1, 1967, when the follow-up period was at least 5 years (range 5-8 years). This 1967 evaluation shows that 23 of the original 45 patients are still living and 10 of these have stabilized vision in both eyes for the entire period of follow-up after treatment, the median duration of stabilization being 6 years and 1 month. An additional 3 living patients show stabilization of vision in one eye alone, the median duration of stabilization being the same. An additional 10 patients are still living, but their visual acuity has decreased so that they now show, in 1967, loss of their original vision, which usually occurred within 18 months after treatment. Twenty-two of the patients had died; a large number of these showed stabilization of vision in both eyes or in one eye up until the time of their death (median survival 33 months). The large number of deaths is not surprising since most of the patients had retinitis proliferans, a form of retinitis in which connective tissue and new vessel formation extends into the vitreous. Root et al.[4] have shown the very high mortality rate in diabetic patients after diagnosis of proliferative diabetic retinopathy because of its association with lethal

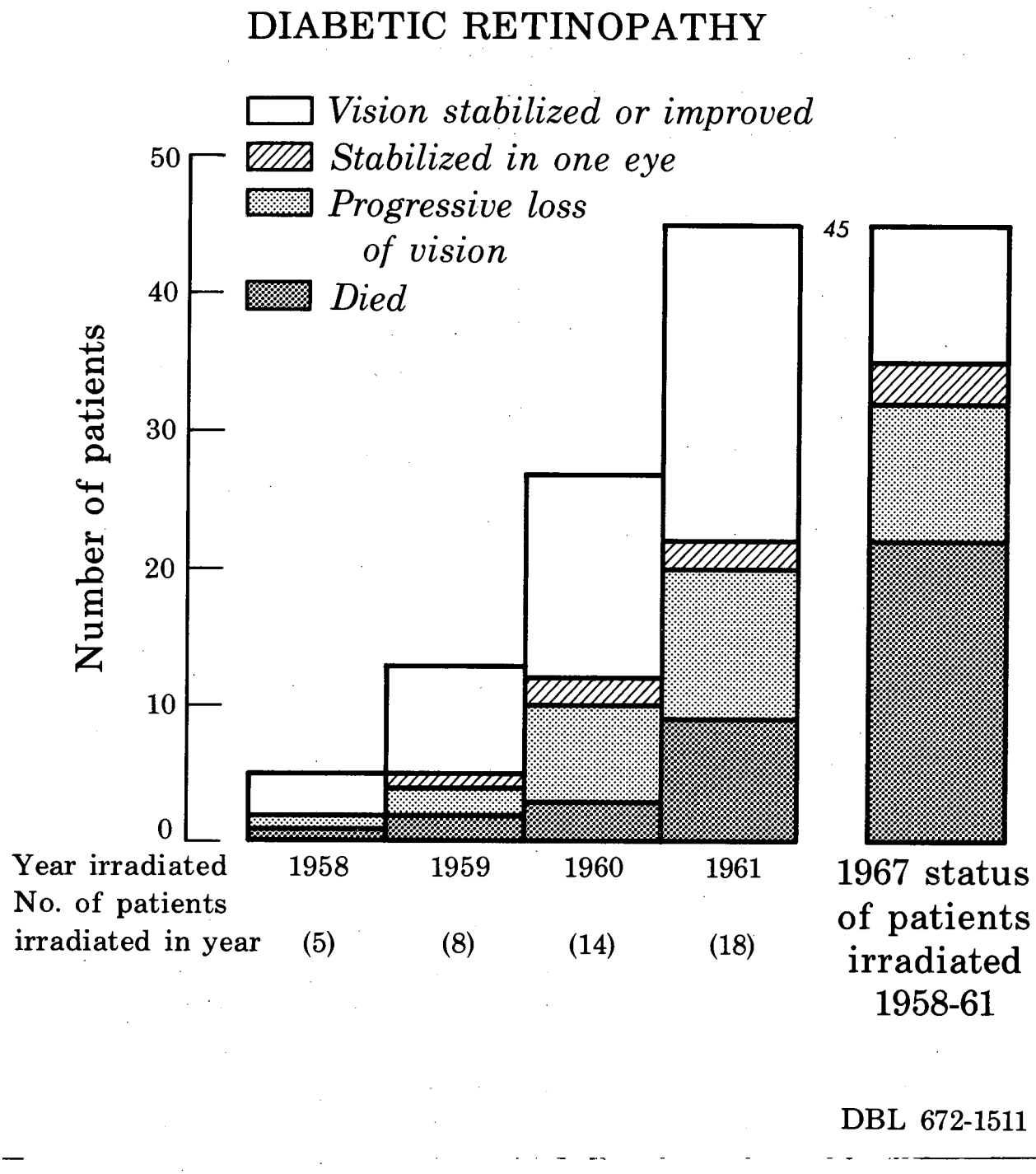


Fig. 1. Cumulative evaluation of visual status of all 45 patients with diabetic retinopathy who received heavy-particle pituitary irradiation from 1958-1961, with current status of all 45 patients in January 1967.

renal and cardiovascular disease. Their statistics show only 64% are alive after 5 years. The crucial problem of selection of patients for treatment then is, if possible, to hopefully select patients with the type of retinopathy which is not associated with advancing cardiovascular-renal disease.

Of more interest and of greater significance is Table I which shows the long periods of time during which many of these patients have maintained usable vision in both eyes or in one eye alone—these periods extending up to as much as 8 years.

The most significant group for study with respect to trying to find factors to use in selecting patients for this treatment are those patients who had survived 5 years or more and maintained stable vision in both eyes. There are 12 such patients in this group and these are cross-hatched in Table I. Ten of these, still alive, continue to have stabilized vision in both eyes: 4 at 5 years, 4 at 6 years, and 2 at 8 years post-irradiation. There is one other patient whose vision remained stabilized until the time of death 7 years after pituitary irradiation; in another patient who is still living, the vision was stabilized for 5 years before progressing to some loss of vision, in this instance as a result of acute hemorrhage.

It is of specific interest to look at the 10 living patients in this group who showed unequivocal long-term preservation of vision in both eyes ranging from 5 to 8 years in duration, as several factors are observed which may be pertinent to their response to treatment. Of these 10, 7 had proliferative diabetic retinopathy (PDR) prior to treatment, and it was further noted that all but one of these 7 had growth-onset diabetes. The remaining 3 patients in this group had no prior evidence of PDR in either eye, and all 3 of these were maturity-onset diabetics. In view of the facts that the overwhelming majority of all 45 patients irradiated in this series had PDR, and that the series was almost equally divided between growth- and maturity-onset types of diabetics (Table II), it would seem warranted to conclude even on this small series that growth-onset diabetics with PDR seem to show some amelioriation of their disease process sufficient for the stabilization of their vision, whereas those diabetics with mature onset of their disease seemed to show long-term benefit primarily when they did not have PDR at the time of treatment (Fig. 2). Part of this latter observation may be due to the fact that patients with later onset of their diabetes show a higher rate of

Table I. Visual status in January 1967 of 45 patients who received heavy-particle pituitary irradiation 1958-1961.

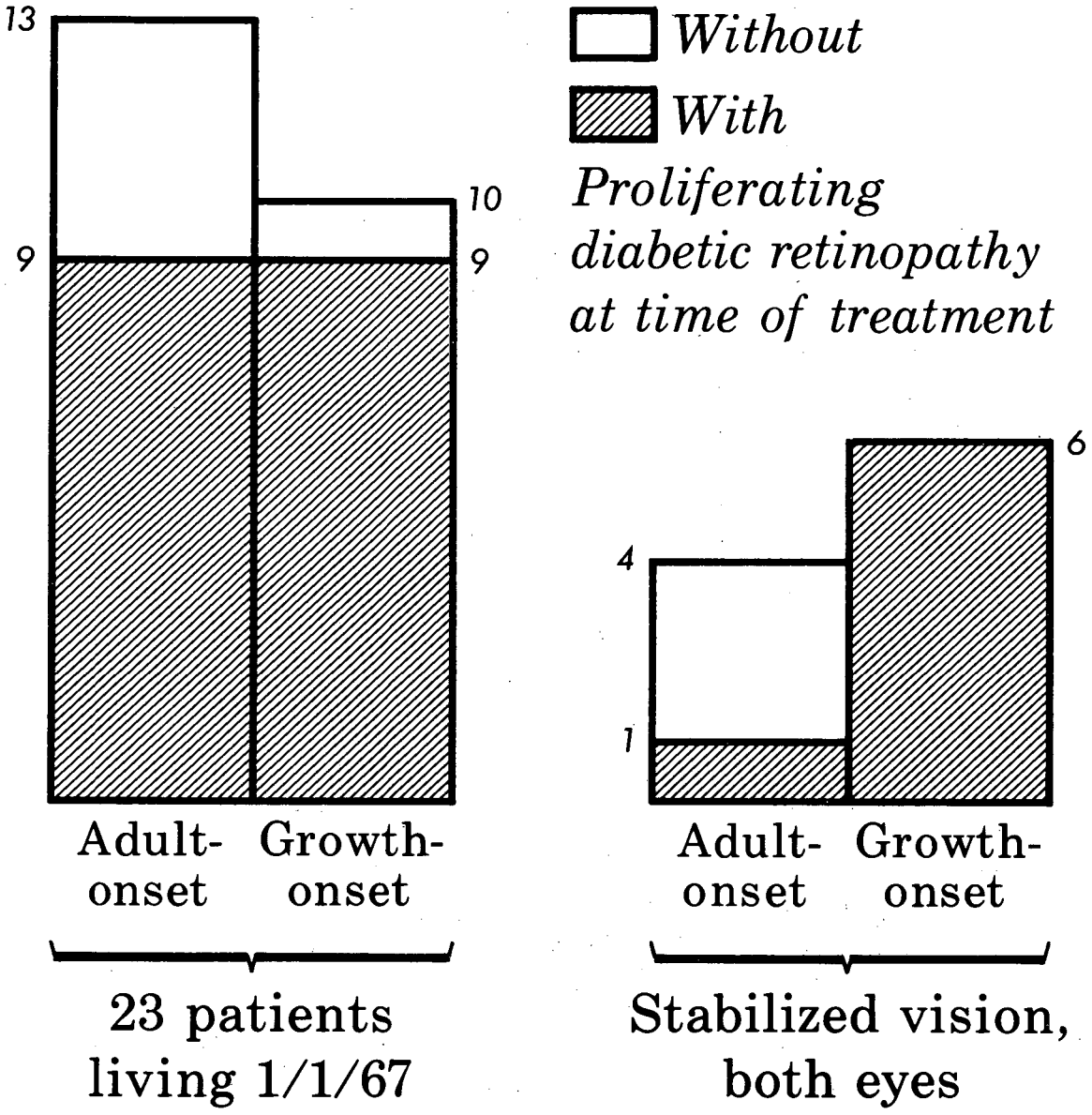
Evaluation of visual status	Number of patients		Duration of observation post-irradiation (years)							
			1	2	3	4	5	6	7	8
Maintained pre-irradiation visual status in both eyes	Living	10					4	4		2
	Deceased	7	1	1	3	1			1	
Maintained pre-irradiation visual status in one eye	Living	3					1	1	1	
	Deceased	5	2			1	2			
Progression of visual loss in both eyes	Living	10	7	1	1		1			
	Deceased	10	7	3						

Table II. Proliferating diabetic retinopathy in growth-onset diabetics and maturity-onset diabetics prior to heavy-particle pituitary irradiation administered 1958-1961.

Class of diabetic	Number of patients	Proliferating diabetic retinopathy		
		Present	Absent	Not known
Growth-onset	21	18	1	2
Maturity-onset	24	15	5	4
Totals	45	33	6	6

deterioration of vision. Caird[1] has pointed out that the estimated rates for eyes with retinopathy increase steadily with increasing age-at-diagnosis of diabetes. In the few maturity-onset diabetics ho had PDR and showed unequivocal benefit, it is noted that the onset of their diabetes was in all instances before age 40 (range 23-38 years).

In looking for some effective means to evaluate whether or not pituitary irradiation was beneficial in arresting the progress of diabetic retinopathy, it is necessary to look to the article of Beetham.[2] Beetham, in his paper on the Visual Prognosis of Proliferating Diabetic Retinopathy, gives us data on patients who had proliferating disease but had not been subjected to ablative or suppressive pituitary procedure, and thus these data are of great value in the present analysis. In 2 series of cases totaling 351 patients, he is able to point out that only 8 patients with PDR have shown a quiescent fundus picture for more than 4 years, and that 27 others show a similar ophthalmological picture, but these have been observed for shorter periods of time (6 for 3 years, 3 for 2 years, and 18 for 1 year), making a total of 35 or about 10% of the total group. One of the sub-groups which he details shows the degree in rate of visual loss in 242 patients who had PDR present at first observation. In his series it is clearly evident that at least 61, and probably a much greater number than this, have been followed for periods in excess of 5 years. Considering our group of 45 patients (33 with known PDR), 26 (21 with PDR) survived to be followed for 5 years or more; there



DBL 672-1502

Fig. 2. Responses obtained from adult-onset and growth-onset diabetics 5-8 years following pituitary irradiation.

are 9 with PDR who have shown a quiescent fundus picture for more than 5 years. The details of these patients are shown in Table III. This represents in our series 27% who have done well as against the 10% reported by him with a much broader group, including many short-term follow-up patients. We are cognizant that we have not evaluated other aspects of retinopathy which may further aid in the selection of patients, such as the presence or absence of hemorrhage, exudates, microaneurysms, neovascularization, and fibrosis, and those considerations relative to these factors.

It must be further considered, though not definitely concluded, that heavy-particle pituitary irradiation may have a general beneficial effect on diabetic patients through lowering the insulin requirements (. necessary for control of their diabetes where complications of advancing cardiovascular-renal disease are not present.

Summary and Conclusions

A preliminary report is presented on 45 patients with diabetic retinopathy receiving heavy-particle pituitary irradiation between 1958-1961 with the objective of determining factors pertinent to the selection of future patients. A sample analysis of the data at this time leads us to conclude that the optimum candidates for this type of procedure are:

(1) Growth-onset diabetics: Those with or without proliferating diabetic retinitis with little or no cardiovascular or renal disease.

(2) Maturity-onset diabetics: Those with no proliferating diabetic retinitis and with little or no cardiovascular or renal disease. Less than optimum but still possible for consideration would be those with proliferating diabetic retinopathy provided the onset of their diabetes was before age 40.

References

1. F. I. Caird and C. J. Garret, Prognosis for Vision in Diabetic Retinopathy, Diabetes 12, 389-397 (1963).
2. W. P. Beetham, Visual Prognosis of Proliferating Diabetic Retinopathy, Brit. J. Ophthalmol. 47, 611-619 (1963).
3. R. P. Kling and J. A. Linfoot, Preliminary Observations on an Untreated Control Series in Diabetic Retinopathy (in this volume).
4. H. F. Root, S. Mirsky, and J. Ditzel, Proliferative Retinopathy in Diabetes Mellitus: Review of 847 Cases, J. Am. Med. Assoc. 169, 903 (1959).

Table III. Patients with proliferating diabetic retinopathy who maintained stable visual status for 5 years or more following treatment.

Case No.	Period of observation (years)	Onset of diabetes to onset of retinopathy (years)	Duration of diabetes at time of treatment (years)	Duration of diabetes when last observed (years)	Visual acuity when last seen (better eye)
1	8	24	27	35	20/20
2	8	21	22	30	20/50
3	7[a]	13	14	21	20/50
4	6	20	26	32	8/100
5	6	20	22	28	20/20
6	6	13	21	27	HM
7	5	11	23	28	20/20
8	5	10	18	23	20/20
9	5[a]	9	10	15	20/100

[a]Deceased.

RADIATION STUDIES IN PARKINSON'S DISEASE

R. Tym* and R. Weyand*

The triumph of heavy particle radiation techniques for obliterative operations on the pituitary gland and its associated tumors has been well attended to by other contributors. From a practical neurosurgical viewpoint these methods have set new, almost impossibly high standards of safety and effectiveness for what, at open operations, are procedures forever fraught with set risks of brain damage and hemorrhage. The introduction of further improvements in alpha particle beam collimation has now made it possible to extend the facility to the treatment of movement disorders such as Parkinson's disease.

In the generalized brain diseases that give rise to movement disorders, the destruction of from 0.03 to 0.3 cc of the ventrolateral nucleus of the thalamus of the brain will tend to the abolition of the unwanted movement of the limbs and the re-establishment of normal control. In general neurosurgical practice, this selective destruction of brain was impossible until the introduction of stereotactic surgery 12 years ago. This form of surgery now allows an electrode or cold probe to be passed through a hole in the skull and, by remote control, on through the brain to the target nucleus in the thalamus. The brain around the tip of the probe is coagulated either by heat or cold.

Some advantages accrue from the possibility, by selective placing of the probe and by incremental coagulation, of tailoring the site and the size of the lesion to the improvement in the individual patient. But there is not an inconsiderable morbidity and mortality associated with blind passage of the probe through the brain and the reactive swelling around the acute lesion at its tip. In old patients this morbidity and mortality is increased by their more atrophic brains and the fragility of their cerebral blood vessels.

---

*Consultants in neurosurgery to the Donner Laboratory of the Lawrence Radiation Laboratory, Berkeley, California.

In placing a radiation lesion of fixed size in a predetermined site by using an external beam of heavy particles, there is no opportunity to tailor the site or size of the lesion to the improvement of the patient at the time of the lesion. As with all radiation damage, there is a prolonged interval between the deposition of the radiation energy and the appearance of functional biological damage. There is no opportunity to stimulate the deeply placed neurons with radiation. Yet exactly this pragmatic approach is, in fact, advocated by many "conventional" stereotactic neurosurgeons using electrodes or cold probes. In their hands, all patients thought suitable for operation have a lesion of predetermined size placed routinely at the most probable site of the target nucleus of the thalamus, and the clinical result is accepted without modification.

We in Berkeley, therefore, do not think it unreasonable to join the pragmatists in occasionally advocating a lesion of predetermined size placed in anatomical abstraction. And in addition, our radiation lesion confers the advantage of not opening the skull or traversing the brain with a probe. We confine radiation lesions to older patients in whom we judge the rate of deterioration of movement caused by the disease to be slow.

Case Report: Mr. G. L. C.

A white, now 73 year old retired farmer developed signs of left-sided Parkinsonism 13 years ago. The disease process progressed slowly until he was severely incapacitated 5 years ago. At that time a "conventional" stereotactic thalamotomy was performed on his right thalamus by using an injection of alcohol to coagulate the target nucleus. There was gratifying improvement in the control of his limbs and a virtual disappearance of the unwanted abnormal movement.

Six months after the successful thalamotomy, Parkinsonism appeared in his right limbs and progressed more rapidly than it had in his left. One year ago he was again severely disabled. He was very reluctant to go through further conventional surgery at his age and he requested to be considered for heavy particle beam therapy.

He was considered in every way suitable for this procedure and was admitted to the Donner Pavilion. He underwent initial pneumoencephalographic studies to determine the three coordinates of the target nucleus with reference to a detachable stereotactic head holder fixed to three

steel screws temporarily placed in the outer table of the skull under local anesthesia. Three days later he was taken to the medical cave of the 184-inch cyclotron. The stereotactic head holder was reattached to the steel screws and his head was thereby aligned with the beam aperture coinciding with the coordinates of the target nucleus. His head was held to the head-rotation mechanism by the usual face mask used for pituitary radiation, which enabled, in this instance, his head to be rotated about the lower pole of the ventrolateral nucleus of the thalamus.

The beam of 986 MeV alpha particles emerged through a circular aperture 6.00 mm in diameter. With the head rotation used, the isodose planes described a prolate spheroid with the long axis in the coronal plane of the head. A central volume of 0.032 cc received a radiation dose of 9000 rads and a volume of 0.27 cc received 4500 rads. The radiation time was 40 minutes and immediately this was over he was allowed to go home--the small placement screws having been removed.

Nine months after the radiation treatment it was reported that all progression of the signs and symptoms of the disease had ceased and that for a significant period of each day the hitherto obtrusive tremor was entirely absent.

It is expected that over the succeeding 9 months he will continue to improve but at a decreasing rate. The attractive theoretical possibility exists that as the disease process slowly progresses so will the size of the lesion slowly progress to keep in check the appearance of more unwelcome signs of the Parkinsonism.

Future improvements in collimation will enable a higher control dose to be given to the target nucleus without increasing the volume receiving 4500 rads. There will then be a more rapid appearance of clinical effect without any sacrifice of safety. The scope of heavy particle radiation treatment of movement disorders will then be vastly increased.

Heavy particle radiation lesions will soon be used in the treatment of temporal lobe seizures and in severe grand mal seizures with generalized electroencephalographic abnormalities by the placement of small, bilateral, diencephalic lesions.

As with certain of the pituitary tumors, where the heavy particle radiation treatment is now second to none throughout the world, there is to be a gradual encroachment of radiosurgery into the control and treatment of generalized brain disorders.

APPLICATION OF THE OMNITRON TO RESEARCH IN NEUROLOGICAL SURGERY

R. A. Fink*

The opportunities for advances in research in neurological surgery and applied basic science, utilizing the equipment available at the Lawrence Radiation Laboratory, Berkeley, are legion. Work done in the past includes use of the heavy particle beam in the treatment of disorders of the pituitary gland and related structures. The present program includes therapy for such conditions as diabetic retinopathy, pituitary tumors, and metastatic carcinoma of the breast with emphasis upon hypophyseal ablation. At present, emphasis is being placed upon improvement of the present techniques, and for the extension of the basic method into other areas of the nervous system dysfunction.

The creation of deep cerebral lesions has been a problem which has been dealt with at great lengths by workers in the neurological sciences for a number of years. The field of stereotactic neurosurgery has been well advanced, utilizing the orthodox techniques for production of lesions within brain matter. These methods have included electrocoagulation, chemical destruction, cryonecrosis, and the implantation of radioactive substances, notably beta emitters, into the brain. All of these methods have as their one disadvantage the fact that a surgical invasion of the intracranial cavity is necessary, usually by means of trephine openings or, in some cases, by actual craniotomy. The heavy particle beam, in the form expected to be available with the development of the Omnitron facility, would seem to be of great help to the furthering of methods in stereotactic neurosurgery to the above-listed disease conditions and also to other conditions which had previously been inaccessible to the stereotactic surgeon's attack. These new applications will include such conditions as deep, midline, inoperable brain tumors, vascular malformations, and physiological attacks on various nerve fibers and tracts lying deep within the brain and inaccessible to the standard stereotactic approaches.

*Neurological Fellow of the Donner Laboratory.

With the neuroanatomical knowledge, such as that obtained in previous, orthodox stereotactic neurosurgical approaches, the heavy particle beam, of the new Omnitron, could be made to perform some of these tasks which had previously either been inconvenient or impossible to perform. Thus, surgery for inoperable intracranial tumors and vascular malformations could be rendered feasible by judicious application of the heavy particle beam.

Finally, utilizing the positron camera, exact plots of the position of the lesion and of the associated Bragg-peak effect could be made by monitoring positron-emitting induced activity in the brain.

HEAVY ION EFFECTS ON ASCITES TUMOR CELLS

J. M. Feola

Ascites tumors are those in which active multiplication of free neoplastic cells and/or cell complexes can be shown to occur in the peritoneal fluid, leading to a high absolute and relative concentration of tumor cells, that is to say, to a nearly pure culture.[1]

Besides this unique feature, work with ascites tumors offers a number of advantages for basic studies in radiobiology: easy transplantation, preservation of transmissibility upon cold storage, exact inoculum dosage, correlation between the number of cells inoculated and survival time, growth in cell suspension and in cell cultures.

Quantitative studies of mammalian cellular radiobiology were limited to the precise techniques of culture in vitro until the methods first reported by Hewitt[2] and Hewitt and Wilson[3] allowed for measurement in vivo of "cell viability" by using cell dilution techniques.

Although the greater biological effectiveness of heavy particles over electromagnetic radiation has been studied by J. H. Lawrence in normal tissue[4,5] as well as in neoplasms in animals,[6,7,8] the need for a better understanding of the mechanisms of action of densely ionizing radiation as well as the necessity of more precise measurement of the decrease of the oxygen effect with increasing LET, stimulated the initiation of a program of research making use of the new tools and methods available.

Sillesen et al.[9] initiated this series of studies with two different tumors: a lymphoma (near diploid, 41 chromosomes) and a mammary carcinoma (aneuploid), both of which "take" in the several lines of mice used after intraperitoneal injection of from 1 to 20 cells. Both of these transplantable tumors have characteristics similar to the transplantable lymphoma[10,11] and mammary carcinoma[6] used in the early studies with heavy particles in 1935.[7,12]

Different, but genetically related strains of mice, such as A/Heston, LAF_1, A/JAX, and CAF_1 were used. The tumors were passed every 6 or 7 days in ascites form by intraperitoneal inoculation for lymphoma and every

10 to 12 days for the slower-growing mammary carcinoma (TA_3 tumor). A week after inoculation the lymphoma mice can be used as donors; mammary carcinoma mice can be used after 12 days.

The cells, contained in ascitic fluid, were placed in thin chambers and exposed to varying doses of radiation by using 910 MeV alpha-particles from the 184-inch synchrocyclotron. The LET of these particles in tissue is 1.7 keV/μ. In order to irradiate the cells with a higher LET a 2.176-inch copper absorber was placed in front of the irradiation chamber, thus making the Bragg peak available. The LET in this case is not well defined; due to the initial energy spread of the beam and the range-straggling of the particles as they pass through an absorbing medium, the cells are exposed to a spectrum of LET's ranging from 10 to 250 keV/μ. In order to irradiate the cells with a more precisely defined LET, experiments were carried out using the heavy ion linear accelerator (Hilac). Special techniques were used to spread out the cells in a very thin layer and irradiate them with the shank, or plateau, of the heavy ions, avoiding the Bragg peak and the corresponding spread in LET values. 200 kV X-rays were used as the baseline for the comparison of various LET's as related to RBE.

Following exposures, the cells were counted, serially diluted, and then injected intraperitoneally into the mice in groups of five animals for each dose and titration level. The mice were then followed for lethality, the follow-up period lasting about eight weeks for the lymphoma and 12 weeks for the mammary carcinoma.

LD_{50}'s (the number of cells necessary to kill 50% of the animals), and surviving fractions (the ratio of the LD_{50} for the control group to those of the irradiated samples), were calculated by using the methods of Reed and Muench[13] and Hewitt and Wilson.[3]

The RBE for the Bragg peak of 910 MeV alpha-particle beam as compared with 200 kV X-rays was found to be:

For TA_3 : 1.9

For lymphoma: 1.8.

Preliminary studies on the oxygen effect were done by irradiating the lymphoma cells in oxygen and nitrogen environments. Results obtained with three heavy ions are shown in Fig. 1. These results with ^{7}Li, ^{11}B and ^{20}Me ions show a decreasing oxygen effect. However, and due to the experimental difficulties

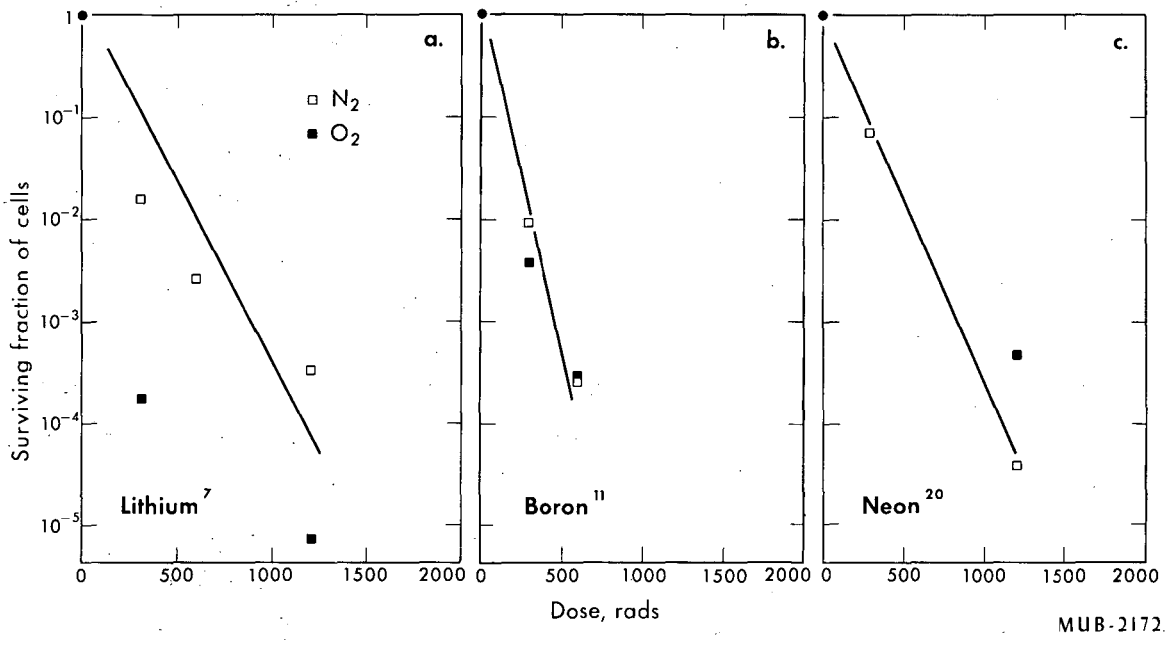


Fig. 1. Surviving fractions of lymphoma cells irradiated with ions from the Hilac, using both nitrogen and oxygen atmospheres, and then injected intraperitoneally into LAF_1 mice.

found by the authors, this work cannot be considered quantitative.

Tobias and Todd[14] and Todd[15] have shown that when cultures are irradiated with heavy ions there is a steady reduction in the effect of the presence of oxygen with increasing LET until its apparent abolition with $dE/dx = 3000$ MeV-cm^2/g or greater. Whether or not an extrapolation of these results to an in vivo situation may be done is still a subject for discussion, and this is one of the reasons why more experimentation in vivo is desirable.

Schmidlin et al.[16] extended the work initiated by Sillesen et al. The in vitro irradiation technique was improved by using very thin Lucite chambers 0.125 mm deep. The cells in the chambers were covered with dialyzing paper, which when kept moist permits water-saturated gas to pass through. The gas is continuously flushed through a gas chamber that fits over the Lucite chamber and is covered with Mylar. By using oxygen or nitrogen for flushing, it was hoped to study the difference in radiosensitivities of oxygenated and anoxic cells. In these experiments only the lymphoma cells were used and only one strain of mice, namely, the LAF_1, which is a hybrid between $C_{57}L$ and A/Heston. Besides the irradiations performed in vitro, Schmidlin irradiated the cells in vivo in either adult or infant mice under three different conditions: (a) the mice were irradiated while breathing air, (b) the mice were sacrificed a few minutes prior to irradiation in order to have anoxic tumor cells, (c) the mice were irradiated after injection of H_2O_2 intraperitoneally to provide well-oxygenated neoplastic cells.

Schmidlin's results were obtained by using protons from the 88-inch cyclotron at LET's of 1.5 to 2.0 keV/μ (plateau) and 3 to 7 keV/μ (peak) as well as alpha-particles from the Hilac at an LET of 18.6 keV/μ and comparing with control experiments using 200 kV X-rays. His results follow qualitatively what is theoretically expected, and show that quantitative results in vivo are possible, provided that a more powerful analytical technique as well as a better control of the variables involved can be obtained.

Because of the low precision of the accumulation method of Reed and Muench[13] and the serious doubts raised regarding its appropriateness,[17,18] Feola et al.[19] decided to use Litchfield and Wilcoxon's method[20] to obtain the LD_{50}'s and their 95% confidence interval with occasional control of results by means of probit analysis.[21]

The small number of animals per dilution and the flushing system proved to be the main sources of error in previous experiments. By increasing the number of animals injected to 10 per dilution and by careful control of the flushing system, reliable and reproducible results have been obtained, as shown in a typical situation in Fig. 2. Points at the same dose level are from independent experiments.

Previous results with the lymphoma cells irradiated in vitro and grown in vivo have already been given.[19] Those experiments, performed with 230 kV X-rays (HVL = 1.6 mm Cu), and the alpha particles produced by the 184-inch synchrocyclotron and by the 88-inch cyclotron have been completed and results are presented in Table I. Figures obtained for the alpha-particles from the 184-inch synchrocyclotron are of particular interest in view of the extensive use of the Bragg peak in therapy.[22,23] The "gain factor," that is, the ratio of the oxygen enhancement factor for X-rays to that for alpha-particles, as defined by Fowler and Morgan,[24] indicates that for equal injury to well-oxygenated tissues, the effect on anoxic cells is increased as if the dose to these cells only had been increased in the ratios given. This decrease in the oxygen effect has been calculated by Tobias and Manney,[25] and although the values observed for the gain factor are still smaller than the theoretical ideal values (2.5 to 3.5) for total destruction of the anoxic cells, they show what might be expected of beams of heavier ions of adequate energies and intensities like the Omnitron would be able to provide.

X-ray irradiations in vivo have been performed at different tumor ages starting with 3-day-old tumors. A high degree of anoxia have been observed only for 8- and 9-day-old tumors. The D_0 for 7-day-old tumors is 260±50 rads. This value is closer to the anoxic curve obtained for irradiations in vitro than it is to the hyperoxic curve. Although these experiments are still in progress, the same type of anomalous results as obtained by Belli and Andrews[26] with the 3-day-old tumors has been observed. However, not enough evidence has been found to support the idea of a population of mixed sensitivity.

As the milieu in which ascites tumors evolve seems to be suitable for measurements of oxygen tension, a series of experiments will be performed to correlate tumor age with oxygen pressure by measuring it in vivo

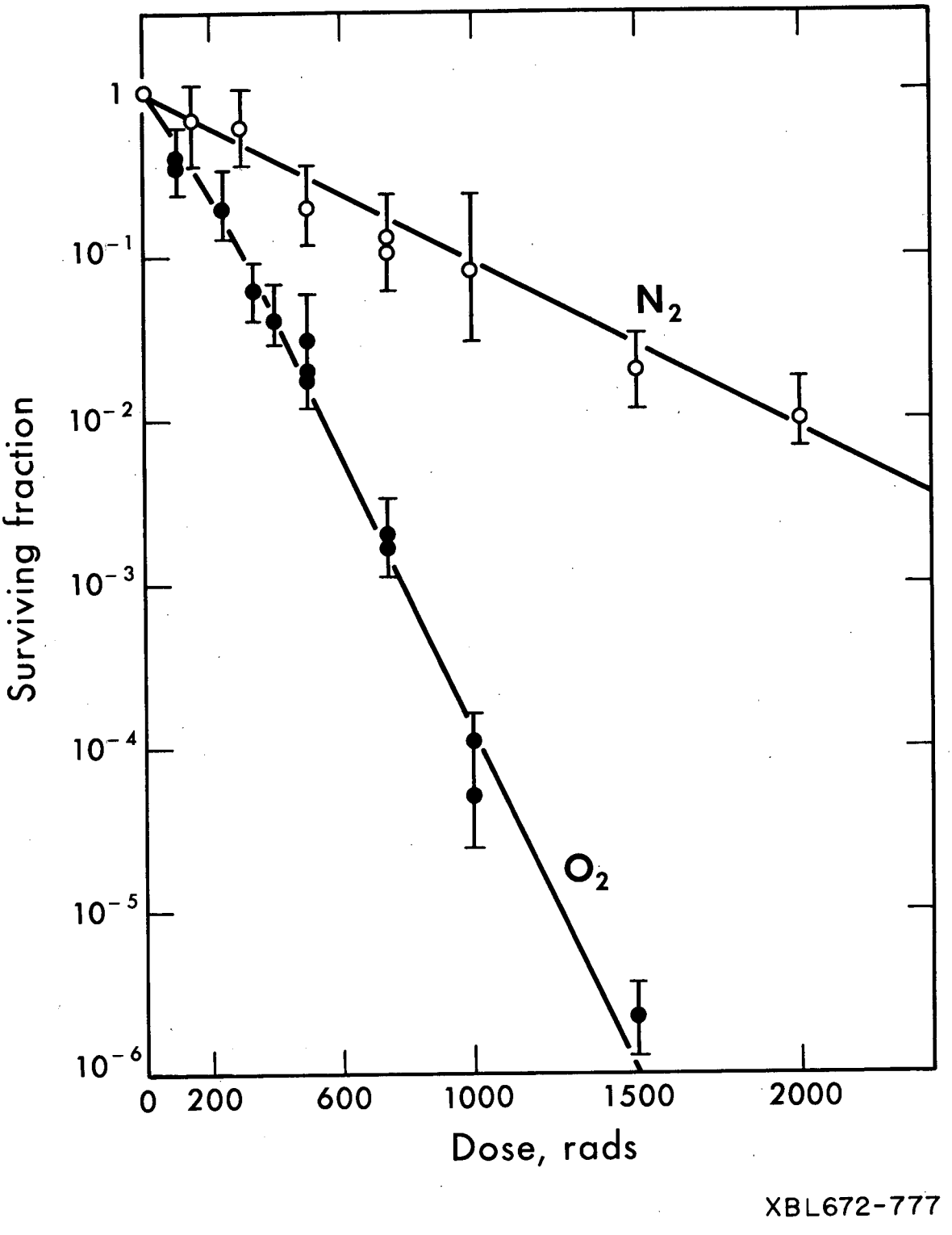


Fig. 2. Lymphoma cells irradiated with 230 kV X-rays under hypoxic and hyperoxic conditions. Standard errors are shown. Points at the same dose level show degree of reproducibility obtained.

Table I. Effects of alpha-particles and X-rays on lymphoma cells irradiated in vitro and grown in vivo.

Accelerator	Radiation	LET (keV/μ)	Number of animals[b]	D_0 (N_2)	D_0 (O_2)	$\frac{D_0(N_2)}{D_0(O_2)}$[a, c]	RBE (N_2)[c]	RBE (O_2)[c]	Gain factor[c, e]
250 kV X-rays	230 kV X-rays	1.5-3	1500	380±50	100±10	3.8±0.9	1	1	
184-inch synchro-cyclotron	910 MeV α-particles, plateau	1.7	500	330±50	130±30	2.5±1.0	1.1±0.3	0.8±0.3	1.5±1.0
184-inch synchro-cyclotron	85 MeV α-particles, peak[d]	10[d]	1000	210±30	100±20	2.1±0.7	1.8±0.5	1.0±0.3	1.8±1.0
88-inch cyclotron	118 MeV α-particles, plateau	8	1000	330±70	115±25	2.0±1.2	1.1±0.4	0.9±0.3	1.9±1.1
88-inch cyclotron	38 MeV α-particles, near peak	21	1000	200±20	110±20	1.8±0.5	1.9±0.4	0.9±0.3	2.1±1.1

[a] Oxygen enhancement ratio.

[b] Each experiment involves about 250 mice.

[c] Errors have been propagated.

[d] Averages of energy and LET distributions are indicated.

[e] The ratio of the oxygen enhancement factor for X-rays to that for α-particles.

with a microelectrode. It is expected that with these measurements a more quantitative approach to the oxygen effect will be possible.

Work with the TA_3 cells has progressed on the same lines as the research done with lymphoma cells. Preliminary experiments show that these cells are more radiosensitive than the lymphoma cells. These results are in agreement with predictions made by Tobias and Manney[25] that a small but definite differential may exist in the radiosensitivity of aneuploid tumor cells and that of cells of normal diploid state.

The TA_3 cells grow well *in vitro*, maintaining a high plating efficiency, and experiments are in progress to compare the effects of heavy ions *in vivo* and *in vitro*. Although this has been extremely difficult due to the fact that these cells lose their tumor-forming ability quite rapidly when maintained in cultures, it has opened new possibilities to study the reasons for these changes.

Two experiments have been done, irradiating lymphoma cells *in vivo* with a 90 MeV pion beam.[27]

In the first experiment an attempt was made to establish the peak-to-plateau effectiveness in cell-killing as well as the oxygen enhancement ratio by irradiating LAF_1 mice bearing 3-day-old tumors (supposedly well oxygenated) and 7-day-old tumors (supposedly more hypoxic) in the plateau and peak regions of the beam. No significant differences were found due to tumor age, but the surviving fractions for the peak were significantly lower than those for the plateau. These results encouraged the design of the second experiment in which animals bearing 5-day-old tumors at the beginning of irradiation were used for the purpose of comparing the effectiveness of the negative pions at peak with that at plateau. The irradiation took 40 hours and the total doses at plateau ranged from 145 to 250 rads while those at the peak ranged from 220 to 380 rads. A replication experiment with ^{60}Co γ-rays was performed *a posteriori* by using dose rates of 5 R/hr and 12.5 R/hr and maintaining the animals and all other conditions the same as in the pion experiment. Results of these experiments are shown in Fig. 3. Inconsistency in the plateau results prevents a reliable estimation of the D_0 in this region. The value obtained for the peak gives, if γ-rays are used as a baseline, an RBE of 5.4 ± 1.8. If an RBE of 0.8 is assumed for gamma rays relative to X-rays, as has been reported,[28-30] the RBE becomes 4.3 ± 1.8 relative to X-rays.

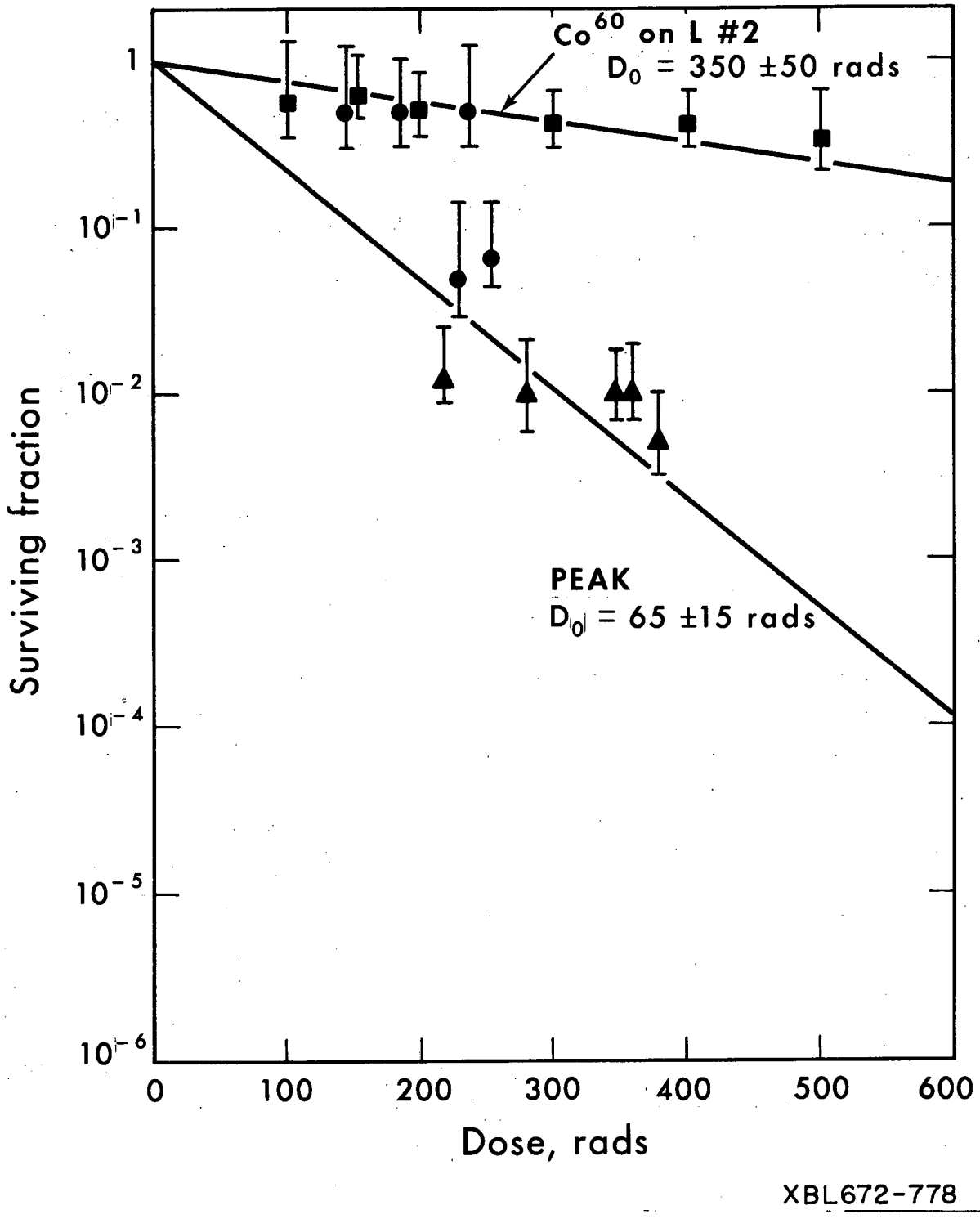


Fig. 3. Survival curves of lymphoma cells irradiated in vivo with a beam of negative pions as compared with ^{60}Co γ-ray irradiation. Circles show inconsistent results obtained at the plateau region of the π^- beam.

This result implies that there is a significant component of high LET radiation in the peak region of a negative pion beam which may be expected to cause a reduction in the oxygen enhancement ratio.

Although more experimentation is needed, it seems that negative pion beams may be more effective in the radiotherapy of tumors than other types of radiation available at present.

References

1. G. Klein and E. Klein, Conversion of Solid Neoplasms into Ascites Tumors, Ann. N. Y. Acad. Sci. 63, 640-661 (1956).
2. H. B. Hewitt, Studies of the Dissemination and Quantitative Transplantation of a Lymphocytic Leukemia of CBA Mice, Brit. J. Cancer 12, 378-401 (1958).
3. H. B. Hewitt and C. W. Wilson, A Survival Curve for Mammalian Leukaemia Cells Irradiated in Vivo (implications for the treatment of mouse leukaemia by whole-body irradiation), Brit. J. Cancer 13, 69-75 (1959).
4. J. H. Lawrence and E. O. Lawrence, The Biological Action of Neutron Rays, Proc. Nat. Acad. Sci. U. S. 22, 124-233 (1936).
5. J. H. Lawrence and R. Tennant, The Comparative Effects of Neutrons and X-rays on the Whole Body, J. Exptl. Med. 66, 667-688 (1937).
6. J. H. Lawrence, R. Horn, and L. C. Strong, Radiation Studies on Mammary Carcinoma of Mice, Yale J. Biol. Med. 10, 145-154 (1938).
7. D. Axelrod, P. C. Aebersold, and J. H. Lawrence, Comparative Effects of Neutrons and X-rays on Three Tumors Irradiated "in Vitro," Proc. Soc. Exptl. Biol. Med. 48, 251-256 (1941).
8. P. C. Aebersold and J. H. Lawrence, Physiological Effects of Neutron Rays, Ann. Rev. Physiol. 4, 25-48 (1942).
9. K. Sillesen, J. H. Lawrence, and J. T. Lyman, Heavy-Particle Ionization (He, Li, B, Ne) and the Proliferative Capacity of Neoplastic Cells "in Vivo," Acta Isotopica 3, 107-126 (1963).
10. J. H. Lawrence and W. U. Gardner, A Transmissible Leukemia in the "A" Strain of Mice, Amer. J. Cancer 33, 112-119 (1938).
11. W. S. Graff, K. G. Scott, and J. H. Lawrence, The Histological Effects of Radiophosphorus on Normal and Lymphomatous Mice, Am. J. Roentgenol., Radium Therapy Nucl. Med. 55, 44-54 (1946).

12. J. H. Lawrence, P. C. Aebersold, and E. O. Lawrence, The Comparative Effects of X-rays and Neutrons on Normal and Tumor Tissue, Proc. Nat. Acad. Sci. U. S. 22, 543-557 (1936).

13. L. J. Reed and H. Muench, A Simple Method of Estimating Fifty Per Cent Endpoints, Amer. J. Hygiene 27, 493-497 (1938).

14. C. A. Tobias and P. Todd, Analysis of the Effects of High LET Radiations on Various Strains of Cells, in Biological Effects of Neutron and Proton Radiations (International Atomic Energy Agency, Vienna, 1964), Vol. 2, pp. 410-418.

15. P. W. Todd, Reversible and Irreversible Effects of Densely Ionizing Radiations upon the Reproductive Capacity of Cultured Human Cells, Med. College of Virginia Quarterly, Vol. 1, 2-14 (1966).

16. Y. Schmidlin, J. H. Lawrence, K. Sillesen, G. Welch, and J. Lyman, Effect of Heavy Particles on the Proliferative Capacity of Ascites Tumor Cells (Lymphoma) Grown In Vivo, in Semiannual Report Biology and Medicine, Lawrence Radiation Laboratory Report UCRL-11833 (1964), pp. 80-94.

17. G. G. Meynell, Inherently Low Precision of Infectivity Titrations Using a Quantal Response, Biometrics 13, 149-163 (1957).

18. D. J. Finney, Statistical Method in Biological Assay, 2nd ed. (Hafner Publishing Co., New York, 1964).

19. J. M. Feola, J. H. Lawrence, and G. Welch, The Measurement of the Effects of X-rays and Alpha Particles on the Proliferative Capacity of Lymphoma Ascites Tumor Cells in Vivo, in Biomedical Program Directors Meeting, Berkeley, Feb. 7-8, 1966 (Lawrence Radiation Laboratory, Berkeley, 1966), p. 120.

20. J. T. Litchfield and F. Wilcoxon, A Simplified Method of Evaluating Dose-Effect Experiments, J. Pharmacol. Exptl. Therap. 96, 99-113 (1949).

21. D. J. Finney, Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve, 2nd ed. (Cambridge Univ. Press, London, 1952).

22. J. H. Lawrence, C. A. Tobias, J. A. Linfoot, J. L. Born, E. Manougian, and J. Lyman, Heavy Particles and the Bragg Peak in Therapy, Ann. Internal Med. 62, 400-407 (1965).

23. J. H. Lawrence and C. A. Tobias, Heavy Particles in Medicine, in Progress in Atomic Medicine, Ch. 6 (Grune and Stratton, Inc., 1965).

24. J. F. Fowler and R. L. Morgan, Pre-therapeutic Experiments with the Fast Neutron Beam from the Medical Research Council Cyclotron, Brit. J. Radiol. 36, 115-121 (1963).

25. C. A. Tobias and T. Manney, Some Molecular and Cellular Effects of Heavily Ionizing Radiations, Ann. N. Y. Acad. Sci. 114, 16-31 (1964).

26. J. A. Belli and J. R. Andrews, Relationship Between Tumor Growth and Radiosensitivity, J. Natl. Cancer Inst. 31, 689-703 (1963).

27. J. M. Feola, C. Richman, M. R. Raju, and J. H. Lawrence, Effect of Negative Pions on the Proliferative Capacity of Ascites Tumor Cells (Lymphoma) Grown in Vivo (to be published).

28. J. B. Storer, P. S. Harris, J. E. Furchner, and W. H. Langham, The Relative Biological Effectiveness of Various Ionizing Radiations in Mammalian Systems, Radiation Res. 6, 188-288 (1957).

29. W. K. Sinclair, S. E. Gunter, and A. Cole, The Relative Biological Effectiveness of 200-kvp X-rays, Co^{60}-rays and 22 Mevp X-rays Determined from the Dose-Survival Curve of Saccharomyces cerevisae, Radiation Res. 10, 418-432 (1959).

30. E. J. Hall, The Relative Biological Efficiency of X-rays Generated at 220 kVp and Gamma Radiation from a Cobalt-60 Therapy Unit, Brit. J. Radiol. 34, 313-317 (1961).

THERAPEUTIC POSSIBILITIES WITH HEAVY PARTICLES

G. J. D'Angio*

It is now almost three decades since John Lawrence became the first to investigate the biological effects of the then new and strange subatomic particles being produced at the cyclotron in Berkeley, California.

Certain features of heavy atomic particles such as protons and alpha particles make them especially interesting to the medical investigator and and clinician. High-velocity heavy particles do not scatter readily in their passage through matter and therefore produce intense excitation and ionization along their path. They give off energy in small amounts until, as their velocities approach zero, the ionization density rises very steeply at the so-called Bragg peak. These features, first described by Bragg,[1] are very different from those of the more familiar X-ray and γ-ray beams in which the dose drops almost exponentially and without range limit. The heavy-particle beam thus has very favorable qualities. All particles within a homogeneous beam travel in parallel paths and have nearly the same penetration, beyond which the dose drops abruptly (within millimeters or less) to zero. A distinction must be made between the initial or high-velocity part of the heavy-particle beam, and the Bragg peak or low-velocity portion, for each has different properties. The former is superior to electromagnetic (X and γ) radiations chiefly because of geometric considerations. The use of small fields and moving beam techniques allows for extremely sharp and very favorable dose distributions at depth within the body. These advantages are ideally suited to the irradiation of small volumes such as the hypophysis, and much of the attention of the Berkeley group has been directed to this gland. They have achieved total or nearly total hypophysectomy with "radiosurgery" and have applied this to the therapy of mammary carcinoma, diabetic retinopathy, acromegaly, chromophobe adenoma, pituitary basophilism, and malignant exophthalmos.

*Present address: University of Minnesota Medical School.

The low-velocity portion of the alpha and proton beams also is known to have special attributes. At the Bragg peak, near the end of the range, an enormous amount of energy is deposited within a few millimeters of path, after which the beam ends abruptly. Thus, at the Bragg peak there is a high loss of energy per unit length of path, or a so-called high linear energy transfer (LET). Radiations of high LET are more efficient dose-for-dose in biological systems than are X-rays or γ-rays. Further, unlike electromagnetic radiations that depend on the presence of oxygen for maximum effectiveness, radiations of high LET are relatively independent of tissue oxygen concentrations.[2,3] (The latter point becomes important since major portions of many cancers are relatively anoxic and therefore resistant to X-radiations and γ-radiations.) An additional property of high LET beams is the low threshold dose, shown not only in simple biological systems[4] but in an *in vivo* mammalian tissue as well.[5] Finally, tissue subjected to high LET beams would be expected to show less recovery between successive doses in fractionated regimens. Studies of this effect are underway with the skin of the rabbit ear used as the test-object. Other experiments will investigate the oxygen effect in the same experimental system.

Preliminary studies of this kind are necessary before the full usefulness of heavy particles in clinical medicine can be assessed. There are many medical problems for which the special properties of the heavy ion beams would be of a great interest. The finite range, sharp margins, high depth dose, and possible radiobiological advantages at the Bragg peak all could be exploited to good advantage in suitable clinical situations. Proton and alpha-particle beams can produce lamellar lesions which could be used to interrupt nerve tracts "radiosurgically." Thus, if the Bragg peak can be placed with sufficiently exquisite accuracy and control, the resulting destructive lesion could well have a significant role in the therapy of such neurological disorders as paralysis agitans (Parkinson's disease).

The irradiation of the bed of childhood tumors adjacent to growing structures is another example. It is well-known that the developing tissues of a child are unusually sensitive to radiation effects. Some of the results of such irradiation are functional impairment, cancer induction, and growth deformity. If the dose could be sharply curtailed so that normal tissues are excluded from the treatment beam, these untoward effects of therapeutic

irradiation could be reduced. It has therefore been proposed that tumors of the extremities in children be treated with heavy particles. This is a particularly advantageous group of patients to treat because: (1) radiotherapy has a well-defined role to play in the management of these diseases; (2) current means of radiotherapy produce the deleterious effects described above; (3) the tissues are homogeneous so that dose and range calculations are simple and accurate; (4) functional and growth impairments become evident within a few weeks to months; and (5) two-year survival in a child has the same general significance as five-year survival in the adult, so that success or failure can be assessed relatively quickly. Thus, the effects of treatment for children would become rapidly manifest and the efficacy known much sooner than for adults.

The radiobiological properties of high LET beams have not yet been fully investigated because of particle-acceleration limitations. The cyclotron-accelerated α-particle beam can be used for this purpose only at the relatively narrow Bragg-peak region; energies available at the Hilac are such that very heavy ion beams with high LET's at the plateau have extremely short ranges in biological samples. When higher energies per nucleon become possible, living mammalian tissues can be irradiated in vivo.

Treatment beams of this type might be of value in the therapy of such tumors as esophageal cancer. This neoplasm is potentially curable by external radiation. X- and γ-ray beams now available for therapy, however, have been unsatisfactory for this purpose because of the high-volume dose required for effective therapy. The entire esophageal length must be treated so as to encompass the ubiquitous lymph-drainage pathways. In order to accomplish this aim and to include the arcuate course of the esophagus, wide-field, moving-beam, or multiple-port techniques have been employed. The lungs, heart, and spinal cord are therefore included in both entry and exit ports and are irradiated to doses beyond tolerance if ablative doses are given to the primary and secondary esophageal cancer foci. Many patients have been cured of cancer only to succumb of radiation pneumonitis, for example.

With a high LET beam of limited penetration that could be modulated and monitored externally, it should be possible to deliver effective doses to the entire length of the esophagus while sparing much normal tissue, including at least one lung. Doses lower than the six krads of low LET radiation

currently required might yield equally good clinical results if the relative oxygen independence, low or absent threshold, and no recovery characteristics of high LET beams can be realized in clinical practice.

Other possible therapeutic uses of these beams include treatment of patients with central nervous system tumors that disseminate via cerebrospinal fluid pathways, such as the medulloblastoma and ependymoma. These tumors grow in the coverings of the spinal cord, a structure that follows an undulant course within the spinal canal. In order to irradiate the brain and entire spinal axis, elaborately shaped fields with a single posterior port have been employed to date. Dose specification is difficult, and the distribution results in "hot" and "cold" spots. Also, X- and γ-ray beams currently employed irradiate many important structures in the exit beam. These include: (1) the bone marrow, where leukemogenesis as well as simple marrow-function depression constitute problems; (2) the gonads, where infertility and genetic damage result; (3) the thyroid, where oncogenesis has already been reported in two patients surviving treatment of the kind described; and (4) the heart, which can be scarred by modest doses, as reported recently by the Stanford Group. A scanning high LET beam—modulated and monitored externally as in the case of the esophagus—should prove a better therapeutic tool. Not only would a more even dose distribution and a more efficient therapeutic effect result, but also the advantages of limited beam penetration would be enjoyed.

Another example of potential usefulness for the high LET beam is the therapy of patients with extensive skin disorders such as mycosis fungoides and Kaposi's sarcoma. Low LET beams—especially electrons—have proven very useful in the short-term control of patients with these malignant processes, but the diseases recur and retreatment becomes necessary. Before long, normal skin tolerance is reached and further therapy becomes impossible. More efficient long-term control if not total eradication might be possible if high LET beams of suitably limited penetration were employed. These cutaneous malignancies commonly have large areas of necrosis within individual nodules, and it may well be the hypoxic "protected" cells that survive low LET beam therapy and provide the nidus for recurrent growth.

Total body irradiation of patients with chronic lymphatic leukemia has been practiced for years by many (e.g., Osgood[6]). At the University of

Minnesota this approach is being extended to the patient with acute leukemia. Encouraging initial results in the control of the myelo-proliferative stage of the disease have been obtained with cobalt teletherapy. Dose distributions and biological efficiency should be improved by high LET beams, and the total cell-kill increased per rad delivered. Since recurrence of murine leukemia is predictable on a simple numerical cell-replenishment model, longer remissions if not total ablation might be achieved in humans by high LET radiation without increasing damage to normal structures.

The precision of placement, and accurate dosimetry available in facilities using heavy particles provides extremely important information regarding the usefulness of radiant energy in the management of various clinical conditions. For example, it was largely through the efforts of the Donner Laboratory team that the feasibility of pituitary ablation by means of external beams of therapy became known. The basic information was of great value in radiotherapy. Radiotherapy groups were encouraged to investigate whether similar effects could be produced by precision, beam-directed, moving-beam megavoltage techniques; and such studies are under way.[7]

Finally, an understanding of the radiobiological effects of heavy-particle irradiation is of importance in the space effort, since astronauts of the future will be exposed to such radiations from cosmic sources. The radiobiology of single and multiple doses of heavy and very heavy particles, and their carcinogenecity are some of the important phenomena under intensive study.

References

1. W. H. Bragg and R. D. Kleeman, Ionization Curves of Radium, Phil. Mag. 8, 726-738 (1904).
2. P. Howard-Flanders and T. Alper, Sensitivity of Microorganisms to Irradiation Under Controlled Gas Conditions, Rad. Res. 7, 518-540 (1957).
3. G. W. Barendsen, Damage to Reproductive Capacity of Human Cells in Tissue Culture by Ionizing Radiations of Different Linear Energy Transfer, in The Initial Effects of Ionizing Radiations on Cells, ed. by R. J. C. Harris (Academic Press, New York, 1961).

4. P. W. Todd, Reversible and Irreversible Effects of Ionizing Radiations on the Reproductive Integrity of Mammalian Cells Cultured in Vitro (Ph. D. Thesis), Lawrence Radiation Laboratory Report UCRL-11614, Aug. 1964.

5. G. J. D'Angio, J. H. Lawrence, A. Gottschalk, and J. Lyman, Relative Efficiency of High-LET Radiation (Bragg-Peak Lithium Ions) on Normal Rabbit Skin, Using Integral Dose as a Basis for Comparison, Nature 204, 1267-1268 (1964).

6. E. E. Osgood, J. A. Seaman, and R. D. Koler, Results of 15-Year Program of Treatment with Titrated Regularly Spaced Total Body Irradiation with Phosphorous-32 or X-ray, in Proc. 6th International Soc. Hemat., Boston, Aug.-Sept. 1956 (Greene and Stratton, New York, 1958), pp. 44-55.

7. R. M. Benbow, C. F. von Essen, J. W. Rowe, and J. B. Stedeford, Treatment Design for Pituitary Suppression Utilizing 6-MeV Photons, Radiology 87, 64-67 (1966).

PHYSICAL STUDIES WITH NEGATIVE PIONS

C. Richman,* M. R. Raju, and S. B. Curtis

When high energy protons or heavy nuclei strike a target, positive, negative, and neutral pions are produced; if, furthermore, the intensity of the primary beam is high enough, secondary beams of pions can be produced.

For biomedical studies, a beam of negative pions is particularly interesting because of its unusual behavior in tissue. Negative pions satisfy the usual range-energy relationship: The 90 MeV pion beam used in these experiments has a range of about 8-1/2 inches of water. The new phenomena take place when the pions come to the end of their range. Here the pions are captured by oxygen, nitrogen, or carbon nuclei and cause a breakup of these nuclei into short-range alpha particles, protons, and neutrons. The Bragg peak is therefore augmented by the dose resulting from the charged fragments. The dose at the peak has a distribution of LET that is broader and considerably higher than the dose in the plateau. Thus, in effect, a pion beam changes its character abruptly as it passes through tissue and stops. This property of negative pions suggested their use for cancer therapy, and preliminary dosimetric studies have been made with this use in mind.[1]

The composition of the beam has been looked at with a time-of-flight of particles system, and it was found that the beam has a contamination of 10% muons and 25% electrons. It is fortunate that the electrons can be entirely removed with an electrostatic separator if one wishes. The present dosimetric system utilizes lithium-drifted silicon detectors which with suitable amplifiers give a voltage pulse proportional to the energy released in the detector. These semiconductor wafers are well suited for particles that produce both high and low ionization densities. A pulse-height analysis gives a direct picture of the nature of the dose in different regions of the medium.[2] The results show clearly that the pulses in the peak, as expected, extend up to 50 million volts while the pulses in the plateau region are of the order of one million volts.

The dosimetric system has been extended to include an integrator that sums each pulse as it comes along. A study of the Bragg peak for a π^--beam

*Graduate Research Center of the Southwest, Dallas, Texas.

with contaminations on the central axis of the beam gives a peak-to-plateau ratio of about 3 with a FWHM of 4.5 centimeters of water. Such a broad peak with the good peak-to-plateau ratio means that realistic tumors could be irradiated with good tumor-to-skin ratios. For a beam without contaminations the advantages would be greater.

The electronics includes a single-channel analyzer, which makes it possible to get the dose of pulses above any level. Studies of both π^+ and π^- Bragg peaks have emphasized the singular character of the π^- Bragg peak.[2]

Future studies will utilize a Cerenkov counter to count the electrons, and these pulses will be put in anticoincidence with all of the pulses; this will give the dose due to the pions with a small contamination of muons. One of the advantages of pulse dosimetry is that coincidence and anticoincidence techniques can be used.

Future experiments should also include an electrostatic separator to separate the electrons from the negative pions.

The pion beam is made by taking a certain solid angle off the target. The beam is therefore large in area, approximately 4 by 4 inches in the usual arrangement, and this is a good size for therapy. This means, further, that for the biological experiments, detailed spatial dose distributions must be made.

It is a well-understood fact that accurate and detailed knowledge of the doses produced by a negative pion beam and heavy particle beams must be obtained to form the basis of radiobiological experiments.

References

1. C. Richman, H. Aceto, Jr., M. R. Raju, and B. Schwartz, The Radiotherapeutic Possibilities of Negative Pions, Am. J. Roentgenology, Rad. Therapy Nucl. Med. 96, 777-790 (1966).
2. M. R. Raju, H. Aceto, and C. Richman, "Pion Studies with Silicon Detectors," Nucl. Instr. Methods 37, 152-158 (1965).

THE RBE OF NEGATIVE PION BEAMS

W. D. Loughman

The search for more effective modes of radiotherapy has stimulated interest in the potentially useful properties of the negative pi-mesons. This subnuclear particle, like other charged particles, gives up a significant fraction of its kinetic energy in the terminal portion of its range. Throughout the preterminal portion of the particle's path, energy loss per unit path length is relatively less. The result is the familiar "Bragg peak" on a diagram of energy loss per unit path length vs. total path length. The preterminal, low ionizing portion of the particle's path with low linear energy transfer (LET) is named the "plateau" region. The terminal, highly ionizing Bragg-peak portion with high LET is named the "peak" region.

Unlike many other particles with therapeutically useful range in tissue and a Bragg peak, negative pions also interact with atomic nuclei at the end of their range. The resultant nuclear fission produces a number of highly energetic, heavy, short-range, highly ionizing particles. The energy from these particles adds to that released in the peak region from Bragg-peak-type effects.

The therapeutically useful physical properties of the negative pion have been investigated and commented upon by Fowler and Perkins,[1] Fowler,[2] Aceto,[3] and Richman.[4,5] Collectively, they suggest that a beam of negative pions, with the Bragg peak region centered in a tumor, should show a high ratio of tumor dose to surrounding tissue dose. Any radioprotection afforded tumors by their hypoxic state should be minimized by the oxygen-effect independence predicted for the high LET particles in the Bragg peak. In addition, the high LET particles produced in the peak region should produce effects greater than an equivalent dose of X-rays. In other words, the peak region of negative pion beams should have a high relative biological effectiveness (RBE).

Artificially produced beams of negative pions have been available since 1948, but biological experimentation was not reported until 1964. Micke et al.[6,7] utilized the low intensity 7 to 9 BeV negative pion beam

from the Brookhaven alternating gradient synchrocyclotron to determine RBE. Avoiding dose-rate effects by irradiating dormant seeds, they scored Zea mays (maize) seedlings for the presence of a specific radiation-induced visible mutation. For this function, the result of a terminal chromosome break, they determined an RBE of 3.2 for mixed peak and plateau regions of a negative pion beam. However, the energy spread of their pion beam was such that no investigations of peak vs. plateau effects could be performed.

The lower energy but higher intensity negative pion beam of the Berkeley 184-inch synchrocyclotron is more suited for studies of the biological effects of Bragg-peak negative pions. This beam was used by Loughman[8,9] to demonstrate that cytological defects were greater in mammalian cells irradiated in the plateau region of the same negative pion beam. The same demonstration was reported by Richman,[10] who used bean root cells. Neither study reported RBE values for negative pions. The differential effects of peak and plateau negative pions are shown in Fig. 1.

Recently, work at the Donner Laboratory and the Lawrence Radiation Laboratory in Berkeley has produced preliminary estimates of the RBE of negative pions, both in the Bragg-peak region and in the plateau region. Loughman[11] has compared radiation-induced polyploidy, in mammalian tumor cells in vivo, for irradiation by cobalt-60, peak region negative pions, and plateau region negative pions. The plateau region pions were found to have RBE = 1, while Bragg-peak pions' RBE = 2.15, all relative to cobalt-60 gamma radiation. Using appropriate assumptions, the effects of muon and electron contaminants of the negative pion beam were subtracted from the effects of the whole beam. The resulting estimate of RBE = 3.64 from pion-nucleus interactions only, represents an upper limit to the RBE of Bragg-peak negative pions, for polyploidy induction under the conditions used. The data and results are diagrammed in Figs. 2 and 3. Also at Berkeley, Feola[12] has investigated the cell-killing capacity of a negative pion beam, with the same in vivo tumor cell system used by Loughman. In these experiments, tumor cells irradiated in vivo were transplanted to compatible recipients. A single viable tumor cell normally results in death of the recipient mouse. RBE was determined from comparison of curves relating numbers of injected irradiated cells to fraction of recipients surviving. Preliminary results indicate an RBE = 5.4 for "peak" region negative pion beams for cell-killing

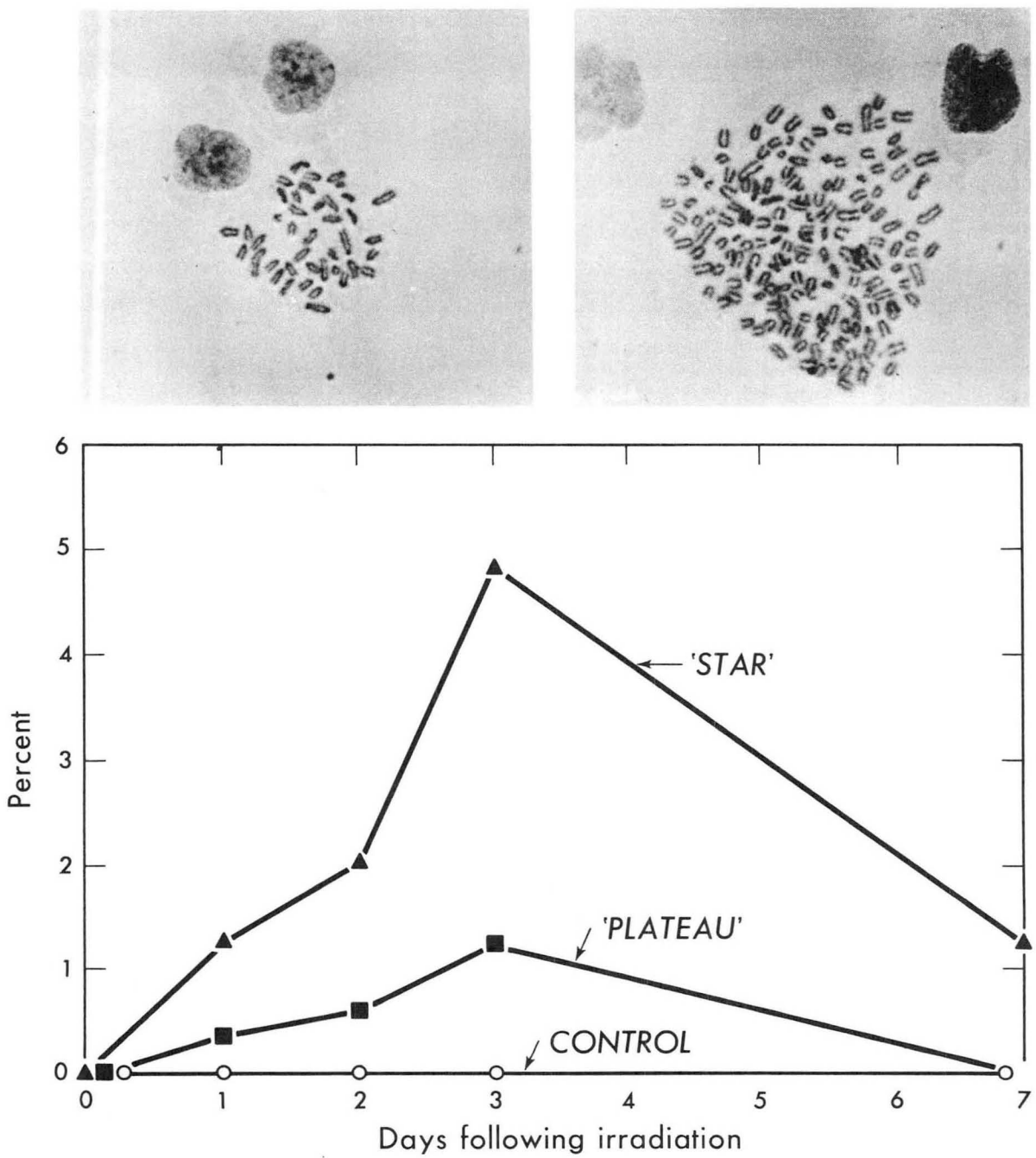


Fig. 1. Polyploid metaphase cells as a percent of all metaphase cells scored in unirradiated control cells, cells exposed in the plateau region of a pi-meson beam, and cells exposed in the "peak" or "star" region. Left photograph = normal Ly-2 metaphase. Right photograph = polyploid metaphase of extreme type.

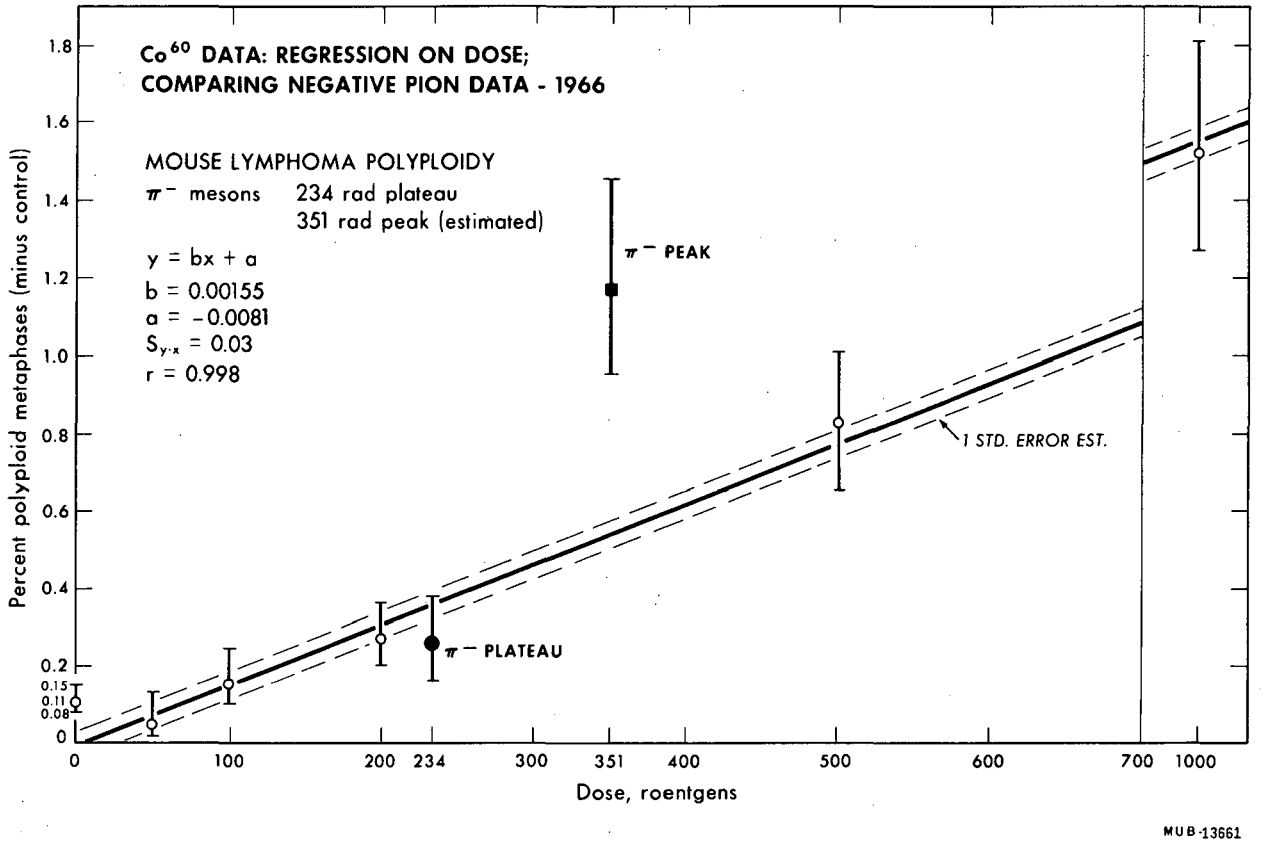


Fig. 2. Polyploidy induction in lymphoma ascites cells as a function of dose: Comparison of data from cobalt-60 gamma irradiation with data from π^--beam irradiation. Open circles = cobalt-60 data. Black circle and square = π^- data. Heavy line is drawn from the equation expressing the regression of polyploidy on dose: (% polyploidy) = [0.00155 (dose) - 0.0081]. Dashed line = regression line ± 1 standard error of the estimate ($S_{y \cdot x} = 0.03$).

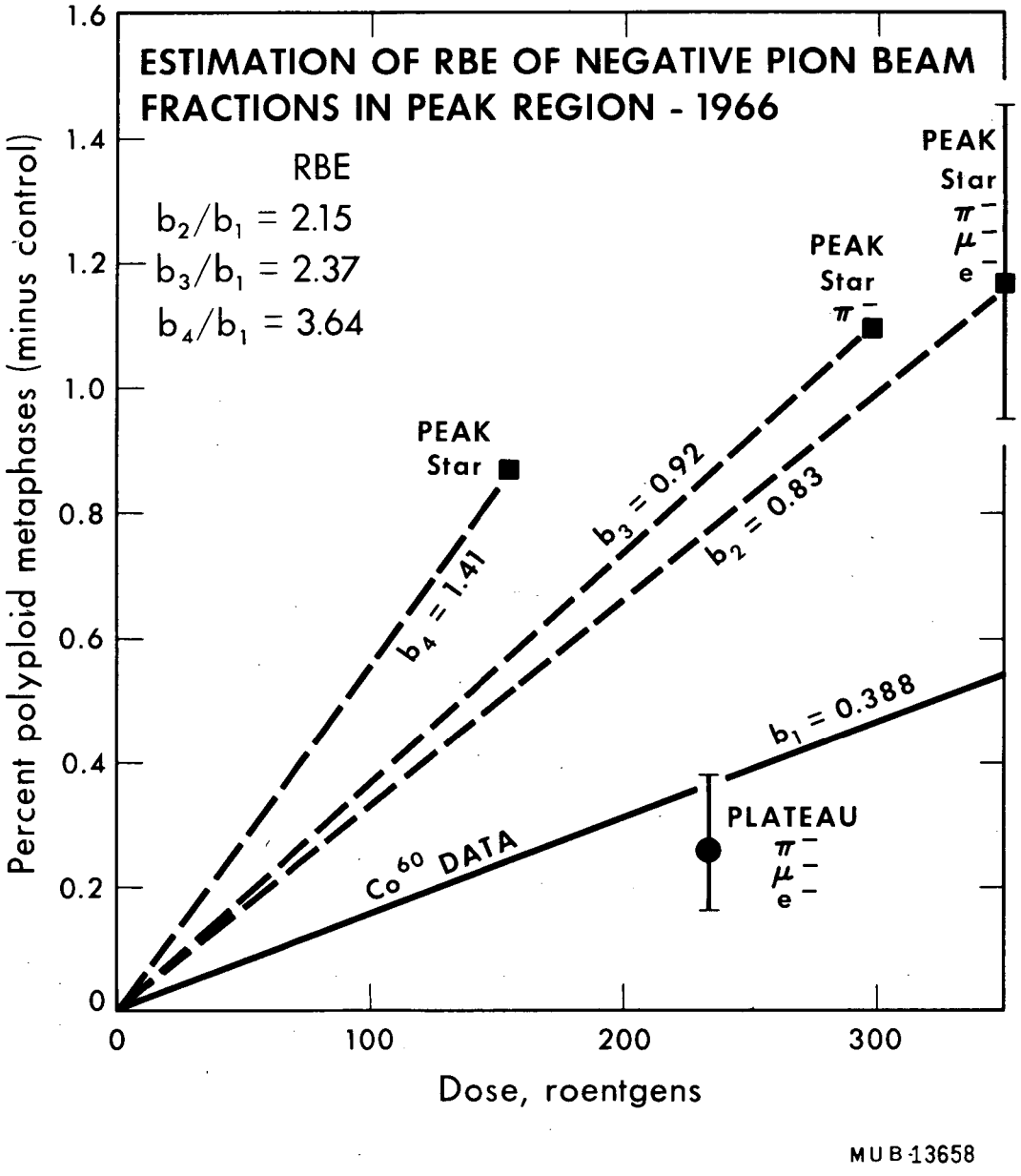


Fig. 3. RBE of fraction of π^- beam. Solid line = regression line from cobalt-60 data. Black squares = values of polyploidy vs. dose for whole peak region π^- beam, pions only in peak region, and "stars" only in peak region. Respective RBE values are ratios of slopes of dashed lines to the solid (cobalt-60) line. Slopes of relevant lines are represented by b_1, b_2, b_3, and b_4.

effects, while for similar cell-killing effects, RBE = 2.4 for the plateau region of that same beam. The peak/plateau effect ratio (or tumor/surrounding tissue effect ratio) of 2.15 found by Loughman for the contaminated pion beam,[11] was similarly obtained by Feola. Other preliminary results of Feola[12] indicate the presence of the predicted oxygen-effect independence of Bragg-peak negative pion beams. Using both induced cytological defects and cell-killing effects, work continues at the Donner Laboratory for confirmation of RBE values, investigation of dose-rate effects, and confirmation of oxygen-effect independence of Bragg-peak negative pions.

A complete understanding of the biological effects of Bragg-peak negative pions depends on accurate pion dosimetry, and on detailed knowledge of the LET spectrum within the Bragg peak. Richman et al.[4] and Raju et al.[13] have investigated these problems, and described the difficulties inherent in their solution. At Berkeley, work is in progress to perfect silicon semiconductor detectors to aid accurate and detailed dosimetry.

Much work remains to be done before negative pions may be considered for radiotherapy. However, the available evidence indicates that negative pion beams may be a more effective radiotherapeutic tool than any other form of external radiation presently available.

References

1. P. H. Fowler and D. H. Perkins, The Possibility of Therapeutic Applications of Beams of Negative π^- Mesons, Nature 189, 524-528 (1961).
2. P. H. Fowler, 1964 Rutherford Memorial Lecture: Pi-Mesons Versus Cancer?, Proc. Phys. Soc. 85, 1051-1066 (1965).
3. H. Aceto, A Feasibility Study of the Therapeutic Possibilities of π^- Mesons, Ph. D. thesis, Lawrence Radiation Laboratory Report UCRL-11482 (June 1964).
4. C. Richman, H. Aceto, Jr., M. R. Raju, B. Schwartz, and M. Weissbluth, On the Dosimetry of Negative Pions with a View Toward Their Trial in Cancer Therapy, in Semiannual Report: Biology and Medicine, Lawrence Radiation Laboratory Report UCRL-11387 (1964), pp. 114-126.
5. C. Richman, H. Aceto, Jr., M. R. Raju, and B. Schwartz, The Radiotherapeutic Possibilities of Negative Pions, Am. J. Roentgenol., Radium Therapy Nucl. Med. 96, 777-790 (1966).

6. A. Micke, H. H. Smith, R. G. Woodley, and A. Maschke, Relative Cytogenetic Efficiency of Muons and Mesons in Zea Mays (L.) and Its Modification by Postirradiation Storage, Rad. Res. 23, 537-550 (1964).
7. A. Micke, H. H. Smith, R. G. Woodley, and A. Mashke, Relative Cytogenetic Efficiency of Muons and π^- Mesons in Zea Mays (L.), Proc. Natl. Acad. Sci. U.S. 52, 219-221 (1964).
8. W. D. Loughman, H. S. Winchell, H. Aceto, C. Richman, M. R. Raju, and J. H. Lawrence, Differential Cytologic Effects of Negative Pion Beams in Plateau and "Star" Regions: Preliminary Report, in Semiannual Report: Biology and Medicine, Lawrence Radiation Laboratory Report UCRL-16246 (1965), pp. 100-102.
9. W. D. Loughman, H. S. Winchell, M. R. Raju, and J. H. Lawrence, A Significant Difference in Mammalian Cell Polyploidy Induction between Plateau and "Star" Regions of a Negative Pion Beam, in Semiannual Report: Biology and Medicine, Lawrence Radiation Laboratory Report UCRL-16898 (1966), pp. 11-13.
10. S. P. Richman, C. Richman, M. R. Raju, and B. Schwartz, Studies of Vicia faba Root Meristems Irradiated with a π^- Beam, in Semiannual Report: Biology and Medicine, Lawrence Radiation Laboratory Report UCRL-16613 (1965), pp. 15-22.
11. W. D. Loughman, manuscript in preparation (1967).
12. J. M. Feola, personal communication.
13. M. R. Raju, H. Aceto, and C. Richman, Pion Studies with Silicon Detectors, Nucl. Instr. Methods 37, 152-158 (1965).

AN INTEGRATED LOW-BACKGROUND COUNTING FACILITY FOR THE OMNITRON

W. E. Siri, D. Van Dyke, and T. W. Sargent

Although the Omnitron possesses the potential for greatly extending the scope of radiobiological research and radiotherapy, the full development of this potential will depend in part on the means available for exploiting the extraordinary versatility of the accelerator. Among the means believed essential for achieving this, a properly designed and instrumented low-background counting facility, contiguous with the target area, is proposed as an integral part of the Omnitron project. The primary function of this facility would be rapid, accurate in vivo counting of short-lived radionuclides, particularly positron emitters.

In much the same way that bubble chambers have served nuclear physics as adjuncts to accelerators, a well-engineered in vivo counting facility will enable improvements to be made in therapeutic applications of high energy Omnitron beams, and will extend the range of biomedical research into areas for which existing facilities and improvised installations are inadequate.

Purposes

The uses for which this facility is intended fall into three major categories that are defined in part by the purpose and in part by the experimental or diagnostic procedure. A fuller discussion of each category and its ramifications is presented in the three sections of this report that follow. The descriptions below are intended only to summarize these categories of applications.

Aid to Radiotherapy and Production of Experimental Lesions with Omnitron High Energy Radiations

For irradiating deep-seated sites for which the beam must traverse bone and soft tissue of varied densities, two procedures should be explored that may significantly improve placement of the Bragg peak and the determination of dose.

The first method is based upon the detection of positron emitters, ^{11}C, ^{13}N, and ^{15}O, produced in tissue along the track of the beam. This procedure, which has been given a successful preliminary test with the Hilac, may permit accurate determination of the Bragg-peak location in vivo. It is not yet clear that the method will yield an accurate measure of radiation dose at the peak.

The second approach calls for an Omnitron beam consisting of an admixture of a short-lived positron emitter. The nuclide most practical for acceleration is ^{18}F (half-life: 112 min) although ^{11}C and others may be possible. Fluorine-18 is readily available from nearby accelerators and could serve as a range marker for a beam of ^{18}O since the mass and e/m are the same. In this procedure, positrons are emitted from the very end of the range where the particles are brought to rest in the tissue, thus marking the location of the Bragg peak and perhaps also permitting a determination of the dose delivered at the peak, through the use of the positron camera.

In both methods, a procedure is envisioned in which a test exposure of low dose is first made and the subject rapidly transferred to the counting facility to determine the actual location of the Bragg peak by in vivo measurement of the positrons. With appropriate corrections in range and position to secure the desired location of the Bragg peak, the subject is quickly repositioned for the prescribed therapeutic or experimental dose. On conclusion of the exposure, the total dose delivered can promptly be verified from the positron activity.

In Vivo Activation Analyses

Exploratory studies on neutron activation in vivo have been conducted by several investigators. The utility and safety of the method, including its use for humans, has been demonstrated but the method's great potential as a research and diagnostic tool has scarcely been touched. In part, its development has been hampered by inadequate facilities for fast, accurate measurement of induced short-lived activities and visualization of regional distributions that can be revealed by positron emitters.

Activation in vivo with charged particle beams offers considerable promise, but to our knowledge has not yet been explored. In contrast with neutron fields, high energy beams of charged particles are sharply defined in range and shape and may therefore have as their most valuable application

the activation of selected tissue volumes and perhaps specific constituents. The use of Omnitron beams for whole-body activation is not precluded, however, since this can be achieved by beam scanning.

A recent study has demonstrated that prompt gamma rays from nuclear interactions produced in vivo by high energy beams may prove a useful method for quantitative determination of tissue constituents. It is proposed to explore further this technique with the Omnitron.

Tracer Applications of Short-Lived Radionuclides

Although ^{11}C and ^{15}O have been employed in significant research for some years, the extensive use of these and other short-lived radionuclides as tracers has been hampered by the scarcity of facilities and the obvious technical difficulties interposed by the need for rapid delivery to the point of use from the site of production. With the proposed counting facility, radionuclides produced at targets in either the high-energy, low-intensity beam or in the intermediate-energy, high-intensity beam would be available for use within tens of seconds after production. The use of a substantial number of short-lived radionuclides would become practicable for investigations of transient, periodic, and steady-flow problems, such as those encountered in lung ventilation, regional blood flow, and rapid metabolic processes.

Basic Requirements of Counting Facility

To serve adequately the purposes outlined above and in the following sections, the counting facility must meet several elementary criteria. Foremost is the need to locate the facility as close as practicable to the target area, subject to limitations imposed by shielding requirements. Closely related to this is the need for rapid transport of irradiated materials, experimental animals, and human subjects to the counting area. Transfer times on the order of tens of seconds would enable the investigator to draw on procedures and radionuclides that are now generally impracticable for biomedical applications.

A low level of background radiation is desirable for most applications envisioned; for some it is essential. Reasonable constancy in the background is also needed to ensure the reliability of low-level counting. The facility should therefore be adequately shielded to minimize the influence of stray radiation fields produced by the Omnitron and neighboring accelerators. Shielding against neutrons may require particular attention to prevent activation of materials and instruments in the counting area.

Design Considerations

The combined requirements of low background and close proximity to the target areas appear to rule out installation of the counting facility within the currently proposed structure of the biomedical wing. Two locations adjacent to the proposed structure, both at right angles to the main axis of the beam, may, however, satisfy the requirements (see Fig. 1).

On the basis of a preliminary examination, the more favorable site is that directly below the target area, separated with sufficient concrete and earth to provide the necessary shielding. Rapid, direct transport of experimental animals or human subjects from their position in the beam to the counting area can be achieved with a fast-operating, platform elevator on which the subject is supported during irradiation.

The alternative location is deep within the rise of ground adjoining the biomedical wing, but on the same elevation as the Omnitron beam. In this configuration, a tracked carriage of suitable design would be desirable for transporting irradiated subjects rapidly from the treatment cell to the counting area. This location, however, appears to introduce more difficult problems of shielding, and it would require traversing the main axis of the Omnitron beam unless significant changes were made in the design of cell arrangements.

Whatever configuration is adopted for the counting facility, there should be provision for rapid delivery, via "rabbit" of short-lived radionuclides produced in both the biomedical wing and the radiochemical wing. Delivery tubes should terminate in a preparatory space within the counting area that is properly shielded from counting instruments and equipped for processing active materials for application.

To carry out the proposed investigations and aids to radiotherapy, the counting facility will need the following primary instruments:

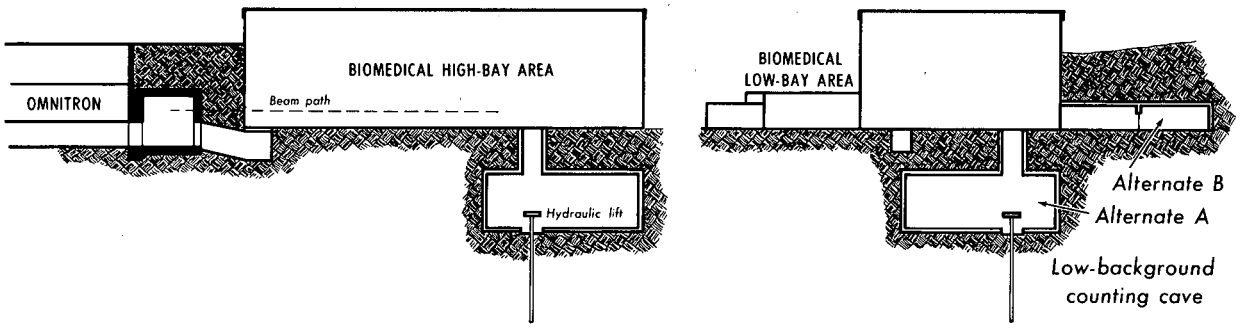
(a) Whole-body counter of the gamma ray spectrometer type consisting of a large Na(Tl)I crystal mounted in a specially shielded vault.

(b) Positron scintillation camera.

(c) Gamma ray *in vivo* counter for localized counting.

(d) Gamma ray spectrometer for sample counting.

Ancillary equipment and the design of the facility are not considered here other than to note that a minimum floor space of 1000 square feet should be provided, consisting of several rooms shielded from one another.



ALTERNATE LOW-BACKGROUND COUNTING FACILITY

DBL 672-1501

Fig. 1

IN VIVO VISUALIZATION OF AN ACCELERATED PARTICLE BEAM USING THE POSITRON SCINTILLATION CAMERA

D. C. Van Dyke

Development of the Omnitron will make available monoenergetic particle beams with energy ranges and Bragg-peak characteristics optimum for irradiation of circumscribed areas at any depth within the body. To fully utilize the unique characteristics of such a beam, one must be able to accurately place the end of the beam path in relation to the organ, tissue, structure, or lesion to be irradiated. If the beam consisted of radioactive accelerated monoenergetic positron-emitting particles, the range of the particles (beam) within the tissue could be visualized with the positron scintillation camera. Resolution of the positron camera is such that not only could maximum depth penetration of the beam be visualized and recorded, but it may be possible to determine approximately the distribution of dose at the end of the range.

Because of the possible advantages in the use of such a technique in facilitating the biomedical uses of accelerated particle beams, this report explores the feasibility of such a combination of facilities.

A patient being given accelerated high energy particle beam irradiation to a small third ventricle tumor, for example, would be positioned for rotation in the beam path with the tumor as the center of rotation. A test dose of positron-emitting particles would be introduced laterally, the patient then rapidly transferred to a positron camera in the low-level counting area, and a picture taken. Correlation of the positron picture with previously obtained tumor-localizing roentgenographic and other studies would show whether the particles are stopping before, in, or beyond the predetermined tumor location. The procedure could, if necessary, be repeated at various angles of rotation to demonstrate the degree of nonuniformity of absorption around the skull. Appropriate absorbers would be positioned around the skull to insure that the range for each port terminated at the tumor, and the beam energy would be adjusted to provide for maximum ionization within the tumor borders. Final preparation would be checked by rapidly rotating the patient through a full arc and obtaining a final in vivo positron camera picture of the beam

penetration pattern. If satisfactory, the desired dose would be given and at completion of the bombardment a final positron picture could be made that would give, when corrected for ionization distribution, not only the pattern of irradiation delivered but an on-the-spot measure of total dose delivered.

Figure 1 shows the anticipated appearance of the beam (camera scope) superimposed on a previously obtained image of the patient's skull. Proper superimposition would be insured by radio-opaque, positron-emitting source markers attached to the patient's head or to the head-holding apparatus.

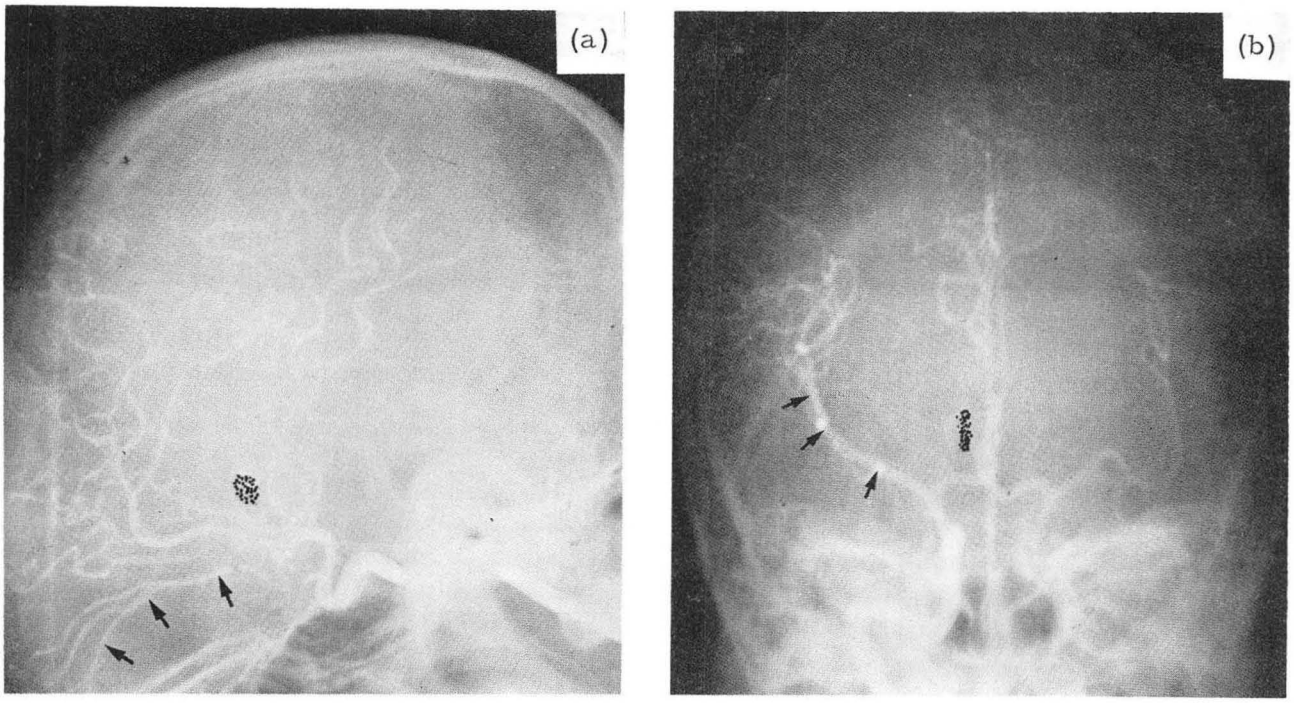
Facilities Required

1. Ability to accelerate positron-emitting particles.
2. Positron scintillation camera located near the treatment cell but well-shielded from radiation fields and particularly activation by neutrons.
3. Facilities for rapid transport of patient to low background counting cave, and for insertion and removal of a positron camera head and focal detector.
4. Correction for non-uniformity. This could be accomplished either by altering the beam energy during rotation of the patient or by an absorber ring surrounding the patient's head.
5. Accelerated positron-emitting particles may be used for the actual irradiation if this is practicable, or for various reasons it may be preferable to use them as markers and perform the actual irradiation with other particles. If used as markers, it may be necessary to change particles rapidly, and the relationship between the penetration characteristics of the positron beam and the therapeutic beam would have to be known in order to make the appropriate corrections. However, the e/m and mass of ^{18}F and ^{15}O would be nearly identical for acceleration and their range would differ only slightly.

Uses

Accurate localization of the end of the beam range is essential to accelerated particle beam radiotherapy and physiologic radio-surgery.

Radiosensitive, deep, midline tumors such as pinealomas, meduloblastomas, third ventricle tumors which are at present inoperable and treatable only by conventional radiotherapy could be given a significantly greater dose of irradiation by this means. This is also true of malignant gliomas situated in the polar areas of the cerebral hemispheres.



BBH 672-36

Fig. 1. An artist's concept of the appearance of the positron camera picture of accelerated particle beam implants of positron-emitting particles superimposed on a previously obtained cerebral arteriogram of the subject. (a) The disc-like appearance of the end of the beam path seen by the camera end-on. (b) The linear deposition of positron-emitting isotopes to be expected from a monoenergetic beam entering laterally. The arrows indicate abnormalities in the arterial pattern.

IN VIVO ACTIVATION ANALYSIS WITH THE OMNITRON

T. W. Sargent

The availability from the Omnitron of beams of nuclei of all the elements at variable energies and in beams which make possible total body irradiation of humans opens up large areas of investigation in activation analysis. Activation analysis to date has been largely confined to small samples irradiated with neutrons in reactors or neutron generators. A much greater variety of reactions and isotope products, in fact all known reactions and many still unknown reactions, and all known isotopes, are conceivably possible to produce with the Omnitron.

The technique which will make possible measurement of the induced activity in humans is the whole-body counter. The great sensitivity of this device, and the gamma-ray spectroscopy which can be done, make it possible to measure several isotopes simultaneously and obtain half-life values by sequential counts. Thus it will be possible not only to determine amounts of known induced activities, but to identify others produced by new and unexpected nuclear reactions.

Published work from this laboratory[1] has demonstrated the production of ^{11}C in humans by the 910 MeV alpha-particle beam during pituitary irradiation, and its measurement in the whole-body counter. It was shown that of the ^{11}C produced, some remained in the body while the remainder was expired in equal portions of CO_2 and CO. The amount of ^{11}C produced was 1.8×10^{-4} μC per gram-rad, which corresponds to production of 0.012 μC ^{11}C with a total-body dose of 1 millirad. This is an amount of ^{11}C readily detectable with a whole-body counter, and the radiation dose is so low as to be acceptable even for normal subjects.

Neutron-activation analysis has been reported, using a neutron generator to deliver a dose of 0.1 rad of fast neutrons to a normal human subject.[2] It was possible to measure whole-body sodium, chlorine, and calcium from the induced ^{24}Na, ^{38}Cl, and ^{49}Ca respectively. A sensitive whole-body counter was necessary to measure the levels of activity here also. If a nuclear reactor were to be used for activation, there would be an accompanying

gamma-ray field that would make the dose to the patient unacceptable. The RBE of fast neutrons is about 1, so the dose delivered to the subject here was 1.0 rem, radiologically acceptable according to the authors, although a lower dose would be desirable.

A method of producing a neutron beam with a lower associated gamma yield will be possible with the Omnitron. Large plates of ^{238}U (a surplus material, depleted of ^{235}U) can be fabricated and scanned with a particle beam, producing increased quantities of neutrons. Of course, other types of targets can also be used for neutron production. Thus neutrons of many energies, with some degree of energy selectivity, will also be available for activation studies with the Omnitron.

If the experience with the 910 MeV alpha beam is any indication of the relative yield in induced activity per rad of dose, the efficiency of particle beams over neutron activation should be considerable. In addition, neutron-deficient isotopes can be produced that are not possible or are more difficult with neutrons. The cross sections and thresholds for nuclear reactions with protons, deuterons, and alphas as a function of energy are only poorly known because of the lack of variable-energy accelerators; the same parameters for reactions of heavier particles are almost completely unexplored. Using heavier nuclei as accelerated particles, many reactions are possible—such as spallation, fusion, and transfer reactions—in addition to the more common exchange reactions. Thus by using a variety of different nuclei as particles, it may be possible to produce any of the various isotopes of each element in the human body by choosing the correct bombarding particle and energy. For whole-body measurement of a particular element it will only be necessary to produce one isotope of that element, so a choice may be available, allowing the reaction giving the least dose to the patient to be used. Another advantage that can be utilized is that many of the isotopes that would be produced by activation emit high energy gamma-rays, of 2 MeV or greater, which can be detected easily because the natural background at those energies is quite low.

The half-lives of a great many of the isotopes that will be useful in activation analysis are quite short, from tens of seconds to tens of minutes, so it will be essential that the whole-body counter be close to the Omnitron. It will be essential, in fact, that a transport device be available to move the patient rapidly from the irradiation site to the whole-body counter. The necessity of the counter's being close to the Omnitron will further necessitate considerable shielding between the two, because of the very penetrating neutrons and other scattered radiation from the accelerator. This could be best accomplished by placing the counter within earth shielding, either below the beam level with access by elevator, or horizontally into the hill with a track-and-car type of transport. In any case, such placement would be coordinated with the other planned facilities requiring low background, the gamma-ray camera and short-lived isotope tracer units, which also require rapid patient transport from the beam area.

The applications of in vivo activation analysis to biomedical research have only begun to be investigated,[1, 2] but from these beginnings some projections can be made. The primary application is in determination of the amount of any particular element in an entire person or animal, or in selected areas of the body, without appreciably disturbing the tissue. Isotope dilution techniques measure only the amount of exchangeable ions and if there are several compartments or the exchange-ability is very slow, these measurements give only partial answers. In many cases the dilution technique cannot be used at all, because the compartment or physiological area involved cannot be sampled without injuring or killing the subject. With activation analysis the only disturbance to the organism is a radiation dose comparable to or less than other radiology procedures.

Some of the elements it would be desirable to study, and some of the associated disease states, can be briefly described.

Sodium is an element intimately involved with electrolyte balance and nerve conduction in the body. Abnormalities in sodium content of the body are observed in hypertension, cardiac disease, and psychiatric disorders (manic-depressive psychosis). Total sodium has been measured by neutron activation analysis and found to differ from the usually measured dilution analysis.[2] Activation isotopes that might be produced would include, in addition to the usual 15 hour ^{24}Na: 11 second ^{23}Mg, 7.6 second ^{25}Al, and 60 second ^{25}Na.

Total phosphorus is very difficult to measure, but activation production of 2.5 minute ^{30}P should be feasible with n2n, αn, or ppn reactions. ^{34}Cl, with a 32 minute half-life, could be produced by $^{31}P(\alpha, n)^{34}Cl$.

Chlorine is also important in electrolyte balance and has been studied[2] by $^{37}Cl(n, \gamma)^{38}Cl$. $^{37}Cl(t, p)^{39}Cl$ is also a possibility.

Calcium is of course of great importance in bone metabolism and diseases of the bone, and isotope dilution studies of calcium would be greatly assisted by total calcium measurements. $^{48}Ca(n, \gamma)^{49}Ca$ has been[2] used to measure total calcium, and many isotopes of scandium would be produced by pn reactions, and of titanium by αn reactions. Calcium metabolism is presently being studied in this laboratory with isotope tracer techniques.

Carbon, oxygen, and nitrogen can yield ^{11}C, ^{15}O, ^{13}N, and ^{18}F by a variety of reactions, and have been found by using in vivo activation by an alpha beam.[1] These reactions would have to be studied in some detail in order to know which target element was actually being studied. Probably beams of several different particles, each with a measured cross section for each reaction, could be used in measuring the yield of each isotope and solving simultaneous equations for the amount of stable carbon, oxygen, and nitrogen present.

Manganese is known to accumulate in the brains of persons suffering from Parkinson's disease, but nothing is known of the role played by this metal, although it is known to be required for the function of several anzymes. ^{56}Mn would be produced by a $^{55}Mn(n, \gamma)$ reaction, or a variety of cobalt isotopes by αn reactions.

Total body iron is impossible to measure at present and is an important factor in clinical management of several diseases of iron storage, as well as in more basic research related to iron metabolism. In addition to ^{59}Fe by nγ reaction, cobalt and nickel isotopes would be produced by pn and αn reactions. Iron turnover and loss are already being extensively studied at this laboratory by other isotopic techniques.

Cobalt is vital to body function, its lack as a vitamin B_{12} deficiency producing pernicious anemia and neurological deterioration. Because it is stored for a long time in the liver, onset of the disease is insidious and could be detected early in suspected cases by measurement of total liver cobalt. This should be quite easy by production of 10.5 minute $^{60}Co^{m}$ from

the $^{59}Co(n,\gamma)$ reaction, which has a very high cross section.

Copper is known to be involved in Wilson's disease, a crippling neurological disorder, and removal of excess copper is used as therapy. Total-copper determination would aid in diagnosis, treatment and study of this disease. There are many potential activation products of copper: ^{64}Cu, ^{66}Cu, ^{63}Zn, and many gallium isotopes.

Zinc is present in the body in relatively large amounts, yet very little is known of its function. Isotope dilution, localization, and whole-body retention studies already under way at this laboratory would be complemented by total-zinc measurements. There are numerous possible activation isotopes.

The above summary is only a brief survey of the possible elements and methods of activation possible. As stated earlier, the kinds of activation reactions which may occur can hardly even be predicted at this time, because with heavier particle beams nuclear reactions may occur with unexpectedly high cross sections for certain unique combinations of odd- or even-mass nuclei or nuclei with magic-number structure, in either the target, the bombarding particle, or both. Some of the elements may not occur in enough abundance to measure, but in special disease states may be sufficiently concentrated in specific organs that these can be separately irradiated and measured. Another possible type of experiment would be to label in vivo by activation, a fairly long-lived isotope; this is the reverse of the usual dilution experiment, in that it begins with uniform labeling rather than ending with it, and by mathematical solution of equations for transfer and dilution should yield results not obtainable by classical dilutions experiments.

The nuclei of radioactive isotopes could even be accelerated in the Omnitron and be implanted in any desired location in the body. The subsequent diffusion and whole-body retention could then be followed. This idea is so new that applications of it have not yet been explored, except as a method of identifying the position in vivo of the Bragg peak, described elsewhere.

To summarize, the combination of the Omnitron with a closely accessible whole-body counter will open up an entire new field of in vivo activation analysis, with important applications in biomedical research.

References

1. T. W. Sargent, Metabolic Studies with Fe^{59}, Ca^{47} and C^{11} in Various Diseases, in Whole Body Counting, (IAEA, Vienna, 1962); also, Lawrence Radiation Laboratory Report UCRL-9718 (May 1961).
2. J. Anderson, S. B. Osborn, R. W. S. Tomlinson, D. Newton, J. Rundo, L. Salmon, and J. W. Smith, Neutron-Activation Analysis in Man in Vivo, The Lancet, No. 7371, 1201 (1964).

BIOMEDICAL APPLICATIONS OF SHORT-LIVED RADIONUCLIDES

W. E. Siri

Short-lived isotopes offer significant advantages in many diagnostic and experimental applications to biology and medicine. For the biologically important elements oxygen and nitrogen, radioisotopes of only short half life exist. Moreover, many of the positron emitters currently employed and others that are potentially useful are short-lived nuclides. More than a hundred such nuclides exist, most of which should prove useful for biological and medical research when adequate facilities for their use become available. A partial listing of nuclides with half lives ranging from one minute to about six hours is given for positron emitters (Table I) and beta-gamma-ray emitters (Table II). Omitted from the tables are those nuclides that can be obtained from "cows" of longer lived parent nuclides.

Thus far relatively few short-lived radionuclides have seen extensive use. Carbon-11 and oxygen-15 have perhaps been employed more than others but even their use has been confined to a few laboratories. The inclination to use short-lived nuclides has been inhibited by technical difficulties, and, in general, improvised facilities in the immediate vicinity of accelerators have been less than satisfactory. It is reasonable to expect, however, that a properly designed and equipped counting facility associated with the Omnitron will permit all the nuclides listed in the tables to be used effectively.

Several practical advantages favor the use of short-lived nuclides in some diagnostic and experimental tests that now employ tracers of relatively long life. Two of the principal advantages are the frequency with which tests can be repeated in the same subject, and the substantial reduction in the radiation dose that is incurred. The value of the former is particularly evident when observing changes in function and when following dynamic processes. The measurement of red blood cell volume, for example, is completed in about 15 minutes, yet the isotopes commonly used, ^{32}P, ^{51}Cr, and ^{131}I, persist as active labels for several days to a month. Chromium-49 (T = 42 min) or one of the short-lived isotopes of iodine may more effectively serve this purpose when repeated determinations are needed or when for

Table I. Short-lived positron emitters.

Nuclide	Half life	Nuclide	Half life
^{11}C	20.4 min	^{60}Cu	23 min
^{13}N	10 min	^{61}Cu	3.2 h
^{14}O	72 sec	^{61}Zn	1.5 min
^{15}O	2 min	^{62}Zn	9.3 min
^{17}F	66 sec	^{63}Zn	38 min
^{18}F	112 min	^{70}As	52 min
^{21}Na	23 sec	^{75}Br	1.6 h
^{30}P	2.6 min	^{77}Dr	1.1 h
^{34m}Cl	32.4 min	^{81}Rb	4.7 h
^{38}K	7.7 min	^{82m}Rb	6.3 h
^{43}Sc	3.9 h	^{106}Ag	24 min
^{47}V	31 min	^{121}I	2 h
^{49}Cr	42 min	^{123}Xe	2.1 h
^{51}Mn	45 min	^{125}Cs	45 min
^{52}Fe	7.8 h	^{127}Cs	6.3 h
^{53}Fe	8.9 min	^{129}Ba	2.5 h

other reasons the accumulated radiation dose must be minimized. In general, reduction in radiation dose of nearly an order of magnitude may often be possible.

Biological and medical investigations involving transient, periodic, and steady-state processes with time constants ranging from a few seconds to several hours are often best served with short-lived nuclides, and particularly positron emitters. Obvious examples of these are pulmonary

Table II. Short-lived beta-gamma-ray emitters.

Nuclide	Half life	Nuclide	Half life
^{24}Ne	3.9 min	^{61}Co	99 min
^{25}Na	60 sec	^{62}Co	13.9 min
^{25}Mg	9.5 min	^{66}Cu	5.1 min
^{28}Al	2.3 min	^{69}Zn	57 min
^{29}Al	5.6 min	^{79}As	91 min
^{31}Sc	2.6 h	^{83}Br	2.3 h
^{34}P	12.4 sec	^{84}Br	30 min
^{37}S	5 min	^{87}Kr	78 min
^{38}S	2.9 min	^{88}Rb	17.8 min
^{38}Cl	37.3 min	^{92}Sr	2.6 h
^{39}Cl	55.5 min	^{101}Tc	14 min
^{44}K	22 min	^{112}Ag	3.2 h
^{49}Ca	8.8 min	^{128}I	25 min
^{52}V	3.8 min	^{132}I	2.3 h
^{55}Cr	3.5 min	^{138}Cs	32.2 min
^{56}Mn	2.6 h	^{139}Ba	84 min

function, gas exchange, regional blood flow, oxygen metabolism, and some aspects of renal and liver function. However, the scope of the applications is in no sense limited. Exploratory tests at Donner Laboratory and elsewhere have clearly demonstrated that short-lived nuclides in general, and positron emitters in particular, will serve as powerful diagnostic and investigative tools for a great many diseases.

THE THERAPY OF NEOPLASMS AND THE OXYGEN EFFECT

C. A. Tobias and J. H. Lawrence

Accelerated heavy ion radiation may be used in two general ways for cancer therapy. We have for a number of years in this laboratory attempted the indirect method of therapy, which appears to be useful in many cases. The general rationale for this is to interfere with the control processes of the body. There are some instances of endocrine-related neoplasms where irradiation of the pituitary gland may sufficiently decrease the hormones needed for growth and proliferation so that the tumor tissue is unable to continue proliferation. Indirect approaches are not necessarily exhausted by pituitary irradiation. For example, another principle that may be subject to eventual experimental test is the possible interference by local radiation with the systemic control of antibody production. It is well known that there is in mammals a mild degree of immune reaction to various neoplasms, and it is also believed that the degree of antibody production may be controlled from the hypothalamus. Eventually hypothalamic irradiation might be of some interest among indirect therapeutic approaches to cancer.

Unfortunately, many neoplasms are not endocrine related and they have become sufficiently undifferentiated so that they do not depend on the central or systemic control mechanisms in the body. In this case, direct local irradiation therapy is applied. There are examples of direct radiation therapy with heavy particles; among these are trials in our own laboratory of treatment of mammary carcinoma when the location of a metastatic lesion gave promise for successful application of local irradiation.[1] At the University of Uppsala, Naeslund and associates[2] have carried out a series of irradiations with high energy protons on metastatic uterine carcinoma.

A number of conditions are important for successful local therapy. Among these are diagnostic knowledge of the location of cancer cells, delivery of a dose to the malignant cells sufficient to stop their proliferation and later metastasis and with minimal harm to normal cells in the vicinity, and finally protection of the skin and other sensitive tissues that may be located on the pathway of the irradiation or near the neoplasms. The fact that the

depth-dose profiles obtainable with heavy particles are better than those from any other available radiation has been often adequately discussed.[3]

In recent years, particularly due to the pioneer work of Gray[4] and his associates in England, and Churchill-Davidson[5] and others, more and more evidence has become available to show that some rapidly proliferating neoplastic lesions frequently grow under relatively anaerobic conditions, whereas normal cells and tissues of the body need adequate oxygen, supplied by the circulating blood, for their sustained proliferation and growth. The ability to grow under anaerobiosis not only gives a selective advantage to neoplastic tissue, but also it has been shown by Barendsen,[6] Deering,[7] and Todd,[8,9] working with mammalian tissue cells that these are 2 or 3 times more resistant to low LET radiation (X-rays and gamma-rays) when they are anaerobic than when they are oxygenated. If the oxygenation of normal and of malignant cells would be comparable to each other, the radiation effects on normal and on malignant cells would be similar and tumor therapy might be more effective in the cases of those tumors having a poor blood supply and thus being relatively hypoxic. This has led to therapeutic trials under high pressure oxygen: the so-called hyperbaric therapy, initiated by Churchill-Davidson.[5]

It has been known for some time that the oxygen effect of high LET radiation is less than at low LET radiation; Gray et al.[10] have demonstrated such effects on chromosome breaks and on cell survival. At the Hammersmith Hospital in London such experiments became the basis for therapeutic trials of fast-neutron irradiation.[17]

In our laboratory, studies were made of the LET dependence of the oxygen effect in mammalian cells in culture utilizing either human or hamster kidney cells[8,9] or ascites tumor cells.[11] Fairly accurate data have been obtained over the whole LET domain available to us with the heavy particles. The dose reduction factor due to oxygen becomes very small, 10% or less, only at very high LET above 100 keV/micron. In the domain where fast neutrons would be used, which operate chiefly by their proton and alpha particle recoils, the oxygen effect is between 1.5 and 2.0. Implications of these findings have been discussed by Tobias anf Todd[12] who pointed out that in dose fractionation the oxygen effect is different from that found in single exposures. For the present we wish to state some of our current conclusions in summary form.

(1) In order to utilize heavy accelerated ions for the investigation of their possible increased efficiency in the therapy of certain neoplasms, sufficiently heavy particles of considerable energy must be employed. Figure 1 shows a graph of the residual range of various particles that is above 100 keV/micron and above 50 keV/micron. It is seen from this that neon or argon ions, or even heavier ions, might be much more useful than protons, alpha particles, or neutrons. The Omnitron particles will have sufficient depth doses for effective application of these principles.

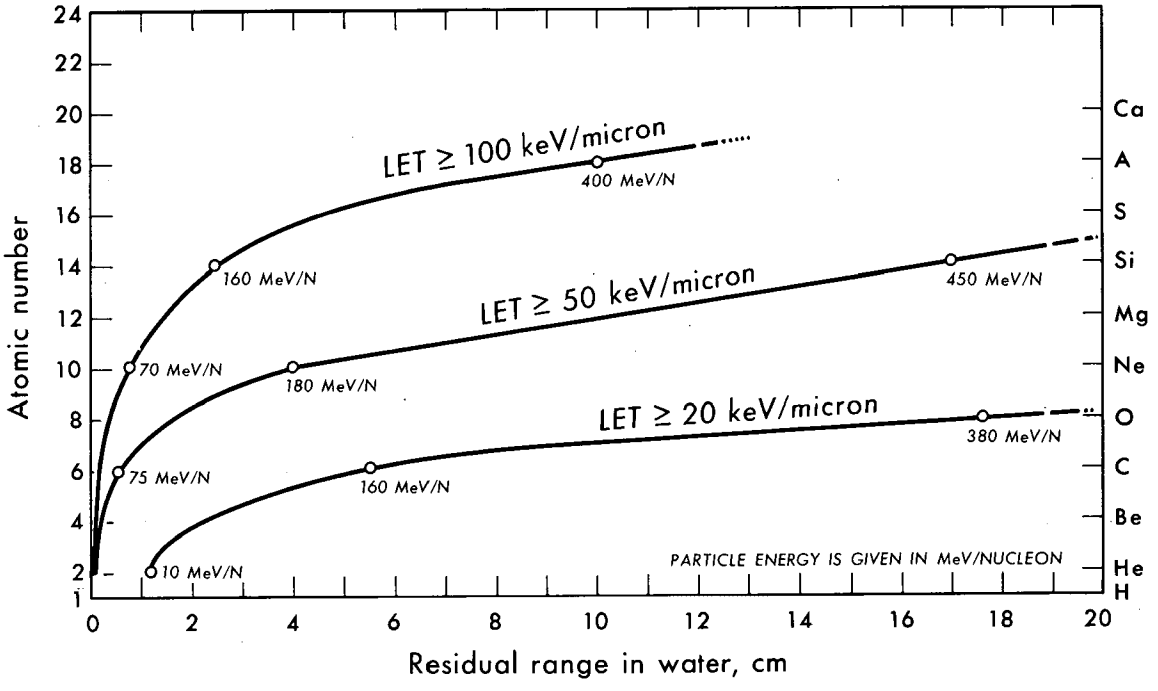
(2) There is preliminary work available now, particularly from the investigations of Skarsgard et al.[13] and in progress in our laboratory, which is indicative of the fact that mammalian cells have great variations in their sensitivity to radiation in different stages of the cell-division cycle.

There is greater sensitivity for cells in mitosis and in midinterphase when presumably new DNA synthesis begins; cells in G_1 and G_2 phases have less sensitivity.[16] One of the consequences that appears following a single dose of X or gamma irradiation is that cells become somewhat synchronized with more of them remaining in radiation-resistant stages.

With heavy ions at high LET it is found that the variations in radiation sensitivity are less during cell division, as indicated by data of Skarsgard et al.[13] Therefore, in the therapeutic investigation of high LET particles, less variation in radiosensitivity should occur than with low LET radiation.

(3) The sensitivity of the cells to the high LET radiation is directly related to the size of the cell nucleus; we may assume that the size and number of chromosomes are determining factors. In many malignant conditions cells are aneuploid and particularly it is frequent to find them with higher than normal ploidy, meaning that they have greater than normal amount of nuclear material present. Because of this, the effect of high LET radiation may be somewhat greater on tumor cells than on normal cells. We know that this effect is not very large, that is, perhaps only a factor of 10 to 50% in radiosensitivity. However, even a small factor may be sufficient to cause a differential effect between tumor tissue and normal tissue.

(4) Several experimental approaches have been applied, intended to decrease the radiobiological oxygen effect on cells. For several years, it was believed that high intensity radiation given in extremely short pulses (as in pulse radiolysis) might eliminate part of the oxygen effect. Preliminary



DBL 672-1530

Fig. 1

data were published by Dewey and Boag.[14] It was argued that the oxygen enhancement of radiosensitivity may diminish if the pulsed radiation would cause depletion of dissolved oxygen in tissue by free radical interactions. If a sufficiently large number of ion pairs would be produced sufficiently close to each other in a short time interval by the pulsed electron beam, the radiobiological effect would be similar to that from high LET particles.

A number of studies are in progress at this moment. Preliminary reports indicate from the work of Gregg and associates, Western Reserve University (private communication), that doses usually employed in the case of ascites tumor cells when given in short pulses of 50 nanoseconds do not appreciably alter the oxygen ratio. Todd and Winchell in our laboratory have attempted to show a change in oxygen effect on kidney cells in culture and have also found lack of evidence for the decrease in oxygen effect (private communication). Calculations show that oxygen depletion should become effective at doses of 20000 rads or more, which are greater than normally used dose levels in radiotherapy. In another set of experiments, attempts were made to decrease the oxygen effect by utilizing the synergism between DNA analog chemicals and radiation. The application of Iodouracil as shown by Tym and Todd[15] does alter the survival curves to radiation. However, the oxygen effect is not appreciably altered by synergistic application of drugs and radiation.

(5) Our knowledge of the manner in which therapeutic radiation can lead to the arrest and regression of tumors is not yet complete. We know that it is not necessary for the radiation to kill each and every cancer cell to cause a successful regression. Investigations were done by the Cohens[18] on this point, who find that the effectiveness of radiation is enhanced by tissue reaction which is probably due to the immune defense mechanism of the body. It is important to administer radiation in such a manner that the immune defense mechanisms are not adversely affected. We do not know at present whether the application of heavy ions will enhance or retard immune reaction against tumors. However, due to the advantageous localization properties of heavy ion beams, it would appear that a large dose of high LET radiation can be delivered to tumor regions while at the same time adjacent regions receive a much smaller dose, much of it at lower LET. It would appear reasonable to think that the immunological defenses which rely on the presence

of normal tissue adjacent to tumor tissue would be less affected by high LET radiation than by low LET radiation.

In conclusion, although hyperbaric oxygen therapy for neoplastic disease has been used by many groups throughout the world, it has not yet provided the answer to the question, "are better results being achieved?"; the sufficiently heavy penetrating and densely ionizing particles which would be available from the proposed Omnitron have enough additional radiobiologic characteristics that they also must be tried in the experimental therapy of certain neoplasms.

References

1. J. H. Lawrence, C. A. Tobias, J. L. Born, A. Gottschalk, J. A. Linfoot, R. P. Kling, Alpha Particle and Proton Beams in Therapy, J. Am. Med. Assoc. 186, 236-245 (1963).
2. S. Falkmer, B. Fors, B. Larsson, A. Lindell, J. Naeslund, and S. Stenson, Piolet Study on Proton Irradiation of Human Carcinoma, Acta. Radiol. 58, 33-51 (1962).
3. C. A. Tobias, J. H. Lawrence, J. T. Lyman, J. L. Born, A. Gottschalk, J. A. Linfoot, J. McDonald, Progress Report on Pituitary Irradiation, in Response of the Nervous System to Ionizing Radiation, ed. by T. J. Haley (Little, Brown and Company, Boston, 1964).
4. L. H. Gray, Radiation Biology, ed. by J. H. Martin (Butterworth, 1959).
5. I. Churchill-Davidson, C. Sanger, and R. H. Tomlinson, Brit. J. Radiol. 30, 406-422 (1957).
6. G. W. Barendsen, H. D. M. Walter, J. F. Fowler, and D. K. Bewley, Effects of Different Ionizing Radiations on Human Cells in Tissue Culture. III. Experiments with Cyclotron Accelerated Alpha Particles and Deuterons, Radiation Res. 18, 106 (1963).
7. R. A. Deering and R. Rice, Jr., Heavy Ion Irradiation of HeLa Cells, Radiation Res. 17, 774 (1962).
8. P. W. Todd, Reversible and Irreversible Effects of Ionizing Radiations on the Reproductive Integrity of Mammalian Cells Cultured in vitro, (Ph. D. thesis), Lawrence Radiation Laboratory Report UCRL-11614, August 1964.
9. P. W. Todd, Cellular Radiobiology of Heavy Ions: Mammalian Cells, p. 91 of this volume.

10. L. H. Gray and O. C. A. Scott, Oxygen Tension and the Radiosensitivity of Tumors, in Oxygen in the Animal Organism (Pergamon Press, Ltd., New York, 1964), pp. 537-553.
11. J. M. Feola, Heavy Ion Effects on Ascites Tumor Cells, p. 246 of this volume.
12. C. A. Tobias and P. W. Todd, Heavy Charged Particles in Cancer Therapy, National Cancer Institute Monograph No. 24, to be published 1967.
13. L. D. Skarsgard, B. A. Kihlman, L. Parker, C. M. Pujara, and S. Richardson, Survival, Chromosome Abnormalities, and Recovery in Heavy Ion and X-Irradiated Mammalian Cells, Radiation Res. Suppl. (1966). Workshop Conference on Space Radiation Biology (to be published).
14. D. L. Dewey and J. W. Boag, Nature 183, 1450 (1959).
15. R. Tym and P. W. Todd, The Sensitization by Iododeoxyuridine of Cultured Human Cells to the Lethal Effects of X Rays and Heavy Ions, Intern. J. Radiation Biol. 8, 589-603 (1964).
16. T. Teresima and L. T. Tolmach, Science 140, 490-492 (1963).
17. J. F. Fowler, R. L. Morgan, and C. A. P. Wood, The Biological and Physical Advantages and Problems of Neutron Therapy, Brit. J. Radiol. 36, 77-121 (1963).
18. A. Cohen and L. Cohen, Radiobiology of the C_3H Mouse Mammary Carcinoma, Brit. J. Cancer 8, 303-312 (1954).

EDUCATIONAL ACTIVITIES AT THE DONNER LABORATORY AND POSSIBLE FUTURE INTERNATIONAL COOPERATION

J. H. Lawrence and C. A. Tobias

One of the advantages of the Lawrence Radiation Laboratory and the Donner Laboratory is that they are located on the campus of a large university. We can profit by consultation and collaboration with faculty members in many specialities and can also offer educational opportunities for those interested in a specialized field of science. Many of the staff members of the Donner Laboratory are also faculty members in the Medical Physics Division of the Physics Department of the University (see appendix). This department was initiated in 1947 and it was instrumental in the introduction of a teaching program toward a Ph. D. in Biophysics and Medical Physics at the University. Currently these doctoral programs are administered by an interdepartmental group of faculty members, and approximately 85 graduate students in Biophysics and 10 graduate students with a M. D. degree in Medical Physics belong to the program. This is currently the largest Ph. D. program in the Biophysical sciences in the United States. The Biophysical degrees are broad programs in which can be accommodated not only those who are interested in radiation physics, radiation biology, or nuclear medicine, but also those interested in a number of other specialities in Biophysics.

The program of the Medical Physics Division has grown an average of 18% during the past 6 years, resulting in overall growth of 285%. The number of Ph. D. degrees awarded in the Biophysics program since its conception is 105, and in Medical Physics 15 Ph. D.'s were awarded. Approximately 70% of the recipients of these degrees are now on the Faculty of some university or college, and many of these men are at National Laboratories; about 30% of the total number of theses have dealt with a topic in radiation biology or nuclear medicine.

The Donner Laboratory in cooperation with the Lawrence Radiation Laboratory is also offering a M. A. in Bioradiology for outstanding students who wish to become acquainted with the principles of radiobiology and

radiological physics. This program is aided by fellowships of the U. S. Atomic Energy Commission. In addition to these programs, the Laboratory has been very active in the postdoctoral field, and for a number of years a significant number of Fellows spent one or more years with us working on specialized research problems. Agencies who have supported this program with special fellowships include AEC, NIH, NSF, International Atomic Energy Agency, the Donner Foundation, and a number of others. Currently there are 12 postdoctoral fellows and visiting faculty members in the Donner Laboratory.

Many of the Fellows are drawn here by the availability of specialized facilities and methods. With the completion of the Omnitron, it may be logical to broaden the training program. Intense interest is evident not only from other universities and national laboratories in the United States, but also from a number of other countries. Already a running cooperative postdoctoral research effort is in effect with scientists from certain countries, particularly Japan, Norway, and England.

With the unusual properties of the Omnitron we have received advance indications that the Atomic Energy Agencies of several countries might become interested to participate in a postdoctoral educational and research program and that they might help to defray the costs of such a program by paying for the expenses of research teams that may be sent from their own country. This kind of international cooperation is beneficial not only because it aids development of basic research in a field important to the health and welfare of people in every country, but also because among the visiting scientists there would be qualified teachers and some postdoctoral fellows eager to continue their educational experience. For this reason we are planning to expand the postdoctoral fellowship program. An advanced educational institute in the field of radiation sciences might be established in the Donner Laboratory, particularly in radiation physics and radiation biology, and in all of the branches of nuclear medicine.

Appendix

Medical Physics Division: Regular and Affiliated Faculty for 1966/67

Regular Faculty:

John W. Gofman, M. D., Ph. D.
Hardin B. Jones, Ph. D.
John H. Lawrence, M. D., Sc. D.
Robert K. Mortimer, Ph. D.
Cornelius A. Tobias, Ph. D.
John H. Northrop, Ph. D., Sc. D., LLD.
Robert H. Haynes, Ph. D.
Howard C. Mel, Ph. D.
Alexander V. Nichols, Ph. D.
Robert M. Glaeser, Ph. D.

Affiliated Faculty:

Henry Borsook, Ph. D., M. B., M. D.
Hans J. Bremermann, Ph. D.
Kenneth S. Cole, Ph. D., Sc. D.
Ernest L. Dobson, Ph. D.
Thomas L. Hayes, Ph. D.
Thomas H. Jukes, Ph. D.
Aharon Katchalsky, Ph. D.
Lola S. Kelly, Ph. D.
Joseph S. Krakow, Ph. D.
A. Douglas McLaren, Ph. D.
Donald J. Rosenthal, M. D.
Roger W. Wallace, Ph. D.
Harry S. Winchell, M. D., Ph. D.
Hiroshi Yoshikawa, Ph. D.

AUTHOR INDEX

Aceto, H. 175
Ashikawa, J. K. 121
Born, J. L. 200, 223, 232
Brustad, T. 68
Calvin, M. 101
Cheung, C. Y. 200, 223
Curtis, S. B. 31, 264
D'Angio, G. J. 258
Feola, J. M. 246
Fink, R. A. 200, 244
Freeman, N. K. 79
Gaffey, C. T. 155
Garcia, J. F. 200, 213
Glaeser, R. M. 108
Hayes, T. L. 108
Haymaker, W. 126
Haynes, R. H. 86
Henriksen, T. 58
Inch, W. R. 86
Jones, W. B. G. 58
King, J. L. 124
Klein, M. P. 101
Kling, R. P. 200, 230, 232
Kruger, L. 136
Lawrence, J. H. 1, 200, 223, 232, 290, 297
Lemmon, R. M. 101
Lindgren, F. T. 79
Linfoot, J. A. 200, 223, 230, 232
Litton, G. M. 18
Loughman, W. D. 266
Luce, J. R. 189
Lyman, J. T. 42, 81, 86, 189
Maccabee, H. D. 38, 108
Manougian, E. 200, 219, 223 232
McDonald, L. W. 159
Mortimer, R. K. 105
Nichols, A. V. 79
Powers, E. L. 81
Raju, M. R. 47, 264
Richman, C. 264
Sargent, T. W. 273, 281
Schoenbrun, R. L. 164
Singman, D. L. 146
Siri, W. E. 273, 287
Steward, P. G. 8
Tobias, C. A. 1, 18, 81, 108, 189, 197, 200, 223, 290, 297
Todd, P. W. 91
Tym, R. 241
Van Dyke, D. C. 146, 197, 273, 278
Wallace, R. W. 18
Welch, G. P. 146
Weyand, R. 241