Symposium on Radiobiology

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The Basic Aspects
of Radiation Effects
on Living Systems

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The Basic Aspects of Radiation Effects on Living Systems

OBERLIN COLLEGE June 14–18, 1950

Edited by JAMES J. NICKSON

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Foreword

The Subcommittee on Radiobiology of the Committee on Nuclear Science of the National Research Council had, for some time, considered the need for a symposium on radiobiology. At the first meeting of the special committee, appointed to consider the nature and scope of the symposium, the need and desirability of such a symposium were very thoroughly reviewed. It was readily apparent that radiological and other societies had been conducting and were continuing to conduct meetings in which various aspects of radiobiology had definite representation. In addition to this, the Atomic Energy Commission was holding meetings on a regular schedule at its various facilities in which current work and progress in radiobiology were presented. It was the consensus of the committee that additional meetings of the character outlined above would serve little purpose and could hardly augment these meetings in any substantial fashion. In this regard it was felt that the Subcommittee on Radiobiology might properly recommend to the Atomic Energy Commission, to the Radiological Societies, and to the Federated Societies for Experimental Biology a continuation of their usual conferences and programs at their regularly established meetings.

The committee turned its attention in another direction and after long deliberation and examination of the various facets of the problem concluded that a symposium, if it were to be held, should concern itself with the basic aspects of the radiation effects on living cells. It was decided that the objective would be a thorough examination of the fundamental concepts that exist in radiobiology. Since the interaction of ionizing radiation in living matter should be considered in as orderly a manner as possible, it was further decided that the subdivisions and their order would be essentially as follows:

First, a survey of the physical interaction of ionizing radiation and matter. Second, a discussion and elucidation of the chemical

changes arising from the transfer of this physical energy. Third, an examination of the biochemical effects; and, finally, a discussion of the changes occurring in living tissue. Much attention was centered upon the manner in which radiation effects in living tissue should be surveyed. It appeared desirable to avoid numerous subdivisions in order to facilitate the handling of this problem. The committee concluded, therefore, that it should first center on the simplest living unit — the cell — and then transfer directly to the complex living system. The mammalian organism, which would in almost all circumstances be our eventual target, was therefore chosen. The agenda followed closely upon these deliberations.

It was proposed that the essayists would present the information available on each of these subjects in its proper background and perspective, giving full development to the subject from its basic aspects through to the most complex. The presentation of original data was to be minimized, such data being utilized only to develop and expound the main thesis. With the material presented in this fashion it was felt that avenues to the solution of existing problems might be pointed up and hiatuses in our present knowledge would be more certainly delineated. Our objectives were fundamental concepts and ideas rather than isolated information which had not yet found its proper position in radiobiology.

Although the symposium may not have achieved this ambitious goal, the participants established a very sound basis for further developments and, in a large measure, aided in defining the field of radiobiology.

Whatever success the symposium may have achieved is due to the essayists, but special mention of their efforts is hardly necessary since their approbation will come from the readers who will have an opportunity to examine their collective work. It appears appropriate, however, again to extend our thanks to the foreign scientists, Dr. Walter M. Dale, Dr. George Hevesy, and Dr. Raymond Latarjet, who came long distances at the expenditure of considerable time and effort. The labors and unflagging zeal of the members of the special symposium committee, Drs. R. E. Zirkle, A. K. Solomon, J. J. Nickson, M. D. Kamen, H. J. Curtis, and A. M. Brues, were particularly important and contributed greatly to the organization of the symposium. The symposium program was arranged under the five subdivisions already men-

tioned. The presiding chairmen for these sessions were, respectively, G. Failla, S. C. Lind, A. M. Brues, Karl Sax, and A. H. Dowdy, whose stimulating guidance in moderating the various phases of the program was greatly appreciated. The committee is especially indebted to its executive secretary, Dr. Harvey M. Patt, who implemented the arrangements for the symposium to the smallest detail and devoted the greater part of a year to its preparation.

The hospitality of Oberlin College, made available through the good offices of Mr. Donald Love, secretary of the college, and President William Stevenson, contributed immeasurably to the success of the meeting.

The symposium committee is also deeply appreciative of the efforts of Dr. Joseph G. Hamilton, Chairman of the Subcommittee on Radiobiology, of Dr. L. F. Curtiss, Chairman of the Committee on Nuclear Science, and of Dr. R. C. Gibbs, Chairman of the Division of Physical Sciences, of the National Research Council, who gave constant encouragement and smoothed the road to the final culmination at Oberlin.

The symposium was honored by the attendance of Dr. Detlev W. Bronk, Chairman of the National Research Council, who addressed the assembly on the nature and scope of the Council's activities and called attention to the opportunities and responsibilities of scientists, as set forth in its charter, to serve in an advisory capacity to agencies of the government in matters pertaining to science.

The subcommittee acknowledges with deep appreciation the support of the symposium by the Atomic Energy Commission and the Office of Naval Research through contracts with the National Academy of Sciences.

H. L. Friedell, *Chairman*
Special Symposium Committee

January, 1952

Preface

This volume reports the papers and discussions presented at the Oberlin Symposium on Radiobiology in June of 1950. As Dr. Friedell has said in the Foreword, the purpose of the meeting was to present in orderly fashion the state of knowledge of the field at that time, and further to indicate the hiatuses that exist in our knowledge, particularly in areas that seemed susceptible of investigative attack.

It is believed that the volume to a considerable degree fulfills the purpose set forth above. It is unfortunate that publication was delayed, but the vagaries of authors, publication committees, and the international situation were such that our deadlines were largely honored in the breach.

The chairman of the publication committee wishes here to record his appreciation for the wholehearted cooperation of Drs. P. Morrison, M. Burton, E. S. G. Barron, G. Failla, and H. M. Patt, who were responsible for the editing of the material of the five divisions of the meeting. In addition the efforts of Dr. I. Rachwalsky and the Misses E. Tyree and E. Sobel in my office were invaluable in preparing the material for publication.

J. J. Nickson

January, 1952

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1.

Radiation in Living Matter: The Physical Processes

P. MORRISON

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Radiation as a Localized Reagent

The beam of radiation, whatever its type, is a source of energy in highly available form. One can estimate roughly the free energy of such a beam, taking into account both the energy it contains and its entropy, or high-degree order. For a typical x-ray beam, say 50 r per min at 1 mev, it is easy to show that one is dealing with a sample of radiation at a temperature of about 10^8 degrees Kelvin. A few minutes of exposure to such a beam corresponds to the introduction into the irradiated volume of free energy fully comparable to that made available by the injection of a strong reagent like nitric acid, up to a concentration of, say, 10^{-2} molar. It is no wonder that rather small total energies have widespread biological effects. It is likewise evident that the history of this free energy, between its introduction in such potency and its final expression in the reaction of an organism, is bound to be a long and complex story, which the 5 days of our symposium will by no means be able to detail.

Examination of a typical cell, say an individual of *E. coli*, on the atomic scale will help fix the space-time picture of the radiation interaction in recognizable terms. Such a cell is a wonderfully organized collection of some 10^{11} atoms, mostly in the molecules H_2O, of course, with many others. About 10^4 ion pairs within the cell produced by x-radiation are enough to lower by a good factor its chances of indefinitely multiplying. (See Fig. 1.) Those ions are formed not at a constant rate, but in short bursts of a hundred at a time, bunched in 10^{-12} sec or less, and spread out along a tortuous and branching path crossing the cell volume. With alpha particles the same effect on multiplication requires a few dozen alphas crossing the cell one by one, leaving in their roughly straight wakes similar short bursts of ionic produce, arranged in columns with nearly every molecule on the path seriously disturbed.

With ultraviolet a similar effect would be produced by a steady rain of quanta, with a few million photons independently absorbed more or less uniformly in the protoplasmic molecules, excluding the water but somewhat concentrated in the side chains of the nucleoproteins. It is no wonder that these very different first steps in the distribution of the available energy lead to differing mechanisms of the consequent effects; it is perhaps more remarkable that the final effects can be so similar. Doubtless this simplicity is in large part an artifact of our gross means of observation.

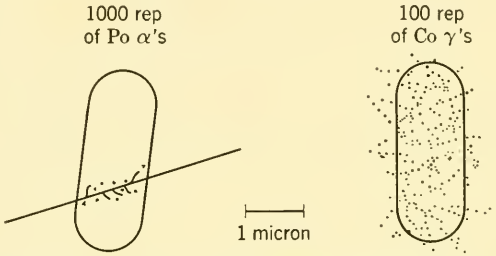

FIG. 1. The distribution of disturbed atoms immediately after irradiation of an *E. coli* cell with dosages indicated. Note the great difference in spatial distribution and the various delta rays, scattered electron paths, etc.

The history of radiobiology has led to the emphasis on ionization as the measure of absorbed energy, since, in the x-ray domain especially, the means of measurement depended on the easy collection of ions in air. The roentgen and its definition in terms of ion pairs produced have fixed this point of view; but we shall use the roentgen, or perhaps better the so-called rep, as a measure of the density of energy absorbed. The generality of the definition is evidently helpful; to say that when 93 ergs per gram of ordinary tissue is the energy density absorbed we have 1 rep is to connect this physical factor with all the biological experience. But it must be evident that the description of the complex class of radiation reagents by the absorbed energy density alone is inadequate, even if we have already far extended the ion-pair definition of the radiologists. We know that qualitative differences can exist among radiations and their effects, even for identical gross energy transfer. The examples above for *E. coli* colony growth corresponded in energy units to about 5000 rep for the x-rays, 20,000 rep for the alphas, and roughly half a million rep if we stretch the concept to include the non-ionizing ultraviolet. The last figure is equivalent to a temperature change of about 1°.

With this general picture in mind, we shall here attempt to outline the principal mechanisms for the transfer of energy from the hot beam of

radiation to the atomic and molecular structures of the tissue. It is hard, of course, to separate cleanly the job of this panel from that of our colleagues the chemists. Very roughly, we quit when we have given the energy to an atom or even an electron whose mean velocity is not too much greater than that of the thermal motion, and which has settled down to a recognizable state which may persist through at least a few atomic collisions. Our story is much longer to tell than to watch, I am afraid, by a factor of some 10^{14}.

Ionizing Radiations

The characteristic physical tool of the radiobiologist is actually an ionizing particle. Excluding the ultraviolet region, which from its high molecular specificity is the proper province of the photochemist, most of the effects of radiations of every kind, from x-rays to neutrons, are due to ionizing particles. This does not mean that most of the effects are due to ionization itself; quite the contrary. But the initial transfer of energy comes to most atoms through the more or less close approach of a charged particle, whose electrostatic repulsion or attraction for the nucleus itself and for the electrons of the atomic shells is the mechanism of energy transfer. The disturbed atoms are very frequently ionized, and because of its adaptability to electrical measurement it is the ionization which is for the physicist the most conspicuous effect of the passage of the particle.

In the materials of interest, the electrons of the atomic shells move with rather low velocities. Even the fastest electrons in the main atoms of biological materials have energies of only a few thousand electron volts; by and large they are much slower. If the velocity of the incoming fast particle is considered (not its energy, but its *velocity* matters), calculations on classical mechanical lines are adequate. One simply considers the hyperbolic orbit of the motion of the incident particle in the inverse-square electrostatic force field of the atomic electron; the whole collision, which is an intimate one, more or less head-on, will be over and done before the atomic electron has had a chance to move in its slow orbital motion about the nucleus. As long as the energy transfer in collision is large compared to the energy by which the electron is bound to its atom, the effect of the atomic binding forces can be neglected safely. The incident particle may be appreciably deflected. The diagram of Fig. 1 sketches the mechanical relations in such a collision (1).

Such close collisions are often called knock-on collisions. They may be handled with high accuracy. The spectrum of secondary electrons

falls off with energy as $1/E^2$. The secondaries can ionize in their turn, and the total energy removed from particle motion converted by such collisions can be computed. For such encounters, one electron is like another, and the energy removed is independent of the kind of atom traversed, except in so far as the composition determines the total electron density of the medium. The space rate of energy loss depends, for knock-ons, only on the velocity—not the mass—of the incoming particle and increases as the square of its charge. If the incident particle is an alpha, or an even heavier ion, like the recoil C^{14} nucleus of slow neutron capture in nitrogen, the charge will not remain constant. Passage of the heavy ion through the atoms of the material can be regarded in a different frame of reference as a kind of bombardment of a stationary ion by the electrons of the matter; sometimes the not completely stripped ion will lose an electron by ionization; sometimes the ion will pick up an electron moving just in its direction, and the charge will be reduced. The process reaches a kind of slowly shifting equilibrium as the particle slows down. It is most important for slow and heavy ions, for which the simple considerations will no longer be adequate, and ionizations may proceed strongly even if the ion is on the average nearly neutral.

Further to complicate the problem there are the very important collisions, often called glancing collisions, in which the ionizing particle does not approach any electron very closely. It may pass through the atom, or even many angstroms away. The distinguishing feature of these collisions is a small energy transfer, not overriding the atomic binding forces, and corresponding to a very slight deflection of the incident particle. The effect of such an undeflected and more or less remote charge sweeping by can to a good approximation be replaced by a strong, rapid, electric pulse, very like a burst of light uniformly distributed in frequency. The equivalent electromagnetic radiation may excite or even ionize the atom as a whole, just as a beam of real photons would do. For this type of collision the simple mechanics of electrostatic forces is entirely inadequate; the effect of light of all colors on the atom must be known. Evidently the problem is more complex and demands a full knowledge of the quantum mechanics of atomic structure (2). In particular, we must recall the following:

1. The atomic electrons now move during these relatively slow and weak encounters. The accurate treatment of the whole process requires a knowledge of the electron orbits so complete that the effect has been worked out in detail only for hydrogen atoms. We have to depend upon this calculation for a guide and supplement it with observed semi-empirical regularities (3).

2. It is by no means obvious that collisions like the glancing ones, which are sometimes quite distant, can be treated as the direct interaction of only two systems: the moving particle and the struck atom. Surrounding or intervening atoms may be polarized by the varying electric fields. Their distorted charge distributions will in turn affect the net force felt by the atom under consideration. Thus the energy loss will be dependent on the chemical composition, and not simply on the electron density. This effect seems rather small for overall energy loss, with a notable exception in the case of very fast particles, where the difference between gases and solids is very marked (4).

3. When quantum effects play a role, it becomes clear that the simple picture in which an electron is either removed from its bond entirely, forming an ion pair, or is simply shaken up and allowed to return to its original state without any energy gain, is not correct. In fact, the energy lost by the incident particle must eventually appear in one of three forms; excitation of the atoms or molecules of the material to discrete excited states; ionization into the continuum; or kinetic energy of secondaries too small to excite any more atoms. Clearly the last form will be negligible in complex material like protoplasm or even water; whereas the first form may be of great importance and may lead to irreversible chemical change, not merely to thermal motion of the stopping material. Radiation of light quanta may intervene, and is in fact to be expected for fast-moving particles.

Of the relative importance of ions and excited atoms Fano will have more to say; it is enough to observe here that for every ion pair formed one will expect two or three excited atoms. The precise kind and number of excitations will in general depend on the stopping material, but not much on the velocity of the particle, so long as it still moves fairly fast compared to the atomic electrons.

In spite of these difficulties we can give a pretty fair account of the space rate at which energy is lost from a particle in tissue. The accompanying graph (Fig. 2) is a fair sample of the dependence on velocity and charge; we summarize (Fig. 3) [after L. H. Gray (5)] the energy loss per micron of path for typical radiation types.

The energy set free on ionization may, of course, include considerable kinetic energy given to the newly freed electrons. Most of this energy appears in electrons of rather low energy, which are capable in their subsequent motion of exciting and ionizing a few more atoms themselves. Thus very frequently the ions are produced not in single pairs but in little clusters of some two to four or five pairs with the corresponding excited atoms. Once such a secondary electron has received, as it occasionally will, sufficient energy to produce more than these few

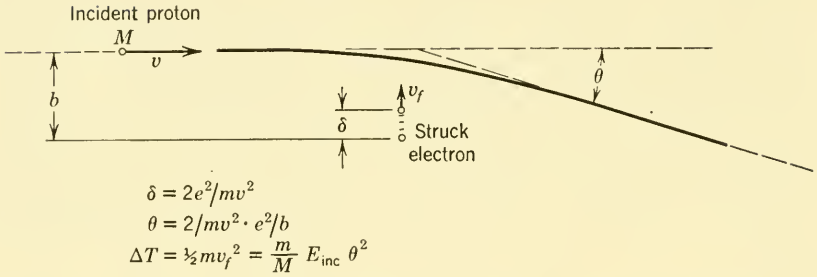

FIG. 2. Approximate mechanics of simple knock-on collision. The distance the struck electron is displaced during the encounter, perpendicular to the path of the incident proton, is called δ; the angle of deflection of the incident proton (considered small) is θ; the energy transfer to the initially stationary electron which moves finally with velocity v_f is ΔT, and $E_{inc} = Mv^2/2$ is the initial energy of the incoming proton. The distance b, often called the impact parameter, is the distance at which the incident particle would pass if there were no force of attraction. Small b means good aim. Note especially how large energy transfers are associated with large angles of deflection, small impact parameters, and big displacements, δ. The faster the incident particle, the greater the energy transfer for a definite deflection, but the smaller must be the impact parameter, and therefore the better the "aim." High energy transfers are evidently less likely.

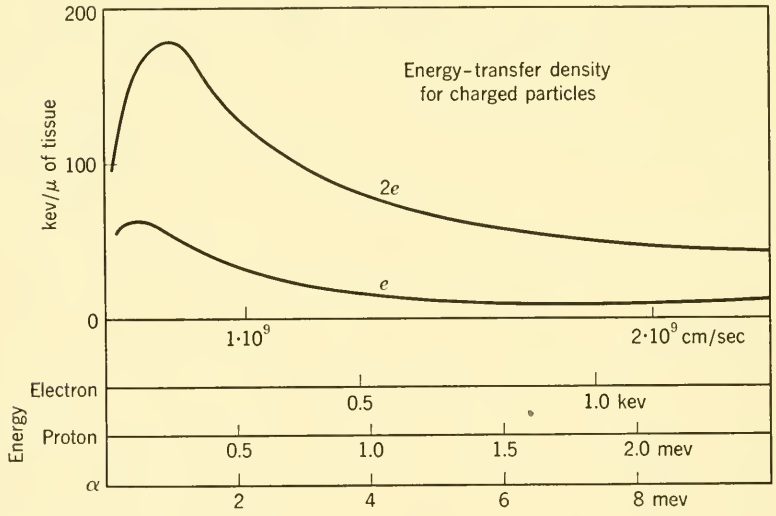

FIG. 3. Space rate of energy transfer, measured in kev per micron of tissue, for various charged particles as a function of energy.

pairs, it will form a secondary track, far from straight, tortuously branching off from the main primary track. Such recognizable secondary electron tracks are called delta rays, distinguished from the secondary ion pairs made close to the primary path by low-energy secondaries *only* by their length. Delta rays are responsible for about half of the energy transfer for all ionizing particles, except the slowest electrons, and extend considerably the volume affected by the concentrated ionizing forces of a heavy alpha particle. Lea [(6), esp. pp. 26 ff.] has estimated that delta rays extend the length of the alpha-ionized column by a factor of 2 or 3, that of recoil protons by 20 per cent or so, that of fast electrons very little. This means that the ions and excited atoms lie in a narrow column a few atom-diameters wide along the actual path of an ionizing particle, but out of this main more or less linear spine there come numerous short branches, producing a feathery structure, especially for the heavier particles. (See Fig. 4.)

Energy-Transfer Density, kev/μ tissue

Minimum ionization particles	0.22 kev/μ
20-mev betatron gamma rays	0.28
Cobalt gamma rays	0.42
1-mev supervoltage x-rays	0.5
200-kev deep-therapy rays	2.8
X-ray region	3.5
12-mev protons	10.0
Cyclotron neutrons: Be + D	23.0
Thermal-neutron capture recoil ions	$\sim$100.0
Polonium alphas	150.0
U fission fragments	$\sim$4.0 mev

Fig. 4. Space rate of energy transfer for a variety of types of beam. Note the wide variation possible.

In the time that any such projectile crosses the cell, some 10^{-14} sec, the main energy transfer takes place. Out of the atoms in the wake of the particle there then come secondaries, while molecular transitions and "free-radical" formation take place in the excited atoms. This stage takes perhaps even less time, say about the time of a few electron orbit passages. Some molecular disassociation, now already sure to occur, will not be complete for a considerably longer period, since the nuclei must move. Meanwhile the secondaries are moving out, most of them slowly, and ionizing and exciting as they go, again for such a period of time. After not more than 10^{-12} sec, then, there is a feathery arrangement of excited molecules and atoms with free electrons and positive ions, none of them possessing enough energy to ionize further,

though they may still excite complex molecules and transfer their kinetic energy in elastic collision into simple heat motion. We are almost at the point of chemistry. Loss by elastic collision would require perhaps 10^{-10} sec, but much molecular excitation might take place earlier. Now the free electrons will become captured by a molecule of the medium. In water one will most frequently find this capture leading to a free negative hydroxyl radical, setting free a neutral H atom as well

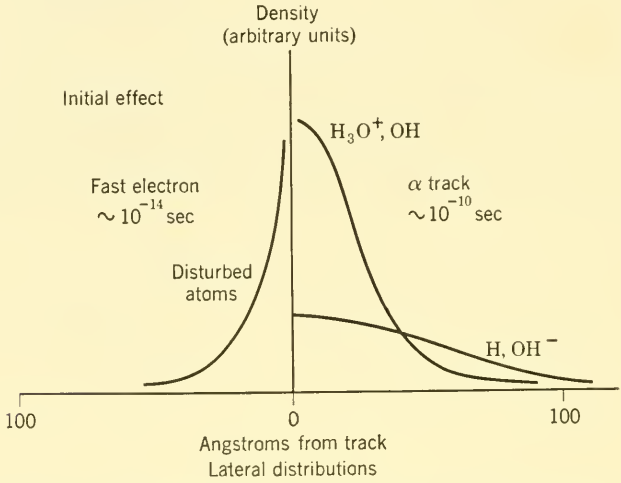

FIG. 5. Qualitative representation of the lateral distribution of disturbed atoms and molecules across an ionizing track. To the left is shown the distribution immediately after passage of a fast electron; to the right, the distribution of diffusing positive and negative ions after an alpha particle has traversed water, about 10^{-10} sec earlier. Note the charge separation due to the faster motion of the secondary electrons which are captured some distance from the track. [After Lea (6).]

in the reaction. What the spatial distribution of these initial products will be is a little obscure. The feathery track will be smeared out by the diffusion processes, but the caging effect of the surrounding medium and the frequently high density of ions along the track itself will combat this free diffusion by making recombination easy, and even by the overall electric field which attracts negative ions toward the generally more concentrated positive-ion core. (Figure 5 suggests the situation.) It is most doubtful that any of the quantitative treatments yet given [see (6), p. 59, and (7)] are adequate for the complex problem involved here. It can be said that the diffusion from a lightly ionizing track may spread the radicals over sizable distances, even a tenth micron or two, whereas in the alpha case it seems likely that most radicals will recombine before some 10^{-8} sec, have passed, in a distance of only a couple of

hundred angstroms. Of course, the proximity of any molecular system more complex than the expected water polymer—such as a long nucleoprotein cylinder—will locally much modify this picture. But these problems belong to the chemists.

Even after the initial particle or its electron secondaries have lost so much energy that they are unable to ionize at all, and their passage is invisible to the cloud chamber or the ionization-sensitive device, they will have a possibly important effect in the stopping material. Heavy particles, however, can ionize appreciably, even after they have become neutral, by capture, though eventually they slow down so far that they cannot transfer to a single electron enough energy to ionize or, finally, even to excite an atom. This limiting energy is not so small, since the maximum transferred energy depends on the velocity of the heavy particle. A carbon recoil from neutron capture in nitrogen might possibly spend a large part of its energy without making a single ion pair. This effect may be of some importance in special cases; it is apparently observed in the effects of bombardment on solid materials. The theory here is far from complete (8).

It is plain that the transfer of momentum to the stopping atoms does not always take place wholly along the direction of motion. The primary particles are deflected by these collisions. The many small-energy-transfer collisions impose on a heavy particle a successive series of angular deflections to all sides of the path. For fairly fast, heavy particles the mean square displacement angle will grow proportionally with path traversed; this will give rise to a statistical distribution of energies after a fixed straight-line segment of path. Such struggling can be important at the end of a high-energy track, as Wilson will show. Electrons and very slow, heavier particles will be scattered more drastically; their paths may, in general, differ widely from a straight line. Sufficiently slow electrons will appear to diffuse from collision to collision. In tissue all electrons below some hundreds of kev will be very greatly deviated from a straight-line path.

Electromagnetic Radiation

The effect of electromagnetic radiation cannot, of course, be described for the whole spectrum at once. For the usual range of interest it is, however, fairly satisfactory to observe that every quantum absorbed can affect at most one *primary* absorbing atom. The secondary product of the absorption, an electron ordinarily, will then be set free to repeat the history of an ionizing particle in living matter. For the typical x-ray at, say, 100 kev, the ionizing events due to these electron secondaries

are about 600 times as numerous as the atoms which directly interact with radiation. The effect of radiation here, then, is just the effect of randomly originating ionizing recoil electrons. We can distinguish a few important quantum energies in the whole spectrum as marking different régimes of radiation:

1. *Near 4–5 ev.* This ultraviolet region is marked by strong selective absorption in specific atomic and molecular structures. Only the direct action of the quantum is important here, as indeed for lower-frequency visible light, where the biological effects of radiation are the most important of all—photosynthesis.

2. *Up to 50 or 60 kev.* In this region of soft and medium x-rays, the principal process of energy transfer is through the photoelectric absorption of the quantum by an inner electron of some atom. All the quantum energy appears in the ejected electron, mostly as its kinetic energy, but partly in the potential energy gain of ionizing the inner atomic shell. Here specific heavy atoms may somewhat affect the probability of the process, though, of course, the photoelectrons are responsible for the great bulk of all energy transferred to the tissue.

3. *Up to about 20 mev.* In this domain of gamma rays, high-energy therapy machines, and the betatron, the principal transfer process is the Compton process, in which the gamma ray is absorbed by an essentially free electron, and both a recoil electron and a secondary gamma emerge. The recoil electrons have a wide range in energy but are invariably fast from the point of view of their stopping effects. Only electron density counts in this region; no specific atomic effects are to be expected.

4. *Above 20 mev, up to about 100 mev.* Here the formation of positron-electron pairs is more important than the Compton effect. This essentially means that the quantum energy is converted to that of two electrons, of widely distributed, very roughly equal energies.

5. *Beyond 100 mev.* Here the cascade region is reached. A single quantum absorbed will lead to a whole chain of new quanta and electrons, dividing the energy up among many fast electrons in the end. The spatial distribution of the energy transferred will be markedly different from that in the other regions; in general the depth dose will exceed the entry dose. On the cellular, fundamental level, of course, all these radiations above the ultraviolet should have qualitatively similar effects: those of fast secondary electrons. But much fundamental dosimetric work still remains in the high-energy field, especially with multicellular organisms.

Neutrons and Nuclear Collisions

The irradiation of tissue with neutrons provides one more example of the deviousness of the path by which energy transfer finally is made by ionizing particles. Neutrons, having no electric charge, interact only negligibly with the atomic shell electrons. They collide, then, only with the nuclei; their mean free paths between collisions are measured in centimeters rather than in angstroms, because of the great difference in size between nuclear and atomic structure. A fast neutron collides almost entirely with the H atoms of tissue, setting them into rapid motion, with any energy between the incident E_0 and 0. These secondary protons—for they will be generally stripped by the shock of collision—then move through the material, ionizing as they go. This makes protons of low range important agents of biological irradiations. Inelastic collisions of neutrons with nuclei are quite frequent as well, with very fast neutrons and heavier atoms. The energy lost to the motion is converted to a gamma ray or even two or three. When the neutron is moving so slowly that it can no longer cause strongly ionizing recoils, it still sets free neutral atoms to make more collisions, disturbing chemical bonds. Even after it has dropped below the excitation region for molecular transitions it will still persist until it reaches thermal equilibrium. Finally it will be captured by a nucleus, in tissue generally by N^{14}, to yield two recoil ions, a proton with less than 1 mev and a slow, heavy recoil C^{14} atom. These projectiles are especially important for thermal neutron effects, where only capture gammas and capture recoil ions can transfer appreciable energy.

High-energy protons or mesons also yield nuclear reactions. Some of these will be sources of multiple highly ionizing tracks. Such star events, while not major contributors to overall energy transfer, may possibly turn out to be sources of specially observable effects, because a single cell may with one event be heavily damaged. Even the products of neutron or charged-particle nuclear reactions, which in general will be radioactive nuclei, may have a role. There is some evidence that the effect of the recoil energy and chemical change subsequent to a radioactive disintegration of a bound atom of P^{32} may have a biological consequence considerably more important than that of the same ionization energy delivered external to the binding molecule. The possibilities here are notable, and suggest caution in the use of energy-absorption data alone as a predictor of biological effect for specific tracer activities.

Enough has been said in this very cursory survey to demonstrate that even within the first hundredth of a microsecond or less, the physicist's

domain, the actual energy transfer from radiation to tissue matter is not simple in nature. The discussion of such a complex physicochemical system in detail is beyond us; add the evident subtlety of the biological problem, and you will see why facile all-embracing explanations find little favor with physicists. But it is equally clear that the construction of models, like the very important and general target model, and the elaboration of such models both in concept and by experimental changes, are the only means of progress. By the steady growth and test of our oversimple ideas we will weed out of the whole picture those features which are decisive in each of the many problems which interest the radiobiologist. We know already the importance of energy density for many processes; we know the importance of diffusing products for others. It is the hope of the physics panel, as the other essayists continue to fill in the details of the physical picture I have sketched, that from this snapshot of the events within an atomic collection workers in the other fields will be able to create a colorful and penetrating set of artistic and convincing portraits.

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2.

Secondary Electrons: Average Energy Loss per Ionization

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Following Morrison's picture of the physical action of ionizing radiations on matter, I should like to elaborate a little on some details. As a part of our general program, I shall deal with two particular topics, namely: (1) the distribution of radiation energy by secondary electrons, and (2) the factors that control the amount of ionization produced in matter, but particularly in gases, per unit amount of energy distributed by radiation.

Spectrum of Secondary Electrons

Secondary electrons are ejected from atoms under the impact of other fast charged particles. They are ejected with a kinetic energy that may be anywhere between zero and a certain upper limit.

The upper limit is set by the conservation of momentum and energy in the collision [(1), p. 494]. If the incident particle is a heavy one (proton, alpha particle, etc.), it cannot impart to a secondary electron more than twice its own speed. Thus, for example, if the incident proton has an energy of 1 mev, the maximum energy of secondary electrons equals 2200 ev. On the contrary, if the incident particle is itself an electron, it may share any fraction of its energy with an atomic electron in the course of a collision. When the two energies are comparable after the collision, one usually calls "primary" the faster of the two electrons and "secondary" the slower one; this convention amounts to fixing the upper limit to the energy of the secondary electrons at one-half the energy of the primary.

Even though the upper limit to the energy of the secondary electrons depends on the nature and energy of the primary particle, the energy distribution of the secondaries depends but little, on the whole, on these conditions. The reason is that the energy distribution is completely

skew; for example, many more secondary electrons have an energy between 10 and 20 ev than between 210 and 220 ev, and still fewer have an energy between 410 and 420 ev. Therefore the location of the upper limit determines only the cut-off point of the far tail of the energy distribution. Compare Fig. 1.

The shape of the energy distribution can be discussed qualitatively on the basis of the classification of the collisions of the primary particle into two classes, namely, "glancing" and "knock-on" collisions. This

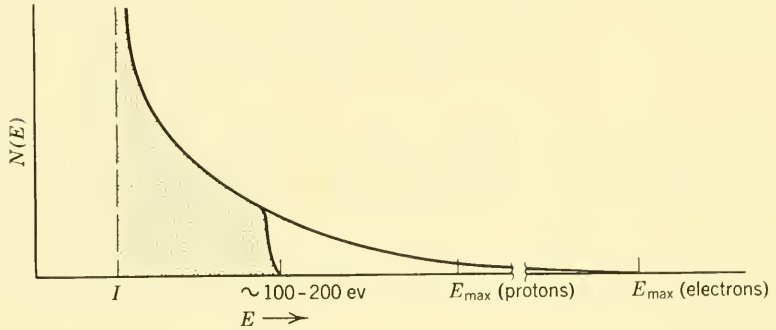

FIG. 1. Number of secondary electrons per unit energy, $N(E)$, receiving total energy E from an incident ionizing particle, plotted against E. Note that every secondary must get at least the ionization energy, I, if it is to leave the atom. The great bulk of the energy transfers occur at low energy, and thus the position of the maximum energy transfer, E_{max}, which may vary greatly with type of incoming particle, has in spite of the great variation no large effect on the distribution of secondary energies. The shaded region locates the region important for total energy loss.

classification has already been explained to you by Morrison. Glancing collisions are much (about 8–10 times) more frequent than knock-on collisions in typical cases.

One may wish to characterize the shape of the energy distribution by its slope n on a logarithmic plot, that is by assuming a distribution law of the type $N(E)\,dE = dE/E^n$. Now, if there were only glancing collisions, the slope n would be roughly 4.5. If there were only knock-on collisions, n would be equal to 2. Therefore the glancing collisions, even though by far the most frequent, are much more unlikely to produce high-energy secondaries than the knock-on collisions [(1), pp. 515 *ff.*].

Low-energy secondaries, say up to 50–100 ev, are due overwhelmingly to glancing collisions. In this energy range the slope n of the logarithmic plot of the spectrum should be of the order of 4. High-energy secondaries are due to knock-on collisions. Above 100–200 ev the slope n should approximate 2. (Figure 2 graphs these relations.) These qualitative

theoretical predictions are confirmed by the available experimental evidence. However, this evidence is not very abundant.

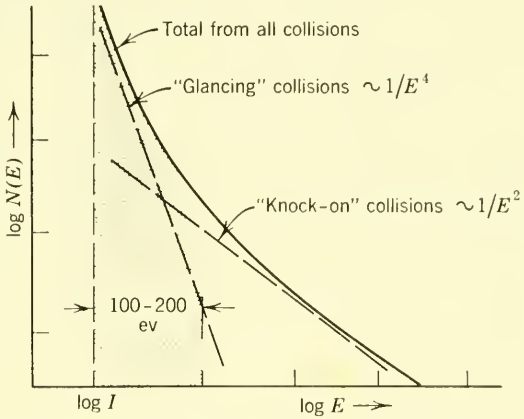

FIG. 2. The energy distribution among secondary electrons, plotted now on a log-log scale. Note that the shaded region, which again represents the bulk of the secondaries, comes mainly from the glancing collisions. The knock-on collisions become dominant only for the infrequent but relatively energetic encounters. The slope of the skew distribution is much steeper for the glancing than for the knock-on collisions, as explained in the text.

It would be helpful to have reliable and detailed tables of the energy distribution of secondary electrons, but such tables do not seem to be available at this time.

ENERGY DISSIPATION BY SECONDARY ELECTRONS

As we have seen, the great majority of the secondary electrons have a rather low energy, even though their aggregate energy amounts to about two-thirds of the energy lost by a fast particle. Electrons whose energy amounts to no more than 100 or 200 ev can transfer energy only to the external electrons of atoms, and this only when passing right through or very close to an atom. On the other hand, every passage in the proximity of an atom has a fair chance of leading to a collision with energy transfer. Also, low-energy electrons experience frequent, repeated, large-angle deflections.

Low-energy secondaries dissipate most of their energy within a short distance from their point of origin. This distance is of the order of 10 Å in solid or liquid materials and about 1000 times as large in gases at atmospheric pressure. This energy is dealt out in the form of activations (excitations or ionizations) at points irregularly scattered in the proximity

of the atoms from which each electron was ejected. These activations are said to form a "cluster" (2). Some of the activations in a cluster are produced by the secondary electron which originates the cluster, but some are produced by other electrons ejected with sufficient energy as a result of ionizing collisions within the same cluster.

Electrons that have spent nearly all their energy usually wander around in a kind of diffusion path, undergoing numerous elastic collisions, until finally they are captured by some neutral atom or molecule to form a *negative ion.* Cloud-chamber pictures, which provide much of the scanty observational evidence on clusters, show the position of negative as well as of positive ions. A large proportion of the clusters appear to consist of a single pair of ions, corresponding to energy-poor secondary electrons.

The larger the initial energy of a secondary electron, the longer and the more nearly straight is its path. The transition from cluster formation to an arrangement of activations along a clear track takes place gradually, of course, as the energy increases from about 100 to about 500–1000 ev. Secondary electrons whose energy amounts to at least several hundred volts are loosely called *delta rays.*

We can now form the following picture of the action of primary fast charged particles. The tracks of fast electrons are marked by a series of variously spaced clusters of various sizes and by a few delta rays. Occasional delta-ray tracks of unusually high energy may fork out from the main track.

Heavy particles of energy up to about 10 mev undergo collisions so frequently that the clusters of activations produced by their secondary electrons merge and blend to form a sort of "column."

Thus the mapping of the energy distribution by secondary electrons appears to be qualitatively understood. Nevertheless a detailed quantitative picture of this process is still missing. To produce a detailed mapping would constitute a fairly laborious task. [This spatial distribution of ions represents a quantity which is under rough control. By varying type and energy of incident particles the mean spacing can be varied over very wide limits. If the effects of individual ionizations, and accompanying excitations, turn out to be independent, the spatial size of the structures involved must be large compared to the mean ion spacing. If the biological structures are not large compared to the ion spacing, correlations will be found. The high-density columns of ionization will almost always be expected to show correlation effects. This type of analysis is, of course, greatly oversimplified. It is in particu-

lar not clear that different biological effects could not originate from ion clusters of different size, even with fixed mean spacing. In such a case, the whole correlation analysis would be washed out by the ion-cluster-size distribution. MORRISON]

IONIZATION YIELD

Ionization constitutes a particularly drastic form of molecular activation. When an electron is ejected from an atom, the resulting separation of electric charges lasts for a much longer time than the minor dislocations of atomic electrons which accompany simple excitations. It is uncertain whether the somewhat larger energy involved in ionization processes than in excitations and the greater permanency of charge separation have a particularly great significance in relation to biological effectiveness.

The separation of charges which results from ionization processes in gases affords a convenient and sensitive method for the physical measurement of radiation effects. It is frequently assumed, on somewhat uncertain grounds, that essentially equal amounts of ionization are produced in a given amount of matter whether the matter is in gaseous, liquid, or solid state. Our information on the subject of ionization concerns mostly the occurrence of this phenomenon in gases.

The amount of ionization produced in a gas generally serves as an index of the total energy dissipation. The main reason for this stems from the following considerations. I shall be speaking primarily about the effect of glancing collisions, but the smaller number of knock-on collisions does not modify the qualitative conclusions.

Some of the glancing collisions merely raise the external electrons of an atom or molecule to an excited state; others transfer more energy and lead to an ionization. The relative frequency of occurrence of transitions to different levels of excitation and ionization can be inferred from theoretical or experimental data on the absorption spectrum of the particular atom or molecule, since the glancing collision has the same effect as an electromagnetic radiation with a continuous spectrum of uniform intensity.

Loosely attached external electrons, that is electrons with a low ionization potential, are generally apt to oscillate with comparatively low frequency and with great intensity while being raised to low excited states, whereas the opposite is true for electrons that are stiffly held. Therefore, excitations are relatively more probable than ionizations

just in those atoms and molecules which require least energy to be ionized. In other words, *just in those substances where an ionization can be produced cheaply*, in terms of energy, *a large amount of energy has to be spent in excitations.* (In substances whose ionization potential has a typical average value, around 10 ev, the relative frequency of excitations and ionizations is of the order of 2 to 1.)

As a result the ratio of the energy delivered to a material to the number of ionizations produced varies within remarkably narrow limits from one substance to another. This ratio is also, of course, essentially the same for different ionizing radiations, since most excitations and ionizations are produced through glancing collisions affecting the surface layers of atoms. Its numerical value for most gases is in the neighborhood of 30–35 ev per ionization. Because of this circumstance, the number of ionizations produced in a gas is very frequently taken as a measure of the energy spent by a radiation within it.

All these considerations pertain to the action of very fast charged particles. As a particle begins to slow down, the excess of glancing collisions with respect to knock-on collisions is no longer very large. Accordingly, the ionization yield increases a little, because every knock-on collision produces an ionization, whereas glancing collisions produce excitations as well.

The whole picture changes substantially when a particle slows down to and below the velocity of atomic electrons. (A heavy particle can move more slowly than an atomic electron and still have a substantial kinetic energy. This is the case, for example, for a proton of 20 kev or a nitrogen atom of 300 kev.) Then the particle becomes unable first to ionize and then to excite at all. The residual energy is presumably dissipated through bodily impacts against atoms which can no longer be easily penetrated. The energy dissipated in this manner probably escapes detection by ordinary radiation-measuring devices. Nevertheless, it may well cause a very substantial dislocation in the structure of matter and thereby acquire a particular biological significance.

The final products of ionization in a substance like water will include negative ions, like the hydrated OH^- formed by electron capture. The subsequent chemical action of such molecules may be of high importance in biological material.

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DISCUSSION OF MORRISON'S AND FANO'S PAPERS

ZIRKLE:

Is the probability of capture of electrons by the oxygen atom markedly greater than the probability of capture of the electron by water?

FANO:

The oxygen atom in the water molecule is the capturing agent.

LATARJET:

Fano stated that atoms with low ionization potential, such as lithium and cesium, are more easily excited, rather than ionized, as compared to atoms with a higher potential. Would this remark be of general value and be applicable to light atoms in the liquid or solid state, that is to living tissues?

FANO:

Yes. It is pointed out, however, that the elements in biological systems are all in the same bracket of ionization potential, except for the few metallic atoms.

LATARJET:

The ionization potential in this range varies from 9 to 20 volts.

FANO:

Only xenon and helium have ionization potentials of about 9 volts. If negative ions, the probability of excitation is greater if excitation is to occur at all.

BURTON:

I wish to comment on the previous two questions (by Zirkle and Latarjet) and on the replies.

It is undoubtedly true that the O_2 molecule itself can capture thermal electrons to give negative ions and that this is an important process in the gaseous state. Threshold energy for the capture of electrons by water in the gaseous state makes the cross section of this process so high that it does not compete effectively with capture by positive ions. In the liquid, however, solvation of the OH^- contributes much energy. The potential-energy curve is so displaced that H_2O now captures an electron in a dissociative process to yield OH^- (aq) without threshold. The cross section for such a process is, of course, lower than the cross section for capture by positive ions, but the concentration of water molecules is so overwhelmingly higher than the concentration of positive-ion species present that the process $e + H_2O \cdot \text{ aq } H + OH^- \cdot \text{ aq}$ becomes very important.

In regard to the question of the relative probability of formation of two species of positive ions with distinctly different ionization potentials, it is important to remember that, although both will be formed initially, the ion of higher ionization potential transfers its charge to the other species. There are

two effects. The ion species of lower ionization potential predominates, and the difference in energy becomes available for excitation.

FANO:

I should like to ask Burton whether he would expect the cross section to change markedly.

BURTON:

There is competition between the positive and negative ions after they are thermalized. The probability of capture increases as the electron gets nearer and nearer thermal energies, but we must consider also the probability of positive-ion capture against the probability of negative-ion capture.

PLATZMAN:

I think that at the present time the effect of the efficiency of ionization by slowly moving positive ions cannot be predicted adequately by existing theory. It may be that such ions are ionized with greater efficiency than heretofore realized. Our knowledge of the efficiency of ionization by ions whose velocity is in the region of orbital velocity or $\frac{1}{10}$ of that value is most inadequate.

FANO:

I have discussed this matter with London, who doesn't see how this could be.

PLATZMAN:

The efficiency of ionization of a gas by a heavy particle penetrating with velocity comparable to or lower than the velocities of valence electrons in atoms of the gas is a question of very considerable importance which is often dismissed inadequately or erroneously and which merits brief mention here.

To say that a heavy particle of velocity v_0 ($v_0 = c/137$ = velocity of the electron in the lowest orbit of the hydrogen atom), which has a most appreciable energy (25 kev for a proton, 99 kev for an alpha particle), *does not* ionize with good efficiency is not justified by any established knowledge and is probably also incorrect. The basis for this frequently encountered statement lies in the fact that, as the velocity of the particle decreases, for values below v_0, the particle spends an increasing proportion of its time as a neutral atom; effects on atoms of the gas therefore tend to be limited to direct encounters, in which colliding and struck atoms interpenetrate, and such collisions take on an increasingly adiabatic character as the collision velocity falls. (Some ionization, but with extremely small yield, is known to occur even at the lowest velocities.) Thus, at very low particle velocities, the probability of excitation or ionization of the gas atom will be small, whereas the probability of scattering—deflection of the particle, with resultant energy less as direct momentum transfer to the atom as a whole—will be great. At *sufficiently low* particle velocity, therefore, the simple "nuclear collision" is the dominant mode of energy loss. Just how low this velocity must be is an important question. Unfortunately, the theory of penetration phenomena has thus far been unable to cope with the problem, at least

quantitatively. And, although some relevant experimental data are available, they are meager indeed and not entirely concordant. However, it does appear that W, the mean energy required to produce an ion pair, starts to rise for a particle velocity somewhat lower than v_0, and thereafter increases steadily as the velocity declines (cf. the work of Madsen*). The functional dependence of W on the particle velocity, in this velocity domain, is still largely unknown. The critical velocity, namely that for which W starts to rise appreciably, appears to be considerably *smaller* than v_0.

That the overall ionization by slower ions is *not* as inefficient as might be anticipated if the energy loss were simply a competition between nuclear collisions, which lead to virtually no ionization, and familiar excitation and ionization, which are often assumed to correspond to the same value of W as for higher velocities (an unjustified extrapolation), might possibly arise from the extremely important contribution to the energy loss, in the velocity domain under consideration, of capture and loss of electrons by the positive ion—a process often erroneously ignored in discussions of this problem. This process might quite possibly prove to have a value of W *smaller* than that for excitation and ionization by high-speed particles, and thus tend to compensate to some extent for the energy lost in nuclear collisions and therefore wasted as far as ionization is concerned.

These matters are also discussed briefly in a later contribution to this volume ("On the Primary Processes in Radiation Chemistry and Biology," p. 97), and a detailed study of the problem by the writer is now in progress.

MORGAN:

My question is directed to Fano. In the case of a fast neutron (with an energy of, say, 2 mev) colliding with an oxygen atom, the most probable energy given to the oxygen atom is approximately 0.2 mev. This corresponds to the energy of an electron of about 8 ev if the electron has the same velocity as the 0.2-mev oxygen atom. In other words, a 0.2-mev oxygen atom would not be expected to produce much, if any, ionization in tissue. It is my understanding from Fano's discussion that he advises including this energy loss of such heavy ions (to energy exchanges other than ionization) in the calculation of the maximum permissible flux for fast neutrons.

FAILLA:

It depends on the energy of the neutrons whether or not this effect is going to be an appreciable fraction of the total. I would say that for permissible limits the figures currently at hand have such a large factor of uncertainty that, in general, this would not be a very important matter.

MORGAN:

It is true that most (or greater than 95 per cent) of the fast neutron energy is lost to hydrogen in the proton production, and from this point of view the energy

* B. Madsen, *Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd.*, **23**: No. 8, 1945.

loss of the recoil oxygen, nitrogen, and carbon atoms of tissue is negligible. However, in some cases, such as with epithermal neutrons, this may not be true.

FANO:

I think that in the epithermal region the secondary reactions are the most important.

FAILLA:

There may be a very narrow region in which the effect discussed by Morgan would be an important factor. I think that, in general, for the very high-energy neutrons most of the energy is transferred to the protons. For very low energies, nuclear reactions which give off radiation probably will set the limit. However, perhaps there is some narrow region in which the effect under discussion might be an important factor.

LOEVINGER:

Morrison has pointed out that ionization may well not be the main mechanism by which radiation produces a biological effect. Yet all dosage computations are based on ionization measurements in air. One uses the average energy per ion pair in air and the relative stopping power to compute energy absorbed by the tissue or organism. Thus, there is the implicit assumption that the biologically important events in tissue are proportional to the ionization in air. Is, then, ionization in air to be considered a satisfactory physical quantity to measure for dosage purposes, or is there hope of finding a better physical quantity to measure for these purposes?

MORRISON:

As I understand it, that was a point of Fano's discussions several years ago which was re-emphasized here; it was just the special property of ionization by glancing collisions in gases that gives a good proportionality between energy lost, the rep, and its measure in the gaseous ionization chamber. I think that it does mean, however, that if we use the very useful parameter of the rep to represent the energy-density distribution for various kinds of radiation, we must expect that 1000 rep may produce very different effects in different biological systems. However, I think that the rep is a very convenient physical unit, primarily because of the excitation-ionization relationship which Fano just showed.

FAILLA:

The way in which the absolute energy is calculated by ionization measurements involves the total energy. Since the number of ion pairs is divided by the total energy of the particle, the average value per ion pair is for the total energy lost and not the energy to produce the ion pair alone. Thus the excitation energy and energy lost by other means are averaged into the energy associated with the production of each ion pair.

BURTON:

Ionization potentials are really different in the gas phase and in the liquid phase. It is very likely that they are lower in the latter merely because of the effect of dielectric constant—a very substantial matter in aqueous systems. Thus, statements of yield per ion pair based on the assumption of, for example, 32.5 ev required per ion pair are definitely wrong. I think that we may object to the usage even if we recall that calculated ion-pair yields are merely a convention, for the numbers we thus derive are definitely prejudicial to the theory. During the war years on the Atomic Energy Project we adopted, instead, the convention of 100-ev yield, the number of molecules converted per electron volt absorbed. The convention has the merit that ordinary 100-ev yields in simple cases without important chain and without important back reactions turn out to be of the order of unity (that is, up to 6 or some such figure). Another merit is that no one is tempted to place any inherent theoretical emphasis on a number so calculated.

AEBERSOLD:

Morrison has given us a very excellent summary of the state of knowledge of the physical processes resulting in matter from ionizing radiations. I was particularly interested in his remarks concerning the time scale of the sequences that follow the passage of an ionizing particle. Before World War II those of us who were interested in comparing the results of different types of ionizing radiation kept in mind the immediate physical picture, as developed by Lea and others, of the ion clusters produced by the particles. This, I gather from Morrison, is the picture at 10^{-14} to 10^{-12} sec after passage of the particle, and that actually the more important picture for comparative purposes is the position of the affected atoms and molecules at later times, say 10^{-12} to 10^{-8} sec. Would Morrison care to review for us the comparative picture of the state and position of the affected molecules and atoms resulting from passage of a 1-mev beta particle and a 1-mev proton at these later times?

MORRISON:

This is a difficult subject, since, in the subsequent motion, caging, recombination, and other factors are involved. Our knowledge of what occurs in the columns of ionization from different particles with varying ionization densities is not adequate. We know the events up to 10^{-12} sec in gases. The question of the conversion of electron energy and excitation in complex molecules is not clear at the present time.

TOBIAS:

I should like to make a comment and ask a question. It was implied in the foregoing discussion that ionization of the less abundant cell constituents may be neglected on account of the small quantity of these elements. In this connection one should mention that an atom of an element with high atomic number will, on the average, more frequently become ionized than an atom with low

atomic number, since the electronic stopping power does not vary rapidly with Z. For example, in an atom of zinc there are 30 electrons; if all these electrons are regarded as free, the Zn atom would have about 30 times higher chance of becoming ionized than a hydrogen atom. On the other hand, one should also remember that some of the essential trace elements are located in very important spots in the cells: zinc, for example, is an essential component of some enzymes.

The question I want to ask is: Are reliable estimates available for the chance of an atom becoming doubly ionized when a charged particle flies by, particularly if the latter has a high rate of energy loss (for example, a low-energy alpha particle)?

Morrison:

Under these circumstances, internal conversion may be appreciable.

Fano:

If one considers the situation electron by electron, all take the same amount of energy, but strongly tied electrons take more energy and are less likely to be ionized. That is to say, zinc, with atomic number 31, does not have 30 times the probability of ionization as compared with the hydrogen atom.

3 ·

Beams of High-Energy Particles

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Introduction

Nuclear physicists, in their quest for the ultimate elementary particles of nature, have constructed larger and larger accelerating machines. Of the fruits of this research, perhaps the most important is the availability of radiologically usable beams of nearly all the known particles. Thus, by means of a cyclotron, a betatron, a synchrotron, or a linear accelerator, one can get a well-collimated beam of high-energy protons, electrons, photons, neutrons, alpha particles, and, indeed, even of nuclei such as Be or C. The use of beams of mesons, the new particles intermediate in mass between electrons and protons, may soon be practical. This sudden wealth of unexploited radiological tools should be of considerable usefulness in the research of the radiobiologist, and in this paper will be described the general characteristics of such beams.

A beam of nuclear particles is characterized by its range, ionization density, and homogeneity. The range of a beam is here given in terms of the distance in centimeters that it can penetrate tissue. The ionization density is the number of ions per cubic centimeter produced on the average at a given point along the beam. To be distinguished from this is the perhaps more important concept of *specific ionization*, namely the number of ions per centimeter along the track of a single particle. The statistical nature of the loss of the energy of the beam introduces inhomogeneities into the beam: inhomogeneities of energy, of direction, and of penetration. This straggling, as it is called, causes a spreading of the beam and a corresponding decrease in ionization density. Nuclear interactions between the particle and then stopping medium can also become important at high energies and also contribute to the inhomogeneity of the beam. These effects will be discussed in more detail as each particle is taken up in turn.

Photons

Let me begin with photon beams. You are probably more familiar than I with the low-energy x-rays, that is, a few hundred kev. Such radiations can be well collimated using lead slits but are rapidly absorbed in tissue. The x-rays, of course, act in the tissue when they are absorbed to form low-energy electrons, the electrons having a range of

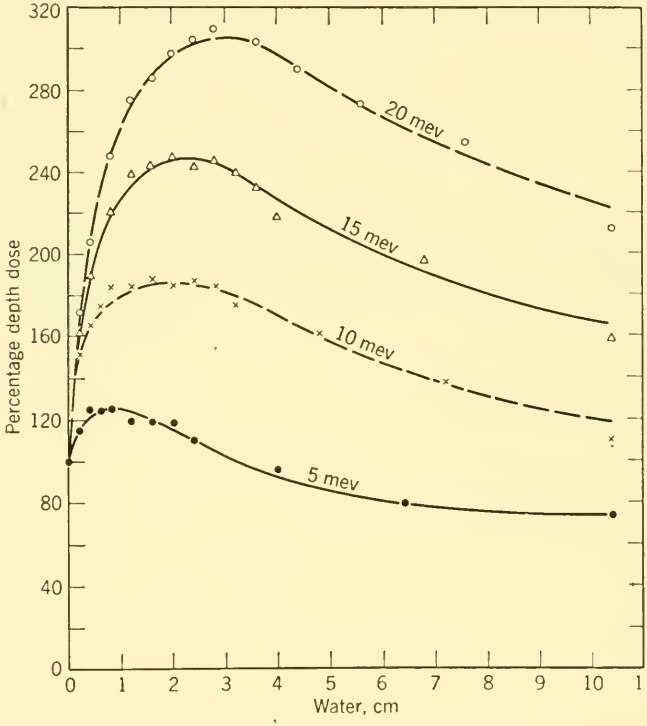

Fig. 1. Isodose contours in phantom, using 16-mev electrons from betatron.

much less than 1 mm. As the energy of the x-rays is raised to the order of 1 mev, the character of the absorption process begins to change. The penetration becomes greater and the electrons formed now begin to have a range in tissue of several millimeters. Additionally, these electrons can now radiate part of their energy into secondary x-rays which in turn can be absorbed farther on in the tissue, and we now begin to see an increase in the dosage with depth. As the energy is increased further, this maximum in the depth-dosage curve becomes more pronounced and its position occurs at a depth below the surface which has been found to increase almost linearly with energy: the maximum dosage comes at

3-cm depth when 20-mev electrons are used (1). (See Fig. 1.) The peaks are quite broad, however, and the intensity falls off very slowly thereafter. At energies higher than about 20 mev the exit dose will be almost as large as that received at the maximum position. Betatrons and synchrotrons now give energies in excess of 300 mev, but there seems little advantage from a radiological point of view in using such high-energy x-rays; the penetration is far too great. At all such high energies the biological effects of the x-rays should be roughly the same, inasmuch as the specific ionization density is nearly independent of the energy, for energies higher than 1 mev.

At very high photon energies, nuclear explosions or stars are induced by the photons. In such stars, several nuclear particles are emitted, thus offering a mechanism for producing high specific ionization density in the tissue. The phenomenon is not well studied as yet, but it does not seem to be large enough to be biologically significant.

Electrons

A real step forward was made by Kerst and his group at Illinois when the electrons were brought directly out of the betatron. An exposure to homogeneous high-energy electrons can now be made directly, without the usual transition of the electrons inside the betatron to a degraded x-ray or bremsstrahlung spectrum and then the additional transition and further degrading of this spectrum back to electrons in the tissue.

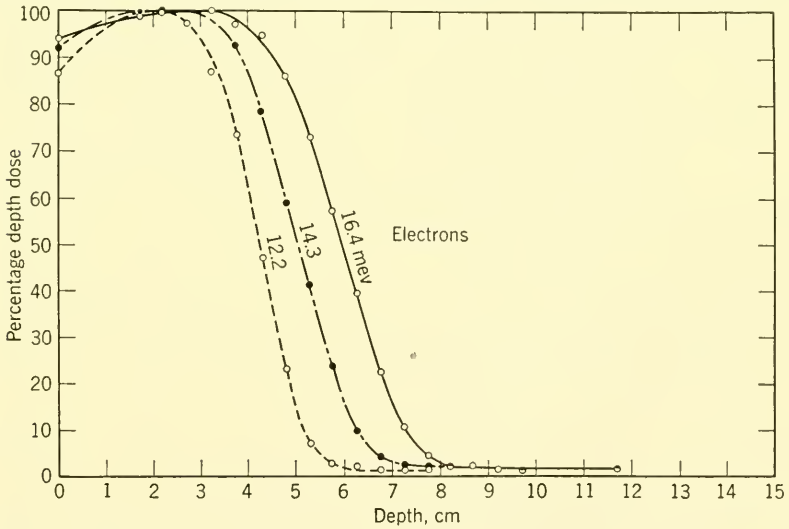

Fig. 2. Depth-dose measurements with electron beam from betatron, in phantom.

Ideally the homogeneous electron beam from the betatron travels straight into the tissue a distance equal to the electron range and then stops. Since the ionization density along an electron track is nearly constant above 1 mev, we might expect nearly constant dosage out to the end of the range and then zero dosage beyond, possibly a slight increase just at the end.

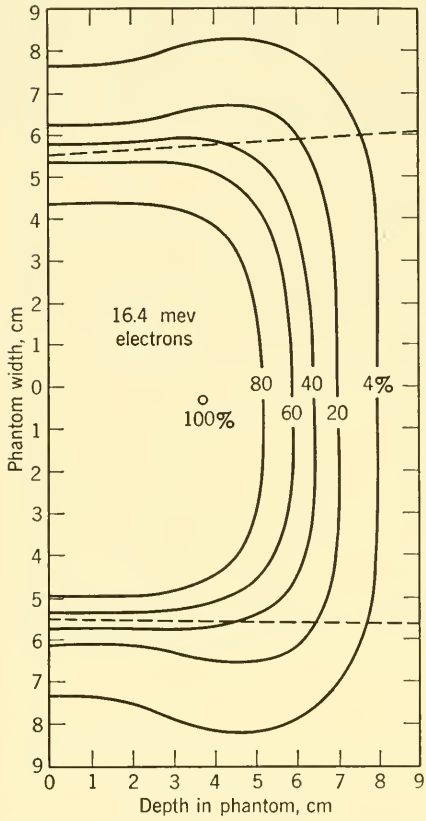

Fig. 3. Depth-dose measurements with x-ray beam from betatron, at various electron energies. The target-to-surface distance was 45 cm in each case.

This simple picture must be modified somewhat. In passing through the tissue, the electrons can radiate secondary photons having a large fraction or all of the energy of the electron. This causes a large straggling in the range of the electrons which tends to give a decreasing ionization density at increasing depths. Furthermore, the radiated secondary x-rays now pass beyond the range of the electrons and, upon absorption, contribute to the dosage there. Fortunately, the secondary photons are so penetrating that the residual dosage is very small beyond the end of the electron beam. The largest modification comes about because of multiple scattering caused by many very small deflections suffered by the electrons as they pass through the atoms of the tissue. The many scatterings add up to very large angular deviations, and near the end of the range the electron motion is more or less a random diffusion. There are a disadvantage and an advantage to this diffusion. It adds to the straggling in range caused by radiation and so brings the total average straggling to about 30 per cent of the mean range. This tends to reduce the ionization density deep in the tissue compared to that at the surface. The scattering also causes the beam to spread out laterally by several centimeters. Thus, if one directs a needle-like beam into the tissue, the ionization density will be very low near the end of the

range of the electrons. On the other hand, and this is the advantage, if one uses a beam of large cross section, the lateral motion will tend to increase the ionization density near the end of the range, for the scattering gives the electron an oblique and hence longer path in a given increment of depth.

The measurements of Skaggs (2), using an 11-cm-diameter beam of 16-mev electrons, show that the latter effect predominates, so that a slight maximum is observed (Figs. 2 and 3). Skaggs finds also that the extrapolated range in centimeters in H_2O is about one-half the energy in mev minus 0.5 cm. A 40-mev betatron would be about right for radiological work.

Nuclear disintegrations are induced by such electrons, but the number of disintegrations is far too small to be biologically significant.

Protons

High-energy proton beams obtainable from synchro-cyclotrons offer considerably higher precision in delivering a large dose to a small volume without overexposing neighboring tissue. Whereas the electron-specific

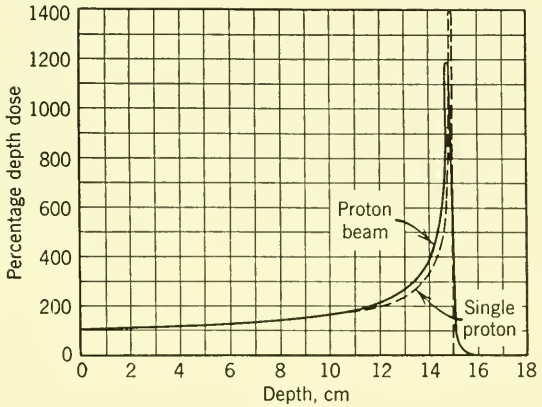

Fig. 4. Calculated depth dose due to protons. The dotted curve shows the effect of a single 140-mev proton in tissue; the full line, the estimated depth dose for a well-collimated beam. The difference shows the effect of straggling and scatter. Reproduced by permission from *Radiology*, **47**: 487, 1946.

ionization is nearly constant for the energies we are considering here, the proton-specific ionization decreases nearly inversely with energy. (See Fig. 4.) The reason is that the electron motion is completely relativistic, that is energy large compared to that of the rest mass (0.5 mev), whereas the proton energy (150 mev) is small compared to

its rest mass (934 mev). Also, because of its large mass, the proton does not radiate secondary x-rays; consequently the straggling in range is much less than for an electron. Similarly, multiple scattering is small for protons. Thus a 150-mev proton has a range of 16 cm in tissue, the mean range straggling is about 0.3 cm, and the mean lateral spreading is about 0.6 cm^3. Accordingly, it should be possible with 150-mev protons to give a spherical volume of 1-cm diameter located 16 cm deep in tissue several times the dosage of any of the neighboring tissue. It is a radiological problem whether the much higher specific ionization at the end of the proton tracks is advantageous or not. It should be emphasized that at the peak of the ionization curve the protons have a broad energy distribution, the mean energy being about 20 mev. The specific ionization of such protons is only several times that of fast electrons. Thus one should not compare the radiological effects to those of recoil protons produced by neutrons, for such protons have very much smaller energies, less than 1 mev.

Nuclear effects become pronounced at these energies. The proton has a considerable chance of impinging on a nucleus of one of the atoms of the tissue before coming to rest (about 30 per cent chance in going 15 cm). In that case it may go right on through, for nuclei are partially transparent at these energies; it may exchange its charge with a neutron and so become a neutron of the same energy and direction as the proton before the collision; it may be scattered; or it may be absorbed. The proton, in going past a nucleus, may also be diffracted or scattered through a small angle. Quantum mechanically, the motion of a proton is described by an associated wave, and the diffraction of this wave is is exactly like that of sound or light around an obstacle. At these energies the wave length of the proton is small compared to the size of the nucleus and the scattering is predominantly forward.

The nuclear effects all tend to flatten the sharp maximum in ionization density that would obtain if only atomic straggling were effective. Thus the protons absorbed along the way excite the nuclei to such a high state of energy that several short-ranged particles may come off, and these will add to the ionization density at the point of disintegration. If the proton exchanges into a neutron, that neutron may exchange back into a proton farther on in the tissue and so contribute ionization beyond the sharp cut-off—a small effect at energies near 150 mev. Large-angle scattering of the proton is not too important; an occasional proton leaving the beam cannot contribute much ionization elsewhere; but its absence at the end of the range decreases the Bragg peak there. Diffraction scattering can be the most important of the nuclear effects if one is interested in confining a dose to the smallest volume possible.

With beams of large cross section, on the other hand, diffraction scattering produces no effect, for it is predominantly forward and is elastic so that the protons penetrate just as far, ending up with a slight lateral displacement. Even with beams of small cross section diffraction scattering is not too serious. About 10 per cent of the initial protons of a 15-cm beam are scattered out of the beam, and these are spread more or less uniformly over an area of about 10 cm^2, so that the density of pro-

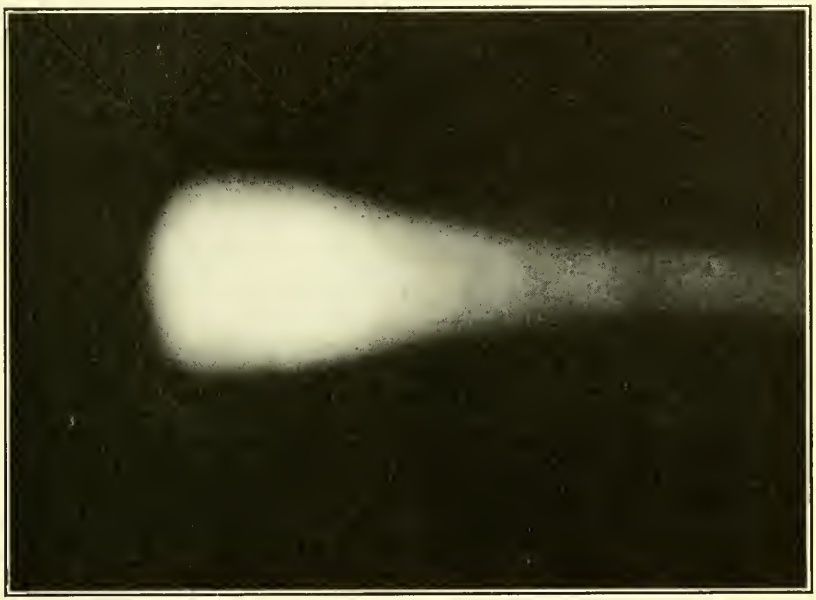

FIG. 5. Photographic plate irradiated under water by a beam of 190-mev deuterons. Note spreading at end of beam and increased ionization.

tons outside the beam drops to about 1 per cent of its value in the beam.

Tobias and Auger (6) have made experimental studies using 190-mev deuterons, which are similar to protons. Figure 5 shows a direct picture of the beam taken by allowing the deuteron to pass through a photographic film which had been immersed in water. One can observe the spreading at the end of the beam and also the increased ionization density. Figure 6 shows quantitatively the differences in ionization density or dose characteristics among x-rays, electrons, and protons. Figure 7 is a typical isodose curve for a beam of 190- mev deuterons.

There are a few things to emphasize in the use of protons. Higher energies than necessary should not be employed. It is true that the protons can be slowed down in some other material outside the tissue,

but the additional multiple scattering introduced can become serious very rapidly. Furthermore, the products of nuclear reactions occurring in the initial stopping material can enter the tissue, thus destroying the initial homogeneity of the beam. Another necessary precaution is to make sure that the protons enter the tissue immediately on leaving the vacuum chamber. Even a thin foil can cause an appreciable multiple scattering that will diverge the beam rapidly in air.

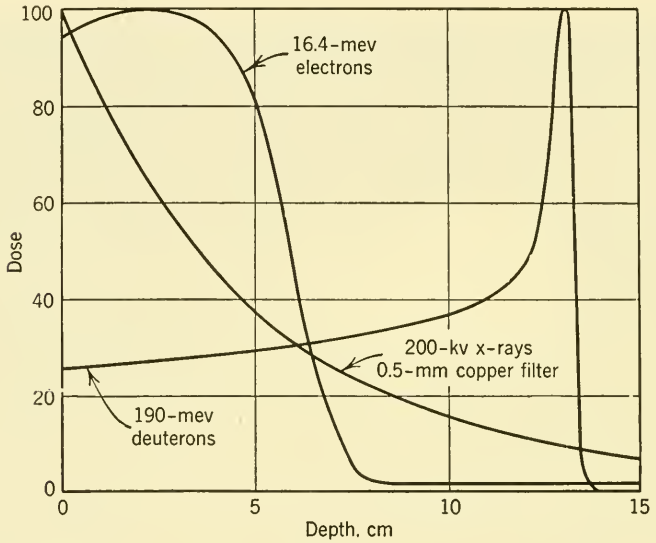

FIG. 6. Comparison of depth-dose curves in water for various kinds of beam, all adjusted to same maximum dosage density.

It is interesting to consider just how the precision of a proton beam depends upon the initial energy. Precision of the beam here means the percentage spreading and straggling. To a first approximation, there is no variation of the precision with energy or range. More accurately, nuclear scattering causes the beam to spread out a bit more at high energies—an effect already discussed—but this is offset, in part at least, by the fact that the percentage spreading and straggling decrease very slowly with initial proton energy. Thus a 150-mev proton beam has a root-mean-square straggling of 0.94 per cent, while for 10 mev straggling is 1.2 per cent. The percentage spreading varies similarly.

Figure 1, showing the specific ionization of a single proton, would indicate that the ratio of the ionization density at the Bragg peak to that at the beginning of the range would be much greater for high energies. It is true that the ion density decreases just as indicated, but the ioniza-

tion density at the peak also decreases, for the increase in straggling of the beam spreads the region of high specific ionization over a great volume. Actually nuclear absorption and scattering of the protons reduce the ratio obtainable at high energies.

Because the protons are charged, it should be easy to pass a divergent or parallel beam through a magnetic lens and so produce a convergent beam whose point of convergence comes in the tissue at a depth equal to the proton range. This is equivalent to cross fire and should produce

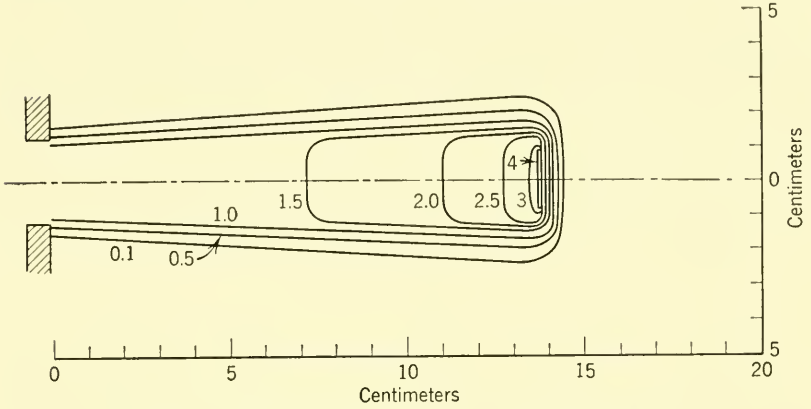

Fig. 7. Isodose curves for 190-mev deuterons in water.

similar spectacular results in reducing the dosage in the surrounding tissue. The same idea could be used with any charged particles, such as electrons.

Heavier Particles

Heavier particles such as deuterons and alpha particles, or even nuclei of atoms, have some advantages over protons. Multiple scattering and struggling decrease as the square root of the mass. Hence the extremes of ionization density will more closely resemble the ideal specific ionization. These extremes are even more emphasized because of the well-known specific ionization dependence on the square of the charge of the particles. The dependence is not quite as good as it first sounds, for, as the particles slow down to velocities approaching those of the atomic electrons, electrons become attached to the particle and so reduce the effective nuclear charge. Eventually the principal loss of energy comes about because of nuclear collisions, and such collisions increase the straggling.

Cyclotrons that accelerate deuterons can also accelerate without readjustment heavier particles such as the nuclei of carbon, the energy being greater in proportion to the increase in mass. Thus, in a 200-mev deuteron cyclotron, one could readily get small beams of 12-bev carbon nuclei. Apart from the factors just mentioned, the specific ionization would be increased by more than 36 times that of the deuterons. The range, however, would be reduced from 16 cm to about 2.5 cm—still usable—and the precision better by a factor of 3. Li^7 nuclei would have an energy of 700 mev, a range of about 6 cm, and a specific ionization greater than protons by a factor of 9. Such beams should be particularly valuable in precision research work on small organisms. The larger accelerators now under construction at Brookhaven National Laboratory and at Berkeley will give greater penetration for these heavy nuclei and make it possible to use even heavier atoms.

The nuclear effects will be somewhat enhanced, but will probably not be large enough to make the use of such beams impractical.

Mesons

Figure 8 shows the track of a negative meson in a photographic plate. At the end of its path, the meson comes to rest and is absorbed by a nucleus. An energy equivalent to the meson rest mass (140 mev) is then liberated, some of which appears in the charged fragments of the nucleus seen in the typical "star" at the end of the track. Thus we have a mechanism for depositing a large amount of ionization at the end of the range of these particles. However, since their mass is nearly one-tenth that of the proton, multiple scattering is very large and may vitiate any concentration of ionization by the star. Our understanding of mesons is still developing. It seems now that, if mesons become plentiful, they may seriously compete with neutrons, but do not seem to have any advantages over, say, a high-energy alpha-particle beam.

Neutrons

Solomon will discuss low-energy neutrons. It is possible to make reasonably homogeneous beams of high-energy neutrons by a process called stripping, but there seems to be little radiological interest in them, for the neutrons become effective in tissue by the production of recoil protons which are now more easily and more homogeneously available directly.

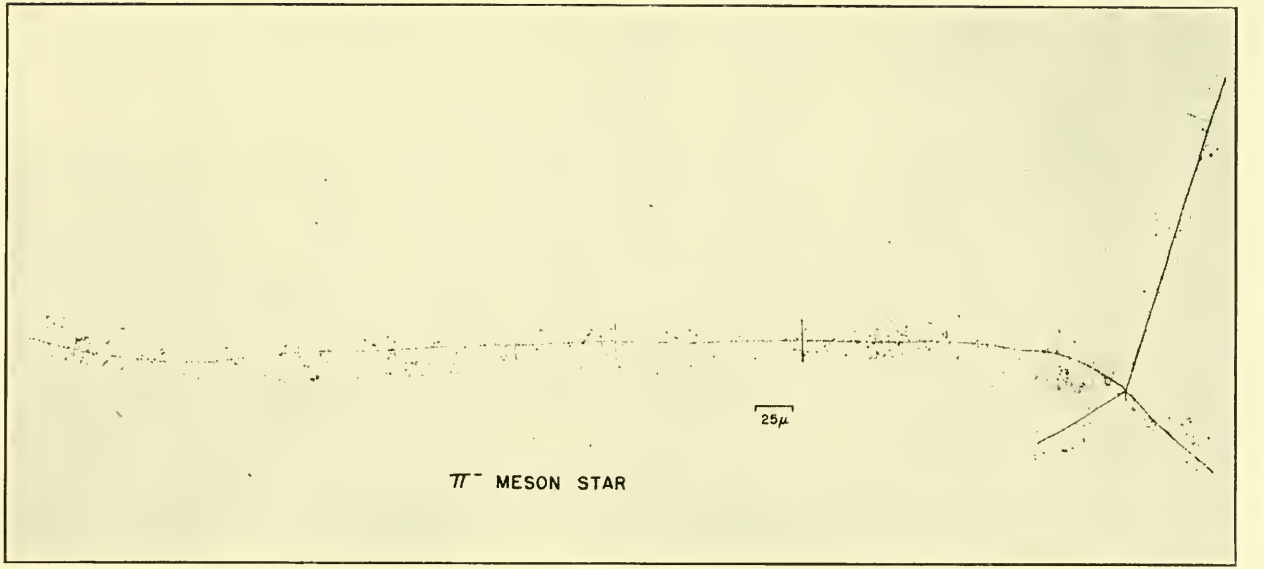

FIG. 8. A meson track in photographic emulsion. The meson enters at the left, comes to rest and is absorbed by a nucleus at the right, and then makes the four-pronged star or nuclear explosion.

Availability and Cost

Electrons and photons for radiological work are cheapest. Betatrons of adequate energy (about 30–50 mev) are commercially available and cost about $100,000, installed. Such machines are not much larger than conventional x-ray machines. Linear accelerators which are being developed to accelerate electrons may be even cheaper.

Medically useful sources of protons would cost at least $1,000,000. As the technology improves, the cost of commercially available synchro-cyclotrons may come down. Per roentgen, the cyclotron may be cheaper, because of its larger beam. This suggests the use of cyclotrons at centers where one cyclotron beam could be piped to, and readily serve, say as many as 100 treatment cubicles at once.

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DISCUSSION

Quastler:

I am afraid that two complications must not be omitted from a consideration of high-energy rays. One is of a biological nature. It is true that the elementary responses to all ionizing radiations are about the same. However, the relative efficiencies of two kinds of radiations, in producing different responses, are not constant. Thus, if doses of two kinds of radiations are matched with respect to one response, the other response might be elicited in a quite different strength. For instance, doses of different radiations, matched so as to produce equal percentages of killing in plant seedlings, will not produce equal amounts of stunting in the survivors. In general, if one considers the whole complex of responses elicited by irradiation of a complicated organism, he will find that the relative strength of the various responses, and hence the total picture of the reaction, depend on the quality of radiation used.

The other complication enters into the evaluation of depth-dose characteristics. It is not realistic to consider a single beam only; one has to study systems of cross firing. Under this point of view, the large exit dose of high-energy photons is not much worse than the high entrance dose of high-energy electrons: besides, the photons have the advantage of much better collimation. The depth-dose curve of high-energy protons looks exceedingly attractive; but as a rule we do not shoot at areas 2 mm in diameter, situated 10 cm in the depth.

If one superimposes fields so as to cover an area of the dimensions met with in practice, he will find that the distribution becomes poorer, by addition of the doses between surface and maximum.

WILSON:

Cross fire with electron beams, will, of course, not influence the scattering at the end of the range inherent in electron beams. This is also true for protons.

TOBIAS:

It is admirable that Wilson was able to calculate the exact range and ionization properties of high-energy protons and predict their radiological application some 2 years before such beams were experimentally produced. His ideas inspired the group in Berkely to carry out the initial experiments. We have now some 2 years of experience with 190-mev deuterons and their effect on animals. I can state that, as far as acute lethal effects of this radiation on mice go, they are very similar in timing and energy dosage to the effects of 200-kev$_7$ x-rays. Initial application to experimental mouse-tumor therapy convinced those doing the work that such beams can be beneficial in deep tumor therapy far beyond the range of usefulness of low-energy x-rays. It is clear, however, that before large-scale-human applications are made, one should find out much more about the effects of local irradiation of animals, acute and delayed, and about biological effectiveness of the different portions of these beams which have different rates of energy loss in tissue. In these connections the high-energy ion beams have become useful tools in radiobiology, in an extended study of the physiological changes produced by localized irradiation. May I suggest that Bond, who has been working on this problem, make a comment.

BOND:

The nature of the work that Miss Marguerite Swift and I have been doing with Tobias is such that the reporting of most of it will be more appropriate during later discussions in the symposium. A few remarks, however, are pertinent to the possible use of high-energy particles in radiation therapy.

We have taken advantage of the lack of lateral scatter from the deuteron beam to achieve highly selective irradiation in the rat. The beam traversed the entire width of the animal, and thus we took no advantage of the increased ion density at the "tail" of the Bragg curve.

A good deal of difficulty in reproducing results was encountered when irradiation was confined to portions of the abdomen. This led us to determine, by means of sectioning frozen animals, exactly which organs were contained in the volume of tissue through which the beam passed. With rats of nearly identical body weight, a good deal of variation both in the type of organ and in the fraction of a given organ contained in the irradiated volume was noticed; hence the possibility of accurately localizing such irradiation to a given body region by means of external markings seems remote.

In addition, it was noted that many animals surviving the acute effects of irradiation localized to the abdomen exhibited, within about 2 months, discrete,

localized, annular, tumor-like masses situated in the small bowel. These apparently are of the type previously described by Shields Warren and seem to represent an exaggerated fibroblastic reaction to local tissue destruction and infection. This reaction was observed at doses of the order of 2000 rep.

BRUES:

It should be emphasized, for the enlightenment of those who have not been concerned (as have practicing radiotherapists) with the localization of structures within the human body, that it is at times very difficult to know where they are or to be sure they stay there. Some attention will need to be paid to physical and other means of establishing the positions of tumors and other structures inside the human body before extreme depth localization of radiation will, in general, be profitable.

FAILLA:

Definition of the tumor volume clinically is very difficult. In practice it is necessary to radiate a margin of normal tissue in addition to the volume thought to contain the tumor. For this reason highly defined beams cannot now be used to maximal advantage. The use of such beams must await solution of the problem of definition of tumor volume.

FRIEDELL:

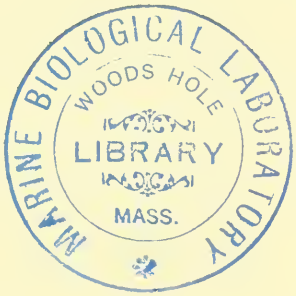
There appears to be some overemphasis on beams by localized radiation to small volumes a few centimeters or even a few millimeters in diameter. Localization has been practiced by radiobiologists and radiation therapists for some time by the use of interstitial radiation in the form of radon or radium needles, cobalt pellets, interstitial colloidal gold, etc. It is doubtful that localization becomes critical, at least from the point of view of specific biological reactions, until we can localize radiation to the small volumes which correspond to cells or portions of cells, such as the nucleus or the nucleolus within the nucleus.

LOW-BEER:

Attention should be called to two factors in the use of any type and energy of ionizing radiation in clinical therapy. One is the time factor between the exposure and the reaction chosen for observational inferences. Morrison said that he was describing only in the first one-hundredth of a microsecond following radiation exposure. Fano said that he is interested in the subsequent fractions of a second. The clinical radiologist must stay with his patient for weeks, months, and often for years. Both early acute and late chronic radiation reactions are manifested by the composite tissues of the patient. The two reactions differ in appearance and also in significance. For the same quantity of various types and/or energies of radiation the early reactions may be similar, exceptions granted. The acute reaction gradually increases, reaches a peak, and disappears again gradually in a period varying from 3 to 10 weeks. Acute radiation reaction is a reversible process. Reactions months or years later—that

is, in the chronic period—often differ considerably in the rate of development and the degree of manifestation. Late radiation reactions appear to be irreversible.

The second factor I wish to emphasize is the importance of the tumor bed in the therapeutic irradiation of a tumor. Consideration of adequate protection for the vascular-connective-tissue tumor bed is as important as destruction of the tumor itself.



4.

Neutrons and Their Special Effects: Recoil Effects

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The neutron is a unique tool for the production of ionization, being set apart from the more familar alpha, beta, gamma, and x-radiation both in its history and in its properties. Its existence was predicted by Rutherford in his famous Bakerian lecture in 1920; indeed, he was so convinced of its existence that he spent some time in a fruitless search for it in electric discharges. It was not until 12 years later that Chadwick, hot on the trail of an aberrant gamma ray, discovered the neutron. It is a radioactive particle, of charge zero, and mass a little greater than the proton. It decays, with a half life of 10–20 min, to a proton and an electron with a maximum energy of 760 kev.

The interaction of the neutron depends very much on its kinetic energy; indeed, as shown by Fermi in 1934, slow neutrons are in general much more effective than fast ones in the production of artificial radioactivity. For our purposes fast neutrons may be classed as those with energy greater than tens of thousands of electron volts. The remainder, the slow neutrons, include the special category of thermal neutrons which have only the energy available at room temperature, approximately 0.025 ev. The average thermal-neutron velocity is 2200 meters per second.

Neutrons are formed in nuclear reactions with energies of the order of millions of volts; to produce slow neutrons it is necessary to strip the fast ones of most of their energy. This may be done by passing fast neutrons through a scatterer, called a moderator, as in a nuclear pile. However, since the neutron has no charge, it can neither give up its energy by direct reaction with the extranuclear electrons nor ionize or excite other atoms directly. It must give up its energy through the agency of another nucleon, either by collision or by reaction, and thus relies on secondary effects to produce its ionization.

Let us examine these processes in some detail in order to derive a few relationships which may help our understanding of the biological action resulting from neutron irradiation. Collision of a neutron with another nucleus results in deflection of the incident particle, along with a transfer of part of the energy of the neutron to the struck nucleus. When the process follows the simple billiard ball laws of classical particle physics and does not raise the *nuclear* energy levels of the struck nucleus, it is called elastic scattering.

The loss of energy by elastic scattering with other nuclei is dependent on the size of the colliding nucleus and the kind of collision, whether glancing or head-on. The neutron will transmit the maximum amount of its energy if it collides head-on with a proton, far less if it just bounces off a heavy nucleus; the exact equations can be derived from the laws of conservation of energy and momentum. On the average, the following simple approximate expression gives the mean ratio of the energy of a neutron after collision (E_2) to its energy before collision (E_1): $E_2 = E_1 e^{-[2/(M+1)]}$, where M is the mass of the struck nucleus. Each collision with a hydrogen atom reduces the average energy of the neutron by the factor $1/e$, or roughly $\frac{1}{3}$. On the other hand, collision with a carbon or nitrogen atom reduces the average energy of the neutron by only about 15 per cent, and collision with oxygen, phosphorus, and sulfur by even smaller amounts. For a 1-mev neutron, 18 collisions with protons are needed to slow the neutron down to thermal energy, a figure which can be compared with 110 collisions required with carbon.

Neutron Interactions with Hydrogen and Tissue

It is helpful to examine the scattering process in detail from the point of view of collisions of a neutron with a proton, because of both the predominance of hydrogen in tissue and the importance of neutron-proton forces in nuclear theory. It is customary to consider the relative importance of several competing nuclear reactions in terms of their cross section, the apparent cross-sectional area that the target nucleus offers to the impinging particle, in this case the neutron. The accepted unit of cross section, 10^{-24} cm^2, is called, in physics jargon, a barn. The radius of the hydrogen nucleus is about 1.5×10^{-13} cm, and it should, therefore, have a cross section on geometrical grounds alone of 0.07 barn for fast neutrons. For neutrons of energies of 10–20 mev, the cross section has been experimentally determined to be about 0.5 barn. From this low value the cross section rises to a plateau of about 20 barns which is maintained in the region from 10,000 ev to 1 ev. At energies below 1 ev, the cross section rises again and reaches a value of about 75 barns at the

lowest measured energy of 0.005 ev, corresponding to a temperature of −216° C.

Theory expects that for elastic scattering up to energies of about 20 mev the scattered particle would have no preferred direction, but would have a perfectly symmetrical distribution in the center of mass system, an expectation which has been verified experimentally. However, in dealing with the total scattering cross section, theory, at first, agreed well with experiment only for fast neutrons. For thermal neutrons, it predicted a cross section of 2.4 barns, which is to be compared with the observed experimental value of 58 barns. This discrepancy was overcome by altering the theory in two ways: first, by considering the spin relationship between the neutron and proton, and, second, by taking into account the chemical binding of the proton. It was shown by Fermi that the scattering cross section for protons bound in molecules was about 4 times larger than that for free protons. Two reasons which contribute to this higher cross section are: (1) the apparent mass of the proton is increased because it is firmly tied to its molecule and (2) for thermal-neutron energies, the molecular vibrational motion of the proton cannot be neglected.

Thus far we have a picture of a neutron entering paraffin or tissue and slowing down in a series of collisions, each of which transfers part of the neutron energy to another nucleus, causing chemical excitation or ionization, but never transferring enough energy to cause nuclear excitation of the struck nucleus. Furthermore, the cross section presented by the proton increases as the neutron slows down, making the target larger, the slower the neutron. We have still to consider the eventual fate of the neutron, the reaction by which it is removed from circulation. Since the half life of a free neutron is of the order of 15 min, whereas its half life in paraffin is about 10^{-4} sec, we must look to a nuclear reaction to account for its capture.

There are only two possible nuclear reactions between a neutron below 100 mev and a light nucleus: simple capture and inelastic scattering. That is, a neutron may be captured by the nucleus, and the excess energy given off as gamma radiation, or else the neutron may be captured and re-emitted, leaving the struck nucleus in an excited state, from which it returns by emission of a gamma ray. Since this process of inelastic scattering always involves nuclear excitation, it can easily be differentiated from elastic scattering. In the light elements, inelastic scattering is very improbable for neutrons of 1-mev energy, which is the range that interests us.

It was shown in 1934 by Chadwick and Goldhaber that a natural gamma ray from thorium C′ could cause a deuteron to disintegrate into

a neutron and a proton. In 1935, Lea observed the inverse (n, γ) process of neutron capture followed by gamma-ray emission, and the gamma-ray energy was subsequently measured as 2.2 mev. This means that every neutron impinging on tissue and ultimately captured by a proton releases 2.2 mev by a nuclear process in addition to the elastic-scattering losses already discussed.

For fast neutrons the cross section $(\sigma_{n,\gamma})$ for nuclear capture and gamma-ray emission may be given by $\sigma_{n,\gamma} = \pi R^2 \xi f(\Gamma)$, where πR^2 is the geometrical area of the struck nucleus, ξ is an empirical factor called the sticking factor, and $f(\Gamma)$ is the relative probability of gamma emission compared to emission of other charged particles. The sticking factor, which is the probability that a neutron, once having hit a nucleus, will stick, must by definition be less than or equal to 1. Also, the relative probability for gamma emission must be less than or equal to 1, so for a fast neutron, the (n, γ) cross section must be less than or equal to the geometrical cross section, which, as we have seen, is 0.07 barn for hydrogen.

At low energies the cross section for capture is inversely proportional to the neutron velocity. This proportionality, called the $1/v$ law, holds in the case of light nuclei up to appreciable energies, as illustrated by the B^{10} (n, γ) reaction, where it is followed up to 50 kev. In the thermal-neutron region the capture cross section for hydrogen is 0.30 barn, in good agreement with theory. This value is low compared to the total thermal scattering cross section of 59 barns, but is about 100 times greater than the capture cross sections for other near-by elements such as deuterium and carbon. As a consequence, heavy water and carbon are used as moderators in nuclear piles; ordinary water removes too many neutrons from circulation.

Thermal neutrons can also be captured by nitrogen; in addition to (n, γ) capture, N^{14} can also capture a neutron and emit an 0.58-mev proton to form radioactive C^{14}. Such an (n, p) reaction produced by thermal neutrons is possible only for light nuclei and takes place in only a few cases. Both (n, p) and (n, γ) processes contribute to the total nitrogen thermal-neutron capture cross section of 2.15 barns (1).* Very little additional energy is contributed to the process by the radioactive C^{14}, since its half life is 5700 years (2) and its maximum beta-ray energy is 154 kev.

* It is pointed out by Zirkle in his discussion that the relative effectiveness in small animals of the (n, p) reaction on N^{14} and the (n, γ) reaction on H could not be determined only from the relative energies of the proton and gamma ray, because the 0.58-mev proton is completely absorbed in a short distance, whereas the 2.2-mev gamma ray may well pass out of the tissue before it has lost a large part of its energy.

The predominant part played by hydrogen in the interaction of neutrons with tissue is amply confirmed by Table 1, which shows the approximate percentage composition of the human body, together with the total neutron (scattering and capture) cross section for the element where available for both thermal and 10-ev neutrons. In general, the

TABLE 1

APPROXIMATE COMPOSITION OF HUMAN BODY AND APPROXIMATE THERMAL-NEUTRON CROSS SECTIONS

Element	Gram Atoms/ 100 g	Gram-Atom Per Cent	Total Neutron Cross Section, barns		Thermal-Neutron Capture Cross Section × Gram-Atom Per Cent
			Thermal	10 ev	
H	10.29	64.3	59	21	19.3
O	4.14	25.8	4.2	3.7	
C	1.38	8.56	5	~4.5	
N	0.18	1.12	11	10.4	2.41
Ca	0.064	0.04	....	....	
P	0.048	0.03	4.5	3.4	
K	0.009	0.006	....	....	
S	0.008	0.005	1.7	1.3	
Na	0.006	0.004	....	....	
Cl	0.005	0.003	54	....	
Mg	0.002	0.001	3	3.4	
Fe	Trace	.....	13.4	....	
I	Trace	.....	....	....	

total cross sections remain about the same between 10 and 100 ev. Further, in the last column the gram-atom per cent of H and N has been multiplied by the thermal-neutron capture cross section for each of these elements, thus providing an index of their relative importance in neutron capture. It can be seen that nitrogen accounts for only about 11 per cent of the neutrons.

A few more approximations will fill out and complete our picture of the neutron, which, as we have seen so far, enters tissue with energies in the million-volt range and is slowed down predominantly by collision with protons until it is finally captured, usually to form a deuteron with the emission of a 2.2-mev gamma ray. From its mean life of about 10^{-4} sec and average velocity of 2200 meters per sec we can calculate that the average thermal neutron traverses a total tortuous path of 22 cm; and further, from elementary diffusion theory, we learn that it makes about one collision per centimeter of path.

Neutron Interactions with Lithium and Boron

Among the light elements, lithium and boron are unique in that they can capture slow neutrons and give off alpha particles. The cross section for this reaction is abnormally large, being at ordinary thermal energy about 900 barns for Li^6 (70 barns for the natural isotopic mixture of Li^6 and Li^7) and 3800 barns for B^{10} (710 barns for the natural mixture). The Li^6 alpha particle is ejected with an energy of 4.6 mev, and the B^{10} alpha particle with an energy of about 2.5 mev. The high, slow neutron cross section, coupled with the short path length and high ionization density of the alpha particles, makes these reactions attractive for the production of local dense ionization.

Kruger (3) and Zahl and his collaborators (4) have attempted to make use of these reactions in selective tumor irradiation. Kruger found that the high ionization density worked well with tumors soaked in boric acid *in vitro*, but neither group was able to devise a method by which the boron or the lithium could be selectively absorbed by the living tumor.

Recoil Effects Following Neutron Capture

The 2.2-mev gamma ray given off when a proton captures a slow neutron causes the deuteron to recoil with considerable energy. This energy is at a maximum when a single quantum is emitted after neutron capture, the usual case for light nuclei. Under these conditions the recoil energy (E_r in mev) from a single gamma-ray emission is given by the expression $E_r = 536 \times 10^{-6}\ E^2/M$, where E is the gamma-ray energy in mev, and M the mass of the recoiling atom in atomic mass units. The deuteron will recoil with an energy of 1300 ev. This tremendous energy can be compared with the ionization potential for hydrogen of 13.6 ev. In slow neutron capture by C^{12}, the product nucleus has a recoil energy of 945 ev from the 4.1-mev gamma ray, to be compared with the carbon-hydrogen bond strength of 3.8 ev. The bond strength of the C—O bond is 3.0 ev; the C—C bond 2.6 ev; and the C—N bond 2.1 ev.

Szilard and Chalmers (5) in 1934 made use of these high recoil energies to obtain radioactive halogen atoms of high specific activity. When a pure liquid hydrocarbon halide is exposed to slow neutron bombardment, the halogen bond is ruptured by recoil, and the halogen can then be extracted by water. However, it has been observed that even though more than enough energy is available to break the bonds, some of the halogen is retained by the parent liquid (6). The fraction retained in the organic layer, called the retention, varies from 31 per cent in irradi-

ated $C_2H_4Br_2$ to 75 per cent in C_2H_5Br. By far the largest part of the radioactivity retained is found in the initial parent molecule. To account for these high retentions, it is necessary to assume that the "hot" recoiling atom re-enters the parent molecule by exchange. When the target halide is diluted by substances whose atomic weight is far different from that of the halide, the retention decreases, and, as might be expected, approaches zero when the target is in the gas phase.

Libby and Miller *et al.* (6) have developed approximate theories to account for some of the simple recoil processes. Their theory is based on two assumptions: first, that every neutron captured leads to bond rupture; and, second, that the collisions of the "hot" recoiling atoms are non-ionizing and elastic. Slow neutron bombardment of a pure liquid, say C_2H_5Br, produces as primary product radioactive bromine because the thermal-neutron capture cross sections are high, 1.11 barns for the production of 34-hour Br^{81}, with equally high ones for the production of other radioactive bromine isotopes. The "hot" radioactive bromine will then lose its recoil energy by elastic collisions. To give up a large part of its recoil energy in a single impact, it is necessary for the bromine to collide with another bromine atom, for the C and H atoms are far too light to carry away enough energy. If the bromine is to re-enter the parent C_2H_5Br, it must satisfy two conditions: first, the "hot" bromine ion must have enough energy, v, so that it can knock off a cold bromine from the molecule, leaving a free radical; and, second, the "hot" ion must be left with so little energy, ϵ, that it cannot escape from the "cage" of surrounding molecules.

The caged ion will then give up the rest of its excess energy by knocking against the walls of the cage until it can easily combine with the free radical. Ions left with energy less than v are removed from further consideration because they cannot again form a free radical. The theoretical expression for the retention R is then given by $R = \epsilon/v$, and ϵ is found to lie, for organic halides, in the range of 0.8–1.9 ev (bond strength 2.6 ev).

Later experiments by Miller and Dodson (7) show that the theory has to be modified in important respects when more than one reacting substance competes for the "hot" atom. Experiments with mixtures of CCl_4 and $SiCl_4$ give good agreement with theory for the variation of retention with composition. However, as soon as a hydrocarbon replaces $SiCl_4$ as the diluent, sharp disagreements with theory arise. In order to reconcile experiment with theory, it is necessary to postulate that every "hot" recoil atom first undergoes reaction with the hydrocarbon to form an unstable intermediate, which may subsequently decompose to free the radioactive chlorine atom for entrance into CCl_4.

Other competing reactions are possible, including the formation of a chlorine hydrocarbon. As Miller and Dodson point out, the system may also be treated solely as a set of competing chemical reactions, as is usual for thermal kinetic systems. Williams and Hamill (8), who have used such a treatment in study of the pressure dependence of neutron capture by bromine in mixtures of HBr, C_2H_4, and C_2H_5Br, were able to account qualitatively for their effects by a kinetic scheme. The "hot" atom reactions may differ widely from those that take place at thermal energies, as was shown by Miller and Dodson, who were unable to detect any change in yields by a 25° decrease in temperature.

The experiments of Süe and Kayas (9) cast doubt on the theoretical assumption that the initial act, in all cases, is that of bond rupture. They have shown that, in a series of cobalt complex compounds, the retention of free cobalt atoms increases markedly as the central cobalt atom is protected by a more complex external structure. The retention of free radioactive cobalt by a solution of $Co(NH_3)_6(NO_3)_3$ is 14 per cent, compared with a retention of 90 per cent by a solution of $Co(NH_2CH_2CH_2NHCH_2CH_2NH_2)_2(NO_3)_3$. Control experiments showed that the retention of the latter salt was not increased when it was irradiated with an equal amount of cobalt nitrate, thus indicating that the additional "hot" Co atoms from the cobalt nitrate could not enter the complex. Within the limits of the control, it appears that in large molecules the struck atom often never severs (in any permanent sense) its chemical bonds.

The process of "hot" atom exchange can also be used in chemical synthesis, as illustrated by the production of radioactive CS_2 from C_2Cl_6 [see Edwards, Nesbett, and Solomon (10)]. In a pile, S^{35} can be prepared with a much higher yield by an (n, p) reaction on chlorine than by an (n, γ) reaction on sulfur, the second important example of this (n, p) process in light elements. Preliminary cyclotron experiments showed that "hot" S^{35} atoms from C_2Cl_6 would exchange with CS_2 dissolved in the hexachloroethane. A solution of 1 gram of C_2Cl_6 in 1 ml CS_2 was then bombarded in the Oak Ridge pile for 1 month. At the end of this time 12 per cent of the radioactive sulfur was found in the CS_2, yielding a product with a specific activity of 1 millicurie per gram.

Ball, Rodkey, Cooper, Davison, and Solomon (11) have investigated the possibility of utilizing inverse Szilard-Chalmers processes for the production of radioactive compounds. The crystalline amino acid cystine was chosen for preliminary studies. Table 2 shows its composition, and the relative neutron capture cross sections of the sulfur and other atoms. Of the total sulfur capture cross section of 1.6 barns, only 0.011 barn is effective in the production of S^{35}; in view of the small total

capture cross section of carbon (0.0049 barn), a much smaller cross section was expected for the formation of C^{14}.

TABLE 2

Insulin and Cystine Composition and Cross Section

	Atoms/ Molecule (15) *	Cross Section for Thermal Capture, barns (1, 12, 13, 14, 15)	Total Atom Capture Cross Section/ Molecule
Cystine			
C	6	0.0049	0.029
H	14	0.30	4.2
O	4	4×10^{-7}	1.6×10^{-6}
N	2	2.15	4.3
S	2	1.6	3.2
Insulin			
C	1581	0.0049	7.75
H	2361	0.30	710
O	475	4×10^{-7}	1.9×10^{-4}
N	412	2.15	885
S	35	1.6	56.0

* Results corrected to insulin molecular weight of 36,000.

Bombardment of the first few samples was disappointing because the cystine was returned so charred by the heat in the pile that it could not be used. Finally, a cool enough place was found, and the returned sample was subjected to an exhausting variety of chemical tests which proved that all the radioactivity was present as S^{35} and that it was contained in a molecule which could be chemically characterized as cystine. This proves that the cross section for (n, γ) capture by carbon leading to formation of C^{14} has a very low value, probably less than 0.0004 barn.

Although exact figures of neutron flux are unavailable, approximate calculations show that about 6.8 per cent of the S^{35} radioactivity was retained in the cystine. The final product had a specific activity of 0.7 microcurie per millimole of cystine.

This result was so promising that it was decided to bombard crystalline insulin, a relatively heat-stable protein containing 12 per cent cystine. The cystine plays an important role in the structure of insulin, for its sulfur atoms serve as the cross links which hold the protein chains together. It is to be expected that insulin, as shown in Table 2, would be considerably damaged by gamma recoil effects, for the preponderance of hydrogen and carbon would cause the disruption of a great many chemical bonds. No appreciable yield of C^{14} was anticipated. Insulin

has a molecular weight of 36,000 and it was hoped that in this large molecule some bonds would not be severed by recoil from S^{35} formation and that an appreciable fraction of all the broken bonds would rejoin.

As a control, cystine was bombarded in another sealed tube in the same aluminum container that held the insulin. The cystine, when purified, had an activity of 170 cpmin per mg compared to 15,500 cpmin per mg of the insulin, thus proving that the large protein held on to its activity as tenaciously as had been hoped. Half-life and absorption measurements show that most of the radioactivity is due to S^{35}.

A series of physical and chemical tests was then carried out to see whether the insulin had been badly damaged. The ultraviolet absorption spectrum of the irradiated material appeared unchanged except for a slightly increased background absorption. None of the radioactivity could be removed by dialysis. However, it soon became clear that the material was impure because it was not possible to crystallize the bombarded insulin, though fibrils could be formed with a recovery of up to 65 per cent of the original nitrogen. The material purified by fibril formation had a specific activity of 19,000 cpmin per mg, higher than the original. As final confirmation, a run on the ultracentrifuge showed that the initial pure compound had returned as a material of mixed molecular weights, so impure that no single peak could be observed in the ultracentrifuge.

The cystine, which contains all the sulfur in the insulin molecule, should be separable on acid hydrolysis; the cystine from the irradiated insulin brought with it only a small portion of the total radioactivity, thus indicating that many of the sulfur bonds had been broken, with consequent reattachment of the sulfur elsewhere in the molecule.

The most interesting finding that has emerged from this study is the relatively high biological activity of the degraded molecular mixture. The irradiated material has been found to have 25 per cent of the biological activity of the initial pure insulin. It is apparent that in this respect neutron bombardment serves as a new physical means of protein fractionation, making it possible to convert the molecule into fractions different from those usually obtained by the classical methods of chemical degradation.

Recoil Effects from Beta Decay and Gamma Emission

The recoil effects following beta decay are even more complex; not only is a neutrino of small but unknown mass emitted simultaneously with the electron, but also the angular correlation of the direction of emission of the neutrino and the electron is virtually unknown. How-

ever, in the specific case of P^{32}, Sherwin (18) has carried out experiments which show that the neutrino probably enters the same hemisphere as the electron. In general, three limiting cases can be considered: the neutrino and the electron can be emitted in the same direction, they can be emitted in opposite directions, or there can be no correlation at all. The recoil effect is maximum in the first case, minimum in the second, and intermediate in the third. The relative probabilities of recoil

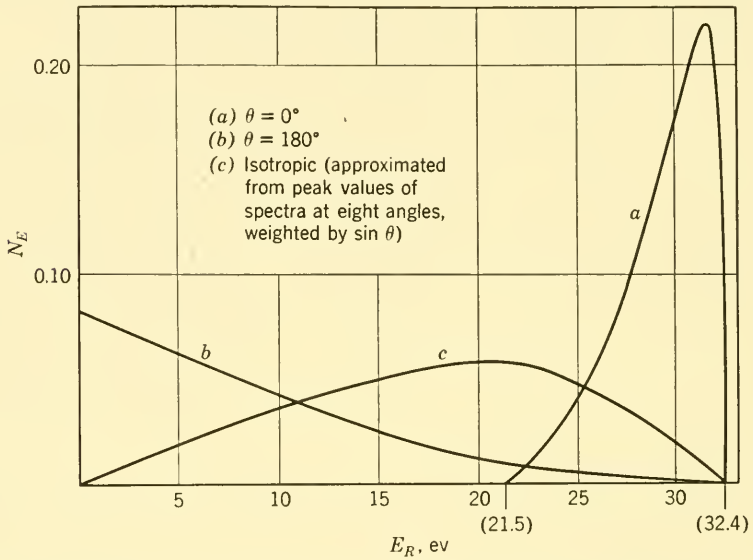

FIG. 1. Recoil spectra for varying angular correlations of electron and neutrino, for hypothetical nucleus of 100 atomic mass units with maximum beta-ray energy of 2.0 mev. [From Edwards and Davies (17).]

energies for a hypothetical nucleus of mass 100, giving off a 2-mev beta particle, have been calculated by Edwards and Davies (17) and are shown in Fig. 1. The maximum recoil energy for C^{14} is 6.9 ev; for P^{32} it reaches the sizable amount of 76.6 ev. The amount of this recoil energy (E_r in electron volts) available for bond strain or rupture is given by $E_i = m/(M + m)E_r$, where E_i is the bond strain energy in electron volts and M and m are the masses of the recoiling atom and the rest of the molecule, respectively. Thus, the energy available for bond rupture following beta decay is large except when the rest of the molecule has a mass small compared with that of the recoiling atom.

It is not entirely clear whether orbital electrons are lost after beta emission. If they are not, the increase in nuclear charge should correspond to a chemical oxidation of one unit. Gest, Edwards, and Davies

(19) report that, when trivalent La^{143} decays to Ce^{143}, 60 per cent of the Ce is found in the tetravalent state; likewise when Se^{83} in $Se^{83}O_3^{=}$ decays to Br^{83}, 35 per cent of the Br^{83} is found as BrO_3^-. In view of the electronic excitation of the recoil atom that is caused by the beta-decay process, these orderly oxidations seem surprising and may very well be the final state of a complex molecular reorganization.

The recoil energy that follows the gamma emission accompanying isomeric transition can be calculated from the expression used for obtaining the (n, γ) recoil energy, and the fraction available for breaking bonds can be obtained from the expression above. In some cases the gamma-ray energy is sufficient to break chemical bonds directly, causing Szilard-Chalmers reactions as already discussed. In others, as for example Br^{80}, the 48.9-kev gamma ray imparts a recoil energy of no more than 0.016 ev. The percentage of the recoil energy available for bond rupture is only 1.2 per cent in the case of Br^{80} in HBr, compared with 26.7 per cent for Br^{80} in C_2H_5Br. The subsequent rupture of the chemical bond must therefore be due to internal conversion of the gamma ray, with the consequent emission of electrons in the K or L shell. This process coupled with the Auger effect leads to the loss of many electrons; for Br^{80}, calculations predict that 60 per cent of the recoil atoms will lose four or more electrons.

Seaborg, Friedlander, and Kennedy (20) have shown experimentally that, in the case of zinc and tellurium, internal conversion is necessary for bond rupture. The gamma ray emitted in the isomeric transition of Zn^{69} is unconverted and more energetic than the gamma rays emitted in the isomeric transition of Te^{127} and Te^{129}. However, the tellurium gamma rays are largely converted, and Seaborg *et al.* were able to observe bond rupture in radioactive tellurium diethyl, whereas they could find none in zinc diethyl observed under the same conditions.

In sum, we can see that recoil effects, whether from beta decay or gamma emission, almost inevitably disrupt chemical bonds and increase the disorder of the system. In the whole spectrum of such effects resistance to rupture or recombination must be viewed as a rare and occasional process.

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DISCUSSION

ZIRKLE:

Solomon has pointed out that, when thermal neutrons bombard average animal tissue, about eight are captured by hydrogen for each one captured by nitrogen. I should like to add a note about the relative amounts of radiobiological action due to these two capture processes. The additional factors to be considered are:

(*a*) *The relative amounts of energy carried by the ionizing agents.* The gamma photon emitted by H^1 (n, γ) H^2 is 2 mev; the proton and the C^{14} nucleus resulting from N^{14} (n, p) C^{14} share about 0.6 mev. Accordingly the hydrogen reaction predominates in energy by roughly 4 to 1.

(*b*) *The relative biological effectiveness (RBE) of the ionizing particles.* This varies widely among various radiobiological actions, but, as a rough average,

we might set the effectiveness of the protons as 4 times that of the gamma rays. Accordingly, this factor approximately cancels factor (*a*).

(*c*) *The relative fractions of the emitted energy absorbed by the biological object.* Since the range of the proton is of the order of 10 microns, the fraction of its energy absorbed is practically 100 per cent even in very small objects. On the other hand, the fraction of gamma-ray energy absorbed depends greatly on the size of the object and is quite low even for an object 1 cm in diameter.

Accordingly, the relative biological action due to the two capture reactions is the product of the relative number of neutrons absorbed (8 in H to 1 in N) times the fraction of the gamma energy absorbed in the particular biological object. This product, even for an object as large as a mouse, indicates that the N capture predominates radiobiologically over the H capture. In larger objects, of course, the H reaction increases in importance because of increase in the fraction of gamma energy absorbed.

KAMEN:

The analysis of beta recoil processes based on the simple picture of an isolated nucleus is not adequate for complex molecules. The main difficulty is ignorance about what it is that recoils: thus, a P^{32} atom in a nucleoprotein is linked in a variety of ways through oxygen bridges to organic moieties, and it is not certain whether only one or two oxygen atoms, or also a portion of the nucleotide and protein, recoils along with the residual S atom. Thus, the mass effective in the recoil energy is unknown. It must also be noted that the recoiling S atom is imbedded in an atomic matrix with innumerable degrees of freedom, so that by a collision of the second kind, or by internal conversion, a large amount of the initial recoil energy can be degraded or transferred through a large portion of the protein, involving a general excitation of the whole molecule. Another difficulty, namely uncertainty about the neutrino distribution, has been mentioned by Solomon.

Perhaps an adequate analysis is not available at the present time. Nevertheless, it is badly needed because, as will be pointed out in the panel on biochemical processes, data are available which would permit conclusions about the radiosensitivity of specific sites in biologically important molecules to be drawn, provided such an analysis were possible.

MAGEE:

Two questions have particularly bothered me in connection with "hot" atom effects following the (n, γ) process, such as are being studied in our Radiation Chemistry Laboratory at Notre Dame by Hamill and Williams.

(*a*) Is the gamma energy given off in one quantum or several? This bears directly on the energy given the recoiling atom, since the resulting momentum of several quanta will result in partial cancellation. The assumption has apparently been made in Solomon's paper that the energy is given off in one quantum in all cases.

(*b*) What is the probability for the conversion of a gamma ray in these processes? The chemical effects resulting from this process may well be more important than the mechanical effects of the recoil.

SOLOMON:

With middle- and high-atomic-number atoms, frequent multiple processes yielding more than one photon are likely; with low-atomic-number atoms, a single photon is more probable.

MORRISON:

Very recent work at Chalk River indicates that, at least with N^{14}, capture results in a mixture of gammas, principally 4 gammas from two cascades, all between 4 and 6 mev.*

PLATZMAN:

Magee is certainly correct in calling attention to the fact that further complexity in the spectrum of recoil energies associated with capture of thermal neutrons is contributed by internal conversion of the capture gamma rays. There is no reason, of course, why capture gamma rays should exhibit internal conversion phenomena at all different from those of gamma rays of any other nuclear origin. [Except for the high conversion associated with the slow radiation of isomers. A rather special circumstance—that capture gamma rays commonly involve the consecutive emission of several "cascade" photons from the same nucleus—will not introduce any special effect, because, just for a gamma-ray transition which has appreciable conversion coefficient, the average time elapsing before emission of the photon will be longer than that required for refilling a depleted inner electronic shell of the atom; the *inner* part of the atom, which is the only portion relevant for the internal conversion, reverts to normalcy after each stage of the cascade. Quite obviously, complexity of the gamma-ray spectrum, and angular correlation between some of the successive photons, will lead to a most intricate spectrum of recoil energies. ED.]

Only very little information on the spectra of capture gamma rays has at the present time been attained. The few cases which have been studied show very complex capture gamma-ray spectra, apparently even for atoms of fairly low atomic weight. The only instance in which internal conversion of capture gamma rays has been studied involves bromine,† and here the effect was indeed found. This experiment is, incidentally, a difficult one. The results indicate at least 0.15–0.40 conversion electron per neutron captured, but they cannot be interpreted adequately because the spectrum of the capture gamma rays is not fully known. On general grounds, one must expect that the average conversion coefficient of all capture gamma rays for any one nucleus will in most cases be small, because the nucleus, in its transformation from the initial, highly excited state following neutron capture to its ground state, will pursue a path from one

* S. Wexler and T. H. Davies, *Report* BNL-C-7, p. 82, 1948.

† B. B. Kinsey *et al.*, *Phys. Rev.*, **77**: 723(L), 1950.

energy level to another involving just the *fastest* transitions, and these are the ones with the smallest internal conversion. Even if there should be a slow step in the sequence, this would usually have appreciable probability of conversion only if it were also a low-energy transition, so that the recoil energy even from internal conversion would be small and would influence the final spectrum of recoil energies (which almost always include the effects of some high-energy gamma rays) in only a minor way. To summarize, internal conversion of capture gamma rays is a factor which will certainly affect the distribution of recoil energies resulting from neutron capture; whether it is an *important* factor for any atom is not yet known.

5.

General Statements about Chemical Reactions Induced by Ionizing Radiation

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Introduction

Molecules, in order to react, must be activated. If a certain system reacts only very slowly, its rate of reaction will be greatly increased by a moderate increase in the temperature of the system. In such a system of thermal reactions, the energy of activation is obtained from the ordinary thermal energies of the molecules. Only that small fraction of all the molecules, which (by the Boltzmann distribution) have energies greatly in excess of the average, will be able to react. When such a slowly reacting system is illuminated with light of wave lengths which are absorbed by one or more of its components, there is a chance that the rate of the reaction will be accelerated. The energy of activation of such photochemical reactions is provided by the energy of electronic excitation of the molecules which have absorbed photons. In radiation chemistry there are two paths of activation. Some of the molecules are electronically excited whereas others are ionized. Both the excited molecules and the ions are capable of undergoing further reaction.

As a consequence of electronic excitation, the molecules may dissociate directly into radicals or may undergo a process of internal conversion (1), that is, transfer of the excitation energy of the electronic system into oscillational energy of the atomic constituents of the molecule. Thus the molecule will oscillate like a "hot" molecule and will undergo chemical reactions in some respects like a molecule at high temperatures. These reactions may involve other molecules, or the original excited molecule may break up into radicals or stable molecules.

* The author is greatly indebted to Professor James Franck for his encouragement and advice. The general outline of the paper and any new concepts which it may contain are due entirely to Professor Franck.

The thermal reactions of these radicals and molecules will then determine the course of the overall reaction. Ions similarly produce radicals or molecular products, either directly or as a result of recombination.

At the present time it is generally believed that the results of all radiochemical reactions (at least for non-vital systems) can be explained as the consequence of a set of reaction steps, similar in nature to those observed in ordinary photochemical and thermal reactions. Since 1936 (2), when this view was first clearly stated, there has been such rapid progress (3) in the field of radiation chemistry, especially during and subsequent to World War II, that we are sometimes inclined to overlook the important contributions which Lind and Mund made before 1936. It should be worth while, therefore, to review briefly some of these earlier results and the hypotheses which were suggested to interpret them. Bragg (4) in 1907 noticed that the number of molecules of liquid water decomposed by radon was equal to the number of ions which the same amount of radon would produce in air and referred to this relation as "a curious parallelism in numbers." Three years later LeBlanc (5) interpreted this parallelism as an analog to Faraday's law, and referred to the radiochemical decomposition of water as "electrolysis without electrodes." These ideas were extended and systematized in 1918 by Lind (6), who presented the formal or "stoichiometric" cluster hypothesis in an attempt to explain the constancy of the ion-pair yield observed for the formation of water from its elements. As experimental evidence accumulated, it was found that the measured values of the ion-pair yields of the oxidation of carbon monoxide and of methane (including the sensitizing action of inert gases), of cracking reactions of simple saturated hydrocarbons, and (possibly) of the decomposition of nitrous oxide were all consistent with the simple or formal cluster hypothesis. However, the large ion-pair yields observed in the polymerization of unsaturates (especially acetylene) demanded the assumption of large clusters, and accordingly a modified "physical" or "dynamic" cluster hypothesis was suggested by Mund (7). The results of the measurements of the decomposition of ammonia required the addition of an arbitrary (but not unreasonable) assumption to render these data compatible with the cluster theory. Finally, the experimental results for the formation of hydrogen chloride and of hydrogen bromide from their elements, as well as results for the *ortho-para*-hydrogen conversion, were completely divergent from predictions based upon the simple cluster theory. Eyring, Hirschfelder, and Taylor (2) published in 1936 the first attempt to interpret the observed rates of radiochemical reactions in terms of ordinary reaction kinetics. Their analysis of the *ortho-para*-hydrogen conversion

data, and the interpretation of the results on the oxidation of carbon monoxide, which was published by Hirschfelder and Taylor (8) in 1938, appear entirely satisfactory and prove, at least for these cases, that it is unnecessary to assume that there is any special or unusual characteristic of the kinetics of radiation chemistry.

To sum up, information which was not available when the cluster hypothesis was first introduced by Lind is sufficient to enable us to reject the assumption that the formation of clusters plays any important role in the kinetics of radiochemical reactions. We are not justified in believing, however, that the part played by ions is necessarily a minor one. Although the historical reason for reporting the results of radiochemical reactions in terms of ion-pair yields is certainly untenable, the use of the ion-pair yield, at least for gas reactions such as the oxidation of CO, is a convenient and reasonable one. It is, of course, for just these systems that the ion-pair yield and the energy yield (for example, the yield per 100 ev) are closely proportional to one another. It must be remembered that, when the ion-pair yield is used for reactions occurring in condensed systems, the number of ion pairs is not directly determined but is extrapolated from gas-phase measurements. The similarity between the ion-pair yield and the quantum yield of photochemistry should not be too heavily stressed, since it is likely to bias one's thinking about such reactions.

The earlier classical work on radiation chemistry was largely a study of reactions involving simple molecules in the gas phase. Recently, because of the obvious biological implications of complex molecules, for practical reasons, and on account of the greater simplicity, in at least one sense, in interpreting the results, the tendency amongst radiation chemists has been to study chiefly reactions involving complex molecules and, frequently, condensed systems. Although undoubtedly these studies are of vital importance, it is somewhat to be regretted that the investigation of gaseous systems of simple molecules has been allowed to lie dormant. The newer viewpoints and the additional information which has been gained, partly from mass spectrographic studies, about the nature of the reactions involving simple ions should permit much more rapid and sound progress to be made in the interpretation of these simple processes. For example, the published data (6) of the water-formation reaction still exist as a challenge to any theoretical student of radiation chemistry. As Franck has stated several times in the last few years, it should also be interesting to study the products of radiochemical reactions involving simple gaseous compounds of carbon, hydrogen, and nitrogen. The mystery of the origin of complex organic material upon the earth might be solved by such experiments, since it is reasonable to

assume that the first complex organic ring structure (such as porphyrins) might have been formed in the atmosphere of the primitive earth by brush discharges.

In photochemistry it has long been recognized (9) that it is useful to separate the reaction steps which constitute the overall process into primary and secondary reactions. The primary reactions are those which the light-absorbing molecule undergoes immediately after capturing a photon. The secondary reactions are thermal steps involving the radicals or other products of the primary reaction steps. Very few, if any (10), chemical reactions are truly simple in nature. With few exceptions, all those which have been analyzed are the result of a combination of a number of consecutive and simultaneous reaction steps. Most commonly, these steps are bimolecular reactions between stable molecules, a molecule and a radical, or two radicals. Less frequently, monomolecular reaction steps, consisting of spontaneous rearrangements or dissociations, are involved. In some cases, chiefly the recombination of atoms or small radicals, reaction steps may be termolecular. Reaction steps of higher order than third probably never occur. In radiation chemistry reaction steps may involve ions.

Chain reactions are of special interest. They are distinguished by high quantum, or ion-pair, yields. During the course of these reactions, some of the reaction steps, in addition to producing the final products, return to the system the radicals or atoms which were formed by the primary process. In this way a single excitation or ionization act may induce the reaction of many thousands of molecules.

Excitation

The following review of electronic excitation is chiefly a restatement of well-known classical principles. However, in a few instances speculations about the nature of specific processes have been introduced.

If no chemical process such as delayed dissociation or rearrangement takes place, an isolated excited molecule will have a mean life of not less than 10^{-9} sec, and it will lose its energy of excitation by emitting a photon. Commonly this fluorescent light will have a longer wave length than that of the absorbed radiation. The longer the normal life of the excited state, the smaller will be the absorption coefficient for the light. For instance, the direct photochemical excitation of an ordinary stable molecule (with a ground singlet state) to an excited metastable state (with a triplet state, which would have a lifetime of about 10^{-2} sec) is so improbable that the corresponding absorption can be detected only

under quite special conditions. The transition probabilities for both absorption and emission are determined by selection rules and by the Franck-Condon principle. For large or even moderately complex molecules the selection rule which holds most generally is that which "forbids" transitions between states of different multiplicities. It is, for instance, the reason that a transition from a singlet to a triplet state is 10^{-6} times less probable than otherwise similar transitions which do not involve changes in multiplicity. The Franck-Condon principle states that during an electronic transition the positions of the heavy nuclei and their kinetic energies practically cannot change. Thus the absorption spectrum permits conclusions about the position of the nuclei at the moment of the absorption act. The heavy nuclei can, however, gain potential energy by the electronic transition, and will therefore start to oscillate if their equilibrium position in the excited state is different from that in the ground state of the electronic system. Usually excitation weakens the binding energy between nuclei and thereby enlarges their equilibrium separation. If this difference is sufficiently great, as it is for the hydrogen iodide molecule, an application of the Franck-Condon principle shows that photochemical excitation results almost exclusively in the formation of an electronically excited molecule with oscillational energy greater than its energy of dissociation. Accordingly, it will dissociate after a single vibration (about 10^{-13} sec) into radicals. Under these conditions the gas is, of course, non-fluorescent and photochemical reactions are very probable.

A process called predissociation (1) is of importance for complex molecules, including moderately complex compounds, such as nitrogen dioxide. Whenever two electronic states of a molecule have the same nuclear configuration and total energy, there is a finite probability that a molecule which is in one of these states will "cross over" into the other. The value of this probability depends on certain selection rules and upon the time the molecule spends in the configuration which is common to both states. As a result of this process, a molecule, in an excited state in which it does not have enough oscillational energy to dissociate, may after a lapse of time cross over into a second excited state in which it is unstable. This second state either may be less stable than the first or may be a completely repulsive state. Depending upon the probability of transfer, the mean life of the excited molecule may be anything between the period of a single vibration (10^{-13} sec) and the normal life of the excited state (10^{-8} sec). The occurrence of predissociation is marked by the appearance of photochemical action, by a decrease in the intensity of fluorescence, and by the disappearance of the rotational structure of the absorption bands.

An excited molecule may also lose energy (11) by a collision of the second kind. In such a process the excited molecule or atom gives up its electronic energy of excitation to its collision partner, and this energy may appear as electronic or oscillational energy of the second molecule or even as translational energy of the system. The amount of energy which appears as either oscillational energy or as kinetic energy of the system is small (not much greater than $\frac{1}{2}kT$); and, therefore, the bulk of the energy has to be transferred into the electronic system of the collision partner. If, as a result, the collision partner has an excited electronic system which is able to emit light, the process is called sensitized fluorescence. If, on the other hand, changes of the electronic system occur like transitions of electrons from bonding to antibonding, that is, a singlet-triplet transition causing dissociation, we speak of a sensitized photochemical process. If the atoms in the colliding molecules come into positions during the collision in which by an electronic transition an atom can switch from the first to the second molecule, such processes will occur with great probability, provided the energy originally absorbed by the first molecule is sufficient. One of the most thoroughly studied examples of this type is the interaction of a normal hydrogen molecule with an excited ($6\,^3P_1$) mercury atom, resulting in the formation of a H atom and a HgH molecule.

In addition to emission (fluorescence), direct optical dissociation, simple predissociation, and collisions of the second kind, complex molecules may lose energy of excitation as a result of the occurrence of *internal conversion* (1). This process, like predissociation, depends upon the existence of two electronic levels having in common the same total energy and nuclear configuration. Since there are so many more atoms and degrees of freedom in a complex molecule, it should be expected that the time required for the excited molecule to attain the appropriate configuration will be much greater than the minimum times observed for predissociation in simpler molecules. The usual result of an act of internal conversion will be the transfer of the electronic energy of excitation (in all or in part) into generalized oscillational energy of some lower electronic state. The occurrence of internal conversion is very probably the explanation of the absence of fluorescence of many complex molecules, even when they are in dilute solution or the gaseous state. If the molecule were completely isolated it would, if it did not dissociate, eventually return to its original state and emit (fluorescent) light. In practice, complete isolation is never attained, and during the long time it takes to reverse internal conversion, the molecule will lose energy by impact with others, thereby losing its ability to come back to the fluorescent state. Momentarily after occurrence of internal conversion the molecule

is thermally activated; that is, it is a "hot" molecule. As such it can undergo chemical changes typical of thermal reactions. It may dissociate into two radicals or into two stable molecules. Decarboxylation is a reaction of the latter type. In giant molecules, such as proteins, it is probable that the oscillational energy would not be evenly distributed over all the degrees of freedom of the molecule, but would for a time be confined to one segment of the molecule. Although energy which is distributed over many degrees of freedom cannot break ordinary chemical bonds, it could be sufficient to break weak linkages such as hydrogen bonds. In this way internal conversion might easily be responsible for reversible denaturation of proteins.

The fate of an excited molecule can be profoundly influenced by its environment. A simple molecule, such as hydrogen iodide, when dissolved in a chemically inert solvent can be photochemically dissociated just as it is in the gas phase. However, the resultant atoms will be caged in by surrounding solvent molecules. Before they can escape from this cage they will undergo many collisions with one another. As a result there is a considerable probability that they will recombine before they can separate. This *Franck-Rabinowitch* (12) effect can be responsible for a noticeable decrease in the quantum yield of a dissociation process occurring in a solution. The probability of escape from the cage is greater if the atoms are small and if they are initially endowed with high kinetic energy.

When an excited complex molecule in a solution undergoes an act of internal conversion, the probability of its losing its oscillational energy is greatly increased, since it is constantly in a state of multiple collision with the solvent molecules. Should the excited complex molecule be dissociated, its radicals will be hemmed in by the solvent cage. The recombination of complex radicals frequently requires some energy of activation. Furthermore, complex radicals must be in a definite orientation relative to one another before they can recombine. These steric and energetic requirements greatly reduce the rate of recombination of complex radicals and probably more than counterbalance their decreased rate of escape due to their size. It should be expected, therefore, that the cage effect will be less efficient in preventing the separation of complex radicals than of atoms or simple radicals. The dominant factor influencing the decomposition of excited complex molecules in a condensed system is most likely the rapid removal of their oscillational energy after the act of internal conversion.

Excitation energy may migrate through many molecules in a crystal in which the binding forces are strong and in which a very good resonance exists between the neighboring fundamental units of the crystal. This

process of exciton migration probably plays an important role in ionic crystals, but is of less importance in crystals made up of organic molecules. Crystals in which a strong exciton migration occurs have absorption spectra which differ typically from the spectra (13) of the compound in the gas phase. The normal molecular spectrum will be replaced by a much narrower, strong absorption region. This criterion leads to the conclusion (14) that transfer of energy of excitation by exciton migration is important in the micelles of cyanine dyes but is of little consequence in crystals like naphthalene. The well-known fact (14) that energy given to the lattice of naphthalene by absorption of radiation is largely re-emitted by naphthacene (present as a trace impurity) has probably little to do with exciton migration. It may be better explained by the process of sensitized fluorescence or, to use a more general term, "classical resonance" (15). This process allows transfer of excitation energy between molecules separated by distances which are great relative to their collision diameters. It has been discussed chiefly in connection with the self-quenching and the depolarization of the fluorescence of solutions of dyes. Its occurrence is most probable when the emission spectrum of the excited molecule overlaps the absorption spectrum of the receiver. However, there is a finite probability of its happening even when the excited molecule is non-fluorescent. It appears to be of importance (14) for intramolecular, as well as for intermolecular, transfer of excitation in complex molecules.

From this viewpoint of radiation chemistry, the most important mode of excitation is the impact of charged particles upon molecules. The specific characteristics of the excitation by the several charged and uncharged particles have been discussed by the physics panel. Direct excitation by impact is in many respects similar to photoexcitation. The Franck-Condon principle still applies, but some of the selection rules are different. The direct transition from a ground singlet state to a repulsive triplet level due to the absorption of a photon is forbidden, but the corresponding transition may be produced by some types of impacts of charged particles. Impact excitation can produce this type of direct dissociation in addition to the other methods of dissociation which are also produced by the absorption of a photon. In some cases the impact excitation of the molecule may be followed by light emission together with a dissociation process. The continuous emission spectrum of a hydrogen arc is an example of this kind (16). The hydrogen molecule, normally in a singlet state, is raised to an excited stable triplet state by electric impact and then falls to a lower repulsive triplet state, emitting a photon and dissociating into atoms. Either predissociation or internal conversion may follow excitation by impact just as it follows

photoexcitation. Indeed, internal conversion appears to be of dominant importance in determining the course of the radiation chemistry of complex molecules (17).

Excited molecules may also be formed by the recombination of ion pairs. Since the energy of ionization exceeds that required for the dissociation of a chemical bond, it should be expected in most cases that dissociation will follow the recombination of an ion pair. The behavior of certain complex molecules (18), particularly aromatic hydrocarbons, is an interesting exception to this general rule. A positive ion may be neutralized by either a negative ion or an electron. There are probably no important restrictions upon the recombination of positive and negative ions, in either condensed or gaseous systems. The capture by an isolated simple molecule of an electron accompanied by the emission of a photon is a very inefficient process (19). However, in gases even at relatively low pressures (say, 50 mm), the system, ion and electron, is on the average so coupled with neighboring molecules that recombination to a highly excited state of the molecule should occur with a high yield by what is effectively a triple collision.

Ionization

As has been discussed by the physics panel, molecules can be ionized by impact (3) with charged particles such as alpha particles, beta particles, protons, and electrons. Molecules are also ionized by interaction with high-energy photons, x-rays, or gamma rays. High-velocity neutrons also indirectly induce ionization. In addition to impact ionization, a molecule containing high enough excitation energy may spontaneously ionize by a process (20) analogous to predissociation. This phenomenon is called preionization when it involves the valence electrons and the Auger effect when it is in the x-ray region. Molecules may also be ionized by thermal impact with an excited molecule or atom (for example, $2\ ^1S$ He), provided the energy of excitation is greater than the energy of ionization of the molecule concerned. Processes such as the combination of two excited atoms (for example, $6\ ^3P_1$ Hg) to form an ionized molecule (Hg_2^+) and an electron have also been observed.

A wide variety of ionized molecules can be produced from a single compound (21) by electron impact if the energy of the electron is sufficient. As has been shown by mass spectrographic studies, single ionization of the original molecule is usually the most probable process at reasonably low electron energies. However, multiple ionization, as

well as ionization accompanied by dissociation of the molecule, also occurs in a variety of ways. Some ions are metastable and dissociate spontaneously after a short lapse of time.

Negative ions (22) can be formed by the capture of an electron by a neutral molecule or atom. Whereas most atoms and radicals have positive electron affinities and can therefore form stable negative ions, many simple molecules (especially those which have a Σ ground state) cannot form stable negative ions. Other molecules, particularly those which are strongly polarizable or have permanent dipole moments, form stable negative ions but usually have small electron affinities. In addition to the simple capture of an electron, negative ions may be formed by capture accompanied by dissociation. For example, a hydrogen bromide molecule can interact with a slow electron to yield a hydrogen atom and a bromine negative ion.

Ionization may migrate through a system either by simple exchange of charge between molecules or because of the conductivity of the medium. Measurements of the effect of traces of impurities upon the mobility of positive ions in gases (23) (such as helium) demonstrate that the exchange of charge between unlike molecules occurs effectively at ordinary pressure. Lind's demonstration (6, 24) that radiochemical reactions can be sensitized by inert gases is additional independent evidence that the exchange of ionization takes place readily, at least under the conditions of the experiments. A similar exchange of charge between a negative ion and a neutral molecule is to be expected. Failure to take the exchange of ionization into account is likely to invalidate any analysis (25) of the kinetics of radiochemical reactions. In solution, particularly aqueous, the solvation of the ions may influence profoundly the rate of exchange. Fortunately the rates of such solution reactions involving electrolytic ions are subject to direct study.

Migration of either positive or negative charges in a crystalline solid (26) may occur readily by electron exchange or by electron migration in the conductivity bands of the crystal. Although such conductivity is observed most readily in ionic crystals, it should also occur in atomic lattices (like diamonds) and in molecular lattices. However, in crystals made up from organic molecules there will be little electron migration on account of the frequent disturbances by thermal vibrations of the lattice and the molecules. Electron conductivity is also to be expected in liquids such as water, but here it should be limited to quite short distances corresponding to the short range order of liquids.

Reactions between ions and molecules are of importance in radiation chemistry. Except for the existence of the charge on one of the reactants, these reactions are in every way similar to the kinetic reaction steps of

ordinary reactions. The following three equations (8, 25) are possible examples of such reactions. The third of these may require some energy of activation.

$$Br_2^+ + H \rightarrow HBr^+ + HBr$$

$$H_2^+ + Br_2 \rightarrow H + Br^+ + HBr$$

$$CO^+ + CO \rightarrow C^+ + CO_2$$

Similar processes undoubtedly occur in solution, but here their energies are greatly affected by the solvation of ions.

Solvent Effects in Aqueous Solution

Radiation chemistry of aqueous solutions differs in many important respects from the corresponding chemistry of gaseous systems. The energy of solvation greatly changes the energy of ionic reactions in aqueous solutions. For example, the heat of recombination of hydrogen and hydroxyl ions in a gas is about 350 kcal per mole. For the same reaction of the solvated ions in aqueous solution, the heat is about 14 kcal per mole. Ions which are formed by impact in a solution will ordinarily exist long enough to come into equilibrium with the surrounding solvent molecules, before they can diffuse together and neutralize one another. For this reason they have some of the properties of ordinary electrolytic ions.

The following two equations (27) represent the reactions which largely determine the chemical characteristics of irradiated aqueous solutions.

$$H_2O + H_2O^+ \rightarrow H_3O^+ + OH$$

$$H_2O + e^- \rightarrow OH^- + H$$

The resulting H and OH radicals react readily with oxidizing and reducing agents, respectively. However, in the absence of such reagents, recombination of the radicals greatly reduces the ion-pair yield. Hydrogen peroxide can be formed by combination of two hydroxyl radicals or, more efficiently, if dissolved oxygen is present, by a reaction between a hydrogen atom and an oxygen molecule to form the perhydroxyl radical. Since hydrogen peroxide is a relatively stable but reactive oxidizing agent, its presence undoubtedly influences the properties of irradiated water. However, the primary radicals, H, OH, and HO_2 are very probably of greater importance.

It is convenient and informative to consider hydrated ions as complex molecules. As Franck has pointed out (28), the photochemical and radiochemical properties of such ions are to a large extent determined by the acts of internal conversion which they can undergo. For example, when a hydrated ferrous ion absorbs a photon, an electron is ejected into the shell of water molecules which surround the central ion. The resulting electronically excited complex molecule can undergo a process of internal conversion and so produce a complex molecule with a large amount of oscillational energy. It may lose this energy as heat to the solvent or, less probably, eject a hydrogen atom leaving a stable hydroxy ferric complex ion. As may be predicted on the basis of this mechanism, the absorption of more energetic photons increases the probability of the escape of the hydrogen atom. High concentrations of oxidizing agents also favor the production of the ferric ion. It is an interesting fact, and one which is consistent with a more detailed analysis of this reaction, that the quantum yield of oxygen production due to the illumination of a solution containing ferric ions is less than 10^{-5}, but in the presence of certain reducing agents the photochemical reduction of ferric ion approaches unity.

When an alpha particle is absorbed in liquid water a concentrated column of ions is formed. Momentarily, the core of this column consists of positive ions surrounded by a cylindrical shell of negative ions produced by electrons which were ejected with relatively high velocities. The localized concentrations of hydronium and hydroxyl ions which are so produced exceed the concentrations of the same ions which are obtainable in even concentrated basic or acidic solutions. Franck has recently suggested the interesting idea that these short-lived regions containing high concentrations of hydroxyl or hydronium ions could well be responsible for the denaturation of a protein molecule or the breaking of a chromosome chain which suffers a near miss by an alpha particle.

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DISCUSSION

BURTON:

In amplification of points raised by Livingston we may note that in photochemical reactions energy absorbed electronically at a particular locus may be internally converted to vibrational energy at another favored locus. In such event rupture of one particular bond, or a particular rearrangement decomposition, may be specially favored. In thermal reactions the energy is initially distributed in many degrees of freedom, and, in general, the most probable primary reaction is that most favored by the frequency factor and particularly by the activation energy. In contrast, in photochemical reactions the process of lowest activation energy is not necessarily the most likely to occur. In radiation chemistry there is evidence for a great variety of possible products, related undoubtedly to the fact that primary ionization and primary excitation (both of which are produced by the ionizing radiation) are not restricted to one part of the molecule. Resultant chemical events are shaped by the nature of the initial physical events peculiar to radiation chemistry. The situation is not comparable to the photochemical case. In radiation chemistry one must reckon

with ionization transfer between molecules and also with ionization transfer within the molecule. Before a process of thermal degradation of energy occurs there is perhaps a situation not too different from that in a photochemical process. However, the process of thermal excitation is not to be compared with that in reactions induced photochemically or by ionizing radiations.

LIVINGSTON:

There are cases in radiation chemistry where the products are like those produced in thermal reactions. Examples of this type are the decarboxylation of organic acids and the splitting of hydrocarbons into two stable molecules. On the other hand, no one can deny that there are photochemical and radiation chemical reactions whose products are predetermined by the manner of activation. The interesting thing is not that there are these differences, but that the reaction products of complex molecules are so often independent of the manner of activation.

MAGEE:

It is somewhat unfortunate that there is so much talk of ionization of the individual atoms of a molecule. Ultimately the charge always resides in the valence electrons and must belong to the molecule as a whole, not a single constituent atom, except in special cases. However, there are differences in the distribution of charge in a molecule ion characteristic of the particular energy state involved in the ionization. It is this average depletion of electron charge in a part of the molecule to which reference is properly made by the expression that a particular group or atom in the molecule is ionized.

Concerning the breakdown of selection rules in impact processes it is, of course, true that almost all selection rules are violated for very close collisions of any fast particle. However, most of the effect on the matter in any irradiation is due to the relatively slow secondary electrons. Here exchange effects must be considered between the secondary electron and the electrons of the molecule with which it collides. The ordinarily forbidden singlet-triplet transitions occur with high probability.

SOLOMON:

I should like to point out that the process of "flow" of ionization is an important one in the operation of Geiger counters, where it is relied upon in order to quench the pulse. Perhaps one could use the quenching time in a Geiger counter to obtain further information on how quickly the ionization flows.

DALE:

Is the theory that the hydroxyl and hydrogen ions are responsible for the denaturation of proteins meant to substitute for the theory of radicals?

LIVINGSTON:

It is probable that both means of denaturation are effective. Their relative importance is not known.

6.

Chemical Reactions in the Gas Phase Connected with Ionization

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The previous papers in this symposium have dealt with the physical theory basic to the study of the interaction of high-velocity particles with matter. We wish to take the first step toward the consideration of complex systems, to consider processes sufficiently simple so that the application of approximate quantum mechanical and statistical calculations is possible, yet sufficiently complex to point the way toward a correct discussion of systems of biological interest. Thus, this report will deal largely with the effect of bombardment of isolated molecules by electrons, under such conditions that no secondary reactions occur between the products of the bombardment.

Most of the subsequent discussion will be based upon data obtained with a mass spectrometer. Let us consider just what information can be so obtained. Almost all the mass spectrometric data now available have been obtained with instruments of the Dempster-Nier type. Here the substance to be studied, which must have a vapor pressure of at least a millimeter or so at a reasonable temperature, is introduced into the ionization region through a capillary leak to give a pressure of about 10^{-4} mm. It is there bombarded by a beam of electrons of known energy, commonly variable over the range 0–100 volts, and the positive ions formed are accelerated by a small field, a few volts per centimeter, toward a slit. Those that pass through are accelerated by a "high potential" of 300–5000 volts and focussed on a second slit, the entrance slit to the analyzer section. Depending upon the type of instrument, 180, 90, or 60°, the ions diverging from the entrance slit pass through a magnetic field in which they follow circular paths of radius

$$R = \frac{C}{H}\sqrt{2\frac{m}{e}V}$$

for the angular deviation given above. Those ions having the appropriate radius are refocussed on an exit slit behind which is a collector electrode connected to a sensitive electrometer circuit. The current in the electron beam is usually about 10 microamp. Total ionization is the order of 10^{-8} or 10^{-9} amp. A very strong peak might give an ion current of 10^{-10} amp. The size of the minimum peak detectable will depend upon the sensitivity and the noise level of the electrometer circuit; 10^{-15} amp is a reasonable lower limit for most instruments.

As is indicated by the above, in the modern mass spectrometer the current density in the electron beam and the gas density in the ionization region are sufficiently low to assure that all processes are the result of single electron impacts upon isolated gas molecules and that all subsequent breakups and rearrangements of the molecules are unimolecular [only exceptions reported: formation of H_3^+ and HCO_2^+ (1)]. Proper location of the filament and the pumping lead essentially prevents the diffusion of products formed by thermal cracking at the filament back into the ionizing region. Thus, by varying either the accelerating voltage or the magnetic field, one can successively collect and measure the ions of each m/e ratio formed from a given molecule by collision with electrons of known energy, without the complications that arise from secondary reactions in gases at higher pressures or in condensed phases.

One major trouble in the interpretation of data arises from the complete lack of direct information regarding the neutral fragments formed along with the ions. This matter will be discussed in some detail in subsequent sections of this report. A second serious complication is that the simple "single-focussing" mass spectrometer is designed to focus and collect efficiently only those ions formed with essentially zero kinetic energy (of the order of translational thermal energy at 200° C, 0.06 volt). By applying a retarding potential to the final ion collector one can determine the amount or distribution of excess kinetic energy possessed by the ions of any given mass number, but the geometry of the spectrometer tube is such that the vast majority of ions with appreciable (over about 1 volt) translational energy will strike the sides of the tube long before reaching the ion collector (2).

Monatomic Gases

The simplest substances for study in the mass spectrometer are the monatomic gases. Here, all the data obtainable can be represented by a set of "ionization-efficiency" curves for the ions obtained by removing one or more electrons from the atom. These curves, in which the magnitude of the ion current is plotted against the energy of the electron

beam, are all of the same general shape; a curved "foot," a very nearly linear section, and a flat maximum after which the ion current drops off inversely with the increase in the electron voltage. The minimum electron potential which yields a given ion should correspond to the spectroscopic ionization potential. However, this minimum potential cannot be measured directly, as there are always contact potentials of unknown and appreciable magnitude associated with a hot filament. In addition, the electrons emitted by the filament have a thermal energy spread of several tenths of a volt. Also, the electron beam is of finite thickness, and in the ionization region there is an electric field perpendicular to the electron beam. These factors contribute to the shape of the foot of the ionization-efficiency curve, but if such experimental factors were alone responsible all curves should have identical feet. Experimentally, this is not the case (3). Moreover, the values for "appearance potentials," the minimum electron voltages at which given ions are formed, obtained by noting the first upward breaks from the axis, give differences in agreement with the spectroscopic ionization values for A^+ and Ne^+ and for A^+ and A^{++}. If instead one assumes that the initial curved portions of the curves arise from experimental factors and so extrapolates to zero ion current the linear portions of the curves, the resulting differences are not in agreement with the spectroscopic data. This is unfortunate, since the "linear extrapolation" is usually simple and objective whereas the point of the "initial break" is subjective (6) and also in some cases depends greatly upon the sensitivity of the instrument (8). The approximations usually made in obtaining ionization cross sections by quantum mechanical calculations break down completely near the appearance potential, so that the theoretical shape of the curve is unknown. Several articles in the literature discuss the significance of these two methods of determining appearance potentials for molecules (3, 4, 5), and other methods of determining appearance potentials have been proposed in which a correction is made for the electron energy spread (6, 7). The situation is not satisfactory. The best method at present seems to be the initial break or "vanishing current" method, with the voltage scale corrected for contact potentials by mixing a gas of known ionization potential, usually neon or argon, with the gas under investigation.

Diatomic Molecules

The effect of electron bombardment on diatomic molecules has been carefully studied for many such molecules (2, 4, 9, 10, 11). We shall here make no attempt at completeness but only consider those aspects useful

in the discussion of the processes occurring in more complex molecules. The velocity of an electron accelerated by a voltage V is approximately $6 \times 10^7 \sqrt{V}$ cm per sec, or $6 \times 10^{15} \sqrt{V}$ Å per sec. Thus the time of interaction of even a 1-volt electron with a small molecule is of the order of 10^{-15} sec, and for electrons of the voltages generally used the time of interaction will be much less than this value for molecules of molecular weight up to several hundred. The highest vibrational frequencies of molecules (other than the H_2 molecule) are of the order of 3000 cm^{-1}, or 9.10^{13} sec^{-1}; most frequencies are one-third this value or less. It is seen that the time of interaction of an electron of energy greater than 10 volts with a molecule is at most one-thirtieth of the shortest period of vibration of the molecule. The electron is too light to transfer appreciable energy directly to the nuclei. We can safely apply the Franck-Condon approximations (12) and state that the first direct result of the electron impact is to raise the molecule to an excited electronic state without change in either the internuclear distance or the nuclear momentum. If we are to observe the results of this collision in the mass spectrometer, it is, of course, necessary that the excited state be an ionized state. It can be either the ground state or an excited state of the ion.

A set of possible potential functions for a diatomic molecule is given in Fig. 1. Here, curve I represents the ground state of the molecule, curves II and III are attractive states, and curve IV is a repulsive state which dissociates to the lowest state for the separated atom plus ion; curves V and VI represent two of the many possible states formed in first approximation from excited states of the atom and ion. In accordance with the Franck-Condon principle, transitions are probable only to those parts of the potential curves between the lines *a–a* and *b–b*. We note several possibilities. Transitions to state II will give only molecule ions, and the minimum electron potential at which such ions appear should be an accurate measure of the ionization potential of the molecule. Transitions to state III can yield either molecule ions AB^+ or atom ions A^+; the appearance potential for AB^+ will be definitely larger than the ionization potential of AB (to give the ion in this particular state), but the appearance potential for A^+ should give an accurate value for $I_{(AB)} + D_{(AB^+)} = D_{(AB)} + I_{(A)}$, and the ions A^+ will be formed with low kinetic energy irrespective of the magnitude of the electron energy. To obtain transitions to state IV will require an electron energy several volts greater than $D_{(AB)} + I_{(A)}$, and the resulting ions A^+ will have the fraction

$$\frac{m_{(B)}}{m_{(A)} + m_{(B)}}$$

of this excess as translational energy. The efficiency of collection of such ions will be very low in most mass spectrometers (2), and those that appear will come at an accelerating potential or magnetic field

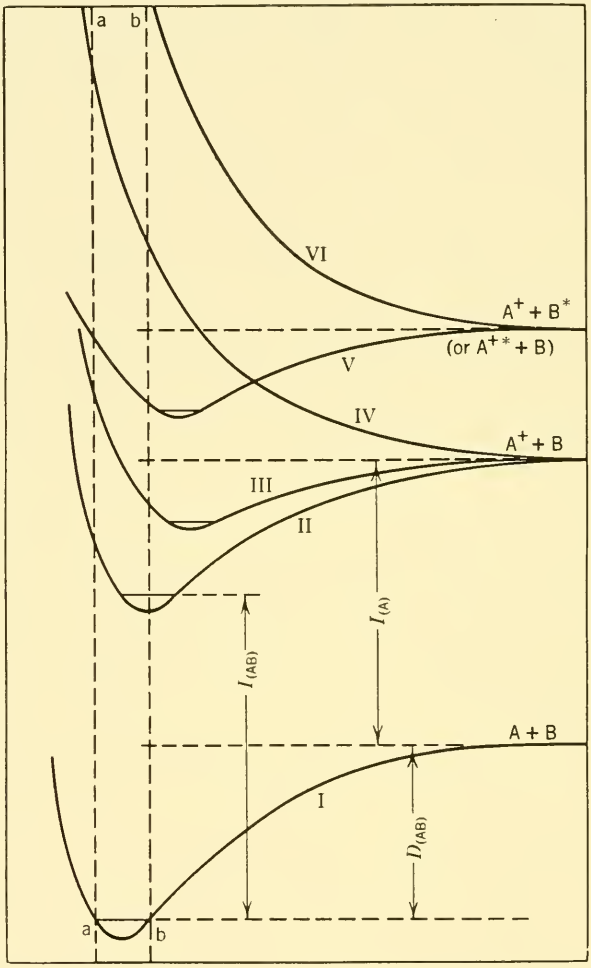


FIG. 1. Potential energy functions for a few possible states of a diatomic molecule, indicating some of the dissociation processes and products which can result from a single electron impact.

corresponding to a mass slightly greater than the true value. Transitions to state V, if the Franck-Condon rule is strictly applicable, can yield directly only molecular ions, but as this state is above the asymptote for formation of $A^+ + B$, secondary reactions can occur: (*a*) there

can be radiative transitions to states such as II (with formation of stable AB^+) and III (with formation either of AB^+ or A^+ with low kinetic energy); the half life for state V for such transitions, by the usual spectroscopic selection rules, will be of the order of 10^{-7} sec; (*b*) there can be radiationless transition to state IV, with formation of A^+ ions with some intermediate amount of kinetic energy; the half life for this transition will depend greatly on the magnitude of the interaction between the two states and can be anything between $<10^{-13}$ sec to >1 sec. The time spent by an ion in the ionization region before collection by the ion "draw-out" potential is the order of 10^{-6} sec. Hence, ions initially formed in a state such as V will normally undergo an electronic transition, with or without dissociation, before acceleration and collection. Lastly, transitions to state VI will be followed immediately by dissociation to give A^+ with excess kinetic energy.

A set of potential-energy curves such as those indicated therefore gives rise to AB^+, A^+ (low KE), and A^+ (high KE). Certainly there will also be states yielding B^+, both without and with kinetic energy. Also, to be complete, one must include double ionization, the formation of AB^{++}, with possible subsequent dissociation most likely to $A^+ + B^+$, but also sometimes to $A^{++} + B$ and $A + B^{++}$. There is also the possibility of forming an excited neutral molecule AB* in such a state that it dissociates: $AB^* \rightarrow A^+ + B^-$. All these possibilities have been observed (2).

In H_2^+, the two lowest states are located relative to the ground state of H_2 in similar fashion to states III and IV of Fig. 1. The observed mass spectral data agree completely with those expected from the above discussion in regard to appearance potentials and kinetic energy of the H^+ ions. Also, a calculation based upon the simple application of the Franck-Condon principle leads to a value of the rates of H^+/H_2^+ to D^+/D_2^+ in essential agreement with experiment (13).

A detailed discussion for diatomic molecules of the dependence of peak shapes as observed with the mass spectrometer upon the relative shapes of the ion and ground state curves has been given by Hagstrum and Tate, with special reference to CO, NO, N_2, O_2 (2). They find, for instance, that both C^+ and O^+ are obtained from attractive states of CO^+, the state yielding C^+ having its minimum a little further out than the curve for state III, Fig. 1, so that some C^+ are formed with appreciable kinetic energy, and the state yielding O^+ similar but with its minimum much further out, so that practically all the CO^+ ions formed in this state dissociate with appreciable kinetic energy.

It is probably not necessary to point out that an appearance potential for an ion, since it corresponds to a "vertical" transition on a potential-

energy curve diagram, gives only an upper limit for the amount of energy necessary for a given process. That is, referring again to Fig. 1,

$$A_{(A^+)} \geq D_{(AB)} + I_{(A)} = I_{(AB)} + D_{(AB^+)}$$

In recent years, workers in mass spectra have succeeded remarkably well in building instruments in which second-order reactions involving ions and molecules are almost completely absent. This was not the case in older instruments, and indeed some instruments were constructed especially for the study of secondary reactions by differential pumping arrangements which permitted independent control of the gas pressure in the analyzer regions (14). The essential result of such work is that the probability of charge exchange reactions,

$$A^+ + B \rightarrow A + B^+$$

varies inversely with the size of $I_{(A)} - I_{(B)}$, and only on the absolute value of this difference if A^+ has much kinetic energy. It may be very large for $I_{(A)} - I_{(B)} \approx 0$. For the special case of charge exchange where A and B are the same, as

$$N_2^+ + N_2 \rightarrow N_2 + N_2^+$$

the cross section for the reaction can be orders of magnitude greater than expected from the kinetic theory diameters.

Interesting applications of mass spectrometric data in combination with thermal and kinetic data are contained in several papers by Eyring, Hirschfelder, and Taylor (15). In the *ortho-para*-hydrogen conversion by alpha particles, 700–1000 molecules are converted per ion pair formed (16). The ionization by alpha particles is actually largely due to the secondary electrons of energies comparable to electron energies in the mass spectrometer. From the ratio of ion pairs produced to alpha-particle energy, and the mass spectrometric data on hydrogen, it is deduced that the primary effect of the secondary electrons is to produce approximately equal amounts of (2H) and H_2^+, and much smaller amount of H^+. Absolute reaction rate calculations indicate that the reaction

$$H_2^+ + H_2 = H_3^+ + H$$

is very rapid, and that the reaction

$$H_2 + H_2^+ = H_2 + H + H^+$$

is of no importance. The formation of "clusters," the formation of H^- and H_2^-, and the *ortho-para* conversion directly by alpha particles and by H_2^+, H_3^+, and H are shown to be negligible. Recombination of H_3^+

with an electron will give an average yield of between two and three H atoms per H_3^+. This is the principal neutralization reaction. Combining it with the previous arguments, one obtains a total H-atom yield of about 6 per ion pair produced. The remaining reactions to be considered are

$$H + H = H_2$$

and

$$H + H_{2(p)} = H_{2(o)} + H$$

The first reaction requires a third body and occurs essentially only on the walls of the vessel. The second reaction is very rapid and effective in causing the conversion. Calculations based upon the known diffusion constant of H atoms in H_2 and the rate of the last reaction above give results in good agreement with experiment.

The synthesis and decomposition of hydrogen bromide by alpha particles in hydrogen-bromine-hydrogen bromide mixtures were treated in similar fashion (17). The primary ionization processes were obtained from mass spectral data. Of the large number of secondary reactions possible, many were immediately discarded as too slow to be important, and an analysis of the kinetics of the system was made in terms of the remaining reactions. It was found that the data could be satisfactorily explained with the assignment of reasonable values to the few unknown rate constants. A similar treatment of the alpha-particle-induced reactions in carbon monoxide-oxygen-carbon dioxide systems has been made by Hirschfelder and Taylor (18).

Polyatomic Molecules

One is naturally inclined to attempt to extrapolate from the reasonably well understood actions of diatomic molecules to polyatomic molecules (19). There are many points of similarity. Each ion formed from a polyatomic molecule has a well-defined appearance potential, and the ionization-efficiency curve for the production of an ion is of the same general shape as similar curves for diatomic molecules. With small polyatomic molecules such as CO_2 and CH_4, one finds ions formed with appreciable amounts of translational energy. However, when we examine the mass spectra of hydrocarbons the size of propane and larger, we find the spectra show certain distinctive features different from small polyatomic and diatomic molecules.

The mass peaks for ions obtained from large molecules are almost invariably symmetrical and of a shape determined only by the characteristics of the instrument. This fact, important in permitting the determi-

nation of relative intensities by the simple measurement of peak heights, also indicates that the production of ions having appreciable kinetic energy is far less frequent here than in diatomic molecules. Very careful measurements (20) have shown that all the members of any one isomeric series of molecules, such as the octanes, show the same total ionization when measured under the same conditions. This can be interpreted in two ways. Either there are essentially no products formed with kinetic energy, or every isomer in the series produces about the same amount of products with kinetic energy. In view of the striking differences in mass spectra of a series like the octanes this second alternative seems far less likely. (See Table 1.)

When an attempt is made to correlate appearance potentials with thermal data on bond dissociation energies, the agreement is generally fair (21, 22). The discrepancies, as might be expected, are all in a direction which indicates that the products of dissociation have a certain amount of excess energy of the order of a few tenths of a volt. This energy could conceivably be either vibration-rotation energy or kinetic energy of translation. In view of the previous discussion it seems likely that it is mostly vibrational, and indeed the apparent magnitudes of the excess energy for heavy molecules seem never to be too large to be interpreted in this way. It is frequently necessary in small molecules such as methane to assume such dissociations as (21)

$$CH_4^+ \rightarrow C^+ + 4H$$

in which the uncharged fragments come off as atoms. However, in a paper by Delfosse and Bleakney (22) on the mass spectra of propane, propylene, and allene it is shown that here no such assumption is necessary for any of the ions measured, which include all the important ions. Every appearance potential measured is best explained by assuming that the minimum energy process possible occurs. That is, wherever possible, the uncharged fragments come off as molecules.

Also, in the mass spectra of all large hydrocarbon molecules one finds a number of metastable peaks (23, 24). These metastable peaks constitute a large fraction of the diffuse peaks occurring at non-integral masses. These peaks have been shown to be formed by delayed dissociations which occur after the original ion has been accelerated but before or immediately after its entry into the analyzer (25, 26). Examination of these peaks in a very large number of hydrocarbons (23, 24) shows that the uncharged fragments of the dissociation almost always are in the correct ratio to form a stable molecule. For most of these molecules, we have no way of knowing the state of association or dissociation of the neutral part, but the fact that the fragments are of such

TABLE 1

Mass Spectra of Octane *

Compound	Percentage of Maximum Peak at n/e Values Indicated																
	114	112	99	85	84	71	70	69	57	56	55	43	42	41	29	27	15
(1) *n*-Octane	6.9	0.02	0.1	30.0	6.0	23.5	12.2	1.3	34.2	18.1	10.2	100	15.8	38.9	34.5	26.2	2.2
(2) 2-Me heptane	4.9	0.04	12.6	1.8	0.8	12.9	17.2	1.3	73.3	8.1	11.1	100	41.9	38.2	27.7	25.3	3.1
(3) 3-Me heptane	3.0	0.02	0.8	49.0	27.3	3.1	2.5	2.7	67.5	38.3	11.2	100	7.9	45.9	40.7	27.8	2.8
(4) 2,4-Me_2 hexane	1.7	....	1.1	46.1	9.0	14.9	9.8	4.2	72.8	29.6	9.9	100	11.0	43.4	32.4	24.2	3.6
(5) 2,5-Me_2 hexane	3.8	2.02	17.3	0.7	0.2	18.8	9.7	1.4	80.2	7.6	10.9	100	34.0	38.2	21.1	23.3	4.4
(6) 3,4-Me_2 hexane	2.2	....	0.3	38.1	7.3	1.5	1.9	3.3	79.2	100	9.9	68.1	4.0	56.5	48.0	24.5	2.7
(7) 3-Et hexane	1.6	....	0.1	28.5	22.6	13.5	12.9	2.5	12.4	5.9	11.6	100	6.7	22.8	19.8	19.3	1.7
(8) 2-Me, 3-Et pentane	1.3	....	0.1	18.1	4.3	24.7	50.0	3.3	14.8	2.9	17.9	100	14.2	27.0	18.7	19.2	2.2
(9) 3-Me, 3-Et pentane	0.00	....	1.9	64.3	18.8	10.5	0.6	5.8	27.0	2.6	8.0	100	2.4	25.0	21.2	17.5	1.7
(10) 2,2-Me_2 hexane	0.03	....	5.6	0.02	0.01	0.7	0.3	0.8	100	32.2	4.6	15.9	1.8	26.3	18.3	11.3	2.4
(11) 2,2,3-Me_3 pentane	0.03	0.01	3.0	2.9	0.2	0.4	0.5	1.2	100	57.5	5.8	22.8	2.2	33.4	23.0	11.4	2.9
(12) 2,2,4-Me_3 pentane	0.02	....	4.7	0.01	0.05	0.8	0.2	0.5	100	32.7	4.0	23.1	2.0	27.4	15.4	10.7	3.6
(13) 2,2,3,3-Me_4 butane	0.03	....	6.2	0.03	0.3	0.3	0.1	1.3	100	27.3	4.5	17.7	1.5	28.2	16.2	7.0	4.7
(14) 3,3-Me_2 hexane	0.01	0.01	5.1	36.1	7.6	47.0	17.1	3.5	40.7	5.3	11.9	100	3.7	29.4	22.8	20.8	3.0
(15) 2,3,3-Me_3 pentane	0.01	....	3.5	25.0	3.5	45.3	35.5	3.5	35.8	1.5	15.7	100	5.4	28.6	16.0	18.2	3.2
(16) 4-Me heptane	3.1	0.02	0.9	4.5	3.0	52.7	46.1	1.3	14.4	2.4	15.3	100	13.5	26.6	22.8	22.5	2.4
(17) 2,3-Me_2 hexane	1.7	....	0.4	1.5	0.4	46.4	58.3	1.6	16.7	1.6	19.8	100	18.9	28.6	19.0	21.6	2.9
(18) 2,3,4-Me_3 pentane	0.3	....	0.2	0.2	1	61.9	40.6	0.9	16.3	2.0	17.1	100	7.4	24.5	13.6	18.2	3.3

* Bloom, Mohler, Lengel, and Wise, *J. Research Natl. Bur. Standards*, p. 129, 1948.

a character seems significant nevertheless. For the butanes the appearance potentials for certain metastable ion transitions have been determined (27) and require that the uncharged fragments be the molecules H_2 and CH_4.

A characteristic of all the experimental facts that have been presented here is that they may all be interpreted by assuming that the dissociation processes are essentially thermal in nature. By this we mean that the molecules gain, in the course of ionization, various amounts of vibrational energy and subsequently dissociate, as a sufficient amount of this energy becomes concentrated in some bond or other. This assumption is very strikingly borne out by the actual appearance of the spectra of the octanes (Tables 1 and 2), where one finds a great deal of correlation between the structure of the molecules, in terms of bond energies, and the mass spectra.

Let us now consider in some detail the basis for this interpretation. It is surely reasonable to assume that in saturated hydrocarbons the binding energies of the electrons in all C—C and all C—H bonds are about the same, and since the normal electron beam energies are from 5 to 10 times the ionization potential of the molecule, which electron is removed from the molecule on ionization will be largely a matter of chance, with perhaps a slight weighting factor in favor of electron removal from a C—C bond. That is, to a first approximation, the numbers of ions formed by removal of an electron from different bonds will be about the same. If there is any validity to the approximation that the bonding electrons in the molecule are localized in pairs in the various bonds, the removal of an electron must leave the ion with a one-electron bond, a bond far weaker than all other bonds in the molecule. If we then assume that ionization is followed immediately by rupture at the bond from which the electron has been removed, we expect the mass spectra of all members of an isomeric series to have patterns obviously dependent on the carbon skeleton. For example, in the mass spectra of the octanes, the amount of $C_7H_{15}^+$ should increase as the number of branched methyl groups increases, since there would be more opportunity for methyl groups to be lost. A glance at Table 2 shows that, far from exhibiting such patterns, the octane spectra depend on structure in an entirely different fashion. This dependence seems to be related directly to the energies of the various types of bonds in the molecule. For example, those octanes having a tertiary butyl group at the end of the chain show only a very few per cent of ions with carbon number greater than 4. (See Table 2.) This parallels the known low energy of this bond in comparison to the other carbon-carbon bonds present (28). In 4-methyl heptane, where splitting at the weakest bonds will allow

TABLE 2

Ion Intensity in Percentage of Total Ionization by Number of Carbon Atoms in the Ion

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
(1) C—C—C—C—C—C—C—C	0.7	18.4	44.5	16.9	9.4	9.1	~0	1.7
(2) C—C(—C)—C—C—C—C—C	1.0	14.9	47.9	23.1	7.5	0.6	3.8	1.2
(3) C—C—C(—C)—C—C—C—C	0.6	17.2	36.3	25.2	1.7	15.7	0.3	0.6
(4) C—C—C—C(—C)—C—C—C	0.9	14.6	43.6	9.8	27.7	2.1	0.4	0.9
(5) C—C—C(—C)—C(—C)—C—C	0.8	15.1	46.5	13.1	8.9	17.0	~0	0.5
(6) C(—C)—C—C—C—C	1.3	14.8	22.5	58.1	0.7	~0	2.4	~0
(7) C—C(—C)—C(—C)—C—C	1.0	12.6	43.0	14.7	27.7	0.5	0.1	0.4
(8) C—C(—C)—C—C(—C)—C	1.1	14.6	39.0	26.2	6.4	12.2	0.3	0.4
(9) C—C(—C)—C—C(—C)—C	1.4	13.0	47.7	25.5	7.4	0.2	4.5	1.5
(10) C—C(—C)—C—C—C	1.1	13.5	38.8	16.2	17.7	11.3	1.4	~0
(11) C—C—C(—C)—C—C—C	0.8	17.6	30.3	40.2	1.3	9.3	~0	0.4
(12) C—C(—C)—C(—C)—C	0.9	12.4	45.3	11.5	22.6	6.4	~0	0.4
(13) C—C(—C)—C—C—C	0.7	14.7	43.4	32.3	2.1	25.3	0.6	~0
(14) C(—C)(—C)—C—C—C	1.3	13.9	24.1	57.6	0.7	1.1	1.0	~0
(15) C(—C)(—C)—C—C—C	1.8	12.2	26.3	57.1	0.7	~0	1.9	0.8
(16) C—C(—C)—C—C—C	1.3	11.0	40.8	15.3	22.9	7.8	1.0	~0
(17) C—C—C(—C)—C—C	1.0	11.5	44.4	11.9	31.1	~0	~0	0.1
(18) C(—C)(—C)—C—C	2.5	11.4	24.9	57.7	0.8	0.2	2.7	~0

primary formation of C_5 and C_3 ions, there is very little formation of heavier fragments. In ethane, the $C_2H_5^+$ ion accounts for 10 per cent of the total ionization, but in the butanes the amount of $C_4H_9^+$ ions is about 1 per cent and in the octanes the amount of $C_8H_{17}^+$ ions is of the order of 0.01 per cent (23).

We need to explain why these apparently straightforward assumptions seem to yield answers in contradiction to the data; this interpretation also must explain the relative intensities of those ions whose formation requires the breaking of several bonds in the original molecule, occasionally with extensive rearrangements occurring in the process. In small molecules, especially diatomic molecules, the number of electronic states of the ion lying within, say, 50 volts of the molecule ground state will be relatively small. This is not the case in larger molecules of low symmetry. If we consider the number of states possible for a collection of atoms having n valence electrons, all in their lowest states for widely separated atoms, the number is found to be 2^n (29). Many of these are degenerate; the number of independent eigenvalues for the energy is $\frac{n!}{(n/2!)^2}$, but this when evaluated by Stirling's approximation gives $\sqrt{(2/\pi n)}2^n$, of the same order of magnitude. Thus, even for propane, where $n = 20$, $2^n = 1.0 \times 10^6$, and $\sqrt{(2/\pi n)}2^n = 1.8 \times 10^5$. All these states lie within 100 volts of the ground state, making the average between states about 2 millivolts. We are actually more interested in the states of the ion, but the number of states with only one electron less will still be astronomical, especially for larger molecules like the octanes, where $n = 50$. Moreover, overlapping these states will be an equally large number of other states formed from the lower excited states of the separated atoms. The separation between adjacent states is so small that they approximate a continuum.

All these states are accessible to the molecule under electron impact. The selection rules that hold for optical spectra where the wave length is long compared to molecular dimensions do not apply here, as the de Broglie wave length associated with a 50-volt electron is 1.8 Å, of the order of a bond length.

With electronic states so dense and with the dependence of potential energy on nuclear configuration different for the different states, intersection of the potential-energy surfaces in regions accessible to the ion with small amounts of vibrational energy will be very frequent. Radiationless transitions, of the type indicated for diatomic molecules by transitions from state V to IV, Fig. 1 (p. 74), will be sufficiently common to permit rapid shifting of the ion between electronic states. This will

give rise to rapid shifting of the electrons from one bond to another and also rapid interconversion of electronic and vibrational energy. Some of these states will be highly repulsive and cause the immediate dissociation of the ion with no opportunity for "wandering" of the electronic energy and with the production of fragment ions having appreciable kinetic energy. As has been mentioned, the evidence supports the conclusion that relatively few ions are produced with high kinetic energy. We conclude that the majority of the tremendous number of possible electronic states to which transitions are probable are at most "weakly repulsive." That is, although the potential energy surface may have a "pass" through which dissociation can occur with little or no energy of activation, the pass will be narrow and the height of the asymptotic region outside the pass will be at most only slightly lower than the potential surface height corresponding to the normal configuration of the ion. Under such circumstances, the parent ion (and then the fragment ions) will, in general, not dissociate immediately on formation, but will "wander" around through various electronic states until it happens into one where its nuclear configuration and momenta are appropriate for dissociation. The dissociation will then occur without removing appreciably more energy from the system than is essential; then the ion fragment will repeat the process and dissociate further, until its energy is too low to cause further bond breakage.

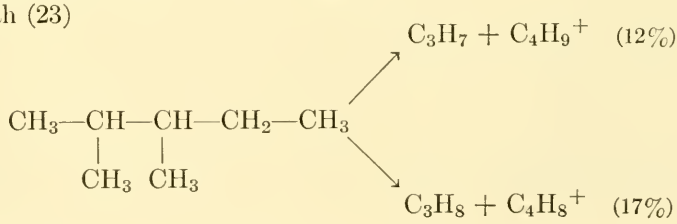
We wish to make still another argument based upon the existence of this large number of states. Transition probabilities from the ground state of the molecule to the states of the ion will no doubt vary over an enormous range. There will be some molecules where the transition probability to one or to a very small number of states will be comparable to the sum of all other transition probabilities. This might arise in a highly unsaturated, conjugated hydrocarbon or in a molecule containing a heavy, easily ionized atom. More generally, however, there will be a large number of states to which the transition probabilities will be large and of the same order of magnitude. For a molecule as light as propane, if this group were to constitute only one-tenth of 1 per cent of the total it would still contain about a thousand states. These states will tend to be concentrated among the lower states of the ion, since transitions to the upper states will, in general, require change of a large number of electron spins. Except for this requirement there is no reason to expect these states with high transition probabilities to be distributed in any other than a uniform manner among all the states of the ion. The probability of transition to states of the ion will tend to cluster about a mean value, the density of states being greatest for energies near this mean and least near the energies corresponding to the most stable and

the most completely antibonding states of the ion. The distribution function which gives the number of molecular ions with a given energy cannot be given as a mathematical expression at this time. A great deal of work has been done (35) on the theory of collisions between electrons, but thus far no satisfactory treatment is available for collisions with electrons bound in a molecule. It is possible to derive a fairly simple expression for the probability of a free electron losing a given amount of energy in a single collision. In the present case, however, an electron must necessarily undergo at least one ionizing and one or more non-ionizing collisions in passing through a given molecule in order to give it enough energy to cause the observed dissociation. This requirement follows from the fact that the experimental conditions are such as to make the collision of two electrons with the same molecules highly improbable.

The spectra of the octane isomers have previously been referred to, and it was pointed out that, where there existed one C—C bond appreciably weaker than the other C—C bonds in the molecule, few fragments larger than the largest formed by breaking this weakest bond were found. The same rule holds for the other saturated hydrocarbons. There is low probability of splitting off a CH_3 from the straight-chain hydrocarbon ion; this probability is greatly increased in molecules with the $(CH_3)_2CH$— group, the factor ranging from 5 (hexanes) to 150 (octanes) and 50 (nonanes) (23), while the factor assuming equal bond-breaking probabilities will be 1.5. We lack the space necessary to point out the many regularities of this sort in detail. The most important rules regarding the initial cracking and subsequent decomposition, consistent with the idea that one initially has a group of high-temperature ions, we believe to be the following:

(*a*) The first bond broken in the parent ion is a C—C bond, and the relative probabilities of breaking the various C—C bonds lie in the same order as the bond strengths in the un-ionized molecules.

(*b*) At times a simultaneous transfer of a hydrogen atom from the ion to the uncharged fragment occurs along with the bond breaking described immediately above, producing an olefinic ion and a neutral, saturated molecule. The probability of this occurrence is usually about ½ to ⅙ that of the process (*a*), but on occasion it is more important than (*a*), as with (23)



(*c*) The ion formed in this initial break will probably have energy enough to break down further. Loss of fragment C_2H_4, C_3H_6, etc., is highly probable, the weakest C—C bond breaking. Also very highly probable is the loss of hydrogens in pairs, a process for which ΔH is only about 1½ volts, although there is certainly an activation energy associated with the process of forming the H_2 molecule.

(*d*) In each breakdown of the ion, there is a chance of the charge going with either fragment, the relative probabilities of obtaining the two sets of products being given by the usual Boltzmann factor involving the difference in ionization energy for the two fragments. If this difference in ionization potential is over a few tenths of a volt, only the more easily ionized fragment will be found. For example, the metastable transition $C_4H_{10}^+ \rightarrow C_2H_4^+ + C_2H_6$ is observed, but the transition $C_4H_{10}^+ \rightarrow C_2H_4 + C_2H_6^+$ is not observed, either metastable or otherwise.

(*e*) A few small peaks that must be due to doubly charged ions are found; a much larger fraction of the doubly charged ions formed surely immediately dissociates to give two singly charged ions. Although the number of ions so produced will be but a small fraction of the total ionization, it probably accounts for much of the relatively constant amount of CH_3 produced from all large hydrocarbons.

It is obvious that the complexity of the situation makes these rules have only the barest qualitative significance and that they are only of limited applicability. The mass spectrum of cyclohexane (23) can reasonably be interpreted in terms of these rules, but the mass spectrum of benzene cannot without special consideration of the effect of the large number of electrons.

To sum up, the essential idea in the foregoing discussion is that the process of ionization of the molecule is accompanied by the simultaneous transfer of excess energy to the other electrons of the molecule, and that instead of an immediate dissociation occurring this energy undergoes a process of rearrangement, being transferred from the electronic to the vibrational states of the molecular ion. As has already been mentioned, we have at present no way of knowing the distribution function for this excess energy. Moreover, we cannot be certain of how much of this energy is transferred to the vibrational states of the molecule. It is likely that at least some of the radiationless transitions are very rapid, but there may, as already mentioned, be some transitions which are much slower than the total lifetime of a molecule in the mass spectrometer. The fact remains, however, that there will be some sort of distribution function for the vibrational energy and that in view of the very large number of electronic states this function will approach a

continuous one. Even without this distribution function in explicit form it is possible to formulate a fairly simple theory of the decomposition of these molecules which, although it oversimplifies the situation, serves to provide a reasonable picture of the processes involved.

As has already been done by Kassel (36), we shall use as a model to represent the molecule a collection of harmonic oscillators coupled by forces which are sufficiently large to allow the energy to pass freely from one to the other but small enough so that the energy of a group of such oscillators may be expressed as a sum of squares of their coordinates and momenta. Also to further simplify the problem we shall assume that the harmonic oscillators all have the same frequency, ν.

We shall let $P(E_i)$ be the probability that after ionization the molecule has acquired, in the manner discussed above, an amount of vibrational energy E_i and shall assume that this energy is randomly distributed among the vibrational degrees of freedom of the molecule. It now becomes necessary to formulate an expression for the specific rate of a particular reaction in which molecules, each with energy E_i, decompose.

A molecule with total vibrational energy E_i will contain

$$n = \frac{E_i}{h\nu} \tag{1}$$

quanta. The molecule, as already mentioned, will be represented by harmonic oscillators corresponding to the vibrational degrees of freedom of the molecule.

Consider a single possible way of arranging these n quanta in the s oscillators such that there are n_1 quanta in the first oscillator, n_2 in the second, and so on up to n_s quanta in the last oscillator. This set of n's obviously must conserve energy, that is,

$$\sum_r n_r = n \tag{2}$$

We shall consider that the slow step in the decomposition process is the transfer of energy to the oscillator corresponding to the reaction coordinate which is to rupture in a particular reaction. In the following formulation we shall consider the reaction to be governed by accumulation of a critical number of quanta, n^*, in a single oscillator. The number of quanta, l_{kj}, which when transferred from the kth to the jth oscillator will cause a break must satisfy the relation $l_{kj} \geq n_j^* - n_j$, where n_j is the number of quanta in the reacting oscillator at the start of the reaction. We now define a transmission coefficient,

$$\gamma(n, n_1, n_2, \cdots n_r, \cdots n_{s-1}, l_{kj})$$

and a frequency, ν, such that

$$\nu\gamma(n, n_1, n_2, \cdots n_{s-1}, l_{kj}) \tag{3}$$

is the rate at which molecules transfer l_{kj} quanta from the kth to the jth oscillator. The total number of ways that n quanta can be distributed among s distinguishable oscillators is

$$\frac{(n + s - 1)!}{n!(s - 1)!}$$

The reciprocal of this quantity will be indicated by the letter C. Thus

$$C \sum_{l_{kj}=n_j^*-n_j}^{n_k} \nu\gamma(n, n_1, n_2, \cdots n_{s-1}, l_{kj}) \tag{4}$$

represents the number of reactions per second due to the transfer of l_{kj} quanta from the kth to the jth oscillator. The specific rate for this distribution can then be written as

$$k_{(n, n_1, n_2, \cdots n_{s-1})j} = C \sum_k \sum_{l_{kj}=n_j^*-n_j}^{n_k} \nu\gamma(n, n_1, n_2, \cdots n_{s-1}, l_{kj}) \tag{5}$$

where $\sum_k$ sums over the $s - 1$ oscillators which can feed energy into the reacting oscillator j. This method of expressing the rate omits the possibility that the l quanta might come from two or more different oscillators into the jth one. Such occurrences will be neglected. For a given n the total rate of decomposition at the jth oscillator will be

$$k_{nj} = C \sum_{n_r} \sum_k \sum_{l_{kj}=n_j^*-n_j}^{n_k} \nu\gamma(n, n_1, n_2, \cdots n_{s-1}, l_{kj}) \tag{6}$$

where $\sum_{n_r}$ is the summation over all sets of n's consistent with the conservation of energy, $\sum_r n_r = n$, and the condition that no oscillator have enough quanta to dissociate them, that is, $n_r < n_r^*$, where n_r^* is the number of quanta required for dissociation at the rth oscillator. We may define an average $\bar{\gamma}$ such that

$$\sum_{n_r'} \gamma(n, n_1, n_2, \cdots n_{s-1}, l_{kj}) = \frac{(n - n_k - n_j + s - 3)!}{(n - n_k - n_j)!(s - 3)!} \bar{\gamma}(n, n_k, n_j, l_{kj}) \tag{7}$$

where the summation includes all ways of distributing n quanta in the molecule such that the j and k degrees of freedom have the fixed values n_k and n_j. The rate then becomes

$$k_{nj} = C \sum_k \sum_{n_k=0}^{n_k^*-1} \sum_{n_j=0}^{n_j^*-1} \sum_{l_{kj}=n_j^*-n_j}^{n_k} \frac{(n-n_k-n_j+s-3)!}{(n-n_k-n_j)!(s-3)!} \nu\bar{\gamma}(n, n_k, n_j, l_{kj}) \tag{8}$$

Actually we should exclude from the terms

$$\frac{(n-n_k-n_j+s-3)!}{(n-n_k-n_j)!(s-3)!}$$

those states which correspond to dissociation, that is, $n_r \geq n_r^*$. However, this would only decrease the expression slightly at the price of great complication.

Expression 8 is apparently quite complicated and in this form would be difficult to evaluate. Actually, however, it is almost certainly true that the sums involved will contain a large number of terms equal to or very near zero. First of all, $\bar{\gamma}$ will be strongly dependent uopn l_{kj}, the number of quanta which are transferred from oscillator k to oscillator j in a single period of the frequency factor. In fact it seems likely that the probability of transferring more than one quantum in time $1/\nu$ may be negligible. This means that all terms are essentially zero unless $l_{kj} = 1$. If this is true then we must have

$$n_j^* - n_j = 1$$

so that only those molecules having $n_j = n_j^* - 1$ can decompose in time $1/\nu$. This reduces the summation over l_{kj} to a single term $l_{kj} = 1$. Since in the summation over n_j the lower limit is $n_j = n_j^* - l_{kj}$, this now becomes $n_j = n_j^* - 1$, and the summation over n_j is thus also reduced to a single term. In the molecule, energy transfer is principally between adjacent bonds; the summation over k can be taken as zero except for three or four terms. The rate then reduces to

$$k_{nj} = C \sum_k \sum_{n_k=0}^{n_k^*-1} \frac{[n-n_k-(n_j^*-1)+s-3]!}{[n-n_k-(n_j^*-1)]!(s-3)!} \nu\bar{\gamma}[n, n_k, (n_j^*-1), 1] \tag{9}$$

For quanta corresponding to $\nu_{osc} = 1000\ cm^{-1}$, a reasonable value for a hydrocarbon molecule, the factorial term will decrease rapidly in size. For example, if $n - n_k - (n_j^* - 1) = 12$ and $s - 3 = 52$ (values which are approximately correct for a molecule like hexane), the factorial term is 3.3×10^{12}; and for the next term, where $n - n_k - (n_j^*) = 11$, its value is 6.2×10^{11}. The ratio of the second term to the first is approximately 0.2. The transmission coefficient $\bar{\gamma}$ will increase as n_k becomes larger. There will therefore be a maximum term in the summation over n_k. Assuming this maximum to be strong allows us to discard the

summation over n_k except for the term where $n_k = n_{k_{\max}}$. The rate then finally becomes

$$k_{nj} = C \sum_k \frac{[n - n_{k_{\max}} - (n_j^* - 1) + s - 3]!}{[n - n_{k_{\max}} - (n_j^* - 1)]!(s-3)!} \nu\bar{\gamma}[n, n_{k_{\max}}, (n_j^* - 1), 1] \tag{10}$$

In order to evaluate k_{nj} it will be necessary to find a value for $\bar{\gamma}$. Assuming $\nu = 10^{13}$ (approximately the vibration frequency of one of the oscillators), one term of k_{nj} would be of the order of 10^9 to 10^{12} if $\bar{\gamma} = 1$. The half life of an ion in the mass spectrometer is of the order of 10^{-6} sec, so that $\bar{\gamma}$ can be no smaller than about 10^{-2} to 10^{-5}. These values for $\bar{\gamma}$ seem reasonable when we consider that it is the probability of transferring energy from an oscillator having lower energy than the one which is to receive the energy.

It is now possible to discuss the general nature of the mass spectrum of a molecule. In a molecular ion with a given amount of vibrational energy a number of different reactions may be possible. Moreover, if the energy is sufficient the products of the initial dissociation of the ionized molecule may undergo one or more successive breaks. The probability that a given molecule will break at two points simultaneously is small. We shall therefore consider that the mass spectrum results from a number of successive decompositions and that the problem of calculating the spectrum can be discussed in terms of a set of competing reactions which have reached a steady state. For a given molecule the fraction f_{nj} of products of decomposition which will be formed by initial break of the parent ion will be

$$f_{nj} = P(E_i) \frac{k_{nj}}{\sum_j k_{nj}} \tag{11}$$

where $\sum_j$ is the summation over all possible single decompositions of the parent ion. (We shall ignore the uncharged fragments here, although their number and energy distribution could also be calculated.) The fraction f_{nj} represents the fractional number of ions of species j resulting from the break of a parent ion with energy $nh\nu = E_i$. The total fraction of all parent ions which yield this species will be

$$f_j = \sum_n f_{nj} = \sum_n P(n) \frac{k_{nj}}{\sum_j k_{nj}} \tag{12}$$

where now $P(n)$ has been written for $P(E_i)$ and the summation extends from the minimum energy necessary to produce species j to the maximum energy of the impacting electrons. The sum over j will, in general, be

different for different ranges of n, since k_{nj} is zero for molecules having $n < n_j^*$. The fraction f_j given by expression 12 does not represent the number of species j ions which will be found in the mass spectrum. For values of n sufficient to break two or more bonds in the molecule the chance exists that the initial break will leave the n_j ion with enough energy to break a second time. The resulting fragment may also decompose further.

In order to simplify the discussion it will be convenient to break the energy distribution into a number of more or less arbitrary ranges. We shall suppose that there is some definite range $E_i = E^{(1)}$ to $E_i = E^{(2)}$ within which the energy is sufficient to break only one bond in the parent ion. A second range $E^{(2)}$ to $E^{(3)}$ is defined such that the products of the first dissociation of the parent ion will have between them enough energy to cause one more dissociation but not enough for two. Higher ranges can be similarly defined. Now the fraction of parent ions which do not dissociate at all is

$$f_p = \sum_{E_i=0}^{E^{(1)}} \frac{P(E_i)}{\sum_{0}^{E_{\max}} P(E_i)} \tag{13}$$

and the fractional amount of a product j resulting from parent ions having energies in the first range is

$$f_j^{(1)-(2)} = \sum_{n=n^{(1)}}^{n^{(2)}} P(n) \frac{k_{nj}}{\sum_j k_{nj}} \tag{14}$$

A similar expression can be written for the j ion produced in the second energy range, but now the situation becomes more complicated. For each j ion produced in this range we must consider the chance that at the time of the initial break enough of the energy not concentrated in the breaking bond will be in the charged product of this break to cause subsequent dissociation of this fragment. That is, if the j ion which is formed by the initial break has q oscillators, then we must calculate the chance that these q oscillators will, at the instant of the first break, contain a number of quanta $n_j^{*\prime}$ or more, where $n_j^{*\prime}$ is the minimum energy necessary for the j ion to decompose to give a fragment j'. This chance is given by

$$A_n = \sum_{n'=n_j^{*\prime}}^{n} \frac{\dfrac{(n - n_j^* - n' + s - q - 2)!}{(n - n_j^* - n')!(s - q - 2)!} \cdot \dfrac{(n' + q - 1)!}{n'!(q - 1)!}}{\dfrac{(n - n_j^* + s - 2)!}{(n - n_j^*)!(s - 2)!}} \tag{15}$$

Such a term as this should multiply each term of $\sum_k$ in k_{nj}, but since $n_{k_{\max}}$ will be only slightly different in each term it will probably be sufficient to write for those j ions which have sufficient energy for a second break

$$f_j^{(2)-(3)}\ (\text{unstable}) = \sum_{n=n^{(2)}}^{n^{(3)}} P(n) \frac{k_{nj}}{\sum_j k_{nj}} a_{k_{\max}} A_n \tag{16}$$

where $a_{k_{\max}}$ is the number of terms in the sum over k in k_{nj}. It follows immediately that the fraction of stable j ions in this energy range is

$$f_j^{(2)-(3)}\ (\text{stable}) = \sum_{n=n^{(2)}}^{n^{(3)}} P(n) \frac{k_{nj}}{\sum_j k_{nj}} a_{k_{\max}} (1 - A_n) \tag{17}$$

We can now calculate the amounts of the various breakdown products of the j ion. To do this we require the energy distribution of the j ions formed in the first break. This distribution is given by the terms in A_n, since each term gives the fractional number of j ions having energy n'. Thus the interesting fact emerges that the distribution function, $P(n)$, once determined, is sufficient to determine also the energy distributions for all secondary and later ions. The expression for the fractional number of j' ions from j ions formed from parent ions in the initial energy range n' to n is

$$f_{j'}^{(2)-(3)} = \sum_{n'=n_j^*}^{n^{(3)}} A_{nn'} \frac{k_{n'j'}}{\sum_{j'} k_{n'j'}} \tag{18}$$

The further calculation of the spectrum is simply a repetition of the calculations indicated before.

In spite of the simple model employed in the discussion it is obvious that, even if accurate data were available for the dissociation energies and if the values of $\bar{\gamma}$ were known, the calculations would be extremely involved. Such calculations would also be expected to yield answers with only the general outlines of the experimental spectrum. A better approximation would require taking two or three different frequencies for the oscillators, but this would even further complicate the formulation. It appears that numerical calculation must await further refinement of the theory.

It remains to consider how much decomposition will occur in a molecule in solution when struck by radiation. The well-known formula

$$T = \frac{Q}{8\rho c(\pi\kappa t)^{3/2}} e^{-r^2/4\kappa t} \tag{19}$$

gives the temperature t at a distance r from the point of absorption of the energy after a lapse of time t. Here κ, the diffusivity, is the thermal conductivity, k, divided by the heat capacity per cubic centimeter of substance. ρ and c are the density and the heat capacity per gram, respectively. For a hydrocarbon such as pentane $k = 0.00032$ while $\rho c \approx 1$. For water $k = 0.00143$ and $\rho c \approx 1$. Thus the rate at which temperature drops off is approximately the same for different substances. If we substitute the constants for water into expression 19, it becomes immediately obvious that for periods of 10^{-12} sec or greater and for molecular distances of $r = 3.3 \times 10^{-8}$ or less the exponential factor is unity. If it be further assumed that 100,000 cal per mole is delivered by the radiation, one finds that after 10^{-12} sec the temperature of the struck molecule is 6900° C above its original temperature. After 10^{-10} sec it has dropped to 7° above the original temperature. A C—C bond oscillates about 3×10^{13} times per second, while a CH bond oscillates about 9×10^{13} times per second. Thus, in the very unusual circumstances that enough energy is delivered to a bond to break it, dissociation will ensue. The earlier considerations of the mass spectrographic data indicate that 10^{-9} sec is a more probable half life for molecules. These processes with half lives of 10^{-9} sec are completely quenched in the liquid state. Even if the molecule should decompose, it still must escape the Franck-Rabinowitch cage before recombination or there will be no reaction. Thus the viscosities of pure liquids are given with some accuracy by the equation

$$\eta = \frac{Nh}{V} e^{\Delta H_{\text{vap}}/2.5RT} = \frac{RT}{Vk'}$$

where η, N, h, V, ΔH_{vap}, R, T, and k' are the viscosity, Avogadro's number, Planck's constant, molal volume, heat of vaporization, gas constant, temperature, and rate of jumping out of the cage, respectively. The constant 2.5 in this equation is the ratio of the heat of vaporization of the liquid to the free energy of activation of the flow process (30). Remembering that pure liquids have a viscosity at these melting points of about 0.02 poise, one finds that a molecule has a half life inside the Franck-Rabinowitch cage of about 2×10^{-12} sec at room temperature. Hot molecules or molecules dissociating with large kinetic energies can escape from the cage even more quickly. Thus the rapid chilling of molecules has much more to do with preventing decomposition than does the entrapment in a cage of their neighbors.

The main source of destruction of large molecules by direct hits will be by the ejection of an electron and the reaction of the positive ion

with the solvent. Much more frequently decomposition of the enzymes will come from reaction with the H, OH, and HO_2 formed from water and oxygen, as is discussed elsewhere in this volume.

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DISCUSSION

BURTON:

It is important to emphasize the tremendous gap which separates such fundamental studies of isolated molecules as that presented by Eyring, and an understanding of reactions in condensed phases, which include biological material. In attempting to bridge this gap, if only crudely, several questions are significant. What is the quantitative role of the cage effect? A molecule may be in one particular cage for less than 10^{-10} sec after excitation, but the life of that cage in such a case is not of immediate importance. For practical purposes, the molecule is caged until it is deactivated or until it decomposes. If the latter process occurs, the life of the cage thereafter is of great importance. If the radicals can recombine, or otherwise interact, during a time approximating the life of the cage, we have a cage effect. Otherwise, there is no cage effect and the reaction with the solvent dominates. The life of the cage evidently depends not only on the statistical behavior of the radical products and the surrounding molecules but also, as Eyring notes, on the energy with which the radicals are formed. How fast does internal conversion of energy occur as compared with external loss (for example, collisional deactivation)? Can a chemical reaction involving an excited molecule occur? Does internal conversion of energy occur fast enough so that a particular degree of freedom acquires sufficient energy for rupture, or does the molecule, as it were, cool off before this happens? One must consider both the rate at which heat is lost externally and the probability of escape of the radicals from their cage before recombination. In the theory of the radiation chemistry of solutions such factors must be considered in addition to those discussed by Eyring. In the case of a water molecule, caging of the decomposition products need not be considered, since the hydroxyl radical and ionic hydrogen primarily formed do not back-react.

EYRING:

Are there examples of reactions studied in both the gaseous and liquid phases of water? Are the products of reactions in these two phases much the same?

PLATZMAN (Communicated):

There are no examples of aqueous reactions so studied. Radiolysis of water vapor has not yet been investigated, and interest in aqueous solutions has focussed on non-volatile solutes.

LIND:

Eyring will undoubtedly recall the classical experiments of Schoepfle and Fellows in which liquid hydrocarbons were irradiated with high-speed electrons. These experiments showed that the greater the branching of the hydrocarbon,

the more methane was obtained. Since Eyring states the opposite to be true in the gaseous state, I believe we have here an instance of the distinction that Burton has discussed.

EYRING:

We are unable to explain this difference. Certain differences in reaction in the liquid and gaseous states are due to the cage effect and to the fact that the energy has not time to migrate when the irradiation is carried out in the gaseous phase. In the gas the molecule may fly apart when it is hit by the bombarding particle, whereas in the liquid state there is time for some of the energy to be degraded into heat.

BURTON:

The products of irradiation of both liquid and gaseous organic compounds are characterized by their complexity, but there is some indication, as Eyring suggests, of a smaller diversity of products in the liquid phase.

ALLEN:

Previous talks might give the impression that radiation chemistry is such a hopelessly complicated subject as to be of little use to radiobiologists. However, experiments in radiation chemistry frequently give reaction rate laws which appear to be reasonably simple and rational. In solution, especially, the observed phenomena can be correlated in a sensible fashion and certain valid predictions made. As in other fields of chemistry, a good deal can be understood about reactions without attempting to ascertain the complete details of the molecular dynamics of each reaction. In radiation chemistry, the attempt to discuss in full detail the nature of all the types of activation, though of interest to radiation chemists, may well tend to give other people too pessimistic an impression of the values and possibilities of this field of study.

BURTON (Communicated):

The techniques, disciplines, and speculations of radiation chemistry are similar to those of other branches of chemistry. Efforts toward detailed understanding are common to all branches of science, and the existence and relation of such efforts should prove a source of encouragement to those not actively engaged in the field. Such efforts are in the direction of simplification and unification. Detailed understanding and well-developed theory limit the number of facts which must be remembered and indeed make a subject more attractive to the uninitiate.

PLATZMAN:

If chemical reactions—thermal, photochemical, or radiation-chemical—seem to be simpler in the liquid than in the gaseous phase (a debatable impression), it is probably because we know so much less about them that we have inadequate empirical or theoretical information about their complexities.

HART:

The complexity of reactions in aqueous solution is illustrated by the work of Gordon and myself at the Argonne National Laboratory. We have irradiated acid solutions containing dissolved deuterium gas and find that deuterium is converted first to HD and then to H_2. In other words, there is a gradual replacement of the deuterium gas by hydrogen gas. On the other hand, at *p*H 12 there appears to be direct conversion of the deuterium to hydrogen without the formation of HD as an intermediate. This has been interpreted as indicating a reaction between the OH radical and D_2 to yield HOD and D; the free D atom then reacts with an OH^- ion to form HOD^-. The latter ion then exchanges with the hydrogen in H_2O, forming HOH^-, and this decomposes to give free hydrogen atoms which combine to produce molecular hydrogen. In the basic solution at *p*H 12 there is less than 10 per cent as much HD as is obtained in the acidic solution.

SOLOMON:

We have been using a mass spectrograph in the determination of the abundance of deuterium in biological samples, and read the $\frac{3}{2}$ peak ratio with an electron beam at 75 volts. Because of the formation of H_3, it is necessary to extrapolate the readings to zero pressure, as the H_3 is pressure dependent. However, the slope of the curve of pressure dependency does not appear to vary in a discoverable way with the pressure, with the electron density, or with any other known variable. In other words the H_3-ion formation does not seem to be proportional to any known factor in the mass spectrograph. Is there an explanation for this?

EYRING:

No information is available, as far as I know.

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On the Primary Processes in Radiation Chemistry and Biology

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Introduction

The first stage in the interpretation of chemical or biological changes which result from the exposure of any material to radiation of high energy must be the elucidation of the primary physical effects of the radiation.

Radiation chemistry and radiobiology bear a close relationship to photochemistry and "photobiology." In both, radiation from an external source (less commonly, from an internal one) is allowed to impinge upon an aggregate of molecules in stable, or almost stable, states, thereby raising some of the molecules to excited states and permitting chemical interactions which could not proceed between normal molecules. The incident energy functions as activation energy, or as requisite energy for endothermal processes, or both, and is ultimately partitioned between degraded energy (heat), altered chemical (potential) energy of the material, and occasionally also emitted radiation.

The *primary processes* of "high-energy radiation" effects, however, differ from those of photoeffects in their tremendously greater complexity. This distinction is incisive and affects even the simplest reactions induced by high-energy radiations. Its origin lies in the fact that each particle of high-energy radiation has energy of from thousands to many millions of electron volts (ev)*—magnitudes very much greater than the energies of the more probable transitions of a molecule, which are usually less than 15 ev. On the other hand, the energy units absorbed in

* This discussion is for the most part restricted to radiations of energies not greatly in excess of those of common nuclear radiations, for which (with the exception of neutrons) *specific* nuclear interactions are so rare as to have negligible influence on chemical or biological effects. The problems are therefore ones of atomic and molecular physics and of chemistry, and not at all of nuclear physics.

photochemical reactions, commonly those of photons of visible or ultraviolet light and therefore in the region of 2–10 ev, lie just in the range of the most likely transitions of molecular electronic systems. Whereas in photoeffects the quanta are absorbed in single events, in high-energy radiation effects the energy loss occurs gradually, in hundreds or many thousands of steps. Hence use of monochromatic light for a photochemical reaction ensures, in general, the excitation of a unique excited state, but monoenergetic high-energy radiation—for example, homogeneous alpha or beta rays—always produces a very great number of different types of excited and ionized molecules of quite widely varying energy.

Another contribution to the complexity displayed by the primary processes of radiation chemistry and biology arises from the fact that many, and usually the majority, of the products are formed, not directly by the incident radiation, but indirectly by secondary, tertiary, etc., radiations which the primary radiation produces. It would evidently be extremely difficult to gain full knowledge of the products of absorption of even the simplest high-energy radiation.

The nature of the primary processes has already been discussed by the first panel of this symposium. These primary processes are almost all separate excitation or ionization events in isolated atoms or molecules of the medium. The processes can be described in a general way, and such a description is invaluable background for understanding chemical and biological effects of radiation. We may be permitted to emphasize, however, that quantitative prediction of the physical effects is within the realm of possibility, at the present time, only for gaseous media composed of *monatomic* molecules, because a necessary basis for comprehensive treatment of the physics of a radiation process is the knowledge (that is, of constitution, energy, stability, and other specifications) of the possible stationary states of the system. Such information on stationary states, obtained principally from spectroscopic investigation, is available in detail only for *atoms:* excited and ionized states of diatomic molecules are far less extensively known, owing to the overwhelmingly greater difficulty in interpreting the spectra; in the case of polyatomic molecules our knowledge is hardly more than in its infancy, and great progress in the near future is not to be anticipated. Some knowledge of ionized states, but not of excited states, is obtainable from mass spectrographic studies for both atoms and molecules; however, it is of highly restricted content. It would be a sophistry to deny that contemporary radiation physics, although it provides most of the information ordinarily required by the *physicist,* customarily disregards consequences of chemical binding, and is scarcely more than a general guide to the understanding of the details of the primary processes in chemical and

especially in biological systems, the latter being invariably composed of molecular species of more or less great complexity.

Much the same difficulty, indeed, also underlies the interpretation of the effects of light on all but the simplest gaseous systems, and this fact has inhibited serious study of such effects in complicated systems, particularly those in the liquid state. For this reason trustworthy information available from photochemistry is, for complex systems, regrettably meager. Hence the important task of interpreting results of radiation effects—that is, of analyzing the extremely intricate stages between initial absorption acts and ultimate chemical or biological transformation—must usually proceed with inadequate aid (from physics, on the nature and distribution of the primary products, and from chemistry, on their interactions), and is usually exceedingly difficult. In all too many instances in which interpretations have been advanced they involve suspicious radicals or ions, endowed with mainly *ad hoc* properties, which effect remarkably convenient actions under almost no disciplinary control except perhaps an occasional admonition from the laws of thermodynamics.

Despite this discouraging basis, some success has been achieved in the understanding of at least a few of the chemical effects of radiation, and notable progress in the field is now being made. It is even possible to understand some of the less intricate reactions in complex systems—at least semiquantitatively, if not in the same satisfying detail achieved by Eyring, Hirschfelder, and Taylor in their uniquely important studies of several gaseous systems some fourteen years ago. Indeed, some striking inferences may often be drawn from an analysis which penetrates no more deeply into the nature of the primary effects than simply to distinguish between excitations and ionizations. These matters are discussed elsewhere in this volume. We may merely note here the familiar conclusion that the experimental evidence thus far interpreted appears to support the identification of excitation and ionization acts with the predominating primary processes.

Primary Processes Other Than Simple Excitation and Ionization

Nevertheless, it would be incautious at the present time to presume that chemical and biological effects of high-energy radiation are caused *exclusively* by isolated excitation or ionization events in which energy transfers lie in the neighborhood of 10 ev. Whereas these primary processes are surely the ordinarily dominant ones for media composed of simple molecules, complex molecules of high molecular weight may well

be found to differ in important respects. Especially if a complex molecule should be exceptionally resistant to the effects of a small energy transfer, or should have ability to recover from such effects, must allowance for other possibilities be made! (That such behavior may not be uncommon is suggested by both empirical evidence and theory.) Great energy transfer to such a molecule arises, according to the point of view commonly held, only from the accumulated effect of a number of small energy transfers to individual atoms of the molecule considered as isolated entities. The probability of great energy transfer would thus be intimately related to the *specific ionization* at the appropriate point along the path of the incident particle. In the case of densely ionizing particles, such as alpha particles and especially heavier positive ions like recoil atoms or fission fragments, rather great energy transfer to a single large molecule may be achieved, and under favorable circumstances much greater permanent changes than would result from equivalent dosages of less densely ionizing radiations can be anticipated. Of course, the effects of specific ionization or, expressed somewhat more significantly, of the *spatial* distribution of excited and ionized atoms and molecules at the instant after the incident radiation has penetrated the medium, and of the fluctuations in this distribution, are well appreciated and are an important factor in the interpretation of ultimate chemical and biological effects of radiations, although they have not yet been analyzed in any quantitative detail.

However, the fact that the individual component of incident radiation has energy that is orders of magnitude greater than the average energy transfer per excitation or ionization act should always be borne in mind. Relatively great energy transfer from a single particle to a single molecule is certainly infrequent, but it is not impossible. The smallness of the average energy transfer results from the particular nature of the chief interaction between incident particle and molecule, and other types of interaction, usually much less probable, could conceivably lead to energy transfers of different magnitude. [One should not regard as being in this category the great *primary* energy transfers which certainly occur when a very fast secondary electron (delta ray) is produced by a charged particle, or a photon of bremsstrahlung is radiated by a fast electron, for this great quantity of energy is subsequently dissipated in a number of secondary events to many molecules, the average energy transfer per molecule still being in the 10-ev region.] It therefore seems not without importance to examine possibilities of energy transfer other than simple excitation and ionization, and several such are considered below. The discussion is deliberately rather general, but is supplemented by calculations for an illustrative case. The processes all have rather low

probability compared to simple ionization, and thus would seem to offer promise of importance (in the absence of chain reactions in intermediate stages) principally in the case of reactions of low yield per "ion pair" or low "target area." Of the processes one, especially, affords a mechanism of great energy transfer to a small volume—perhaps a single molecule—and appears to have quite appreciable probability in many instances.

Nuclear Collisions

Some fraction of the energy of *any* high-energy radiation penetrating a medium is lost in direct momentum transfers from charged particles to atoms as whole units. The primary process here is customarily called a *nuclear collision*, because the effective interaction is that between the (screened) Coulomb fields of the particle and the nucleus of the atom. An atom experiencing an impact of this type is often ejected from its original position in the medium, and will then usually come to rest elsewhere. If the struck atom acquires sufficiently great kinetic energy, some of this may be lost in excitation and ionization; the remainder, and practically the entire energy of more lightly ejected atoms, is expended in further nuclear collisions. Much of the energy that is transferred by this mechanism goes directly or indirectly into excitation of molecular vibrations and is for the most part ultimately dissipated into heat; some of the original energy loss, however, is preserved as augmented potential energy of the medium, deriving from the altered atomic arrangement. The nuclear collision is known to be the effective process in the observed disordering of the structure of a solid substance by heavy charged particles (the latter being either the primary radiation or secondary particles projected under neutron irradiation). Indeed, this effect is apparently not brought about, at least with appreciable yield, by simple ionization events.

For most of the range of an energetic charged particle this mode of energy loss contributes a very minor fraction—for protons or alpha particles in media composed of light atoms roughly 0.05–0.10 per cent—of the total. For very slow heavy particles, and therefore for initially energetic particles near the end of their ranges, it is the predominant process, but the energy of these particles is so low (of the order of 1 kev for alpha particles) that the total energy transferred to nuclear motion is small. Thus, to cite an example, rough estimate shows that a 10-Mev alpha particle absorbed in air will lose 8 kev in nuclear collisions, of which about 1 kev will be lost at the very end of the range. In light media about two-thirds of the nuclear-collision energy loss will be in collisions violent enough to remove the struck atom from its mole-

cule, and for, say, a 10-Mev alpha particle the total number of ejected atoms (including ejections by the ejected atoms themselves) will be of the order of 100. For high-energy particles the number of ejected atoms is invariably very much smaller than the total number of excited and ionized atoms (as in the example cited, where the ratio is of the order of 10^{-4}), and since the latter entities are usually chemically effective, nuclear collisions are usually unimportant and are properly neglected in considering the primary processes. Whether cases exist in which electronic energy transfer is so ineffectual in causing chemical or biological change that the nuclear collisions are of consequence is not known. It is certainly not inconceivable that in some instances energy transfer to nuclei might play a decisive role in effecting changes in very large, stable molecules, for the ejection of an atom, particularly if this in turn ejects other atoms from the same molecule, will severely and probably permanently damage the molecular structure. It is evidently much less likely, in general, that a molecule will recover from loss of an atom than from loss of an electron.

Fast electrons also may lose energy in nuclear collisions, but even if they have very great energy (their mass then significantly exceeding the small rest mass) the number of collisions sufficiently violent to eject atoms from molecules is extremely small—much smaller, relative to the number of ionizations produced, than for energetic heavy particles. The nuclear-collision mechanism is therefore certainly negligible for electron or gamma-ray irradiation.

For heavy *ions*, such as fission fragments, the energy loss in nuclear collisions is relatively greater than for bare nuclei like protons or alpha particles, because, compared to the ionic charge, the nuclear charge of the particle is higher and the internuclear Coulomb interaction therefore of more consequence. This suggests that for these radiations the process might be more likely to achieve importance in determining chemical and biological transformations. The same conclusion applies for slowly moving recoil ions—for example, some of the recoils from fast neutron collisions—and the effect would seem to merit study, for instance in evaluating dosages for these recoil ions.

In order to provide quantitative illustration of some of the matters discussed above, Figs. 1–3 (pp. 111–112) present information on the nuclear collisions of protons in water, which was chosen for convenience in computation and also because it is a representative medium (in this respect) for radiobiology. The figures, and the calculations upon which they are based, are explained in the appendix below. Similar calculations could be made readily for any medium the atomic composition of which is known.

Multiple Ionization

A primary process not often considered in radiation chemistry and biology is the formation by the incident particle, in a single collision, of a doubly (or, in general, multiply) excited or ionized atom or molecule. Such a process, it will be shown, is probably not of importance in most instances. Nevertheless, it certainly ought not to be completely ignored, for although undoubtedly infrequent it could in some special cases conceivably have much greater permanent effect than a number of isolated single excitations or ionizations. (The latter are usually chemically effective for simple molecules, but, as mentioned above, are perhaps less commonly so for complex molecules.)

Multiple processes produced by consecutive impacts of two different particles of the radiation are, of course, utterly negligible in all cases.

Multiple excitation or ionization in a single event finds perhaps its most conspicuous physical manifestation in the excitation of non-diagram, or satellite, x-ray emission lines, some of which owe their origin to multiple ionization of inner shells of the target atoms by fast electrons.

Unfortunately, contemporary theory does not offer much possibility of accurately calculating the yield for this type of process—for example, the relative probability that a charged particle will in a single collision produce a doubly or singly charged ion—except for the simplest of atomic systems. This is because the approximation that one is ordinarily obliged, for reasons of tractability, to use for the possible stationary states of the affected atom (the so-called single configuration, central-field approximation) is such that multiple excitation and ionization events automatically have zero probability, that is, are inherently neglected. The use of a more realistic atomic model imposes the greatest calculational difficulties, even for single processes; and, moreover, superior models are not generally available for substances of chemical or biological interest. The only theoretical treatment of a multiple collision process thus far accomplished is one for the excitation of some of the x-ray satellite lines mentioned above.

It should be permissible, however, to disregard multiple excitation, since this process seems less likely to play a significant role than does multiple ionization. (By multiple excitation is normally meant the *simultaneous* excitation, by a single passing charged particle, of several electrons in a single atom or in atoms closely coupled together in a molecule. Excitation by a single particle of two or more widely separated atoms in a molecule will more often be important. It can be treated by theory as a special case in the consideration of the spatial distribution of the energy loss. Since the collision time for a fast particle and a not

too large molecule is of the order of 10^{-15} sec, while roughly 10^{-13} sec is required for significant internal reorganization of atoms in a molecule, such multiple excitations are also effectively "simultaneous." This distinction, although not always well defined, is a useful one. Either type of multiple excitation could be important if more energy than is provided by a single excitation is required to disrupt the molecule.)

There does exist some empirical information on multiple ionization by impacts of electrons of low and intermediate speeds, almost all of it for monatomic gases. (Slowly moving charged particles are known, on general grounds, to be much more effective than rapidly moving particles in producing multiple processes, the yield of the latter being weighed relative to that of single processes.) The results on the noble gases, which are the simplest to interpret, indicate a yield for the production of an $(n + 1)$ positively charged ion roughly one-tenth that of the corresponding $+n$ ion for electron energies from several times the ionization potentials up to 500 ev, the upper limit of the experiments. (For helium the relative yield of He^{++} to He^{+} is much smaller—of the order of 10^{-3}.) The yields of multiply charged ions are, however, very much lower at smaller energies (and are, of course, zero below the respective ionization potentials), and this is the region in which most of the secondary electrons ejected by high-energy particles fall. For the few other gases investigated, the relative yields, although not exactly similar, are at least of the same order of magnitude. Thus the over-all yield of initially doubly charged ions produced by high-energy radiation would be expected to be very small—less than 10^{-3} and perhaps less than 10^{-4} of all ions. The contribution of the *primary* ionization should not alter this conclusion, although experimental information on the question is unsatisfactory and is in fact entirely lacking for beta or gamma radiation. Experiments performed some 30 years ago failed to detect multiple ions, either primary or secondary, in yields greater than about 1 per cent, produced by alpha particles in a variety of gases. (Some results indicating 5–15 per cent yield of He^{++} relative to He^{+} in helium gas, by alpha particles at various speeds, attracted much attention at the time. They have been interpreted as proving that in helium half of the primary ionization acts near the maximum of the Bragg curve create double ions. If true these results are remarkable and merit further study. They are not relevant in the present considerations, however, and the evidence on other gases is clear.)

Virtually nothing is known about multiple processes in molecular systems of chemical or biological importance. A conservative estimate suggests 10^{-3} as an upper limit for the relative yield of doubly ionized atoms or small molecules produced directly in single events by most

varieties of radiation. For radiations of high specific ionization the relative yield will be greater, but it would be difficult to treat this situation even semiquantitatively.

That multiple ionization must be extremely effective chemically can be concluded from the fact that even doubly ionized molecules tend to be highly unstable; they are, for example, observed only rarely in mass spectrometric studies. The basis for this instability is clear: even if the doubly ionized molecule should be formed in a stable state, the potential surface for this state will in general cross that of the repulsive state formed from two singly ionized radicals. The latter state always lies lower than the former at great nuclear separation because more energy is required to doubly ionize one atom than to singly ionize two. This instability can be pictured very crudely as resulting from the capture by a doubly ionized atom of an electron from an adjacent neutral atom in the molecule, the two singly ionized atoms then dissociating the molecule by their repulsion. (Recall that two electronic charges separated by a distance of 1 Å, the C—H bond distance, repel each other with an energy of 14 ev.) The problems posed by multiple ionization of complex molecules are obviously much too elaborate to permit their discussion here. (For the lines along which an analysis should proceed, cf. the contributions to this volume by Eyring *et al.* and by Livingston.)

We note finally that multiple ionization is to be expected for all high-energy radiation and for all media except hydrogen and helium as the result of Auger processes following ionization in inner electron shells. The yield relative to single ionization events, for biological media, should be of the order of 10^{-2} to 10^{-4}, and will usually exceed that of direct multiple ionization. These effects and their consequences are discussed in detail below.

Capture and Loss of Electrons by Positive Ions

Another mode of energy loss, possible only for a positively charged particle, is the capture of an electron from a molecule of the medium into a discrete orbit about the particle and its subsequent loss in a later collision. This pair of events occurs a few thousand times, for example, in the absorption of a single alpha particle. It is important, for light ions, only when they are slow—and thus for an energetic particle only near the end of its range. Indeed, it is a principal mode of energy loss for an alpha particle of energy between (roughly) 1 and 500 kev. The net effect of such a pair of events is simply the ionization of a single molecule of the medium, the positive ion and electron being formed, however, at a distance from each other. This should be an unimportant dis-

tinction, which fact—together with the small total energy loss by this mechanism—will make the process unimportant in the interpretation of most chemical and biological effects.

A possible exception to the last conclusion might arise in the case of irradiation with rather slow neutrons, for which the recoil ions will often have velocities in the capture-loss region. Nevertheless even here the *spatial distribution* of ions will not be very abnormal, because just for those particle velocities for which capture and loss predominates, the cross sections for the two processes approximate geometrical cross sections, so that positive ion and electron originate at *almost* the same place. The *total ionization* in this velocity region, however, may differ considerably from that anticipated on the basis of extrapolation of knowledge for high velocities (the partition of the total energy loss between excitation and ionization may be quite different at low from that at high energies, and nuclear collisions have an important effect). There is as yet very little information on this question, and the possibility of peculiar effects should not be discounted.

Auger Disruptions

Although most of the energy lost by high-energy radiation is transferred to valence electrons of molecules of the medium, a not inappreciable portion is absorbed by inner electrons of the atoms. The remarkable effects to be anticipated for this part of the energy loss apparently have not been pointed out before.

The media of importance in radiobiology, and in much of radiation chemistry as well, are composed almost entirely of two types of atoms: hydrogen, having only a valence electron, and carbon, nitrogen, and oxygen, which have inner (K) electrons in addition to the outer ones. (Presence in the medium of small amounts of heavier atoms will not affect any of the conclusions to be drawn.) An important and representative medium is water, and, as an example, Fig. 2 illustrates the total fraction of the energy of penetrating protons which is transferred in primary collisions to the K electrons of oxygen atoms when the protons are completely absorbed in water. (Figure 1 shows the manner in which this portion of the energy loss is distributed along the range of the protons.) The fraction, although small, is by no means negligible. In the case cited, for example, it is 4 per cent at 3 Mev and increases with the proton energy. Transfer of energy to a K electron almost invariably ejects that electron from its atom, an ion with an inner vacancy thus being formed. The average energy transferred in such processes is several times the K ionization energy (which is 531 ev for oxygen)

and varies somewhat with the velocity of the particle. Ionization of K levels by secondary electrons is entirely negligible. The ejection of inner electrons by swift charged particles—both electrons and heavy particles—has been verified experimentally in the observation of characteristic x-rays emitted by atoms during irradiation with these particles.

Where a K shell of a C, N, or O atom has been ionized, an energy transfer of some hundreds of electron volts to the molecule containing the atom has occurred. (The exact values are 284 ev for C, 400 ev for N, and 531 ev for O.) If the molecule is moderately or very large, it will retain all this energy! Creation of a K-shell vacancy in these very light atoms is *not* followed by emission of an x-ray photon, as is the case for a heavy atom. There ensues instead a radiationless transition in which an L electron drops into the vacancy and a second L electron is ejected from the atom. Thus a doubly ionized atom results. (Before emitting a photon of K radiation, an atom persists in its excited state for a period of the order of $10^{-8}Z^{-4}$ sec, where Z is its atomic number. For very light atoms this is so much longer than the time required for radiationless transition that the latter almost always occurs first. The yield of radiation from C, N, or O is certainly smaller than 1 per cent.) Such a process, commonly called an Auger transition, takes place within a time interval of about 10^{-15} sec. It is so much faster than any possible motions of atomic nuclei in the molecule that, as follows from the Franck-Condon principle, the nuclei cannot respond appreciably until after the second electron has left. Whether the ejected electron must be one belonging to the same atom that suffered K ionization, or may originate in an atom bonded to it, is not known, and the question, moreover, is not free from ambiguity. In any case the distinction is not very important, for the molecular ion will have ample time for electronic readjustments before dissociation proceeds. It is also worthy of mention that in some instances not only one but several Auger transitions might follow a single K ionization, for very much more than sufficient energy is available to ionize several valence electrons at or near the site of the original vacancy.

The great quantity of energy transferred to a single atom when one of its inner electrons is ejected will not remain in that atom, of course—except for the minor portion retained by virtue of the ionization (and perhaps also excitation) of the valence shell. Where the atom is bound in a large molecule, however, the energy carried away by the Auger electron(s) will not escape, for such electrons dissipate their energy in excitations and ionizations within a distance of less than about 10 Å. (Indeed, it may be proper to raise the question whether, in the case of polyatomic molecules, it is valid to consider the Auger transition and

the reabsorption of the Auger electron(s) as distinct events: these processes may in fact be coupled together to some extent, at least for that portion of the energy reabsorbed by an adjacent atom. In this connection it is of interest to note that an Auger electron of 100-ev energy has a speed of about 10^9 cm per sec, and therefore is "reabsorbed" by the molecule in a time of the order of 10^{-15} sec or less. This is very much shorter than the time required for molecular rearrangement. Regrettably little is known about Auger transitions in very light atoms, experimental investigation being hampered by extreme difficulties of a practical nature. The structural changes consequent to Auger effects in light atoms bound in molecules can, however, be investigated more readily. Such studies have only recently been initiated. They should ultimately provide much information of interest.)

Although the exact series of mechanisms cannot be mapped with certainty, there can be no doubt that, wherever a K ionization occurs, a relatively great amount of energy is communicated to a small region of space—perhaps a single molecule or a portion of a very large molecule. The "primary process" embraces the effects of the initially ejected K electron, of the Auger electron(s), and of the multiple ionization, all centering at the atom originally affected. This energy, transferred in a single primary encounter, will soon be converted to molecular *potential energy* by electronic "rearrangements" (including internal conversion) and will then usually shatter the molecule by a complex polyatomic dissociation, the latter occurring rather slowly, that is, not as a direct primary effect. (Indeed, the process could result in the disordering of a solid—even by electron bombardment, for which the amount of dislocation by direct momentum transfer is very small.) The concentration of absorbed energy will in fact exceed that arising *on the average* from direct energy transfer to electrons in the penetration of the medium by a beta or gamma ray, and may even exceed that sustained in penetration by radiation of such high specific ionization as an alpha particle. Its possible importance for effects not induced by small energy transfer is therefore impressive. Such effects are customarily ascribed exclusively to delta rays, which transfer relatively much energy to a small volume. The relative importance of the two mechanisms cannot be elucidated without a much more detailed analysis; however, an approach to this analysis could readily be based on a simplified model, and such a study would doubtless prove most interesting. It is evident that the relative contribution differs for different types of irradiation and different media. The mechanisms are intrinsically distinct from the physical view: in one case energy is transferred only as direct momentum transfer to an electron; in the other, it is transferred to the electronic system of a single

atom and is subsequently communicated to the neighborhood of the atom.

The connotation for interpretations in radiation chemistry and biology is obvious. Many effects are known which have yields, per "total" number of ionization acts, of magnitude 10^{-2} or less. The possible role of "Auger disruptions" should merit earnest consideration in some of these cases. Effective dosages, in any instance where this mechanism is operative—if any such be found—would be illusory if computed on the basis of *total* ionization. There the number of K ionizations of C, N, or O would be the relevant measure.

It is possible to calculate this number purely from theory. Figure 3 presents the results of calculations, for protons penetrating water, which, it is hoped, may be useful for comparison with practical cases for which this mechanism may be considered.

For non-aqueous media studied in radiation chemistry more elaborate calculations would have to be made, account being taken in the case of atoms of intermediate or high atomic number of electron ejection and subsequent Auger effects in other inner shells as well. With increasing atomic number the yield of ejections from any given inner shell decreases, the fraction of ejections which lead to Auger transitions also decreases, but the energy transfer per ejection increases; indeed, a cascade of successive Auger effects should occur, leading in some instances to an atom or molecule with *many* electrons missing. Because of the importance, for very swift charged particles penetrating atoms of intermediate or high atomic number, of energy transfer to electronic shells other than the valence shell, Auger processes might well have conspicuous effects in media in which such atoms preponderate.

For biological media the yield of Auger disruptions is greater than is suggested by the values given for water, for two reasons. First, the effect increases, other factors being the same, with the ratio of the total number of K electrons in C, N, and O to the total number of electrons other than K electrons in atoms of the medium. This ratio is greater for biological media than for water: for water it is 1:4; for carbohydrates and for glycine it is 1:3; for dry virus it is about 1:2.9. Second, and more important, the probability for ejection of a K electron from an atom of atomic number Z by a charged particle increases *rapidly* as Z decreases: for high particle energies this increase is as Z^{-2}; for very low (heavy) particle energies it is as Z^{-12}. The total number of Auger disruptions for any medium can be computed approximately from theory if the atomic composition of the medium is known. It is notable, and merits emphasis, that the effect for the K shell is relatively great (the ratio of number of Auger disruptions to total number of ionization acts

being, in favorable cases, as high as 10^{-2} to 10^{-3}) just for atomic numbers in the region near C, N, and O: for larger Z the probability of K excitation is very small, and also the probability of x-ray emission competes more favorably with the Auger process.

Summary

The primary processes in the absorption of high-energy radiations by matter are considered in relation to the chemical and biological effects of the radiations. The importance of achieving a detailed understanding of these processes is discussed, and the reasons for the extreme complexity of the problem analyzed. Although simple isolated excitation and ionization events are the preponderant primary process, the possibility of greater chemical effectiveness, especially in complex molecules, of *rarer* events—particularly those involving greater-than-average energy transfer—suggests examination of less probable primary processes. Several relatively infrequent processes are, therefore, investigated. Of these, the nuclear collision may well be of chemical consequence in some cases; direct multiple excitation or ionization is probably unimportant; capture-and-loss of an electron is certainly unimportant as a distinct process, although it may well influence the partition of the energy loss between excitation and ionization; ejection by a swiftly moving charged particle of an inner atomic electron, followed by Auger "disruption" of the molecule, is a process, in effect one involving a great energy transfer, which is distinct from great energy transfer to a secondary electron, and, although no specific application is offered, it is concluded that this process might in some instances play a significant role. The results of detailed calculations of the extent of some of these processes in a typical case of interest (protons penetrating water) are presented.

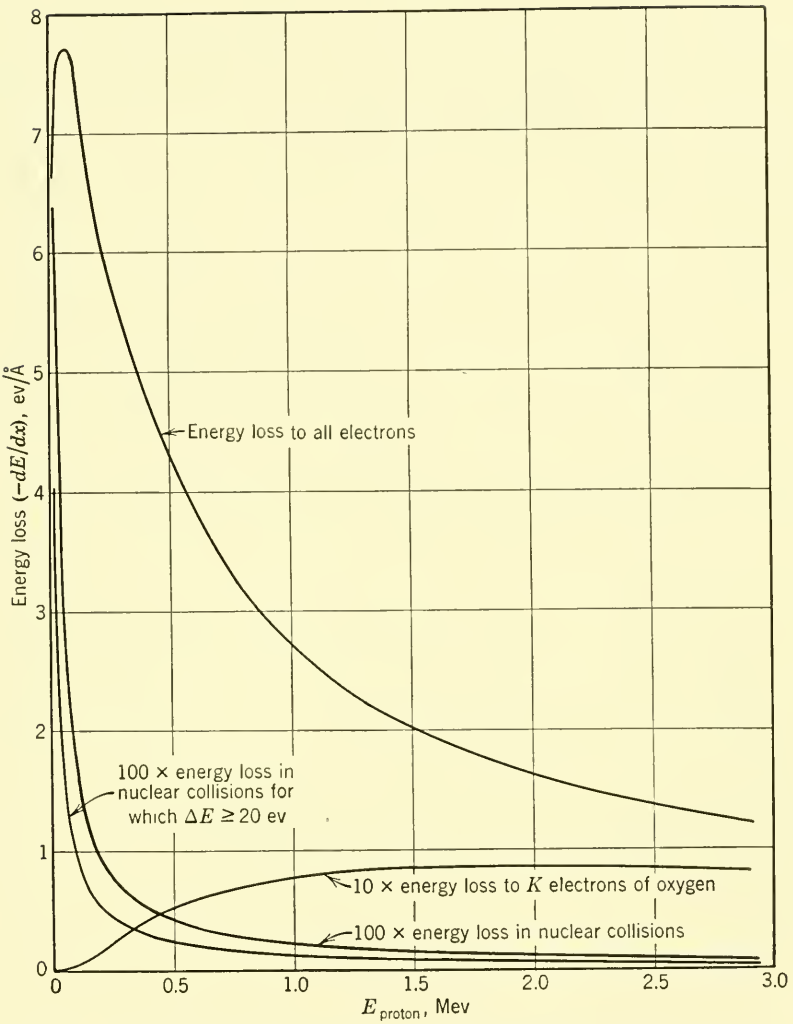

FIG. 1. Various modes of energy loss (stopping power) of protons in water.

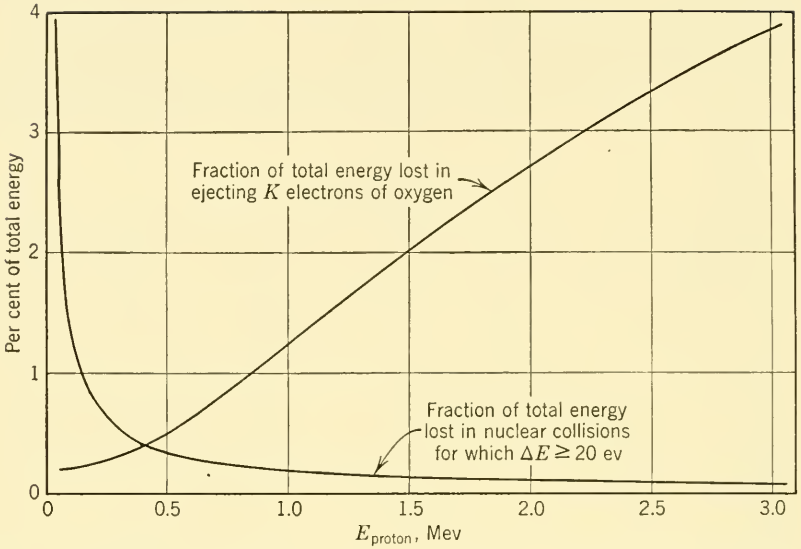

FIG. 2. Fraction of total energy of protons penetrating water which is lost in ejecting K electrons of oxygen, and in "violent" nuclear collisions.

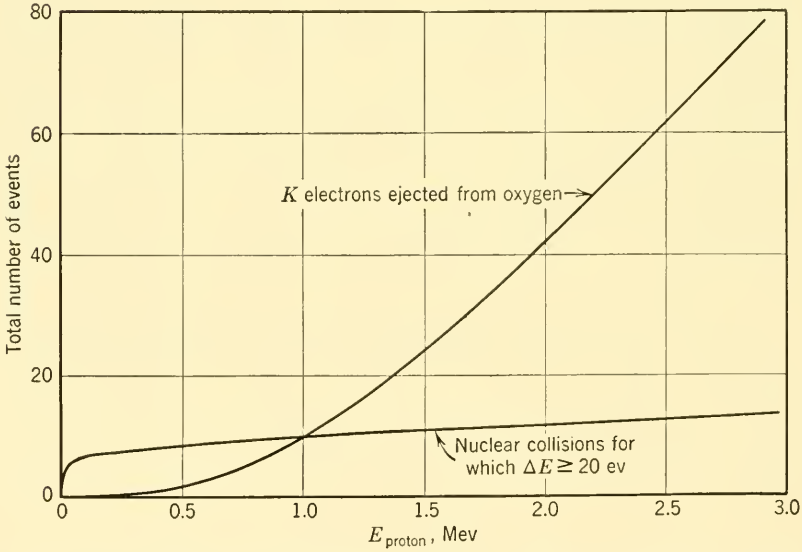

FIG. 3. Total number of K electrons ejected from oxygen atoms, and of "violent" nuclear collisions, for protons penetrating water.

APPENDIX. EXPLANATION OF THE FIGURES

Fig. 1. Various Modes of Energy Loss of Protons in Water

(*a*) The total electronic energy loss (stopping power), newly recalculated, is plotted as a function of proton energy. For more suggestive reference in interpretations of radiation chemistry and biology, the stopping power is expressed in units of electron volts of energy loss per angstrom of distance traversed, for water of density 1.00. (The distance between nearest neighbor molecules in water is about 3 Å.)

The basis for the calculation of these values of the stopping power is discussed in detail in reference 7. Briefly, it is:

1. The stopping power of water is assumed to be equal to the sum of the stopping powers of its constituent atoms.

2. The Born approximation is presumed to be valid, and the theory of Bethe (6) is therefore applied.

3. The simple Bethe stopping-power formula is, however, corrected for its premise that the orbital velocities of the various atomic electrons are negligibly small compared to the proton velocity: for the K electrons of oxygen, by the accurate method developed by Bethe (6) and recently modified (2), and for the electron of hydrogen, and the L electrons of oxygen, by a prescription of yet unestablished validity proposed by Hirschfelder and Magee (5).

4. The empirical constants representing the mean excitation potentials of hydrogen and oxygen atoms are redetermined from data on the stopping powers of the elements and some of their compounds.

5. At low energies—specifically, for proton energies less than about 0.3 Mev—no competent theory exists. The stopping power in this region is therefore estimated as well as possible, using experimental data on water vapor obtained by Crenshaw (3).

The results cannot be considered trustworthy in this region. Because of some of the approximations used, and the absence of adequate experimental information on the stopping power of water itself, the data are presented with no assurance that they are more than a qualitative guide (7).

Values of the stopping power of water for *alpha particles* can be obtained from those for protons by use of the familiar relation:

$$\left(\frac{dE'}{dx}\right) \text{(for alpha of energy } E') = 4\left(\frac{dE}{dx}\right) \text{(for proton of energy } E = 0.2517E') \qquad [E' > 1 \text{ Mev}]$$

For proton energies greater than about 3 Mev (but not so great that relativistic corrections are demanded) the energy loss may be computed directly from the formula:

$$\left(-\frac{dE}{dx}\right) = \frac{1.841}{E}(1.525 + \log_{10} E) \text{ ev/Å} \qquad [E \text{ in Mev}]$$

(*b*) A second curve presents the contribution to the stopping power of energy loss to the K electrons of oxygen, and is calculated from results of Brown (2). Values for the corresponding energy loss for alpha particles may be obtained from those for protons by a relation similar to that given above. For extremely great values of E, the energy loss to the K electrons approaches 19 per cent of the total energy loss; for the energy region treated here, however, this contribution is smaller, being 7 per cent at 3 Mev, for example.

(*c*) A third curve gives the energy loss in nuclear collisions, calculated by methods developed by Bohr (1). Since this treatment uses a Thomas-Fermi approximation to the screening, it will be somewhat inaccurate for these light atoms; however, the error should be slight.

(*d*) Finally, there are presented values of that contribution to the stopping power which derives exclusively from the more violent nuclear collisions. By a "violent" collision is meant (here) one in which the struck ("recoil") atom is ejected from its molecule. For convenience in calculation, violent collisions are assumed to be those in which more than 20 ev is transferred to the (H *or* O) recoil atom. Such a model contains the effect of chemical binding on atom ejection—very crudely, to be sure. The *20 ev* is an estimate; the conclusions, however, are not very sensitive to the value of this quantity. It will be seen that such violent collisions contribute about one-half of the total nuclear-collision stopping power. (Although less numerous, the events involve greater energy transfers.)

Fig. 2. Fraction of Total Energy of Protons Penetrating Water Which Is Lost in Ejecting K Electrons of Oxygen, and in "Violent" Nuclear Collisions

This information is obtained by numerical integration of appropriate data from Fig. 1, and is subject to the same limitations mentioned above.

Fig. 3. Total Number of K Electrons Ejected from Oxygen Atoms, and of "Violent" Nuclear Collisions, for Protons Penetrating Water

(*a*) The cross section for ejection by a proton of a K electron from an oxygen atom is computed from results of Henneberg (4). Since he calculated the effect of screening for a case somewhat different from that of oxygen, values for the number of K ejections computed from his cross sections and presented in Fig. 3 are in error, but are too *low*. However, they are still trustworthy approximate values. A more accurate calculation could be made readily by numerical integration of the transition probabilities given by Bethe (6), which are valid for oxygen.

The total number of K ejections from oxygen atoms is computed by numerical integration from values of the cross section for K ejection and of the total stopping power of water (from Fig. 1).

(*b*) The total number of violent nuclear collisions (cf. above for definition of "violent") is computed by numerical integration from the simple Rutherford

cross section (which is valid for such collisions) and the total stopping power of water (from Fig. 1). Note, however, that this gives only the number of violent *primary* collisions; the total number of atoms ejected will be greater—often as much as twice as great—because some of the recoils eject other atoms. The figure shows clearly that at low proton energy (for water, below 1 Mev) most of the ejections occur at the end of the range, whereas at high energy most of them are distributed along the range, in approximately constant proportion to the total (that is, electronic) energy loss.

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DISCUSSION

FANO:

I sympathize very much with the general idea of emphasizing the limitations to the help that physics can give in these matters. At the same time I wonder whether Platzman's remarks might not cause undue concern in the opposite direction. On the whole, it would seem that physical theory does provide a reliable guide on how to analyze most of the practical questions relating to the physical action of radiation. True enough, the theory does not yield quantitative predictions as accurate as one might wish, but this lack of accuracy does not seem to me to be too critical at the present time.

PLATZMAN:

I quite agree with Fano that radiation physics has contributed immensely by making possible the treatment of most of what he terms the "practical questions" of radiation action. There was no intention of depreciating this contribution. Rather, I have sought to stress how radiation physics has been inadequately developed, thus far, as an aid in understanding the fundamental chemical mechanisms of radiation effects.

MAGEE:

I should like to say a word about the chemical effect following the Auger process. It is my opinion that to consider the chemical effect as the result of electrostatic repulsion between the two charges is viewing the situation too simply. Considerable work has been done at Notre Dame on the isomeric transitions in hydrogen bromide and deuterium bromide. Theory indicates that the

atoms may receive an average charge exceeding 4; nevertheless, it is found experimentally that as many as 60–70 per cent of the molecules will not rupture their bonds. According to a simple electrostatic repulsion model, the bromine ion in this case would capture all available electrons, and, as a result, the positive hydrogen and the still positive bromine would repel each other with 100 per cent probability of decomposition. The model obviously fails for this simple case, and there is no reason to believe that it applies in any more complicated one.

Morrison:

Platzman has brought out in interesting detail the role of what he has termed the nuclear collision in the energy loss of charged particles moving through matter. By this designation he referred to the way in which energy was transferred to the mass motion of the atom as a whole. That is to say, the collision arises from an interaction between the charged particle and the electrostatic field of the nucleus. In other words, the nucleus is here regarded not as a sticky point, but as center of an electrostatic field. This kind of reaction does occur, and it is to be clearly distinguished from the sort of collision discussed by Tobias and Wilson, in which there is a nuclear-force interaction between the nucleus and very high-energy protons or deuterons. It is interesting to compare the two for 200-Mev deuteron beams. Most such particles traverse their range without making a single nuclear collision of the sort I have described, that is, a nuclear-force collision. There is about one chance in three that such a particle will make a nuclear-force collision. When it does, it transfers a considerable amount of its energy, giving rise to a many-pronged star from which several columns of heavy ionization start out in several directions. If any large-scale structure of micron size responsible for radiobiological effects is located in one of these stars of ionization, the stars might give rise to biological effects. But the sort of nuclear collision that Platzman has been discussing, that is, the electrostatic field collision, might occur in the order of 100 times, along the deuteron path, while each energy transfer would involve only enough energy to disrupt a few molecules. The difference between these two types of characteristic nuclear events is worth mentioning.

8.

Elementary Chemical Processes in Radiobiological Reactions *

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The elementary chemical processes of radiobiology are the elementary chemical effects of high-energy radiation on aqueous solutions containing oxygen, on pure organic matter, and on organic matter suspended in aqueous solution. In the aqueous layer the important primary physical process is ionization; in the organic portion both ionization and excitation must be considered. In biological systems the active entities in the aqueous layer are principally OH and HO_2. The presence of the latter radical increases the volume of the effective aqueous layer around the biological particle.† The effectiveness of a hit in the organic material is determined in part by the properties of the surrounding cage and depends, among other factors, on the size of the biological particle. The effect of a hit may be propagated by ionization transfer, by free-radical diffusion, by a chain reaction, or by change in local *p*H. Furthermore, free H and resultant HO_2 are formed in the ambient liquid even when the hit is in the particle itself. In view of these elementary processes the biological particle cannot be uniformly sensitive to radiation over its entire volume, the target of "target theory" is not to be identified with the biological particle, a single ionization act is not necessarily lethal, and the target dimension is not simply related to the true size of the biological particle.

From the chemical viewpoint the elementary processes of radiobiology are those which can be expected in aqueous systems containing dissolved and suspended organic compounds. For simplicity we may consider first the general nature of the elementary processes which can occur.

* A contribution from the Radiation Chemistry Project, operated by the University of Notre Dame, under Atomic Energy Commission Contract No. AT(11-1)-38.

† In this paper the term biological particle refers to the microscopic unit of interest in target theory, such as a cell or a virus unit.

Elementary Chemical Processes Characteristic of Water

In pure water the primary processes are usually written

$$H_2O \rightsquigarrow H_2O^+ + e \tag{1}$$

$$H_2O^+ + aq \rightarrow H_3O^+ + OH \tag{2}$$

$$H_2O + aq + e \rightarrow H + OH^- \cdot aq \tag{3}$$

Processes involving excitation of water in the primary physical act are usually neglected because it is the general opinion that the Franck-Rabinowitch cage either prevents formation of free hydrogen atoms and hydroxyl radicals or causes their immediate recombination without consequent secondary chemical effects; that is,

$$H_2O \rightsquigarrow H_2O^* \rightarrow H + OH \rightarrow H_2O \tag{4}$$

A feature peculiar to the set of reactions 4 is that, unlike the first three listed, they occur without any local changes of hydrogen- or hydroxyl-ion concentration. The reactions ensuant on reactions 1 to 3 depend on the relative proximity to each other of the products of reactions 2 and 3.

In fast-particle irradiation (that is, high-energy electrons, x-rays, etc.) the processes of reaction 1 may occur several hundred molecules apart, whereas in slow-particle irradiation (that is, the usual alpha and neutron processes) the distances between adjacent hydroxyl radicals, formed by the successive reactions 1 and 2, may be less than 10 molecules. Reaction 3 usually occurs a considerable distance, perhaps as much as 10 molecules, from the locale of origination of the electron involved. Thus, we may expect a distribution of atoms and radicals somewhat isotropic for a fairly homogeneous beam of fast-particle irradiation, definitely anisotropic for slow-particle irradiation.* In the latter case each ionization column consists essentially of a core of hydroxyl radicals and oxonium ions surrounded by a sheath of free hydrogen atoms and hydroxyl ions.

* *Note added in proof* (*August 29, 1951*): In a rapidly developing field, new facts are found and new ideas develop in the course of a year. This picture and its consequences have now been greatly modified. In both fast-particle and slow-particle irradiation, about three-quarters of the primary physical effects are in spurs which contain approximately the same number of ions approximately similarly distributed in both cases. The important parameter, which must account for the difference in the effects of the two types of radiation, is, therefore, the distance between spurs, which is relatively large for fast particles. In further development of the theory this fact must be carefully considered (cf. forthcoming paper in *Nucleonics* by Burton and Magee).

Thus, reaction between hydroxyl radicals

$$OH + OH \rightarrow H_2O_2 \tag{5}$$

is quite probable when they are formed close together, as in slow-particle irradiation. The reaction

$$OH + OH \rightarrow H_2O + O \tag{6}$$

might also occur under such conditions. However, although reaction 6 is $\sim$9 kcal mole^{-1} exothermal, the difference in activation energies $E_6 - E_5$ may favor reaction 5. An unpublished estimate indicates that $E_5 \lesssim 4$ kcal mole^{-1}.

The reactions

$$H + H \rightarrow H_2 \tag{7}$$

$$H + OH \rightarrow H_2O \tag{8}$$

occur readily under any circumstances, and the reaction

$$OH + H_2 \rightarrow H_2O + H \tag{9}$$

which is $\sim$9 kcal mole^{-1} exothermal, is an important back reaction which serves to decrease the yield of electrolytic gas in pure water. According to Allen (1, 2), reaction 9 is one step of a two-step chain, of which reaction 10

$$H + H_2O_2 \rightarrow H_2O + OH \tag{10}$$

is the second, which in general reduces gas production under gamma, x-ray, and fast-electron irradiation to barely detectable levels. Ghormley and Allen (3) have shown, however, that with slow-electron irradiation, as with 5.6-kev betas from tritium, the yield of electrolytic gas resembles what might be expected from our knowledge of effects of alpha-particle irradiation.

Introduction of impurities into the water may have a considerable effect on the yield of gas. Such anions as $SO_4^{=}$, $PO_4^{\equiv}$, and Cl^- are without effect, but Br^- and I^- are successively more effective in inducing production of gas. The obvious point noted by many acquainted with these facts is that the electron affinity of the negative ion involved relative to hydroxyl ion is the decisive factor. Where we can write, for aqueous systems,

$$X^- + OH \rightarrow X + OH^- \tag{11}$$

the anion X^- is effective in production of electrolytic gas to an extent related to the weakness of its electron affinity. Of course, in reaction 11 the electron affinities involved pertain to the solvated ions. However, the

explanation of the effect was not so readily apparent. Many efforts were made to explain the result in terms of a chain involving X and X^-. Allen (1, 2) suggested the explanation that the redox potential of reaction 11 controls the steady-state concentration of free radical OH and thus determines the effectiveness of the back-reaction sequence 9 and 10. It is, as a matter of fact, known that with sufficient Br^- or I^- present the rate of gas production is proportional to the intensity of irradiation and that the concentration of Br^- or I^- determines the maximum rate of such production, I^- being the more effective. So far as this author is informed, Allen's theory of this effect has not been subjected to quantitative tests. For the purpose of this presentation it is sufficient to note that the presence of anions of low electron affinity reduces the free-hydroxyl-radical concentration at any intensity level of irradiation and thus decreases the effectiveness of steps 9 and 10 for the back reaction. As a result, H_2 gas escapes from the liquid. The H_2O_2 which escapes reaction 10 may, however, decompose by an overall reaction we note simply as

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2 \tag{12}$$

Allen (1, 2) has suggested as the chief mechanism of this reaction the chain

$$H_2O_2 + OH \rightarrow H_2O + HO_2 \tag{13}$$

$$H_2O_2 + HO_2 \rightarrow O_2 + H_2O + OH \tag{14}$$

$$OH + HO_2 \rightarrow H_2O + O_2 \tag{15}$$

Presence of oxygen also assists production of electrolytic gas under fast-particle irradiation. In this case the effective reaction presumably involves formation of free hydroperoxyl radical

$$H + O_2 \rightarrow HO_2 \tag{16}$$

The process reduces the free-hydrogen-atom concentration (and thus the effectiveness of the back sequence 9 and 10) and also provides a substance itself capable of capturing hydrogen atoms.

$$HO_2 + H \rightarrow H_2O_2 \tag{17}$$

The simplest satisfactory explanation of the effectiveness of dissolved oxygen in assisting production of electrolytic gas and hydrogen peroxide lies in those two facts. However, as Allen has noted, the hydroperoxyl radical enters also into its own back-reaction sequence (13 and 17) and the total effect consequently depends in quite complicated fashion on intensity of irradiation, concentration of dissolved oxygen, pressure above the liquid, and relative volumes of gas and liquid.

In summation, irradiation of water results ultimately in formation of hydrogen, oxygen, and hydrogen peroxide. With slow-particle irradiation, the yields are determined by the dosage. With fast-particle irradiation, yields are barely detectable without special provision for study. With such irradiation, yields of the order of those obtained under slow-particle irradiation may be obtained when oxygen or anions of suitably low electron affinity are present. The results are understood in the framework of a mechanism involving primary formation of free hydrogen atoms and hydroxyl radicals and, when oxygen is present, secondary formation of free hydroperoxyl radicals. According to Allen, the number of moles of hydrogen gas formed per 100 ev in water is very much the same with various dissolved anions [Br^-, I^-, NO_2^-, $SeO_3^=$, $AsO_3^=$, $Fe(CN)_6^=$] present but is affected by the velocity of the impingent particle.

Obviously, any dissolved substance substantially affected by free atomic hydrogen or by free radicals will change the nature of the gaseous product and may, at the same time, be itself permanently affected.* Fricke, Hart, and Smith (5) studied the products formed by irradiation of aqueous solutions of organic compounds, and a number of workers (6–11) have been investigating the mechanisms of these processes in a detailed way. In the early work of Fricke and his coworkers the results were ascribed to the action of "activated water." Presently, the "activated water" is believed to be free hydrogen atoms and hydroxyl radicals. When oxygen is present, the free hydroperoxyl radical may be added to this list.

Behavior of Organic Compounds

Elementary processes of radiation chemistry in organic compounds may be understood in the light of the Eyring, Hirschfelder, and Taylor (12) (EHT) mechanism as it has been interpreted by this writer (13–16) and by Magee and Burton (17, 18). We may write for the processes involving ionization †

$$A \rightsquigarrow A^+ + e \qquad (I)$$

$$A^+ + e \rightarrow A^* \qquad (II_a)$$

* Such an effect has been known for many years. For example, A. Kailan (4) noted that during the fumaric-maleic acid isomerization reaction in water there occurred a simultaneous reaction which he at that time ascribed to intermediary of hydrogen peroxide.

† Processes involving excitation have already been discussed in this symposium by Livingston. Here, we dismiss them temporarily with the statement that they are akin to the phenomena of photochemistry.

or

$$M + e \rightarrow M^- \qquad (IIb)$$

$$A^+ + M^- \begin{cases} \nearrow A^* + M & (IIc) \\ \searrow \text{Other reactions} & (IId) \end{cases}$$

$$A^* \begin{cases} \nearrow R + X & (IIIa) \\ \searrow B + C & (IIIb) \end{cases}$$

In this scheme A^+ as it reacts in reaction II*a* is probably in a more stable configuration than the ion in the instant of its production in step I; A* is an excited molecule; M may be the same as or different from A; R and X represent free atoms or radicals; B and C designate stable molecules formed in one elementary act. In an interpretation of part of this mechanism based essentially on a careful study of the reasonably calculable aspects of the reactions of hydrogen, Magee and Burton (17) concluded that (in those cases where reaction II*b* and its ensuant reactions can be ignored) the most probable path of reaction involves production of free atom or radical partners III*a*, one of which is excited. In the liquid state, probability of rearrangement of an excited particle to yield two ultimate molecules is considerably increased (16, 17). Since, of all the possible rearrangements which may occur, one usually goes by an energetically lowest path, it may be expected (16, 17) and it has actually been found in certain cases (19, 20) that a particular rearrangement decomposition is so highly favored as to preclude the probability of any other such process.

In the EHT mechanism as just outlined we have omitted consideration of possible decomposition of the ion A^+ itself. Since this subject is discussed in this symposium by Eyring *et al.*, we note only that two paths of decomposition are open

$$A^+ \begin{cases} \nearrow R^+ + X & (Ia) \\ \searrow B^+ + C & (Ib) \end{cases}$$

where the letters have the significance already noted. Of the two reactions, one involving decomposition into a free radical and a free-radical ion and the other decomposition into a molecule and a molecule ion, the first may usually be expected to require more energy. Thus, unless the initial ionization is to a point on an attractive curve above the dissociation limit for rupture as indicated in I*a*, decomposition of type I*b* alone occurs, even though it may take a considerable time (of the order of

10^{-8} sec or more). However, the initial ionization may be to an electronically excited state of the ion A^+. Ensuant internal conversion to a lower state of A^+ and decomposition (that is, predissociation), as by either of the reactions I*a* and I*b*, may likewise take a long time. The time involved will govern the phenomena observed. For example, in mass spectrometry we detect all manner of peaks with masses less than the parent peak. Some must have resulted from *rearrangement* in a time short compared with that required to move from the slit system to the magnetic field ($\ll 10^{-6}$ sec). On the other hand, the ghost peaks in mass spectrometry have been attributed by Hipple and his coworkers (21) to decomposition of the metastable ions while within the accelerating field; for example,

$$C_4H_{10}^+ \rightarrow C_3H_7^+ + CH_3 \qquad (2 \times 10^{-6} \text{ sec})$$

$$C_4H_{10}^+ \rightarrow C_3H_6^+ + CH_4 \qquad (1.7 \times 10^{-6} \text{ sec})$$

In this discussion of radiation chemical processes in organic compounds such entities as R, X, B, and C and their ions have not been identified as other than possible decomposition products of A which may be radicals or molecules. In a complicated or large molecule a great variety of possibilities exists. In a homologous series many possible paths of decomposition are equally probable. Thus, we have a principle (14) which has been found to apply fairly well for straight-chain paraffins and their normal carboxylic acids:

> Where the special chemistry of the substances involved does not play a significant role, both nature and relative concentration of products are determined by nature and relative frequency of occurrence of parent groups in the affected molecule.

This principle is particularly noteworthy for its limitations. In very few cases can chemistry be neglected. We might expect, on such a basis, that all compounds are equally sensitive to the effect of ionizing radiation. However, as a matter of fact, we know that aromatic compounds in particular are quite resistant to effects of radiation as compared to aliphatic compounds (13, 14). It is a matter of particular interest for radiobiologists that the aromatic group may protect the side chain. Hentz and Burton (22) have shown that such compounds as toluene, mesitylene, and ethylbenzene are more easily decomposed than benzene but are far more resistant to radiation (that is, by a factor of 10) than alkanes. On the other hand, another feature interesting to radiobiologists is that energy liberated in the aromatic group may be effective for decomposition of the side chain (22).

Some Important Aspects of the Elementary Physical Processes

For practical purposes, of course, organic compounds of interest to the radiobiologist are rarely pure. Mostly, they are dissolved or suspended in water. Consequently, consideration of the reciprocal effects of nature of the ionization process and nature of the mixture is here desirable.

Effect of ionization potential and negative-ion formation

In a mixture of possible reactants, one will have the lowest ionization potential, or, since we wish some rigor and the case may be complicated, a process involving one of them will yield a positive ion at a lowest energy level. Schematically, we may say that in a mixture A, B, C, etc., a process such as

$$A \rightarrow A^+ + e$$

or

$$A \rightarrow D^+ + E + e$$

proceeds at minimum energy. In aqueous systems, because of the high solvation energy of the H^+ ion, the process involving ionization of the water

$$H_2O\ (aq) \rightarrow H_3O^+ + OH + e \qquad (18)$$

requires only about 7.4 ev at a maximum.* This value is quite low, perhaps lower than that for any process involving ionization of an organic compound dissolved or suspended in water. An accurate statement cannot be made because the only available data (25) refer to the gaseous state of relatively simple "complex molecules."

It can be said that irrespective of the primary ionization process in a mixture of gases the ionization is with high probability transferred to that entity ionization of which requires the minimum energy.

In liquid mixtures, such as those of interest to the radiobiologist, water preponderates. It is most likely to be ionized in the first instance simply because its mass concentration so much exceeds that of any solute or suspensoid. Any solute molecule or suspended particle is practically constantly in collision with the ambient water. Thus, even if the primary process involves ionization of such an entity, we may expect that the resultant effect may be very much as if only the water molecule had been primarily affected.

* Calculated from data of F. R. Bichowsky and F. D. Rossini (23) and O. K. Rice (24).

The fate of the electron in an aqueous system is determined by the electron affinity of the various species present. Magee and Burton (18) have shown that the electron must, with high probability, be thermalized before it can be captured. Furthermore, the threshold energy for capture must be practically zero in order that negative-ion formation compete effectively with capture by positive ions. The threshold energy for capture by water is practically zero; the electron affinity of the hydroxyl ion plus its heat of solvation makes the reaction

$$H_2O + aq + e \rightarrow H + OH^- \cdot aq \qquad (3)$$

of significant importance. In general, it is improbable (though not impossible) that some dissolved or suspended species can capture thermal electrons to form negative ions. Since water preponderates, we may once again expect that on this basis alone it will provide the major trap for free thermal electrons. Possibility of the competitive reaction

$$H_3O^+ + e \rightarrow H + H_2O \qquad (19)$$

must not be ignored. However, its effect, like that of reaction 3, is to yield free hydrogen atoms in the ambient layer and to increase the local *p*H of the solution.

Biological Systems

The major conclusion from our brief consideration of elementary physical processes is that in aqueous systems of interest to radiobiologists the initial radiation chemical processes are much the same as they are in pure water. We may now review the major important features.

DISTRIBUTION OF EFFECTS

For the most part the radiant energy affects the water itself. The first chemically important elementary processes are reactions 1, 2, and 3. *Reaction 3, or its equivalent (19), may occur in the aqueous layer even when the primary effect of the radiation is in the biological particle itself.* The distribution of primary products depends on the nature of the incident radiation. For particles of the same charge and energy, velocity and mass are related by the expression

$$\text{velocity} \propto \text{mass}^{-0.5}$$

The frequency of production of excited molecules or ions by action of the impinging particle is inversely related to its velocity. Thus, slow particles produce reactions such as (1) relatively close together, whereas fast particles tend to produce a relatively isotropic distribution of free hydrogen atoms and hydroxyl radicals. High-energy gamma and x-radi-

ation (which produce Compton recoils) and electrons are included among the fast group. On the other hand, alphas, deuterons, and protons of energies usually employed belong in the slow group. Since in hydrogenous material the major effect of an incident neutron is the liberation of energetic protons, the neutron may also be placed in the slow group.

In summation, slow particles produce relatively high densities of ionization and of hydroxyl radicals along the ion track. A beam of fast particles produces a relatively isotropic distribution of a mixture of free hydrogen atoms and hydroxyl radicals.

*p*H EFFECTS

It may be remarked also that *p*H is markedly changed in the neighborhood of an ion track. In the track itself *p*H decreases; in the envelope immediately surrounding the track *p*H increases. The *p*H values locally attained may be very much less or very much greater than those which are common to biological systems. In the neighborhood of the track of a heavy particle this effect may be very much exaggerated. In a private discussion with the participants in this symposium Franck has pointed out that since biological systems are notoriously sensitive to variations in *p*H this *p*H effect itself can have profound significance for radiobiology.

EFFECT OF OXYGEN

It is usually maintained that in the radiation chemistry of aqueous systems the principal competitive processes are the diffusion of free H and OH toward each other and their diffusion toward the other reactive species present. Biological systems are marked principally by the presence of impurity, the outstanding example of which is oxygen. When the latter is present, an important fate of the free H atoms is the formation of hydroperoxyl radicals, HO_2, as by reaction 16. Although these radicals may themselves react with free OH, reaction 15, we may expect that such a process will have a distinct activation energy and steric factor. Meanwhile, since both H and H_2 concentrations are reduced, probability of reactions 8 and 9 decreases. The effect of the presence of oxygen is thus not only to convert a reducing agent, free H atom, into a persistent radical, HO_2, but at the same time to increase the life span of free OH radicals. *It must be emphasized that reaction 3 or 19 very probably occurs in the water even when the primary ionization effect of the radiation is on the biological material. Thus, there is a high probability, if oxygen is present in the system, that the entity* HO_2 *will be formed sufficiently close to a biological particle to have a chemical effect on it even when the initial ionization does not. In such case, ionization of the biological particle is effectively destructive, whereas excitation may not be.*

Thus, presence of dissolved oxygen in a biological system sensitive to oxidizing agents makes that system more sensitive to the effects of radiation. An antidote to oxygen in biological systems involves the incorporation in such systems of strong (non-toxic) reducing agents not quite capable, however, of direct reaction with oxygen. This latter requirement is not essentially a thermodynamic one (for example, the redox potential might even permit a direct reaction with oxygen) but in reality one of kinetics (that is, the reaction with oxygen simply should not proceed under the particular conditions). Choice of suitable compounds depends primarily on an experimental search.

Oxygen, however, does not always play a special role. Imagine an alpha-irradiation process in which a hit is scored directly within the biological particle. Since the alpha is relatively slow moving, there will be a number of accompanying hits in, and close to, the particle. As a result, some free radicals are necessarily produced so close to the biological particle that their only fate can be to react with it. Consequently, it would not be inconsistent with these simple concepts to discover that presence or absence of oxygen is without discernible effect on the results obtained in alpha irradiation of biological systems.

CHAIN REACTIONS

When a radical such as OH or HO_2 reacts with an organic compound, it either breaks a single bond or opens a double bond. In either event a new free radical is produced, the fate of which depends on its specific chemical properties. It is important to appreciate that under suitable conditions (for example, when the initial process is distinctly exothermal) this free radical may itself enter into chain reactions. One such type of chain is common to pyrolytic reactions:

$$\mathrm{R{-}} + \mathrm{AH} \rightarrow \mathrm{RH} + \mathrm{A{-}}$$

$$\mathrm{A{-}} \rightarrow \mathrm{R{-}} + \mathrm{S}$$

The radical R reacts with species AH in a reaction which requires activation energy. Decomposition of A into R plus a stable product S may also require activation energy. Such chains would tend to be very short near room temperature and would probably not be very important in biological processes. Another type of chain is characteristic of the growth of polymer molecules:

$$\mathrm{R{-}} + \mathrm{A} \rightarrow \mathrm{RA{-}}$$

$$\mathrm{RA{-}} + \mathrm{A} \rightarrow \mathrm{RA_2{-}}$$

$$\mathrm{RA_n{-}} + \mathrm{A} \rightarrow \mathrm{RA_{n+1}{-}}$$

with a final chain-termination step which involves another radical. Only the simplest type has been indicated. Polymer chains involving two or more kinds of molecules are also possible. In general, such free-radical chain reactions can proceed readily at room temperature and the chains may be 1000 or more molecules long. An interesting example of radiation-induced polymerization is afforded by the work of Dainton (26) on alpha-ray-induced polymerization of acrylonitrile in deaerated water. In that work he gave good evidence that the chain starter is free OH. The possibility of such chain-propagated effects of radiation-produced free radicals certainly may be quite important for radiobiological processes.

A converse phenomenon, perhaps equally important for radiobiology, is suggested by the work of Grassie and Melville (27), who report free-radical-induced depolymerization. A simple illustration of such a phenomenon is afforded by unpublished work of Sworski, Gordon, and Burton which has led to speculation that acetylene production in benzene radiolysis proceeds via the steps

$$C_6H_6 + H \rightarrow -CH_2{=}CH_2-CH_2{=}CH_2-CH_2-CH_3$$
$$\swarrow$$
$$CH_2{=}CH_2 + -CH_2{=}CH_2-CH_2-CH_3$$
$$\swarrow$$
$$CH_2{=}CH_2 + -CH_2-CH_3, \text{ etc.}$$

in a sort of "peeling-off" reaction. The first step may involve some considerable activation energy, but the interesting feature is that links in a chain of similarly linked units break successively so that a large molecule is degraded, as the result of one primary step, into a number of smaller ones. Such a process may also be operative in radiobiology and could account for sensitivity of biological particles to energetic free radicals.

DIRECT HITS

We have seen that radiation may act on biological material indirectly through its action on ambient water. Even when the primary effect of the radiation is on the biological material, free H and resultant HO_2 necessarily formed in the ambient layer (via reaction 3 or 19) may have an important chemical effect. However, direct action on such material is not necessarily precluded. Indeed, on desiccated biological material radiation must act directly. Consequently, it is important to note that the remarks concerning the effects of radiation on organic compounds may have considerable significance for radiobiology. Two classes of effects may occur. Either the primary chemical effect occurs at or near

the locus of energy absorption, or it may occur at a more remote region. The latter phenomenon occurs with aromatic compounds. Energy absorbed in the ring may split off a methyl hydrogen from toluene or mesitylene or a methyl group from ethylbenzene (22). Perhaps, in certain conjugated structures, an even more remote split is possible. Thus, we may conclude that in biological material energy need not necessarily be absorbed in the prosthetic group in order for it to have a devastating effect there. On the other hand, there is no assurance that a purely random hit is necessarily damaging to the prosthetic group. Indeed, we may visualize the possibility that a significant fraction of the volume of a biological material may be damaged (either temporarily or permanently) without effect on the prosthetic group; that is, without lethal effect as we might measure it. We might expect, consequently, that the probability of a chemically effective hit increases with the number of nearly simultaneous hits made on a particle of biological material. Seemingly, our best evidence (28, pp. 111 *et seq.*) is that in many cases a single hit is all that is necessary. A multiplicity of hits, as by an alpha particle, seems to be no more effective than a single hit, as by an electron. However, this conclusion is based on calculation and surmise—certainly not on direct visual observation—and there is no requirement that the hit be within the biological particle. A very careful analysis of the implications of this so-called *target theory* in the light of our knowledge of the elementary processes of radiation chemistry is necessary for an understanding of the data and of the attendant processes.

SOME REMARKS ON THE TARGET THEORY

It is by no means the function of this paper to attempt a critical evaluation of the target theory, details of which have been presented so ably by Lea (28). Rather, I would prefer to interpret certain aspects in the light of our general knowledge of radiation chemistry. In his presentation Lea recognized quite clearly that the not-necessarily spherical target had dimensions which were not necessarily identical with the particle of biological material (28, pp. 93 *et seq.*). Since ionization in the water immediately surrounding the particle could produce free H atoms and OH radicals capable of reaction with the biological material, the target size according to L. H. Gray is effectively increased by an amount related to the diffusion distance of those active particles (28, pp. 66, 67). Lea stated that this distance could be 150 Å for H atoms but only 20 or 30 Å for OH radicals. He neglected mention of the diffusion distance of the very much more persistent HO_2 radicals. However, perhaps the best way to see the target is to look at it as a whole, that is, the biological particle and its environment.

Figure 1 is a schematic representation of a biological particle in its aqueous sheath. The region ABC within the solid line is the particle itself. The region D includes all that aqueous layer in which primary creation of ions has a resultant chemical effect on the particle. The region AB (not necessarily continuous) is the sensitive part of the particle. It includes (or may be) the group injury to which is made apparent by a change in the detectable behavior of the particle. The region C is effectively inert; that is, injury, *or a hit,* within it is not made

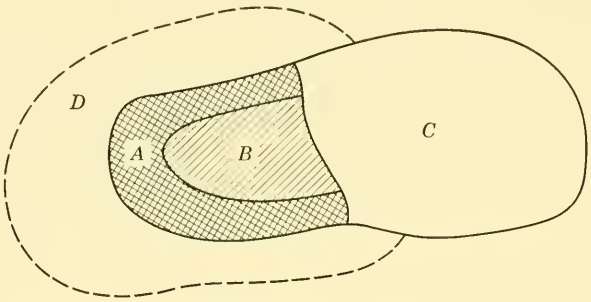

FIG. 1. Schematic representation of a biological entity. A, Hits in this region are always effective; B, hits in this region are sometimes effective; C, hits in this region are never effective; D, ambient layer of fluid in which hits may be effective.

apparent by a change in detectable behavior; as a matter of experimental fact it may be non-existent. The region A includes several portions:

(a) That volume which may be directly affected by radicals produced in the layer D or by changes in the pH of that layer.

(b) That volume which may transfer ionic charge to the layer D and thus make it chemically active.

(c) That volume in which untransferred ionic charge leads every time to damage in the region A or B.

In reference to this last point we must make some note of the elementary processes of the Franck-Rabinowitch cage effect. Neutralization of an ion deep in the cage will not necessarily result in decomposition; that is, the ion-pair yield may be less than unity both because of energy dissipation (from the excited molecule A*) without primary decomposition and because of primary recombination of radical (as distinguished from molecule) products while still within their mutual range of influence. In the region B the ion-pair yield is less than unity. On the other hand, as intimated in (c) above, part of the decomposition in B may be the result of a primary physical effect in A; cf. the effect of absorption of energy in an aromatic ring on decomposition in a side chain.

A feature to be emphasized in consideration of Fig. 1 is the precise

meaning of a hit. Lea (28, pp. 66, 67) defines a hit as the production of ionization within the target. From the point of view of our knowledge of the elementary processes of radiation chemistry as they relate to organic compounds such a definition appears unnecessarily restrictive. Production of excitation within the biological particle, depending on the locale of such excitation, can produce chemical change just as ultraviolet radiation might. The number of such primary excitations within the target always exceeds the number of primary ionizations. Thus, the conclusion, based on preoccupation with ionization processes, that in many cases a single hit is all that is necessary for production of a lethal effect is fundamentally misleading and obviously in error. If our ideas of the elementary processes of the radiation chemistry of organic compounds are correct, such a computation emphasizes a fact not otherwise apparent; namely, in the target theory there is no special virtue in a single hit if the hit is presumed to be in the organic material itself. Indeed, the best data so far used to support that notion offer clear evidence that it cannot be correct for targets of the size assumed.

On the other hand, there is almost inevitable formation of HO_2 (via reaction 3 or 19) in the ambient layer whenever primary ionization occurs in the biological particle. Such HO_2 may be more virulent in its effects than either a primary ionization or a primary excitation. Under such circumstance, we may expect that ionization deep within the particle may be more probably associated with damage than would similar excitation.

A question that must arise regarding application of Fig. 1 to any particular case concerns the volume ratio of regions $A + B$ to C. This is a matter regarding which the chemist certainly can make no general statement. Another question concerns a possibility that this same ratio may be a function of particle size; for example, the larger the particle the greater is the probability that a significant portion of it (that is, C) is effectively inert to the radiation. From the purely chemical viewpoint such an assumption may be justifiable in a homologous series. I am unable to judge its worth biologically. However, it may be profitable to examine the more obvious consequence of this assumption. That consequence simply is that the larger the particle the greater becomes the probability of an ineffective hit. The further consequence is that with large particles an increasingly large negative deviation of the calculated size (as determined from naive target theory) from the true geometric size (as determined from electron diffraction microphotographs of the dry material) is to be expected.

The significance of elementary processes in radiation chemistry for radiobiological reactions can be summed up by consideration of "target size" as affected by each one of the elementary processes.

1. If a spherical target have radiosensitive dimensions corresponding to its geometric dimensions and if each hit be lethal, the probability that a hit at any distance x from the center would produce a lethal effect would be unity within the radius r and zero outside. Figure 2 illustrates

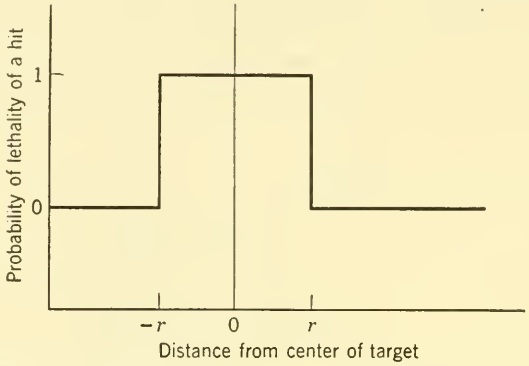

FIG. 2. Lethality of a hit as a function of distance from center of target on basis of simple target theory.

this situation. On the same model, geometric dimensions and dimension computed from radiobiological effect would be identical for all sizes of biological particle. The "ideal" line 1 of Fig. 3 shows this effect.

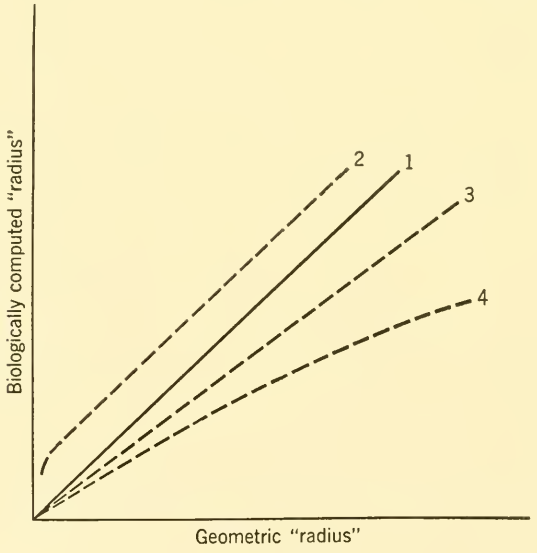

FIG. 3. Effect of various factors on biologically computed "radius" of target. 1, Ideal; 2, ideal + contribution from region D; 3, $C/(A + B)$ = constant > 0; 4, $C/(A + B)$ increases with size.

2. Free radicals as well as sharp variations of pH in the region D, both resultant from hits in that region, ionization transferred to that region, or electron capture therein, may (but do not necessarily) produce a lethal effect in the region A. The effect is to make the computed particle size larger than the geometric size. This contribution of the region D is shown qualitatively by the difference between lines 1 and 2 in Fig. 3. The probability of lethality is shown by Fig. 4.

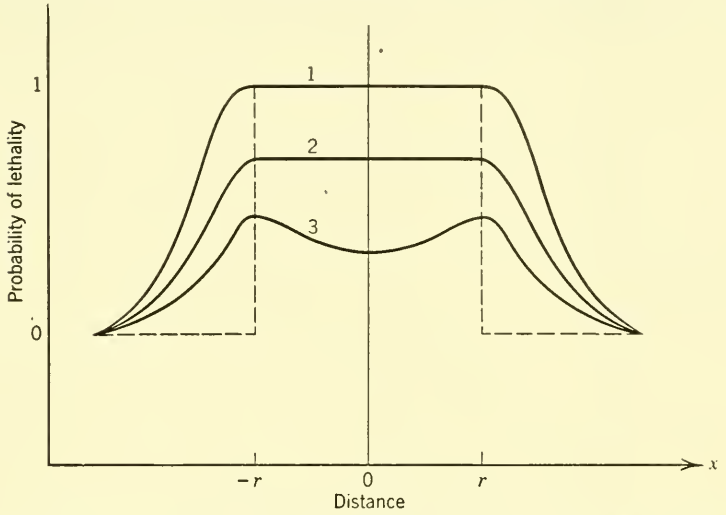

FIG. 4. Lethality of a hit as a function of distance from center of target on basis of various modifications of target theory.

3. The existence of a region C decreases the biologically computed size below the geometric size. If the ratio $C/(A + B)$ is constant, line 3 in Fig. 3 shows this effect. If the ratio increases with size, line 4 shows the effect. The probability curve now has no simple shape but depends on the distribution of C relative to A and B. If the ratio $C/(A + B)$ is a constant greater than zero and C is isotropically distributed, the probability of lethality of a hit within the particle is less than unity and the effectiveness of a hit within the diffusion distance in the ambient layer D may be decreased. The effect is shown roughly by line 2 in Fig. 4.

If the ratio $C/(A + B)$ increases with geometric size, the probability of an effective hit within the biological particle simply decreases with size. This statement means that for a large particle the plateau of line 2 in Fig. 4 would be lower than for a small particle.

4. The region A is confined to the surface of the particle. A hit within that region gives a rupture (or other decomposition) and the cage effect

is nil. On the other hand, in *B* there is a cage effect and that effect becomes bigger the larger the size of the particle. This effect is also shown by a line like 4 in Fig. 3. The probability of lethality of a hit is a maximum in or near the surface. The effect is shown by line 3 in Fig. 4.

The conclusion from these considerations is that the relationship between biologically computed target radius and geometric radius even for a spherical particle is not simple. For real particles of such ideal shape the qualitative nature of the relationship must be as shown in Fig. 5. The fact that the computed radius is so nearly like the geometric

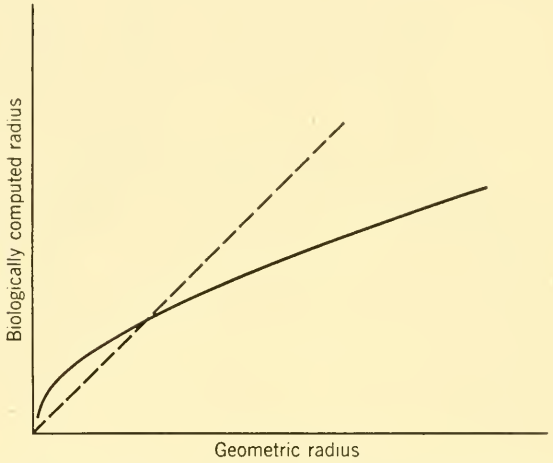

FIG. 5. Relation between effective radius of target and actual dimensions of biological entity.

radius transpires to be an interesting consequence of the elementary processes involved in radiobiological reactions. It is a relationship which has, for some, emphasized the naive features of target theory and really beclouded the processes involved. The happy fact is that investigators in the field were actually not led astray in spite of the terminology employed. Lea (28) himself emphasized the lack of a definite boundary. In a study of the effect of deuteron bombardment on bacteriophage, Pollard and Forro (29) have shown that a target exists which is smaller than the phage itself but that nevertheless the computed target size is increased because a deuteron whose path actually misses the phage can nevertheless inactivate it.

In conclusion one fact not heretofore mentioned, so far as I know, bears repeated emphasis. Free H and resultant HO_2 are very probably produced in the ambient liquid around a particle even when the hit, as in x-irradiation, may be directly and exclusively in the particle. It is

the formation of these active entities which may be largely responsible for effects heretofore attributed to the hit itself.

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DISCUSSION

KORNBERG:

Burton differentiated between the production of OH radicals, etc., and the direct effect of radiation on solute molecules with resultant activation followed in certain cases by decomposition. Perhaps, if the ratio of the two effects were known in some simple but fairly representative biological medium, a reasonable

basis, not wholly dependent on ionization in gases, for estimation of number of primary physical processes might evolve. Of course, this is in addition to the contribution such knowledge could make to the mechanism of damage.

BURTON:

Study of the effect of radiation on a mixture of benzene and cyclohexane yields information which is of much general interest and which may also have some bearing on Kornberg's comment. The ionization potential of the benzene molecule is less than 10 ev; that of cyclohexane is somewhat higher. If a mixture of the two compounds is irradiated, the ionization will tend to reside ultimately in the benzene. One might therefore expect that only benzene will be chemically affected: the benzene should protect the cyclohexane. The experimental evidence, however, indicates appreciable decomposition of the cyclohexane. It would thus appear that not quite all of the energy is transferred to the benzene; some decomposition has occurred before the energy is transferred. Primarily excited molecules may not transfer their energy like the ions, and it may be such cyclohexane molecules that decompose. Perhaps the chemical data may give some clue as to the relative probabilities of ionization and excitation transfer. However, we should note the complicating possibility of some transfer of energy from benzene to cyclohexane in a sensitization process.

LIND:

Is there transfer of energy in the liquid system?

BURTON:

I should have stated that the experiments were performed on benzene-cyclohexane mixtures in the liquid state.

MORRISON:

In a field as complex as the one under discussion at this symposium it is both useful and necessary to set up simple models. The target theory is such a model, and during the past two decades much useful experimental work has been based upon this model. It is important that future models should define a situation in an operational way, as the target theory has done. I wonder whether the model set up by Burton defines the situation from this point of view.

BURTON (Communicated):

Morrison is correct in implying that the proffered model is an oversimplification. The purpose of this model, as of any other, is to assist in correlation of results of research. Without question it will be modified as the facts demand. The mathematics of the picture I have suggested is, I believe, the mathematics of the target theory. All that I have done is to define a possible target in the light of our present knowledge of the radiation chemistry of aqueous systems. That target is clearly something different from the biological entity. The model is suggestive of the mechanism of the action of oxygen and of experiments which may be performed to test that mechanism. It also indicates why the

apparent target size may differ from the size of the biological entity even in the dry state.

MORRISON:

I do not believe that ion migration in the organic molecule is a very likely event.

BURTON:

It is the electron migration, and not the ion migration, that is important. The electron migrates; and, when it is thermalized, if it is thermalized near the water, it is much more likely to be captured by water than by an organic molecule or by a positive ion.

MORRISON:

Is there any available estimate of the probability of electron escape without damage to the organic molecule?

BURTON:

Not that I know of.

APPLEYARD:

I do not wish to defend a target theory necessarily as a main mechanism of radiobiological action. It can, however, certainly be of use as an experimental procedure. Pollard's group at Yale has tried to work under experimental conditions which give such a target theory an optimal chance of application, namely, irradiation of dried materials with densely ionizing particles. Under these conditions, for both enzymes and viruses, "operational" target sizes are found which, while often less than the known sizes of these materials, appear to mean something in the sense of defining a region with a high degree of molecular organization.

BURTON:

I should have referred to the work of Pollard's group at Yale. Did not some of the experimental evidence indicate a target size somewhat larger than the geometrical size of the biological unit in question?

APPLEYARD:

No. To the best of my knowledge Pollard and Forro have never found target sizes for phage larger than the size of the phage as evaluated by other methods.

BURTON (Communicated):

My question was founded on a misinterpretation of some data in the literature. However, I should like to re-emphasize a point which should not be forgotten: when dry biological material is irradiated the biological particle and the target of target theory are identical. Any size discrepancy between calculated and geometrical target is a reflection of inertness of a portion of the particle.

ABELSON:

Since the hit theory has come under discussion, I wish to point out a case in which the single-hit target theory seems to be relatively meaningless. Roberts at the Carnegie Institution of Washington has shown that small differences in biological manipulation can give large changes in the apparent multiplicity of hits. By small variations in time of incubation in a saline solution after irradiation, either single-hit or multiple-hit curves may be obtained for the survival of *E. coli* irradiated by ultraviolet light.

BURTON:

The target theory in its original form identifies a hit with an ionization. The target theory does not demand that a single hit be effective. A single hit has been found, by analysis of empirical results, to be effective in certain cases (for example, viruses and chromosomes), but multiple hits may be required in other cases.

HART:

Oxygen molecules play an important role in the radiolysis by x-rays of aqueous solutions of formic acid and hydrogen peroxide. In the absence of oxygen, a chain oxidation of formic acid takes place, resulting in the overall reaction:

$$HCOOH + H_2O_2 = 2H_2O + CO_2$$

Oxygen is an excellent inhibitor for this reaction. The hydrogen atoms produced during irradiation react with oxygen in preference to formic acid even under conditions where the ratio of formic acid to oxygen is 10,000 to 1. In the absence of oxygen and hydrogen peroxide, the hydrogen atoms do, however, react with formic acid to produce molecular hydrogen. This has been demonstrated by the irradiation of DCOOH in aqueous solution. HD is a primary product of this reaction. Therefore it is apparent that hydrogen atoms do not require oxygen in order to promote chemical changes in solute molecules.

BURTON (Communicated):

I did not intend to imply, in my paper, that hydrogen atoms require oxygen in order to be effective. As a matter of fact, we can guess that the activation energies of processes (in biological systems) involving atomic hydrogen will, in general, be lower than those of processes involving HO_2. This fact is precisely why HO_2 survives longer and diffuses farther in such systems, and serves to explain the role of oxygen in processes in which the hit may not be in or near the target. In the interesting case described by Hart the atomic hydrogen reacts with formic acid whenever it is not removed by some other process.

9.

Influences of Details of Electronic Binding on Penetration Phenomena, and the Penetration of Energetic Charged Particles through Liquid Water

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I. Introduction

Liquid water is perhaps the most important inorganic chemical substance and is certainly the fundamental biological material. The interaction of high-energy radiations with water is thus a subject of the greatest consequence for studies of radiation action. Yet almost nothing is known directly about the phenomena attending passage of swiftly moving charged atomic particles through liquid water. The importance of this problem in radiobiology is emphasized by the reminder that the phenomena referred to are chemically non-specific: the penetrating particle affects atoms and molecules encountered along its path simply in approximate proportion to the populations of the various species. Hence the *primary* effects in biological systems are intimately related to the effects in pure water and, indeed, are often roughly identical with them.

Although we have today a wide knowledge of, and deep insight into, high-energy penetration phenomena for gases, particularly monatomic gases, we know less about the corresponding phenomena for polyatomic gases, very little indeed about solids, and almost nothing concerning liquids. Admittedly, the quantitative differences in the phenomena for the last three cases—when the material is compared with a hypothetical mixture of monatomic gases of identical over-all chemical composition—have up to the present time usually been comparable to or smaller than the relevant experimental uncertainties or the accuracies demanded in common applications. Indeed, it is to this lack of obvious *practical* importance that neglect of the fundamental aspects of the question is

probably to be ascribed. In the case of liquid water, however, the consequence in theoretical radiobiology and radiation chemistry of appreciable manifestation of *intra*molecular binding and *inter*molecular interaction is potentially so great that further disregard of the possibilities can no longer be tolerated.

Formidable experimental difficulties are responsible for the fact that only very few experimental studies relevant to this problem have been made. Thus, neither the primary nor the total *ionization* produced in liquid water by any type of radiation has ever been measured, no method having yet been advanced by means of which the ionization can be directly observed. The stopping power of liquid water for energetic charged particles has been determined only once: the difficulty here lies in preparing a section of water which is sufficiently thin (for the energy region hitherto accessible). There exist also three measurements of the *range* of natural alpha particles in water. The results are not in accordance with one another, and it is sufficient at this point to note that they do not much clarify the basic questions. Moreover, no theoretical treatment of penetration phenomena for the specific case of liquid water has heretofore been given. This inattention should be attributed to the *opinion* that liquid water must behave toward high-energy radiation very much like a gas, or to the barrier presented by lack of any detailed knowledge of the electronic properties of liquid water.

In the present paper we shall review the appropriate experimental results, consider the theoretical problems involved, and also point out directions which future investigation might pursue. It will prove gratifying if the discussion should serve to emphasize our ignorance of many of the fundamental aspects of the subject and help to stimulate their study.

The subject of high-energy radiation effects in water is, of course, a great one, embracing a number of diverse phenomena. It is therefore necessary to restrict the present discussion to a few aspects which are of foremost practical importance, or which seem most likely to shed light on the question of possible peculiarities of liquid water. Topics which merit particular attention are the stopping power and range of swiftly moving charged particles, effects of electrons and positive ions of intermediate and low velocity, and the mean over-all efficiency of ionization.

II. Review of Experimental Information

A. Michl (1914) (1)

In this first measurement on liquid water a thin platinum wire covered with a very thin layer of polonium rested on a photographic plate, the

whole being immersed in water, and the range of the Po alpha particles was determined from the contour of blackening of the emulsion. A careful study under different experimental circumstances led to a value for the range of Po alpha particles (5.298 Mev) of 32.0 ± 0.5 microns in liquid water. Michl dealt as best he could with a number of possible complications—among them swelling of the gelatin and depression of the gelatin by the wire, as well as solubility of the Po in water—but it is virtually impossible today to assess the accuracy of his final result.

Michl's value for the *range* in liquid water is 20 per cent *smaller* than that calculated for water vapor at (hypothetically) equal density using modern data (cf. Table 1, p. 143).

The ranges of Po alpha particles in alcohol and in six other organic liquids were also determined. All ranges were smaller (by 10–20 per cent) than values predicted for the corresponding vapor, reduced to the same density.

B. PHILIPP (1923) (2, 3)

By means of visual scintillation observation, with the radioactive source mounted below a water surface and the fluorescent screen just above it, the range of RaC′ alpha particles (7.680 Mev) in liquid water was found to be 59.5 ± 0.8 microns, 16 per cent smaller than that calculated for the vapor (Table 1). Ranges in alcohol and in two other organic liquids were also determined. Philipp measured the ranges in the corresponding vapors as well. For water vapor of density 0.532 mg per cm^3 he found an extrapolated range of 13.0 cm, which corresponds to a mean range of approximately 12.7 cm; the above value for liquid water, when reduced to the same density, is 12 per cent smaller than this. In contrast to the abnormally small range in the liquid, which also was found to occur for alcohol (for which the difference was 11 per cent), measurements on aniline and pyridine gave more closely equivalent ranges for the liquid and vapor. Philipp attributed this difference to the circumstance that, whereas the latter liquids are "normal," both water and alcohol are "associated." Even if his results are correct, this is a correlation but certainly not an explanation.

C. APPLEYARD (1949) (4, 5)

In this ingenious experiment a very dilute solution of polonium in 0.5–1.5 *N* HCl acted as a thick source, the alpha particles being counted by a thin-window Geiger counter mounted at varying distances in air above the liquid. From the variation of the number of alpha-particle counts with distance, the stopping power corresponding to an energy close to the initial energy of the alpha particles could be determined.

This is the only determination yet made of the true *stopping power* of liquid water; the other measurements (1, 3, 6) cited all yield ranges. Appleyard found the stopping power corresponding to an alpha-particle energy of roughly 4.5 Mev to be 1.71 ± 0.05 (per molecule of H_2O relative to one "atom" of air). (The uncertainty in energy is unimportant, since the *relative* stopping power is known to be rather insensitive to energy variations in *this* energy region; cf. Fig. 1, p. 165.) This value is 15 per cent higher than the theoretical one for water vapor (cf. Table 1).

D. DE CARVALHO AND YAGODA (1950) (6, 7)

In this measurement, the photographic emulsion technique was again employed. The source of alpha rays was a tiny particle ("radiocolloid") of polonium or radium sulfate; many such particles were sprinkled over the surface of the emulsion, which was then immersed in water. The procedure was in a sense a modern refinement of Michl's method. Those alpha particles emitted by a radiocolloid particle almost tangentially to the emulsion struck the latter when they were very close to the ends of their ranges and hence delineated the range in *water*.

The experiments yielded values of the ranges of Po and of RaC′ alpha particles in vapor, liquid, and solid water which, when reduced to the same density, agreed closely with each other (to within 1 per cent) and with the value calculated for the vapor (cf. Table 1). The results are thus in utter disagreement with those of the three other investigations.

It might be noted that de Carvalho (6) states that his emulsions are sensitive only to alpha-particle energies greater than 0.2 Mev. This should make his ranges too *short* in the liquid, but this difference is not great and he is able to correct for it. [Similarly, the scintillation method, employed by Philipp, has a visual threshold of between 0.13 and 0.17 Mev (8).]

E. DISCUSSION OF THE EXPERIMENTAL INFORMATION

The experimental results are summarized in Table 1. The semitheoretical values for water vapor listed there are taken from a new calculation, the results of which are presented in Figs. 1 and 2 (pp. 165 and 166); the basis for this calculation will be discussed in Section VI below. The quantity S_R should not be confused with s. Whereas s is the true stopping power, relative to that of air at the same particle energy and for the same density of atoms, $1/S_R$ is the average reciprocal stopping power (the average being over all values of particle energy as the particle is progressively slowed down) relative to the corresponding average in air for a particle of the same initial energy. More simply, $1/S_R$ is

the *range* of the alpha particle in water divided by its range in air, corrected for the difference in atomic densities. Thus, although S_R is more directly a relative range, it is often called the average or mean relative stopping power and sometimes even *the* relative stopping power. However, S_R and s would be equal only if s were independent of particle

TABLE 1

SUMMARY OF EXPERIMENTAL INFORMATION ON RANGE AND STOPPING POWER: ALPHA PARTICLES IN LIQUID WATER

Source of Data	4.5-Mev Alpha Particle	Po Alpha Particle (5.30 Mev)		RaC′ Alpha Particle (7.68 Mev)	
	s	R in μ	S_R	R in μ	S_R
Michl (1914) (1)	....	32.0	1.83	....	....
Philipp (1923) (3)	....	....	....	59.5	1.77
Appleyard (1949) (4)	1.71	....	....	....	....
de Carvalho and Yagoda (1950) (7)	....	38.1	1.54	67.2	1.57
From semitheoretical calculation	1.49 ± 0.05	[40]	[1.48]	[71]	[1.47]

Definitions:

s = stopping power, per molecule of water, relative to that of one "atom" of air

R = range in water of density 1 gm per cm^3, in microns

S_R = reciprocal of range in water, relative to range in air, reduced to equal numbers of molecules (H_2O) and "atoms" (air) per unit volume
$= 1.525 \cdot 10^{-3} R_{air}/R_{H_2O}$

NOTE: for explanation of uncertainties in calculated values of R and S_R indicated by brackets, cf. Section VI.

energy. This is occasionally approximately but never exactly the case (cf. Fig. 1). The quantities S_R and s are often confused in the literature.

It is a striking fact that both the older measurements of S_R by Michl and by Philipp and the later one of s by Appleyard give values approximately 15–20 per cent *higher* for the liquid than those calculated theoretically for the vapor. The approximate agreement between these

three "discrepancies" is impressive. (One would not expect them to be identical, even if the experimental errors were negligible, for the measurements yield three closely related but essentially different quantities.*)

It merits mention here that the reality of the discrepancies found by Michl and by Philipp has never been generally accepted by workers in the field. Thus Rutherford (9), in 1930, dismisses the effect as "small and difficult to account for." Gray (10), in an authoritative review published in 1944, also discounts the direct measurements on liquids, implying that they are in error in some unknown way(s); generalizing from an analysis of the stopping powers of a number of compounds, mostly of C, H, and O and almost all in the vapor phase, he concludes that possible effects of chemical binding and state of aggregation on the stopping power for fast particles are at most of magnitude ± 1 per cent. As far as applications are concerned, it has always been the custom, in any considerations into which the stopping power or range of energetic charged particles in liquid water enters, to presume the water to have behavior identical with that of a mixture of hydrogen and oxygen gas having the same composition and density as the liquid.

We now have two new and different measurements, performed with modern techniques. The work of Appleyard, particularly in its semiquantitative agreement with the earlier work, undermines the complacency sketched above, although a *great* difference between liquid and vapor is so remarkable in the light of theory, as will be discussed below, that still another, independent confirmation would appear to be demanded, and more accurate data are necessary in any event. However, the conclusion of de Carvalho and Yagoda that S_R is the same for liquid and vapor (and solid) throws a cloud of uncertainty over the entire situation. It is clearly imperative to have a vigorous attack on the experimental problem, preferably from several different directions, not only to establish the correct results, but also to identify the nature of the various errors.

In view of this situation, it does not seem propitious to investigate extensively the theoretical aspects of the problem. Only a qualitative

* Some doubt may also be raised that the medium in any of the experiments was truly "water." Thus Appleyard used rather concentrated electrolytic solutions, in which the ions were separated, on the average, by only about 3–4 water molecules. And in all the experiments the distance traversed by the alpha particle in the liquid was small—of the order of magnitude of 10^5 molecular diameters of water in the experiments of Michl, of Philipp, and of de Carvalho-Yagoda, and even less in that of Appleyard—and the water layer might have had structural abnormality (of surface in the experiments of Philipp and of Appleyard, of intersurface in those of Michl and de Carvalho-Yagoda). It has not been possible, however, to invoke a plausible reason why such peculiarities might account for the observations.

(but, it is hoped, complete) survey of possible effects of the finer details of electronic binding will therefore be given. This suffices to demonstrate that these effects are by no means all negligible, and that some are of extreme importance.

In order to discuss the stopping-power effects more meaningfully, it will first be necessary to review and evaluate such aspects of contemporary knowledge and understanding of stopping powers as relate to the problems under consideration. This critical evaluation will, indeed, be one major objective of the present study, and should, it is hoped, prove helpful quite independently of the problem of liquid water.

III. Résumé of Stopping-Power Theory for a Monatomic Gas

The stopping power of a medium for a swiftly moving charged particle of energy E is defined as the ratio of the energy lost by the particle $(-\Delta E)$ in penetrating a very small distance (Δx) into that medium, to Δx. Thus, stopping power equals $-\Delta E/\Delta x$, and equals $-dE/dx$ in the limit of infinitesimal penetration. (Treatment of E as a continuously declining function of x is a valid approximation because the magnitude of ΔE which corresponds to a Δx of atomic dimensions is of the order of electronic binding energies, that is, a few electron volts, and hence is extremely small compared to E for high-energy particles.)

The stopping power is a well-defined parameter of the physical situation and is a compound of the probabilities of numerous possibilities of energy loss. (The stopping power has in fact a probability distribution, but only its *average* value will be considered in this paper.) It depends in general on the nature (charge and mass) and velocity of the particle, and on some of the properties of the medium. The stopping power of a particular medium is often expressed (as in Section II) in dimensionless form by its ratio to the corresponding stopping power of air (that is, for the same particle at the same velocity, and for air at such a density that the number of air "atoms" per unit volume is the same as the number of molecules of the medium per unit volume). This *relative* stopping power is simply the stopping power of a single molecule of the medium divided by that of one-half of an "average" air molecule. Stopping powers of air, for various particles over a wide range of velocities, are well established, largely through the work of Bethe, and are available in convenient graphical form (11, 12, 30). The relative stopping power, which is denoted by s, is convenient in that it is highly insensitive to the charge and mass of the penetrating particle; indeed, it is known from both experiment and theory that for "fast" particles s depends only on the *velocity* of the particle and on the nature of the medium, and

in fact varies rather slowly with the velocity. By a fast particle is meant in this paper one with velocity great compared to v_0, the orbital velocity of an electron in the normal Bohr orbit of the hydrogen atom (v_0 is a fundamental atomic constant compounded of universal constants: $v_0 = e^2/\hbar = 2.188 \times 10^8$ cm per sec $= c/137$, where the symbols e, $h = 2\pi\hbar$, and c have their customary meanings). At the velocity v_0 an electron has energy 13.6 ev, a proton 25 kev, a deuteron 50 kev, an alpha particle 99 kev, and a fission fragment (of mass 120) 3 Mev. For fast particles the stopping power arises almost entirely from individual acts of energy transfer from the particle to electrons of atoms close to the path of the particle; these transfers range in magnitude from small ones, of roughly 5–15 ev, which excite the atom, to greater ones, of energy ranging from the ionization potential P upward, which result in a free electron and a positive ion—and so on, with decreasing probability, to very great energy transfers which ionize the atom and produce a very fast secondary electron. The *mean* energy transfer is always of the order of magnitude of 20 ev.

Theoretical treatment of the stopping power for fast particles has been developed in satisfactory detail, thus far, only for the case of a medium composed of isolated atoms of low atomic number. (In this respect it is fortunate that only light atoms are important for radiobiology.) Indeed, a variety of different modes of treatment is available. A particularly illuminating approach is the so-called "method of impact parameters," which will now be sketched very briefly for later reference. (An alternative model will be demonstrated in Section IV.) We restrict the discussion to fast particles having small positive charge and mass of atomic magnitude, of which the only ones now experimentally accessible are alpha particles and ions of hydrogen (H, D, or T). The other practically important cases, namely fast electrons and fast *heavy* ions ("recoils," fission fragments), will be mentioned later.

For these heavy particles the momentum is extremely great compared to the momentum change in any collision in which momentum is transferred to an atomic electron of the medium. Therefore the motion of the heavy particle is only insignificantly affected by any one momentum (energy) transfer, and the excitation or ionization act can be treated as caused by a uniformly moving Coulomb center of force. If we consider two concentric cylinders with radii p and $p + dp$ and axes on the path of the particle, we easily find from the laws of classical dynamics the energy transfer to electrons initially lying *between* the cylinders:

$$-dE_p = \frac{4\pi z^2 e^4}{mv^2} n \frac{p\,dp}{p^2 + R^2} dx$$

where ze is the charge of the particle, $-e$ that of an electron, m the electronic mass, v the velocity of the particle, and n the number of electrons per unit volume; R is a parameter called the collision radius which measures the "size" of the Coulomb field and is equal to ze^2/mv^2. To find the stopping power we simply integrate over all permitted values of p:

$$-\frac{dE}{dx} = 2\kappa n \int_{p_{\min}}^{p_{\max}} \frac{p\,dp}{p^2 + R^2} = 2\kappa n \ln \left(\frac{p^2_{\max} + R^2}{p^2_{\min} + R^2} \right)^{\frac{1}{2}}$$

The quantity κ, defined as $2\pi z^2 e^4/mv^2$, is called the *stopping parameter*. The factor $2\kappa n$ appears in all formulae for stopping power, for all media. It determines the order of magnitude of the stopping power; all details, however, are contained in the balance of the expression, in which our interest will therefore be centered exclusively. Many of the intricacies of the problem lie in the determination of $p_{\max}$ and $p_{\min}$.

Although superficial examination might suggest that $p_{\min} = 0$, application of the laws of quantum mechanics shows that, if one uses the above, essentially classical, formulation, one must set $p_{\min} = \hbar/mv$. (Thus $p_{\min}$ is just the wave length $\lambda\!\!\!^{-}_e$ which an atomic *electron* has in the coordinate system in which the *particle* is at rest, and the electron therefore moves with velocity v; the position of the electron is "uncertain" by $\lambda\!\!\!^{-}_e$, which suggests that $p_{\min}$ cannot be smaller than $\lambda\!\!\!^{-}_e$, but the fact that $p_{\min} = \lambda\!\!\!^{-}_e$ actually requires a careful justification which will not be given here.) Since $\lambda\!\!\!^{-}_e = \hbar/mv = R(v/zv_0)$, it follows that for fast particles $p_{\min} \gg R$, and

$$-\frac{dE}{dx} = 2\kappa n \ln \frac{p_{\max}}{p_{\min}} \tag{1}$$

It is obviously absurd to set $p_{\max} = \infty$, for this would predict infinite stopping power. Bohr pointed out in 1913 that an upper limit to p is established by a kind of "dynamic" screening arising from the binding of the electrons in atoms of the medium: a bound electron located at a very large distance p from the path of the particle is perturbed adiabatically and no energy is transferred to it. If we consider first that an atom contains one electron bound with frequency $\omega/2\pi$ (binding energy $= \varepsilon = \hbar\omega$), the condition for adiabatic perturbation is "duration of collision" $\approx p/v \approx 1/\omega$, so that $p_{\max} \approx v/\omega$. This value of $p_{\max}$ is correct in quantum theory as well as in classical theory except for a numerical factor, which detailed calculation determines as 2, so that $p_{\max} = 2v/\omega$. Hence, for fast particles,

$$-\frac{dE}{dx} = 2\kappa n \ln \frac{2mv^2}{\varepsilon} \tag{2}$$

The considerations above were oversimplified in one important respect: it was assumed that there is a unique binding frequency $\omega/2\pi$. The quantum theory of dispersion shows, however, that even for the hydrogen atom, with its single electron, it is necessary to treat the atom as an assembly of an infinite number of different "virtual" oscillating electrons; each type has effective number (or oscillator strength) f_n and frequency $2\pi\omega_n = \varepsilon_n/h$. Here, $\sum_n f_n = 1$: the total effective number of such oscillators corresponds to just a single electron. Hence, for the stopping power of a gas containing N hydrogen *atoms* per unit volume, we have

$$-\frac{dE}{dx} = \sum_n f_n \left(2\kappa N \ln \frac{2mv^2}{\varepsilon_n}\right) = 2\kappa N \ln \frac{2mv^2}{I}$$

In this last expression I is defined by

$$\ln I = \sum_n f_n \ln \varepsilon_n \tag{3}$$

The sum should be understood as embracing both the summation over discrete and the integration over ionization states. The quantity I is called the *mean excitation* energy or mean excitation potential. It is in effect a geometrical mean of all possible excitation and ionization energies of the atomic system, each weighted by the corresponding oscillator strength. For hydrogen atoms an exact calculation can be carried through and leads to the value $I = 15.00$ ev, some 10 per cent greater than the ionization potential (13.60 ev).

For atoms with more than one electron the same treatment is applicable, although it must cope with the vastly more complex dispersion model for the electronic frequencies. There is one important restriction, however: in order for the final formula given below to be valid, it is necessary for the incident particle to be "fast" not only with respect to v_0, but also with respect to all orbital electron velocities in an atom of the medium—or, in effect, to the greatest of these, namely that of a K electron, approximately Zv_0. This is a most severe restriction. For oxygen, as an example, $Z = 8$, and at a velocity of $8v_0$ a proton has energy 1.6 Mev, a deuteron 3.2 Mev, an alpha particle 6.4 Mev. If, however, the particle *is* fast compared to Zv_0, the stopping power is given by the celebrated formula of Bethe:

$$-\frac{dE}{dx} = 2\kappa NZ \ln \frac{2mv^2}{I} \tag{4}$$

where N is again the number of atoms of the stopping medium per unit volume, Z the number of electrons per atom (atomic number), and I

the mean excitation energy for *all* the atomic electrons. Unfortunately, it has not yet been possible, except for $Z = 1$, 2, 3, or 4, to calculate I on the basis of theory alone, because of inadequacy of present knowledge of the dispersive properties of the atoms, and this constant must be determined for each value of Z from experimental stopping-power data.* Once I is so established for any atomic medium, the stopping power can be computed at once for any sufficiently swift particle by Eq. 4.†

The restriction of the stopping-power formula to particle velocities great compared to Zv_0 robs it of much of its potential usefulness by removing from its domain of applicability the majority of cases heretofore of practical interest. (Work with particles in the 20- to 1000-Mev energy region is only just beginning.) Thus, even for a medium composed of oxygen atoms, the formula is not valid for any natural alpha particles. Fortunately, it is possible to deduce from theory the necessary correction to the formula, at least in some instances. For intermediate and heavy atoms this is a highly complicated problem which has not yet been very much developed. For light atoms, for which, in the case of all but very slow particles, only the K electrons do not satisfy the velocity criterion, the correction for the modified contribution to the stopping power of the K electrons when v is not great compared to Zv_0 has been calculated quantitatively by Bethe (11) and by Brown (13). This means that the stopping power so corrected is valid for particle velocities great compared to the orbital velocity of L electrons in an atom of the medium, and this is a very much less stringent restriction, so that the formula thus corrected covers a much broader region of applicability.

The above considerations apply strictly only to media which are monatomic gases. Experimentally, this entails limitation to the noble gases He, Ne, A, etc. Metal vapors, *when monatomic*—for example, Hg or Na—would be interesting cases, but their stopping powers have not

* It is possible, on the basis of the Thomas-Fermi model for the atomic frequencies, to deduce from theory an expression giving I of any atom, complete except for a single constant (which is calculable in principle but must at present be obtained empirically). The usefulness of this result has been limited by the fact that the Thomas-Fermi model is trustworthy only for heavier atoms, for which, at familiar particle energies, the Bethe formula does not apply because of the velocity restriction.

† It must be stated that the proof of Eq. 4 has been accomplished only for the special model in which all atomic electrons are assumed to be hydrogen-like. This fact, together with the absence of a purely theoretical calculation of I for a complex atom, has in a sense reduced the formula, which is of the greatest fundamental importance and which must be very accurate for atomic hydrogen, to the status of a semiempirical formula. As such it has been extremely valuable in practice, but it cannot be said to have been rigorously tested as yet by accurate experimental data for a single particle and medium over a broad energy region.

yet been studied. To this list might be added atomic hydrogen, which can be treated very accurately by theory but which is not amenable to experimental observation. To summarize, for all monatomic gases we have an accurate, simple formula for the stopping power, involving one empirical constant for each gas, which is, however, applicable to a successively smaller domain of velocities, the larger is Z. For light monatomic gases there are available numerical values of the stopping power, again requiring an empirically determined constant, which span practically the entire useful range of velocities. For atomic hydrogen and helium we have an exact, purely theoretical formula of very wide applicability. Empirically, however, we possess only a moderate amount of information about the stopping powers of He and A, and rather less about Ne, Kr, and Xe.

IV. Stopping Power of a Polyatomic Gas

No detailed theoretical treatment of the stopping power of a diatomic or polyatomic gas has yet been achieved. There is available, however, a wealth of experimental data on diatomic gases (notably H_2, N_2, O_2, and air) and also some information on a few polyatomic gases.

It must be stated at once that the binding of atoms in molecules does not alter their stopping powers to any great extent. Little is known about the changes that do occur. The problem has not been one of great interest as far as collision theory is concerned—it is at least as much a problem involving the electronic structure of molecules—and since the advance of our understanding of penetration phenomena has, historically, stemmed largely from the practical needs of nuclear physics, the topic has been a neglected one. Yet it surely is important. Not only have such small alterations an intrinsic practical consequence for accurate work; they also may afford valuable clues to far greater effects of molecular structure on other aspects of penetration phenomena; and they are in themselves of much fundamental interest. In this section we shall first appraise the present status of the empirical information and then analyze the molecular problem theoretically.

To illustrate the effect of chemical binding on the stopping power we need only consider the case of hydrogen; here we have experimental data for H_2 and trustworthy theory for H. The empirical data can be fitted to formula 4, with the quantity N interpreted as the number of molecules per unit volume and Z as the number of electrons per molecule (that is, 2). The data are not at all certain enough to determine the value of I as accurately as one might wish, but values in the neighborhood of 18 ev are usually obtained. However, the theoretical value for isolated hy-

drogen atoms is 15.00 ev, and this must be very accurate. The difference between these two values of I is equivalent to a difference in the stopping powers for alpha particles of 3 per cent at 10 Mev, 4 per cent at 5 Mev, and 5 per cent at 1 Mev.

In lieu of theory, interpretation of the stopping phenomena of polyatomic gases has always been based on the so-called "Bragg rule," which supposes each type of atom to have a definite stopping power for fast particles which is a function of particle charge and velocity, and the stopping powers of atoms bound together in a molecule to be *additive*. In practice this rule works surprisingly well. Thus, for example, stopping powers of H, N, and O have been obtained by taking one-half of the empirical stopping power of the corresponding diatomic gas, and that for C by difference from the stopping power of any of a number of gases (or solids) containing carbon. In this way a basic table for H, C, N, and O, and for other atoms as well, is compiled, and is tested with data from as many different compounds as possible, or is used to predict the stopping power of a compound not yet studied experimentally. The Bragg rule can be expressed equivalently—and more conveniently—in terms of the *relative* stopping powers of the elements. However, ranges can in general be measured much more accurately than can stopping powers (although even for range determinations there has been an abundance of overoptimistic assessment of experimental error), and there have been many more measurements of ranges than of stopping powers. For this reason the Bragg rule has often been tested by the additivity of values of S_R, rather than of s. (The distinction between s and S_R was explained above.) But additivity of S_R is not the same thing as additivity of s, because of the variable dependence of these quantities on the particle velocity. The statement of the rule which we have adopted, that is, additivity of values of s, is the more fundamental, and is equivalent to additivity of S_R if, and (barring highly unlikely accidental compensations) only if, the ratios of all s values for atoms in the molecule, at all velocities less than the initial particle velocity, are velocity-independent. This tacit assumption is never exactly valid, and is more in error, the greater the disparity in atomic numbers of the atoms in the molecule, and the smaller the initial particle velocity.

However, it should always be borne in mind that the Bragg rule does not rest on any quantitative theoretical basis. As data on stopping power improve in accuracy we must anticipate ever more numerous and more consequential departures from the rule. At the present time, because of inaccuracies in stopping-power data, the observed departures are not certain enough to be considered as established, far less to have revealed any pronounced regularities. The smallness of the departures

does not mean, of course, that binding of an atom in a molecule has *no* effect on its stopping power. Rather, it implies that the binding of an atom in different molecules has approximately the same effect on its stopping power in each. Since the demands of valence lead to an approximate uniformity in conditions of binding, this is certainly reasonable.

If one would search for a disagreement with the Bragg rule, he should investigate different compounds of an atom in which the nature of the chemical binding is known to differ greatly. Furthermore, the atom should be as light as possible, for binding affects only the valence electrons, so that it is for the lightest atoms that the effect will be most pronounced. How great the effect may be is easily estimated to order of magnitude but is not known with any certainty. As mentioned above, theory has thus far provided no quantitative information, and, moreover, experimental stopping-power data are not yet accurate enough to shed light on the question. As a rule, investigators have greatly underestimated the systematic errors in their measurements; and in interpretations such vital factors as the velocity dependence of the relative stopping power, the distinction between relative range and relative stopping power, and necessary corrections to the Bethe stopping-power formula have all too often been disregarded or dealt with inadequately. The writer views with some skepticism the claims of many stopping-power data to an accuracy of ± 1 per cent or better. Even the accepted values for the stopping powers of air, surely the most carefully investigated of any substance, have changed as much as 5 per cent between 1937 (11) and 1950 (12). In perhaps the most thorough analysis of the validity of the Bragg rule yet made, Gray (10) concluded that the atomic S_R values are additive in molecules in almost all cases to within ± 1 per cent. This conclusion, based upon data for alpha particles in the 5- to 9-Mev energy region, would probably be equally valid for the true stopping powers. However, it is, perhaps, also an underestimate. Whereas in many cases the Bragg-rule discrepancy may indeed be 1 per cent or smaller, in others it may ultimately be found to be somewhat greater—perhaps 2 or 3 or even 5 per cent. That it will ever be found to be very much greater than the last figure for any gas (except at very low particle velocities) is most unlikely. The entire problem is certainly ripe for refined reinvestigation. It would seem that among the most promising cases are those for which large discrepancies have already been reported. Thus, to cite only a few: Schmieder (14) found "the" stopping powers of N_2O to be 92 per cent, of NO to be 109 per cent, and of NO_2 to be 113 per cent, of the respective Bragg-rule predictions; data of Gibson and Eyring (15) lead to a stopping power of

azomethane which is 4 per cent "too low"; Förster (16) found H_2O vapor to have 3 per cent smaller stopping power than an equivalent mixture of H_2 and O_2. Such results are usually discounted [for instance, cf. Gray (10)].

Turning now to theory, we may recognize several more or less distinct factors which should cause the stopping power of a diatomic or polyatomic molecule to differ to some extent from the simple sum of stopping powers of the isolated atoms of its constituents. They are:

1. Binding of atoms in a molecule changes the values of the possible excitation energies (ε_n) of the system, and also of the associated oscillator strengths (f_n).
2. The incident particle may transfer small quantities of energy to vibrational modes of the molecule.
3. Energy may be transferred to rotational modes of the molecule.

For the last two effects the existence of a permanent dipole moment of the molecule plays a decisive role; for the first also it has, in a sense, an indirect influence. We shall consider each of these factors, briefly, in turn.

The first factor is undoubtedly the most, and except at low particle energies is probably the only, important one. It affects the stopping power by altering the value for the molecule of the mean excitation energy I, as may be seen from Eq. 3.* Insight into the working of this influence is gained by considering the Williams-Weizsäcker method of treating the collision problem (which was discussed above in terms of impact parameters). For collisions in which the energy transfer to an electron is great compared to the ionization potential of the latter the

* Mention must again be made that the Bethe stopping-power formula 4 has not been demonstrated to apply exactly in the case of a molecular medium. Conceivably, two sorts of complication may exist. One, that the spatial distribution of atoms is not random, as inherently assumed in the derivation of formula 4, is almost always without influence. (It might have an effect for radiations of extremely great specific ionization.) The other, that the valence electrons are *necessarily* far from hydrogen-like, is in a general way covered by the alteration of ε_n, f_n values discussed above, provided that formula 3, as properly generalized for a many-electron system, is valid. This effect has not been studied. However, Williams (17, cf. p. 24) has mentioned the possibility that deviations from hydrogen-like binding of the electrons in helium might be responsible for a 10 per cent departure in stopping power from the prediction of the hydrogen-like model, the latter yielding too great a stopping power. Such an effect in He would be a close analog to the effect in a valence bond of a molecule. It would be of great interest to compare accurate stopping-power data for He (which are not available at present) with the results of a refined theoretical calculation based on an accurate dispersion model, such as is provided by the work of Vinti (18) and of Huang (19). (The former has shown, for example, that 2 per cent of the total oscillator strength of the He electrons arises from double excitations, which are automatically ignored in the hydrogen-like approximation.)

difference between molecular and atomic binding of the electron is certainly insignificant. But of approximately equal importance for the total stopping power are those collisions in which the energy transfer is of the same order of magnitude as the ionization potential. For such collisions the penetrating particle has an effect equivalent to electromagnetic radiation, the frequency distribution of which corresponds to a Fourier analysis of the impulsive field of the passing particle. The energy transfers are thus closely related to the optical absorption spectrum, both discrete and continuous. (This relation was encountered, in the discussion above, as the dependence of I on the dispersion.) Now molecular binding has a decisive effect on the discrete spectrum and would without doubt be known to have as great an influence on the continuous absorption in the vicinity of the absorption edge, were our knowledge of the continuous absorption in the case of molecules sufficiently advanced. We must thus anticipate a pronounced alteration by molecular binding of the details of energy loss in these "lighter" collisions, a somewhat smaller influence on the over-all energy loss, and a still smaller but nevertheless appreciable influence on the stopping power, which, as may be recognized by inspection of formula 4, depends chiefly on $2\kappa NZ$ and less sensitively on details of the binding of the electrons.

Molecular binding will also influence, to some extent, the oscillator strengths, but not appreciably the binding energies, of *inner* electrons. This is related to the fact that the oscillator strength of an inner electron is smaller than unity, because its transitions to occupied discrete states are not possible. For a molecule there is, in crude terms, a fuller occupancy of the lowest discrete states, thus decreasing the total oscillator strength of an inner electron. This decrease must be compensated by an augmentation of oscillator strengths of outer electrons, so that one might expect I in molecules to be decreased and the stopping power therefore increased by this effect. However, the character and positions of the energy levels are so altered in the molecule that it is not certain whether this conclusion has any validity.

The second and third factors listed above are simply additional modes of energy loss, and must be added to the energy loss to electrons in order to obtain the total stopping power. Some energy transfer to vibrational modes will in general accompany electronic excitation, of course; this follows from the Franck-Condon principle and the fact that bond distances in excited states usually differ from those in the ground state. However, energy transfer to vibrational and rotational modes can occur independently of electronic excitation, as is seen at once by considering the equivalent radiation field of the particle. This view also shows that, as in the corresponding optical effects, the energy transfer will be neg-

ligible unless the molecule possesses a permanent electric dipole moment.* The stopping power arising from these two types of energy loss has not been calculated.† However, we may easily deduce the following approximate expressions which must be correct, at least in order of magnitude, and which suffice for the present discussion:

$$-\left(\frac{dE}{dx}\right)_{\text{vib}} \approx \kappa N \left(\frac{w_{\text{vib}}}{me^4/2\hbar^2}\right)\left(\frac{\mu}{\hbar^2/me}\right)^2 \ln \frac{2mv^2}{w_{\text{vib}}} \tag{5}$$

$$-\left(\frac{dE}{dx}\right)_{\text{rot}} \approx \kappa N \left(\frac{w_{\text{rot}}}{me^4/2\hbar^2}\right)\left(\frac{\mu}{\hbar^2/me}\right)^2 \ln \frac{2mv^2}{w_{\text{rot}}} \tag{6}$$

Here w_{vib} and w_{rot} are the energies of the first molecular vibrational and rotational levels, respectively. Note that this contribution to the stopping power is not of order of magnitude κN: the great magnitude of atomic relative to electronic mass enters through the small value of w (in atomic units). The equations show at once that these contributions are small compared to the electronic stopping power for fast particles, being roughly 10^{-2} and 10^{-3} of the total, respectively: the small factor $w/(me^4/2\hbar^2)$ dominates the greater logarithmic factor. In the language of the method of impact parameters, the energy loss is smaller because of the greater mass (atomic rather than electronic) which must be set in motion, although this is partly compensated by the greater $p_{\max}$—greater because the dynamic screening is less stringent and permits energy transfer to greater distances. In the language of the Williams-Weizsäcker method, the energy transfer by emission and absorption of virtual, infrared quanta is smaller, although the smaller energy in such quanta is partly compensated by their greater abundance in the Fourier spectrum.

The magnitude of these two additional contributions to the stopping power, relative to the total, is not, however, independent of the energy

* There is always a small probability of vibrational excitation arising from collisions in which the particle actually passes through the molecule. This probability is small compared to that for "indirect" excitation in the case of polar molecules, and for fast particles is always insignificant in its contribution to the total stopping power. For extremely slow particles (for example, electrons of a few-electron-volt energy) it is known to be appreciable, *even for homopolar molecules*, but this case does not concern us in the present study.

† Massey (20) has derived an expression for the cross section for rotational excitation which leads at once to our expression (Eq. 6) for the stopping power, but which is valid only if the dipole moment μ is small compared to one-half of an atomic unit ($\hbar^2/me$). However, Eq. 6 is still approximately correct for greater values of μ, a fact worth noting because μ is comparable to $\hbar^2/me$ for many molecules of practical importance. Cf. also Wu (21).

of the particle. Both increase, relatively, as the energy declines. The contribution of rotational excitation is, of course, much the smaller of the two. Although for fast particles they are beyond the discrimination of present stopping-power data, it is quite possible that excitation of vibrations may play a detectable role for particles having velocity of the order of magnitude of, or only a few times greater than, v_0.

We shall now examine a specific and most important example, molecular hydrogen. The difference in stopping power between the molecule and the extreme of *isolated atoms* has already been emphasized, for $I_{\text{isolated}} = 15.00$ ev, whereas $I_{\text{bound}} \approx 18$ ev, the former being an exact value from theory and the latter a very approximate one from experiment. A theoretical treatment of the stopping power of molecular hydrogen, or, in effect, a theoretical calculation of I_{bound}, has not yet been achieved. It would be a valuable contribution and appears to lie within the realm of possibility. Values of ε_n for the molecule are, of course, known with great accuracy from analysis of the molecular spectrum. They show a crude, but necessarily far from close, similarity to the corresponding values for the hydrogen atom. The disparity, which arises chiefly from the difference in binding energies for the different states, is enhanced by the fact that transitions induced by the passing particle may not appreciably alter the internuclear separation—this being the demand of the Franck-Condon principle—and hence, because all excited states have considerably greater equilibrium separations than the ground state, always leave the excited molecule in a high vibrational level. This coupled vibrational excitation is about $\frac{1}{2} - 1$ ev for most excited (attractive) states. For all ionization processes it is slightly greater than 1 ev. For "repulsive" excited states, of course, a great additional amount of energy is transferred as momentary potential energy of the molecule.

Although a detailed calculation of I_{bound} for molecular hydrogen will not be attempted here, a rough estimate may readily be given. We write Eq. 3, appropriately generalized to the many-electron case, in the form

$$\ln \frac{I}{P} = \frac{1}{Z} \sum_n f_n \ln \frac{\varepsilon_n}{P} \tag{7}$$

where P is the ionization potential. In the single instance in which this expression can be evaluated exactly, namely atomic hydrogen, it is found that $I/P = 1.102$, and it has been common practice to assume that this ratio has the same value for certain other related atoms and molecules; thus, for molecular hydrogen (for which the ionization potential is 15.43 ev), it is assumed that $I = 1.10P = 17$ ev. This pre-

scription is not correct, however. Exchange effects in atoms other than hydrogen, or in molecules, effectively "tighten" the binding, and this may be considered to have two more or less distinguishable results: the ionization potential P, and the various excitation energies ε_n *relative to* P, become *greater* than the predictions of the hydrogen-like model; and the ratio I/P is increased.* The last-mentioned effect derives in part from the higher values of excitation energies (even when expressed relative to P), and in part from a shifting of oscillator strengths toward higher excitations and especially ionizations. (In general, the total fraction of oscillator strengths residing in the continuum is greater, the more saturated is the character of the valence-shell binding.) It is obviously necessary to take both effects into account. Thus, for hydrogen, $P_{\text{at}} = 13.60$ ev and $P_{\text{mol}} = 15.43$ ev. The first excited atomic level ($2p$) has $\varepsilon/P_{\text{at}} = 0.75$, but the corresponding molecular levels ($2p$, $^1\Sigma_u^+$ and $2p$, $^1\Pi_u$) are located at about $\varepsilon/P_{\text{mol}} = 0.81$. Similarly, higher molecular levels also exceed corresponding atomic levels in their values of ε_n/P. This analysis is impeded, however, by the fact that not much is known, either empirically or from theory, about the detailed dispersive properties of molecular hydrogen. However, a preliminary study has been reported by Mulliken and Rieke (22). Their results give a total f of 0.31 for the first excited levels (specified above), per atom, compared to 0.42 for the corresponding atomic level. The second group of levels also has lower f for the molecule. Thus both the effects of departure from hydrogen-like binding are apparent in the case of H_2. We therefore conclude that the ratio I/P should be greater for the molecule than for the atom. Unfortunately, in the absence of theoretical information concerning oscillator strengths for transitions to the continuum (and of empirical information on the continuous absorption of H_2) this analysis cannot be carried much further with confidence. A crude estimate leads to $I_{\text{bound}} \approx 1.2P_{\text{mol}} \approx 19$ ev. The effects under discussion all tend to make the stopping power of the molecule *smaller* than that of the separated atoms. Thus, the above estimate suggests a decrease of about 5 per cent for a 5-Mev alpha particle. It would be of great interest to construct an approximate but complete model for the dispersion of molecular hydrogen, carry through the analysis sketched above, and then compare the conclusion with accurate empirical stopping-power data which will eventually become available. (Those now at hand give merely $I/P \approx 1.1 \pm 0.1$.)

The rather great molecular effect indicated above will not enter in the application of the Bragg rule to practical cases involving *hydrogen*, be-

* An extreme example is helium, for which the values of ε_n/P *greatly* exceed hydrogen-like values, and I/P has a value of about 1.8.

cause atomic hydrogen is not accessible to stopping-power studies, and the binding of hydrogen in different molecules is of a particularly uniform character. One would anticipate only minor deviations (ordinarily smaller than 1 per cent) in the contribution of hydrogen to the stopping power for fast particles of various gaseous compounds, the mean value probably being close to the value for $\frac{1}{2}H_2$. Nevertheless the analysis for H_2, the simplest of all molecules, is of interest as a guide to the understanding of more complex molecules.

For such molecules the alterations in f_n, ε_n values arising from molecular binding will be vastly more complex, and there is hardly hope that they will yield to detailed theoretical analysis. Of course, there may be discovered some method of deducing the effect of chemical binding on the stopping power in a general way, without elaborate analysis of the f_n, ε_n values. Some promise is also offered by the possibility of determining and analyzing the ultraviolet absorption spectra (including continua).

Only transitions involving a valence electron will be strongly affected by molecular binding. Thus deviations are likely to be suppressed for all but the lightest elements, notably C, N, O, and F. Because the character of the valence bonding in molecules containing these atoms can vary most markedly, significant deviations from the Bragg rule should be anticipated. Double bonds, triple bonds, and resonating-group structures (such as benzene) seem especially suggestive in this respect. Because of the inert character of the inner electrons, however, deviations exceeding about 5 per cent will be rare. Molecules, such as NO and NO_2, having an odd electron, may present unusual features.

V. Stopping Power of a Solid or Liquid

The Bragg rule of additivity of stopping powers is usually presumed to be applicable to liquids and solids as well as to polyatomic gases. One would anticipate, however, that the rule should in most circumstances be even more in error for condensed media, in so far as the contribution to the stopping power by outer electrons is concerned, because in addition to effects of chemical binding there may enter effects of interatomic or intermolecular interactions involving several or many units of the medium.

Such "collective" effects have been investigated only * for the special and important case of metallic conductors. Here the valence electrons

* In this paper we exclude problems of relativistic velocities, at which there occurs a *decrease* in stopping power, arising from screening of the field of the moving particle by the excited and ionized atoms in its wake, that may be very important for condensed substances. This effect, which has attracted much attention in recent

are essentially "free," and naive application of the method of impact parameters, and Eq. 1 with $p_{\max} = 2v/\omega$, would lead to a paradox for these electrons, for which $\omega \sim 0$. Resolution of this paradox, and formulation of the theory for the contribution to the stopping power by conduction electrons, have been given by Kramers (23) [cf. also A. Bohr (24)]. In essence, it is found that the effective "radius of action" of the field of the moving charged particle is, after the polarization of the medium by the passage of the particle has been taken into account, not simply $p_{\max} = 2v/\omega$, but rather $p_{\max} = 2v(\omega^2 + 4\pi e^2 n/m)^{-\frac{1}{2}}$. (Here n is the number of electrons per unit volume; thus $p_{\max}$ depends on the *density*.) For all media except metallic conductors this quantity is effectively the same as $2v/\omega$, since $4\pi e^2 n/m$ is always smaller than ω^2 and may be very much smaller, and the state of aggregation is consequently without influence. For metals the dynamic screening (by conduction electrons) does not exist, for $v/\omega \sim \infty$, and it is $p_{\max} = 2v(4\pi e^2 n/m)^{-\frac{1}{2}}$ that must be used in Eq. 1. This leads at once to a stopping power for valence electrons different in the gaseous and metallic states.

Beryllium is the only substance in which this effect has, thus far, been demonstrated. Beryllium vapor cannot be studied, but A. Bohr (24) has computed its stopping power for 1-Mev protons and also that of metallic Be, the latter by adding to the stopping power of the K shell the stopping power of the conduction electrons calculated by the theory of Kramers. The result, which is *smaller* by 7.4 per cent than the theoretical value for the vapor, agrees very well with accurate measurements on Be foils by Madsen and Venkateswarlu (25), who found a difference of 9.1 ± 1.8 per cent.

As always, the influence of inner electrons, which are essentially unaffected by changes in the state of aggregation of the atoms, is such as to suppress the influence of valence electrons, which are so affected, and with presently achievable experimental accuracy the polarization effect is detectable only for metals of low atomic weight. Metallic lithium should exhibit an effect, but one less pronounced than that in Be because it has only one conduction electron. Recent data do provide a value of I for lithium, but a detailed analysis has not yet been given.

In the case of non-metallic solids and liquids, virtually nothing is known, either from experiment or theory, about possible effects of state of aggregation on the stopping power. Such effects again involve only

years, has been treated theoretically by Fermi, Halpern and Hall, Wick, and others. It is negligible (that is, of the order of magnitude 0.1 per cent, at most) in practice for fast (but non-relativistic) particles—particles having velocity v in the range $v_0 \ll v \ll c$—except in the case of metals, discussed above.

the outer atomic electrons. They must also influence the ultraviolet absorption spectrum, but about the latter, too, very little is generally known. It has been observed that dissolving a substance in a *non-polar* solvent generally affects its electronic absorption spectrum only slightly, and this fact might seem to point to only small differences between the stopping power of a molecular solid or liquid and the corresponding vapor. It should be realized, however, that such observations have been restricted, in effect, to the portion of the spectrum readily accessible, that is, that in the visible and near ultraviolet regions. Light absorption to higher levels, and especially to ionized states, *would undoubtedly be strongly modified.* And it is just these higher excitation processes which are decisive in determining the stopping power. (The pronounced effect on the stopping power of an alteration in the distribution of oscillator strengths throughout the continuum has been noted previously—for example, in connection with helium.) The absence of empirical studies or theoretical analysis of this alteration * evidently calls for some caution in assuming the accurate applicability of the Bragg rule to molecular solids or liquids of low molecular weight.

The above remarks view the influence of the medium as a perturbation acting on a single atomic or molecular entity. In the case of a condensed substance one must also deal with collective effects arising from the mutual coupling of several or many such entities. To such effects are attributed the fact that the excited energy levels of a crystalline solid are vastly different from those of the corresponding vapor. (The alteration exceeds that arising from binding in a polyatomic molecule.) The electronic levels of liquids are virtually unknown, but would without doubt be rather similar to those of analogous solids. Admittedly, liquids possess a lower degree of order (although not greatly lower), but this is not very important. (Thus liquid metals exhibit almost as great electronic conductivity as the corresponding solids.)

For polar media the influences referred to above are greater, and it is surprising that the behavior of such media with respect to penetrating radiation has practically never been studied. The alkali halide crystals would seem an especially attractive case for study, for their dispersive properties have been extensively investigated. Lithium fluoride, in particular, is likely to exhibit pronounced deviation from the Bragg rule,†

* Instructive attempts to understand the modification of *lower* transitions have been made. An outstanding instance is the red shift of the center of gravity of the resonance absorption line of mercury atoms dissolved in water. Cf., for example, the note of Phibbs (26) and literature there cited.

† As an hypothetical and extreme model one might consider a medium of hydrogen in which the constituents are alternately H^+ and H^-. Here the electronic stopping power for fast particles would exceed that of H_2 by roughly 50 per cent!

and measurement of its stopping power (which could readily be accomplished, for example, using the extremely fast particles provided by the new accelerators), and a parallel theoretical study based on its known optical properties, would appear to be equally feasible and straightforward, and a promising subject for investigation.

It deserves mention that stopping-power and range data for several solid organic substances and for mica have been accumulated, and—in those cases in which the data have been "interpreted"—the Bragg rule apparently confirmed. It would appear, however, that the uncertainties in the component atomic stopping powers as well as in the experimental data do not permit a clear analysis of the quantitative extent of the agreement.

A qualitative analysis of the difference in stopping powers of a molecular solid or liquid and its vapor can be given readily. In the condensed state the intermolecular fields alter the excitation levels in two ways which can be distinguished formally: the levels are *broadened* and may be split into a number of components. The latter effect results essentially in lower energies of excitation for a given electron. (This shifts an absorption line "toward the red," an effect often observed, and is to be understood as a consequence of the fact that an excited electron is always *attracted* in the internal field of a neighboring molecule.) Such an alteration results in an increased stopping power; this can be seen directly from formula 3, in which I has been decreased, or more physically from the Williams-Weizsäcker view in terms of the increase in number of virtual radiation quanta for the greater wave length. The magnitude of this shift will be greater, the higher the level of excitation. (This statement can be understood simply as following from the greater size of the orbit of a more highly excited electron, with greater consequent penetration of neighboring molecules.) The continuum will, indeed, be more radically altered. But the broadening of the energy levels by the strong fields present will also have an effect on the stopping power; even a *symmetrical* broadening will lower the stopping power slightly (cf. again Eq. 3). It is concluded that the stopping power of the condensed state must always be greater than that of the vapor. These effects will be discussed again in Section VII with specific reference to liquid water. It would be a valuable advance if a general, semiquantitative theory for them could be developed, based upon a simple model.

VI. Stopping Power and Range in Water Vapor

The object of this section is the assembly of data on stopping-power and range relations for water vapor, with as great accuracy as may at present be achieved, using both experimental and theoretical information. The difficulties which this comparatively simple problem presents, and the unsatisfactory and inaccurate way in which many of these difficulties must be met, vividly underscore some of the *quantitative* shortcomings in contemporary understanding of energy-loss problems. The results which are obtained, uncertain as they are, are nevertheless useful as a basis for discussion of the phenomena for the liquid state and will doubtless prove helpful in the future as a starting point for more accurate experimental or theoretical work.

The Bethe stopping-power formula 4 and a modified application of the Bragg rule compose the basis for the calculations. Of course, Eq. 4 gives the stopping power directly, in the energy region in which it is valid, once the mean excitation energy for isolated water molecules, $I_{H_2O}(g)$, is known. In the extensive energy region in which it is not valid, resort must be made to approximations, some moderately satisfactory, others highly unsatisfactory. The various complications, and the manner in which they are met, are now itemized and explained.

1. The Bragg rule predicts that $I_{H_2O}(g) = (I_H^2 I_O^8)^{1/10}$. Very accurate values of I_H and I_O are not available, however, and there is sensible disagreement between various "adopted" values in the literature. Thus, data given by Livingston and Bethe (11, Table XLIX, p. 272) lead to $I_H = 14$; Joliot-Curie *et al.* (27, p. 19) give $I_H = 16.0$, $I_O = 100$; from S_R values adopted by Gray (10) we calculate $I_H = 18$, $I_O = 95$; Hirschfelder and Magee (28) give $I_H = 17.93$, $I_O = 98.9$. (All values of I are in electron volts.)

We adopt the values: $I_H = 18$, $I_O = 94$. From these values the Bragg rule predicts that $I_{H_2O}(g) = 68$ ev. We estimate the corresponding uncertainty as ± 3 ev.

2. The actual value of $I_{H_2O}(g)$ must depart to some extent from the prediction of the Bragg rule. This departure is not known and cannot even be estimated with any confidence. Considered very crudely, the binding is "looser" in H_2O than in $(2H_2 + O_2)$, as evidenced, for example, by the low ionization potential, and one might suppose $I_{H_2O}(g)$ to be slightly smaller than the Bragg-rule prediction. However, the possible influence of the permanent electric dipole moment of H_2O on the ultraviolet dispersion, and hence on $I_{H_2O}(g)$, is not known, so that very little weight should be given to this reasoning. Also, Förster (16) found the stopping power of water vapor for alpha particles, averaged

over the energy interval from 5.1 to 2.7 Mev, to be 2.4 ± 0.4 per cent smaller * than the corresponding average for an equivalent mixture of hydrogen and oxygen, thus leading to an $I_{H_2O}(g)$ *greater* than the Bragg-law prediction by about 8 per cent, a deviation of opposite sense to that suggested above. We shall not take this single measurement into account, but it is obviously important that the experiment be repeated and a result established. The only other reported measurements of the stopping power (in distinction to the range or to S_R) are those of Crenshaw (29). However, Crenshaw's data all lie at low energy, the highest being at 0.17 Mev (protons). At this energy the theory is so very untrustworthy that the experimental data cannot be translated into a value of I with any certainty whatever.

We adopt the value: $I_{H_2O}(g) = 65$ ev and estimate the error as ±6 ev.

3. At moderate and low energies the Bethe formula fails to account properly for the contribution to the stopping power of the K electrons of oxygen. However, a satisfactory method for correcting the formula has been given by Livingston and Bethe (11) and more recently by Brown (13). We have used the results of Brown to calculate this correction, which amounts to about 3 per cent of the stopping power at 2 Mev and 2 per cent at 4 Mev (for protons).

4. At low energies the Bethe formula, corrected for the contribution of the oxygen K shell, fails to account properly for the contributions of the remaining 8 electrons. There is no satisfactory way at present to correct for this failure. We therefore employ a prescription devised by Hirschfelder and Magee (28), which advocates:

(*a*) Assumption of the Bragg rule (that is, assumption that the correction is the same as that for 2 hydrogen K electrons and 6 oxygen L electrons).

(*b*) Assumption that the correction for the hydrogen K electrons is given by the same theory (and has the same analytic form) as that for K electrons of heavier atoms.

(*c*) Assumption that the contribution for the oxygen L electrons is also of the same analytic form.

The objections to this procedure are:

(*a*) That the Bragg rule is certainly less valid at low energies than at high.

(*b*) That the Livingston-Bethe and Brown correction is based on use of the Born approximation, which for these low energies is equivalent to the assumption that the charge of the passing particle is *very small*

* We have corrected Förster's quoted result, 3.0 ± 0.5 per cent, for a temperature variation that he apparently neglected.

compared to the nuclear charge of the atom under consideration. This is clearly not the case for hydrogen and must lead to an error which may be serious and is as yet completely unknown.

(*c*) That this assumption too has never been tested and must be in error. (The calculation of the corrections is based on the use of hydrogen-like wave functions, and the L wave functions are far from hydrogen-like.)

There being no alternative way of effecting these corrections theoretically, and no empirical data to aid in doing so, we adopt the scheme of Hirschfelder and Magee, utilizing, however, the K-correction function of Brown.

5. At low energies (below about 2.5 Mev for alpha particles, 0.6 Mev for protons), the phenomenon of capture-and-loss of electrons by the penetrating particle modifies the stopping power, both by modifying the normal mode of energy loss to electrons, and by itself contributing an additional energy loss. There is at present no way of estimating these effects with meaningful accuracy. However, in view of deficiencies in our treatment underlined under 4 above, to do so would not appreciably improve the stopping-power values at low energies. We therefore ignore them.

Crenshaw (29) has given stopping-power data at low energies (cf. Fig. 1). We have accordingly drawn the stopping-power curve at low energies to agree with these data, and otherwise to follow the general course of the calculated values.

6. At very low energies the stopping power considered above is augmented by an additional contribution arising from energy transfers to vibrational and rotational modes of the molecule. However, very rough estimate shows this contribution to the stopping power to be only 0.5 per cent for 1-Mev protons and 1 per cent for 300-kev protons. It is never great and is appreciable only at energies so small that our stopping-power values are for other reasons grossly in error. We therefore neglect it.

The results of the calculations are presented as follows:

Figure 1 gives values of s, the stopping power of water vapor relative to that of air. Values of the stopping power of air were obtained from semiempirical data given by Bethe (30). For a proton of energy greater than about 0.6 Mev the value of s is the same as that for an alpha particle of the same velocity (or energy approximately 4 times the proton energy). As the energy decreases the values of s become progressively less certain, and the error is different for protons and alpha particles: at low energies the s curve should separate into two curves, one for protons and one for alpha particles. Experimental points determined by Cren-

shaw are indicated in the figure. The peculiar shape of the curve at low energies is anticipated, since the "Bragg"-type curves of water and of air have their maxima at different energies. As the energy of the particle increases indefinitely, the value of s must approach the ratio of total number of electrons in water and in air, that is, 10/7.22 = 1.39. The curve exhibits a slow approach to this value.

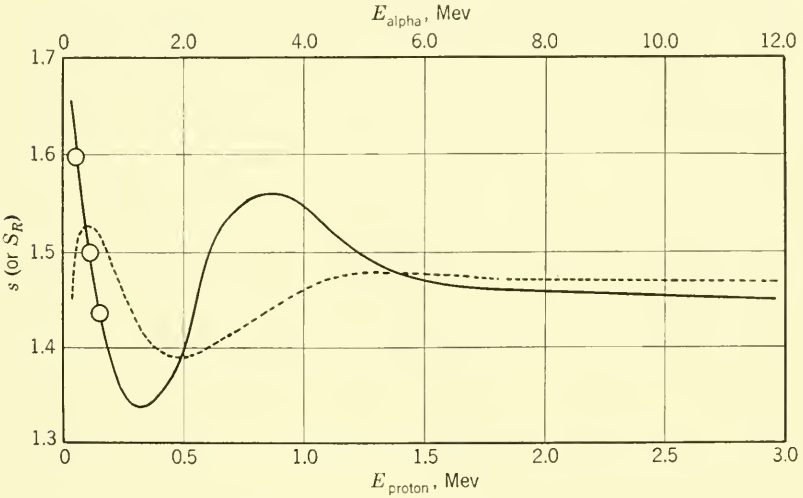

FIG. 1. Stopping power of water for protons and alpha particles, per molecule, relative to one "atom" of air. (Dotted curve is $S_R = R_{air}N_{air}/R_{H_2O}N_{H_2O}$.) The circles are experimental points of Crenshaw (29), whose data were used to establish the low-energy region of the stopping-power curve.

Figure 1 also presents values of the relative ranges in water and air, as represented by $S_R = R_{air}N_{air}/R_{H_2O}N_{H_2O}$. These are more uncertain, at proton energies less than several Mev, than values of s. The error in S_R is different for the two particles; thus the S_R curve should in fact separate into individual curves for protons and alpha particles, and at a greater energy than that at which the individual s curves separate.

Figure 2 gives values of the range of protons and alpha particles in water vapor, calculated for a density of 1 gm per cm^3. In any application the appropriate range from the figure must be divided by the density of the vapor. The range-energy relation was obtained by numerical integration of the stopping-power data. For proton energies greater than about 2 Mev it should be a trustworthy guide, within the limitations set forth above, *except* for an unknown additive constant which arises from the erroneous stopping powers at low energies. This unknown additive constant is different for protons and alpha particles.

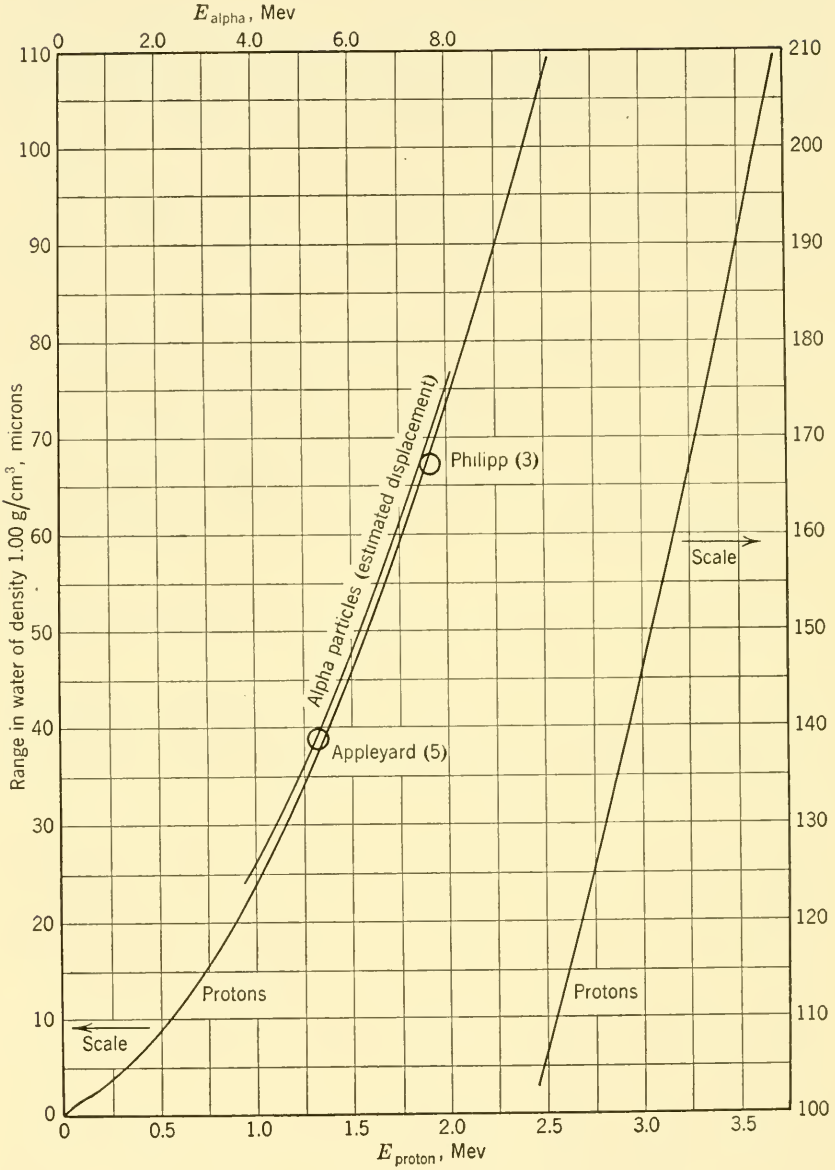

FIG. 2. Range in water of protons and alpha particles.

In effect, then, the curve must be shifted up or down by an unknown amount, different for the two particles. We estimate this shift as probably smaller than 2 microns. At high energies its role is negligible; at lower energies differences in ranges for two different energies are predicted more accurately than either range itself.

Only two empirical range data for water vapor have been reported: a value for RaC′ alpha particles by Philipp (3) and one for Po alpha particles by Appleyard (5). The corresponding points are indicated in Fig. 2. Both agree fairly well with our calculated curve; neither is considered trustworthy enough to justify its use in establishing the curve (or in estimating the unknown correction to the range for alpha particles).

VII. Stopping Power and Range in Liquid Water

The various factors to which any difference in stopping power between liquid and vapor water must be ascribed have been discussed qualitatively in Section V. The stopping power of the liquid must indeed be greater than that of the vapor, because the dominant change will be the depression of excited energy levels by the internal electrical fields of adjacent molecules. That this perturbation is great for *all* excited states of H_2O (except possibly the lowest triplet state, which may be strongly excited as an intercombination permitted in the external electrical field) can be seen from a crude model in which the excited electron is considered to move in a hydrogen-like orbit with effective nuclear charge of roughly e. Then the lowest excited state has $n = 3$, and corresponds to a Bohr orbit of radius approximately equal to $9(\hbar^2/me^2) = 4.8$ Å, whereas one-half the intermolecular distance (in liquid water) is 1.5 Å. Thus the interaction is always strong and all excited states are strongly perturbed. Moreover, water possesses a remarkably great proportion of weakly bound electrons: six of the ten have ionization potentials in the range from 12 to 17 ev!

Even an approximate theoretical calculation of these effects and the resultant change in the mean excitation energy I would be exceedingly difficult and will not be attempted here. (It would require not only estimation of the changes in ε_n but also of those in the f_n values, particularly the f_n pattern in the continua.) We can only repeat that the theoretically anticipated shift of I is of the correct *sense*. Whether it is of the correct magnitude can be answered only by a detailed theoretical analysis, and also only after the conflict in experimental results is resolved.

It should be mentioned here that the change in I has sometimes been stated to be approximately equal to the energy of vaporization—for

water, about 0.4 ev. This is a fallacy. The ground state is shifted by this amount, but the shifts in *excited* levels are virtually unrelated to it.

The rather close agreement between the older range determinations by Michl and by Philipp and the recent stopping-power measurement by Appleyard has already been stressed. This is important (to the extent that any credence can be granted to the old experiments) because of the weakness in the theoretical basis for the calculation of the absolute range: the phenomena at low energy—for example, capture-and-loss of electrons—are not in the least understood quantitatively. It is interesting to note that the difference in ranges of Po and RaC′ alpha particles, calculated from the measurements of Michl and of Philipp (27.5 μ), agrees much more closely with the result of de Carvalho and Yagoda (29.1 μ) than do the absolute measurements, suggesting the possibility that at least part of the disparity lies at the end of the range, where the experiments may measure something different from what is claimed. Some disagreement for the high-energy portion of the range remains, however. We may summarize the experimental results by presenting values of $I_{H_2O}(l)$ calculated, respectively, from the Po, RaC′ range differences determined by Michl and by Philipp, from the same range differences determined by de Carvalho and Yagoda, and from the stopping power determined by Appleyard. These results are presented in Table 2. In addition to $I_{H_2O}(l)$ is given the corresponding value of

TABLE 2

Summary of Values for the Mean Excitation Energy of Water

Medium	Origin of Data	$I_{H_2O}(l)$, ev	I', ev
Liquid	Michl (1); Philipp (3)	40 ± 6	20 ± 4
Liquid	Appleyard (4)	42 ± 5	21 ± 3
Liquid	de Carvalho-Yagoda (7)	48 ± 4	25 ± 3
Vapor	Bragg rule (cf. Section VI)	68 ± 3	38 ± 2
Vapor	Estimated (cf. Section VI)	65 ± 6	36 ± 4

I', the contribution to $I_{H_2O}(l)$ of the eight *outer* electrons of water, calculated from the formula

$$I' = (I_{H_2O}{}^{10} I_K{}^{-1.89})^{1/8.11}$$

where I_K is the effective excitation energy of the K shell (31). Analogous quantities for the vapor are included to facilitate comparison. The data for I' show at once the unsatisfactory state of affairs. This has a two-fold basis: not only are the experiments with the liquid in disagreement, but the uncertainties in the deviation from the Bragg rule and in the adopted values of I_H and I_O preclude a trustworthy comparison of

liquid and vapor. It is evident that there is very little at the present time in the way of a quantitative basis for theoretical study of the effects of the liquid state. One may conclude that there is moderately strong evidence for a significant decrease in I' in the liquid state, and that at present the most likely estimate of the extent of this decrease gives about 20–30 per cent. In view of the important fact that the value of I' in the vapor is so much greater than the relevant ionization potentials, such a decrease in the liquid state must be regarded as fairly plausible.

VIII. Ionization in Liquid Water

Even if the anomalously great stopping power of liquid water should ultimately prove spurious or greatly exaggerated, this would not evidence that liquid water must behave like a gas in respect to other penetration phenomena—for example, and especially, in respect to the total ionization. And, if a small stopping-power anomaly should remain, this may imply, and provide a key to the understanding of, *great* anomalies in other properties.

The total ionization is most conveniently measured by the mean energy required to form an ion pair, commonly denoted by W. The dependence of the ionization of a gas on molecular structure is familiar from a great number of studies of gaseous systems. Indeed, Bragg, in 1913, in his statement of the additivity rule for molecular stopping powers, pointed out that additivity does not hold for the total ionization. Nevertheless, the patently naive statement that *liquid* water does exhibit behavior similar to a gas in respect to total ionization is very commonly encountered in publications concerned with interpretations of radiation effects on chemical and biological systems. Such an assumption may lead to grave errors.

As mentioned above, the ionization of liquid water by high-energy radiations has never been directly studied; the natural conductivity is too great to permit ion collection by any conventional means. Modern techniques of rapid ion collection may offer some hope of collecting the electrons produced, however, although the slowest electrons might still interact too quickly with the medium. (The interaction of very slow electrons with liquid water is by no means well understood, but recent advances in the interpretation of the interaction of electrons with crystals may provide a valuable basis for analyzing the corresponding problem with water.) The positive ions must interact extremely rapidly, dissociating to $OH + H^+$, the latter "hydrating" in a period of the order of magnitude of the dielectric relaxation time for water, that is, 10^{-11} sec.

Some studies of the ionization by high-energy radiations of non-polar liquids such as carbon disulfide [cf., for example, the work of L. S. Taylor (32)] have sometimes been cited in support of the view that the total ionization in liquids is not much different from that in gases. Passing over the difficulties in experimental measurements and in the interpretations thereof, which are formidable, we must state most emphatically that the assertion that water behaves like a non-polar liquid such as CS_2 is unjustified, to say the least.

Of decisive importance in interpretations of chemical and biological effects of radiations is the spatial distribution of the H and OH radicals which are the secondary products of the energy transferred from the radiation. In essence, it is believed that a pair of radicals formed by the dissociation of an excited H_2O molecule will most probably recombine inside their so-called "liquid cage," whereas such a pair formed subsequent to an ionization act (namely, by dissociation of H_2O^+ and independent dissociative capture of the electron by H_2O) is separated by a distance equal to that traversed by the ionized electron; this distance is in general quite great enough so that the radicals will survive for subsequent reactivity. Thus, grossly speaking, ionization acts are expected to have chemical effectiveness, but excitation acts not. We must recall, however, that the excited levels are all broadened in the liquid, so that higher states (perhaps all but the lowest triplet) are actually continuous and what is an excited state for a free molecule may correspond to an "ionized" state in the liquid. (This phenomenon is familiar in the spectra of gases as a reduction of the effective ionization potential by a strong external electric field.) Indeed, because of the pronounced quasi-crystalline structure of liquid water, the interaction of the excited molecule may be with a *number* of closely coupled water molecules, so that the energy level may approach in properties a conductivity band of short-range order. Thus the "excited" electron may move through many molecular diameters before being trapped—a phenomenon which has been called *internal ionization*. The consequent chemical (and biological) effects of such a process should closely approach those of a normal ionization act. We thus are led to expect an *effective* W (in the sense here adopted) much smaller than that for the vapor. For the vapor, W has been found by Appleyard (33) to be 30 ± 1 ev. For the liquid we should predict a value of the order of magnitude of half of this—roughly 15 ± 5 ev. [It will not be as small as the lowest excitation energy ($\sim$7 ev) because of the generous energy loss to vibrational modes and perhaps also to the chemically non-effective lowest triplet state.]

Considerations such as the above, with refinement of the meaning of excitation and ionization acts in the liquid state and analysis of the

ultimate sums for energy loss, must be of the utmost importance in the formulation of interpretations of mechanisms of radiation action. They may even have serious repercussions in dosimetry—for instance, in the conversion of density of energy loss to density of "ionization." This is true for all types of high-energy radiation, including x-rays, and there may exist unknown dependencies on energy or character of radiation. Even the common practice of equating two different types of radiation, or radiations of different energy, on the basis of equivalent total gaseous ionization, is suspect. The possible anomalies of stopping power may enter too, of course (for instance, in the conversion of roentgens to *density* of energy loss). The possibility of serious error in conventional means of treating these problems based on gas-like models would appear to demand careful study of the problems here posed.

IX. Penetration Effects of Low-Energy Particles

Very little can be said at the present time about the energy loss and ionization by particles of velocity of the order of magnitude of v_0 or less except in emphasis of the fact that very little indeed is known. To this circumstance has already been attributed the impossibility of any sort of theoretical calculation of the stopping power of water—liquid or vapor—for heavy charged particles of low energy, and the consequent importance of realizing that theoretical ranges such as those in Fig. 2 are uncertain by a *constant* additive amount (probably at most of order of magnitude 2 microns) at higher energies and virtually meaningless at low energies (below about 200 kev for protons).

The lack of understanding of penetration phenomena of low-energy particles in gases is enhanced in the case of a condensed medium. Specific effects of importance in liquid water are as follows:

1. Capture-and-loss of electrons will be different in liquid and vapor states, since these phenomena are very sensitive to details of the binding of the valence electrons.

2. Energy loss by excitation of vibrational and rotational degrees of freedom plays an important role at low velocities.

3. The polarization of the medium by the penetrating particle (Fermi effect) influences the energy loss at low energies.

4. The details of electronic binding are of paramount importance for the energy loss to electrons of the medium by slowly moving particles. Thus the Bragg rule will break down completely at sufficiently low velocities. This tendency has been discussed in Section IV. For example, it is responsible for the familiar and important fact, already mentioned, that the total ionization of gases by high-energy radiations *is* strongly

dependent on the molecular constitution; it exerts its influence on that portion of the ionization caused by slow secondary electrons.

The paucity of information in this energy domain is in many respects a result of the greater experimental difficulties which investigations with slower particles entail, and in a practical sense reflects also the lesser importance of this energy region in experiments of nuclear physics. However, these phenomena are of extreme importance in radiobiology, for many important studies involve intimately the effects of slow particles (for instance, secondary electrons from any high-energy radiation, and recoils from neutron irradiation), and almost all studies involve them to some extent.

X. Suggestions for Further Study

An important purpose of this paper is the examination of lacunae in the contemporary understanding of the fundamental physical processes underlying the interpretation of radiation effects on chemical and biological systems involving liquid water. In this section there are accordingly collected specific suggestions for theoretical or experimental investigations bearing, directly or indirectly, on the interactions of ionizing radiations with liquid water. Most of them have been analyzed in more or less detail in the paragraphs above.

1. *Measurement of the stopping power of liquid water* for extremely fast protons or alpha particles, using one of the new high-energy accelerators. This is a relatively simple experiment and should lead to an accurate value for $I_{H_2O}(l)$.

2. *Further experimental study of the range of natural alpha particles in liquid water,* and also of the stopping power of water. Such investigation is important, not only to clarify the bases of previous experimental errors, but also because theory, even after $I_{H_2O}(l)$ is known, cannot provide the needed stopping-power data in the moderate- and low-energy regions. It is likely that Am^{241} would provide a better source than Po, for practical reasons.

3. *Study of the electronic energy levels of liquid water,* using any or all relevant methods; for example, the far-ultraviolet and soft-x-ray spectra, photochemical and radiation-chemical effects, and theory.

4. *Refined investigation of the deviations from the Bragg rule.* For the simplest molecule, H_2, both theoretical and experimental work are required. Studies on more complex molecules, and most particularly on water vapor, would also be valuable. An accurate experimental value of $I_{H_2O}(g)$ is, of course, necessary for the theoretical interpretation of $I_{H_2O}(l)$.

5. *Study of the ionization in liquid water.* It would be extremely helpful to have some direct experimental information, especially on slow electrons, but work with all high-energy radiations would be very important. A theoretical investigation is also demanded.

6. *Study of the stopping power of an ionic crystal.* (Cf. Section V.)

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The writer wishes to express his appreciation to Drs. R. K. Appleyard, H. de Carvalho, and H. Yagoda for most generously furnishing detailed information regarding their respective experimental investigations in advance of publication. He is also deeply grateful to Professor James Franck for the privilege of many invaluable discussions.

Summary

The few and discordant experimental studies of the penetration of natural alpha particles into liquid water are reviewed critically. As a necessary background for their possible interpretation, the Bragg rule, which claims additivity of atomic stopping powers for all media, is analyzed in the light of available experimental data and also in the light of theory. The analysis discloses a variety of unsolved problems concerning penetration processes in complex systems. A purely theoretical approach to an important and tractable instance in which to study the Bragg rule—the stopping power of molecular hydrogen as compared to that of atomic hydrogen—is discussed qualitatively. A new calculation of stopping-power and range data for swiftly moving charged particles penetrating water, presuming only a small departure from the Bragg rule, is presented, and the assumptions underlying the calculation evaluated. Possible causes for failure of the Bragg rule in the case of liquid water are examined, and it is concluded that theory predicts an abnormally great stopping power, in the same sense as has been several times observed; however, the simple theoretical considerations here given do not permit an estimate of the magnitude of this effect, so that the reality of the reported results cannot yet be decided on a theoretical basis. Some possible consequences of the effects of liquid state on interpretations of radiation effects in water and aqueous systems are suggested, and, finally, some promising lines for future investigation proposed.

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DISCUSSION

EYRING:

Various mixtures of gases, and molecules having the same composition as a mixture of gaseous elements, did not, in studies we carried out many years ago, give differences of the order of 20 per cent in their stopping powers for the same ionizing radiation. If these results are to be accepted—namely, that there is no substantial contribution to the stopping power of the binding energies—then one must speculate as to the differences between water in the liquid state and water in the gaseous state. This, Platzman has done. I find it difficult to believe that there would be differences as great as 20 per cent between these two states. I

would be inclined to feel that differences of the order of 1 per cent may well be present.

ALLEN:

I agree with Platzman that the concept of ionization in liquid water is a very vague one and that conclusions drawn on this basis are apt to be incorrect. The effect of phase on the stopping power of water is hard to explain. One would expect from the theory that any differences would be more pronounced in ionic solids than in liquids. It is my impression that the stopping power of mica from experiment agrees well with that predicted by the theory. I should like to ask Platzman if there is any theoretical reason to feel that water is abnormal from this point of view.

PLATZMAN:

The problem is not one of invoking a reason, but of assessing its magnitude: we know that the effects being discussed exist, but we do not possess unquestionable knowledge concerning their magnitudes. The case of mica cited by Allen is important, for it is the only one in which the stopping power of an inorganic, non-metallic solid has been accurately measured. We may regard it as throwing additional doubt on the great abnormality in water. Yet even here there is room for question: the cooperative effects, involving as they do only the valence electrons, are progressively more suppressed as the mean atomic weight of the atoms of the medium increases (it is much greater for mica than for water); and the individual stopping powers of the atoms constituting mica, and the velocity dependences of these stopping powers, are hardly known with sufficient certainty to permit a conclusive statement on the magnitude of the deviation from the additivity rule.

EYRING:

One would expect less straggling in liquids than in gases.

APPLEYARD:

I should like to draw attention to some aspects of the "cooperative effect" in water—if it exists.

1. In the early work reported by Platzman, it was reported that other associated, polar liquids show similar, though smaller, effects.

2. Water vapor appears to have the same stopping power as a stoichiometric mixture of hydrogen and oxygen. The Bragg law is therefore not called into question here.

3. Any such anomaly of the magnitude generally indicated by all the experiments preceding those of de Carvalho would correspond to a decrease in the parameter (the mean excitation energy I) characterizing the stopping power of the outer electrons of the molecule by a factor of nearly 2, because the stopping power is rather insensitive to I. The effect would be much smaller for faster ionizing particles which lose more of their energy by head-on collisions whose behavior is independent of the medium. In the absorption of ultraviolet light,

however, we have a process corresponding to only the "glancing" collisions of the theory of stopping. This suggests that a fruitful experiment might be a comparison of the spectroscopic ultraviolet oscillator strengths of liquid and vapor water. To be of maximal value such a comparison would have to be extended to as short a wave length as possible—perhaps even down to 200 Å. Such oscillator strengths would in any case represent information of the greatest radiobiological value.

PLATZMAN (Communicated):

The spectroscopic information on water which Appleyard requests would certainly be of the greatest interest. Indeed, the corresponding information for *any* substance would be invaluable for the understanding of its stopping power. Such information is, however, not available. Nor is it likely to be readily forthcoming; it requires investigation in one of the most difficult of wave-length regions, and, moreover, demands absolute intensity determinations. But this statement should not be taken to imply that the experimental problem is hopeless. In fact, an attractive preliminary approach would be simply the *comparison* of the continuous absorption spectra of liquid and vapor water in the accessible Schumann ultraviolet.

LIND:

It is evident, I think, from the papers and the discussion today that more information on the effects of ionizing radiation in liquids, both aqueous and non-aqueous, is very much needed before we will be able to think in a general way about the effects of ionizing radiations in the non-gaseous state.

10 ·

Some Aspects of the Biochemical Effects of Ionizing Radiations

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It is the ultimate aim of research in the field of radiation biology to follow the fate of the absorbed radiation energy through all phases, from the primary physical act to its effect on cells, tissues, and the whole organism. Unfortunately, however, our knowledge of the biochemical processes within the cell, with which this section deals, is the weakest link in the chain of events. It is probably the most inaccessible problem from the point of view of experimental evidence, and we are forced, at least for the time being, to deduce mainly from test-tube experiments what may happen within the intact cell. The conclusions from such experiments are therefore to a certain extent speculative; but, if they carry some core of truth, they may open up possible approaches to the difficult problems to be solved.

Mode of Action of Ionizing Radiations

When we consider the developments which have taken place during the past 15–20 years in our views of the mode of action of ionizing radiations, we find as a prominent feature the clear recognition, due to the pioneer work of Risse (1) and Fricke (2), of the fact that these radiations act on aqueous systems not only directly, as pointed out by Burton in his paper, by release of energy within the solute molecules but also indirectly by a change of the water molecule, a change which is transmitted to the solute molecule. The mode of this transmission is still not fully understood, but many of the experimental facts can be interpreted in terms of H atoms and OH radicals being formed from the water molecule. Although quite a number of authors are aware that these radicals may originate from excitation as well as from ionization, the accent on ionization is only recently becoming weaker, because for some reactions the number of radical pairs, formed by dissociation or neutralization of charged molecules, is less than the number of solute

molecules transformed. There are even reactions in which ionization plus excitation may not be adequate to account for the magnitude of the effect and one may be led to invoke chain reactions (5).

Further progress has been made by recognizing the dependence of biological and biochemical effects on the specific ionization along the tracks of the ionizing particles (electrons, protons, alpha particles) and on the separation of these tracks. This dependence has been investigated for solutions and radiation effects in tissues by comparing the efficiency of alpha and of x-radiation. In the not very numerous experiments with solutions it was nearly always found that the ionic yield for alpha radiation was considerably lower than that for x-radiation; in fact, so much lower that the delta rays may account for the whole effect (3). Similar results were obtained in some biological experiments; in others, however, the efficiency of alpha radiation proved to be greater than that of x-radiation (4). In the latter case biologists usually postulate the necessity for producing within a particular molecule multiple local high spots of energy which the densely ionizing alpha rays can provide. One has also to think of a possible H_2O_2 effect, since H_2O_2 is generated in much higher concentration in the vicinity of the alpha-ray track than in the case of x-rays.

If we now deal with the direct action first, that is the released energy within any molecule, we can say that the biochemical effect is unpredictable in the sense that we do not know where and in what way the molecule will be affected. It is, however, worth mentioning that in the deamination of glycine (5) the ionic yield is approximately the same for the dry material as the yield for an only 20 per cent solution (Fig. 1). One cannot at present obtain absolute values for each of the two modes of action (direct and indirect) in solutions experimentally. The only way of arriving at an estimate is via the hypothesis that the dry material when dissolved does not change its properties and therefore its response to a direct hit. But is this assumption justified? One could be tempted to argue (6) whether a hydration shell could raise the direct-action ionic yield of a dissolved substance so that the combined indirect- and direct-action yields for the dissolved substance apparently equal the ionic yield of the dry substance. What really matters, however, from the biochemical point of view is the combined effect, since dry substances do not occur in living tissues. On the contrary, the average water content is of the order of 75–80 per cent, and for young and embryonic tissues much higher still. If one allows for the inhomogeneity of the cell content, some components, for example the nucleus, granules, and mitochondriae, probably contain less water. How far water of hydration and non-solvent water play a special part remains a problem to be solved

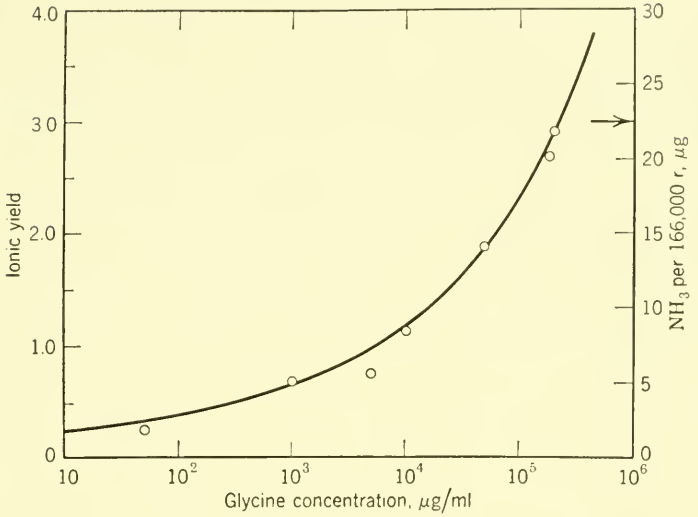

FIG. 1. Ionic and absolute NH_3 yield from irradiation of glycine by 166,000 r. Points are experimental; curve is theoretical. Arrow represents ionic yield of dry glycine.

by the physical chemist. Since water is so preponderant in the composition of living matter, it is reasonable to assume that the indirect mode of action of radiation is of importance, and in fact the majority of experiments have been carried out with solutions.

It will facilitate the later discussions to recall the main features of the indirect action. They consist of the following characteristics. At very high dilutions the ionic yield D/C is smaller than for moderate concentrations, probably because of competitive recombination of radicals (Fig. 2). At moderate concentrations up to about 15 per cent so-

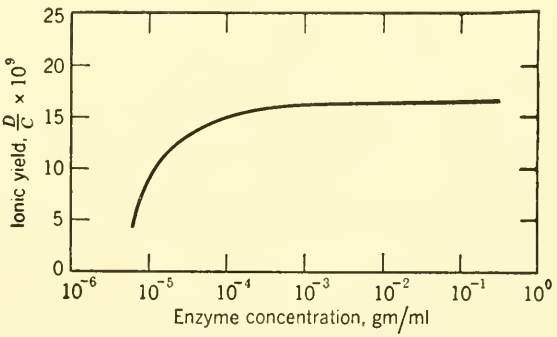

FIG. 2. Relative ionic yield for different concentrations of carboxy-peptidase irradiated by x-rays.

lutions, that is for a very wide range of concentrations, the effect of competitive recombination is so negligible that the ionic yield is constant. Beyond 15 per cent the direct action would become noticeable. A further characteristic of the indirect action on complex organic substances is the exponential curve obtained when the ratio of the concentration of biologically active material remaining after irradiation, to the initial concentration, is plotted against the radiation dose. This is due to the ever-growing competition for radicals by the increasing fraction of already inactivated solute. In fact this competition is a manifestation of the so-called protection effect, that is the sharing of radicals by two or more solutes which takes place according to the relative concentrations and abilities of these solutes to react with radicals, thus leading to a mutual reduction of the radiation effect. Last but not least, evidence is accumulating that the indirect action can be very *p*H dependent. These relationships have been established for a number of biologically active substances, for example enzymes, viruses, and toxins. It is, however, justified to focus attention in the first place on enzymes which occur in an abundance of varieties within cells but individually in minute amounts. Their importance lies in the fact that no meta- or anabolic change can take place without their help. At the same time they are substances which allow of quantitative determination of radiation effects. Furthermore, enzymes are, apart from their prosthetic groups, proteins, and results obtained with them are one aspect of the response to radiation of molecules of high molecular weight and intricate composition.

Inactivation of Enzymes

What is actually measured in such experiments is only the deactivation of the enzymatic activity, generally without reference to the chemical reaction underlying this. There have been, however, two attempts to define more precisely the process of inactivation. One is the extensive work done on the class of so-called SH— enzymes (7). In this particular class some of the sulfhydryl groups of the protein are essential for enzymatic activity, and it could be shown that these were oxidized by radiation and thereby inactivated, unless they were shielded against radiation by linkage to SH— reagents. Sometimes it was possible to restore the enzymatic activity fully by subsequent addition of a fresh supply of SH— groups in the form of glutathione. Barron and his collaborators have been prominent in this line of research.

The other attempt has been an investigation with the enzyme *d*-amino acid oxidase, which can be split into two essential components, the prosthetic group alloxazine adenine dinucleotide and the specific protein (8).

It was possible in this way to examine the effect of radiation on the components separately and when combined together. Both moieties were radiosensitive, and a summation of deactivation occurred when each component was irradiated singly and then joined after irradiation to form the complete enzyme. The protein appeared to be more radiosensitive than the prosthetic group, weight for weight. If, however, the difference in the size of the respective molecules is taken into account and the results are calculated in terms of collision frequencies with radicals, the prosthetic group shows the greater radiosensitivity of the two. We have seen in these two examples that the inactivation of enzymes by radiation can be caused by different chemical reactions. Generally the type of reaction, as well as the size of the macromolecule, may be a determining factor for variations in the radiosensitivity. Carboxy-peptidase has an ionic yield of 0.18, which is 6 times greater than that of ribonuclease, which has the lowest figure of the literature.

Enzymes as Indicators

Experiments mentioned so far aimed at establishing quantitative relationships between radiation and its effect on the enzymes themselves. Another type of experiment uses the enzymes as indicators to investigate radiation effects on other non-enzymatic substances via the protection effect, and this kind of experiment has served to reveal the remarkable specificity of the indirect action of radiation on substances of special chemical composition. The basic idea is that, on adding the substance to be tested for its ability to react with radicals to the solution of the indicator substance (enzyme), the indicator will be protected and thus require a bigger dose of radiation in order to be inactivated to the same extent as in the absence of protector. From experiments of this kind it has been found that the protective power can vary over a ten-thousand-fold range for different substances (Table 1). Of these, compounds containing sulfur in organic linkage are at the top of the scale. This fact, taken together with the important role of sulfur groups in proteins, the occurrence of sulfur in higher proportion in the skin, and the further fact that the experiments made by the Chicago Laboratory (9), in which the injection of cysteine increases the mean lethal dose for mice by more than 50 per cent, seems to be a useful opening for additional research which may become of practical importance. The further analysis of the protection effect has shown that present concepts of radical action and the formulae developed in the past for a straightforward sharing mechanism of radiation energy are not adequate, without modification, to cover all phenomena observed.

TABLE 1

PROTECTIVE POWER PER MOLECULE OF VARIOUS SUBSTANCES RELATIVE TO CARBOXY-PEPTIDASE (CP) AND DINUCLEOTIDE (DN)

Protector	Molecular Weight	Protective Power with CP	Protective Power with DN	Ratio of Protective Power with DN and CP
Thiourea	76	4.7	2.1	2.2
Sodium formate	68	3.1	0.74	4.2
Dimethylthiourea	104	1.9	....	...
Glucose	180	0.79	0.21	3.8
Dimethylurea	88	0.4	....	...
Egg albumin	~40,000	0.24	0.21	1.1
Alloxan	142	2.1×10^{-2}		
Sodium mesoxalate	152	2.4×10^{-2}		
Sodium oxalate	134	2.0×10^{-3}		
Urea	60	7.5×10^{-4}		

It has been found that the protective power per unit mass of protector is sometimes not a constant but declines relatively with increasing concentrations of the protector (Fig. 3). I shall return to this point later.

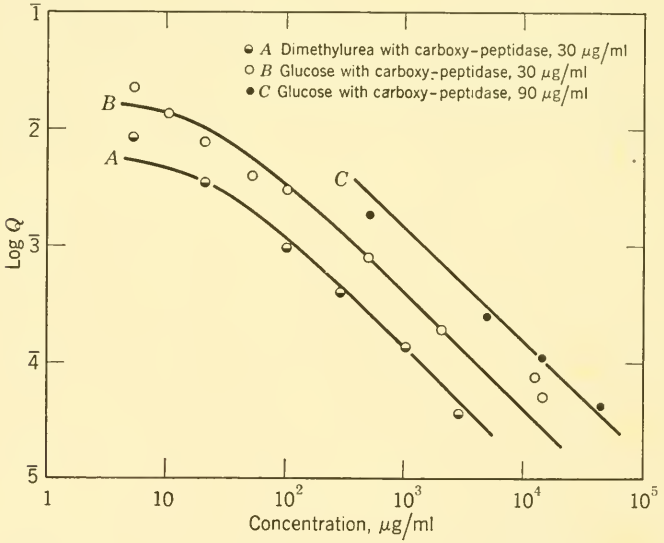

FIG. 3. Protective power of various substances per unit of mass protector. Reproduced by permission from *Brit. J. Cancer*, **3**: 31, 1949.

The specificity of the indirect action could also be demonstrated for the deamination reaction (5) in which the deamination varies in relation to the configuration of the remainder of the amino acids. These experi-

ments have also shown the stability of the peptide linkage in polypeptides. The practical value of all these experiments—and a great deal still remains to be done on these lines—is that they furnish an indication of the likelihood or otherwise of reactions which could occur within the cell.

Nucleic Acid and Irradiation

I now turn to investigations of radiation effects on the important cell constituent, nucleic acid. Euler and Hevesy (10) studied the formation of desoxyribonucleic acid in unirradiated and irradiated Jensen Sarcoma of rats with the aid of P^{32} and found a retardation of its formation within ½ hr after a dose of 1000 r. This effect declined with time, two-thirds of the inhibition disappearing within 2 hr after irradiation. A possible mechanism of the inhibition is suggested by the work of Mitchell (11), who followed the nucleic acid metabolism in proliferating and incompletely differentiated cells. According to Mitchell, the drop in desoxyribonucleic acid content is due to an inhibition of the synthesis of this compound in the nucleus and the accumulation of ribonucleotides in the cytoplasm, based on the inability of the irradiated cell to reduce ribonucleotide to desoxyribonucleotides in the nucleus. This very probably means that the radiation has interfered with an enzymatic reaction. Holmes (12), however, found ribonucleotides unaltered or diminished in the cytoplasm of Jensen rat sarcoma after irradiation. Hevesy (13) has also proved the x-ray effect on the formation of desoxyribonucleic acid by following the uptake of C^{14} into this substance. In other experiments the uptake of P^{32} in the phosphatides of the liver or of the intestinal mucosa of irradiated mice was shown to be unaffected by irradiation. These metabolic changes appear to stress the importance of nucleic acid metabolism and are examples of experiments *in vivo*.

There is, however, an investigation on nucleic acid done *in vitro* which I should like to mention because it will lead us on to another aspect of radiation effects which in my opinion should attract more attention than it has received so far. Hollaender, Greenstein, and Taylor (14) investigated the change in the viscosity of sodium thymus nucleate occurring with x-radiation. They also found a change in the ability of the solution to precipitate out in 95 per cent alcohol in the presence of salt. This ability was completely lost in the irradiated material, but they did not find any change in the chemical properties of the nucleic acid molecule or in its digestibility by the enzyme desoxyribonuclease. These carefully conducted experiments led the authors to the conclusion that the effect was apparently due to breaking up of the particles into shorter

fragments of variable dimensions. It seems to me that there may be a possibility of a different interpretation of their experimental results. One would not expect a demonstrable change in the chemical properties of the 3 per cent solution with the dose of x-rays applied. The marked effect, however, of the change in viscosity seems to point rather to a self-propagating reaction of a physicochemical nature whereby the frictional resistance of the dispersed phase is governed by a zeta potential undergoing changes in response to radiation. In that case the viscosity of the nucleic acid particles is a result of the charged water layer, and the change in viscosity would not necessarily be due to a change in the ratio of the longitudinal and transverse axes of the particle; furthermore, the loss of streaming birefringence which was observed could be attributed to the inability of orientation under the influence of changes of charge. In the same direction of a colloidal phenomenon lies the change in the ability to precipitate out of alcohol solutions. In short, the solution of nucleic acid would have undergone a change in the direction of becoming more hydrophil.

This interpretation of these experiments on the viscosity of nucleic acid leads to the consideration of a more physicochemical nature, referring to colloidal systems, surface membranes, and cell structures, all of which may play a vital part from the biochemical point of view. We do not know enough about the state in which enzymes occur within cells, but we do know that certain enzymes are firmly linked to surfaces and cannot be removed, whereas others are easily extracted. We can be pretty certain that some enzymatic reactions proceed on active surfaces, and it seems to me necessary to try to get much more information about the effect of radiation on surface-catalyzed reactions as well as the effect on the surfaces themselves. I would also mention in this respect the well-known hemolytic action of x-radiation on red-blood corpuscles, which obviously means some alteration of their protein-lipoid envelope, and the puzzling investigations by Crowther and Liebmann (15) and Gray, Read, and Liebmann (16) on the effect of radiation on graphite suspensions and colloidal gold solutions. They observed, after irradiation with a few roentgens, precipitation followed by alternate zones of suspension and precipitation of the particles when the radiation dose was increased.

Before I proceed to draw special conclusions on what may happen within the cell, I want to summarize briefly what I have mentioned so far. We have dealt mainly with the results of test-tube experiments on the direct and indirect action on vital substances occurring in cells, such as enzymes, nucleic acids, amino acids, and proteins. We have seen that as a result of irradiation the destruction of the activity of

enzymes occurs. We have further mentioned the high specificity of the indirect action in its relationship to the chemical structure of the molecules concerned, and we have also seen that the present concept of radical mechanisms may need modification in the light of more recent developments. Finally we have considered the more physicochemical aspects of radiation effects. This may enable us now to discuss tentatively the consequences of irradiation on the biochemical phenomena affecting tissues.

Biochemical Phenomena and Biological Systems

To start with, one has to distinguish between substances split off and residues remaining therefrom, and the mere depletion, even though it be local and temporary only, of necessary substances occurring in minute amounts. All these factors, singly or together, may play a part. At the time when the dilution and protection effects were established it was natural to speculate on possible consequences in biological systems (cells). The question arose whether these phenomena could account for radiation effects on living cells, and it was tentatively assumed that the inhomogeneity of the cell interior could provide the necessary regions of dilution through which solutes (for example enzymes) had to pass on their way to their places of reaction. Then when the solutes were decimated in these zones by incident radiation the usual delicately poised balance of reaction was upset. This upset would, according to its shorter or longer duration, either only disturb or completely and irreversibly destroy cell activity. Against that it can be said that the presence of other radiosensitive solutes might be sufficiently protective to minimize the dilution effect. Later experiments have shown that the protective effect is by no means always proportional to the amount of protector but relatively diminishes with increasing concentrations of protector. Furthermore, examples of reactions have been found in which an ionic yield for indirect action of 3 and more than 3 is obtained on increasing the concentration of solute. These findings could be of particular relevance for the biochemical effect in cells.

In addition to the loss of essential solute through irradiation the formation of new products from the solvent or solute has to be considered. With regard to the solvent (water) the formation of H_2O_2 as a result of further interactions of H atoms and OH radicals, partly in reaction with dissolved oxygen, has been assumed to be the actual agent of the radiation effect in cells, a reaction recently more stressed by the French school. There is a real danger of an undue emphasis on the effect of H_2O_2, and the pitfall has to be avoided of thinking that the formation

of H_2O_2 is *the* mechanism of biochemical effects, lest progress made in other directions be obscured or even entirely lost. There is no doubt that H_2O_2 in certain cases can account for some of the effects (17), and there the action of H_2O_2 is a factor which tends to complicate the unraveling of the mechanisms. There are, however, sufficient examples of substances not sensitive to H_2O_2 which show marked radiosensitivity, and again examples where it can be shown that H_2O_2 formed by the dose applied can only to a minor part account for the end effect. Moreover, catalase, one of the most powerful enzymes ubiquitously present in tissue, should take care of H_2O_2 formed unless the H_2O_2 reacts before it can be destroyed, or else the catalase would be destroyed by radiation in preference to H_2O_2. If one wishes to consider further examples of possible "poisons" formed by radiation, one could think of ammonia, which can be formed with a high ionic yield from proteins and amino acids, or the formation of H_2S from cysteine and glutathione, recently discovered by us (19), which could have toxic effects on biological components. One has, of course, to be careful not to regard any new *single* reaction as of overriding importance; rather the contrary is probably nearer the truth, that is that the sum total of the derangement of the balance and interplay of cellular processes is what matters. On the other hand, the possibility that one "poison" or another may play a vital part is supported by the more recent discovery of mitotic and radiomimetic substances. But these can be introduced into the cell interior only with difficulty and in a limited way, whereas radiation knows of no permeability barrier and can perform with ease chemical reactions within the cell.

I think what I have said has shown that the solution of the problem of the biochemical effects of ionizing radiations is still in its initial stage. Are we justified in looking for a few key reactions, or are the effects rather the sum of a multitude of processes? Only more intensive work can decide this question. A review by Monné of the progress made in the elucidation of the structure of protoplasm pictures a highly organized system of fibrils consisting of minute granules, chromedia, which alternate with interchromedia, the whole sustained in a dynamic equilibrium. These chromedia consist partly of enzymes and can be separated, according to Monné (18), from minced cells and analyzed by exact chemical methods. There would thus be a chance of testing radiation effects by isolating the chromedia before and after irradiation. We may hope that the cooperation of cytologists and cytochemists will help to put some of our speculative conclusions on a firm basis.

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DISCUSSION

BURTON:

It appears that in my paper I meant to ignore the indirect effect. This I certainly did not mean to do, and Dale has very thoroughly showed the existence of the indirect effect.

KAMEN:

About 10 years ago Chalmers, co-discoverer of the Szilard-Chalmers reaction, called attention to the alternate flocculation and solution of radioactive gold solutions with differing radiation dosage. He proposed that this might be used as a means of estimating radiation dosage. I should like to know whether any further work has been done using this system.

DALE:

Not so far as I know.

SOLOMON:

Is there any theoretical explanation for the wide variety of compounds which give protection?

DALE:

No. The problem of specificity of protection is one in which much future work must be done. It may be that the effect is associated with electron linkage, where

the radical may be able to break certain vital linkages in some compounds, whereas in other compounds no vital linkage is affected.

FAILLA:

T. C. Evans, about 10 years ago, demonstrated dilution and protection effects with *Arbacia* sperm, which is a very different thing from the molecules of the chemists and biochemists. It is interesting that the effects discussed by Dale can also be detected with a living system.

DALE:

Evans' work is most interesting. I have discussed the matter with him here, and we feel that the results indicate a surface effect on the organism. Evans' work clearly points to further research in this field.

11.

Ionizing Radiation and Cellular Metabolism

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It has been known for many years that ionizing radiation interferes only to a minor extent with respiration and glycolysis, whereas it markedly influences mitotic processes. Though developments have shown that the first-mentioned effects are far from being negligible, the most conspicuous effect still remains the action of ionizing radiation on cell division, which will be considered first. Radiation causes a drop in the rate of mitosis, followed by an attempt at recovery accompanied by the appearance of degenerate cells. For example, 5 min after exposure of cultures of avian fibroblasts to a total of 1000 r UX beta radiation, the mitotic figures were found to be reduced from 100 to 87; after 30 min to 15. After 9 hr, however, mitosis was increased (1). When leukocytes from patients with chronic myeloid leukemia were exposed to x-rays in tissue cultures, mitosis was temporarily inhibited by a dose of 100 r, and apparently permanently inhibited by 1000 r (95).

Irradiation may influence the rate of mitosis not only of cells in an advanced stage of the mitotic cycle, but also of those in an early or the earliest period of interphase.

Since in the mitotic process the cell nuclei are primarily involved, irradiation can be expected to influence the cell nucleus. This was shown to be the case in numerous investigations. For example, Henshaw (2) found that, when nucleated and enucleated egg fragments of *Arbacia* were x-rayed and then fertilized with normal sperm, delayed and abnormal development was found only in nucleated egg fragments.

Of the nuclear constituents, desoxyribonucleoproteins, having a molecular weight of about 1 million and comprising a large part of the nuclear material, may be the most important. Ionizing radiation, possibly through the intermediary of active radicals produced in the tissue water or other tissue constituents, interferes with the synthesis assembly of

nuclear constituents. The same is true for mustard gas, ethylenimines, and numerous other compounds (3, 110, 114). The extent of the interference due to ionizing radiation depends partly on the dose and partly on the density of ionization temporarily produced in the tissue. Interference with cell division may result, followed by mutation and cell degeneration.

The formation of desoxyribonucleoprotein molecules involves the participation of histone or other proteins, of desoxypentose, of purine, of pyrimidine, and of phosphate groups. It is easiest to assume that the assembly and possibly even the synthesis of some of the constituents involved in the synthesis take place at an interphase. The synthetic process requires energy expenditure, the energy most likely being supplied through carbohydrate metabolism. Thus, in addition to synthesis of desoxyribonucleoproteins, the above-mentioned constituents require the presence of sugar (glycogen), oxygen, and an intact enzyme system.

Energy Supply and Mitotic Division

Bullough (4) in the study of diurnal mitotic rhythm of the female mouse has shown that mitosis could not occur unless there was an adequate concentration of sugar or glycogen in the tissue. He suggested that the function of this carbohydrate is to supply the energy for cell division and showed that in the vagina of the mouse the glycogen disappears as mitosis proceeds. Sugar lack, however, depresses mitosis. In conditions of sudden acute hypoglycemia induced by insulin, all those divisions which had already reached the prophase were found to be normally completed. From these observations it follows that the depressing effect of sugar lack is felt only during the transition of a resting cell to the prophase. Bullough defines the precise time at which the glucose and oxygen exert their effect. It is a matter of minutes before the prophase becomes recognizable, in a stage of division which may be called the antiphase. Any division which has proceeded beyond the antiphase can pass to completion in the absence of both glucose and oxygen. Also, almost all the mitoses present at the time of death due to hypoglycemia continue without interruption and not even at a significantly delayed rate (5). That cells which have passed through the critical phase complete mitosis was observed by Koller (122).

In conditions of sugar lack few cells enter prophase but those which do so complete their division at about normal speed. Conversely, it has also been shown by Bullough that with an abundance of sugar a greater number of resting cells were stimulated to divide, although here too the time for a mitotic division remained normal. Hence, it may be

concluded that within wide limits the number of resting cells which enter prophase is in direct proportion to the sugar concentration, whereas for the completion of these divisions sugar is much less important.

The importance of carbohydrate metabolism for desoxyribonucleic acid formation follows also from the observation of Roberts (6). The incorporation of P^{32} into desoxyribonucleic acid in *E. coli* is very markedly reduced (by a factor of 7) when the cells are partially depleted of potassium. Potassium is involved in the glucose cycle, and interference with the cycle is presumably responsible for the reduction in the rate of desoxyribonucleic acid formation.

The use of narcotics, such as nembutal, which among other effects reduces oxygen consumption, was found to partially inhibit the incorporation of P^{32} into the Jensen sarcoma of the rat (17).

In this connection, the investigation of Medawar (7) should be mentioned as well. Working with epidermis *in vitro*, he found that an increase in oxygen concentration resulted in increased mitotic activity, and conversely that strictly anaerobic conditions prevented all mitosis, although the cells might survive for a week.

Oxygen and enzymes involved in sugar metabolism are, therefore, required for supplying the energy used in the mitotic process, especially at the onset. The doubling of the nucleic acid and other cellular constituents must involve considerable amounts of work, in which a significant quantity of sugar and oxygen may be involved.

Runnström (8) and Brachet (9), working with sea-urchin eggs and amphibian eggs, respectively, found a peak in oxidation during prophase. Zeuthen (13), however, working on a number of different eggs such as those of the frog and sea urchin found in all cases a slight continuous rise in respiration beginning during the first part of mitosis, followed by a subsequent decrease. The respiratory peak was around metaphase or anaphase. Thus, respiration proceeded at an increasing speed during the period of synthetic activity.

In developing anthers, Erickson (10) observed a pronounced fall in oxygen consumption during the transition from the interphase to the prophase. Other evidence (11) has been presented of a drop in respiration during this transition. Furthermore, Zeuthen (12, 13, 14) found, in the interval of the development of egg cells in which interphase is practically absent, that the respiration rate remained almost constant. After approximately six divisions had taken place the mitotic rate slowed down, presumably because, at least partly, of the appearance of interphases. By this time, when interphases became visible, respiration increased markedly in a stepwise manner, each step coinciding with a mitotic cycle. How far interphase and how far prophase is responsible

for the increase in oxygen consumption cannot, however, be concluded from these observations. This is a point of interest, among others, in view of a possible connection between desoxyribonucleic acid synthesis and oxygen consumption.

Effect on P^{32} Incorporation into Nucleic Acid

From the viewpoint of the chemist cell division is necessitated by the spectacular accumulation of synthetic products in the nucleus. By interfering with mitosis, ionizing radiation can be expected to interfere with many of the synthetic and rearrangement processes involved in cell division. When comparing the rate of incorporation of labeled phosphate into the desoxyribonucleic acid extracted from the growing Jensen sarcoma of irradiated and of control rats (15, 16, 16*a*), it was found that irradiation with an x-ray dose of a few hundred roentgens or more reduced the rate of formation of desoxyribonucleic acid in the sarcoma. Assuming that the intracellular inorganic P or organic phosphate, which comes comparatively rapidly into exchange equilibrium with inorganic phosphate (96, 97), was the significant precursor of the desoxyribonucleic acid phosphorus of the tumor (should this assumption not be tenable, the renewal figures would be still higher than stated above), the percentage renewal of the desoxyribonucleic acid P of the sarcoma was calculated to be about 2.5 per cent in 2 hr in the controls, while for the irradiated sarcoma half that value was found. In experiments taking 1 hr or less Holmes (17) observed irradiation with 2000 r to reduce the rate of incorporation of P^{32} into the desoxyribonucleic acid of the sarcoma to one-half of that found in the controls.

As irradiation may influence the permeability of phase boundaries, one may be tempted to interpret the decreased rate of incorporation of P^{32} in the desoxyribonucleic acid molecule of irradiated tumors as caused by a diminished rate of passage of labeled phosphate into the tissue cells of the irradiated sarcoma. That this is not the case can be shown by a comparison of the ratio of the specific activities of the plasma inorganic P and tumor inorganic P in the controls and in the animals with irradiated sarcomas. A difference in the permeability is indicated by the results obtained. This is, however, insufficient to explain the depressed rate of incorporation of P^{32} into the desoxyribonucleic acid of the irradiated sarcoma. Furthermore, Holmes (17) found irradiation to influence to only a minor extent the rate of incorporation of P^{32} into ribonucleic acid in contrast to its incorporation into desoxyribonucleic acid of the tumor. This result is not compatible with the assumption that the effect of irradiation on the rate of formation of

labeled desoxyribonucleic acid results from a change in the rate of intrusion of labeled phosphate into the tumor cells.

In contrast to the incorporation of P^{32}, the incorporation of C^{14} into desoxyribonucleic acid (88), and into ribosenucleic acid (124) (the rate of incorporation into the purines of these compounds being investigated) was found to be depressed, although the latter to a smaller extent than the former.

Holmes (98) recently investigated the effect of irradiation on the incorporation of S^{35} and of N^{15} into the protein moiety of nucleoproteins. No interference was observed.

When comparing the effect of irradiation on the incorporation of P^{32} or C^{14} into desoxyribonucleic acid and into ribonucleic acid the following consideration may be of interest. In the tumor a large part (one-half or less) of the labeled desoxyribonucleic acid molecules represents additional material due to growth (16). From the labeled ribonucleic acid molecules a much smaller amount (one-sixth or less) (18, 19) represents only additional material due to the growth, the rest being due to a renewal process which may take place without involving such fundamental steps as does the formation of additional molecules. The blockage of any of these steps due to irradiation will markedly influence the formation of labeled desoxyribonucleic acid molecules. As to the effect of irradiation on the formation of ribosenucleic acid, an early observation obtained by applying the ultraviolet technique indicated that formation is promoted (61, 119). The results obtained in the study of P^{32} incorporation into ribosenucleic acid of irradiated sarcoma did not, however, indicate an increased formation, while C^{14} incorporation into purines of both desoxyribo- and ribonucleic acid was found to be depressed by irradiation.

In a study Jones (20) compared the extent of incorporation of administered P^{32} into the tumor desoxyribonucleic acid of rats receiving 60 r whole-body irradiation with the incorporation in non-irradiated controls. The difference was found to be 0.18 per cent depression of P^{32} incorporation per roentgen, which is the same order of magnitude as the depression effect of radiation on red- and white-corpuscle formation. This coincidence is interpreted by assuming the synthesis of desoxyribonucleic acid to be a function of the mitotic activity of the tissue, so that this also represents the effect of radiation on a proliferative process.

Isotopic tracers can advantageously be applied in this type of investigation. Not only is it difficult by the usual chemical methods to determine a change of only 1 per cent in the desoxyribonucleic acid content of a tumor, but even if such a difference was ascertained—Stowell (21)

found in transplantable mammary carcinomas that, whereas 4000 r produced a mean reduction of 5.4 per cent in the desoxyribonucleic acid content per cell, 2000 r caused no significant changes—the question would still remain whether a decrease in the nucleic acid content due to irradiation should be explained by a diminution in the rate of formation of nucleic acid molecules or by degradation of some of the nucleic acid molecules present. Istopic indicators make it possible to distinguish between new and old molecules, and the experiments show that it is mainly the rate of formation of new molecules which is diminished by the action of ionizing radiation.

Investigations comparing P^{32} content of desoxyribonucleic acid and of inorganic phosphates in various tissues after administration of labeled phosphate clearly indicate a close connection between incorporation of P^{32} into desoxyribonucleic acid of the tissue and cell division. Only minute amounts of P^{32} were found in the desoxyribonucleic acid of organs which showed a very low mitotic index, such as the liver and kidney of grown rats. On the other hand a marked P^{32} content was found in the desoxyribonucleic acid of corresponding organs of newly born animals or in the liver of the partially regenerating hepatectomized adult rat (22). Similar results were demonstrated in investigations in which the incorporation of N^{15} into desoxyribonucleic acid was studied (23, 24*a*). In organs of adult animals which show a high percentage of mitotic figures, such as the bone marrow, the intestinal mucosa, or the spleen, the desoxyribonucleic acid has been found to take up an appreciable fraction of the admistered labeled phosphate (25). It is also of interest to note that the desoxyribonucleic acid P of white corpuscles (26) and nucleated erythrocytes (27) in which mitosis is absent does not interchange with P^{32} present in the circulation.

In these investigations the desoxyribonucleic acid was isolated from the total organ or from the total nuclei of the organ, and its P^{32} or its N^{15} content determined. Such investigations did not reveal the phase of the mitotic cycle wherein the incorporation of P^{32} into the desoxyribonucleic acid molecule actually occurs. In some preliminary work utilizing Jensen rat sarcoma, however, P^{32} intake was measured and also the cells in mitosis were counted. These experiments indicated, 1 hr after irradiation, when nucleic acid synthesis was observed to be minimal, that most of the cells found in mitosis (almost all the mitoses were abnormal) must have formed their nucleic acid before irradiation (17). Further information on the above point can be obtained by using autoradiographs as a cytochemical tool, as was done by Howard and Pelc (28). These experimenters have grown roots of *Vicia faba* for periods of 2–48 hr in solutions containing labeled sodium phosphate.

Stripping film autoradiographs were made of squashes and sectioned material from the meristem and from proximal segments where cell division is rare. The observation of Howard and Pelc that no nucleus in division, not even those in early prophase, showed an autoradiograph at 6 hr, but all did at 24 hr, is interpreted to indicate that the incorporation of P^{32} into nuclear compounds stops some time before visible prophase, and that there is no synthesis during the actual division. According to Caspersson's observations, however, a certain increase in nucleic acid formation during the very earliest prophase does take place (99).

Cells when sufficiently irradiated even in an early interphase fail to divide, or else divide abnormally (29). This fact suggests that irradiation must interfere with the mechanism involved in the synthesis of nuclear constituents of cells, which are present even in that early stage of their development.

That irradiation depresses the formation of desoxyribonucleic acid is clearly brought out by the various observations discussed above. Cell division is a result of intense accumulation of pertinent cellular constituents, and if the accumulation is obstructed by irradiation, cell division will not occur. That the accumulation of free purines and nucletide takes place during the growth lag, their conversion into nucleotides and nucleic acids starting towards the end of the lag, is suggested by results obtained in the study of ultraviolet absorption (118) and of phosphorus uptake (120) by *Bact. lactis aerogenes.*

Although irradiation interferes with the *formation* of desoxyribonucleic acid, it should be pointed out that doses which produce hemolysis of blood corpuscles and thus make the nucleic acid content of the nucleated blood corpuscles available to the effect of degrading enzymes will lead to a removal of much of the preformed nucleic acid. In a similar way doses which produce far-reaching changes in the bone marrow, making it semifluid, may degrade much of the nucleic acid (123).

Inactivation of Enzymes

As previously stated, the synthesis of desoxyribonucleic acid necessitates the participation of numerous agencies. The absence of one or more of these may interfere with the formation of desoxyribonucleic acid molecules. For example, the inactivation of enzymes involved in carbohydrate metabolism could interfere with the formation of desoxyribonucleic acid. In numerous experiments diluted solutions of different types of enzymes were irradiated with restricted doses of ionizing radiation. Frequently an inactivation of the enzymes of different types was observed.

In this manner evidence was presented of the reversible inhibition of sulfhydryl enzymes on irradiation with x-rays and further irreversible inhibition with increasing doses of radiation (30). Immediately after irradiation the formation of desoxyribonucleic acid both *in vitro* and in surviving tissue slices (126) is found to be depressed. However, Richmond (127) observed recently that, shortly after irradiation with a similar x-ray dose, hemin formation in bone marrow or spleen homogenate increases three-fold, as does oxygen uptake (128). After the lapse of 2 days hemin formation in the bone marrow homogenate (not, however, in spleen homogenate) is found to be markedly depressed below the value found in controls.

One hundred roentgens of x-rays produced a 21 per cent and 500 r a 94 per cent inhibition of phosphoglyceraldehyde dehydrogenase. When the enzyme was 21 per cent inhibited, there was complete reactivation on addition of glutathione. Presumably, the inhibition was due to the following or similar reversible oxidation:

$$2RSH + 2OH \rightleftharpoons RS + 2OH + 2H$$

Yeast hexokinase, which catalyzes the reaction glucose + ATP $\rightleftharpoons$ glucose-6-phosphate + ADP, was inhibited only 13 per cent by irradiation with 1000 r.

Adenosinetriphosphatase at *p*H 9.1 could be 10 per cent inactivated by the remarkably low dose of 10 r and 95 per cent inactivated with 1000 r. Enzyme inhibition is almost completely reversed on addition of glutathione.

There are two kinds of —SH groups of proteins: the easily oxidizing, and the sluggish groups which are not oxidized by mild agents but which do react with mercaptide-forming groups (Hg, As, etc.). Evidence has been presented that only the first-mentioned groups are oxidized by a moderate dose of ionizing radiation. In the case of hexokinase evidence came from the fact that iodosobenzoate inhibited only 15 per cent of the total —SH groups. It was concluded that the rest of the —SH groups are of the sluggish type. Inhibition of the enzyme with x-rays was low, as was expected, viz., 12–18 per cent only.

The specific action of irradiation on —SH groups was also shown in experiments in which urease was inhibited by gamma-ray doses of 25–200 r. If enzyme inhibition by gamma rays were due only to oxidation of the —SH groups by the oxidizing products or irradiated water, there would be no inhibition on irradiation of urease if the —SH groups were protected by a *p*-chloromercuribenzoate. If inhibition were due to destruction or denaturation of the enzyme, it would occur even after con-

version of the —SH groups to the R-S-Hg-benzoate. Urease with glutathione was inhibited by gamma rays to the same extent as the enzyme alone. When glutathione was added to the irradiated enzyme containing *p*-Cl-Hg-benzoate, the enzyme activity was restored completely. Protection of the —SH groups by formation of the reversible mercaptile compound protected the enzyme from the inhibiting action of gamma rays.

That H_2O_2 plays an important role in the process of inhibiting —SH groups by radiation was suggested by the protective action of small amounts of catalase which frustrate the inactivation of phosphoglyceraldehyde dehydrogenase, for example, by alpha rays (31, 31*a*).

Inactivation of enzymes, may, however, be more difficult to produce *in vivo* than *in vitro* because numerous tissue components may compete (32, 33) for the radiation products, such as OH, O_2H, and H_2O_2 (34) which are formed from water, or for other inactivating radicals.

The hydrogen peroxide concentration is reduced to half its initial value by the action of catalase of 1×10^{-6} molar concentration, as found in the liver in as short a time as approximately 0.02 sec (37). The localization of catalase within the various types of cells is not sufficiently understood, and in some cells or cell districts the lifetime of hydrogen peroxide may be much longer than that which follows from the figure stated above. Also, organic peroxides of long lifetime may be formed under the effect of radiation. Organic peroxides have recently been shown to be mutagenic and to induce chromosome abnormalities in *Vicia faba* (37*a*). Furthermore, morphology on the molecular scale may have much influence on radiosensitivity (116). Radiosensitivity may be very pronounced *in vivo* as well; a dose of 0.01 r suffices to influence the growth rate of a single cell of the mold *Phycomyces* (36).

Many of the decomposition products of water have a lifetime of only a fraction of 1 sec. Indications are not lacking, however, for the existence of inactivating products of longer and even of much longer lifetime.

Indirect Effects

When one sarcoma of a rat was irradiated while the other was shielded, the rate of incorporation of the administered labeled phosphate not only into the desoxyribonucleic acid of the irradiated but also into that of the shielded sarcoma was found to be reduced (23*a*, 25). The lifetime of the inactivation products, presumably responsible for the above-mentioned indirect radiation effect, was estimated to be about 1 hr (17). Attempts were made to demonstrate the existence of a circulating substance produced by irradiation in the rabbit. The existence of

such a circulating substance is suggested by the observation that the replacement of a substantial part of the blood of a non-irradiated rabbit by blood of an irradiated rabbit, followed by administration of labeled phosphate, leads to the formation in the kidneys of desoxyribonucleic acid of lower P^{32} content than that formed in controls (24).

In experiments on human beings, when a dose of x-rays was given to two small areas of skin separated by a varying width of untreated protected skin, the reactions produced were significantly larger than that over a single area of skin exposed to the same dose of radiation. The length over which the hypothetical substance responsible for indirect radiation effect diffuses was found to be 2 cm (100).

Jones (20) irradiated rat muscles by injection of radioactive yttrium colloid interstitially and observed a significant depression in the incorporation of administered P^{32} both into the liver and into the tumor. The effect obviously must have been indirect as the liver and tumor were unirradiated. He found the rate of P^{32} incorporation into desoxyribonucleic acid of the liver to be depressed by 0.28 per cent per roentgen and by 0.16 per cent per rep after whole-body x-ray irradiation and specific-muscle beta-ray (yttrium colloid) irradiation, respectively. The first-mentioned figure compares well with the effect of whole-body x-ray irradiation on the depression of white-cell count (0.23 per cent) or red-cell formation (0.3 per cent per r).

There have been many reports of the indirect effects of radiation. The x-ray dose when the tumor was directly irradiated sufficed to produce regression of 75 out of 100 Jensen rat sarcomas and was found to be effective in 35 cases out of 100 when only the bed of the sarcoma was x-rayed (22*a*).

Van Dyke and Huff have shown that when a parabiot is irradiated with 900 r, one member being shielded, both become depilated, and the non-irradiated member is relatively more depilated than the other (36*a*).

Protection

Protective substances can reduce radiation effects *in vivo* as well. Thioglycolic acid was found to be very effective in protecting bacteriophage T_2 from the effects of irradiation (37). When cysteine or thioglycolic acid was added to the nutrient solution of *Propioni-bacterium pentosaceum* and the solution then irradiated, the radiation damage was found to be only one-half of that observed in controls (38). The growth rate and surviving percentage of *Allium cepa* roots are 2–3 times as great if the irradiation with 500 r takes place in the presence of cysteine (137). Mice are significantly protected by thiourea or dithiophos-

phonate intraperitoneally administered a few minutes before irradiation (101).

Rats are partially protected from radiation effects by intravenous administration of cysteine (39, 39*a*, 113). Cysteine injected into the skin of guinea pigs partially protects the hair follicle from radiation effects (38). SH groups disappear in the skin of the guinea pig after irradiation (109). Massive doses of glucose (20 g) have a similar effect on the hair follicles of the rabbit (40). Hollaender (133) has shown that bacteria previously grown in broth containing glucose are thereafter less radiosensitive than bacteria grown in ordinary broth. Latarjet (37) interprets these results as due to the loss under irradiation of glucose of some hydrogen which combines with the oxidizing agents to produce H_2O_2, and thus prevents these agents from combining with the sensitive molecules. Latarjet has in certain cases observed that an increase in H_2O_2 production was accompanied by a decrease in the biological effect of irradiation.

Cysteine may exert its protective action in different ways. In the leaf of the zinc-deficient walnut tree cell division is stopped in the prophase, quinone being formed in these leaves by oxidation of dihydroxyphenols. The mitotic index increases by addition of cysteine. The effect of cysteine has been ascribed by Reed (40*a*) to the fact that cysteine contains the group R—SH, which can donate H, thereby enabling the cells to keep the dihydroxyphenols in a reduced state, whereas they would otherwise be oxidized to quinones by the polyphenoloxidase. Normally, tissue would contain enough reducing substances to reduce quinones back to dihydroxyphenol derivatives, but not, however, when grown on a zinc-deficient soil.

Besides the above-mentioned protecting effect of substances containing —SH groups, other agencies affecting cellular oxidation may also produce a protective action.

Intraperitoneal injection of 0.1 mg sodium cyanide immediately before irradiation was found to protect 50–80 per cent (41, 42, 43, 112, 125), and injection of 0.1 mg sodium nitrite to protect 20 per cent, of irradiated mice from the lethal effect of x-radiation. Only a small protective effect was observed if the injection (which took 6–7 min) was given immediately after the irradiation, while all protective effect was absent if the injection took place 15 min after irradiation. The protective effect was observed only if the dose administered did not differ widely from the lethal dose of about 700 r. If 900 r was given, only 20 per cent of the mice were protected by NaCN injection, and protection was not demonstrated if the dose was as high as 1200 r, or if a daily dose of 84 r was given for 12 days.

Irradiation leads to enzyme inactivation, followed by partial or total blockage of some normal, and a simultaneous opening of other, possibly noxious, pathways. Cyanide inactivates enzymes, but interferes primarily with other processes, as do the decomposition products of water, which are responsible for much of the irradiation effect. It is possible that cyanide, by blocking the noxious pathways, makes the x-ray effect less lethal. If the x-ray does not exceed 700 r the noxious pathway is intercepted. The primary x-ray lesion has now sufficient time to be healed. A somewhat different explanation of the protecting effect of cyanide is discussed on p. 202.

Irradiation with a lethal dose is followed among other processes by formation of fatty liver in the mouse as a result of interference with the normal path of carbohydrate and/or fat metabolism. The liver of cyanide-injected irradiated animals shows a much less pronounced fat deposition (43). It is also of interest to remark that acute death in the chick is associated with rapid accumulation of uric acid in blood and tissues (44). We have here an example of a toxic picture due to the insult of materials discharged from injured cells at a rate which the organism is unable to handle in a normal fashion.

The period of 50 per cent survival in mice given 350 r of x-rays was doubled by 22 daily injections of 15 μg of folic acid, beginning 7 days before irradiation. Pyridoxine had a similar effect (45).

A protective effect produced by numerous other substances, as, for example, desoxycorticosterone (46) or atropin, was reported as well (47). Even changes in the diet have been reported to influence the mortality rate of the guinea pig due to irradiation with 100–600 r (48).

Ionizing radiation produces a contracture of the stimulated frog muscle. Bacq (49) attributed this effect to the inactivation of the enzyme systems involved in carbohydrate metabolism in muscle. Another explanation has been advanced by Barron (50), who feels that oxidation of the —SH groups of myosin is responsible for the observed inactivation.

Irradiation and small amounts of mustard and of some other compounds (3, 110) all influence the mitotic cycle in a similar way and are, among other factors, very effective in activation of the phosphate-transferring —SH enzyme phosphokinase. Therefore, the possible effect of the inactivation of this enzyme on desoxyribonucleic acid synthesis, among others, has to be considered (51).

Also included in the enzymes to be considered is adenosinetriphosphatase, which can be inactivated *in vitro* by irradiation with very modest doses (31), though only in the absence of protecting substances.

Inactivation of this enzyme may interfere with carbohydrate metabolism, but it is also conceivable that adenosinetriphosphatase is involved

in the transfer of the phosphate groups into the desoxyribonucleic acid molecules, its inactivation frustrating this process.

In the study of the effect of the ionizing radiation on cellular metabolism it may be a profitable line of attack to compare the activity of enzymes involved in carbohydrate metabolism and also of other enzymes extracted from irradiated growing tissues and from growing tissues of controls. Phosphatase activity in Kato's rabbit sarcoma upon irradiation with 600 r was found to decrease after the lapse of only 7 days (52). A decrease in the activity of alkaline phosphatase present in the plasma of the rat occurs after x-irradiation (53).

The anomalies observed in the distribution of desoxyribonucleic acid were found to be those produced in the distribution of phosphatase (102). Parallelism between the intensity of the nuclear alkaline phosphatase reactions and the turnover rate of desoxyribonucleic acid was emphasized by Brachet (54). This observation enhances interest in the study of the inactivation of alkaline phosphatase.

A reason why the inactivation of enzymes may perhaps be considered the primary seat of inactivation is the low concentration of enzymes in the tissues. The decomposition products of water or other tissue constituents, which on their way to the enzyme escape reaction with the numerous "protecting" molecules present in the tissue, may reach and inactivate enzymes. The number of such successful molecules may, however, not suffice to inactivate more common tissue constituents present in larger amounts. This line of thought is based on experiments on enzyme inactivation *in vitro*. The probability of inactivation decreases with increasing concentration of the product to be inactivated (32).

From the fact that inactivation is produced in some cases by very restricted doses we have to conclude not only that some enzymes are easily inactivated, or their new formation is easily prevented, but also that the cellular placing of these enzymes must be such that they are easily reached by the inactivating radicals (38*a*). The possibility cannot, however, be excluded that sulfhydryl groups, for example, present in numerous compounds involved in mitosis and other processes, and present in a much higher concentration than those of —SH enzymes, are oxidized by ionizing radiation and thus set out of action (31, 31*a*, 50).

Quite apart from the important problem as to which is or are the sensitive spots in desoxyribonucleic acid synthesis, and possibly in that of other nuclear constituents as well, the sensitivity of the mitotic process to the effect of ionizing radiation can to some extent be ascribed to the great speed with which the synthesis of highly complicated molecules takes place. Ample evidence is available that the reduction of

the rate of nuclear synthesis due to lack of oxygen or low temperature often reduces considerably the sensitivity of the tissue to the effects of irradiation.

Barley seeds show a reduced sensitivity when irradiated in vacuum (103). Inhibition of the growth of *Vicia faba* root tips is materially reduced if the exposure is made under anaerobic conditions (55). Similar results obtained by other experimenters will be mentioned later. *Torula cremonas* is more sensitive to x-rays in the presence of oxygen (56). Five times as many roentgens are necessary to produce an equivalent killing effect when irradiation of bacteria is done in the absence of oxygen than when oxygen is present (104). Cells forming the margins of carcinomatous cell masses are more sensitive to gamma radiation than the central cells. A reasonable explanation for this fact is that the marginal cells, being near the blood vessels, have a more abundant oxygen supply and are, therefore, more radiosensitive. Treatment of mice with thyroxin greatly increases the lethality of a given dose (105). Cells under anaerobiosis are relatively insensitive to radiation (57, 139, 140). Variations in blood supply, and hence in oxygen supply, control radiosensitivity of tumors. Embryos become more radiosensitive as soon as a blood supply is established (56*a*, 57, 136). There is a striking reduction in the number of recessive lethal mutations induced in *Drosophila* males when they are exposed to x-rays while in an atmosphere of low oxygen concentration (76*a*). The protecting effect of cyanide mentioned on p. 200 may also be interpreted as due to a reduced oxygen consumption. To protect against radiation damage, the radiation has to take place shortly after the administration of cyanide, thus when oxygen consumption is markedly reduced. The protecting effect is only minute when the cyanide is injected 15 min after irradiation (41). The protecting effect of cysteine may also be partly or wholly ascribed to oxygen starvation produced by the easily oxidized cysteine.

Only when the solution contained oxygen did Butler and Conway (87) observe an after-effect of irradiation with 7000 r on a thymus nucleic acid solution. They found the effect not to be due to the formation of hydrogen peroxide, but possibly to the HO_2 radical.

Studies on inactivation of some enzymes *in vitro* (106) show no influence of the oxygen concentration of the medium on their radiosensitivity. In the case of ribonuclease (107), which is inactivated under the action of hydroxyl radicals in the medium, dissolved oxygen was found not to play an essential part. Reduced oxygen supply may slow down the metabolic rate, reducing the rate of formation of noxious products or giving more time to wipe out lesions. Reduced oxygen supply can, however, interfere with radiation sensitivity in different

ways as well (133). As already mentioned, H_2O_2 and possibly other inactivating peroxides are not formed under the action of x-rays in the absence of oxygen.

Not less spectacular is the effect of low temperature on radiosensitivity. When eggs of *Drosophila* are exposed to x-rays at 13°, 23°, and 28° C and incubated at room temperature, greatest injury (failure of hatching) is found in eggs exposed to radiation at higher temperatures. When the eggs are irradiated at room temperature and then incubated at 18° and 28°, greater injury is again observed at the higher temperature (58).

At low temperature or in the absence of oxygen, cell division is stopped and the formation of additional cellular components is not required; enzyme inactivation remains thus without far-reaching consequences. If, however, the supply of pertinent components is suppressed in the course of an intense synthetic process, such a suppression may lead to a lethal or other far-reaching effect.

Tissue injury of the skin of 1-day-old rats irradiated at 0–5° C with dosages ranging from 300 to 3000 r is comparable to that of the rat treated with only 300–600 r at 30° (59). When newly born mice are kept for 10 min at 0° and irradiated for 1 min with 1500 r, they develop normally, in contrast to mice irradiated at room temperature. Anesthesia with nitrogen or carbon dioxide was found by Lacassagne to have the same effect as has cooling mice (60). These and numerous similar results clearly bring out some connection between the intensity of nuclear synthesis and radiosensitivity. In frogs, however, toxicity was found by Patt (83) not to be influenced by altering their body temperature during the first 24 hr after total-body irradiation with 1000 or 3000 r. Survival is greatly enhanced as long as the animals are kept in the cold (5–6° C) continuously after the exposure. This altered sensitivity is interpreted to be due to a prolongation of the latent period (cf. also 108). A still greater difference with regard to the effect of temperature is shown by plants. In *Vicia faba* Mottram found many years ago that roots irradiated when at 0° C are markedly more sensitive than those irradiated at 24° C (141).

Chromosome Rupture

In the above consideration an attempt was made to explain the effects of ionizing radiation on cell division as an interference with synthetic processes taking place in the tissue cells, which ultimately induce the cells to divide. Another type of interference is, however, to be considered also. If under the action of ionizing particles chromosome

threads are broken, synthetic processes taking place on the thread surface may also be influenced or interrupted. The possibility has also to be envisaged that enzymatic processes taking place in the cytoplasm are governed by the happenings in the nucleus (129, 138). In that case a chromosome break may also have far-reaching consequences.

It is known that massive doses of roentgen rays in the range of 1,000,000 r may not only depolymerize desoxyribonucleic acid, but lead to some fission of glycosidic linkages with the liberation of purine bases, to some breaking of the ester linkages, to splitting of the internucleotid linkages, and so on (62). Doses of the order of 50,000 r markedly decrease the viscosity and thus influence the degree of polymerization of desoxyribonucleic acid (63). The rigidity of nucleoprotein thread is diminished under the action of a few thousand roentgens (64). Dielectric investigations also indicate the depolymerizing effect of irradiation. Dielectric dispersion curves for aqueous solutions of desoxyribonucleic acid irradiated with 5000 r differ from those of the controls (65). It is conceivable that irradiation with even a few hundred roentgens produces some depolymerization of desoxyribonucleic acid which facilitates the breaking of chromosomes. Chromosome constituents other than desoxyribonucleic acid may also be changed in chemical composition, the change leading to a breakage of the chromosome thread.

The effects of hard roentgen rays, soft roentgen rays, neutrons, and alpha particles on chromosomes in microspores of *Tradescantia bracteata* can be accounted for (66) by assuming that all observed aberrations arise from chromosome or chromatid breaks primarily produced by the passage of an ionizing particle through the thread at the locus of the break (67, 117). It is not without interest, however, to note that the aberrations seen at metaphase 24 hr after a roentgen-ray dose of 150 r were found by Catcheside to arise entirely from chromatid injuries in early prophase when intense nuclear synthesis sets in (68).

Furthermore, chromosome fragility is generally greatest at the end of the resting stage and in the most actively growing and dividing cells. It is virtually absent in the heterochromatin and in the prophase chromosomes (69). Death of cells was interpreted by Koller as a loss of chromosome fragments at the ensuing mitosis (70, 115). The chromosome breakage observed in the roots of *Vicia faba* and *Allium cepa* was also interpreted as resulting from changes taking place before the formation of the chromosome matrix in the interphase and prophase (70*a*).

Above several cases were mentioned in which the absence of oxygen during irradiation markedly reduced radiosensitivity. Giles and Riley (71, 72) studied the effect of oxygen on the frequency of x-ray-induced chromosomal aberrations of *Tradescantia* microspores observed at a

4-to-5-day interval following irradiation. Initial comparative exposures made in air, in nitrogen, and in oxygen demonstrated that aberration frequencies were markedly reduced in nitrogen and increased in oxygen as compared with air (cf. 139). It was also shown that the effect of oxygen in increasing aberration frequency was exerted on the initial breakage mechanism rather than on the recovery process. Similar behavior was shown by *Drosophila* irradiated in the presence or absence of oxygen (89).

The frequency of aberrant cells in *Vicia faba* is much higher when a particular dose of x-rays is administered in the presence of oxygen than it is when the oxygen is replaced by nitrogen. On the other hand, oxygen has little, if any, influence on the effectiveness of alpha rays (73). That the number of structural changes produced by a given dose of alpha radiation can be much larger than that produced by the same dose of x-rays, gamma rays, or neutrons has been repeatedly observed. For example it is true for *Tradescantia bracteata* (75). A possible interpretation of these observations is the following. The primary action responsible for radiation lesion is due to the effect of H_2O_2, or products formed from H_2O_2. Whereas H_2O_2 is formed in alpha-ray-irradiated water even in the absence of oxygen, its formation under the effect of x-rays requires the presence of oxygen in the water (74).

That ionizing radiation can disrupt bonds in the absence of all metabolic influence follows from the above-mentioned experiments in which irradiation *in vitro* was found to depolymerize desoxyribonucleic acid. Simultaneous treatment of *Tradescantia* with x-rays (250 r) and sonic energy (9100 cycles per second) increases the yield of x-ray-induced chromosomal aberrations by approximately 1.3 times the yield obtained with the same amount of x-rays (76). It also follows from the fact that chromosome breakage is observed after irradiation at low temperature and under other conditions of almost imperceptible metabolic activity. Fabergé (77) observed chromosome breaks in *Tradescantia* pollen grains at $-192°$ C. Finally, chromosome breakage was shown very spectacularly in experiments where hemocyanine was irradiated with alpha rays at the temperature of liquid air. Under these conditions a marked depolymerization of the highly polymerized molecule was observed (78).

In the above discussions we considered mostly the mitotic effects of ionizing radiation. We do not lack indications, however, that other than mitotic effects may be decisive for the histological response of tissue to radiation. The lymphoid tissue and intestinal epithelium, which are among the most radiosensitive tissues in the body, show less interference of mitotic activity after exposure to x-rays than do the less radiosensitive skin and adrenal gland (79). We may attempt to make a less pro-

nounced regenerative power of the first-mentioned tissues responsible for their greater radiosensitivity. It is more difficult to interpret on such lines the finding of Jacobson that erythroblast vulnerability to irradiation injury is not enhanced by increased mitotic activity (80). The power of the tissue to wipe out changes produced by irradiation is as important a fact in determining the radiosensitivity of the tissues as its mitotic response to radiation.

In all those cases in which the target theory is considered to be applicable, as, for example, in the production of gene mutation, the changes effected by irradiation are assumed to be irreversible. Work of Spencer (81) and Bonnier (82) indicates, however, that besides the irreversible direct hit action of ionizing radiation there is an additional reversible effect. This additional effect becomes negligible as soon as the x-ray dose amounts to a few hundred roentgens. Carlson (130) found a gamma-ray dose of 128 r to be more effective in depressing mitosis of the grasshopper neuroblast if given at 32 r per minute than at 2 r per minute.

In the adult organism the oxygen consumption due to mitotic processes is an almost negligible fraction of the total oxygen uptake. In such an organism a significant change of oxygen consumption due to irradiation must be ascribed to interference of the irradiation with other than mitotic processes. Measurements of both the oxygen consumption and carbon dioxide output by irradiated animals indicate such an interference.

Application of Radioactive Indicators

Numerous and partly contradictory data are available on the effect of irradiation on oxygen consumption. In a recent investigation on the frog, after irradiation with 3000 r there was found a slight early decrease in oxygen consumption with maximal depression of 34 per cent 15 days after exposure. This was followed by return to control levels about 25 days after exposure (83). Rats, after total-body irradiation of 809–972 r, showed, on the other side, an elevation of oxygen consumption of approximately 35 per cent occurring within 24 hr (84). When C^{14}-labeled glycine was injected into the rat 48 hr after exposure to roentgen rays, a striking increase in the C^{14} activity of the expired CO_2 was observed by Altman (85). A depressing effect of irradiation on the metabolic rate was observed by Barron in investigations in which the effect of x-rays on tissue slices was studied (89*a*).

The effect of irradiation on metabolic processes taking place *in vivo* in a single organ can be studied by comparing the rate of incorporation of labeled atoms into tissue fraction in irradiated animals and in controls.

The administration of a radioactive indicator of one of the main body constituents is generally followed by a marked decrease in the specific activity of the labeled compound. After the injection, for example, of labeled sodium phosphate into the circulation, the specific activity of the plasma inorganic P rapidly decreases as the radioactive phosphate penetrates into the tissue cells and is incorporated into organic P compounds and into the mineral constituents of the skeleton, some P^{32} being excreted. Because of these processes the sensitivity of P^{32} as an indicator markedly increases during the experiment. If at the start of the experiment the presence of 1 microcurie of P^{32} in the plasma inorganic P indicates the presence of 1 mg P^{31}, in a later stage of the experiment the same activity will indicate, quite apart from the decay of the activity of P^{32} with time, the presence of much more that 1 mg of inorganic P.

Consider, for example, the formation of labeled liver lecithin following intravenous injection of P^{32}. In the first day as much lecithin will be turned over as in the second one, but because of the decrease in P^{32} in the inorganic phosphate pool with time the incorporation of the same amount of phosphorus into liver lecithin will be followed in the second day by the incorporation of appreciably less P^{32} than in the first day. One week before the administration of P^{32} one leg of a mouse was irradiated with roentgen rays, and the irradiated leg was found to take up only 70 per cent of the amount of P^{32} incorporated into the non-irradiated leg (86). Thus the amount of P^{32} removed from the inorganic phosphate pool was reduced by altering the metabolic rate, in this instance by exposure to ionizing radiation. Correspondingly the liver lecithin of an irradiated mouse will incorporate more P^{32} in the course of a given time than does liver lecithin in a control mouse. The increased specific activity of the lecithin P in the irradiated animal will not be due, as one may be inclined to interpret, to an increased lecithin turnover in the liver of the irradiated animal. It is due to an interference of irradiation with the turnover rate of the mineral constituents of the skeleton, which makes P^{32} a less sensitive indicator of phosphorus in the irradiated mouse than in the control.

Bicarbonate, acetate, succinate, and a great number of other carbon compounds are metabolized at a spectacular rate. For example, in the mouse less than 1 per cent of labeled acetate is present 24 hr after injection of acetate, labeled in the carboxyl group. Interference with the normal metabolic pattern can thus be expected to lead to a change of the activity level of the respiratory CO_2. Such a change can be expected to reflect itself, for example, in a change of the activity level of glycogen carbon.

In an investigation carried out by Forssberg and the writer (92), administration of cyanide to mice led to a marked depression of the amount of CO_2 exhaled and, correspondingly, to an increase in the activity level of exhaled CO_2. The latter reflects an increased C^{14} incorporation into glycogen, which after administration of labeled bicarbonate and non-labeled glucose mainly takes place, according to Wood (111), in the 3,4 position of the molecule. The increased incorporation of C^{14} into liver-glycogen carbon in mice previously injected with cyanide is not due to an increased glycogen formation, but to the increased activity level of the expiratory CO_2. Increased C^{14} incorporation into liver glycogen of irradiated rats has correspondingly to be interpreted as due to a metabolic depression.

The writer, in the investigation of the effects of irradiation on the incorporation of C^{14}-labeled acetate on the various tissue fractions (dry tissue, *total* fat, and proteins) in 200 *fed* mice, studied in ten groups, found that, though the mean value of C^{14} incorporation into all but the protein fraction of the intestinal mucosa was higher in the irradiated rats than in the controls, these differences cannot, in many instances, be considered significant. An increase, apparently significant, in the incorporation of C^{14} into dried brain tissue (34 per cent; P less than 0.03 per cent), into brain proteins (16 per cent; P less than 0.05 per cent), brain fats (31 per cent; P less than 0.015 per cent), dried plasma (12 per cent; P less than 0.01 per cent), liver proteins (12 per cent; P less than 0.04 per cent) in the irradiated mice was, however, observed.

Forssberg and the writer found (92), when investigating the effect of irradiation with 900 r on 340 *fasting* mice, no significant change in the incorporation of acetate C^{14} into liver and other investigated protein fractions. A significantly reduced incorporation (12 per cent; P equal to 0.001 per cent) into the fatty acids was, however, observed.

Under the effect of an x-ray dose of 300 r or more, the rate of absorption of the alpha-carbon-labeled glycine from the intestinal tract of the rat was found to be markedly reduced (85). Other constituents of the diet, as glucose (132), may be reabsorbed from the intestinal tract of the irradiated animal at a slower rate as well. A depressing effect of irradiation on the metabolic rate was furthermore observed by Barron (89*a*).

That, after administration of labeled glycine, more C^{14} was taken up by acutely starved rats (90) than by the controls is possibly also due to a decrease in the sensitivity of the radioactive indicator in starving rats. Salomon (93) found irradiation with 600 r to increase the C^{14} ratio of hemin to globulin. In these experiments, glycine labeled in the methylene group was administered to rats. A greatly increased incor-

poration of C^{14} into the free carboxyl groups of the amino acids in liver protein hydrolysates was observed by Hempelmann (94) after administration of carboxyl-labeled alanine to mice.

Not all differences found in the rate of C^{14} incorporation due to irradiation are to be explained by a change in the sensitivity of the radioactive indicator. For example, because of decreased rate of incorporation of glucose into liver glycogen of the irradiated guinea pig, as found by Lourau (131, 132), less glucose C^{14} is expected to be incorporated into liver glycogen in the irradiated animal.

The application of C^{14} as an indicator reveals a great variety of indirect effects. Interference with the normal steps of the Krebs cycle in the liver may lead to a changed activity level of the circulating CO_2, which may reflect itself in the extent of C^{14} incorporation into muscle glycogen. Interference with the formation rate of labeled liver protein, due to changes in the sensitivity of the indicator or to changed turnover rate, will influence the C^{14} content of plasma proteins and of proteins of organs which take up proteins from the plasma. Acetoacetate, which is one of the main fuels of muscle metabolism, is not synthesized in that tissue (91) but is presumably carried into the muscle cells from the liver or the intestinal mucosa. The effect of irradiation on the last-mentioned organs must therefore reflect itself in the extent of C^{14} incorporation into muscle constituents. We meet here a great variety of indirect effects of irradiation, some of which are discussed on p. 197 and in H. Jones's paper.

The mapping out of a metabolic route by the use of C^{14} as an indicator is an arduous task. The task will be even more arduous if the effect of irradiation on this route is to be elucidated. Changes produced in one organ may be reflected very markedly in reactions taking place in another organ. Changes in hormone production produced by irradiation may, furthermore, exert a pronounced influence on metabolic pathways. In spite of these facts, the application of C^{14} to radiation studies may open a profitable line of attack.

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DISCUSSION

PATT:

Concerning the relationship between the rate of metabolism and radiosensitivity, I should like to point out that, although there is considerable evidence which indicates that the level of metabolism after irradiation influences the effect, evidence relating to the influence of metabolic rate during irradiation is equivocal. In the frog, for example, altering temperature during irradiation does not influence the response. Likewise, depriving the frog of oxygen before and during exposure to x-rays is without effect.

Regarding the protective action of cysteine, I believe that we must not lose sight of the fact that cysteine protection against x-rays may not necessarily be related to the neutralization of free radicals. It is not unlikely that cysteine and anoxia effects in mammals are similar and are related to some change in the pathways of metabolism. In view of the discrepancy between the frog and the rat in regard to the influence of temperature and anoxia, we plan to determine whether cysteine will protect the frog.*

HEVESY:

Patt's experiments are most interesting and show that the frog is different from the mouse with regard to the temperature effect. The frog has a low metabolic rate. In mammals with a high metabolic rate the situation is fundamentally different, and I feel that the initial metabolic rate in the mammal has some effect on the response to radiation. In the frog, if the temperature is

* A protective effect of anoxia has been demonstrated in recent studies with frogs (D. E. Smith, E. B. Tyree, and H. M. Patt, unpublished data). The early failure to induce resistance in this species with anoxia may have been due to some latent infection in the animals, since protection was observed subsequently when streptomycin was added to the water or when the radiation dosage was decreased.

lowered in the postradiation state, the appearance of radiation damage is delayed until the animal has been warmed up again.

DOWDY:

I feel that we should distinguish between oxygen presence and oxygen utilization or metabolic rate, particularly in the warm-blooded mammals. Bennett, in our laboratory, has demonstrated very well the protection obtained from anoxic anoxia in rabbits, rats, and mice. The degree of anoxia permissible is dependent upon a very fine balance. For example, the rat tolerates an atmosphere of 95 per cent nitrogen and 5 per cent oxygen. The adult mouse, however, will succumb to such a low atmosphere of oxygen. With 7 per cent oxygen in the inspired air the mouse will live and if irradiated at this time will derive a relative degree of protection. There is little or no protection when the mouse is irradiated in an atmosphere of 10 per cent oxygen. When rats are irradiated in an atmosphere of 5 per cent oxygen, there is complete protection from 600 r total-body radiation for an indefinite period of time. The 30-day LD_{50} for rats in our laboratory is approximately 600 r. By these acute anoxic experiments we can raise the 30-day LD_{50} in rats from 600 r to between 1200 and 1400 r total-body radiation. However, at 1000 r and above, the rats die between 60 and 250 days, depending upon the total dose of radiation. This late death is entirely different from what we are accustomed to see.

Bennett also tied up the respiratory enzymes with cyanide without protection to acute total-body radiation. Patt and his associates have demonstrated that, if frogs are irradiated at a low temperature, while this low temperature is maintained the animals appear normal; but once they are brought back to normal temperatures destructive changes take place and the animals die similarly to the controls. Haley, in association with our group in our laboratory, has failed to obtain protection in rats who were thyroidectomized. Large doses of thyroxin, on the other hand, accelerated mortality in irradiated rats.

It therefore seems that the presence of the oxygen molecule in the tissues at the time of irradiation permits certain chemical reactions to take place. These reactions may be slowed down or accelerated, depending upon the metabolic rate. If the oxygen molecules are greatly reduced in the tissues, certain chemical reactions either do not take place or occur at a greatly reduced frequency. It seems obvious, however, that certain chemical reactions occur which do not require the presence of molecular oxygen and are manifested by late deaths 60–250 days later.

TAHMISIAN:

I wish to bring up the question of metabolism. We have irradiated grasshopper eggs with high doses such as 25,000-200,000 r. Grasshoppers normally have a stage known as diapause. When they enter this stage there is no further development unless this block is broken in nature by the onset of winter or by chilling to 0° C in the laboratory for a period of 3 months. When eggs at this stage are irradiated, immediately after irradiation, even after 200,000 r, no morphological or cytological differences are observed. One cannot tell a control

from an experimental embryo. If the embryos are allowed to metabolize at room temperature, those that receive 25,000 r undergo a negative growth. The negative growth is less pronounced with higher doses, so that with 200,000 r there is no morphological change. Cytologically every nucleus becomes pycnotic after doses of from 25,000 to 200,000 r. The respiration, however, of embryos that receive 25,000 r is approximately 50 per cent higher than those of the control, whereas the respiration of those that receive 200,000 r gradually diminishes.

This indicates that to bring about a change after irradiation in a cell two processes, namely anabolism and catabolism, must be considered. With lower doses only the anabolic processes are injured, whereas with higher doses anabolic and catabolic processes are injured. After irradiation, if experimental eggs are placed in an icebox for as long as 6 months and then observed after removal from the icebox, no damage is seen. However, if these eggs are maintained at a metabolic temperature, the injury shows up as if they were taken from the x-ray machine and left at room temperature.

Low-Beer:

I wonder whether Hevesy compared the uptake of P^{32} in Jensen sarcoma with the oxygen consumption after x-ray irradiation. Some years ago Reiss and I found that, when Jensen sarcoma *in vivo* was exposed to approximately 600 r of 0.7 mm Cu, HVL x-rays, the oxygen consumption decreased by a factor of 4 when measurements were made 24–48 hr after irradiation.

As to the use of P^{32} as a metabolic indicator of radiosensitivity, I might mention that uptake of P^{32} is decreased six- to eight-fold in human breast cancer after a tissue dose of approximately 4000 r (1.0 mm Cu HVL) without discernible microscopic changes of the cells when tissue sections are compared before and 4 weeks after irradiation. I should mention that a decrease of P^{32} uptake occurs also in some instances after the administration of estrogens and in some lymphoma group tumors after administration of HN_2. Thus the effect apparently is general metabolic inhibition.

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The Effect of Ionizing Radiations on Some Systems of Biological Importance

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A quarter of a century ago a great controversy arose in the field of cellular respiration. On one side were the partisans of the theory of activation of oxygen as the all-important process, and, on the other, the partisans of the theory of hydrogen activation. The controversy became more and more heated with neither side willing to retreat. Even the late Sir Frederic Hopkins' oration at the Physiology Congress of Stockholm, advocating that both sides were right, did not bring the necessary calm. Years had to pass to demonstrate that, in fact, both theories could be harmonized into one. The same sort of controversy has been going on in the field of ionizing radiations in biology between the partisans of the "target" or "one-hit theory" and those of the "indirect action." As in the case of cellular respiration, indications are that both theories are partly correct. The definite progress made during the last ten years has, in fact, demonstrated that both processes occur when ionizing radiations strike living cells. The cell components may obviously be affected by direct collision with the ionizing radiations. They will also be affected by the products of ionization of water and of oxygen. The contribution of each one of these actions will depend on a number of factors, such as stability of the components, nature of the environment, and reactivity towards the products of ionization of water. In calculating the part played by these two actions, however, it must not be forgotten that water comprises 80 per cent of the total body weight, and that this fluid is permanently saturated with molecular oxygen. The radiochemical reactions of biological importance are, then, those taking place in an aqueous, oxygenated milieu.

Ionizing Radiations and the Thiol Groups of Cells

When in 1943 I was asked to study the mechanism of action of ionizing radiations, I was struck by the similarity of response produced by such seemingly different agents as mustard gas, nitrogen mustard, and x-rays. All three agents produced leukopenia, hemorrhagic manifestations, mutations, and inhibition of mitosis and of cell division. Since the first two have one common chemical property, that of reacting easily with thiol groups to form alkylated compounds, and in 1930 Rapkine (1) had demonstrated that —SH groups are necessary for mitosis and cell division, it was reasonable to postulate that one of the effects of ionizing radiations was the destruction of the thiol groups. Such destruction would be caused by the oxidation of —SH groups to the —S—S bond, a process brought about by the products of irradiation of water: the radical OH, a product of the primary ionization reaction; the radical O_2H; and H_2O_2 formed in the presence of oxygen. Thus, four —SH equivalents would be oxidized per ion pair:

$$2\text{—SH} + 2OH = \text{—S—S—} + 2H_2O$$

$$2\text{—SH} + 2O_2H = \text{—S—S—} + 2H_2O_2$$

$$2\text{—SH} + H_2O_2 = \text{—S—S—} + 2H_2O$$

The first experiments to test the plausibility of the hypothesis were performed on thiol enzymes, that is, those enzymes that require the presence of —SH groups for activity. Thiol enzymes, such as phospho-

TABLE 1

Effect of X-Rays on the Activity of —SH Enzymes

(From Barron *et al.*, *J. Gen. Physiol.*, **32**: 537, 1949)

Enzyme	X-Ray Dose, r	Inhibition, %	Reactivation, %
Phosphoglyceraldehyde dehydrogenase	100	21	Complete
	200	50	62
	500	94	10
Hexokinase (yeast)	2000	19	
Adenosinetriphosphatase (myosin)	10	10	Complete
	100	22	Complete
	500	41	54
	1000	73	22
Succinoxidase (muscle)	5000	75	94

glyceraldehyde dehydrogenase, adenosinetriphosphatase, and succinodehydrogenase, were reversibly inhibited by ionizing radiations (Table 1). Furthermore, the ionic yield was higher than that obtained by Dale on inactivation of non-thiol enzymes (Table 2). Protection and

TABLE 2

Ionic Yields of Enzymes Inhibited by X-Ray Irradiation

Enzyme	Ionic Yield
Phosphoglyceraldehyde dehydrogenase	0.93
Trypsine	0.025
Ribonuclease	0.03
Carboxypeptidase	0.16
d-Amino acid oxidase	0.1
Catalase	0.06

reactivation of the enzyme were achieved by the addition of glutathione. I urged at that time the use of thiols for prevention and treatment of x-ray sickness. Feeble, unsuccessful attempts were made then (1944) at the Metallurgical Laboratory. It was not until 1949 that Patt and his coworkers (2) and Chapman and his coworkers (3) simultaneously succeeded in protecting animals against lethal doses of x-rays by the previous injection of thiol compounds, such as cysteine, BAL, and glutathione.

The importance of this reaction (oxidation of —SH groups by ionizing radiations) is the consequence of the tremendously important role of thiol groups. As soluble thiol compounds (glutathione, cysteine) they are one of the regulatory mechanisms of cellular metabolism (4); they take part—as yet in an unknown manner—in the processes of mitosis and of cell division and cell growth. As fixed thiol groups, attached to the side chains of proteins and of nucleoproteins, they contribute to the activity of enzymes, to the union of muscle proteins, to blood coagulation, and to the flexibility of fibrous proteins, such as those in the skin (kerateins) and in the lens.

I believe that the cataract produced by ionizing radiations is due to oxidation of the thiol groups of the lens proteins; oxidation of the —SH groups would produce intermolecular —S—S bridge formation with consequent polymerization, thickening of the protein molecules, and production of opacity [von Euler (5) and Bellows (6) have both shown that the —SH content of the lens proteins is diminished in cataract]. A similar explanation may be given for the production of chromosome breaks on irradiation. According to Davidson and Lawrie (7), chromosomes contain very few thiol groups. Their oxidation would

result in the formation of —S—S— bridges between molecules with production of asymmetry and "breakage." Thoday and Read (8), it must be recalled, reported that x-ray irradiation of root tips of *Vicia faba* under anaerobic conditions had a much reduced effect on the growth rate; there was also considerable reduction in the number of cells showing chromosome bridges or fragments at anaphase. Lack of oyxgen prevented the formation of the O_2H radicals and of H_2O_2; hence, an inhibition of oxidation processes would be the consequence. I might go as far as to postulate the existence of an —SH enzyme for the synthesis of ribonucleic acid, an enzyme containing freely reacting —SH groups, easily attacked by alkylating and oxidizing agents. This would explain the inhibition of cell growth produced by alkylating agents (mustard, nitrogen mustard) and by oxidizing agents (ionizing radiations). It must be emphasized that, whenever there is a diminished effect in the absence of oxygen, this effect must be attributed to oxidation and not to direct collision or "hit."

The rapid oxidation of thiols by ionizing radiations was shown in experiments in which glutathione, BAL, and other dithiols were irradiated with x-rays, gamma rays, and beta rays. In all cases, the ionic yield was over 3, which means a 75 per cent efficiency of the oxidation reaction (Table 3). It was possible also to measure the part played

TABLE 3

IONIC YIELDS OF THIOL COMPOUNDS OXIDIZED BY X-RAYS AT *p*H 7.0

(From Barron *et al.*, *J. Gen. Physiol.*, **33**: 229, 1950)

Thiol	Ionic Yield
Glutathione	3.35
Propane 1,3-dithiol	3.5
2,3-Dithiopropanol (BAL)	3.72

by the three oxidizing agents produced on irradiation of oxygen-containing water. In the presence of catalase there was 86 per cent oxidation; in the absence of oxygen there was 33 per cent oxidation as compared to the oxidation of control solutions containing oxygen and no catalase. From these two series of experiments it is possible to calculate the efficiency of the three oxidizing agents in oxidation of thiols: H_2O_2 contributes 24 per cent, O_2H 43 per cent, and OH 23 per cent, of the total oxidation in an oxygenated aqueous milieu.

There is no doubt now of the importance of the thiol groups in ionizing radiations. It must be emphasized, however, that not much attention has been paid to the oxidation of the soluble thiol compounds, those

acting as regulators of cellular metabolism and cell division. The thiol reagents increase cell respiration and inhibit cell division when used in small concentrations, whereas they inhibit cell respiration when the concentration is increased. In the same manner, ionizing radiations used in small doses might increase respiration and inhibit cell division. It is quite possible that the effects of small amounts of ionizing radiations, those which produce nuclear changes, inhibition of mitosis, and production of mutations, may be due to the oxidation of the soluble thiol groups, and not to direct effect on the nucleoproteins.

The Oxidation of Substances of Biological Importance

Ascorbic acid is another oxidation-reduction system that exists in the tissues mostly in the reduced state. Anderson and Harrison (9) found it to be oxidized on x-ray irradiation; the ionic yield, however, was much lower than that of glutathione oxidation, 0.7. Reduced cytochrome c is also oxidized on irradiation with x-rays (Fig. 1). Because

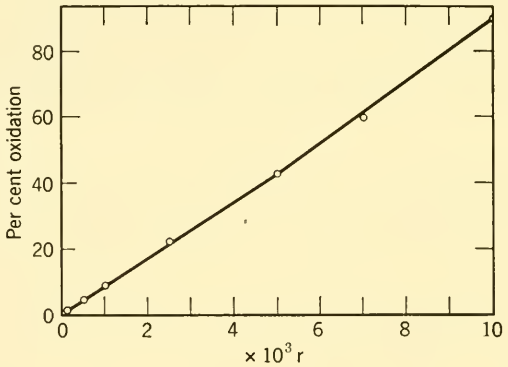

Fig. 1. Oxidation of ferrocytochrome c by x-ray irradiation. Solvent 0.005 *M* phosphate, *p*H 7.0. Cytochrome 1.6×10^{-5} *M*. *Abscissa:* X-ray dose in roentgen units; *ordinate:* per cent oxidation.

of the characteristic absorption spectrum of the different components of the molecule (protein, porphyrin, iron) the effect of irradiation can be easily distinguished. By spectrophotometric measurements it was possible to observe that oxidation of the Fe^{++} cytochrome c occurred before there was any change in the porphyrin or in the protein molecule. Oxidation of ferrocytochrome by ionizing radiations is reversible (Fig. 2). The ionic yield of the oxidation reaction is 0.55. The protein moiety of ferricytochrome c was altered on irradiation with large doses of x-rays, the alteration being more marked in alkaline solutions

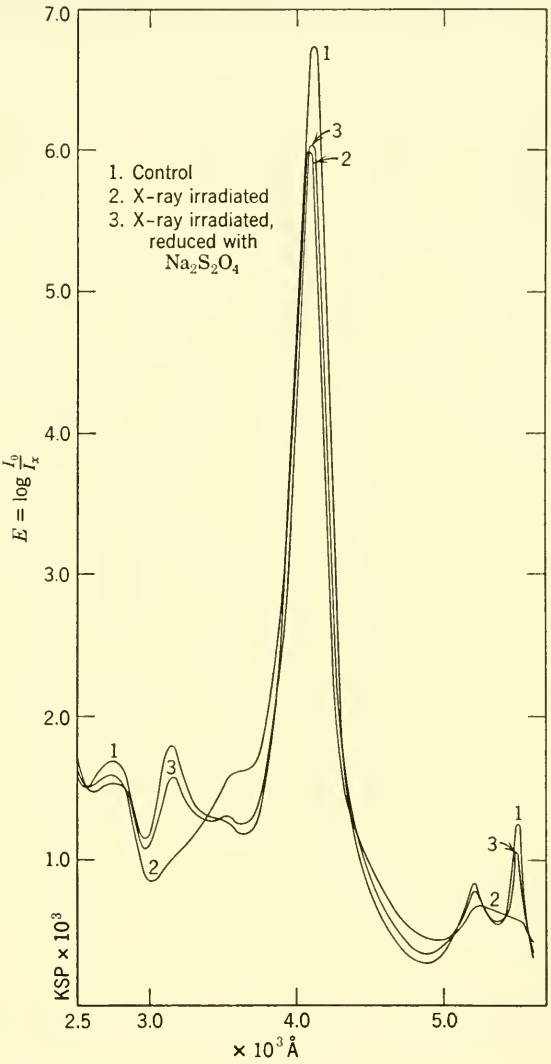

FIG. 2. Reversible oxidation of ferrocytochrome c with x-ray irradiation. X-ray dose, 10,000 r. *Abscissa:* Wavelength in angstrom units; *ordinate:* specific extinction coefficient $\times 10^3 E$.

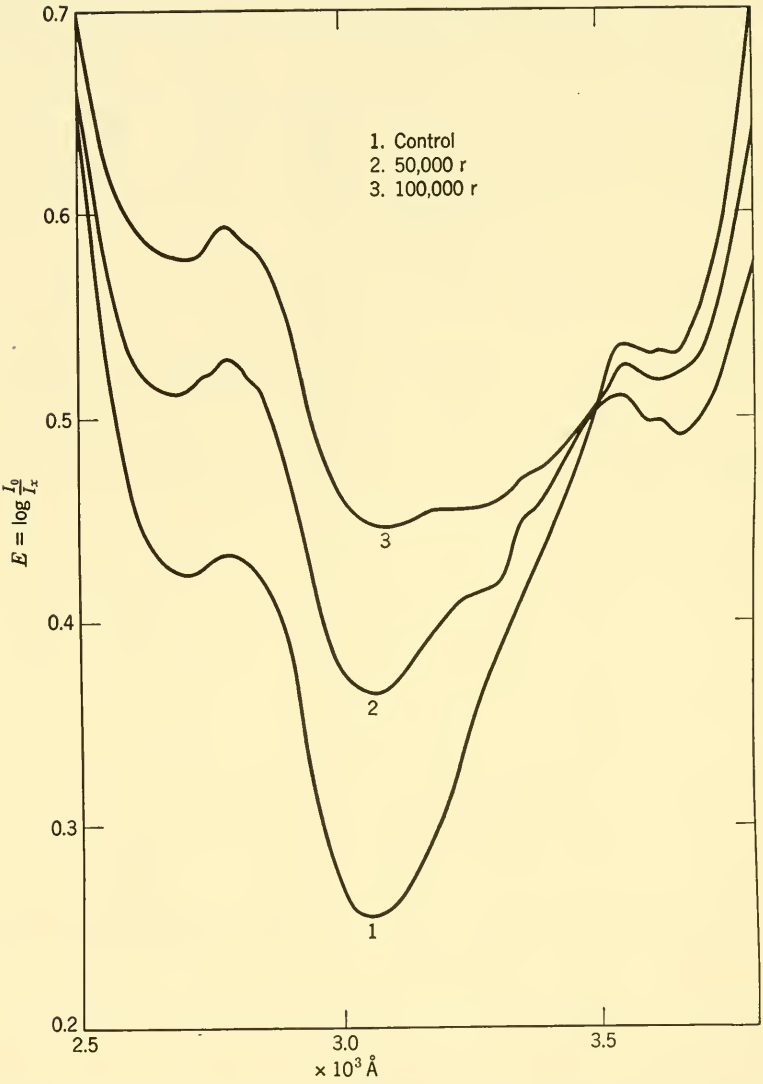

FIG. 3. Effect of x-ray irradiation on the protein moiety of ferricytochrome c, dissolved in 0.005 *M* NaOH. *Abscissa:* Wavelength in angstrom units; *ordinate:* optical density *E*.

(Fig. 3) than in neutral solutions (Fig. 4) or acid solutions (Fig. 5). An examination of the absorption spectrum curves shows that in the region of light absorption by the tyrosine residue (2800 Å) x-rays produced

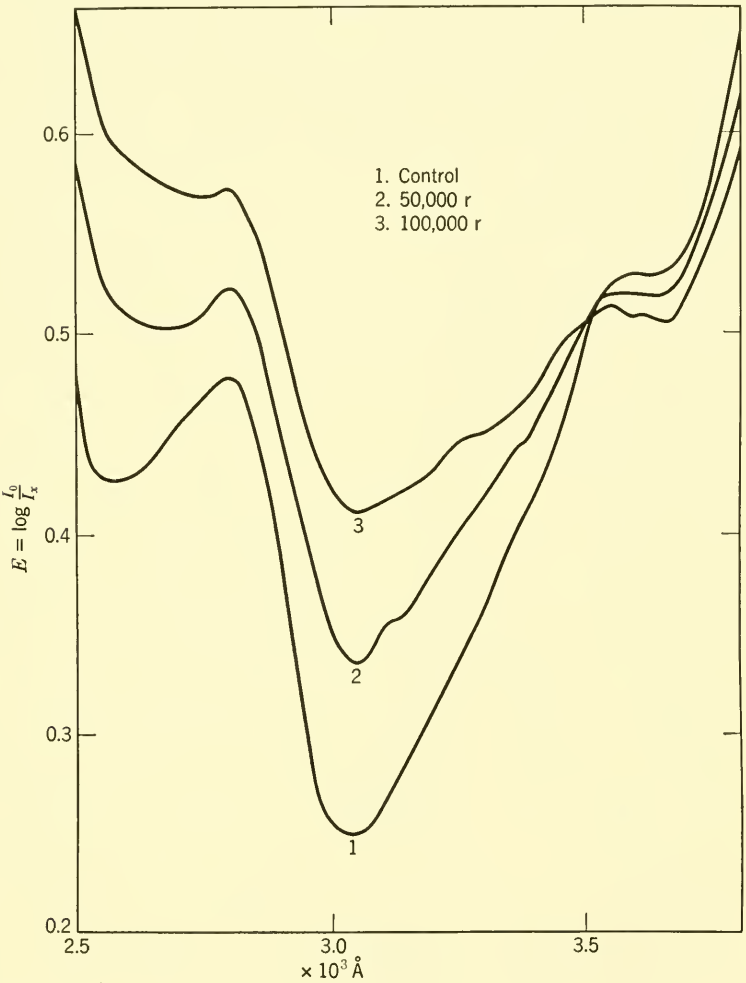

FIG. 4. Effect of x-ray irradiation on the protein moiety of ferricytochrome c, dissolved in 0.005 *M* phosphate buffer, *p*H 7.72. *Abscissa:* Wavelength in angstrom units; *ordinate:* optical density *E*.

an increased absorption. The effect of x-rays on the porphyrin nucleus as observed by light absorption in the ultraviolet was also influenced by the H^+-ion concentration of the solution, as can be seen on observing

the spectrum at 3600 Å. X-ray irradiation reduced the absorption of porphyrin at 4100 Å (the Soret band), the effect being different at

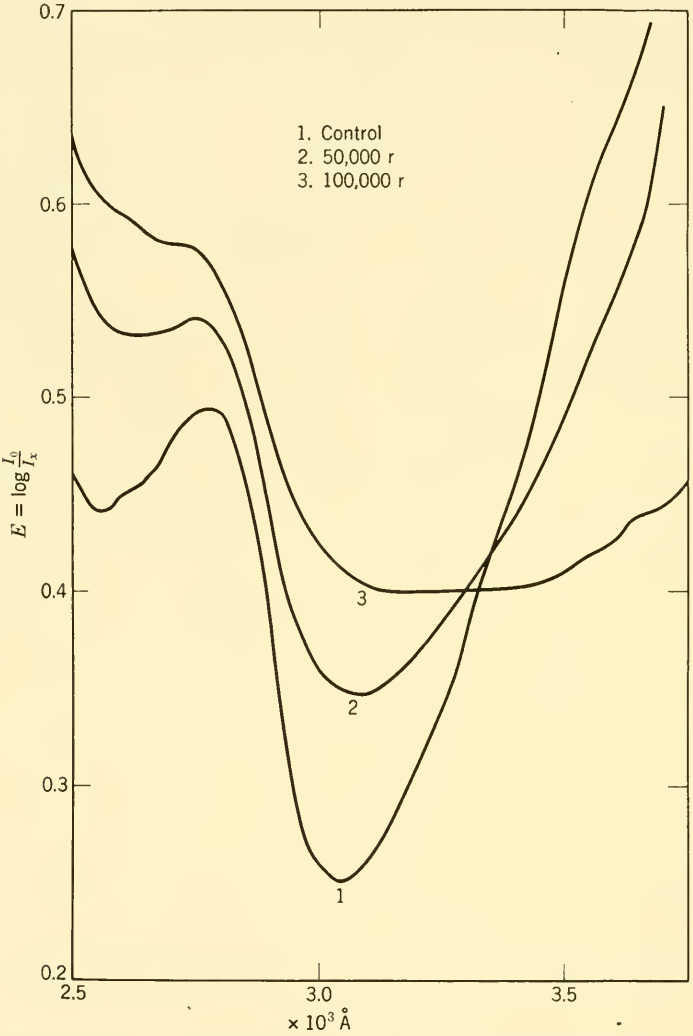

FIG. 5. Effect of x-ray irradiation on the protein moiety of ferricytochrome c, dissolved in 0.005 *M* HCl. *Abscissa:* Wavelength in angstrom units; *ordinate:* optical density *E*.

different *p*H values. In acid solutions, the Soret band almost disappeared on irradiation with 100,000 r (Fig. 6). In alkaline solutions (Fig. 7) and in neutral solutions (Fig. 8) the effect was not as marked

as in acid solutions. The absorption spectrum of ferricytochrome c in the visible light was little changed, and no reduction was observed even

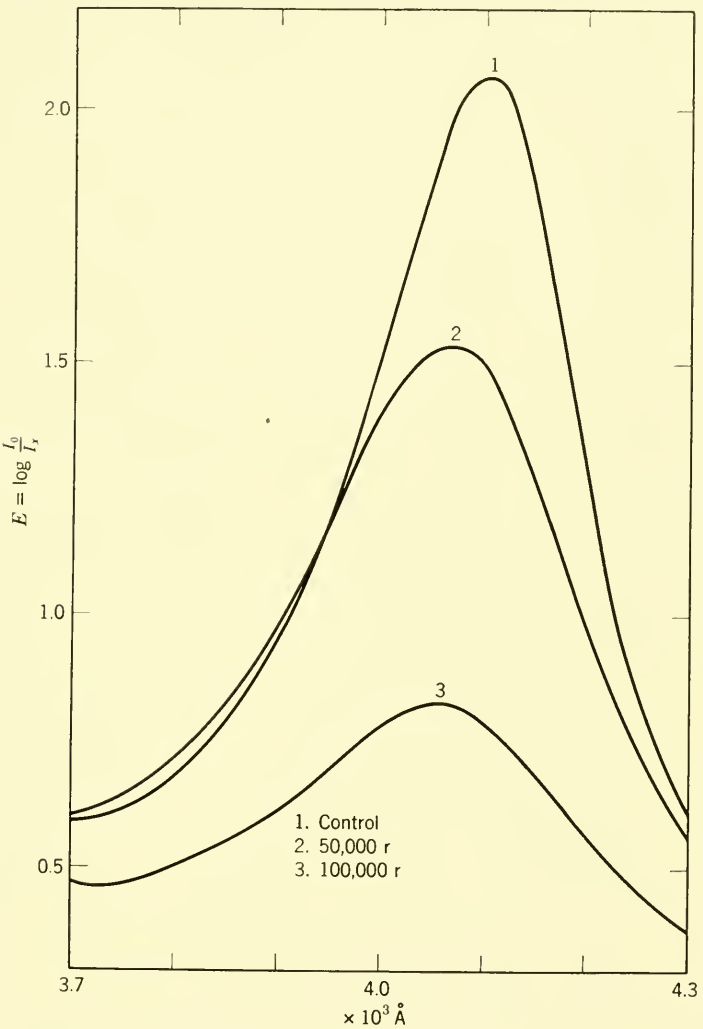

FIG. 6. Effect of x-ray irradiation on the absorption spectrum of ferricytochrome c, dissolved in 0.005 *N* HCl. *The Soret band. Abscissa:* Wavelength in angstrom units; *ordinate:* optical density *E*.

on irradiation with 100,000 r (Fig. 9). Oxidation of Fe^{++} cytochrome c seems to be produced by the OH radicals alone, for neither catalase nor the absence of oxygen had any effect on the ionic yield. The

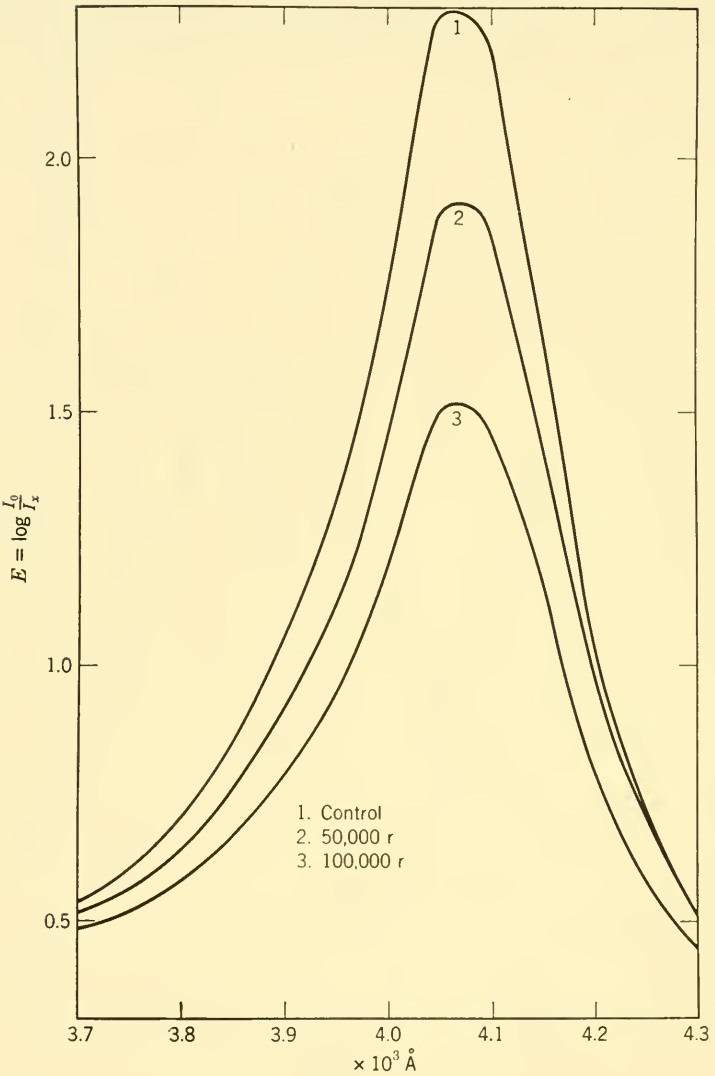

FIG. 7. Effect of x-ray irradiation on the absorption spectrum of ferricytochrome c, dissolved in 0.005 *M* NaOH. *Abscissa:* Wavelength in angstrom units; *ordinate:* optical density *E*.

mechanism of the changes produced in the porphyrin nucleus and in the protein portion is as yet unknown. Perhaps some of these effects are the result of direct collision.

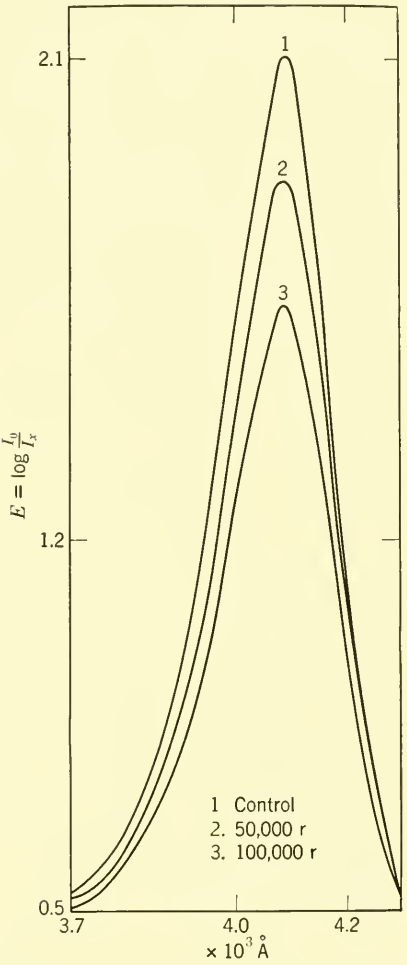

FIG. 8. Effect of x-ray irradiation on the absorption spectrum of ferricytochrome c, dissolved in 0.005 *M* phosphate buffer, *p*H 7.72. *Abscissa:* Wave length in angstrom units; *ordinate:* optical density *E*.

A large number of other systems of biological importance may be oxidized by the products of irradiated water or perhaps directly by the withdrawal of electrons by the ionizing radiation. A systematic study of these oxidations is being made in my laboratory.

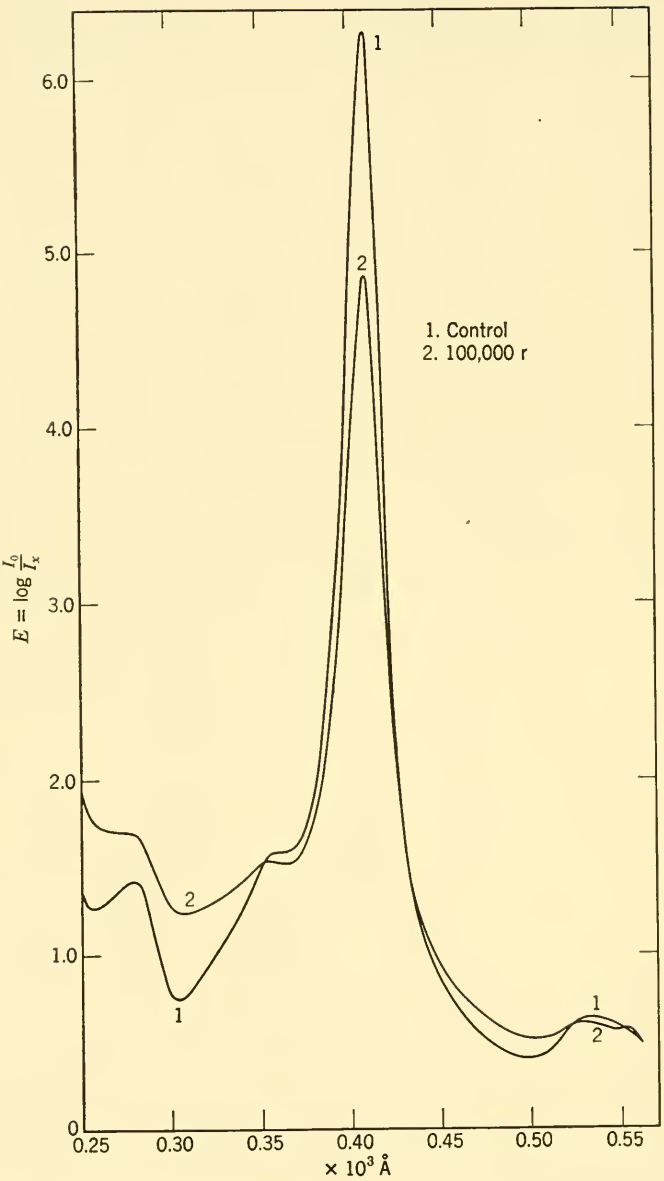

FIG. 9. Effect of x-ray irradiation on absorption spectrum of ferricytochrome c. *Abscissa:* Wavelength in angstrom units; *ordinate:* optical density E.

Ionizing radiations produce also reductions, as shown by the reduction of $KMnO_4$, $Ce(SO_4)_2$, $KClO_3$, KNO_3. Reduction reactions, however, seem to be of no biological importance. In fact, neither oxidized glutathione nor diphosphopyridine nucleotide nor oxidized cytochrome c was reduced on x-ray irradiation.

The Role of H_2O_2

A word may be said about the importance of H_2O_2 on the mechanism of action of ionizing radiations. That H_2O_2 is produced on irradiation of oxygenated water is a well-known fact. As Bonet-Maury and Frilley demonstrated (10), the production of H_2O_2 can be used as a simple method of measurement of x-ray intensity. However, the role of H_2O_2 in the irradiation of living cells has not yet been determined. The amount of H_2O_2 formed in oxygenated aqueous solutions, such as biological fluids, is always less than that formed on irradiation of distilled water because the electrolytes contained in those fluids diminish H_2O_2 formation; furthermore, the universal distribution of catalase will further diminish the amount of H_2O_2. Whether the catalase —H_2O_2 complex will act as an oxidizing agent, as Keilin and Hartree (11) have shown on the oxidation of ethyl alcohol, is not known. The possible role of H_2O_2 could be postulated from the experiments of Evans (12) on inhibition of the fertilizing power of sea-urchin sperm, and from our own experiments on inhibition of respiration (13) when the sperm is suspended in x-ray-irradiated sea water. However, the x-ray dose required to produce such effects is much higher than that required to inhibit both functions (fertilization and respiration) on direct irradiation. The inhibition of respiration produced by x-irradiated water occurred even 2 hr after irradiation. Moreover, the inhibition was not produced by changes in the organic matter, or the salts of sea water, for similar inhibition was observed on irradiation of distilled water and addition of salts afterwards (Table 4). It is obvious that H_2O_2, when added

TABLE 4

Inhibition of Sea-Urchin Sperm Respiration by X-Irradiated Water

(The necessary amount of salts to make artificial sea water was added at different times after irradiation. Irradiation: 100,000 r)

Experimental Condition	Inhibition, %
Soon after irradiation	68
30 min after	65
60 min after	60

in vitro, will oxidize thiol compounds; the experiments in which H_2O_2 or other peroxides produced mutations or chromosome breaks show only that oxidation reactions are responsible for these phenomena. There is no plausible reason, however, to attribute such oxidations to H_2O_2 when there are two other more powerful oxidation products formed on irradiation of water, namely, the radicals OH and O_2H.

Radiations and Coenzymes

Ionizing radiations attack not only easily oxidizable systems, but also a number of substances of biological importance. The adenine residue of adenosinetriphosphoric acid can be oxidized by x-ray irradiation,

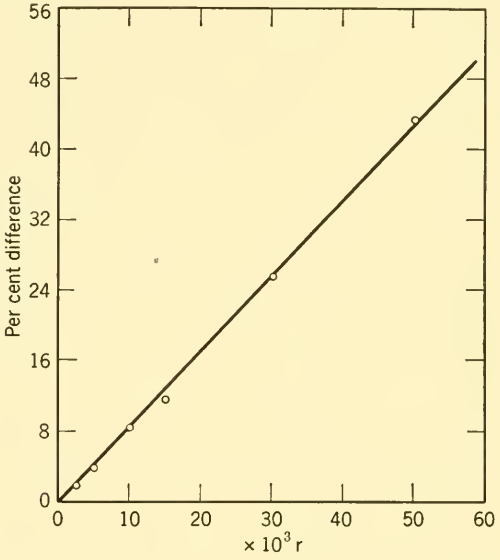

Fig. 10. The x-ray efficiency on the destruction of adenosinetriphosphoric acid as measured by the absorption spectrum at 2600 Å. *Abscissa:* X-ray dose in roentgen units; *ordinate:* per cent difference in the absorption spectrum.

although the ionic yield is rather low, about 0.09 (Fig. 10). That the measured change (height of the absorption spectrum at 2600 Å) (Fig. 11) is due to oxidation is demonstrated by the reduction produced by catalase (46 per cent) and by the exclusion of oxygen (70 per cent). The pyrimidine-pyridine residues of diphosphopyridine nucleotide, however, are more resistant to the action of ionizing radiations (Fig. 12) (ionic yield 0.01). If the intensity of irradiation is increased, there will be more profound alterations, as has been shown by Scholes *et al.* (14)

on irradiation of ribose nucleic acid and thymonucleic acid with 2×10^6 r. However, even in these experiments the part played by oxidation can be determined by the greatly diminished formation of NH_3 when

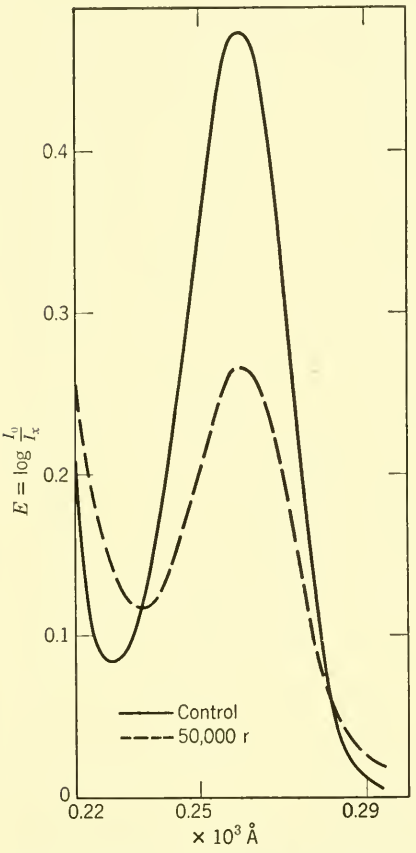

FIG. 11. Effect of x-ray irradiation on the absorption spectrum of adenosinetriphosphoric acid (muscle). *Abscissa:* Wavelength in angstrom units; *ordinate:* optical density E.

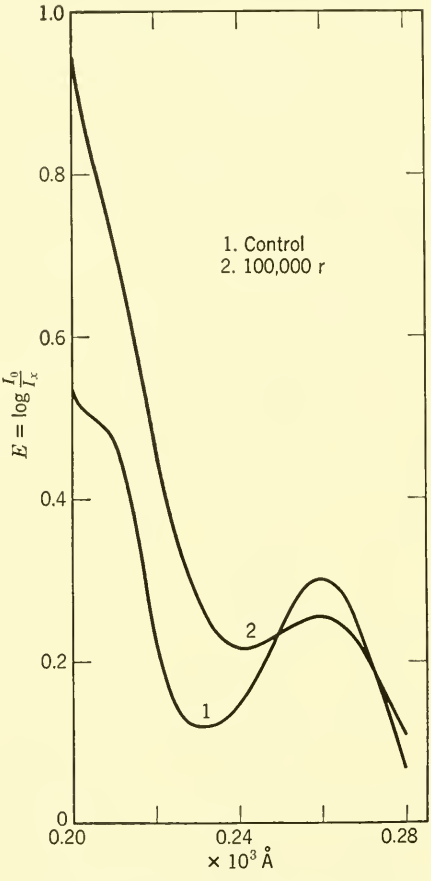

FIG. 12. Effect of x-ray irradiation on the absorption spectrum of diphosphopyridine nucleotide. *Abscissa:* Wavelength in angstrom units; *ordinate:* optical density E.

irradiation was performed in vacuum. The absorption spectrum of sodium desoxyribonucleate was less altered on irradiation with x-rays (Fig. 13). It is quite possible that the primary alteration is in the viscosity when the nucleic acid combines with the basic protein (15). It may be predicted from these studies that the sensitivity to ionizing

radiations of young cells and of cells in division is not due to the effect of ionizing radiations on nucleic acids. It may be predicted furthermore that the part played by direct collision is of less significance than that produced by the products of water radiation.

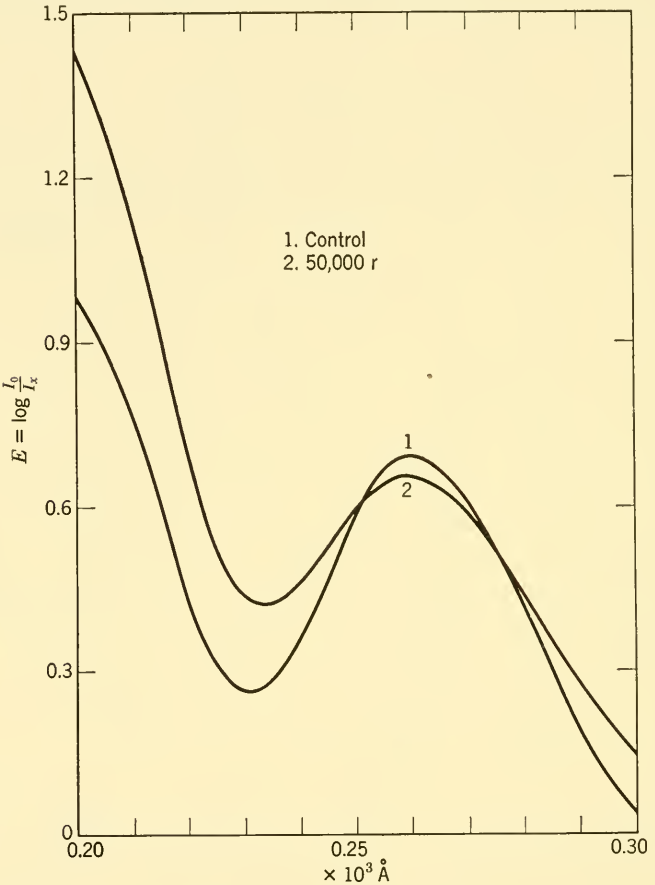

FIG. 13. Effect of x-ray irradiation on the absorption spectrum of sodium desoxyribonucleate (40 micrograms per 1 cc). *Abscissa:* Wavelength in angstrom units; *ordinate:* optical density E.

IONIZING RADIATIONS AND PROTEINS

The effect of ionizing radiations on proteins has been the subject of a large number of investigations. Protein denaturation and precipitation, viscosity decrease, and changes in the absorption spectrum and in the sedimentation constants have been observed when proteins were irradi-

ated with ultraviolet light, x-rays, and alpha rays. However, the mechanism of these changes is still unknown, and most of the early work was done with impure solutions of protein.

Sanigar *et al.* (16) reported that irradiation of proteins with 29,000 r of soft x-rays had no effect at all on the absorption spectrum. Contrary

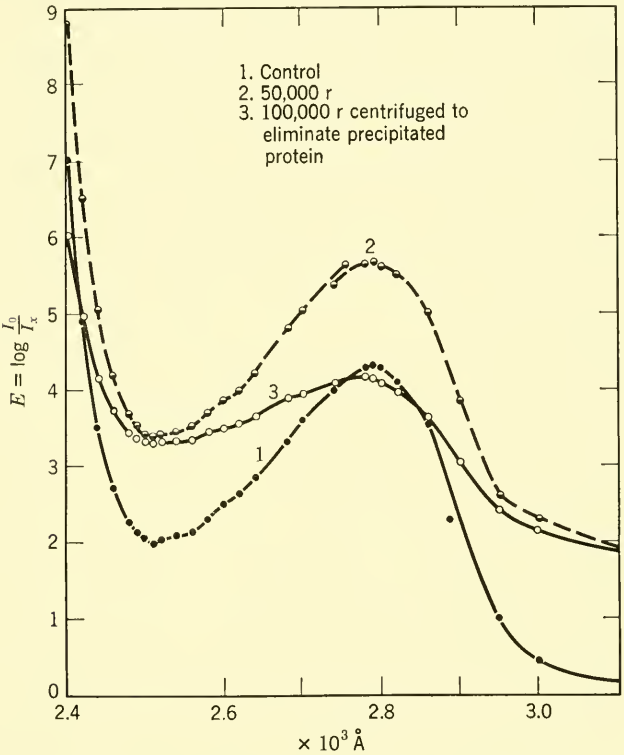

FIG. 14. Effect of x-ray irradiation on the absorption spectrum of bovine serum albumen (1×10^{-5} *M*) dissolved in water. *Abscissa:* Wavelength in angstrom units; *ordinate:* molecular extinction coefficient $\times 10^5 E$. (From Argonne National Laboratory Quarterly Report, May–July 1949, p. 117.)

to this report, we found that x-rays definitely affected the absorption of light by protein solution even after irradiation with so small an x-ray dose as 100 r. There was a general increase in the absorption of ultraviolet light by the irradiated protein, the effect being greatest when the protein was dissolved in water (Fig. 14) and least when the protein was dissolved in acid solutions (Fig. 15) at a *p*H value below the isoelectric point. These changes were less when the protein was irradiated in the relative absence of oxygen.

In spite of oxidation of thiol groups and of tyrosine residues, electrometric titrations have been of no aid, as no difference is observed between the control and the irradiated solutions. Measurements of electrophoretic mobilities were also of no help. Neither the areas nor the

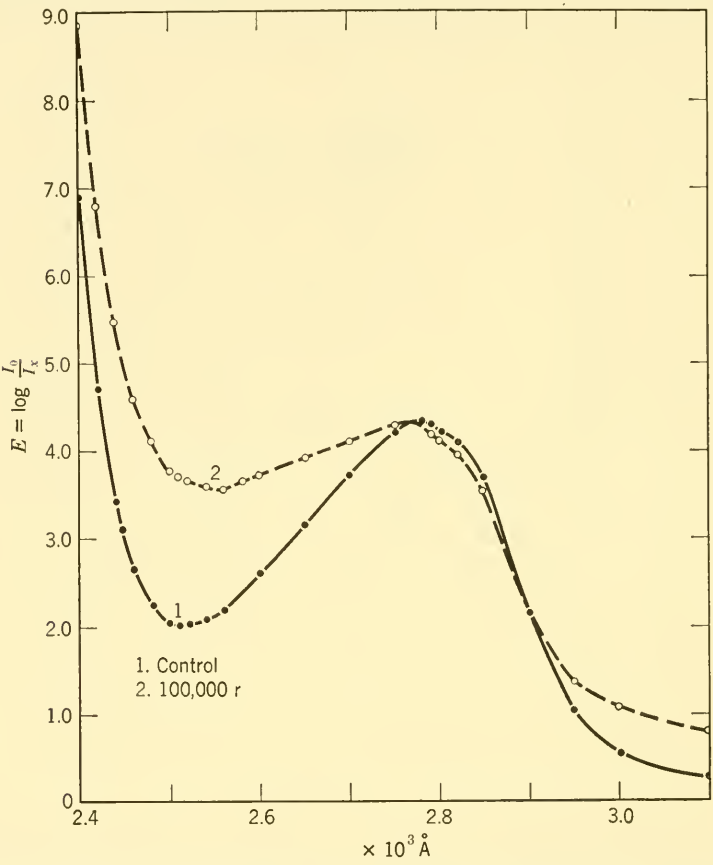

Fig. 15. Effect of x-ray irradiation on the absorption spectrum of bovine serum albumen, dissolved in 0.01 *M* fluoroacetate buffer, *p*H 3.0. *Abscissa:* Wavelength in angstrom units; *ordinate:* molecular extinction coefficient $\times 10^5 E$.

mobilities of the protein solutions (serum albumin and globulin) were altered significantly. Measurement of the sedimentation rate, however, showed the presence of a second, faster-moving component when albumin and globulin were irradiated at *p*H 3. It is quite possible that this is a "dimerized" protein, the result of intermolecular union of two protein molecules through their —S—S— bonds to give

Protein—S—S—Protein

It was possible to prevent formation of this fast-moving component by addition of cysteine.

The protein changes on irradiation are extremely temperature-dependent, especially those which result in denaturation and precipitation. In fact, protein solutions that become cloudy after 2 hr, when irradiated

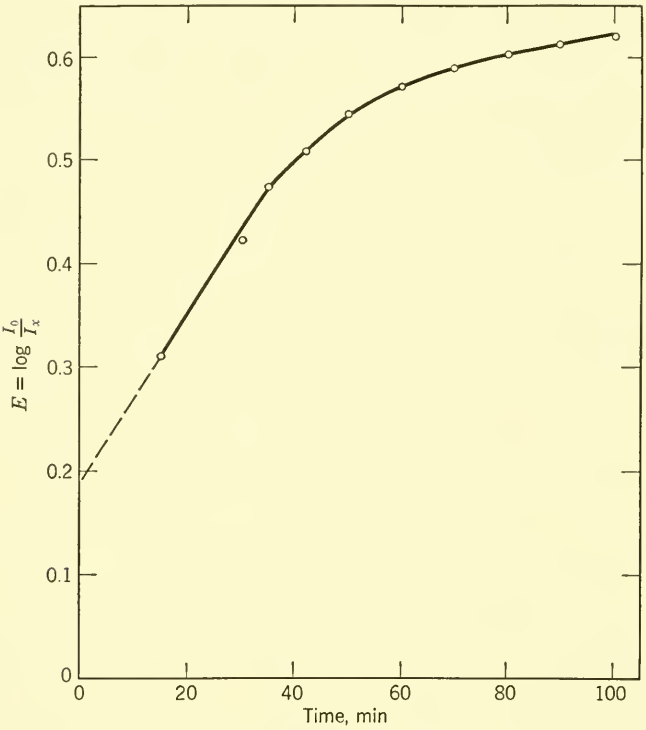

FIG. 16. Protein denaturation and precipitation by x-ray irradiation as measured by light absorption at 2510 Å at different times after irradiation. The E values are the difference between the irradiated and the control. X-ray dose, 71,900 r.

at 10° C with 71,900 r, remain clear for hours when kept at 3°. However, as soon as the irradiated clear protein solution is brought to 27° precipitation occurs. This process can be seen clearly on measuring the absorption of light at 2510 Å. The log (I_0/I_x) (E) values rose steadily with time (Fig. 16). Denaturation and subsequent precipitation not only are temperature-dependent but also are processes requiring a rather long time for completion. Similar protection produced by keeping x-irradiated frogs at a low temperature must be due to this delay in protein denaturation.

Ionizing radiations produce, according to Bacq *et al.* (17), a contracture

of the stimulated frog muscle. The authors attribute this effect to inhibition of glycolysis. A simpler explanation is that of oxidation of the —SH groups of myosin, which are essential not only for the activity of adenosinetriphosphatase, but also for the formation of actomyosin. There is no necessity to bring forth H_2O_2, since the radicals OH and O_2H have a greater oxidizing power towards thiols.

From the definition of the term "ionic yield," $I = M/N$, it will be seen that recognition of changes in the protein molecule, when small doses of irradiation are used, will depend on the method of detection. In fact, the excellent work of Dale on inhibition of enzymes by irradiation with small doses of x-rays was possible because he used amounts of protein in the vicinity of 1×10^{-10} moles.

When the concentration of the protein is increased, and the dose of radiation is increased, the probability of direct collision also increases. Under those conditions we have protein denaturation and precipitation. Drastic changes also occur in the amino acid molecules, as has been observed by Dale and his coworkers on irradiation of glycine and other amino acids. Splitting of the protein molecule on irradiation with high doses of alpha rays was observed by Swedberg. Hemocyanine was split into two. Since the process was temperature-independent, Swedberg believes that splitting was produced by direct collision, or "hit," of the ionizing particle and the hemocyanine molecule. Similar molecular disintegrations were also observed by Sparrow and Rosenfeld (18) on irradiation of thymonucleohistone and thymonucleate with large doses of x-rays. Viscosity and birefringency fell on irradiation. Whether such a drop in viscosity is due to direct collision alone or to reactions produced by the ionization products of water remains to be found.

It is well known that hemorrhages and diminished coagulation time are symptoms of radiation sickness. It is quite possible that the lack of coagulation may be due to oxidation of the —SH groups of the fibrinogen molecule.

In conclusion it may be stated that ionizing radiations may affect proteins either indirectly by the products of water irradiation or directly by collision. The indirect action produces mainly oxidation of polar groups such as —SH and OH groups; the denaturation and precipitation phenomena are perhaps due to rupture of hydrogen bonds by direct collision.

Ionizing Radiations and Cell Metabolism

It has been known for a long time that ionizing radiations inhibit catabolic as well as anabolic reactions, and that, as Hevesy discusses, synthesis reactions are inhibited with relatively small doses of x-rays.

The partisans of the "target theory" derive some of their support from experiments on the death of bacteria by irradiation. What is meant by death is really multiplication of bacterial cells after irradiation, which is a more complex process. According to this theory, although many ionizing particles pass through the bacterium before it is killed, its death, when it does occur, is due to one of these particles alone which chances to pass through an especially sensitive region or "target," the

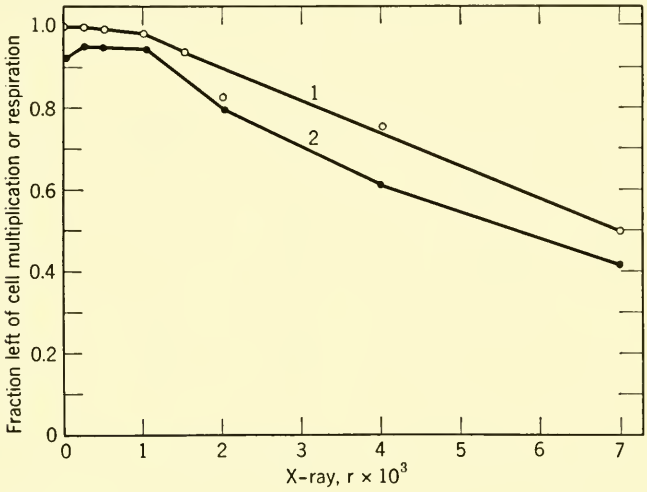

FIG. 17. Effect of x-ray irradiation on the multiplication and respiration of *Corynebacterium creatinovorans*. 1, Fraction left of cell multiplication; 2, fraction left of cell respiration; in both cases after x-ray irradiation of bacteria in its own culture medium.

"zone sensible" of the organism. Bacterial cells are very complicated organisms, and within their small volume will be found, among other things, a large number of enzyme systems possessing the same complexity as that found in mammalian tissues and with varying degrees of sensitivity towards ionizing radiations. The complexity of the cell renders the "target" theory too simple.

A more precise and easier method of determining bacterial multiplication is that of measuring the turbidity of liquid cultures. Between certain limits, turbidity readings in a colorimeter are proportional to the dry weight of bacteria. Simultaneous measurements of turbidity and of respiration of x-irradiated *Corynebacterium creatinivorans* showed that it was possible to depress respiration without altering cell multiplication (Fig. 17). On irradiation of "resting," non-growing cells with 10,000 r, it was found that, although the residual respiration remained

unaffected, the O_2 uptake in the presence of acetate, glutamate, aspartate, lactate, and allantoin was depressed (Table 5). Pyruvate and succinate oxidation was not affected. When irradiation of bacteria was performed in the absence of oxygen, the inhibition of acetate oxidation diminished, evidence that the radiation effect was due to an oxidation process.

TABLE 5

Effect of X-Rays on Some Oxidations Produced by *Corynebacterium creatinovorans* (Washed Bacteria)

(Unpublished experiments)

Substrate	X-Ray Dose, r	Inhibition, %
None	10,000	None
Glucose	10,000	10
Glutamate	10,000	17
Lactate	10,000	12
Allantoin	10,000	12
Pyruvate	10,000	None
Succinate	10,000	None
Acetate	6,000	32

Although we are still far from the desired goal—clear understanding of the mechanism of ionizing radiations in biological systems—I feel that we have made substantial progress. The preponderant role of water and of oxygen has been firmly established. This is obvious in a system like the living cell, containing 80 per cent of water saturated with oxygen. Furthermore, it has also been established that oxidation reactions dominate the picture, oxidations produced mainly by the powerful oxidizing radicals OH and O_2H, and less effectively by H_2O_2 produced on reduction of O_2H. There are good arguments to postulate that, of these oxidations, the oxidation of thiol groups, in particular, is of considerable importance because of the multiple functions of these groups in cell metabolism, in cell division, in cell growth. Work in progress on the effect of ionizing radiations on other systems of biological importance will further clarify the problem.

It must be emphasized, however, that all these studies on the effect of ionizing radiations on systems of biological importance are only a guide—an essential guide—for the search of effects in the living cell. These studies represent somewhat like thermodynamic possibilities. If a biologic system is found to be very sensitive, it is possible that the same changes might occur in the living cell; it may also be possible that such might not be the case because of the presence of other factors

which protect the system from alteration. If a biological system is found to be resistant to the action of ionizing radiations *in vitro,* there is reasonable certainty that it will also be resistant on irradiation in the living cell. Besides these complicating factors of the action of the environment, the complexities increase when mutual interactions are taken into consideration, to wit, the metabolic dynamic equilibrium which allows for continuous resynthesis of the destroyed products, salt and H^+-ion effects, hormonal effects, oxygen tension effects, etc.

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DISCUSSION

TAHMISIAN:

Several years ago Barron and his coworkers found that small concentrations of sulfhydryl reagents increase the oxygen uptake of sea-urchin sperm. When we found that the oxygen uptake of embryos increased on irradiation with 25,000 r, we wanted to test whether the sulfhydryl groups were oxidized. *In vivo,* however, when the sulfhydryl groups were tested with the nitroprusside test, no change could be detected after irradiation even with doses up to 200,000 r. It is very important, as Dale and Barron show, that damaging of molecules *in vitro* can be brought about by irradiation. But making conjecture that this is what happens *in vivo* becomes very dangerous, so that these *in vitro* experiments must also be substantiated experimentally *in vivo.* There is a gradual diminution in

sulfhydryl groups as the embryo is allowed to metabolize. This, however, shows up 10–18 days after irradiation if the eggs are kept at room temperature.

PLOUGH:

I have been very much impressed by Barron's interesting and suggestive analysis and his clear evidence that an important effect of radiation occurs in the oxidation-reduction systems. I am, however, a little disturbed by the fact that all the evidence he has cited for bacteria has to do with very low radiation dosages—as I recall, about 10–50 r. From these results he concludes that radiation has no effects on nucleoproteins. I feel that I must point out that all the data bearing on changes in nucleoproteins, that is, gene mutations, come from the utilization of very much higher dosages. For instance, in our rather extensive work at Amherst on what appear to be gene mutations in the bacterium *Salmonella typhimurium* we get no permanent effects until we reach dosages of 25,000–100,000 r. In this range we get many auxotrophic (or nutrilite-requiring) mutants, and they are roughly in proportion to the dosage. Below 50,000 r the most frequent mutant is a cysteine-requiring strain. Thus it does appear that effects on nucleoproteins do occur, and they have to do with reducing activities.

BARRON:

I should like to know the name of the bacteria Plough worked with.

PLOUGH:

Work with *Salmonella* shows that gene changes appear with higher doses than those used by Barron. This is a common finding.

BARRON:

The statement on nucleoproteins was made to raise comment. Direct effects on the nucleoprotein cannot be ruled out. Denaturation and precipitation may also occur.

13 ·

Some Factors Influencing Cell Radiosensitivity by Acting at the Level of the Primary Biochemical Action

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The primary biochemical processes that result from the interaction of radiation and living tissues are not accessible to direct observation. Various indirect pathways provide some indication concerning these processes. Chemical actions *in vitro* will be considered in later papers; action on isolated cell constituents, such as enzymes, viruses, and chromosomes, and on cellular metabolism, will be presented by other contributors.

Another indirect approach involves the study of factors capable of influencing cellular radiosensitivity. If such a factor alters the sensitivity, this means that it intervenes in some step of the process, and its nature may provide some hint as to the process itself. This report will deal with this indirect pathway by examining several factors which undoubtedly influence sensitivity by acting at the level of the primary biochemical process.* This approach, at the same time, will bear on the problem of cell sensitivity, which greatly depends on the course of this primary process. In that which follows, the terms "lesion," "sensitivity," and "resistance" will be used for "radiolesion," "radiosensitivity," and "radioresistance." They will always concern a well-defined effect in a given type of cell rather than the totality of possibly observable lesions which correspond to unrelated sensitivities.

In this paper I shall develop mainly general considerations, and it will be only in order to substantiate them by experimental facts that I will sometimes cite particular experiments. French works are discussed preferentially, because it seems to me that one of the aims of this symposium is to exchange information about the work going on around us.

* Other such factors will be discussed by Hollaender.

Number of Cellular Units Involved

Any lesion results from the alteration of a certain type of cellular element. Three possibilities must be considered:

(*a*) The element is represented by several units (n), and the considered function acts with an intensity proportional to the number of these units. Thus the lesion shows up progressively according to the number of altered units. An example is cell respiration, in relation to the number of respiratory enzymes, which decreases with increasing dose, as an increasing number of enzymes is altered.

(*b*) The element is again represented by several units (n), but the considered function remains intact as long as n remains greater than a certain threshold value, z. The lesion is then an all-or-non-phenomenon. An example is the infective power of a cell infected with several virus particles.

(*c*) A particular case of (*b*), $n = z = 1$, arises when the element is represented by a single unit, as, for example, a gene in a haploid cell.

In the case of (*b*), which is very frequent, the number n of units present in the cell at the time of irradiation contributes to resistance if the radiation inactivates these units by discontinuous individual actions: the greater this number, the lower the sensitivity. One might be able to relate the rate of production of lesions by a certain dose within a homogeneous population of cells to the number n. When certain conditions are fulfilled—which cannot be discussed here—this relation is given by the classical Poisson formulae. Two experimental examples will illustrate and define quantitatively the influence of this factor on sensitivity.

VIRUS-INFECTED CELLS

Radiations affect many metabolic functions through their action on the enzymes responsible for these functions; thus certain physiological changes in the cell influence sensitivity by modifying conditions of enzymatic activity. It would be interesting to consider here the influence of the number n of molecules of a certain enzyme on the disappearance of the function governed by that enzyme. Hevesy has pointed out that the low concentration of enzymes renders the function that they control particularly sensitive to radiation, and that this sensitivity decreases as the concentration of enzyme increases. This consideration applies to all other constituents controlling well-defined cellular functions. One would like to express this quantitatively. Unfortunately, we cannot determine the number of intracellular molecules of an enzyme

or that of other normal cytoplasmic particles, let alone vary their number in a controlled way. On the other hand, we can enumerate very precisely the particles of certain bacterial viruses (bacteriophages), and infect sensitive cells with known and carefully controlled numbers of these particles. Let us infect the cells of a homogeneous bacterial population with known numbers of a certain virus, so that each cell will contain in average 1, 2, $\cdots$ n particles. Each infected cell represents an infective center in the sense that, transferred to another appropriate medium, it will carry and propagate the virus. By radiation, the infective power of this cell can be suppressed by destroying the intracellular virus; it has been shown that the infective power persists as long as a single intact virus remains in the cell (21). In this case, $z = 1$. If we consider as a lesion the suppression of the infectivity of each cell, we can establish experimentally the "survival curve," showing for every dose of radiation the proportion of cells retaining their infectivity, that is, still containing at least one intact virus capable of multiplication.

We are dealing with a schematic case of a lesion in which all ($z = 1$) the units of a certain intracellular element, whose number n can be set between 1 and about 30, must be affected. We have evidence that each of these particles is inactivated by a single quantum of radiant energy, either ultraviolet photon (18) or ionization (32). The number of effective quanta required to injure each cell is thus in principle equal to n. Classical calculation shows that in theory, that is to say, assuming a discontinuous mode of action of radiation, the lesion curves should follow general formula 1 (Fig. 1):

$$y = 1 - (1 - e^{-\alpha D})^n \tag{1}$$

where y is the proportion of uninjured cells.

D is the dose in number of quanta absorbed per unit volume.

α is the quantum yield.

n is the initial number of particles.

Experiments (21, 15) have given results in agreement with these theoretical predictions. Bacteria (*E. coli*, strain B) in known number were mixed in liquid medium with a known number of particles of bacteriophage T_2 so that the average number n of phage particles absorbed by each bacterium was controlled precisely. Under conditions precluding any intracellular multiplication of the virus, infected bacteria were irradiated either with ultraviolet or x-rays, and the lesion curves were drawn for each value of n. It was found (Fig. 2) that they corresponded satisfactorily with the theoretical curves of Fig. 1.

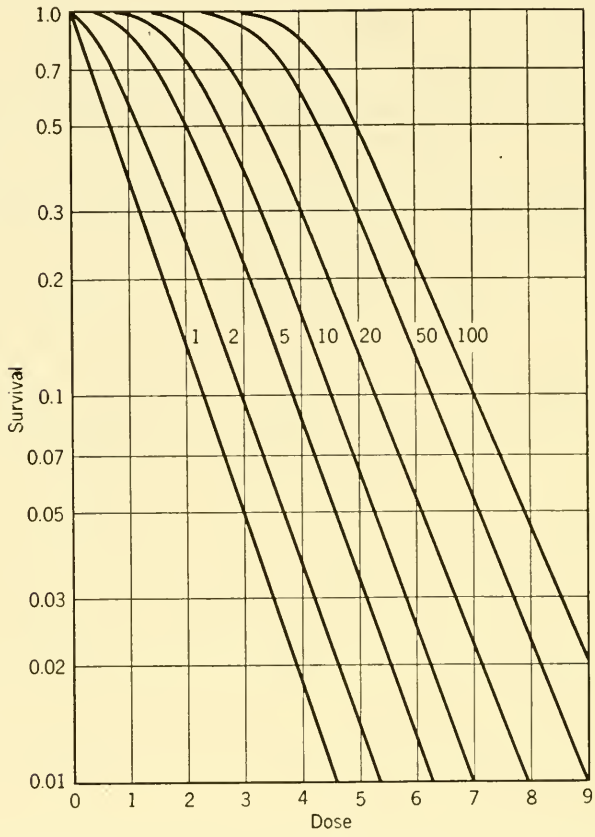

FIG. 1. Theoretical survival curves for different values of the multiplicity from 1 to 100.

This shows that the sensitivity of infected cells decreases regularly as the number of intracellular particles is increased. This decrease is not very pronounced because, in increasing the number of particles, the total volume they offer to radiation is correspondingly increased. Thus, for example, to injure 90 per cent of the cells one needs a dose 3 times as large when they contain 100 particles as when they contain only 1 particle.

Formula 1 and the group of curves derived from it define, in the present case, the quantitative influence of the number of units involved on cellular sensitivity.

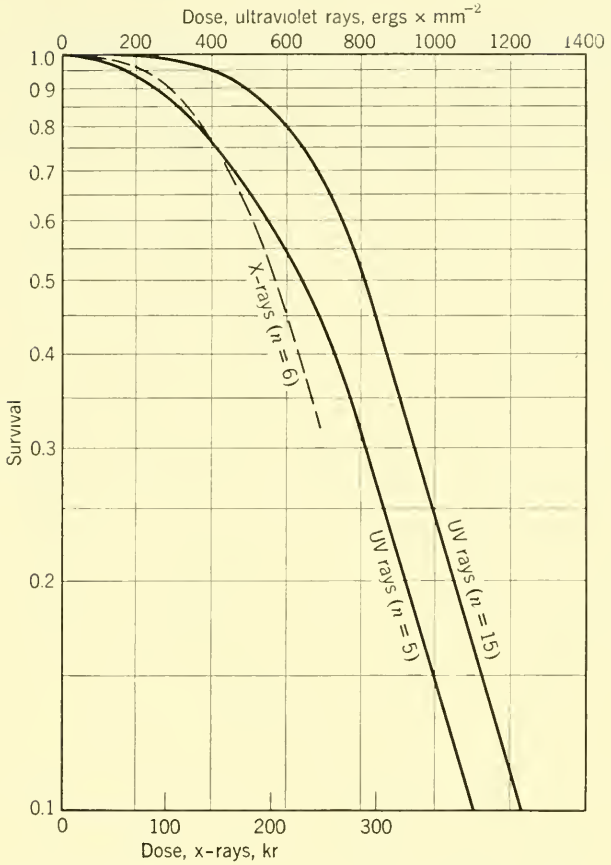

FIG. 2. Survival curves at 5 min in the case of multiple infection system, with ultraviolet and x-rays. [Latarjet (15).]

INFLUENCE OF PLOIDY IN YEAST

We have just observed the influence of multiplicity of units on sensitivity, taking as a criterion injury of cytoplasmic elements. Let us now consider the influence of a nuclear constituent, comparing haploid and diploid cells of the same species of yeast as to inhibition of their division by x-rays.

All the survival curves obtained for yeasts are sigmoid in shape. Especially with x-rays, the most precise determinations give two-hit curves. Using ionizing radiations with increasing ionization density: x-rays of 0.7, 4.15, and 8 Å, and alpha rays of polonium, it has been shown (7) that two distinct units are affected, as all these radiations

give two-hit curves, independently of the ionization density (Fig. 3). As the yeast used in this experiment was diploid, one could wonder whether the involvement of two units might not be linked to diploidy. To answer this question, irradiations with x-rays were carried out comparatively on diploid strains and on haploid lines derived from them by

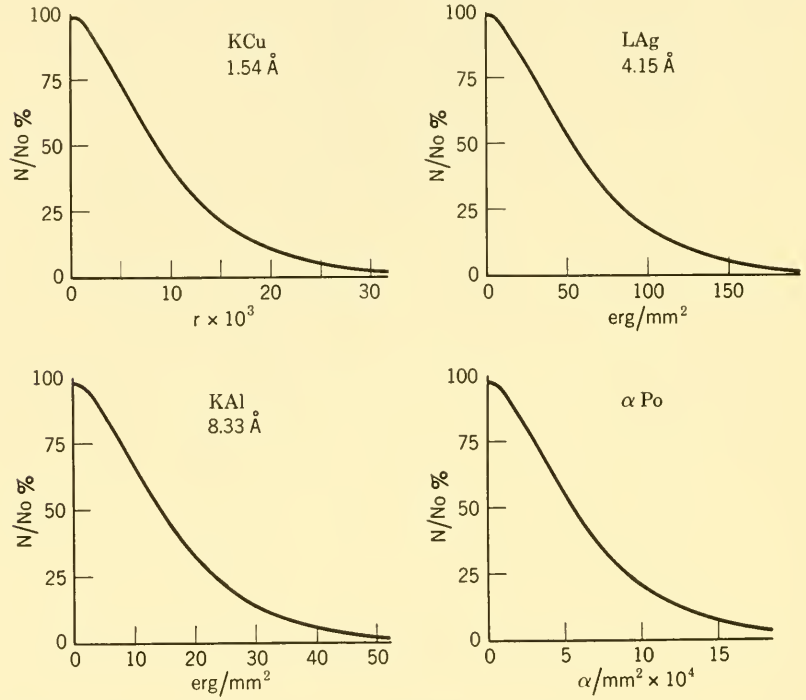

FIG. 3. Survival curves of diploid yeasts after irradiation with several monochromatic radiations (KCu, LAg, KAl) and alpha rays from polonium. All are two-hit curves. [Frilley and Latarjet (7).]

Ephrussi (16). The lesion used as a criterion was the inability to multiply indefinitely (immediate death + delayed death). The results (Fig. 4) show that each diploid line gives a classical two-hit sigmoid survival curve, whereas the haploid lines give one-hit curves and, at the same time, show much greater sensitivity than the diploids. This phenomenon cannot yet be interpreted in terms of a precise mechanism, but there is an undeniable influence of the number of replications of each chromosome.*

* Most of our results have recently been confirmed by Magni in Italy and by Tobias and Zirkle in America (cf. Tobias' paper).

An analogous situation has recently been observed by Atwood and A. Norman (personal communication) in *Neurospora*, where mononucleate cells give one-hit curves with ultraviolet rays, whereas multinucleate cells show greater resistance and give multihit curves whose multiplicity equals that of the nuclei.

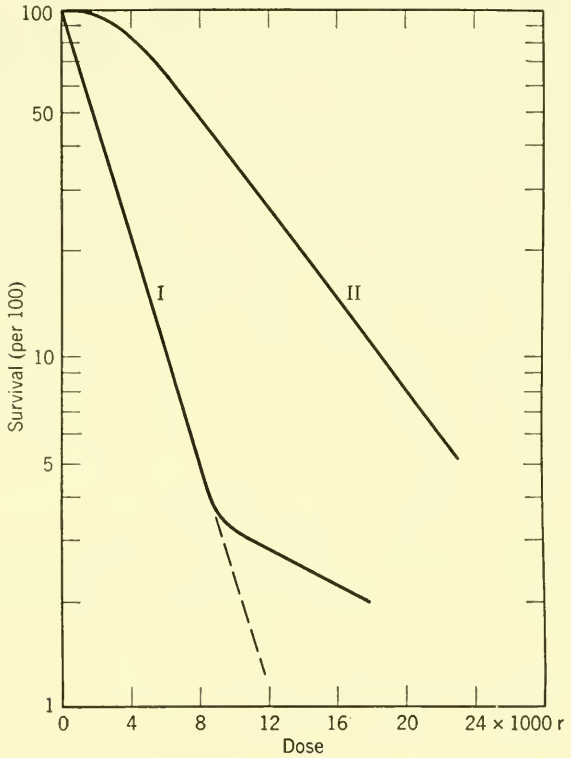

FIG. 4. Survival curves of x-rayed yeasts of the same family. I, Haploids; II, diploids. [Latarjet and Ephrussi (16).]

Another difference, perhaps more important, appears between diploid and haploid cells. Figures observed under the microscope in irradiated haploids (Fig. 5) are limited to normal colonies arising from uninjured cells; single cells, normal in size or enlarged (immediate death); and pairs of enlarged cells (delayed death after one division). These figures are definitive and do not change in the course of prolonged incubation. Haploid yeasts do not recover; their lesions are irreversible.

In the diploids, on the other hand, one sees, besides the preceding figures, numerous recovery figures which develop in the course of incuba-

tion (Fig. 5). On chains of giant cells apparently doomed to die, cells of normal appearance often arise from which normal colonies then develop. In yeasts this ability to recover from radiation injury is thus linked to polyploidy.

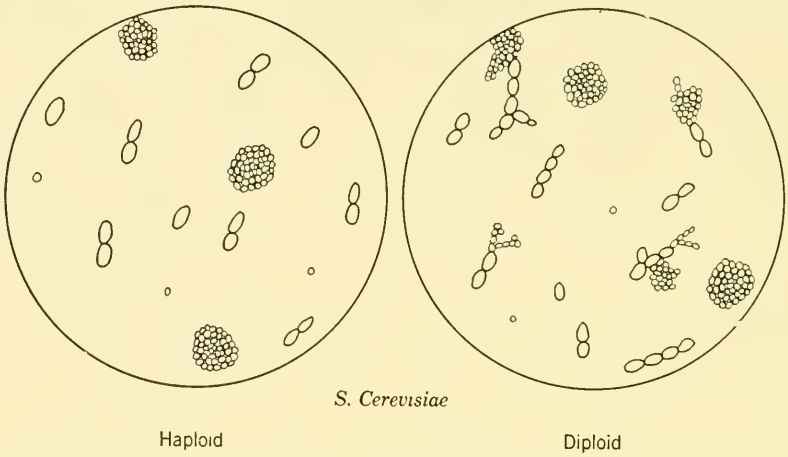

FIG. 5. Aspect of colonies and injured cells of *S. cerevisiae* after x-irradiation.

ACTION OF TEMPERATURE

When temperature influences the production of a lesion, it is difficult to know whether this influence takes place during the primary effect, or later, during the "dark reactions" (chemical reactions initiated by the primary effect). However, in certain cases, an experiment may answer this question.

Radiochemical reactions are usually considered insensitive to temperature. Although rather general, this affirmation should not be accepted in all cases. Thus Schreiber (26), in suppressing the motility of *Sphaerocarpus donellii* spermatozoa by monochromatic ultraviolet rays (2650 and 3025 Å), obtained the following results: with 2650 Å, the efficiency of irradiation remains constant between 0 and 15° C, then increases regularly above 15°; with 3025 Å, the efficiency increases steadily with temperature from 0° on. The temperature coefficient of the phenomenon therefore depends on the wave length; this proves (*a*) that temperature acts on the primary effect rather than on the dark reactions; (*b*) that this primary effect is a photochemical reaction without intermediate; (*c*) that this photochemical reaction is sensitive to temperature. (In this case, a relatively simple experiment informs us of the nature of the primary effect.)

Such effect of temperature on photochemical reactions can be interpreted by a modification of the absorption spectrum of the absorbing molecules. A rise in temperature increases the molecular oscillations and shifts the entire absorption spectrum toward the red. Such radiation, which at a low temperature can only excite the molecule, may dissociate it at higher temperature, the molecule then being more probably in a state of predissociation. The temperature coefficient of the reaction then depends on the wave length. In Schreiber's experiment, in the case of 3025 Å, which is of low efficiency, this efficiency increases steadily with temperature; in the case of 2560 Å, which is more efficient, the energy of oscillation must reach a noticeable value (15°) in order to manifest itself in respect to the actual energy of the photon.

If, as we have just seen, the primary effect is sometimes sensitive to temperature, the latter always accelerates the dark reactions and precipitates the appearance of the lesion. But the earlier appearance of the lesion does not mean a change in sensitivity. What is important here are the frequency and the gravity of the lesions, which temperature may also influence. The unknown processes involved in the dark reactions interfere and compete with the cellular metabolisms, some of which, although more or less disturbed by irradiation, may secure different types of recovery. These recoveries are influenced by temperature in so far as it affects differently the speeds of the chemical reactions put into play, and in the sense of this difference. When there is no such difference, no temperature recovery takes place (6, 24). It may happen, however, that a cooling off during the latent period slows down the process of lesion more strongly, so that it favors recovery and lowers sensitivity.

Strangeways and Fell (28) observed that degenerative modifications produced by x-rays in chicken embryos are lessened and sometimes stopped if embryos are kept for 24 hr at 5° C after irradiation. They concluded that slowing the metabolism gave to the tissues the possibility of repairing the lesions.

Cook (5) confirmed this observation by submitting several groups of *Ascaris* eggs to an x-ray dose of 5000 r. The controls incubated at 25° immediately after irradiation gave 2 per cent normal embryos. The other groups were kept at 5° for increasing periods, then incubated. This exposure to cold increased progressively the proportion of normals: 4 per cent after a period of 1 week, 15 per cent after 4 weeks, 45 per cent after 8 weeks, after which no more recoveries took place.

By irradiating, either with x-rays or with ultraviolet rays, a bacterium (*B. dysenteriae*) and a yeast (*S. ellipoideus*), I noticed (14) that a stay at 5° C before incubation favors recoveries among the irradiated yeast

cells but not among the bacteria (Table 1). The significance of this difference in behavior is not clear, and it probably will be useful to check what happens in haploid yeasts, which (cf. p. 245) give no spontaneous recovery, whereas the above-mentioned diploid yeast gives some.

TABLE 1

Type of Radiation	Yeast		Bacterium	
	A	*B*	*A*	*B*
I. X-ray, λ 1.54 Å (Kα of the curve), intensity 6250 r/min	Dose 12,500 r		Dose 12,500 r	
	0	44.8	0	77.7
	2	22.8	2	80.5
	5	11.2	5	78.6
	.	....	7	83.4
II. Same radiation	Dose 13,100 r		Dose 5,000 r	
	0	37.0	0	55.3
	3	30.0	2	61.6
	4	28.6	7	55.4
	6	22.3	10	68.7
	10	19.0	13	60.0
III. Ultraviolet radiation, λ 2537 Å, intensity 26 ergs/mm^2/sec	Dose 700 ergs/mm^2		Dose 400 ergs/mm^2	
	0	60.0	0	86.9
	2	46.4	3	91.8
	5	32.0	7	90.2
	8	32.4	10	93.4
	9	29.0	..	....

A. Time at 5° C (in days). *B*. Number injured.

Some Problems of Radio-Oxidations

SENSITIZATION BY OXYGEN

According to a very early and well-tested observation (cf. 4, 23) microorganisms capable of living either in aerobiosis or in anaerobiosis are appreciably less sensitive to radiation when they live in the absence of oxygen.* The same observation holds for cells of higher organisms (25). This influence of oxygen is probably most evident when mammals completely deprived of oxygen are irradiated *in toto*. In their experiments, Lacassagne and Latarjet (12, 13) have taken advantage of the remarkable capacity of newborn mice to resist for as long as 20 min complete asphyxia with cessation of circulation and respiration and complete anoxia of tissues. The animals can be irradiated during this

* See Hollaender's paper.

period, and later most of them can be easily revived. Mice asphyxiated by nitrogen or carbon dioxide received soft x-rays (0.8 Å), at a dose of 1500 r, on the whole body. Whereas the normally irradiated controls all died within 12 days, the animals irradiated during anoxia not only all survived, but subsequently grew at the same rate as the non-irradiated

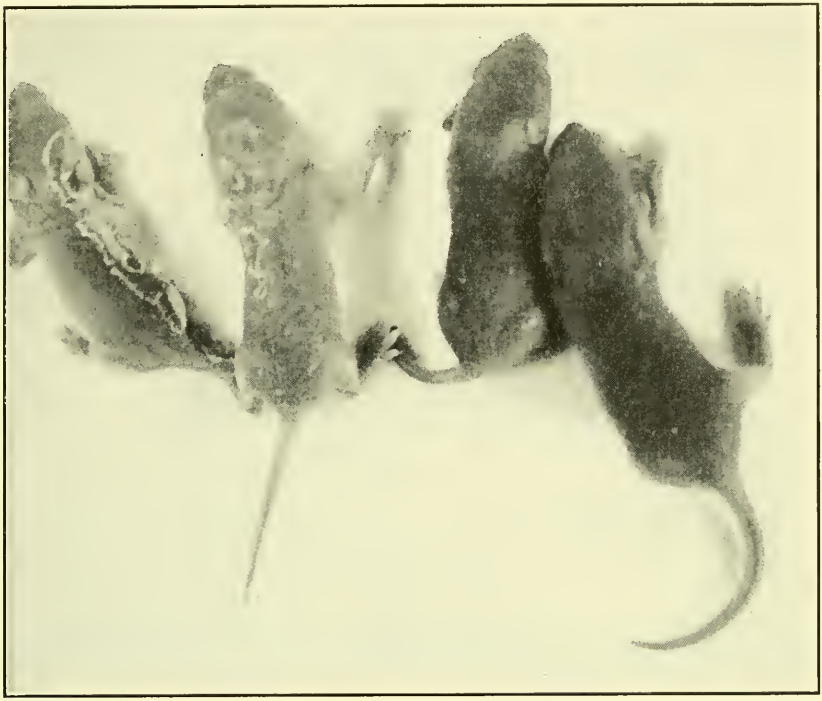

FIG. 6. Effects of anoxic irradiation on newborn mice. *Right to left:* 1, Non-irradiated control; 2, irradiated in anoxia; 3 and 4, normally irradiated.

controls. With ultraviolet rays the difference between normal and oxygen-deprived animals was as notable as with x-rays, with reference both to cutaneous lesions (erythema, epidermitis, deep burns) and to general toxic effects (delay in growth, death). Figure 6 shows, starting at the right, a non-irradiated control, an animal irradiated in anoxia (similar in all respects to the control), and two animals normally exposed to the same dose. The last two show severe cutaneous lesions and distortion due to deep edema. The animal at the left died.

The same influence of oxygen is found at the other end of the organic scale, in a simple radiochemical reaction such as formation of hydrogen peroxide in water irradiated with x-rays or alpha rays. The primary

role played by oxygen in the reaction has been verified (Loiseleur *et al.*), but has not been found in the case of alpha rays. With x-rays, for example, about 50 times less H_2O_2 is obtained in the absence of oxygen. It is mainly in the presence of dissolved oxygen that irradiation provides aqueous media with the properties of an active oxidation system. This fact can be demonstrated directly by recording the platinum potential of an aqueous redox system during irradiation. This was done by Loiseleur and Latarjet (19) by x-raying an aqueous solution of quinhydrone either saturated with or completely deprived of oxygen. The

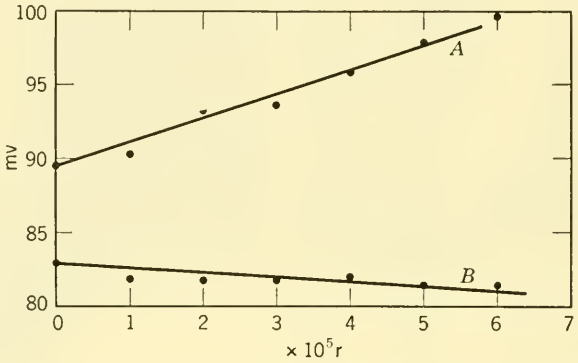

Fig. 7. Conversion of hydroquinone to quinone by irradiation in the presence of oxygen (curve *A*), but not in the absence of oxygen (curve *B*). [Loiseleur and Latarjet (19).]

platinum potential of the solution permits observation of the trend of the system, either towards quinone (oxidation) or towards hydroquinone (reduction). One observes (Fig. 7) a progressive oxidation of the saturated solution, whereas, in the alternate case, no oxidation takes place. This proves that irradiation of water alone does not liberate oxidative radicals in sufficient quantity to displace the equilibrium in the direction of oxidation, whereas dissolved oxygen insures the phenomenon.

Although a mechanism involving free oxygen may not explain all radio-oxidations, this constituent is responsible for a high proportion of them. This is proved by a number of experiments carried out on various biological systems. We may cite, as examples, inactivation of auxine by x-rays (27), and the experiments of Thoday and Read (29) on chromosome alterations produced by x-rays and alpha rays in bean roots suspended in water saturated with nitrogen or oxygen: with x-rays the lesions are more severe in samples irradiated in the presence of oxygen, whereas with alpha rays this gas has almost no effect. Giles and Riley (8) observed in *Tradescantia* that chromosome sensitivity is

markedly decreased in the absence of oxygen, the ratio of sensitivities between O_2 and N_2 being about 5 to 1.*

From the results obtained in these experiments, it is almost certain that this influence of O_2, even in complex systems, is expressed at the level of the primary effect rather than during the dark reactions. Weiss's (30) interpretation of this phenomenon during the primary effect in aqueous systems attributes to the O_2 molecule the role of a buffer in the course of the reactions initiated in water. This interpretation has the advantage of explaining the disappearance of this factor in the case of alpha rays. It is probable, nevertheless, that O_2 acts also by other processes. Direct oxidation of a biological molecule by O_2, after activation of one of these two molecules, also plays an important part, according to the theory of Loiseleur and Latarjet. This second mechanism leads to a better understanding of the decrease of sensitivity caused by oxygen acceptors, a phenomenon which we will now discuss.

OXYGEN ACCEPTORS AS RESISTANCE FACTORS

Whatever the nature of the oxidating agents produced in irradiated medium, whether activated oxygen or free radicals are involved, these agents may reach the oxidizable molecules at the mercy of unknown chemical affinities.

A true competition is established in any complex medium, and the oxidation of a biological molecule has to contend with the presence of other oxidizable molecules which more or less protect it. These protection phenomena have been thoroughly studied, and we will consider them here only in so far as they concern oxygen acceptors (without underestimating all other types of protection which are pointed out by Dale, Hevesy, and Barron).

When desensitization is obtained, by means of prior injection of proteins, serum, or hormones (9), in animals irradiated *in toto*, it is impossible, in the present state of our knowledge, to decide which sensitivity factor is involved. In certain cases, however, the properties of the injected substance may suggest a mechanism. Thus radiologists long ago observed that diabetics are less sensitive than normal subjects. This fact has been experimentally verified in animals (1, 20); in this particular case it was assumed that glucose acted as an oxidation buffer at the level of primary processes.

In fact, working with simple systems, it has been observed that glucose acts as a buffer in that it behaves as a hydrogen donor (17). In water

* Cf. Giles's paper.

irradiated with x-rays, glucose gives up some hydrogen which combines with oxidative agents to form H_2O_2; so doing, it blocks these agents, which are thus prevented from combining with other solutes. I take this opportunity to approve Dale's statement regarding H_2O_2. I should like, however, to point out that if the French school has attached great importance to this substance (cf. 3) it is not always in order to endow it with an active role in the production of the lesions. On the contrary, we have emphasized (17) that H_2O_2 is, in many instances, a subsidiary product whose presence attests that oxidizing agents have been diverted from the lesion process. This is true to such an extent that in certain cases increase in H_2O_2 means a decrease in the yield of the biological reaction.

The decrease in sensitivity of mice injected with α-tocopherol (10) seems also to be connected with an oxidative process since this substance, as well as glucose, is a strong inhibitor of peroxides.

The protection afforded by oxygen acceptors is clearly displayed in irradiation of aqueous solutions of strychnine (20). X-rays inactivate this alkaloid by probable oxidation into genostrychnine with almost complete loss of toxicity. Inactivation is not changed by the presence of non-oxygen-accepting solutes such as NaCl, $NaNO_3$, $Fe_2(SO_4)_3$, $SnCl_4$, and saccharose. On the other hand, almost complete protection is afforded by oxygen acceptors closely related to these solutes, that is, $NaNO_2$, $FeSO_4$, $SnCl_2$, and glucose. This experiment gives strong support to the idea of participation of free oxygen in this inactivation and of a very simple mechanism by which oxygen acceptors assure such protection.

PEROXIDASES AS FACTORS OF RESISTANCE AND RECOVERY

Instead of decreasing sensitivity by diverting oxidating agents toward acceptors, one can obtain the same result by destroying these agents. For this purpose, peroxidases, and in particular catalase, come first in mind. The preliminary results that I am about to describe reveal the great complexity of the primary processes and especially their dependence on cell metabolisms. Chemical destruction of the first irradiation products implies either an immediate contact with the chemical, which therefore must be present in the cell at the time of irradiation, or sufficiently stable radioproducts. We do not yet know the magnitude of the life span of oxidative free radicals, but experiments show that catalase may remain active when added after periods of a minute or even an hour. Accordingly it is possible to conceive of a general method for decreasing certain radiation effects which would consist in treating

with appropriate substances *after irradiation.* This "recovery method" would differ from the "protection method" previously discussed. It may be illustrated by some of the results recently obtained in my laboratory by treatment of ultraviolet-irradiated bacteria with catalase. This study followed an observation by Monod *et al.* (22) that bacteria (*E. coli,* strain K_{12}) irradiated with ultraviolet light can be reactivated by addition of catalase to the culture medium. This phenomenon, which recalls Kelner's photoreactivation (11), seems to depend to a certain extent on the presence of visible light. The preliminary results that we have already obtained with two bacteria (*E. coli,* strains B and K_{12}) can be summarized as follows:

1. Both bacteria, enriched in catalase content by growing in a medium containing a large amount of this enzyme, show increased resistance to ultraviolet light. Survival rates may be 20 times as high as those of the controls. This effect depends on the dose; weak with low doses, it increases with higher doses. The table shows the results of one experiment:

Dose, ergs/ mm^2	Survival Rates		
	Controls I	Catalase II	II/I
800	3×10^{-4}	6.7×10^{-4}	2
1000	3.3×10^{-5}	3.3×10^{-4}	10
1200	5×10^{-7}	1×10^{-5}	20

2. This effect does not occur with x-rays, either in B or in K_{12}. This negative result recalls an observation by Barron *et al.* (2) that catalase does not prevent the inhibiting action of x-rayed water on cellular respiration. With ultraviolet rays, since catalase is present in the cell at the time of irradiation, we may be dealing with either a protection or a recovery effect.

3. According to Monod's observation, the recovery effect appears when K_{12} bacteria grown in synthetic medium and sterilized by ultraviolet radiation are plated with catalase and incubated. The extent of the reactivation varies widely, depending on the dose and on the metabolic conditions of the cell. We did not succeed in controlling the metabolic factors, and results varied greatly between experiments carried on under apparently identical conditions. So far reactivation has occurred sometimes when catalase is administered without any addition of visible light. But it has been a constant and striking phenomenon when, before incubation, the bacteria undergo an exposure to visible light which in itself would produce only a very slight effect.

The following are the results of a typical experiment:

K_{12} irradiated with 1200 ergs/mm^2 (λ 2537 Å)

Number of irradiated bacteria plated per Petri dish:	4×10^5
Number of colonies after 36-hr incubation:	
Irradiated control (dark)	22
Irradiated control (+ visible light)	220
Irradiated control (+ catalase dark)	35
Irradiated control (+ catalase, same dose + visible light)	2500

(Sometimes catalase alone would give 1000 colonies.)

4. This phenomenon seems to require much more stringent conditions than photoreactivation. For example, we were not able to reproduce it in bacterium B under the conditions used with K_{12}, although these conditions allowed B to undergo strong photoreactivation and although this bacterium had shown increased resistance to ultraviolet radiation when grown in the presence of catalase.

5. This recovery takes place in K_{12} even when catalase is added some time after irradiation, the length of the time depending on the temperature. In cultures kept at 37°, reactivation is still possible when catalase is added 2 hr after irradiation. It is thus evident that the action of the enzyme involves relatively stable compounds.

6. No reactivation was obtained after x-irradiation. Such facts might throw some light upon the origin of the radioresistance displayed as a hereditary character by some mutant strains (31).

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DISCUSSION

RUBIN:

I should like to discuss briefly the radiation deaths in various strains of *E. coli*. It has been shown that the B strain, the parent strain, and the B/r, which is derived from the B strain, show different sensitivities to ionizing radiation. It has further been shown that B/r resembles K_{12} and most other strains of *E. coli*. In other words, the B strain tends to be the one that stands by itself rather than the B/r strain. If one examines the data, it would appear to be evident that two different mechanisms of killing both apply to the B strain and only one mechanism of killing to the B/r strain and to the K_{12} strain. It may be that the reason that Latarjet has not found reactivation is that in one strain a certain mechanism is operating and in the other strain another mechanism is operating, so one would not expect to find comparable results in his two strains.

LATARJET:

I am very much interested in this remark. As a matter of fact, K_{12} is significantly more resistant to radiation than B, though still more sensitive than B/r. Therefore, B/r should be tested for catalase reactivation. However, there might remain some differences between K_{12} and B/r, such as recombination, which could account for differences in behavior regarding the catalase phenomenon.

DALE:

I am very interested in the fact that the results varied with the presence or absence of oxygen in these experiments. I should like to ask Latarjet whether in his quinhydrone experiment the control without radiation in the presence of oxygen showed a change to the oxidized form.

LATARJET:

No.

DALE:

The recovery of K_{12} in the presence of catalase may possibly indicate that a highly active, organically bound ion causes of itself some inactivity.

LATARJET:

If catalase is added after the radiation has been done, the factor is controlled.

DALE:

It may be possible that, even if added later, catalase brings about some recovery.

BARRON:

Latarjet's experiment on oxygen is confirmed by our experiment with ferrocytochrome c, in which the inactivation was due to the hydroxyl and HO_2 radicals. Catalase does not protect ferrocytochrome c during exposure to x-rays. Ferrocytochrome c irradiated in the absence of oxygen shows 37 per cent protection, as compared with the results of radiation in the presence of oxygen.

14 ·

On the Localization of Radiation Effects in Molecules of Biological Importance

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A biological system is a theorist's nightmare. Even in its simplest manifestation it consists of a semiliquid, heterogeneous aggregation of molecules of all sizes and complexities, interacting in a precisely ordered manner by means of various mechanisms largely unknown. Study of these systems underlines the embarrassing gaps in our knowledge of the liquid state and of polymer chemistry at the presumably more amenable level of inorganic physical chemistry.

The abstractions derived from the quantum description of molecules like methane or benzene in the gaseous state, or from the radiochemistry of simple molecules in aqueous solutions, are necessary but not sufficient for an understanding of the interaction of radiation with biological systems. At present the only recourse is to grope empirically—at least until we come to grasp an adequate understanding of protein structure, enzyme synthesis, and the mechanism of enzyme action. However, it does not follow that studies of the effect of radiations in complex systems cannot be made and their results correlated with what is already known from the study of simpler systems.

Among the questions which can be posed in such a study is whether, in large molecules of biological importance like protein or nucleoprotein, there is varying radiosensitivity in any particular bonds or regions. Experiments in which external radiation is used may provide data like ionic and excitation yields and quantum efficiencies. But they give no information on this very fundamental point.

It is possible to make an experimental approach to this problem using chemical complexing agents specific for particular atomic groupings, like S—H and free amino groups. A more direct attack, however, would seem to result from placement of unstable isotopes of elements involved in definite bond sites. An example which comes to mind readily is sub-

stitution of radioactive carbon (C^{14}) in the carboxy-peptide linkage of protein. But, if we are to achieve a sufficient number of bond disturbances from the disintegration act, it is more practical to use a relatively short-lived isotope, like P^{32}. Such a short-lived isotope may be used to study the effects of disturbing O—P bonds in phosphorus-containing structures, particularly in nucleoprotein. To exemplify this approach and the manner in which interpretation of results obtained await the solution of purely physical problems, we will consider briefly some of the recent experiments by Hershey, Kennedy, Gest, and the writer.*

When bacteria and bacteriophage are grown in a medium containing phosphate with a high P^{32} content (0.003–0.03 per cent), the viral progeny are unstable and show a progressive loss of infectivity with time. We find that this inactivation is primarily the result of the radioactive decay of the assimilated P, and not of the ionization resulting from passage of the beta particles through the phage. There is a linear relation between the inverse of the P^{32} content for phage and the average survival time. This relation holds over a sufficient range to provide a useful technique for examining distribution of P^{32} atoms among the phage population.

The phage particles studied may be thought of as spherical nucleoprotein macromolecules with a maximal diameter of about 110 millimicrons. Each particle contains $\sim 5 \times 10^5$ P atoms distributed in some unknown manner. Some of these P atoms are radioactive and decay with frequency determined by the disintegration constant, λ. The results of this act in any given decay will be excitation and probably rupture of the O—P bonds, which may initiate a chain of reactions leading to inactivation of the whole molecule.

In analyzing the expected dependence of the phage survival on the P^{32} content, we begin by assuming (*a*) that disintegration of a single atom can occasionally inactivate a phage molecule, (*b*) that all the phage particles are equally radiosensitive, and (*c*) that the P^{32} atoms are distributed at random among the phage particles. From these assumptions, it follows that the change of phage titer with time, $-dS/dt$, is proportional to the number of disintegrations occurring in unit time, which can be shown to equal $3.4 \times 10^{-6} \alpha \lambda N S A_0 e^{-\lambda t}$. In this expression the known quantities are λ, the disintegration constant, or fractional decay per day; N, the total number of P atoms per phage particle; A_0, the

* A detailed presentation of the data and methods involved has appeared since preparation of this article; see A. D. Hershey, J. W. Kennedy, H. Gest, and M. D. Kamen, *J. Gen. Physiol.*, **34**: 305, 1951. The writer wishes to express his gratitude to Dr. Hershey for his willingness to permit this discussion to appear before publication of the formal report.

initial specific P^{32} content (specific radioactivity in curies per gram); and S, the surviving phage titer per milliliter. Thus, α, the efficiency of inactivation, can be determined, knowing the rate of decay of the phage population. Setting $S = S_0$ and $t = 0$ when the initial specific activity is A_0, it follows that

$$\log S - \log S_0 = 1.48 \times 10^{-6} \alpha A_0 N(1 - e^{-\lambda t})$$

Extensive experimental data have been obtained which in all cases agree with this relation in presenting a straight line when the logarithm of the phage surviving is plotted against the function $(1 - e^{-\lambda t})$. The intercept at $t = 0$ agrees with the value $\log S_0$, and the slope is proportional to the initial specific radioactivity A_0. The values of αN derived from these plots vary from 41,000 to 58,000, with a mean of 43,000 for the phage tested (phage type T^4 and three closely related strains were examined). Since N is known to be $5.0 \times 10^5 E$, where E is the efficiency of the counting method (plaque count), the value for α is $0.086/E$ per atomic disintegration. E is believed to be very close to 100 per cent, so that on the average, under the experimental conditions used, a phage particle is inactivated at least once in 11.6 disintegrations.

Now what can be done about interpreting this figure? First it is necessary to examine the efficiency of inactivation to be expected, on the basis that the inactivation results from the passage of beta particles resulting from the P^{32} decay. The average energy of the P^{32} beta particles is 7.0×10^5 ev. The average initial specific ionization is 7.1 ion pairs per mm air. The air-path equivalent of such beta particles in a phage molecule would average 0.04 mm, so that 0.28 ion pair would be produced per phage particle. For the highly energetic P^{32} beta particles this probably represents a maximum, because clustering of ion pairs would be expected to occur rather frequently. Using the value for α derived experimentally, this means 11.6×0.28, or 3.3, ionizations produced by the beta particles inside a phage for inactivation. Thus, the efficiency is 0.3 per ionization.

Actual measurements of inactivation of unlabeled phage placed in labeled phosphate solutions show an efficiency of only 0.009 per ionization. Comparable efficiencies found from perusal of x-ray data in the literature are somewhat higher but still far from the expected figure of 0.3. Hence it is highly improbable that we are dealing with inactivation resulting from ionization by beta particles. It can be concluded that the overwhelming majority of inactivations occur as a consequence of the events following transformation of the P atom itself, and that these experiments bear on the question of the relative radiosensitivity of the O—P bonds in the nucleoprotein.

We must now examine mechanisms available for inactivation following transformation of P atoms. These may be of two types. One involves bond rupture, the other bond excitation.

Many uncertainties arise in attempting to assess the fraction of the phosphate bonds ruptured per disintegration. The bond energies probably range between 10 and 20 ev. If one assumes the nuclear recoil to be taken up by the resultant S atom and one or two of the O atoms, the mass available for the recoil momentum ranges from ~30 to ~60. Still more uncertainty attends this estimate because the angular distribution of the neutrinos emitted in the beta process is not known. If the neutrino and beta particle fly off in the same direction, the residual nucleus can experience its maximum recoil (~100 ev) while showing a continuous spectrum of recoil energies ranging down to some value below 10–20 ev. Other distributions are a consequence of assumptions in which the average angle between neutrino and beta particle is taken as 180° or, what appears to be the most popular value, 135°.

A very rough calculation indicates that for the most part there is sufficient energy to effect a bond rupture for at least 50 per cent of the disintegrations.

Another effect, namely, the replacement of P by S in the nucleoprotein at the site of the disintegration, might be expected to alter radically the functionality of the particular bond involved. This alteration would not be expected to be so much a consequence of the mere replacement of P by S as of the rearrangement of electrons resulting during the transformation. Thus, the overall change would involve only departure of one proton along with the nuclear electron. Little strain on the bridging bonds between the phosphate and the nucleotide moieties would result because the S—O and P—O bond distances are not very different.

It can be appreciated that much uncertainty attaches to the estimate of percentage bond rupture following radioactive decay. It seems reasonable, however, to conclude that any of the processes involved would be quite as effective in immobilizing a portion of the nucleoprotein and, taken in the aggregate, would be more than sufficient to explain the high efficiency of assimilated P^{32} for inactivation as compared to external P^{32} or x-rays. We must suspend, for the time being, the interesting query whether bond rupture is more effective than bond excitation in inactivation of nucleoprotein. Incidentally it would be of interest to see whether properties other than mere inactivation are affected by a few radioactive events.

Although the experimental exploitation of this direct procedure for probing variation in radiosensitivity is still very much in its infancy, it is possible to distinguish certain features of the data which indicate that

specific atom groupings, such as phosphate in nucleoprotein, vary considerably in response to radiation. Thus, data on inactivation of phage—whether derived from x-ray experiments or from experiments in which P^{32} is incorporated into phage—indicate that, although one "hit" or event may be sufficient to inactivate a whole phage molecule, numerous such events occur before inactivation takes place. In x-ray inactivation, for instance, the survival curve shows the "one-hit" type of process, while dosage measurements indicate approximately 75–100 ionizations occurring inside each phage. Experiments employing incorporation of P^{32} directly into the phosphate of phage yield data of a similar nature, but they reveal besides that a few specific bond sites, that is, phosphate groups, cannot be disturbed without inactivation of the molecule as a whole. Only when the structure of nucleoprotein and the disposition of phosphate within the viable molecule are understood will it be possible to propose definite mechanisms for inactivation by radiation interaction with the P atoms of the nucleoprotein.

Some extensions of these procedures to other enzymes and systems come to mind. A few systems which might be examined by these methods include:

1. Inactivation of enzymes containing more or less firmly bound cofactors, that is, triosephosphate oxidase of rabbit muscle with its one molecule of diphosphopyridine nucleotide (1).

2. Changes in physicochemical properties of proteins into which large amounts of phosphate can be incorporated, as in so-called phosphoproteins of yeast.

3. Use of S^{35} in studying radiosensitivity of H—S and S—S bonds in proteins, such as insulin.

4. Possible localization of short-lived C^{11} in carboxy-peptide bonds by biosynthesis of protein from labeled CO_2 or carboxyl-labeled amino acids.

5. Comparison of products obtained by x-ray bombardment of and by P^{32} incorporation into simple molecules like glucose-1-phosphate and adenosinetriphosphate.

Ramifications of considerable potential importance may also be expected from exploitation of the suggestion by Hershey that the relation between survival time and P^{32} content of phage be utilized for distinguishing distributions of P^{32} in heterogeneous phage populations, and in particular for distinguishing parent from progeny phage in experiments designed to establish mechanisms of phage reproduction.

The future exploitation of these methods depends primarily on the availability of radioactive elements with high specific activity. In extending researches of the type described, P^{32} samples with specific

activities greater than 50 curies per gram are required. Such samples can be prepared using the present uranium-pile installation, apparently without marked changes in routine procedures. However, the potentialities of these researches warrant increasing efforts to produce large amounts of the various radioactive isotopes needed with specific activities some orders of magnitude greater than are now available routinely.

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DISCUSSION

RUBIN:

Absorbed P^{32} was found to increase significantly the rate of mutation at a specific locus in *E. coli* (streptomycin resistance).

The calculation of the distribution of P^{32} decays of such low energy as to increase significantly the specific ionization in the cells shows no great difference from Kamen's calculations.

WYSS:

The decay of radioactive P to S might be of primary importance. The phage containing such a substitute for P might readily invade a bacterial cell but could not there reproduce itself because of the absence of identical building blocks, that is, nucleic acids containing S instead of P. In Kamen's experiments such a phage is recorded as being non-infective, and the implication is that it became non-infective during the emanation.

KAMEN:

It is not obvious that a single sulfate radical-containing nucleotide would necessarily be unavailable for synthesis into a nucleic acid. Such a "thionucleotide" would be only a slight modification of the natural nucleotide and might be used to synthesize a slightly modified macromolecule in which only 1 in several 100,000 P atoms was replaced by an S atom. When it is possible to prepare sulfur analogs of nucleotides, it will be interesting to see how well the nucleic acid synthesis system is able to incorporate them into the natural nucleoprotein.

FAILLA:

It could also be assumed that only the low-energy beta particles produce enough ionization in the phage; that is to say, only the low-energy beta particles will have a high probability of effectiveness and of consequent biologic change.

POWERS:

The experiments at Argonne on the phenomenon reported today have given qualitatively the same result.* In *Paramecium aurelia* it has been shown that

* Powers, E. L., *Genetics*, **33**: 120, 1948.

incorporated P^{32} is about 5 times as efficient in inducing death after autogamy as are $Sr^{89,90}Y^{90}$ solutions delivering the same beta-radiation dosage to the cell. In our system the accumulation of phosphorus by the organism is a complicating factor which reduces the calculated efficiency of P^{32} to about 4 times that of the Sr solutions. It should be noted that the *Paramecium* evidence concerns the induction of changes in micronuclei which undergo a series of maturation divisions followed by the fusion of two gametic nuclei before the expression of the effect. The phenomenon has, then, been detected in a system which is genetic in the "gene-chromosome" sense.

The experiments have been extended to radioactive hydrogen. Recent results show that tritium is certainly no more efficient than one would expect on the basis of dosage of ionizing radiation delivered to the cell by the beta particles from the H^3 atoms. It appears that the respective positions of P and H in the structure of the nucleus are markedly different in importance.

SOLOMON:

In addition to the bond rupture around radioactive sulphur which Kamen pointed out, it might also be possible to have the situation complicated by the reactivity of the sulfur which is formed; perhaps it can combine with the oxygen atoms. In our laboratory we have found that sulfur produced from chlorine will exchange with sulfur in the carbon disulfide molecule, which does not in the ordinary way happen.

LATARJET:

What would be the efficiency of an ionization in Kamen's case as compared to the one I obtained with soft x-ray * irradiation of intracellular T_2?

I am attempting at present to induce host range mutations in a bacteriophage by ultraviolet irradiation of the intracellular phage during its growth process.† I should like to ask Kamen whether he thinks that his technique would be suitable for the same kind of experiment with ionizing radiation.

KAMEN:

The effect could be studied. It might be possible to take mutants containing P^{32}, cross them with members of a normal strain, and study the results of hybridization.

LATARJET:

Is α the probability of the efficiency of ionization?

KAMEN:

Yes.

LATARJET:

It is interesting that studies with x-rays on the same phage give values of approximately 0.12, which is in fair agreement with Kamen's value of 0.08.

* *J. Gen. Physiol.*, **31**: 529, 1948.
† *Compt. rend.*, **228**: 1354, 1949.

MULLER:

Is there any reason to believe that 1 in 11 represents the chance that inactivation will result from the conversion of any P atom in the nucleoprotein to S, rather than the proportion of P atoms in the nucleoprotein which are so situated that inactivation will inevitably result from their conversion to S?

KAMEN:

It is not known whether 8 per cent of the phosphorus is in the right portion of the molecule and will have 100 per cent probability of inactivation following disintegration of the P^{32}, or whether distribution of the energy throughout the molecule will give rupture at the appropriate bond in 12 per cent of the cases.

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Recent Evidence on the Mechanism of Chromosome Aberration Production by Ionizing Radiations *

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Understanding the mechanism by which ionizing radiations produce chromosome aberrations is one of the fundamental problems in radiation genetics. The present discussion will deal with recent evidence on this subject, obtained for the most part from experiments with plant chromosomes, especially those of the spiderwort, *Tradescantia*. At the outset it appears desirable to review briefly the interpretation of aberration production in *Tradescantia* as proposed originally by Sax (24, 25) and developed in quantitative form primarily by Lea (20). After this, more recent results, stemming principally from the observations by Thoday and Read (28, 29) on the effect of oxygen on aberration frequency, will be presented. Finally the implications of the oxygen effect for an interpretation of the biochemical mechanism of radiation-induced chromosomal changes will be considered, in particular the extent to which this effect requires a revision of previous views as to the mechanism involved.

The Production of Chromosomal Aberrations in *Tradescantia* by Ionizing Radiations

The general experimental technique for observing the effects of ionizing radiations on *Tradescantia* chromosomes as developed by Sax (24) has been described in detail previously by Sax (25), Catcheside, Lea, and Thoday (6, 7), and Lea (20) and will be considered here only briefly. The typical procedure is to expose entire inflorescences, consisting of several buds containing microspores in various stages of development, to penetrating radiations such as x-rays or fast neutrons. Cytological

* This work was done under Contract W-7405-Eng-26 for the Atomic Energy Commission, Oak Ridge, Tennessee.

examinations are then made at appropriate intervals after treatment to detect aberrations at the first postmeiotic mitosis in the microspore. Two general categories of aberration types are noted: (*a*) chromatid types, resulting from irradiation of chromosomes which are effectively double in prophase, and (*b*) chromosome types, resulting from irradiation of chromosomes which are effectively single in resting stage. In both instances, radiation produces breaks in one or more of the six chromosomes in the nucleus of a microspore, and the resulting broken ends may remain as such, undergo restitution, or rejoin with other broken ends to produce aberrant configurations which are cytologically detectable. The principal configuration types with which we shall be concerned in the experimental results to be discussed in this paper may be designated as chromosome interchanges. These rearrangements, observed in cells examined 4–5 days after irradiation, are either dicentric or ring chromosomes and result from breaks in two separate, undivided chromosomes, or chromosome arms, followed by the reunion of broken ends to give one chromosome with two centromeres, or one continuous ring chromosome, plus an accompanying acentric fragment in each case.

The essential features of the hypothesis developed by Sax, Lea, and others to explain the production of chromosome aberrations in *Tradescantia* may be outlined as follows. Electromagnetic or particulate radiations produce their effects as a consequence of the formation of ion pairs within a chromosome during the passage through the chromosome of either primary or secondary charged particles. Chemical changes resulting from this *direct* ionization of the molecules composing the chromosome lead to the production of chromosome breaks. The resulting broken ends may remain as such, yielding terminal deletions, or undergo restitution, giving rise to apparently normal chromosomes again, or rejoin with other broken ends, producing cytologically visible aberrations. The restitution and reunion processes are competitive and both space and time factors are involved. Several ionizations (between fifteen and twenty) must occur within the chromosome to produce a break, and the factor of major consequence in distinguishing the quantitative effects of various radiations is the difference in ionization distribution along particle tracks. For certain radiations (for example, gamma rays and x-rays) ionization distribution along the tracks (of secondary electrons) is such that the probability of breakage of a chromosome by a traversing particle is considerably less than 1 for most of the length of the track except near the end. As a consequence, such radiations are relatively inefficient in producing breaks, on the basis of total ionization produced per track. Furthermore, breaks in two separate chromosomes (or chromosome arms) are almost always produced by two separate particle

tracks, with the result that the frequency of interchange aberrations (for example, dicentrics and rings) increases as the square of the dose (a two-hit curve) when the time of irradiation is kept constant. Because such aberrations are two-hit phenomena, there is also an intensity effect—the yield of interchanges decreasing when a constant dosage of radiation is administered over periods of increasing duration. The average time during which a break may remain "open" before restitution or reunion occurs (that is, its half life) is at least 4 min.

For other radiations (for example, recoil protons from fast neutrons), ionization distribution along tracks is such that the probability of breakage of a chromosome by a traversing particle is close to 1, and as a consequence the efficiency of such radiations in producing chromosomal changes is very high compared to those of gamma or x-rays. In addition, both the breaks in the two separate chromosomes (or chromosome arms) taking part in an interchange are usually produced by a single particle track, resulting in a linear relationship between interchange frequency and dose (a one-hit curve) and the absence of an intensity effect.

In addition to aberrations resulting from breaks in two separate chromosomes, certain types are produced by breaks in a single chromosome arm (either divided or single). The majority of these aberrations are one-hit types resulting from the passage of a single ionizing particle, and exhibit no intensity effect with any type of radiation.

The interpretation of chromosome-aberration production as just outlined has been quite generally successful in accounting for most of the quantitative results of radiation experiments with *Tradescantia*. However, experimental data of two sorts have been obtained which indicate that this hypothesis in its simplest form is not entirely adequate. The first evidence was that obtained by Kotval and Gray (17) in their studies with alpha particles. On the basis of comparative ionization distribution and particle numbers, the hypothesis predicts that a given amount of ionization produced by alpha particles should be considerably less efficient in producing chromosome breaks than an equal ionization dose produced by fast neutrons, whereas the experimental results indicate that for equal ionization doses alpha particles are somewhat more efficient. It was concluded that a proportion of the breaks produced by alpha particles arises from ionization produced in the immediate vicinity of, but not within, a chromosome, thus suggesting the involvement of an *indirect* as well as a direct mechanism. The second, and even more striking, evidence was that obtained by Thoday and Read (28), who noted a pronounced effect of oxygen on the frequency of x-ray-induced aberrations in the root-tip mitoses of the broad bean, *Vicia faba*. Their experiments indicated that the absence of oxygen during irradiation re-

sulted in a marked decrease in aberration frequency. Similar results were obtained by Hayden and Smith (15) in experiments with barley seeds. Experiments on the effect of oxygen have also been performed with *Tradescantia*, and the results of some of these will now be discussed.

The Effect of Oxygen on X-Ray-Induced Chromosomal Rearrangements in *Tradescantia*

The experimental methods used to expose *Tradescantia* inflorescences to x-radiation while in atmospheres containing various percentages of oxygen have been described in some detail by Giles and Riley (11, 12) and will be outlined only briefly here. Inflorescences were placed in an appropriate holder inside an airtight Lucite exposure chamber. This chamber was placed inside the x-ray machine and connected through ports by pressure tubing and appropriate valves to a vacuum pump, a gas pressure cylinder, and a mercury manometer. Air in the chamber could be evacuated and replaced by the appropriate gas or gas mixture from the cylinder. The chamber could also be maintained under vacuum, or under pressures up to 3 atm above normal atmospheric. Rapid introduction or removal of gas could be effected with the apparatus. All these manipulations could be carried out before, during, or after irradiation, depending on the experimental conditions desired. The x-ray intensity for each exposure was determined by means of a Victoreen thimble ionization chamber which could be inserted into the box in the same position as that normally occupied by the inflorescences.

A series of dosage curves was obtained for inflorescences exposed in air, in oxygen, and in nitrogen. The yield of both interchanges (dicentrics and rings) and interstitial deletions was markedly reduced when nitrogen replaced air in the chamber, and increased somewhat when oxygen replaced air. Additional comparative exposures were made in other gases, such as helium and argon, and also under vacuum. In all instances, reduced aberration frequencies similar to those obtained in nitrogen resulted, indicating that the absence of oxygen was responsible for the decrease in radiosensitivity (11). No chromosomal effects were noted in control experiments in which similar exposures to nitrogen and oxygen were made without irradiation. It was clear from these results that the presence of oxygen resulted in a marked increase in aberration frequency.

The next problem was to determine the reason for this effect of oxygen. If it is assumed that all breakage is the result of direct-hit effects, the same number of breaks should be produced in the presence or absence of oxygen by a given x-ray dose. Thus the increased aberration fre-

quency obtained in oxygen might result from an effect of oxygen itself on the recovery process, such that when oxygen is present new reunions of broken ends are favored as opposed to restitution. It seemed possible, for example, that such an effect could result from the stimulation of chromosome movement by oxygen. Another and perhaps more likely possibility appeared to be that the effect of oxygen itself is an indirect one, such that in the presence of dissolved oxygen x-rays produce in cells a certain substance or substances which increase the yield of aberrations. In this event, such an intermediate substance could produce an effect by way of either the recovery or the breakage mechanism. In the former instance, the x-ray breakage of chromosomes would still be considered a direct effect; in the latter, however, the breakage would have to be considered an indirect effect, at least in part.

It appeared feasible to attack some of these problems experimentally in *Tradescantia*, since in this organism the recovery process extends over a considerable period of time (the average time between production of a break and restitution or reunion being at least 4 min). Thus it is possible to separate to a considerable degree the two processes of breakage and recovery and to test the effect of oxygen on each. To do this, inflorescences were exposed to a single dose of 300 r of x-rays in 1 min, either in the presence of pure oxygen or in the absence of oxygen (*in vacuo*). Immediately after the irradiation oxygen was either removed (by evacuation) or introduced (to a positive pressure of 1500 mm of Hg). The exchange of gases, as recorded by the manometer, could be effected quite rapidly, and in this fashion it was possible to have the breakage process occurring in oxygen and recovery largely in its absence, or the reverse. In addition, other experiments were performed in which oxygen was either introduced or removed during part of the x-ray exposure. The results of such comparative exposure are reported in the paper of Giles and Riley (12). Certain other experiments of a similar type have been performed, and these results will be presented here. In the original experiment in which a single exposure of 1 min to 300 r was made in vacuum followed by the immediate introduction of oxygen, no effect on the recovery process was noted. It was decided to increase the possibility of detecting such an effect by fractionating the dose so that a relatively larger portion of the recovery period would take place in oxygen. The following procedure was used. A set of inflorescences was exposed in vacuum for 20 sec at 300 r per min; after the irradiation, oxygen was immediately introduced into the chamber to a positive pressure of 1500 mm of Hg and allowed to remain for 8 min; the chamber was then re-evacuated and another 20-sec exposure made, followed by the reintroduction of oxygen as above; this procedure was repeated 5 times to give a

total dose of 500 r. Two control series were run, in one of which the procedure just described was followed except that helium rather than oxygen was introduced. In the other control a total dose of 500 r was administered in 100 sec of continuous exposure in vacuum; helium was then introduced and remained in the chamber for 8 min after the x-ray exposure. The data from this experiment are presented in Table 1. It

TABLE 1

TEST OF THE EFFECT OF OXYGEN ON THE RECOVERY MECHANISM, USING FRACTIONATED DOSES

All series received a total dose of 500 r at 300 r per min. In series A this dose was delivered in five equal fractions, with irradiation occurring in vacuum, and each intervening recovery period of 8 min occurring in oxygen at a positive pressure of 1500 mm of Hg. Series B was similar, except that the recovery periods occurred in helium. Series C received one continuous exposure in vacuum, with recovery occurring in helium.

Series	Conditions	No. of Cells Examined	Interchanges per Cell	Interstitial Deletions per Cell
A	Fractioned dose; irradiation in vacuum; recovery in oxygen	323	0.23 ± 0.027	0.20 ± 0.025
B	Fractioned dose; irradiation in vacuum; recovery in helium	450	0.28 ± 0.025	0.31 ± 0.026
C	Continuous exposure; irradiation in vacuum; recovery in helium	582	0.31 ± 0.023	0.35 ± 0.024

is evident that there is no increase in aberration frequency in series A, in which oxygen was present during recovery, as compared with B, in which helium was present. The somewhat higher values for C are to be expected, since this was a continuous exposure with no intervening recovery periods.

A second experiment has been performed (in cooperation with A. V. Beatty) to retest the observation of Giles and Riley (12) that the addition of oxygen during irradiation results in an immediate increase in aberration frequency. These data are presented in Table 2. In this instance, essentially the same experimental conditions were utilized as previously, except that a second exposure (series C) was made in which oxygen was present during only the last 15 sec of the total x-ray exposure of 1 min. The results of this experiment are in agreement with the earlier one in indicating that the introduction of oxygen during irradiation results in an immediate increase in aberration frequency.

It is clear from these comparisons (and those reported earlier) that the addition or removal of oxygen immediately after irradiation does not

modify the aberration frequency, thus indicating that oxygen itself has no effect on the recovery process. There seems to be little question that, under the experimental conditions utilized, oxygen diffuses very rapidly into the cells and is present during the recovery process. This is shown by the fact that the introduction of oxygen during irradiation causes a

TABLE 2

EXPERIMENTS ON THE INTRODUCTION OF OXYGEN DURING X-IRRADIATION OF *Tradescantia* INFLORESCENCES

(All exposures of 300 r at 300 r per min)

Series	Pretreatment Conditions	Exposure Conditions	Post-Treatment Conditions	No. of Cells	Interchanges per Cell	Interstitial Deletions per Cell
A	Buds in vacuum	Vacuum	Vacuum—10 min	850	0.11 ± 0.01	0.08 ± 0.01
B	Buds in vacuum	1st 30 sec; vacuum	Evacuation (within 25 sec)	900	0.32 ± 0.02	0.36 ± 0.02
		2nd 30 sec; oxygen introduced (within 3 sec) to 1500 mm of Hg	Vacuum—10 min			
C	Buds in vacuum	1st 45 sec; vacuum	Evacuation (within 25 sec)	600	0.22 ± 0.02	0.26 ± 0.02
		Last 15 sec; oxygen introduced (within 3 sec) to 1500 mm of Hg	Vacuum—10 min			
D	Buds in oxygen	Oxygen at 1500 mm of Hg	Evacuation (within 25 sec)	900	0.61 ± 0.03	0.67 ± 0.03
			Vacuum—10 min			

pronounced increase in aberration frequency. This latter result is also important in providing additional evidence that oxygen, to be effective, must be present during the actual irradiation and that there is little or no latent period between the introduction of the gas and its effect in terms of increased aberration production. Other experiments have also demonstrated that a pre-exposure of buds in pure oxygen before they are irradiated in helium has no effect.

From all these observations, it seems clear that the effect of oxygen itself is an indirect one, presumably arising from the production by x-

rays, when oxygen is present, of some substance which increases the frequency of aberrations. As indicated previously, this effect of such a substance might operate by way of either the breakage or the recovery mechanism. The most likely hypothesis seems to be that such a substance would cause an increased production of chromosome breaks. However, the alternative possibility, that the recovery process is modified, cannot be immediately excluded on the basis of the data just presented. This problem must be attacked by other methods. Experiments designed to determine whether the recovery time is different for breaks produced in the presence and absence of oxygen are under way (Giles and Riley, unpublished). The results to date are compatible with the view that the recovery mechanism is essentially the same under these two conditions. The previously reported differences in the slopes of dosage curves obtained at a constant intensity of 45 r per min in air, in oxygen, and in nitrogen, which at first appeared to be due to an effect on restitution time, can be explained on the basis of an intensity effect resulting from a lesser production of breaks per unit time in nitrogen. It has been shown by Sax (26) that the exponents of dosage curves from chromosome interchanges decrease with decreasing radiation intensity. Evidence has also been obtained (Giles and Beatty, unpublished) that the effect of temperature, which has been previously interpreted as influencing the recovery process (25), is actually, at least to a considerable extent, an indirect effect on oxygen availability. The experiments of Baker and Sgourakis (4) with *Drosophila* have demonstrated that oxygen has a marked effect in increasing the frequency of other types of x-ray-induced genetic changes, sex-linked lethal mutations, where there is no evidence that a recovery process is involved. All these results suggest very strongly that the oxygen effect is on the breakage and not on the recovery mechanism in *Tradescantia.*

In order to provide additional evidence concerning the mechanism of the oxygen effect, it appeared desirable to determine the relationship between the amount of oxygen present during irradiation at a constant dosage and aberration frequency. Consequently, a series of exposures has been made of inflorescences in atmospheres containing different percentages of oxygen (mixed with helium) at a single x-ray dose of 400 r. Some data on this point have already been published; see Giles and Riley (12). Another experiment (Giles and Beatty, unpublished) has been carried out to determine this relationship more precisely. In this instance special attempts were made to free the helium used of any residual oxygen. The percentages of oxygen (2, 5, and 10 per cent) in the other gas mixtures used were accurate to within ± 0.2 per cent, and a larger number of cells was scored to increase the statistical reliability

of the determinations. A graphical summary of the results is presented in Fig. 1, together with the averages of points obtained at higher percentages of oxygen in previous experiments. It is clear that there is still a substantial yield of aberrations even in the complete (or nearly complete) absence of oxygen. When oxygen is present in irradiated cells, there is a rapid rise in aberration frequency above this base level. This

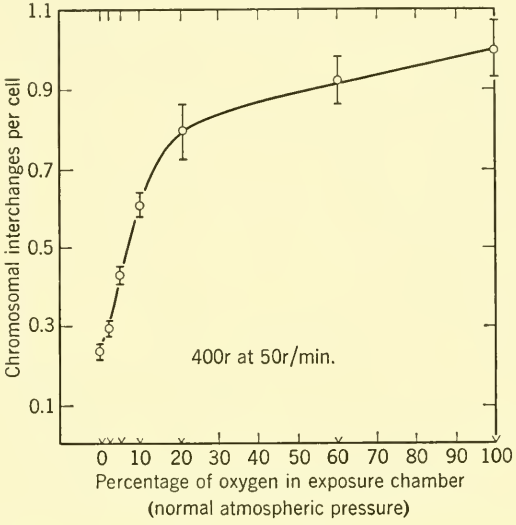

FIG. 1. Reproduced by permission from *Science*, **112**: 643, 1950.

increase is linear between 0 and 10 per cent oxygen, after which the rise is apparently more gradual.

Certain additional experiments (Giles and Beatty, unpublished) provide further evidence that the amount of dissolved oxygen present in the cells is an important factor in determining aberration frequency. In these experiments, a constant percentage of oxygen was used in the exposure chamber, but irradiations were carried out with the inflorescences under pressures up to 3 atm above normal. The data obtained for exposures at 0, 1, 2, and 3 atm above normal pressure (approximately 740 mm of Hg) in 10 per cent oxygen (plus 90 per cent helium) have been indicated in Fig. 2, on the assumption that the amount of effective dissolved oxygen in the cells is directly proportional to the pressure. Control experiments in which comparable exposures were made in helium under pressure indicated that pressure alone did not change the aberration frequency. As can be seen from the graph (Fig. 2) there is good agreement with previous exposures in different percentages of oxygen

at normal atmospheric pressure. As mentioned previously, preliminary experiments have also been completed which strongly suggest that the effect of temperature on aberration frequency is, at least in large part, actually an oxygen effect (Giles and Beatty, unpublished). Similar evidence concerning the temperature effect on sex-linked lethals in *Drosophila* has already been obtained by Baker and Sgourakis (4).

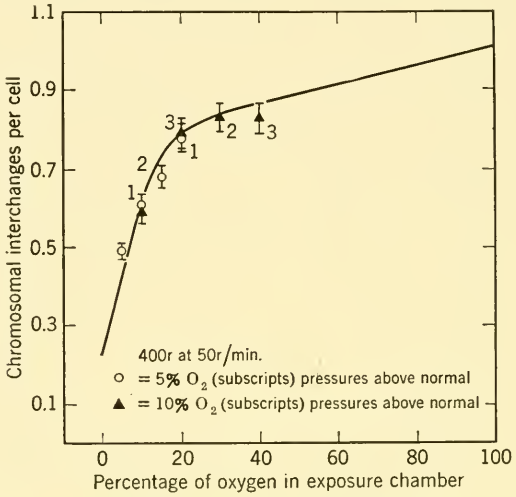

FIG. 2. Reproduced by permission from *Science* **112**: 643, 1950.

The Biochemical Mechanism of the Oxygen Effect

It is clear from the results which have just been presented that oxygen has a marked effect in increasing the radiosensitivity of *Tradescantia* chromosomes, as measured by the frequency of x-ray-induced interchanges and deletions. We shall assume, as most of the evidence seems to indicate, that this effect arises only if oxygen is present in cells during the actual period of irradiation, and results from the production by x-rays of more chromosome breaks under these circumstances. The simplest explanation for this situation would appear to be that, in the presence of oxygen, irradiation results in the production within the nucleus of some substance (or substances) which causes an increase in chromosome breakage and that the amount of this substance produced is positively correlated with the amount of oxygen present. It thus becomes of interest to determine, if possible, what this substance is.

Since these cells are composed largely of water, it seems very probable that the substance is a product of irradiated water, more particularly, a product characteristically formed when oxygen is present in irradiated

water. It has already been suggested by Thoday and Read (28, 29) that this product may be hydrogen peroxide. Additional evidence is now available which supports this conclusion. The results of four entirely unrelated types of experiments furnish evidence favoring or compatible with the H_2O_2 hypothesis. Bonet-Maury and Lefort (5) have investigated the production of H_2O_2 in water irradiated with x-rays and with alpha particles under various conditions, including the effect of oxygen concentration and of temperature. In addition, data are available on the effect of *p*H on peroxide yield; see Loiseleur (21).

At least four striking parallels exist between H_2O_2 production and chromosome-aberration production under varying conditions of irradiation. (*a*) It is found that with x-rays H_2O_2 is not produced in oxygen-free water [or is produced in very small amounts, as shown by Allen (1)], but that when oxygen is present the amount of H_2O_2 produced depends markedly on the concentration of oxygen. The type of curve obtained for the increased yield of H_2O_2 with increasing oxygen concentration at a constant x-ray dose is generally similar to that obtained in the present studies for the relation between aberration frequency and the percentage of oxygen present during x-radiation. (*b*) In oxygen-saturated water, H_2O_2 production by x-rays decreases regularly with decreasing temperature, a definite discontinuity marking the passage from water to ice. Below $-116°$ C no H_2O_2 can be detected. Fabergé (10) has shown that, when *Tradescantia* pollen grains are x-rayed at various temperatures, the general character of the sensitivity curve (as measured by the number of chromosome breaks observed in pollen tube divisions) resembles that for H_2O_2 production. There is a dip in the region of 0° C and a gradual decline thereafter. However, although H_2O_2 production stops at $-116°$ C, chromosome breaks are still produced at $-192°$ C, their frequency being almost one-fifth that at $+25°$ C. (*c*) The *p*H of the solution exerts an effect on the yield of H_2O_2 with x-rays, an alkaline *p*H favoring a lower H_2O_2 concentration. The experiments of Marshak (22) have shown that the frequency of chromosome aberrations observed at anaphase in root tips of *Vicia faba* and *Allium cepa* is markedly reduced when x-radiation is carried out in the presence of penetrating bases, such as ammonium hydroxide. (*d*) Undoubtedly the most cogent evidence obtained to date in favor of the H_2O_2 hypothesis is that derived from a comparison of x-ray and alpha-particle effects in the presence and absence of oxygen. With alpha particles, H_2O_2 production occurs even in oxygen-free water and the addition of oxygen does not increase the yield. Thoday and Read (29) have shown that, for aberrations induced in the root tips of *Vicia faba*, the removal of oxygen results in little if any decrease in aberration fre-

quency when the cells are irradiated with alpha particles, as compared with a very marked decrease when x-rays are used. These observations have been confirmed in preliminary results obtained by Conger (unpublished) in experiments on the irradiation of mature pollen grains of *Tradescantia* with alpha particles.

Although these comparisons suggest very strongly that H_2O_2 may be the product involved in the oxygen effect, they do not establish this point unequivocally. The possibility exists that other products of the radiodecomposition of water may be concerned. The observations of Krenz and Dewhurst (18) on the effect of dissolved oxygen on the oxidation of ferrous sulfate in aqueous solution by gamma rays can apparently best be explained by a mechanism involving the HO_2 radical. The marked similarity between the magnitude of the decreased oxidation of ferrous sulfate in the absence of oxygen (to about one-fourth) and the decrease in aberration frequency obtained in the early experiments with *Tradescantia* is noteworthy. However, the absolute magnitude of the decrease in aberration frequency occurring in the absence of oxygen apparently depends on the dose and the intensity in a particular experiment. Further, the experimental results with alpha particles in the presence and absence of oxygen appear to make it unlikely that HO_2 is the intermediate involved. These results are in agreement with the view, on radiochemical grounds, that H_2O_2 is formed directly in large amounts by alpha particles because of the very close proximity of the OH radicals produced in the center of the particle track [Gray (13)]. The failure of O_2 to increase the yield of H_2O_2 apparently indicates that the usual reaction for peroxide formation by way of the intermediate HO_2 radical is relatively unimportant [Bonet-Maury and LeFort (5)]. It thus appears that H_2O_2, rather than HO_2, may be responsible for chromosome breakage. There are, however, additional mechanisms by which HO_2 may be produced from peroxide, and the possibility cannot be entirely eliminated that this radical may also be to some extent an effective agent in chromosome breakage.

Additional evidence on the presumptive role of H_2O_2 should be obtained from experiments with enzyme inhibitors such as cyanide which should block the action of catalase and permit H_2O_2 accumulation. A suggestive slight mutagenic effect of cyanide alone, which has been interpreted on this basis, has already been reported by Wagner *et al.* (30) in *Neurospora*. The experiments of Mottram (23), which show that cyanide increases the sensitivity of roots of *Vicia faba* to x-rays, as judged by inhibition of growth, also lend support to this possibility. In the *Neurospora* experiments a direct mutagenic effect of H_2O_2 was also noted. In experiments utilizing enzyme poisons to elucidate the mecha-

nisms of the oxygen effect, however, the specificity of the poison would appear to be exceedingly important; otherwise an unequivocal interpretation of the results is not possible. Effects due to cyanide might result from an inhibition of the cytochrome system, thus preventing the utilization of oxygen by the respiratory enzyme systems of the cell. If such a utilization of oxygen is necessary to bring about its effect on radiosensitivity, then it might be expected that cyanide would decrease, rather than increase, radiosensitivity. There is, in fact, one report, that of Bacq *et al.* (3), that cyanide exerts a protective action against the killing of mice by x-rays, but these results are not confirmed in similar experiments by Dowdy *et al.* (9) with rats, in which a clear effect of anoxic anoxia was demonstrated. With respect to the production of chromosomal aberrations, it appears probable that the oxygen effect is produced by oxygen dissolved in the aqueous medium of a cell, but additional experimental evidence on this point is being sought.

There is also the possibility that, even though H_2O_2 may be one of the essentially primary radiation products associated with the oxygen effect, it still may not be the actual mutagen directly responsible for chromosome breakage. It may be only an intermediate in the formation of other substances such as organic peroxides, some of which have been shown by Dickey *et al.* (8) to have marked mutagenic effects in *Neurospora*. It appears likely that the effect of organic peroxides may result from free-radical formation; and, indeed, it is possible that most, if not all, chemical mutagenic effects may be explicable on this basis and thus turn out to be fundamentally related to radiation-induced mutations [Auerbach (2), Dickey *et al.* (8), Jensen *et al.* (16)].

The Relation of the Oxygen Effect to Previous Views on the Mechanism of Chromosome Breakage in *Tradescantia* by Ionizing Radiations

The preceding discussion has indicated the remarkable effect of oxygen in increasing the radiosensitivity of *Tradescantia* chromosomes. This effect can be most easily interpreted as resulting from an increased production of chromosome breaks by x-radiation, the amount of increase being positively correlated with the amount of oxygen present in cells. On the basis of such results, it would appear that the previous hypothesis utilized to explain the production of aberrations in *Tradescantia* must be modified. On this hypothesis, as outlined earlier, chromosome breakage has been considered to result from the direct action of the radiation in ionizing the molecules actually composing a chromosome, as a consequence of the passage through the chromosome of an ionizing particle

[Lea and Catcheside (19)]. It now appears most likely that an indirect mechanism is involved, in which irradiation of the oxygen-containing aqueous medium in the cell leads to the production of some substance which in turn produces chromosome breaks.

It should be recalled, however, that a substantial aberration frequency is still produced by irradiation in the absence of oxygen (at least in so far as oxygen can be removed from these cells). The question thus arises whether there are two mechanisms for chromosome-break production, one involving direct ionization of the chromosome molecules and the other an indirect effect from the irradiated aqueous medium, and further whether the relative importance of the two mechanisms may be judged by the degree of the oxygen effect. That such is the situation is by no means clear. It seems possible, in fact, that at least some of the aberrations induced in the absence of oxygen may also be the result of an indirect effect, being produced by substances other than H_2O_2 or HO_2, such as OH radicals, resulting from the radiodecomposition of essentially oxygen-free water.

Attempts have been made to test this point by experiments (Giles and Beatty, unpublished) designed to minimize the effectiveness of the OH radical by promoting, during irradiation, the back reaction to form H_2O [Allen (1)]. To do this, inflorescences were exposed to 400 r of x-rays in atmospheres of hydrogen, both at normal pressure and at 3 atm above normal. Interchange frequencies were essentially the same for the two exposures, and although both values were somewhat lower than those obtained in comparable exposures in helium or nitrogen, the difference is not significant. If it is valid to assume that hydrogen would in fact react to remove OH radicals formed during irradiation, the failure to detect a reduced aberration yield in these experiments supports the view that chromosome breakage produced by x-rays in the absence of oxygen may all result from direct ionization of the chromosome molecules. It should be pointed out, however, that this conclusion is based on the assumption that reactions leading to H_2O_2 production or suppression in cells from which oxygen has been removed as completely as possible are similar to those occurring in oxygen-free pure water. There is as yet little experimental evidence on this point, and it is quite possible that the complexity of the cellular environment may cause very different reactions to occur.

Unfortunately it does not appear to be experimentally feasible to assess the relative importance of the indirect and direct effects on chromosomes by the method normally employed for enzymes and viruses—that of determining the effect of a constant radiation dose when the solute concentration is varied over a considerable range. The nearest approach

to this situation would appear to be sensitivity tests of cells that vary in water content. This is not possible with developing microspores of the type used in these experiments. It might be feasible with mature pollen grains, however. There are in fact many data which indicate that dry seeds of plants show increased radioresistance, both with respect to killing and to induced genetic changes, genic as well as chromosomal, as compared with hydrated seeds [Gustafsson (14)]. However, it is not always possible from such comparisons to conclude that the sensitivity changes are directly correlated with water content, and do not result from changes in the mitotic state of the nucleus, which are known to affect radiosensitivity.

Regardless of the degree of importance of the direct effect, it appears probable that in *Tradescantia*, at least, the magnitude of the indirect effect is considerably greater. However, it is still possible to interpret the results in terms of target theory, as indicated by Thoday (27). The essential requirement is that the action of ionizing particles, whether direct or indirect, be relatively localized. If the effect is principally indirect, it appears that a substance, such as H_2O_2, must be produced along the track of an ionizing particle and must have a relatively limited effective diffusibility (or short half life). In fact it seems necessary that its effective distribution within the nucleus must correspond in pattern rather closely to that of ionization distribution along particle tracks. Such a localized distribution would appear to be essential, as has been pointed out by Zirkle (31), in order to explain the striking quantitative differences among various radiations, as, for example, the shapes of the dosage curves for interchanges induced by x-rays and alpha particles.

Summary

Recent experiments have demonstrated that oxygen has a marked effect in increasing the sensitivity of chromosomes in *Tradescantia* and other plants to x-rays as measured by the frequency of cytologically detected aberrations. It has been shown that this is not an effect of oxygen itself on the behavior of broken ends of chromosomes. The effect apparently arises from the production by x-rays, as a result of the radiodecomposition of water in cells containing oxygen, of some substance which causes an increase in aberration frequency. Several independent lines of evidence indicate that this substance may be H_2O_2. It appears likely that the increased frequency of aberrations arises from an increased production of chromosome breaks when oxygen is present during irradiation, rather than from a modification of the recovery process. Thus a major fraction of the radiation effect on *Tradescantia* chromo-

somes is to be considered indirect rather than direct. In the absence of oxygen, however, there is still an appreciable aberration frequency and some evidence indicates that this entire fraction may be the result of direct radiation action.

Since the previous hypothesis explaining chromosome breakage in *Tradescantia* assumed that all the effect of the radiation resulted from the direct ionization of the molecules of the chromosome, the demonstration of an indirect effect necessitates a revision of this interpretation. However, it is still possible to interpret the results in terms of target theory. The essential requirement is that the action of ionizing particles, whether direct or indirect, be relatively localized. If the effect is principally indirect, it appears that a substance, such as H_2O_2, must be produced along the track of an ionizing particle and must have a relatively limited effective diffusibility (or short half life). In fact, it seems necessary that its effective distribution within the nucleus must correspond in pattern rather closely to that of ionization distribution along particle tracks.

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DISCUSSION

BARRON:

I had no intention of taking part in this discussion. However, at the suggestion of Eyring, I will re-emphasize the role of oxygen in ionizing radiations, inasmuch as under normal conditions biological fluids are constantly saturated with oxygen. It is well known that the formation of H_2O_2 on irradiation of water with x-rays occurs only when oxygen is present. Furthermore, the atomic hydrogen, formed on the primary ionization of water, may reduce molecular oxygen

to form the powerful radical O_2H: $O_2 + H \rightarrow O_2H$. The presence of oxygen, therefore, increases considerably the oxidizing power of ionizing radiations. Molecular oxygen can be used, for these reasons, as a test for the mechanism of action of ionizing radiations. If the effects obtained on irradiation are greater in the presence of oxygen, there will be no doubt that the effect was due to products of the irradiation of water. The radical OH and atomic hydrogen are formed regardless of the nature of gas dissolved in water; O_2H and H_2O_2, however, are formed only in the presence of oxygen. Thiol compounds, such as glutathione, —SH enzymes, —SH proteins, are oxidized by all three agents, and irradiation will be more effective in the presence of oxygen. Ferrocytochrome c is not oxidized by H_2O_2; in this case the presence of oxygen would increase only oxidation due to the radical O_2H. Finally, there is the possibility, which must be investigated, that the H_2O_2 produced on irradiation may act as an oxidizing agent when combined with catalase.

In summary, the presence of oxygen introduces two more oxidizing agents. It is obvious, therefore, that the absence of oxygen will decrease the toxic effects of ionizing radiations, a decrease which reaches 70 per cent in the case of the oxidation of thiol compounds. If ionizing radiations have the same effect in the presence as well as in the absence of oxygen, oxidation processes cannot be dismissed because the powerful OH radicals are still formed. It would be interesting to study the effect of low oxygen tensions on irradiation of tissues with *small doses* of radiation. It is quite possible that x-irradiation of bacteria at low oxygen tensions, such as those prevailing in the high plateaus of the Andes and the Himalayas, would produce fewer mutations than irradiation at sea level.

16.

Physical and Chemical Factors Modifying the Sensitivity of Cells to High-Energy and Ultraviolet Radiation *

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Microorganisms are in some ways ideal materials for obtaining immediate quantitative information on some of the basic aspects of radiation effects. It is well known that they can often be handled in very large

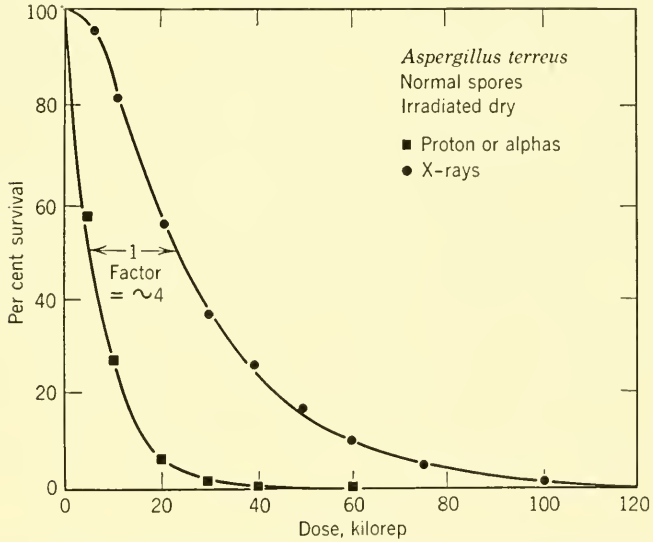

FIG. 1. Comparative lethal action of x-rays (250 kev), of protons, and of alpha particles on dry spores of *Aspergillus terreus*. [From Stapleton and Martin (18).]

numbers, thus giving statistical results on effects which are hardly recognizable on higher organisms. They are, in general, not suitable for

* This work was done under Contract No. W-7405-Eng-26 for the Atomic Energy Commission.

studies of radiation effects on chromosomes, which have been so well demonstrated by Giles, and for most genetical studies, which will be discussed by Muller.

Typical killing curves which have been obtained with fungi in our laboratory are given in Fig. 1. They refer to a comparative study of x-rays, and alpha particles and protons, on *Aspergillus terreus* [Stapleton and Martin (18)]. We are, however, not too enthusiastic about the use of these curves as the only basis for interpretation of the mechanism of lethal action of radiation, since "death" could be caused by a variety of mechanisms which are difficult to untangle.

There are many treatments which in themselves have little or no effect on the radiation sensitivity but, if given in supplement to ionizing radiation, produce striking effects. In general, these supplementary treatments have increased the sensitivity of cells to radiation rather than protected them; however, there are a few exceptions to this.

Heat and Visible Light

The first two factors to be discussed are the effect of heat and light in the treatment after irradiation. Cells are more sensitive to heat after irradiation; this applies to x-rays as well as ultraviolet [Gaulden (8)]. I should like to refer you also to the work of Curran and Evans (5) and of Anderson and Duggar (2). E. H. Anderson (1), in our laboratory, found that a certain strain of *Escherichia coli* can be reactivated after exposure to ultraviolet several hundred-fold, if incubated at 40° C, as compared with incubation at 30° C. This effect can be found to a slight degree after x-raying (about five-fold). However, this heat recovery has been recognized with only strain B of *E. coli*. In this connection it should be mentioned that Hayden and Smith (9), in their work with maize, found heat reactivation of seeds after x-ray exposure. Unfortunately, no repetition of this work has been reported. A careful test in our laboratory has given negative results [Suskind (20)].

Photoreactivation

In 1949 Kelner (14) and Dulbecco (7) first reported "photoreactivation" after exposure to short ultraviolet. Most of the work reported until now has dealt with primary irradiation of 2537 Å. Some work by Carlson and McMaster (4) in our laboratory has shown that, in the nucleolus of the grasshopper neuroblast, the photoreactivation declines after exposure to wave lengths shorter than 2537 Å. The photoreactivation itself is limited to wave-length range of 3650–4500 Å, given im-

mediately after "short ultraviolet" exposure. There is some difference of opinion in regard to the most effective wave length for different organisms or even different strains in one species of organism [Kelner (15), Knowles and Taylor (16)]. No significant photoreactivation has been found after exposure to x-rays. It appears from the present information that photoreactivation either destroys a toxic substance which is formed by irradiation with 2537 Å or reverses the destructive action of an essential compound needed for the survival of the cell.

The fact that photoreactivation is found after exposure to short ultraviolet fits into the general picture of the behavior of cells after irradiation with ultraviolet as compared with x-rays. Bacteria or fungi surviving ultraviolet have a much extended lag phase [Hollaender and Duggar (10)]. This extended lag phase has been determined very carefully, but it can also be recognized by observing plate cultures which have been incubated about 12 hr. There is very little of this extension after x-ray exposure. Our interpretation at present is that the extension of the lag phase is a non-chromosomal effect and that photoreactivation works mostly through the cytoplasm. Such an interpretation seems reasonable at first but would have to be checked experimentally.

Infrared

We reported several years ago, first in cooperation with Kaufmann (12) and later with Swanson (19), that infrared around 10,000 Å given before (*Drosophila, Tradescantia,* and *Aspergillus terreus*) or after (*Tradescantia*) x-radiation will increase the effectiveness of x-radiation in producing chromosomal rearrangements and chromatid breaks and mutations (*Aspergillus terreus*). The effect is somewhat more pronounced in regard to chromatid breaks, as Giles and Beatty (unpublished) have found in our laboratory. The infrared alone, given under carefully controlled conditions, will produce no recognizable chromosome changes. It is important to point out that a carefully designed experimental technique must be used so as not to raise the temperature in these biological materials to a level in which heat damage could appear. This infrared work indicates that, in addition to the usually recognized damage, some "potential" damage must be produced in the chromosomes by x-radiation which is not obvious under normal conditions and which can be repaired if x-radiation is given alone. These additional effects of infrared radiations have also been observed in regard to nitrogen mustards by Swanson and by Kaufmann. These experiments indicate that x-radiation causes an unstable condition in chromosomes which can be made obvious by infrared treatment.

Water Content

I am sure that you are acquainted with the work on the sensitivity of plant viruses to x-rays, depending on the water content of the virus crystals. Similar work has been done by Stapleton in our laboratory in regard to irradiated *Aspergillus terreus* spores, (*a*) suspended in water, (*b*) freshly removed from an agar slant culture containing about 40–50 per cent water, and (*c*) dried and desiccated for 3 days, and containing only about 20–25 per cent water. There is a striking increase of resistance to x-rays of very dry spores. Water apparently sensitizes the spores to x-rays by bringing in direct contact some substances formed in the water by x-rays. However, what we call "dry" spores in our experiments still have a water content which should be an important factor in the sensitivity.

In connection with the irradiation in water, we should discuss the work of the Texas group [Wyss, Stone, and Clark (21)], who found that, when the medium was irradiated with wave lengths shorter than 2537 Å, bacteria and fungi grown in this irradiated medium showed a somewhat increased mutation rate. Hydrogen peroxide produced the same effect. Certain organic peroxides have been found to increase mutation rates, as reported by a California Institute of Technology group [Dickey, Cleland, and Lotz (6)].

These findings point to the possibility that hydrogen peroxide, or certain organic peroxides which may be produced by radiation in the medium or inside living cells, may be an important factor in regard to radiation sensitivity.

Further evidence is also brought out in the following discussion.

Oxygen Tension

The effect of oxygen tension on the sensitivity of chromosomes to x-radiation has been reported by Giles. R. S. Anderson reported in 1941 that yeast irradiated in the presence of oxygen was much more sensitive to x-rays than yeast irradiated in the absence of oxygen. We have carried out similar studies in regard to *Escherichia coli*, the experimental details of which, although important, will be omitted here for the sake of brevity. All data presented are based on plate counts of bacteria grown aerobically after irradiation. Figure 2 illustrates results obtained with aerobically grown bacteria irradiated in oxygen, air, and nitrogen. This graph shows that the ratio of survivors at 60,000 r under two different gases is

$$\text{nitrogen/oxygen} = >1000$$

for aerobically grown *E. coli* (B/r), whereas the relative sensitivity for oxygen-treated samples, as compared with nitrogen-treated ones, is approximately three-fold.

The same modification of sensitivity has been demonstrated on *E. coli*, strain B, which has been reported to be more sensitive to radiation

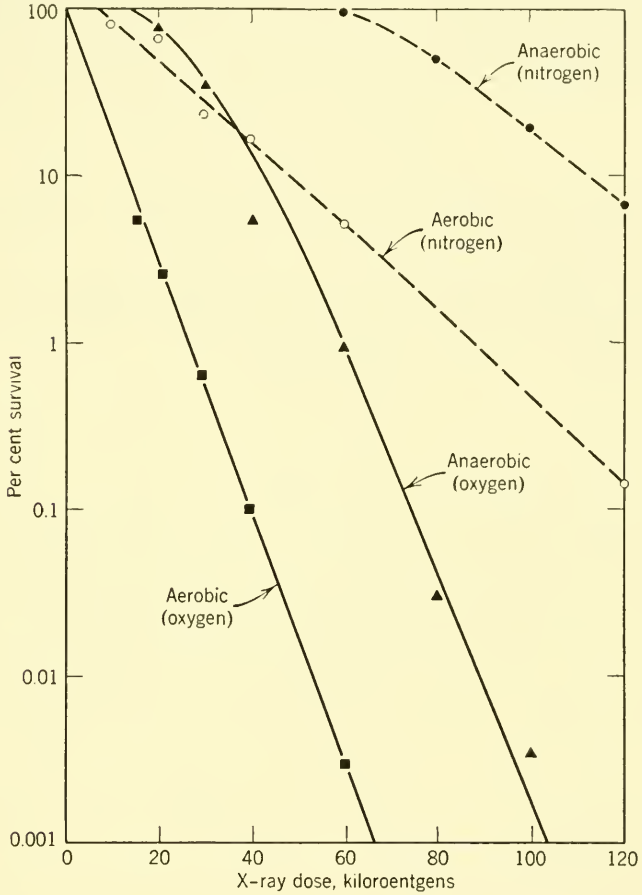

FIG. 2. "Anaerobic" and "aerobic" refer to the mode of growing the organisms. "Oxygen" and "nitrogen" refer to type of suspension in which bacteria were kept during irradiation.

than B/r. Since strain B is more sensitive to x-rays, the rate of killing is greater under both conditions studied; nevertheless the ratio of sensitivity of oxygen-treated cells to nitrogen-treated cells is approximately equal to that found for B/r. Nitrogen can be replaced with helium, hydrogen, or carbon dioxide without significantly changing sensitivity.

SENSITIVITY OF AEROBIC VERSUS ANAEROBIC *Escherichia coli* (B/r)

Since it is well established that *E. coli* is a facultative anaerobe, it was decided to compare the relative sensitivity of this organism grown anaerobically before irradiation with that of the same strain grown under strictly aerobic conditions. The bacteria grown anaerobically before irradiation and irradiated anaerobically were found to be extremely radioresistant.

The ratio of survival for the cells grown anaerobically before radiation is

$$\text{nitrogen/oxygen} = 10^5$$

The ratio of sensitivity between oxygen- and nitrogen-treated anaerobic cells is again, perhaps fortuitously, very close to 3.

If the sensitivity of the extreme cases is compared, that is, cells grown aerobically before irradiation and irradiated in the presence of oxygen, and those grown anaerobically before irradiation and irradiated in the absence of oxygen, a factor of 10 is found.

USE OF ORGANIC COMPOUNDS (AMINO ACIDS) AS PROTECTIVE AGENTS

It was noticed that bacteria (*E. coli*, B/r) were more sensitive to x-rays when exposed in phosphate buffer than in nutrient broth (Difco), 8 gm per liter. Since broth contains a wide variety of amino acids, it was decided to test the protective action of amino acids, first in groups and then individually, if the group tests appeared promising.

The effect of amino acid solutions as protective agents was studied from two points of view: (*a*) the effect of amino acid concentration on bacterial survival; (*b*) the protective action of optimum concentration of amino acids as obtained from survival (*a*) as a function of x-ray dose.

The results indicate that only glutamic acid and cysteine afford increased protection over the range of concentrations used in these experiments (Fig. 3).

It appears from the data presented here that there are two different effects: (1) radiation produces changes in the medium which can be reduced by lowering the oxygen tension of the medium; (2) the studies with anaerobic cells, on the other hand, indicate that essentially complete removal of oxygen from the cells also results in lowering their x-ray sensitivity. The combination of two protective systems results in extreme resistance of the organisms to radiation.

The possibility that respiration is tied up with radiation sensitivity seems to be indicated by these tests.

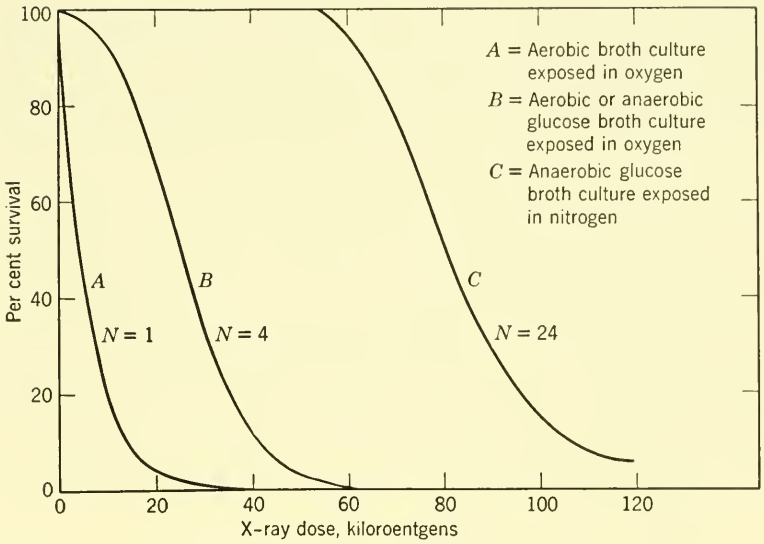

FIG. 3. Data from Fig. 2 recalculated for "target" determination. See discussion in text.

DISCUSSION

The data from Fig. 2 were recalculated on the basis of the target theory [see Lea (17)] and are given in Fig. 4. A purely physical interpretation on the basis of the target theory of the effects of x-rays on *E. coli* leads one to the conclusion that it is possible to vary the number of "targets" at will, by means of adjustment of growth conditions or conditions during irradiation. Whereas this type of physical interpretation may be a helpful tool in the study of radiation effects, the chemical approach appears more promising at the present time. The hypothesis that hydrogen peroxide formation induced by radiation is responsible for radiation effects on chromosomes is in many ways attractive, as Giles has so well shown. However, this hypothesis is less attractive in the interpretation of lethal effects on bacteria and fungi for the following reasons. (*a*) Bacteria suspended in a medium which has been irradiated with x-rays (60,000–80,000 r) will not be killed by this medium. The half life of hydrogen peroxide in buffer solution is of sufficient length that it should show a residual effect. (*b*) Hydrogen peroxide added to the suspension medium becomes toxic to bacteria only if concentrations are used which are far in excess of the ones produced by the usual amounts of radiation (>200,000 r). (*c*) Organic peroxides, as well as hydrogen peroxide, should be produced by irradiation in broth suspen-

sion and amino acid solutions. However, bacteria become more resistant if irradiated in the presence of these compounds. There is a possibility that the hydrogen peroxide attaches itself to the organic compounds, especially amino acids, and thus makes itself less available to the bacteria.

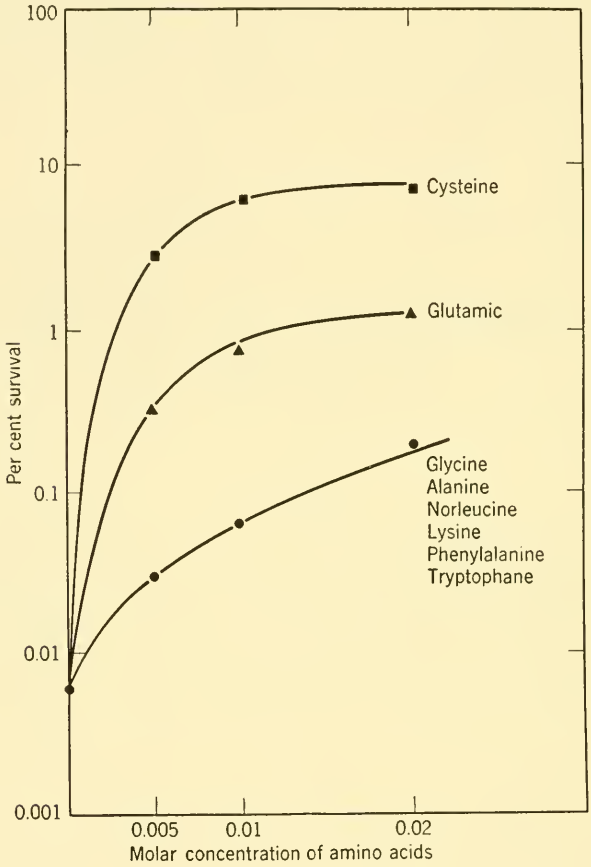

FIG. 4. Effects of amino acids, prepared immediately before irradiation and added to the suspension to be irradiated, on the survival of B/r at 60,000 r. [Hollaender, Stapleton, and Martin (11).]

It appears that radiation probably produces a radical which has a relatively short life but exists long enough to diffuse through the medium and into the bacteria or is produced in the cells themselves and distributed by diffusion. At present this radical could be HO_2. However, there may be other compounds formed by irradiation, the existence of which is not yet established.

We mentioned in the introduction that radiation death in organisms could be effected by many factors. Hydrogen peroxide may be one of the contributing factors, but other radicals found in the decomposition of water by radiation may play a special role in contributing to the killing of the organisms. The effect of oxygen in regard to the sensitivity could be largely explained by such a hypothesis. I feel that we are basing our speculation too much on studies which have been conducted with pure water, whereas in living cells, where really "pure" water does not exist, there must be a loose association of molecules which could be affected by these radiation-produced radicals. A typical example would be a nucleoprotein built into chromatin material. It appears somewhat promising that untangling metabolism mechanisms effected by oxygen under the influence of radiation will give us a clue to this problem. Finally, the inactivation of the sulfhydryl-requiring enzyme systems discussed by Barron may also play an important role.

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DISCUSSION

TAYLOR:

From the standpoint of radiation chemistry, an interesting feature of Hollaender's and Giles' papers is that there is no mention of a marked effect of hydrogen. This would seem to indicate a small importance for H_2O_2, since its concentration is repressed in irradiated aqueous systems by the presence of hydrogen. Definite, quantitative conclusions are made difficult by the relatively small concentration of hydrogen (less than 10^{-3} molar at 1 atm) relative to other solutes, and by the lack of detailed knowledge of radiation chemical effects in solutions (that is, individual radical concentrations, individual reaction rates). One gets, however, the qualitative impression that much of the biological effect occurs through immediate collision of freshly formed oxidizing radicals (for example, OH) with the biological material.

SPARROW:

In cooperation with M. J. Moses we have obtained some information concerning the effect of x-radiation on chemical changes in fixed nuclei. The preliminary results indicate that the desoxypentose nucleic acid of the fixed cells is more susceptible to mild acid hydrolysis after x-irradiation than before. The interpretation is that modifications induced by the irradiation permit hydrolysis to proceed at a faster rate. It is not known whether the changes induced are chemical or physical or a combination of both.

I should also like to mention that we now have data indicating that sensitivity of chromosomes to x-ray breakage may be related to the synthesis of desoxypentose nucleic acid (DNA). Sensitivity increases as DNA synthesis progresses, reaching a maximum where synthesis stops and decreasing to a minimum to the point where synthesis again begins.

PLOUGH:

I should like to comment briefly on Hollaender's discussion of the relation of oxygen tension to the production of mutations by radiation. He and others have said that many other chemical processes must be taken into account in connection with the problem, and I should like to call attention to certain relevant facts from our studies of radiation-induced auxotrophic mutations in *Salmonella*. Just as in *E. coli*, we now have strains of *S. typhimurium* which differ markedly in resistance to radiation. In strain 533 (sensitive) we can isolate after exposure to a dosage of about 50,000 r about 70 per cent of biochemical mutants—after penicillin screening—whereas with strain 519 (resistant) only about 20 per cent of similar mutants appear after the same treatment. Thus the frequency of mutations parallels the resistance to radiation. Yet the optimal oxidation-reduction potential for each of these strains is the same. On the other hand, preliminary tests indicate that *S. newport* grows best at a much lower oxidation potential than does *S. typhimurium* (strain 533), and yet the frequency of radiation-induced mutations in relation to dosage is approximately the same through the range of dosages tried. It would certainly appear from this finding that other factors than available oxygen influence the frequency of radiation-induced mutation.

HOLLAENDER:

I agree with Plough that the need of the organism for oxygen is not the deciding factor as to whether it will be radiation resistant or not. It appears from our findings that oxygen is an important factor in regard to x-ray sensitivity because of (*a*) its prevalence in the medium in which the organisms are suspended during irradiation; and (*b*) the presence of oxygen inside the organism. Absolute resistance of the organism is, of course, also influenced by many factors such as inherited resistance, nutritional background, and growth phase during which the organism is irradiated.

17 ·

Gene Mutations Caused by Radiation

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The Relation of Radiation Mutations to Those of Spontaneous Occurrence

Perhaps the most striking thing about the gene mutations induced by radiation, in organisms in which they have been intensively studied, is the fact that they have thus far been in no wise distinguishable from the so-called spontaneous mutations. Pick out any locus in *Drosophila* in which a spontaneous mutation is known, and the production of a similar-appearing mutation in this locus by means of ionizing radiation may be guaranteed. Moreover, if multiple alleles of the locus are known to have occurred spontaneously, a similar series of multiple alleles can in time be obtained by radiation also. We do not mean to imply here that mutant genes whose effects look alike are necessarily the same in their inner genic structure but only that no consistent differences have been found in the alleles, or series of alleles, arising with and without radiation. It is further to be observed that, if mutations in the reverse direction have been obtained spontaneously, they too can be produced by exposure to radiation. And it is probably true, conversely, that any gene mutation arising as a result of irradiation could be found in untreated material, if a prolonged and intensive search were made.

It must be admitted that it has not yet proved possible to put this comparison into quantitative form. That is, although spontaneous gene mutations in *Drosophila* have been known for over 40 years and those induced by radiation for 24 years, the per-locus rate of mutation, especially that occurring spontaneously, is so low as to have prevented the making of a comparison of the per-locus distribution of mutation rates, or what has been termed the mutational spectrum, in spontaneous and radiation samples. That would require a long-term project, the funds of which were not subject to year-by-year uncertainties, because the so-called "personal equation" is so strong a factor in the detection of visible mutations. Thus in a given series of radiation experiments

the present author found a rate of visible mutations 5 times that found by a group of specially trained graduate students. In view of this, it is evident that unless the same person, who must be one with special aptitude for such work, has had a chance to accumulate data of this kind on spontaneous mutations at specified loci over a period of years, and to obtain corresponding data on mutations induced by radiation, the comparison will be dubious, either on quantitative or on qualitative grounds or both. This then poses a condition which present *Drosophila* projects, with their short terms, can hardly meet. The same stricture applies also to the determination of the spectra of both spontaneous and induced mutations to be obtained under given conditions, as in different cell stages, on application of chosen chemicals, in various physiological states, and in the presence of particular genes.

But, although no such exact comparison of spectra has been possible, nevertheless there is at least rough agreement between spontaneous and irradiated samples in regard to their ratios of visible to lethal gene mutations. Indeed, the data of Timoféeff-Ressovsky (72, 73) on this point appeared to show a really good agreement, but since in this work the results for spontaneous mutations had to be obtained over a considerable period, as is usually the case, the errors due to probable personality differences, referred to above, must make us hesitate to place reliance on the exact figures obtained. To turn back to a consideration of individual loci, although again really quantitative conclusions are impossible, it is in general to be noted that gene mutations which are obtained relatively readily without treatment, such as white eye, garnet eye, and cut wing, are comparatively frequent after irradiation also, whereas certain others, such as scute and achaete, which have seldom been found spontaneously, are similarly rare (although they were carefully looked for) in the irradiated material. At the same time, we must acknowledge that even in untreated material, as in the comparisons of "high-mutation-rate lines" with other lines reported by Neel (60) and by Ives (29), the rates for one or two loci (especially that of yellow body) are sometimes found to have been disproportionately changed. If this can be true even without artificial treatment, and if the result was not caused by unstable alleles of the particular genes in question having been present in some lines (a very likely possibility in these cases), then it would hardly seem likely that irradiation, a much stronger influence, would raise the mutation rates of all loci to just the same extent. This is a matter that merits further analysis, if similar cases of high mutation rate are found in the future, by transference of genes at the loci in question to different genetic backgrounds.

Yet, whatever the answer may be to the question last raised, it is

evident that the gene mutations induced by radiation form no distinctive category. Moreover, the results show that the likelihood of occurrence of mutations at different loci, and of different mutations at a given locus, if not raised quite equally by the application of radiation, must usually be raised at least sub-equally. This is a quite remarkable relationship in view of the fact that the absorbed energy of the radiation "hit" is so inordinately higher—in a considerable proportion of the hits by some two orders of magnitude—than the energy likely to be involved in the course of most spontaneous mutations.

Evidence was presented by Muller and Altenburg (55) in 1919, and confirmed almost a decade after that [Muller (40, 41)], that temperature influences the production of spontaneous mutations in *Drosophila* and acts in fact as a limiting factor in controlling their frequency. The warning was given (1928) that the effect of thermal agitation here might have been indirect, as, for instance, by having caused some special kind of chemical change in the food. For this reason it has been especially desirable to have the necessary large-scale work of this kind repeated with other organisms, and also with other stages of the same organism. Yet the decades have passed without this having been done by anyone. However, as the present writer also pointed out in 1928, the agreement in sign and in general magnitude of the effect with what was to be expected of a simple intervention of thermal agitation in the mutation process itself makes it seem likely that this temperature effect is a direct one.

The above conclusion seemed to be strengthened, after Timoféeff-Ressovsky (71) had obtained similar data on *Drosophila*, by considerations presented by Delbrück (11) concerning the relatively large amount of temperature influence, having a Q_{10} of about 5, to be reckoned for reactions with rates as slow (molecular changes as infrequent) as those here dealt with. The molecular changes in question, here represented by mutations in individual loci, are so infrequent as to result in a half life of some thousands of years for the individual gene, as first shown by Muller and Altenburg (55). It was noteworthy that, in agreement with the calculated Q_{10} of 5 for rates as slow as this, there is in fact an approximately five-fold increase in mutation frequency with a 10° C rise in temperature. The basic assumption in this calculation was that the mutation occurs whenever a certain energy level, which is inferred to be about 1.5 ev, happens to be attained by the mutable material. This supposedly requisite energy level is so high in comparison with the average kinetic energy of the particles in the protoplasmic medium (some 50–70 times as high) that it would be attained very infrequently, giving the reaction the low rate found, yet this rate would be raised by a 10° C

temperature increase by about 5 times instead of by only the 2 or 3 times usual for chemical reactions.

To this latter line of reasoning, however, we must interpose several words of warning. According to one possible criticism, low reaction rates are not always or entirely caused, as assumed above, by the fact that sufficiently high energy levels are so seldom attained by the reactive particles. They may equally well be caused, especially in a complex medium like protoplasm, by infrequency of encounters of just the right structural (that is, "qualitative") types for the production of the reaction, despite the fact that the energy level remains low. This infrequency of effective encounters may simply be due to rarity (low concentration) of the reactive substances or, what amounts to nearly the same thing, to a highly specific and unusual type of encounter being necessary. However, the high temperature coefficient would not be explained in this way without bringing in some accessory assumption, such as that the reactive (mutagenic) substances were present in higher concentration at a higher temperature. As another alternative, a low reaction rate for mutation (long half life) may be due to a coincidence of two or more unusual events being required for its occurrence, even though both these events took place at a relatively low energy level. In that case the temperature coefficient could be as high as the product of the coefficients of the two or more participating events, and so it would simulate the coefficient to be expected for a high-energy-level reaction. Thus we see that, although a high energy level, of some 1.5 ev, seems to be the *simplest* way of interpreting the finding of a low rate of mutation combined with a high temperature coefficient, it is by no means the only plausible possibility.

That the occurrence of mutation does not depend merely upon a given energy level being reached at a given point, but also upon the conformation of the material, is indicated by a comparison of the energy levels for spontaneous and ultraviolet-induced gene mutations. Even the high energy level of some 1.5 ev proposed by Delbrück for spontaneous mutations falls far short of the 10 ev or more (accumulated in units of about 5 ev each) which, as we shall see in the next section, is probably necessary for mutation by ultraviolet. This seems to mean that, from an energetics standpoint, the chemical pathway to mutation by ultraviolet activation of purines or pyrimidines is far less efficient than the spontaneous pathway or pathways.

However, we cannot yet exclude the opposite possibility that, instead of involving lower energy, the spontaneous mutation process may involve even higher energy than that of Delbrück's hypothesis. Living matter can on occasion attain energy levels higher than those found in

the plus "tail" of the statistically random energy distribution which results from the operation of ordinary thermodynamic principles. These high levels are due to mechanisms, utilizing the energy-transferring properties of some nucleotides, which cause an accumulation of the potential energy from multiple quanta, absorbed at different times, and which can then suddenly release this energy at a high level, as occurs, for instance, on a molar scale in electric organs. At present, however, there seems no need to postulate an energy level above 1.5 ev for spontaneous mutations merely because of its being needed for ultraviolet mutations. It seems more plausible to refer the difference to the nature of the chemical pathways and/or to a multiplicity of key events being necessary to effect the occurrence of mutation.

The above diversity of possibilities should show what a high degree of caution is necessary when the attempt is made to interpret biological events on the basis of simple physical principles, without regard to the chemical complexities that may be involved. Nevertheless, despite our uncertainty regarding the energy level necessary for spontaneous mutation and the nature of the chemical steps involved, we can be sure that the random encounters of thermal agitation play an important and necessary part in the process of spontaneous mutation. Moreover, this being the case, it is probable that the increase in spontaneous mutation frequency that accompanies a rise in temperature within the range normal to the organism is, in part at least, caused by the increase in thermal agitation which the higher temperature entails.

That the spontaneous gene-mutation process must usually depend upon thermally activated reactions follows, for one thing, from the calculations and data presented by Muller and Mott-Smith (56), later confirmed by others, which showed that natural radiation is entirely inadequate in amount to be an important cause of spontaneous mutations in *Drosophila* at the rate at which they occur in that material. That these thermally activated reactions which lead to gene mutations are random events beyond individual control by cellular regulative processes and that they are therefore subject to the statistical principles of ordinary thermodynamics is attested to by their sporadic, pointwise distribution in space and time. For one thing, this randomness is of such scope that, as yet, it has not been found possible (at any rate in experiments that have been confirmed) to bias the occurrence of ordinary spontaneous mutations by applying special conditions that would favor one type against another. But, more telling still, the randomness can be shown to exist even in the range of microscopic or submicroscopic dimensions. For, as the present writer pointed out in an early discussion of this question (38, p. 470): "Mutation is due to an event of such minute

proportions, so circumscribed, that it strikes only a single one of two near-by, similar loci in the same nucleus"; that is, when one gene mutates, its allele of identical composition, usually only a small fraction of a micron away, remains unaffected.

Thus the determination of just *which* gene mutates in a given case, and to which allele, must be a consequence of the ultramicroscopic accidents of thermal agitation rather than of the chemical nature of the material reacting with the gene. And it may be concluded [Muller (39), p. 43] that "mutations are not caused by some general pervasive influence, but are due to 'accidents' occurring on a molecular scale. When the molecular or atomic motions chance to take a particular form, to which the gene is vulnerable, then the mutation occurs." Since the time when this statement was made, it has become possible to add that the similarity of radiation mutations to spontaneous mutations, in regard to the kinds of effects produced, their relative numbers, and their random distribution in space and time, lends strong support to this viewpoint. In this connection it is especially noteworthy that in the genesis of the radiation mutations, unlike that of the spontaneous ones, the accidents are initiated by a fast particle the path of which can have had no relation to cellular needs or metabolic processes, and that nevertheless the spontaneous mutations appear as random as those produced by radiation, and essentially similar to them. If in the natural accidents that cause spontaneous gene mutations different kinds of protoplasmic substances differed much from one another in regard to the types of mutations they favored, then we should hardly expect spontaneous gene mutations as a group to agree as much as they do with the group of gene mutations produced by the absorption of high-energy radiation. Hence it seems likely that a thermally occasioned encounter of the right kind to produce one mutation would also, according to which gene and gene-part happened to be involved, have sufficed to produce practically any other mutation. Thus there seems to be very little difference in the type of process, or amount of energy necessary, for the occurrence of different kinds of gene mutations, and the same general sort of chemical substitution may well be involved in all cases. If we had more data on mutational spectra we might make this inference more secure.

Our inference that the mutations of different genes can be occasioned by chemical encounters of the same type by no means implies, conversely, that the type of chemical encounter to which a gene is exposed is of no account in the determination of whether or not a mutation will be produced in it. That is, the occurrence of spontaneous mutations does not depend solely on the energy level reached, as has sometimes been assumed, but also on the energy being conveyed in an appropriate

manner, that is, by given substances and/or with attendant conditions of a suitable kind. This is shown clearly by the great variations in the overall frequency of spontaneous gene mutations found in different experiments, amounting to 10 times or more [Muller (40, 41)]. Not only can genetic differences have effects as great as this [Demerec (12), Neel (60), Ives and Andrews (30)], but also differences in cellular conditions, depending on age and stage of development [Muller (48)]. The action of "temperature shocks" appears to come in the same category. Finally, as has been known since the work of Auerbach and Robson [see summary by Auerbach (2)], chemical influences of specific types are potent causes of mutation, a finding long to have been expected in view of the above facts and considerations.

The question, which the present author raised in 1928 (41, p. 345), as to whether gene mutation occurs by the chemical change of a pre-existing gene or by a misstep in the synthesis of the daughter gene by its mother gene, is one which, for spontaneous mutations, seldom admits of an answer in any given case. However, the intense concentration of spontaneous gene-mutational occurrences in *Drosophila* into those stages of the germ cycle in which gene duplication (as evidenced by mitosis) is going on more actively [Muller (48)], and earlier findings of a similar nature in bacteria by Zamenhof (80) and later by others, strongly suggest that in these cases spontaneous mutations usually take place by misconstruction of the daughter gene. On the other hand, the accumulation of spontaneous mutations in mature, dormant spermatozoa [Muller (48)] indicates that in them it is the old gene which is being transformed. The proof of this will not be complete, however, until it is shown that the mutant offspring from the aged sperm are mutant throughout their bodies. To obtain evidence on this matter for spontaneous mutations, very large-scale work involving the study of certian particular types of mutations, which affect the whole external surface of the body visibly, is required. Yet, however that may be, the answer to the question is already clear so far as the great majority of gene mutations induced by radiation of spermatozoa is concerned: that is, in this case, the pre-existing gene undoubtedly becomes altered in its composition. For, wherever evidence concerning the point at issue is available, for radiation mutations induced in spermatozoa, it is found that the whole body is usually involved.

In view of this apparent difference in the usual method of their origination, the spontaneous mutations studied having more often arisen through the misconstruction of the daughter gene and the radiation mutations studied having more frequently involved changes in the composition of the already finished gene, the previously noted similarity in type of product between the spontaneous and the radiation mutations

is the more interesting. It would indicate that those spots which are more vulnerable in the finished gene are also more prone to be effectively disturbed during the process of gene construction. It is not surprising, however, that the unfinished gene should be more labile in general than the finished one; that is, that it should be more susceptible to having mutational disturbances caused in its synthesis by the relatively mild influences that operate in non-radiated material.*

If the above general view of the spontaneous gene-mutation process has validity, it is not at all strange that high-energy radiation also induces the occurrence of gene mutation, for it not only releases energy at a far higher level than necessary for such a result but in virtually as many different forms as it would naturally be encountered in, and more besides. Indeed, such a chaos of different, molecularly more or less localized reactions must arise in irradiated protoplasm, both as direct results of the ionizations and activations on the molecules hit, and as secondary, tertiary, etc., consequences of the varied combinations into which these products later enter, that it would be strange if those reactions which, in non-radiated material, result in spontaneous mutations were not included among those here arising. In addition, the gene itself could be struck directly by a fast particle, with results that might resemble those brought about by the intermediation of mutagenic substances. In all this welter of effects and of possibilities, the tracing of the more usual trains of mutational processes, in physicochemical terms, is a matter of the greatest difficulty. This field is only now opening up, as a result of studies of chemical influences upon the occurrence of gene mutation, both with and without radiation.

On the Proportionality between Induced Gene Mutations and Ionizations

Before referring to some of the specific findings along these lines, let us consider the question of how the production of mutations is affected by changes in the dose of radiation. Since the doctoral thesis presented

* While this manuscript was in press, results were reported by Novick and Szilard (*Proc. Natl. Acad. Sci.*, **36**: 708–719, 1950), showing that in *Escherichia coli* growing at a given temperature spontaneous mutations continue at the same rate regardless of the rate of growth and multiplication (varied by controlling the amount of some minimal nutrient available for them), provided they are able to multiply at all. Here, then, the changes must be in the "old" gene; however, we do not know how much turnover of material it is undergoing. A paper by Maale and Watson (*Proc. Natl. Acad. Sci.*, **37**: 507–513, 1951) reports results on phage "tagged" with P^{32} which may be interpreted by assuming that at least the phosphorus in the genetic material of the phage does undergo exchange apart from reproduction, but other interpretations of these results are possible.

to the present author by Oliver 20 years ago, in which he reported a simple proportionality of the frequency of induced gene mutations to the dose of x-rays applied, this finding has been ever further substantiated and extended. There have been only such discrepancies as are to be expected in consideration of the nature of the difficulties involved, and of human frailty, and these have in general been rather quickly corrected. The same statement applies to the independence of the frequency of the induced gene mutations from the time distribution or intensity of the dose of radiation. Thus, despite a number of unfortunate publicized statements by non-geneticists, casting doubt on the mutagenic effectiveness of small doses of radiation, the work has gone so far as to make it inconceivable, on physicochemical grounds, for a single ionization track traversing a nucleus to be without its proportionate probability of inducing a gene mutation.

Of course, this does not directly prove that one ionization or activation can cause a mutation, since the ionizations come grouped within tracks, and a good share of them (for the most frequently employed wave lengths about the same share) in tight clusters. This may be one of the bases of Opatowski's (63) mathematically expressed objection to the conclusion that a single ion is effective. It is hardly sufficient to answer that soft x-rays have been found to have the same effectiveness as ordinary x-rays, since the wave lengths tried have seldom been quite long enough to meet possible objections, and the measurement of the amount of penetration of those which are long enough presents great uncertainties. A more telling argument might seem to be the fact that, despite their greater ionization density, neutrons are not more effective, but are in fact somewhat less so, than ordinary x-rays, in inducing separately recordable gene mutations. This, however, proves somewhat too much, for here the ion density is obviously *beyond* the optimal for individual effects.

It might be thought that, if two or more ionizations near together were ordinarily needed for producing a gene mutation, then this would cause the mutation rate to rise more rapidly than the dose. For, although the frequency of clusters located at the ends of tracks and of their branches, as in the course of delta rays, would all increase linearly with the dose, there would in addition be some independently produced ionizations, lying in different tracks, which happened to be as near together as those in the natural clusters, and the frequency of such accidental juxtapositions would rise with the square of the dose for two ionizations, with its cube for three, etc. If, however, we calculate the frequency of such groupings in comparison with that of the natural clusters, for any doses of the size actually used for *Drosophila*, we find

accidental groups to be so rare, even at best, that they could not exert a perceptible influence on the results found. This is the more true the higher the number in the group, but it holds even for groups of two. Thus, even if a gene mutation did require two or more ionizations, such a large proportion of the clusters that produced them would at all doses used be "natural" clusters, the frequency of which varied linearly with the dose, just as that of single ionizations does, that we should obtain no evidence on the matter through mere dosage studies.

Implications of the Results with Ultraviolet

Nevertheless it seems unlikely that more than one ionization should be necessary to produce a gene mutation, in view of the mutagenic action which even ultraviolet exerts, and the fact that this comparatively low-energy agent works through activations which, within any given substance, are distributed at random with regard to one another. Yet it is true that the total amount of energy which must be absorbed for the production of a gene mutation is far higher for ultraviolet than for x-rays or gamma rays. Some work on *Drosophila* by Meyer, E. and L. Altenburg, Edmondson, and Muller shows that, for the most efficient ultraviolet doses which we have thus far worked with, between 100 and 1000 times as much energy must be absorbed by the chromosomes themselves as is absorbed by them when x-rays enough to give the same mutation rate are applied.*

The precise interpretation that should be placed on the mutation frequency-dosage relation found for ultraviolet is subject to much uncertainty because of the complexity of that relation. In both fungi [Hollaender (24, 25), Hollaender, Sansome, Zimmer, and Demerec (27)] and *Drosophila* [Sell-Beleites and Catsch (65), L. and E. Altenburg, Meyer, and Muller (1)] the curve expressing this relation, with mutation frequency as ordinate, after rising for a short space in a more or less linear

* In the paper as presented at the meeting it was stated that approximately equal amounts of energy were absorbed for the production of a gene mutation whether by ultraviolet or by x-rays. This is, however, true only of the energy absorbed by the cells in question (primordial germ cells) as wholes. As the ultraviolet, unlike the x-radiation, is absorbed selectively by the chromatin, which constitutes less than one-hundredth of the cell material, we must reckon the ultraviolet as correspondingly less efficient. (See later discussion on the limitation in the spatial range of mutagenic effectiveness of the energy derived from x-ray absorption.) The treatment of the relation of mutation frequency to dosage of ultraviolet as the latter is varied has also been radically revised in the article as now presented. The author wishes to acknowledge his indebtedness to Dr. C. P. Swanson and Dr. N. H. Giles for having called his attention to some facts in this connection, although these investigators are in no way responsible for the interpretations here presented.

manner (see below), tends gradually to level off and then even to sink at very high dosage levels, levels resulting in a great mortality of the germ cells or offspring. It seems reasonable to infer, as Hollaender has done, that this flagging of the curve is caused, in part at least, by the mortality being selective, for the distribution of ultraviolet can seldom be made very even, and cell susceptibility also may vary. It is to be expected that the deleterious action of the radiation on viability would in general be exerted more heavily against the same cells as have more mutations induced in them, sometimes because these cells have received more radiation and sometimes because they are in a more susceptible condition. Moreover, in some material (that in which the effect of the mutations can show relatively early, before the physiological effect of the ultraviolet on viability has faded away) the mutations themselves would give the cells, in their further development, a greater mortality as compared with non-mutated specimens when they had at the same time received a physiologically more effective dose of ultraviolet; this too would tend to lower the apparent mutation rate more at higher doses. There may in addition have been appreciably more "light reactivation" at the higher doses in some of the experiments, and this would have weakened the effective doses more just when they were intended to be stronger.

It is also conceivable, as an explanation of why the curve becomes convex at higher doses, that ultraviolet of mutagenic wave length exerts a second effect on the mutation process, similar perhaps to that of very long ultraviolet and short visible light, so as to interfere with the production (or to cause the reversal before their completion) of the very mutations which this same mutagenic ultraviolet, presumably through separate quanta, induces. In that case we should have to assume further that this counteracting effect rises more steeply with the dose than the mutagenic primary effect does. This would, for instance, be true if the frequency of mutations primarily induced varied as d^n, that is, as the nth power of the dose, because of n hits being required for a mutation, whereas the final or net frequency of mutations, that remaining after the counteracting quanta had taken effect, varied as the product of d^n times e^{kd} (where k is a constant). This formula would follow the principle which appears to operate in "reactivation" by visible and long ultraviolet light, as judged, for instance, by Novick and Szilard's results (see below), although of course in the case of visible and long ultraviolet light reactivation it would be necessary in this formula to substitute a different letter for the second d, representing the amount of that radiation, since the first d would represent only the amount of mutagenic radiation. It is true that this interpretation has the objec-

tion of requiring a bimodal curve for the spectral distribution of effectiveness of the interfering action, since radiation in the region of 3100 Å has very little such effect, at least in bacteriophage reactivation [Dulbecco (14)]. However, it is difficult to explain on other grounds why the falling off in net mutagenic effectiveness at higher doses should be so widespread and so extreme as it has been observed to be.

But no matter which of the above interpretations, or which combination of them, be adopted, the influence of selective mortality or interfering radiation should be negligible at low doses, and at these doses (that is, nearer the orgin of the frequency-dosage curve) the mode of operation of the positively acting factors should become clear, as is usual for sigmoid curves in general. Moreover, it would become very probable that we had reached low enough doses for judging the action of the positive factors by themselves if we found a portion of the curve (to the left of its convex region, and including the lowest doses for which effects were measured) in which, over a considerable range of dosage, involving, for instance, a dosage variation by a factor of 4, the amount of effect (mutation frequency) was found to be proportional to a constant power of the dose. That is, we could assume that at these dosage levels the interfering action had not yet come into play appreciably and that the observed curve of results reflected the influence of only the factor or factors which induced the mutations, in the virtual absence of the counteracting influences.

Unfortunately, it is extremely difficult to get data of sufficient statistical reliability for such low doses. However, if we examine the most detailed published data approaching these conditions, such as those obtained by Hollaender and Emmons (25) on *Trichophyton*, and by Hollaender, Sansome, Zimmer, and Demerec (27) on *Neurospora*, we find distinct indications that at low doses the mutation frequency rises faster than a simple linear relation to dose would allow. On the other hand, so far as one can judge, the frequency hardly seems to rise faster than the square of the dose, as it would if two activations were required for a mutation. Yet the data are too meager for a decision either on this question or on that of whether low enough doses to rule out the interfering factors have been reached.

Novick and Szilard (61) have reported measurements of the frequency of three specific types of mutation (to phage resistance) in *E. coli* on application of varied doses of ultraviolet. In this work the amount of effect caused by "reactivation" by light of longer than mutagenic wave length was determined at the same time, for each of the types of mutation. It seems unlikely that the viability of mutants of these kinds would have been reduced by the ultraviolet much more than that of

the non-mutants would have been. Although it is hard to rule out differences in susceptibility, there were probably no important differences in the amounts of exposure of the genetic material of the different organisms, since they are so minute and were agitated during treatment. It is true that the individual mutation-frequency determinations were subject to a high statistical error, yet the effect of this is minimized by the fact that the results from all the series studied agree very closely in principle. For, when the mutation frequency is represented on a logarithmic scale as ordinate and the dose on a logarithmic scale of the same magnitude as abscissa, the lines connecting the datum points are found to have the same slope in the case of all six series (a light-treated and a dark series for each of the three mutant types). Moreover, since the effect of the light follows the same formula throughout (see p. 306), it is possible to allow for it and thus to combine the results of the light and dark treatments into one curve for each of the three types of mutations.

When this conversion is carried out it is found that the effective dose (that after correction for light treatment) varied, for two of the mutant types, by a factor of about 3, and for the other by a factor of about 5. There are several scattered datum points along each of the three curves, and each curve is found to form, so far as can be judged, a straight line, on the log-log scheme of representation used. This means that the mutation frequency, over the whole range of doses employed, rises as a sensibly constant power of the dose.

The slope of the straight lines, on this log-log curve, having a tangent of about 2.3, is such as to show that for each small increment of dose there was about 2.3 times as great a relative increment of mutation frequency. This relation, showing that the mutation frequency rises as the 2.3 power of the dose, would ordinarily be interpreted (although the authors do not discuss this matter) as meaning that in the production of each mutation 2–3 independently produced unit elements of the dose, that is, in this case 2–3 activations (sometimes 2 and sometimes 3), usually take part. Indeed it does not seem possible to avoid this conclusion, except by gratuitously assuming still greater complications, with effects canceling one another sufficiently to return the final, net effect to the 2.3-power relationship to dose. In other words, there is evidence for an only slightly higher than two-hit curve. And, since the same curve (although with different absolute frequency of effect) was found in the case of each of the three mutants for four or more points over a three- to five-fold dose range, it does not seem likely that it has at these doses been modified appreciably by those influences above discussed, which in other material tend to cause an apparent leveling off or drop at very high doses. A curve of 2+ "hits" thus seems to represent the process

of ultraviolet mutagenesis here without those complications which so often distort its expression at the higher doses.

That not only ultraviolet but also mustard works through a multihit process is indicated by the synergism between the mutagenic effects of these two agents reported by Swanson *et al.* (68). This makes it the more likely that spontaneous mutations too, since they probably result from changes of still lower energy level, require a concatenation of rare events. The ionizations induced by high-energy radiation rise above this need, perhaps because their higher energy, in its degradation, has the necessary multiple effects, or because it can accomplish, more directly, what the multiple effects in the other cases converge to do.

In the work on ultraviolet mutations in *Drosophila* it has not been feasible to get mutation-frequency data for a number of widely differing doses at levels low enough to make the probable role of differential viability unimportant. Hence we have not been able to determine the probable number of hits participating in a positive way in ultraviolet mutagenesis in this material. Nevertheless we do have, in the data of the group of *Drosophila* workers previously mentioned, a very suggestive point of correspondence with the *E. coli* results, which raises the presumption that essentially the same mechanism may be at work in both. The point in question concerns itself with the amount of absorbed ultraviolet energy necessary to produce a specified type of mutation.

For this purpose we must take into consideration the data for the lowest dose for which significant results were obtained in *Drosophila*, for that is the dose at which the complications of differential viability, etc., which reduce the apparent effectiveness of the dose, are at a minimum. It happens that, judging by the factors of the type of lamp, the distance, and the time, this dose (involving an exposure to a G.E. "germicidal" lamp at 200 cm for 3 min) came within the range used by Novick and Szilard with *E. coli*. Although the intensity of our treatment appeared some 4 times lower than theirs, we have not found such a difference to play a very large role in our own work when the factors of the dose are of the order of magnitude that obtained here. Thus we can arrive at an approximate comparison of the probability of a mutation of a given type (say, to resistance to phage T_4) being produced in *E. coli* at a given dose with the probability of a mutation being produced at an individual locus in *Drosophila* by the same dose. It is true that in this particular *Drosophila* work we have not dealt with individual loci, but the relation between the overall lethal frequency (the modulus used in the present work) and the average per-locus frequency of mutation has been approximately determined in other studies. Thus our present results can be translated into these terms.

Reference to Novick and Szilard's graphs shows that, at the dose in question (without light treatment), *E. coli* has a frequency of about 1 induced mutation in 5000 to T_4 resistance and 1 in 20,000 to T_6 resistance or T_1 resistance, respectively. At this same dose, *Drosophila* gives visible mutations in the specified individual loci studied at the rate of some 1 in 5000, although actually the rate varies from about twice to half this value, according to the locus.*

There are too many sources of error in both the above sets of values for the comparison to be valid except as regards the orders of magnitude involved. Moreover, the assumption has not been proved that the same locus is always involved in the independently arising bacterial mutations that give resistance to the same type of phage. Nevertheless, it is of interest that the results on two such different organisms should agree as closely as those here found seem to do. We are reminded at this point of some similar apparent agreements in per-locus rates of spontaneous and of x-ray mutations that have been reported in other comparisons of widely different organisms. At any rate, in view of the wide range of conceivable values of mutation rate, and of values found under other circumstances, it does not appear very plausible to regard the present agreement as a complete coincidence. If it is not, however, it would indicate a similarity in the quantitative features of the mechanism of mutagenesis, as well as in the nature of the genetic material, in the two cases.

The above considerations appear to favor the conclusion that more often only two and seldom more than three quanta of 2537 Å ultraviolet are involved in the production of a gene mutation. At the same time, however, it can be calculated that there is probably somewhat less than one chance in a thousand of actually getting a mutation, even when as many as three of these ultraviolet quanta have been absorbed by one and the same purine or pyrimidine group of the desoxyribonucleic acid portion of a chromosome. Other "accidental" circumstances, therefore, must determine whether the absorbed energy results in a genetically reproducible change. It is to be noted that the chance is also not much over one in a thousand (about $\frac{1}{700}$ or less, as noted on p. 312), in *Drosophila*, that a mutation will result when an ionization has been produced by x-rays or gamma rays within a chromosome. Yet in a considerable proportion of cases such an ionization involves a much larger energy transfer than would three ultraviolet quanta added together.

The above energy relations make it seem very likely that when x-rays

* The work on the production of mutations in *Drosophila* by ultraviolet, referred to in this section, was supported by a grant from the Public Health Service, with the cooperation of the National Advisory Cancer Council.

or gamma rays are applied a single ionization, if favorably placed, would be sufficient to give rise to a mutation, that is, that an ion cluster would not be required. As regards the effectiveness of the scattered *activations* produced by x-rays or gamma radiation, the conclusion to be drawn is less clear, however. If the matter could be regarded purely quantitatively, it would seem that one activation had little or no chance of being effective unless its energy reached the relatively high level of two 2537 Å ultraviolet quanta, or (supposing the activation to be weaker) unless its effect were combined with that of one or more other activations. However, judging by the infrequency with which such combinations are effective in producing mutations even in the case of ultraviolet quanta, we should hardly expect the far less abundant activations arising from x-rays or gamma rays at the doses ordinarily used to have an appreciable mutagenic influence. (For, as noted above, the x-ray or gamma-ray doses are, in terms of total energy absorbed, several orders of magnitude lower than the ultraviolet doses.) This purely quantitative way of judging the problem represents an oversimplification, since most of the activations produced in protoplasm in the tracks of ionizing radiation are of much more varied types than those arising from 2537 Å ultraviolet (which are in effect confined to the purine and pyrimidine groups), and their potentialities would therefore be less limited. But whether they would, on the average, be more or less effective than those of ultraviolet in producing mutations it is at present impossible to know.

The discussion in the above paragraph concerns itself with the scattered activations produced in the tracks of ionizing particles. There are, however, clusters of activations produced at the ends of the tracks (and of their branches, notably in delta rays), chiefly in the same regions as the clusters of ionizations. If we are right in judging that ultraviolet mutagenesis usually involves the cumulative effect of several near-by activations, then these clusters of activations produced in the tracks of ionizing particles after they have slowed down should give a much higher chance of mutation than the same number of scattered activations would. However, the reckoning of how great that chance actually is, in comparison with that of mutation produced by ionization, could not be carried out without far more detailed knowledge than is now available.

It should also be observed that the ionizations themselves result in activations, in fact in clusters of activations of varying size, depending in part upon the amount of energy transfer the ionization has involved. The comparatively high ionization energy must become degraded, and in this process by no means all of it is translated simply into kinetic energy. Thus even those mutations that result from ionizations may, in part at

least, have done so by the intermediation of the activations, or groups of activations, which the ionizations occasioned. If then such activations were, under other circumstances, induced more directly, the mutations would in that case arise in the absence of ionization.

The Inapplicability of the Target Hypothesis in Its Simplified Form

The target hypothesis, in the simplified form in which it has usually been applied by those sponsoring its application to problems of mutagenesis, has admitted only ionizations, not activations, as causes of the mutations induced by high-energy radiation. As we have seen above, it appears premature at present to assume the effect to be so limited. We may now examine some of the other premises which have been set up on the target hypothesis.

One of these premises is that every ionization within the genetic material results in a gene mutation, thus making it possible to calculate the volume of the gene. When such calculations are made, however, it is found, in *Drosophila*, that only about one in seven hundred or more ionizations occurring within the volume of a chromosome when it is in a condensed stage (a stage of relatively high susceptibility) is followed by a gene mutation of some detectable kind—lethal, detrimental, or visible. A similar figure is arrived at if we estimate the maximum volume of that part of a condensed chromosome including but one gene and then compare the frequency of ionization induced by a given dose within such a volume with the frequency with which those individual genes that have been studied give detectable mutations on application of the same dose. This result would lead, on the target hypothesis, to the conclusion that the genetic material itself occupied only about a seven-hundredth of the volume of the condensed chromosome.

Now such a conclusion appears on the face of it very improbable. Recent work renders it more so, if we agree that the evidence concerning the "transforming substance" in bacteria points strongly to the conclusion that the genetic material is composed, in part at least, of nucleic acid. Not only is the nucleic acid content of a chromosome very high, but the work of Mirsky and Ris has shown that the amount of desoxyribonucleic acid (which usually forms the great bulk of the nucleic acid of condensed chromosomes) is constant and proportional to the number of genomes, throughout the somatic and germinal cells, despite the great variations in amount of other nuclear and cytoplasmic material. In view of this, it would be strange if only a minute fraction of this desoxyribonucleic acid were genetic in nature. But if we discard this alter-

native it would follow that a considerable proportion of the ionizations which occur within the genetic material fail to cause a mutation in it.

What now about the complementary premise of the target hypothesis of radiation mutations, according to which virtually no ionizations or activations induced outside the genetic material result in mutations? In this connection it should be admitted to begin with that, in *Drosophila*, there is good reason to believe most of the mutagenic effect usually to be rather narrowly localized, not merely in a molar but also in a microscopic sense, even though this does not necessarily mean on an atomic or molecular scale. The molar localization was first shown in the experiments of Kerkis (31). These showed that the mutation rate induced in flies which had only the posterior half of the body irradiated with x-rays was the same as in those whose whole body was irradiated, whereas if only the anterior half was irradiated there was no appreciable effect. Later Timoféeff-Ressovsky (72, 73) showed that irradiation of the superficial tissues with x-rays too soft to penetrate to the gonads was without mutagenic influence on the germ cells.

That this principle extends down to a microscopic scale is indicated, for one thing, by the close similarity of the x-ray-induced gene-mutation rate in the relatively condensed chromosomes of late oöcytes and in those of mature spermatozoa in the male [Muller, R. M. Valencia, and J. I. Valencia (58)].* In the oöcytes there is much accessible nucleoplasm and cytoplasm which could serve as absorptive media for the mutagenic effects of the radiation if, indeed, they can be transmitted to the chromosomes over microscopically visible distances. In the sperm, on the other hand, packed tightly as they are, the amount of such extrachromosomal material is far more limited. At the same time, in the sperm, both cell and nuclear boundaries would usually be interposed in the way of the supposed transmission. And since the sperm in this stage are dormant their amount of exchange with the medium is likely to be at a minimum. Similar considerations apply to sperm in the receptacles of the female, which have been found to have about the same mutation rate [Muller (40)].

More critical evidence of the microscopic or ultramicroscopic localization of most of the mutagenic effects of high-energy radiation is given by the results of Zimmer and Timoféeff-Ressovsky (81), showing the lesser effectiveness of neutrons than of x-rays and gamma rays in producing lethal mutations, and the contrasting results of Giles (18, 19),

* The work on the production of mutations in *Drosophila* by high-energy radiation, referred to in this section, was supported by a grant from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.

showing the greater effectiveness of neutrons, and their linear rather than exponential relation to frequency of effect, in producing chromosome rearrangements. All these results were to be expected in consequence of the greater density of ions in the tracks arising from neutron treatment, *provided* only the mutagenic effects remain more or less localized, on a microscopic or ultramicroscopic scale, in the neighborhood of the tracks themselves.*

However, the above-indicated limitation in the range of transmission of most of the mutagenic effects of high-energy radiation in the material studied is entirely consistent with their transmission over a more minute but still significant distance or even, under other circumstances, over a greater distance. That is, it by no means proves the assumption that every mutagenic ionization, or even the great majority of them, must have occurred within the gene. Thus, as Lea (33) himself calculated in 1947, the ions produced by x-rays in water usually travel through what is, in molecular but not in microscopic terms, a quite significant distance before having their charges neutralized. As Fricke and others long ago maintained, and as has been shown with increasing force in recent years, these ions and their reaction products are often very potent chemically. Moreover, whether or not these are the usual initiators of the events which result in radiation mutations, there are now, as we shall see, abundant empirical data which demonstrate the importance that intermediate chemical reactions of one sort or another have in mutagenesis. It is, however, difficult to estimate at present, in terms of atomic distances, the spatial range of the reactions from which the majority of radiation mutations in, for example, *Drosophila*, result. More especially, we do not yet know to what extent these reactions are ordinarily initiated within, and to what extent outside, the visible bulk of the chromosomes, or, more specifically, of the genetic material itself. Yet the mere existence of the intermediate reactions should make us very wary in assuming that the mutagenic effect of each hit is always localized within the very particle that was itself hit. And we should also bear in mind the theoretical possibility, to which some results in radiation genetics have appeared to lend support, that a single ionization may on occasion, by a

* Since the above was written Muller and Valencia (*Rec. Genetics Soc. Amer.*, **20:** 115–116, 1951, and *Genetics*, in press) have reported evidence that the lesser apparent effectiveness of neutrons in producing lethals in *Drosophila* is caused by the genetic effects often being too close together to be recorded separately, but *not* on the same gene. Although, on the one hand, this shows the mutagenic chain of reactions in this material to be highly localized, it also shows that most ionizations within the genetic material fail to be mutagenic, and so it confirms the conclusions arrived at by other methods on the preceding pages.

branching of its effect like that of a bomb [Muller (44)], be able to cause more than one genetic change in its neighborhood.

At this point a few words concerning the history of the target hypothesis may be in place. It does not seem to be generally realized that this hypothesis, although now so popular, is over a quarter of a century old, or that it was originated by the British biophysicist J. A. Crowther. When Crowther (9) proposed it in 1924, he used it as a means of interpreting the relation of the frequency of inhibition of mitosis to the dose of x-rays that had been observed by Strangeways and Oakley in 1923 in cells of tissue cultures. Crowther found that these results might be accounted for by assuming that the inhibition of mitosis was caused by a single ionization produced in a particle about the size of a centrosome. In a later paper (10) he proceeded to show, in connection with results on the survival of x-rayed protozoa, how not only the size of sensitive body but also the number of ionizations in it which are necessary to produce a given effect, when more than one ionization is necessary, can be calculated from the observed frequency-dosage curve. Crowther was, however, duly cautious in the drawing of actual biological conclusions from the use of the brilliant analytical method which he had proposed, for he realized that the premises employed might be inapplicable in the given cases.

It was realized by the present author that this method might be applied to his own and his coworkers' data, for the calculation of gene size, and, in collaboration with Mott-Smith, such calculations were carried out in 1930 [see Muller (45)]. The results were not published at the time, however, since we thought it very unlikely that all the required premises should be valid at once. However, in 1931 and 1932 the physicist O. Blackwood of the University of Pittsburgh published results on the size of the sensitive volume or "nucleus" of the gene, calculated by the application of Crowther's method to the data of the Texas group of *Drosophila* workers.

In and about 1935 Timoféeff-Ressovsky and his coworkers, as well as Haskins and others, revived the method or some modification of it, again using it for the calculation of gene size [see, for instance, Timoféeff-Ressovsky and Zimmer (74)]. The present writer, however, has from the time of the first application of the method to this problem warned against its usage in this connection both in publications (42, 43, 44, 45, 53) and in the Gene Conferences held at Copenhagen (1936) and Spa (1938). After the first of these conferences, Timoféeff and his coworkers greatly moderated their original position, so as to admit the points that the calculated "sensitive volume" changes with conditions outside the gene, with the gene and allele studied, and with the type of mutation

looked for, and that there is no present way of relating this calculated volume to the actual size of the gene. At the same time, however, they did not yet concede the possibility, proposed by the present writer, that a single "hit" might, like a bomb or shell, sometimes give rise to a branched chain of reactions, so as to cause two spatially distinct yet near-by genetic changes.

Despite all the above discussions and publications, a number of other investigators have since 1935 brought up the method again for the finding of the size of the gene or chromonema, or of all the genetic material in a chromosome. The attempts along these lines which have attained most prominence in recent years have been those of Lea and Catcheside (35, 32).

In view of the revival of interest which the last-named workers have aroused in this matter it may be permissible to risk redundancy by restating here that the target hypothesis, as applied to calculations of the size of the gene or even of some supposititious "sensitive" portion of it, must make the following assumptions, *all* of which have to be true at once if the method is to work, but no one of which has yet had critical evidence adduced in its favor. (*a*) Virtually all the radiation-induced mutations result from ionizations rather than mere activations. (*b*) Virtually every ionization within a gene, or within its "sensitive" portion, results in a mutation, no matter what the circumstances. (*c*) These mutations are (at least for the genes studied) all detectible. (*d*) No ionization or activation occurring outside the gene (or its "sensitive" portion) can result in its mutation. That not all these postulates are true at once is directly shown, as stated by the author (44) in 1937, by the variation in gene-mutation rate under different conditions. The more realistic task thus becomes that of throwing "light upon what atoms and atom configurations are the more important ones in the initiation of the mutation and breakage processes, and upon what kind of steps may be involved in the secondary reactions occurring between the ionization and the genetic change itself" [Muller (45), p. 46].

Lines of Attack on the Intermediate Steps of Mutagenesis by Radiation

The discussion in the preceding section has been in a sense negative in its results, in showing the artificiality of postulates which have hitherto been widely adopted, but this may be helpful in clearing the track of investigation for studies of the actual physicochemical mechanisms at work.

The use of the chemical attack for an analysis of the steps involved

in the mutagenic action of radiation may be said to have started in earnest only three years ago, when Stone and his coworkers, Wyss, Clark, Haas, Wagner, Haddox, and Fuerst, succeeded in inducing bacterial mutations by irradiating the medium with ultraviolet, and shortly afterwards adduced evidence that the effect was to be attributed to peroxides, including organic peroxides, formed under the influence of the radiation. This momentous discovery has since been extended to *Neurospora*, both by Dickey, Cleland, and Lotz (13), and by Wagner and others working with Stone (76), and the evidence has made it probable that many peroxides, or perhaps organic peroxides in general, are mutagenic. Moreover, influences which would favor the formation of peroxides by radiation, such as supplying additional oxygen, or blocking the utilization of oxygen in the ordinary metabolic routes, have been found to increase the rate of production of mutations by radiation, whereas, conversely, the opposite conditions depress the process.

The independent discovery by Thoday and Read (69) that the production of chromosome aberrations by radiation is positively correlated with the amount of oxygen present is in striking agreement with the above results. The further finding by Thoday *et al.* (70) that the oxygen has less influence on such changes when radiation is used which gives tracks more densely crowded with ions has been thought to mean that the active radicals were in this case more quickly neutralized by by their complements derived from other ionizations. An alternative or additional reason would seem to be that the denser tracks have concentrations of ions further beyond the optimal concentration for ratio of breakage effects to ionizations: that is, many of the ions in the denser tracks are supernumerary in that they are unable to produce recordable breaks in the given chromosome region because the effectiveness of other ions near-by has pre-empted their field. Thus a reduction in the concentration of active radicals in the latter case (caused by less O_2) has less effect than when the ionizations are more scattered. It will be seen that, in this interpretation, it is taken for granted that there is a certain degree of localization of much of the mutagenic effect, despite the fact that, in Stone's work at least, some part of the effect must have a long range of diffusion. Reasons for inferring this localization were given on p. 313. It is fortunate, however, for purposes of analysis, that not all the effect is so localized, in all material.

The studies of Barron (5, 6) on biochemical and cytoplasmic effects of radiation, and more particularly his discovery of the high radiosensitivity of sulfhydryl groups and of enzymes containing them, appear, both in their essential features and in various details, to fit very well into the same general scheme, for the change induced in the sulfhydryl groups is

a kind of oxidation process involving the removal of hydrogen. And though it seems unlikely, for the reasons given by Dickey, Cleland, and Lotz (13), that the mutagenic change is an ordinary oxidizing process (since cellular oxidations are such a commonplace and organic peroxides are in most connections not very effective oxidants anyway), nevertheless it is now evident that oxygen-carrying radicals of certain kinds can in some way effect mutagenesis and that some of them can also cause alterations in sulfhydryl-containing proteins. At any rate, it is found by Barron that extra sulfhydryl groups, supplied to a medium, tend to protect proteins (enzymes) having organically bound sulfhydryl groups from attack by radiation. This finding has been followed up by the work of Patt *et al.* (64), showing that cysteine tends to protect rodents from radiation sickness, even though Le May (*Proc. Soc. Exptl. Biol. Med.*, **77**: 337–339, 1951) has more recently reported no inhibition of —SH enzymes *in vivo* by x-radiation. The question thus arises, how far is there a parallelism between the processes studied by Barron and those involved in mutagenesis, and to what extent may they be identical? May it not be, for example, as Barron (in a personal communication) has suggested as one possibility, that sulfhydryl groups of the genetic nucleoprotein itself are attacked when a mutation is produced by radiation?

As everyone recognizes, despite the provocative points of agreement in the chemical work carried on by very diverse methods, we still are far from having anything like a complete picture.* There are results which indicate that the reaction chains leading to mutagenesis have various links, and that in fact the chains may branch and/or anastomose. Moreover, there may be a number of different chains, having more or fewer features in common, but all capable of leading to the end result in question—permanent change of the genetic material.

A possible illustration of this complexity is afforded by the finding of Stone and his coworkers that, although very short ultraviolet was effective in making the medium temporarily mutagenic, that of longer wave length was incapable of doing so. Yet this longer ultraviolet was still in the mutagenic range (shorter than 3200 Å): that is, if absorbed directly by the organisms it did produce mutations. It would seem, then, as if the shorter-range effect that is produced by the longer waves

* Just as this proof is being returned to the press, a noteworthy series of findings has been reported by a whole group of workers of the Biological Division at Oak Ridge (see *Rec. Genetics Soc. Amer.*, 1951, and the 1951 *Cold Spring Harbor Symposium Quant. Biol.*) showing the influence of several different kinds of chemicals, as well as of physical conditions, in modifying the effectiveness of ionizing radiation in the production of radiation damage of varied kinds, including gene mutations and chromosome changes, in diverse organisms.

must work through the production of somewhat different substances from those involved in the longer-range effect produced by the shorter waves.

A somewhat similar division of effects, perhaps at a different level, has been found in studies of the "reactivation" or undoing of potential radiation effects by means of visible and long-wave ultraviolet light [see, for instance, Novick and Szilard (61), Watson (77)]. It is found that there is a fixed proportion of the ultraviolet or x-ray effect (varying with the type of radiation) which is not "reactivable." Similarly, in studying the influence of withdrawal of oxygen [Giles and Riley (20)] or of freezing [Fabergé (17)] upon the induction of genetic effects, certain apparently irreducible minima of effects are obtained. These seem to represent changes induced by a rather different mechanism from that involved in those effects which are responsive to changes in the condition in question. They may, of course, involve bound oxygen, yet even in that case it is unlikely that the reaction formulas are identical. Again, in the analysis of radiation effects on phage, Watson finds evidence of several distinct types of changes, the production of each of which is differently influenced by various conditions attendant upon or following the irradiation. The whole problem therefore is, as might be expected, a multiple one. And it is becoming increasingly evident that there are various different pathways leading from ionization or activation to mutation, some shorter and straighter, others longer and more branched and devious.

In the present discussion, attention has been concentrated chiefly on problems of gene mutation, not because structural changes in chromosomes are unimportant, or because they are unrelated to gene mutations, but simply because the topic would then become too diffuse. It is, however, necessary for us to orient the two subjects to one another in respect to two particulars. The first of these concerns itself with the fact that there are good grounds for regarding the distinction between the two categories as valid, even though in many individual cases it is impossible to discriminate between them. Reasons for this have been given elsewhere [Muller (45, 47, 52)] and need not be repeated here. Second, we must clear the ground of the hypothesis [tentatively suggested but abandoned by the present writer many years ago and recently urged by Lea and Catcheside (34), and by Herskowitz (22)] that "gene mutations" are often or usually accompanied by a chromosome breakage near by, which may have been followed by either restitution or recombination. Here again the reader must be referred to other papers [for example, Valencia and Muller (75), Muller (52)] for the evidence that gene mutations unaccompanied by rearrangement are not restitution phenomena, and that the effects resembling gene mutations

which are found near the sites of chromosome structural change are usually position effects of the latter.

It may be added that this problem appears to arise very little except in *Drosophila* and other Diptera, and possibly *Neurospora*. In mammals, for instance, there is evidence that few if any structural changes entail a position effect, for translocations seldom give evidence of lethal or other mutational effects when homozygous. Thus there has been no reason to suppose changes in gene functioning to be in any way connected with chromosome breakage in organisms in general. Nevertheless, in all organisms, small deficiencies, caused by two breaks near together with loss of the interstitial piece, can give effects similar to those of drastic gene mutations. These very similarities in phenotypic effect make it the more important to exert ourselves to maintain our theoretical distinction. And, although there is undoubtedly much in common in the mechanism of production of both types of genetic change by radiation, they must be studied separately, for we cannot now know in what further particulars, besides the somewhat contrasting influence of ultraviolet on the two, they may be found to differ.

The Need for More Exact Knowledge of Gene-Mutation Frequencies

The above discussion has been concerned mainly with questions of the nature of the process whereby mutations are induced by radiation. Equally important, both from a practical standpoint and from the viewpoint of evolutionary and even sociological theory, are the questions concerning the effects of the induction of mutations on populations subjected to such treatment. Now it is evident that one of the first desiderata in the assessment of such effects is a knowledge of the actual frequency of gene mutations in any given population, both in the absence of treatment and after delivery under known conditions of a specified dose of radiation.

It might be thought, in view of the positiveness of our knowledge of the linear relation of induced gene-mutation frequency to dose, discussed in an earlier section, that sufficient information of the requisite kind is already at hand for the making of fairly good estimates of the consequences of applying a given dose. However, even in *Drosophila*, the absolute gene-mutation frequency for a given dose is a matter of considerable uncertainty—much more so than that of the frequency at one dose relative to that at another dose. Now, as we shall see later, the absolute frequency is a most important datum when the effects of a given dose on the population are to be reckoned.

It is true that we can tell fairly exactly, for standard conditions, what total frequency of recessive lethals will be found for a given dose applied to *Drosophila* spermatozoa. But there is still a wide margin of error in determining what proportions of these lethals represent gene mutations, deficiencies, and position effects, respectively. And there is much greater doubt in the estimation of what the ratio is of these recessive lethal mutations to gene mutations in general, chiefly because very little is known about the frequency of mutations of very minute effect, despite their great importance for the population (see p. 322). There is still considerable uncertainty also concerning the ratio of gene mutations induced in *Drosophila* spermatozoa to those induced in ordinary interphase cells, by the same dose, and the latter is the more important quantity in practice. Moreover, estimates of the ratio of the mutation rate induced by a given dose to the spontaneous mutation rate sometimes differ by a factor of more than 5, if, for instance, lethals as a class have been followed in one experiment and visible mutations at specific loci (the determination of which is subject to far greater uncertainties) in another experiment. And yet, in still other work, these two categories of mutations have appeared to vary *pari passu*. Work on the spontaneous mutation rate is a far larger undertaking than that on the induced rate, yet it is equally important for gauging the significance of the results obtained concerning the induced rate. Thus there remains room for far more exact and larger-scale work along these lines than has yet been carried out. Moreover, this work should be carried out under conditions that will make possible the minimizing of so-called "personal equations."

The above is the situation even in *Drosophila*, although this has been, for most purposes of detailed genetic analysis, the pilot form. This situation reflects lack of recognition not only of the needs of such work, but also of its implications. It is fortunate that it has been possible to establish some of the more important principles at work in the field of radiation mutations without having to ascertain the absolute values of the factors involved. However, if estimates are to be made of the genetic effects of radiation in organisms of intrinsic importance, as in man or (as a clue to man) in other mammals, then it will be essential to gain exact data on these quantitative features. But, as a guide to such stupendous investigations, and to avoid a repetition of such costly missteps as have already been made in the work that has been done on mammals by persons with too limited genetic outlook, it is important that such data be obtained first in lower-level pilot forms suitable for exact genetic analysis. At the present day, this still means, in the first place, *Drosophila*.

Now, although so little is yet known of the magnitude of the effect of

radiation in producing gene mutations in mammals, Paula Hertwig's work clearly shows the existence of the effect in mice, and her data indicate it to be not so very different in magnitude from that in *Drosophila*. That is, one gets the impression that the per genome frequency of gene mutations induced by a given dose may be somewhat smaller than in *Drosophila*, but probably not by as much as one order of magnitude. It will be most important, and most difficult, to obtain a more exact estimate in such an organism. We believe, however, that the investigations of Russell and his group, described in a parallel paper in this symposium, will in time supply the first really key data of the kind needed.*

Despite the urgent need for more exact data, however, enough is already known about mutation frequencies to allow the conclusion to be drawn that, if these frequencies per generation, both in untreated material and in that exposed to a given dose of radiation, were similar in man and *Drosophila*, the ultimate effect on human populations of those doses to which they are likely (if present trends continue) to become exposed, would in its totality be very serious. In fact, the same conclusion appears to follow, although with a much greater margin of error, even on the basis of the very meager work with mice already reported. For an understanding of this, it is necessary to consider the manner in which mutations express themselves and undergo elimination, and the character of the consequent effect on the population in general. In this way light will at the same time be thrown on the nature of the mistakes which have been made by prominent non-geneticists, in attempting to give the lay as well as scientific public the impression that, as judged by the results in other forms, as well as by the observations already made at Hiroshima, the genetic effects of radiation would in practice be negligible in human populations.

The Way in Which an Increase in Mutation Rate Will Affect a Population

In considering this matter, a simple and cardinal point to be reckoned with is that each mutation received by an offspring results, on the average, in the genetic death of one descendant genome or individual, no matter how slightly detrimental the effect of the mutant gene may be [Muller (49, 50, 51, 52, 54)]. This paradoxical result is a consequence of the fact that the less detrimental genes will tend to accumulate so

* Results reported by the Russells since the above was written, and now in press in the 1951 *Cold Spring Harbor Symposium Quant. Biol.*, have given definite evidence that the per locus mutation rate induced by a given dose of x-radiation in mice is far higher than in *Drosophila*, perhaps by as much as one order of magnitude.

as to hamper ever more individuals, until they finally make their "kill" and so become eliminated. For this reason the total harm done by a so-called small mutation is in the end as great as that done by a large, fully lethal mutation. It follows that, to know the total damage done to a population by a given mutagenic agent, we have to know the total number of mutations produced, including even the smallest on equal terms with the largest [see Haldane (21)]. The detection of these "small mutations" is, however, a matter of the greatest technical difficulty, if not impossible, except through very special and laborious techniques, some of which should be so set up as to utilize their effects *en masse* and others, as a countercheck, so devised as to analyze a small sample of them individually [Muller (53)]. Nevertheless, it is already evident that these mutations are considerably more numerous than the "large" ones, and that they must therefore contribute correspondingly more to the sum of biological ills.

Another point of importance in regard to the mode of action of mutant genes in affecting a population concerns itself with their degree of dominance. It has not hitherto been widely realized that, although most mutant genes produce far more than twice as much abnormality when homozygous as when heterozygous, and are in this sense recessive, nevertheless they usually produce some slight effect, at any rate, when heterozygous, and that this effect is of major importance. Evidence for this conclusion has long been in existence, but has been generally neglected. This degree of dominance, occurring in man as well as in *Drosophila* [Levit (36), Muller (52, 53, 54)], is usually so small as to be imperceptible to ordinary observation. Nevertheless, it is highly significant because it usually causes elimination of the gene to occur in a heterozygous individual, before it ever gets a chance to show itself in homozygous form. The realization of this situation is making necessary a complete rereckoning of the mutant-gene frequencies to be expected in populations at equilibrium, of the speed of approach to these equilibria, of the prevalent mode of expression of mutant genes in populations, of their extinction rates, etc. In all these respects the supposedly recessive mutant genes are in effect, so far as their major action on the population is concerned, to be reckoned with as dominants. As dominants, however, most of them take their place in the category of mutations with small effects (since the effect on the heterozygote is usually so small), even in those cases in which, owing to their drastic action on the homozygote, they had previously been classed as extreme "freaks" or lethals.

Because of this slight dominance which most mutant genes exert, a mutagenic agent must give rise to a biologically significant depression of vigor and "viability" (survival rate) in the first generation after exposure,

even though the exposed parents had undergone interbreeding in the ordinary more or less random fashion. This does not mean that this detrimental effect would be large enough to be technically perceptible, however; in fact, even in *Drosophila* whose parents had been given very high doses of radiation, such as 5000 r, it would be very difficult to demonstrate directly. But the decreased viability, which we are supposing to have been caused by the exposure of only one generation, would continue over scores of generations of descendants, until it had faded away by reason of the "genetic deaths" which it had occasioned. For this reason the total damage entailed might be very considerable, even though that in any *one* generation was too little, scattered, and intermingled with variations of other origin, to be demonstrable by ordinary means.

If now such effects were, as a matter of policy, judged to be too insignificant to justify the measures needed to avoid them, and the same mutagenic influences were in consequence repeated in every generation, the effects, slight in any one generation, would, as it were, pile up layer on layer, towards an equilibrium at which the effect on the viability of each generation had finally become proportional to the artifically raised mutation rate. Thus if, for example, the mutation rate had been raised by an amount equal to 25 per cent of the spontaneous value, as would happen in *Drosophila* by application of some 25 r per generation to the immature germ cells, then the population, after reaching genetic equilibrium for this dose, persistently repeated, would in each generation have a death rate, due to genetic causes, which was 25 per cent above that existing in non-treated material. As this genetic death rate in ordinary non-treated *Drosophila*, due to spontaneous mutation, appears to be at least 10 per cent (that is, 10 per cent of individuals die or fail to reproduce because of their spontaneous mutations), that in the treated population would in this case rise to some 12½ per cent, the difference, comprising 2.5 deaths per hundred individuals, having been caused by the 25-r exposure.

A figure of this kind has more than academic interest, for it can be shown that, in a population of stable size, the number of "genetic deaths" which are caused in *each* generation by a mutagenic agent that has been applied repeatedly, for an unlimited succession of generations, is in fact the same as the total number of genetic deaths caused throughout future generations, until the effect has faded away, by the same mutagenic agent, if applied in the same strength, but for only one generation. In our example, this would mean that the 25 r if applied to only one generation would eventually cause two and a half deaths, scattered throughout the future, for every hundred members of the one treated generation.

There would of course be a great many more individuals who, though suffering some damage, had not met actual elimination; in fact the number of these in the given case may be estimated at something of the order of one or two hundred descendants slightly affected, for every hundred of the treated generation. These effects then are inappreciable only because they are hidden from us by the veils of space, time, and circumstance.

Even if highly accurate measurements could be made, in organisms with easily controlled breeding, nutrition, etc., of the amount of depression of viability caused in one or in a few generations as a result of one or a few generations of application of a mutagenic agent, this would not give us a reliable means of estimating the total amount of genetic damage done by the given exposure, if all generations were considered. Neither would it allow us to determine what the condition of the population at equilibrium would be, if the agent were applied in the same strength, generation after generation, indefinitely. The answer to these questions depends not only on the total effect that finds expression in one or a few generations after treatment, but also on the amount and type of distribution of the mutational damage among different loci. If, for example, most of the damage was caused by a relatively few mutations, with individually marked expression, the mutant genes concerned would be eliminated more quickly by natural selection and the effects would sooner die away, and so the total damage throughout all generations would be much smaller than if the observed effect had been divided among a great many mutations with individually small action. Keeping watch of the viability for a few generations would hardly give a delicate enough means of measuring the curve of decay of the effect. To obtain the needed information, highly controlled breeding and genetic analysis would be called for, in which a sample of the mutant genes was "caught," as it were, in the homozygous condition, and then carefully tested, with the use of exactly regulated genetic and environmental backgrounds, for the ascertainment of the amount of effect which the given genes had on viability when they were in heterozygous condition [Muller (53)]. This sort of thing has not been done even for *Drosophila*, and would be a major project in that organism.

It might be thought that in view of the uncertainties even in *Drosophila* the situation as regards man would be pure guesswork. Yet the information that has been brought forward by various investigators on spontaneous mutation frequencies at a number of specific loci in man, taken together with a number of considerations of comparative genetics, makes it feasible to form an approximate estimate of the probable spontaneous mutation rate in man, and of the consequent genetic depression

of viability in an equilibrium population [Muller (54)]. According to this estimate, which has a range of error corresponding to a factor of 5, there is one new spontaneous mutation, on the average, for every five offspring, as a minimum rate, up to possibly one mutation for each offspring, on the average, as a maximum rate. It makes a great deal of difference, however, just where within this range the actual rate lies, for with the small size of families obtaining under modern civilized conditions there would not be enough surplus to allow the genetic elimination necessary for the maintenance of genetic equilibrium and for the long-term survival of the species unless the mutation rate were at the minimum end of this range of possibilities. Hence in any case it is probably not far from what may be called the "critical level," the level beyond which selection, so long as it is limited by the existing birth rate, cannot keep up with deterioration.

In view of the above situation, even a comparatively small increase in the mutation rate, artificially induced, might cause it to transgress the critical level, and so, if it were indefinitely continued, it could have disastrous consequences. It could be counteracted only by a corresponding rise in the birth rate, accompanied by more stringent selection. On the other hand, if the level of mutation which was "critical," in relation to the existing level of reproduction and selection, were already being exceeded by the spontaneous rate, then the induced mutations would occasion a correspondingly faster decline.

It is true that human policies can hardly be expected to continue sufficiently unchanged, over so many centuries, as to lead to the long-term results in question. But, as has been pointed out, the results of an agent operating for a briefer time can be measured in a proportionate manner, in terms of those results which would have been produced had it been of longer duration. Moreover, from an ethical standpoint, policies should be framed according to this yardstick.

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DISCUSSION

LATARJET:

(1) It seems to me that the "daughter" conception of spontaneous gene mutations could find some support in the clonal distribution of host range spontaneous mutation in bacteriophage among individual bursts, as has been recently shown by Hershey and by Luria. I should like to ask Muller's opinion on this point.

(2) I don't think that Novick and Szilard's experiments give evidence for a linear relation between dose and ultraviolet-induced mutations in bacteria. These authors repeated the experiments that Demerec and I did previously,* but their main concern dealt with photorecovery. Their dose-effect curves, drawn with few points, fit our delayed-effect curve in the sense of a non-linear relation (their linearity is due to log plotting). I don't think that the mutation from sensitivity to phage resistance may yet be interpreted in terms of a simple gene change.

* *Cold Spring Harbor Symposia Quant. Biol.*, **II**: 38, 1946.

MULLER:

In answer to Latarjet's first question, I feel that the relation of mutation rate growth is suggestive in this matter but not proof positive. Perhaps tagging might help. With regard to the second question, I would agree that the relation is not linear, as I had at first thought it to be.

NIMS:

The long-range effects discussed by Muller in the latter portion of his paper are most important. He has stressed that the radiation-induced mutations do not differ from the spontaneously occurring ones. Since this is so, it could be assumed that all the gene variabilities exist in the human population and are in a steady state. If this is so, all that radiation does is to increase the rate of mutations without greatly disturbing the steady state. That is to say, there would be no wide departure from existing genic patterns, nor would new deleterious genes be introduced into the population.

MULLER:

The answer to this line of reasoning lies in the fact that natural selection throws out the deleterious mutations as fast as they occur in those races that manage to survive. The major mutations are unimportant, as a rule, because of their rarity. They are important only as handles to study genetics. It is the sum of many minor or undetectable changes that gives importance to gene change. The number depends on the spontaneous rate and is proportional to it. If the rate is doubled, the genetic death rate associated with it is also doubled, and twice as heavy a load is placed, so to speak, on the individual who doesn't die. How far the *Drosophila* data can be applied to mammals is not certain. If the data are applicable, then an increase of 25 per cent in the natural mutation rate is far from negligible. A new equilibrium will be established if the exposure is continued. At the present time, equilibrium, in my opinion, is not established, since the rate of radiation exposure is increasing. About 0.01 per cent of the mutations may be beneficial, but the sacrifice of natural selection means that human beings cannot accept the burden of the harmful mutations for the sake of the very occasional beneficial one.

It is possible that in another generation the increase in exposure to radiation may, on an average, equal 25 r per person.

SPARROW:

In plant material the situation does not appear to be as gloomy as Muller suggests for man. When *Tradescantia* are grown under continuous gamma irradiation, the postmeiotic aberration frequency (micronuclei resulting from meiotic chromosome fragmentation) gradually increases and reaches a maximum by 16 days. Further exposure at a given dose rate causes no further increase. If the plants are removed from the radiation field, the aberration frequency returns to normal in a few weeks. In this case dose rate seems to be more important than total cumulative dose once the minimum period of about 16 days is passed. Sax has obtained similar results in his experiments with *Tradescantia.*

SAX:

We have done similar experiments and find that the effects of continuous radiation are not cumulative. Chromosome aberrations increase for several weeks, and then the frequency remains stationary even when radiation is continued for months.

MULLER:

Back mutations, both spontaneous and radiation-induced, have been studied. The likelihood of recovery for gene mutations is negligible. It is emphasized that natural selection is operating on the somatic cells as well as on the gonadal cells where chromosome aberrations are concerned. That is, damaged somatic cells die out and the undamaged normal cells survive. But this effect is practically absent for gene mutations.

18.

Speculations on Cellular Actions of Radiations *

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After the foregoing thorough discussions of radiation physics, radiation chemistry, and biochemical effects of radiations, it would appear that the tasks of the cellular radiobiology panel are essentially as follows: (1) to review briefly the available data on cellular effects, with special reference to quantitative aspects; (2) to attempt to fit the data with theories, old or new, giving special attention to relations between the cellular effects and the physical and chemical effects; (3) to indicate gaps in our present knowledge and understanding, and to suggest possible methods of eliminating them. This paper will be confined to remarks about cellular effects in general. Papers by the other contributors deal in more detail with certain important types of cellular effects, about which we now have considerable quantitative information.

Whenever we carefully ponder the mechanism of any known radiobiological action, we end by admitting that we have substantial direct information only about the very beginning and the very end of the story. The beginning is the act of irradiation, during which charged subatomic particles speed through the biological material, transferring energy to molecules along their paths and thus producing "tracks" of activated (ionized and excited) molecules. All this has been carefully described by the physics panel. The end of the story is the biological end effect, which, if the observations are made on a cellular basis, may be a mutation, a chromosome aberration, an inhibition of cell division, a change in permeability, a change in metabolic rate, or any one of many other deviations from normal structure or function.

These end effects are of such nature that in no case have we been able to see how they can be directly related to the physical events con-

* Many of these speculations have been suggested by experimental work done under Contract No. NR-116-256 with the Office of Naval Research, Department of the Navy, in cooperation with the Atomic Energy Commission.

stituting the beginning of the story. It accordingly would seem that certain (possibly numerous) events must occur between the primary energy transfer and the end effect. The time interval that comprises these events is usually termed the "latent period"—something of a misnomer because this period is obviously one of important activity. So far as I have been able to learn, for no radiobiological action do we have any direct and certain information about the nature of the events of the latent period. However, for some actions we have considerable suggestive and apparently pertinent information; and when we reflect that, if we could discover and connect the main events of the latent period, we would have substantially complete understanding of radiobiological action, it seems worth while to attempt a systematic description of our ignorance, using the suggestive information as a guide for our speculations. Such an attempt will be my chief concern in this paper.

First let us briefly review the currently available facts.

Contributions of Radiation Physics and Chemistry

Physics furnishes information about the amount of energy transferred. Let us consider an x-ray dose of 10,000 r, which will produce many drastic cellular effects. This corresponds to about 840,000 ergs (or 0.020 cal or 5.2×10^{17} ev) per gram of ordinary soft cellular substance. Such a small amount of energy per unit mass, if delivered in the form of heat, would of course have no deleterious effects. If we assume that the average amount of energy required to produce an ion pair in the cell is the same as that in air (32.5 ev), we find that the dose of 10,000 r will produce 1.6×10^{16} ion pairs per gram. The number of excited but not ionized molecules probably would be of the same order. Since average cells contain about 3×10^{22} molecules per gram, it is evident that 10,000 r activate only about one-millionth of the molecules in the cell. Thus, although the ionizing particles have sufficient energy to activate any species of molecule, a species represented by only a few individual molecules per cell (for example, a specific type of gene) stands an excellent chance of escaping a direct activation.

Physics also gives us information about the distribution of the activated molecules. These are not produced singly at random in the cell but are localized along the tracks of the ionizing particles, the tracks being located more or less at random, depending on the technique of irradiation. In gases this is directly revealed by the Wilson cloud chamber; and it would appear that what we see in the cloud chamber gives us indirectly a good qualitative picture, magnified about 800 diameters, of the distribution of activated molecules in tissue. The detailed distribu-

tion of positive ions, negative ions, and excited molecules, at the instant of production and throughout their lifetimes, is not directly observable but must be deduced by the methods described by our physics panel. It is noteworthy that, along the ionization tracks, the activated molecules tend to be formed in small clusters, and that, when the clustering effect is smoothed out, the number of activated molecules produced per unit length of track varies as the square of the charge of the particle and decreases as its instantaneous speed increases. Accordingly, with different types of ionizing particles and different conditions of irradiation, the distribution of activated molecules along the ionization tracks may vary widely. This factor, we shall see, influences most radiobiological actions to a significant degree.

Once the activated molecules are produced, how can they promote a radiobiological action? We have no sure information about this, but radiation chemistry provides certain facts, obtained by experiments on non-living aqueous systems, which indicate some possibilities.* First, it has been found that both water and certain solutes can be altered by irradiation. Second, there is abundant evidence that, in many cases, the solute does not need to be directly activated by the ionizing particles but can be altered indirectly by reaction with activated water. In such a case, if two or more reactive solutes are present, they compete for the activated water molecules. This is usually called the protection effect. Moreover, if only one solute is present, the number of molecules transformed per unit of irradiation is independent of solute concentration. This is known as the dilution effect. Third, there is evidence that H atoms and OH radicals are rapidly formed from the original activated molecules and enter actively into the mechanisms of radiochemical action.

The foregoing facts from radiation chemistry are confirmed by radiation biochemistry. The protection effect and the dilution effect, as well as evidence for the role of H and OH, have all been observed in irradiation studies of the physiological inactivation of important biological substances, particularly enzymes, in aqueous solution. Moreover, there is evidence that, in addition to the "indirect" action on these important solutes through activation of water molecules (possibly followed by the intervention of H and/or OH), there is sometimes a detectable "direct" action initiated by activation of the solute molecules by the original ionizing particles. These biochemical observations greatly encourage us

* For reasons given elsewhere (3), it is assumed that radiation chemistry almost certainly is a basic portion of a radiobiological action. However, Read (4) has recently suggested a mechanism of chromosome breaks which includes no hypothetical chemistry.

to use the findings of radiation chemistry in our attempts to visualize possible mechanisms of radiobiological actions.

Bearing in mind the foregoing facts of radiation physics and chemistry, I shall now present a model of an imaginary mechanism of radiobiological action. For the sake of concreteness I shall first present a special case and then attempt to generalize.

Mechanism of Radiobiological Action

As our special case (Fig. 1), let us consider a known radiobiological action, namely, inhibition of division of a unicellular organism, for example a yeast. We start (at top of Fig. 1) with the normal state (α) of the cell; this state includes the ability of the cell to divide if it is not irradiated. We then imagine various states (β, γ, δ μ, π) through which the cell passes to reach the end effect (ω), which in this case is the failure of the cell to divide. Intervening between these imaginary states are the imaginary processes (A, B, C, M, P, Z) by which the cell passes from state to state. The first process (A) is energy transfer from ionizing particles to water molecules, the cell thus entering a state (β) in which ions derived from water are present and diffusing. (There is, of course, good evidence that this state is not imaginary.) We now assume that OH radicals are formed from the H_2O^+ ions (process B and state γ). Next we imagine a slight complication of mechanism, namely the formation of some sort of peroxide by the reaction of OH with a suitable molecular species (process C and state δ). The peroxide reacts with and inactivates a species of gene (process M and state μ) which normally is essential for the synthesis of an enzyme necessary for accomplishment of cell division. With the genic species inactivated, insufficient enzyme is present (process P, state π), and cell division is inhibited (process Z, state ω).

The processes and states so far mentioned are assumed to be integral parts of the mechanism of the specific radiobiological action under discussion. They are accordingly considered *relevant* to that particular action. In addition, it appears extremely probable that there are numerous states and processes (as β', γ', δ', μ', A', B', C', M') which are *irrelevant* to the action leading to the effect ω (but may be relevant to other actions in the same cell). For instance, process B' (formation of H atoms) very probably occurs if OH radicals are formed (process B). However, if the mechanism of the action under consideration is promoted only through the OH radicals, as we have assumed, then the H atoms are irrelevant to this action, although they may be reasonably expected to promote some other action, which may or may not be observed. In-

deed, in our specific case under discussion, most of the energy absorbed by the cell must ultimately go into irrelevant processes, since the energy passed on to only one or a few genes must be a very small fraction of the total dissipated throughout the cell, even though we know, as noted earlier, that this total energy has a small absolute value.

It will be noticed that the relevant processes are visualized as in *competition* with the irrelevant ones. They may also encounter two other types of competition. The first is from *reverse processes*, as indicated for imaginary processes C and M in Fig. 1. The second is from *restitution* (not indicated in Fig. 1), by which is meant a change from any given state back to an earlier one by any process(es) other than strict reversals of relevant processes.

Among the various relevant states, I have introduced for convenience the notion of a *decisive state* (μ), by which is meant that, once this state has been attained in any individual cell maintained under a given set of conditions, the remaining relevant states and processes, including the end effect ω, are inevitable. In other words, after the decisive state, the relevant processes have no competition from irrelevant processes, from reverse processes, or from restitution. In view of the likelihood of these competitive processes, it may well be that in many radiobiological actions the decisive state is attained only with the end effect and is identical therewith. This, however, does not impair the value of the general concept.

For any radiobiological action, the decisive state in any individual cell must consist of a critical number n (one or more) of *decisive entities* (individual structures of molecular, or greater, size). Each of these decisive entities must have a *precursor*, that is a molecule or group of molecules which has a definite role in normal cell structure, activities, or both.* In our special case, a decisive entity is an inactivated gene, and its precursor is the normal gene. If the cell is haploid, $n = 1$; if the cell is diploid and homozygous, $n = 1$ or 2, depending on whether or not one gene can suffice for the formation of enough enzyme for normal cell operations. The process type M, by which a decisive entity is produced, is termed a *decisive process*. To produce a single decisive entity, individual processes of type M must occur m times ($m \geq 1$). In our special case, M is the reaction of peroxide molecules with the relevant gene. If

* It is evident that the molecules (or groups of molecules) involved in the *predecisive* processes are not, in the non-irradiated cell, essential to the normal biological role of the precursor or to the structure or function whose alteration constitutes the end effect. The precursor is almost certainly a solute; it seems inconceivable, on numerical grounds, that the decisive entities could be water molecules so altered as to render them unfit for the normal biological roles of water.

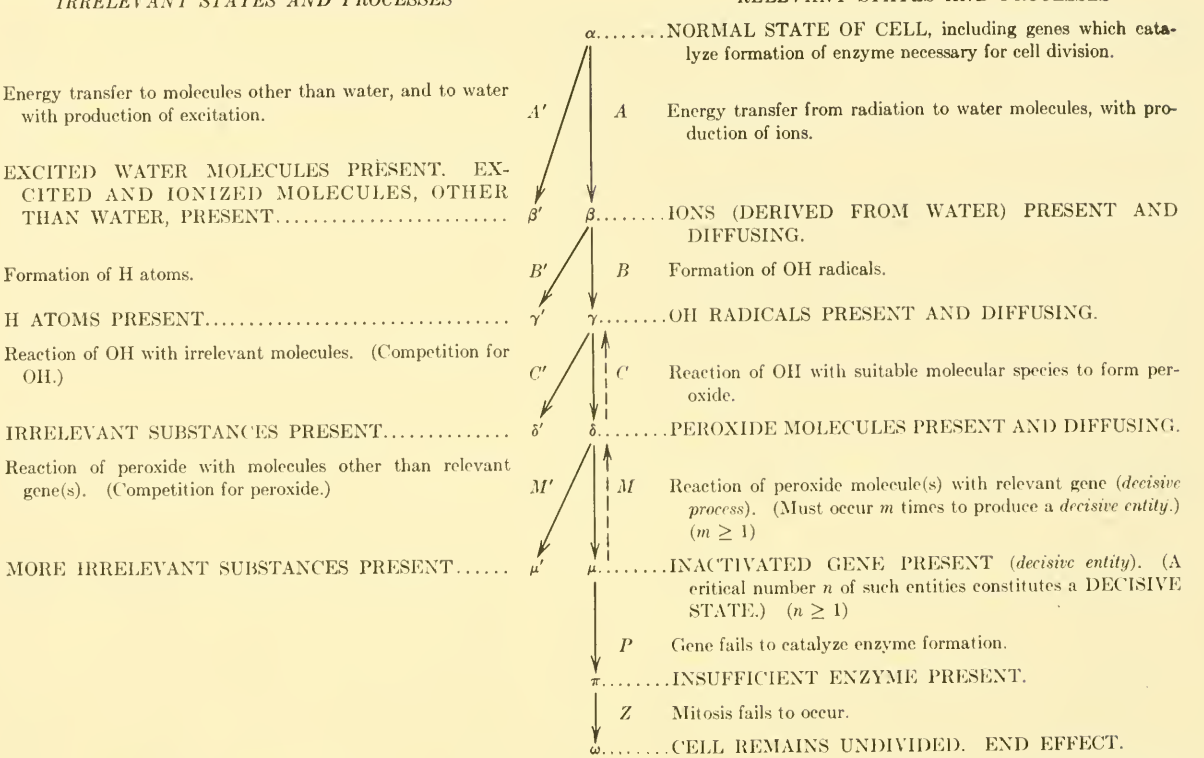

FIG. 1. Model of an imaginary mechanism of cellular radiobiological action resulting in a specific known effect (inhibition of cell division in a yeast). For description see text.

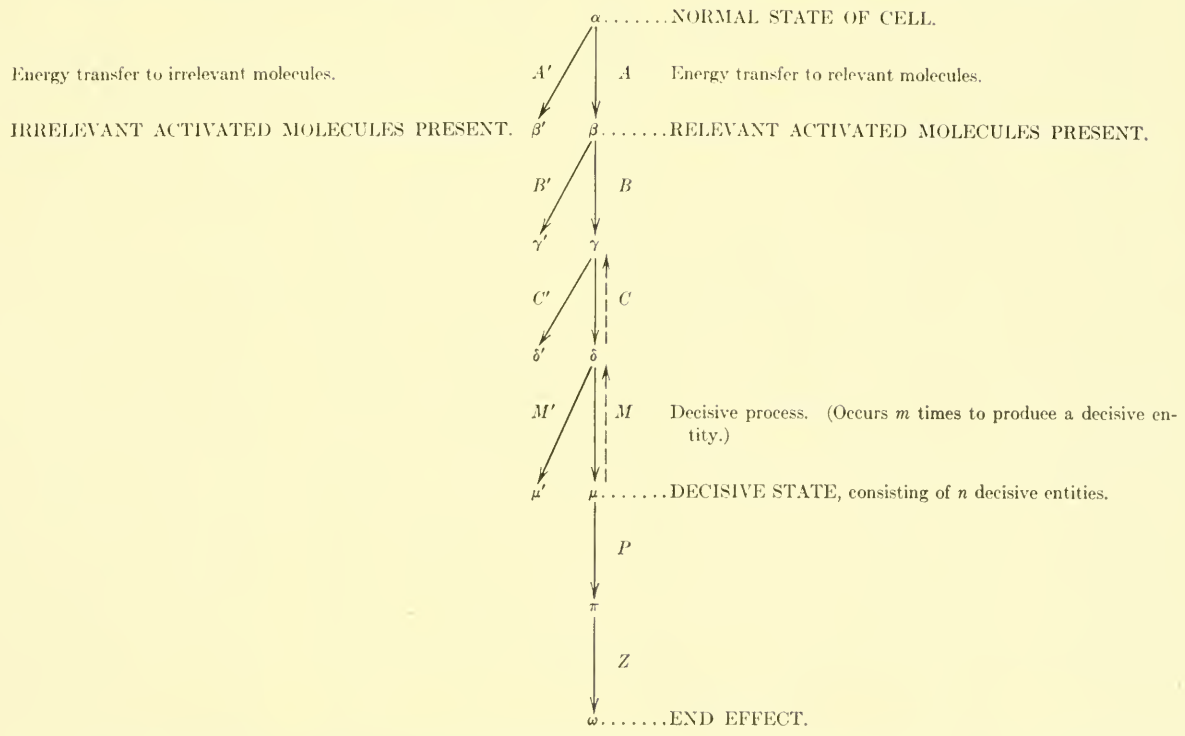

FIG. 2. General model of mechanism of cellular radiobiological action. With suitable insertion of details this model presumably would fit any cellular action. See text.

we consider that a gene might be inactivated by a change in a relatively small chemical group in the gene "molecule," it seems reasonable that m might be quite small, perhaps unity.

Some of the foregoing notions can be visualized qualitatively by reference to Fig. 3. Two ionization tracks are shown crossing a single cell.

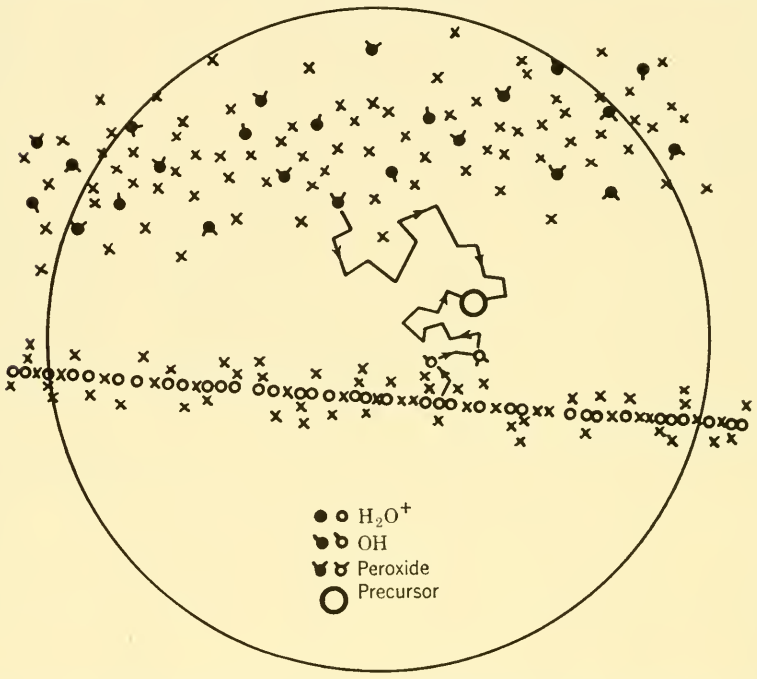

FIG. 3. Qualitative relations between a cell, two ionization tracks, and some relevant entities involved in the model of Fig. 1. See text.

The lower one has just been formed. Ions and excited molecules are present. The H_2O^+ ions, which are assumed relevant to the specific action described in Fig. 1, are designated by circles and are all located directly in the path of the fast ionizing particle. All other entities produced by the ionizing particle (all excited molecules and all ions except H_2O^+) are irrelevant and are designated by crosses. One possible path by which relevant entities may diffuse to the precursor is shown. Note that the H_2O^+ starts to diffuse and reacts to form an OH radical (one-pronged circle). The radical diffuses a bit and reacts to form a molecule of H_2O_2 (two-pronged circle) which finally, by diffusion, chances to collide and react with the precursor (decisive process). The upper track is older than the lower. All the relevant and irrelevant entities are sepa-

rated by diffusion. The ions have reacted to form other entities. A few OH radicals remain at this instant, but most of the surviving relevant entities are H_2O_2 molecules. The ratio of relevant to irrelevant entities is much lower than in the younger (lower) track because many of the potentially relevant entities have been removed by irrelevant processes. The possible diffusion path of one of the H_2O_2 molecules from the older track to the precursor is indicated.

Let us now try to generalize, that is, select from our outline of a special case (Fig. 1) those states and processes which may reasonably be expected to be parts of *any* radiobiological action. These are listed in the skeleton outline of Fig. 2, and the relevant ones are as follows:

α. Normal state of cell.
A. Energy transfer.
β. Activated molecules present.
M. Decisive process.
μ. Decisive state.
ω. End effect.

Of these we can be absolutely certain that α, A, and ω are relevant. We can be certain that state β occurs, but, although its relevance seems highly probable, we cannot be certain that it is relevant to all radiobiological actions. (The relevant mechanism in some actions may not involve chemical changes at all but work through physical processes entirely, as in Read's proposed mechanism of chromosome breaks.) For no radiobiological action do we know the natures of decisive process M and decisive state μ, but I have included them in the general outline (Fig. 2) because I intend to use them as convenient general concepts, even though it is likely that in many mechanisms the decisive state μ is identical with the final state ω.

I shall now briefly consider some of the most prominent radiobiological facts, interpreting them in terms of the foregoing outlines of mechanism.

Quantitative Aspects of Dose-Effect Curves

Let us first consider the quantitative relations between dose and effect. We here imply that both the dose and the effect can be measured. I shall assume that the dose measurement is the affair of the physicists and is well in hand. Some remarks are necessary, however, about measurement of effect. In the first place, since every radiobiological effect is a deviation from a normal condition, various degrees of effect are always expressed in terms of a datum for a normal control population of

cells. In any given case, the deviations from normal are determined in one of the following ways:

1. The observations are made on *groups* of cells of the same species; for example, the respiration rate of a suspension of irradiated yeast cells is measured and expressed as percentage of the rate of a non-irradiated control suspension.

2. The observations are made on the *individual* cells of the sample.

a. The end effect is of the all-or-none type; for example, the cell divides or it does not.

b. The effect is a quantitative modification of a normal structure or activity; for example, the number of fat droplets in the cell is increased, or the respiration rate of the cell is decreased.*

Throughout this paper I shall be discussing primarily the all-or-none effects (type 2*a*), although many of my remarks may well apply to the other two types.

A very common type of experiment consists in dividing a population of similar cells into several samples, giving each of the samples one of a series of graded doses of a given radiation, and subsequently classifying each cell on an all-or-none basis. From these raw data can be calculated the fraction of the cells affected in each sample, and this fraction can then be plotted as a function of dose to obtain a *dose-effect curve.* For convenience we frequently alter this procedure by plotting the fraction *not* affected against dose, thus obtaining a *survival curve.*

If the individual decisive processes occur entirely at random, any survival curve can be theoretically described in terms of m, the number of individual decisive processes of type M which must operate on a precursor to produce a decisive entity; n, the number of decisive entities required to constitute a decisive state; and h, the mean number of individual decisive processes M produced per cell per unit dose. However, in some radiobiological actions, the individual decisive processes operating on a given individual precursor may be linked; that is, r such processes may result from r individual chains of events (Fig. 2) which have their ultimate origin in the same ionization track. Moreover, it is possible that in some actions the decisive processes operating on s (two or more) precursors may also be linked. Accordingly, to make our statements as general as possible, we shall replace m with m/r, the number of *groups* of decisive processes required to change a precursor to a decisive entity; replace n with n/s, the number of groups of decisive en-

* This method is usually laborious and consequently avoided. If desired, the experimental data can usually be handled as all-or-none by using an arbitrary criterion; for example, the cells whose respiration rate is decreased to 50 per cent or less of normal may be scored as affected, the remainder as "survivors."

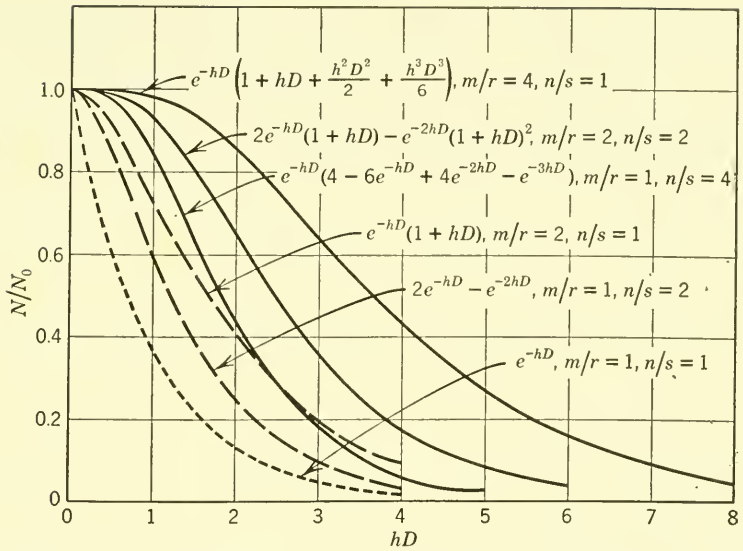

FIG. 4. Theoretical dose-survivor curves described by Eq. 1 when, with h constant, m/r and n/s are assigned various integral values. See text.

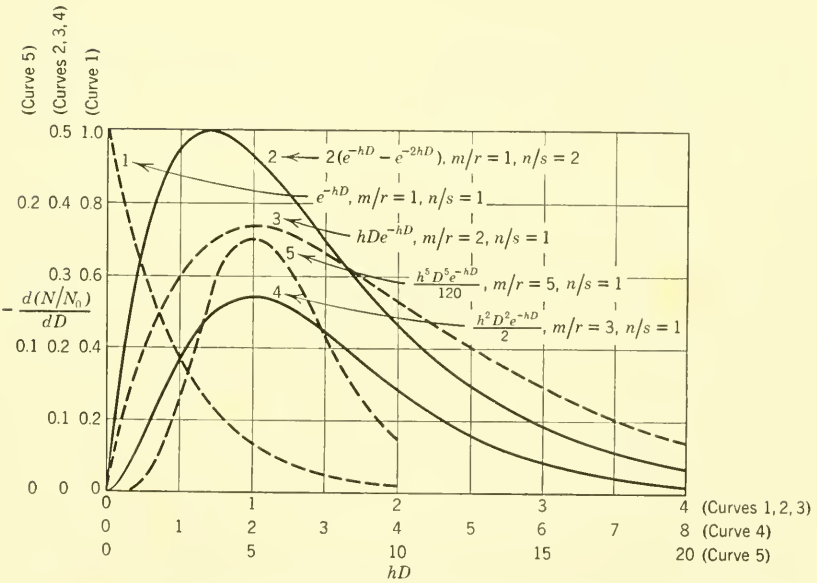

FIG. 5. Frequency distributions necessary to explain shapes of survival curves on basis of variation in radiosensitivity. See text.

tities required for the decisive state; and now regard h as the mean number of groups of decisive processes produced per cell per unit dose. The dose-effect relationships can now be described mathematically by the methods of conventional, formal target theory (1).

When m/r and n/s are constant among the individual cells, the survival curve in general is described by

$$\frac{N}{N_0} = 1 - [1 - Y]^{n/s} \tag{1}$$

where

$$Y = e^{-hD}\left[1 + hD + \frac{(hD)^2}{2!} + \cdots + \frac{(hD)^{\frac{m}{r}-1}}{\left(\frac{m}{r} - 1\right)!}\right] \tag{2}$$

in which expression the first m/r terms are used in any particular case.* When N/N_0, the fraction of cells not affected, is plotted against the dose D, the shape of the resulting survival curve is determined by m/r and n/s, and its slope by h. If $m/r = 1 = n/s$, the survival curve is a simple exponential:

$$\frac{N}{N_0} = e^{-hD} \tag{3}$$

If m/r, n/s, or both are greater than unity, the curve is sigmoid and its precise shape is determined by m/r and n/s. Some examples are shown in Fig. 4, along with the exponential of Eq. 3, the value of h being the same in all cases.

Analogies with Target Theory

At this point we can recognize analogies between some of our concepts and those of conventional target theory. Our precursor is the "target"; the group of r decisive processes is a "hit"; m is the number of individual decisive processes necessary to alter the target, but it is possible that these m processes may be delivered in only m/r "packets"; m/r accordingly is the "hit number" for the change of a single target (precursor) to a decisive entity; n is the number of targets which must be altered to guarantee the end effect (that is, produce the decisive state); if a group

* Equation 1 presupposes the following conditions: (*a*) whenever $m > 1$, the individual decisive processes (M, Fig. 2) operating on an individual precursor occur independently of each other and are equally probable; (*b*) the probability of irrelevant processes is large compared to that of relevant ones and is constant during irradiation.

of s targets can be changed by a sufficiently large group of decisive processes, n/s is the number of such groups of targets which must be transformed into decisive entities to produce the decisive state.

Now it is a fact that experimental survival curves frequently have shapes very similar to those of the theoretical ones, such as shown in Fig. 4. The question arises: how much information can we extract from this comparison of experimental and theoretical curves? If in our experiments we could measure both the effect and the dose to any desired precision, we could determine m/r and n/s forthwith. This, of course, would be a great help. However, in most cases our experimental accuracy is far too low to permit unequivocal determination of these quantities. Let us consider those curves in Fig. 4 which represent the survival function of Eq. 1 for the two simple cases where $m/r = 1$, $n/s = 2$ (curve 2) and $m/r = 2$, $n/s = 1$ (curve 3), the value of h being constant. When we adjust the abscissae to make the 50 per cent survival points coincide, we find that these simple curves have surprisingly similar shapes. In fact, they are so similar that currently available experimental techniques are entirely too inaccurate to enable us to distinguish between them on the basis of shape alone. If h were known, they could be distinguished by the difference in slopes of curves 2 and 3, but unfortunately the evaluation of h involves additional data which almost always are lacking.

The foregoing difficulties are enhanced when we consider that the radiobiological properties of a given cell species may vary quantitatively from individual to individual. In fact, many persons have maintained that the exponential and sigmoid shapes of experimental survival curves are entirely due to such biological variation, that is to a frequency distribution of radiosensitivities. Some notion of the validity of this claim can be gained from consideration of the frequency distributions necessary to account for the shapes of the experimental curves. For convenience let us consider experimental data which fit simple theoretical curves such as shown in Fig. 4. Then the corresponding frequency distributions are readily calculated, since each is the negative derivative of the appropriate special case of the survival function:

$$-\frac{d(N/N_0)}{dD} = -\frac{d}{dD}\left[1 - (1 - Y)^{n/s}\right] \tag{4}$$

where Y is defined by Eq. 2. In Fig. 5 are shown the frequency-distribution curves which result from assigning various small integral values to m/r and n/s. It will be noted that, whenever m/r times n/s is greater than 2, the pertinent frequency distribution has a shape which corresponds reasonably with general observations of variations in biological

magnitudes. If $m/r = 2$ and $n/s = 1$, or vice versa, the frequency distribution looks unfamiliar; and when $m/r = n/s = 1$, the distribution function is an exponential. I have never heard of an *observed* exponential frequency distribution for any biological property, and I believe I am safe in stating that, if any are reported in the literature, they are exceedingly rare. Accordingly it seems to me that frequency distribution of radiosensitivity *cannot* explain exponential survival curves, and that it is an *unlikely* explanation for survival curves formally described by Eq. 1 with $m/r = 2$ and $n/s = 1$, or vice versa. On the other hand, when the experimental data can be fitted by Eq. 1 only by using values of m/r and n/s whose product is greater than 2, our general biological experience does not enable us to rule out the frequency distribution as the cause, or at least the partial cause, of the shape of the survival curve.

However, if we adhere to our scheme of mechanism (Fig. 2), it is evident that, despite biological variation, Eq. 1 is fundamental to the dose-effect relationship. Since the dose-effect curve is determined by m/r, n/s, and h, biological variation can operate only through variations in one or more of these quantities. Any or all of these variations can be taken into theoretical account by substituting suitable summations and integrations for m/r, n/s, and h in Eq. 1. The resulting expressions are, of course, so complicated that at present there is no hope of using them in determining m/r, n/s, or h for any real radiobiological action. However, I should like to emphasize that, since a cell consists of a finite number of molecules, and since irradiation can therefore initiate only a finite number of individual series of relevant processes and states, survival curves can always be described by theoretical equations if sufficient information is available, this despite any biological variation.

Before leaving our analogies with target theory, let us briefly consider the nature of a hit. As noted above, a hit may be identified with our "decisive process" or a group of such processes. I believe that most writers on formal target theory visualize a hit to be an individual energy transfer, or group of transfers, producing one or more ionized or excited molecules, in an especially "sensitive" volume of the cell, which may be roughly identified with our "precursor" of a decisive entity. Referring to Figs. 1 and 2, we can see that a hit (or decisive process) does not have to be an initial energy transfer or an ionization; it may be a chemical reaction. Moreover, it may occur considerably later than the initial energy transfer; thus there may be time for the chemical species involved in the relevant processes to diffuse some distance from the point of energy transfer before the hit (decisive process) occurs. Accordingly the initial energy transfer does not necessarily have to occur in the "sensitive volume" (precursor).

We have just seen that at present the *absolute* shapes and slopes of dose-effect curves can yield information about mechanism only when we are dealing with radiobiological actions in which h is a constant and both m/r and n/s are very small integers. On the other hand, we have seen that in all actions the dose-effect relations must be determined by these three quantities, even though one or more of them may vary from cell to cell. Accordingly, we can often obtain useful information from *relative* slopes and shapes of dose-effect curves when they can be changed by various modifying factors.

Quantitative Changes Produced by Modifying Factors

Referring to Figs. 1 and 2, let us see how a modifying factor could change the dose-effect relations. It is conceivable that it might produce a *qualitative* change in the nature of the decisive process, even though the nature of the end effect might not be changed in any distinguishable way. However, such a drastic modification of mechanism would seem to be quite unlikely. Any changes in dose-effect relations are much more likely to stem from quantitative changes bearing on decisive processes and the decisive state. Let us inquire how such quantitative changes might affect the shape or slope of a dose-effect curve. Since the curve is determined by m/r, n/s, and h, the modifying factor clearly must operate through changes in one or more of these quantities. Both m and n obviously depend only on the natures of the biological object and of the radiobiological end effect. On the other hand, r and s can depend not only on these biological properties but also on the nature of the irradiation. Accordingly m/r and n/s can vary with the nature either of the biological system or of the physical agent. The same may be said of h, since it must be influenced by the morphological and chemical composition of the cell and since, as pointed out when h was defined, it can depend on the values of r and s. Therefore, in summary, any of the parameters m/r, n/s, and h can be changed by any factor which, in any relevant way, changes the morphological or chemical constitution of the cell or affects the nature of the individual energy transfers or their distribution in space and time. In the following paragraphs, let us bear in mind that changes in m/r and n/s are reflected in alterations of the shape of the dose-effect curve and that changes in h affect the slope.

It is often observed that different species of cells, or even different strains of the same species, yield different dose-effect curves, even though the experimental conditions and the end effect studied are identical so far as we can determine. Since even closely related but morphologically or physiologically distinguishable types of cells must differ in chemical

makeup, that is initial state, it is clear that we should not be surprised if any of the parameters m, n, r, s, and h should vary with species or strain, such variation being reflected in differences in shape and/or slope of the survival curve.

Another biological factor known to affect dose-effect relations is phase of mitosis. This factor, like species or strain, involves marked changes in the physical and chemical initial state of the cell, and again it should not be surprising if any of the five parameters varied from phase to phase of mitosis, thus changing either slope or shape of the dose-effect curve or even both.

The concentration of water in the cell is naturally of prime interest if the radiobiological mechanism involves energy transfer to water molecules as in the special model of Fig. 1. With increase in water content, h should increase, and experimentally it has been observed in various radiobiological actions that the slope of the dose-effect curve varies directly (not necessarily linearly) with water concentration.

Quite a list of simple solutes is known to affect dose-effect relations, for instance oxygen, carbon dioxide, and cysteine. Such simple molecules, added to the cell, might conceivably alter the precursor of the decisive entity in such a way as to alter m, or they might change the chemistry of the cell in some fashion to alter n, the number of decisive entities required to constitute a decisive state. (If m or n were altered, then r or s might consequently be altered.) However, in view of the suggestive findings of radiation chemistry, we are much more likely to look for an influence on h exerted through a change in relative probabilities of relevant and of competing processes, perhaps at the level of process B or C (Fig. 2). Unfortunately, in the studies to date on influence of chemical modifiers, complete survival curves have very rarely been worked out. However, complete dose-effect curves might be instructive, because distinct changes in shape would indicate changes in m/r or n/s.

Temperature, in many radiobiological actions, can influence the dose-effect relations. In most of the available papers, the data are not too plentiful, but it appears that the magnitude of the influence differs considerably among various radiobiological mechanisms, and sometimes the direction (decrease or increase) also differs. This state of affairs is not too surprising in view of the following considerations. Temperature could intervene in at least two general ways. First, it could change the chemical composition of the cell. (As a simple example, the respiration rate might change, thus changing the concentration of oxygen or carbon dioxide, and then one of these chemical factors might alter the dose-effect relations as suggested above.) Second, the variation of tempera-

ture might differentially affect the rates of the relevant and of the competing processes, thus altering h and the slope of the dose-effect curve. Since we have seen above that h is also the parameter most likely to be affected by change in chemical makeup of the cell, it appears that, by and large, temperature is much more likely to affect the slope than the shape of the dose-effect curve. Moreover, neither an increase nor a decrease in slope should be very surprising.

Another physical factor which affects dose-effect relations is the spatial distribution of the individual energy transfers from ionizing particles to molecules in the cell. I shall discuss this in terms of the *rate of energy transfer* (*RET*), which is equal to $-dE/dx$, the instantaneous loss of kinetic energy by the ionizing particle per unit length of its path. This quantity is roughly proportional to the ions produced per unit length of path (*specific ionization* or *ion density*) and has been thoroughly discussed by the physics panel.

In examining the possible effects of *RET*, it is essential to bear in mind that, at the very beginning of the action, the individual energy transfers are not spaced singly at random but are grouped along ionization tracks, the degree of grouping being dependent on *RET*. Very quickly, however, the ions and other relevant entities tend, because of diffusion, to become distributed singly at random. Let us now examine two extreme types of action. In the first, the decisive processes occur *late*, that is, after the relevant entities have become distributed singly at random. In this case, $r = 1 = s$, and therefore the shape of the dose-effect curve cannot change with *RET*. On the other hand, it is possible for h to change with *RET*, because of the latter's possible influence on the efficiency of production of earlier relevant entities, and accordingly the slope of the curve may depend on *RET*. In the second case, the decisive processes occur *early*, that is, when the relevant entities involved are not distributed singly at random but are still grouped substantially like the sites of the individual energy transfers. (This, incidentally, is the situation usually discussed by writers on conventional target theory.) In this case, as *RET* increases, r should increase, and therefore m/r should decrease, with unity as the lower limit. (Likewise, but less likely, s could increase and therefore n/s decrease.) Accordingly, with increase in *RET*, the shape of the dose-effect curve should vary toward lower hit number (except, of course, in cases where this is impossible because $m/r = 1 = n/s$ even when *RET* is minimal). The experimental literature contains examples of the foregoing two cases, some of which are summarized in Table 1. Finally, we must recognize that many radiobiological actions may not belong to either of the two extreme groups just discussed, but have properties intermediate between the two.

Another important physical factor is the time pattern of irradiation. I use this general term to include variation in dose rate and also various more or less complicated schemes of fractionated irradiation. Although, among the hundreds of papers on the subject, complete dose-effect curves are all too rarely found, the following general statements may be made (2). (*a*) A large but not overwhelming fraction of the known radiobiological actions depends more or less on the time pattern of irradiation. (*b*) As the time consumed in delivering the dose is lengthened —by decreasing the dose rate, by fractionation, or by combinations of the two—a small fraction of the time-pattern-dependent actions is enhanced; that is, the effectiveness of a given total dose increases. (*c*) On the other hand, for the overwhelming majority of time-pattern-dependent actions, the reverse is true; that is, the effectiveness of a given total dose decreases.

In terms of our model (Fig. 2), it appears that the time pattern might exert its influence in two general ways. First, the biological system might change during irradiation—either because of natural cellular activities or because of some alteration due to irradiation (possibly produced by some of the processes designated as "irrelevant" in Fig. 2)—and such a change in the biological system might develop to greater or less degree as the irradiation is prolonged. In this event, as pointed out in the discussion of influence of other biological factors, such as mitosis, it is possible for any of the parameters m, n, r, s, or h either to increase or to decrease, and accordingly either an increase or decrease of effectiveness might result from prolongation of the total irradiation period.

The second general way in which the time pattern might exert its influence is by changing h through differential alteration of the probabilities of the relevant and of the various competing processes. As in the discussion of *RET*, let us consider this possible mode of influence with respect to the time at which the decisive processes occur. If the decisive processes occur *early*, that is, when the participating entities are localized in or close to the ionization track, then each wave of relevant processes proceeding from a single track acts independently, and the time pattern can exert no influence on any of the parameters of the dose-effect relation, unless the dose rate is varied to such extremely high values that there is appreciable probability that ionization tracks may spatially coincide during their very brief lifetimes. On the other hand, if the decisive processes are *late*, the rates of the relevant and of the competing processes depend on the concentrations of the participating entities when they are distributed singly at random in the cell, and these concentrations obviously can be affected by the time pattern.

TABLE 1

EFFECTS OF RATE OF ENERGY TRANSFER (*RET*) ON PROPERTIES OF DOSE-EFFECT CURVES

Biological Object	End Effect	Variation of Dose-Effect Parameters with Increase in *RET*		Interpretation		References
		m/r and/or n/s	h	Decisive Process	Remarks	
Vegetative bacteria	Inhibition of colony formation	$m/r = 1 = n/s$	Decreases	Early		Wyckoff (5), Lea *et al.* (6)
Bacterial spores	Inhibition of colony formation	$m/r = 1 = n/s$	Constant at low *RET*; increases at high *RET*	Late	*RET* influences early relevant process(es)	Lea *et al.* (6)
Haploid yeast	Inhibition of cell division	$m/r = 1 = n/s$	Constant at low *RET*; increases at high *RET*	Late	*RET* influences early relevant process(es)	Zirkle and Tobias (7)
Diploid yeast	Inhibition of cell division	>1; constant	Constant at low *RET*; increases at high *RET*	Late	*RET* influences early relevant process(es)	Zirkle and Tobias (7)
Tradescantia	Two-break chromosome aberrations	Decrease		Early		Giles (8), Thoday (9), and others
Aspergillus spores	Inhibition of germination	Decrease	Increases	Early		Stapleton *et al.* (10), Zirkle (11)
Fern spores	Inhibition of cell division	>1; constant	Constant at low *RET*; increases at high *RET*	Late	*RET* influences early relevant process(es)	Zirkle (12)

Let us briefly examine this last notion with regard to each of the three types of competition: (*a*) irrelevant processes, (*b*) reverse processes, and (*c*) restitution (Fig. 2). (*a*) If the absolute rates of any relevant process and of the irrelevant process(es) competing for the same relevant entity are both dependent on the first power of the concentration of the relevant entity, then the relative rates of the relevant and irrelevant processes are unaffected by this concentration and therefore by the time pattern. If a more complicated situation prevails—for example, if the relevant process depends on the square, and the irrelevant on the first power, of the concentration of relevant entity, or vice versa—then one process is favored over the other by a change in time pattern, and a change in h occurs. However, there is no way to predict whether the relevant or irrelevant process will be favored and consequently whether h will be increased or decreased. (*b*) The foregoing statements apply also to the influence of time pattern on the relative rates of relevant and reverse processes. (*c*) The situation with regard to restitution is perhaps more specific. Restitution is any process (or series of processes) by which the cell tends to regain the initial state, aside from reverse processes in the strict sense used here. Restitution processes would seem to be of three possible types: (1) processes involving no radiation-produced entities; (2) processes involving relevant entities and *natural* ones; (3) processes involving two or more species of relevant entities. Type 3 would seem to be relatively so improbable that we may neglect it. In processes of type 2 it would appear extremely unlikely that more than one species of relevant entity should be involved, and therefore the reaction rate would depend on the first power of its concentration. If the relevant process involving this entity should depend on a higher power of its concentration, then the effectiveness of the radiation should decrease as the time pattern is prolonged. In restitution of type 1, the rate is completely independent of the time pattern, and accordingly, even if the relevant processes proceed at rates dependent only on the first powers of the concentrations of relevant entities, the effectiveness of the treatment is decreased by prolongation of the time pattern.

In earlier paragraphs we have seen that we cannot predict, as the time pattern is lengthened, the direction in which the irrelevant processes or reverse processes are likely to change the effectiveness. On the other hand, we have just seen that restitution is likely, in all its most probable mechanisms, to *decrease* effectiveness (h) if the time pattern is prolonged. Now we have already noted that, among the large number of radiobiological actions known to be dependent on the time pattern, all but a few decrease in efficiency with extension of the time pattern. This would seem to be an indication that the influence of time pattern is usually

exerted through restitution ("recovery") rather than through the other two types of competing processes.

So far we have seen that a study of dose-effect relations and their modifying factors can yield a limited amount of information about mechanism. In a few favorable cases, the basic shapes of dose-effect curves, and the influence of *RET* and other factors on both shape and slope, give us some ideas about the number of decisive entities and processes and about the time and place(s) at which the decisive processes occur. If water content affects the dose-effect relations, we suspect that the action involves activated water molecules; if oxygen tension has an influence, we have evidence that oxido-reduction reactions are included among the relevant processes; and so on. Undoubtedly much more information of this sort could be and is being obtained by diligent investigation of dose-effect relations and their modification. However, no amount of this type of investigation, without other methods of attack, can give us the main parts of the story: the identification of the physical and chemical natures of the relevant and competing processes and their interrelationships.

Proposals for Future Studies

At present there appear to be two theoretically possible means of accomplishing these ends (3). The first is direct: we work out the detailed history of all the chemical and morphological entities in the cell from the moment of energy transfer to the time when the end effect is observed. This obviously is the theoretically satisfactory way to do the job because, not only would radiobiological mechanism be completely understood, but so would many basic mechanisms of normal cell biology. However, in practice such a method implies advances in techniques and knowledge of cell chemistry and physics which almost certainly will never be attained in our time.

Accordingly I suspect that any advances in the reasonably near future will still be rather haphazard and will be attained largely by indirect methods. To establish by such methods the relevance of a process to any given end effect, the following general procedure would seem to be minimal.

First, a given process must be suspected of possible relevance. Our chief source of suspects naturally is radiation chemistry. Any change observed in a cellular constituent irradiated *in vitro* in an aqueous system may fairly be suspected of relevance to any radiobiological action. We have heard of many interesting examples in this symposium. However, a process which occurs *in vitro* does not necessarily occur in a cell; from

the chemical standpoint a cell is about as impure a system as one is likely to encounter. Accordingly the suspected process must be identified in the living cell. This is usually a difficult task, but with advances in biochemistry, particularly in analyses of intact cells, we may expect more information of this sort.

Unfortunately, even if a suspected process can be demonstrated to occur in the irradiated cell, this is far from adequate to establish its relevance. As we have seen, it seems highly probable that most of the processes initiated by irradiation are irrelevant to any single end effect, although they may be relevant to other effects. Accordingly, the radiobiological process suspected of relevance must be *connected* (3) by some means with other processes and states, especially the observed end effect. Since, as just noted above, we are in practice largely limited to indirect methods, it appears that the modifiers of dose-effect relations are likely to be our chief means of establishing connection. For instance, if we were studying inhibition of cell division and found that peroxide was formed in the irradiated cells before division was affected, and if further it was found that each of the various modifying factors affected peroxide formation and inhibition of cell division in essentially the same manner, we could be reasonably justified in concluding that peroxide formation was relevant to the action on cell division.

In my foregoing remarks I have pointed out certain types of information needed for an understanding of radiobiological mechanisms and some of the general methods which might be employed to obtain such information. However, I have not said much about methods at the laboratory level. There is much room for advance here, as is obvious if we cast a thoughtful glance at Figs. 1 and 2.

In the first place, we have at present only a fragmentary knowledge of the normal state of the cell. This situation will improve as general cellular biology improves. As radiobiologists we have an enormous stake in the advance of general biology.

About energy transfer (process *A*) we can infer quite a lot from observations on gaseous systems, but much valuable information is still lacking, such as (*a*) the average energy required to activate a molecule in the cell; (*b*) the relative numbers of ions and excited molecules produced; (*c*) the detailed distribution of individual energy transfers in the ionization track; (*d*) the lifetimes of the initially activated molecules; (*e*) the possible ways in which energy may be transferred from the initially activated molecules to other molecules.

At the other end of the story there is even more room for improvement. Many of the end effects which we observe and try to measure are probably the results of complicated mechanisms involving relatively

large, and possibly variable, numbers of decisive processes, decisive entities, and perhaps even decisive states. One of our greatest needs is unremitting prospecting for cellular mechanisms of a favorable type. By a favorable mechanism I mean one in which m/r and n/s are very small integers and are constant from cell to cell, in which m/r, n/s, and h can be modified by various factors, and in which the decisive state either is identical with the end effect or is separated from it by relevant processes none of which allows distortion of the dose-effect relations.* Intensive study of a few such mechanisms might do for cellular radiobiology what the exploitation of the simple line spectrum of hydrogen did for spectroscopy.

Between the beginning and the end of the mechanism we have the great gap known as the latent period. Here we face the staggering problems of identifying the physical and chemical natures of the relevant and competing processes, of determining their sequence and where they occur, and of working out the quantitative relations existing among them. As pointed out earlier, it appears at present that our readiest weapons for attack on these problems are radiation chemistry and exploitation of the factors which modify dose-effect relations.

In our present ignorance, it is impossible to state just what we need in the way of radiation chemistry. However, it is clear that we need much more information about the processes induced by radiation in aqueous solutions of cellular constituents. For instance, it would be useful to have reasonably good values for the lifetimes of H and OH in pure water and in the presence of various solutes, and to know the diffusion constants for these radicals. Moreover we need from biochemistry some methods by which we can demonstrate and even measure abnormal processes occurring in the irradiated cell. This is, of course, an exceedingly difficult requirement to meet.

In the exploitation of the modifying factors, the two elementary procedures involved in every experiment are measurement of dose and measurement of end effect. Both these measurements should be rapid and reproducible, and each should be expressible in some one unit under all experimental conditions. In both cases the present methods leave something to be desired. Dose measurements are usually fairly rapid and reproducible, but at present we have no satisfactory, to say nothing of standard, methods of measuring doses of all the high-energy radiations

* The ease with which distortion might occur can be illustrated with Fig. 1. If, despite irradiation, some of the cells should contain enough of the relevant enzyme to permit cell division to occur, even though the relevant gene were inactivated, the count of survivors would be too high and the dose-effect relations accordingly distorted.

in the same unit. This is particularly true of measurements of alpha rays, beta rays, high-energy electrons, fast neutrons, and x-rays and gamma rays of either very low or very high energy. When we consider that in many investigations of rate of energy transfer, one of our most useful modifying factors, some or all of these radiations must be employed, the handicap is obvious, and we must appeal to the physicists to eliminate it.

With regard to measurement of effect, the situation is much worse. In the first place, reproducibility is usually poor; even in favorable cases, reproducibility within 5 per cent may be considered relatively good. This may be improved by better control of environmental and genetic factors and the utilization of more favorable mechanisms, as discussed above, in quantitative studies. In the second place, measurements of effect are usually slow and laborious. This, it seems to me, is one of our chief obstacles in radiobiological research because it forces each investigator to specialize in one or a few end effects and thus narrows his experience and outlook. I have no specific suggestions; I can only urge that time and ingenuity be expended in speeding up the measurement of various effects, so that the output of each available investigator may be multiplied throughout his all-too-brief productive period.

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The Dependence of Some Biological Effects of Radiation on the Rate of Energy Loss

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A review of the published theories on biological effects of radiation reveals varying interpretations. Some people favor the so-called hit theory. Many others are inclined to favor the intermediate action theory of Dale. A small group of investigators, some of whom have expanded their views at this symposium, seek to effect a compromise between the two sets of ideas. Still there are some who see futility in attempting to bring order out of the obviously complex physical phenomena and chemical reactions which take place in the chain of events in the biological cell. These people claim that there is no reason at present to make a theory, since any theory would be an oversimplification and could not hope to explain all the facts. For the sake of discussion, one might mention some general arguments in favor of theories and define the scope of usefulness of theoretical interpretation in biology. Theories are usually proposed to reduce the complexity of observations to a few simple, logical processes. Quantitatively the theory is successful if a number of invariant quantities appear in it and if these quantities can be determined by experimentation. These invariants are constants in the equations describing the theory, and they correspond to more or less general laws or properties of the systems under investigation.

In biology at the present stage of development one must often resort to derivation of theoretical relationships based on experimental observations; thus most of the theories have empirical or semiempirical bases. In a field as highly empirical as biology, one should not immediately aim for theories that have a general validity and that hold true for all living phenomena. Rather, theories should be proposed for a limited range of phenomena and their validity tested with experiments. Thus, a model of the biological effects of radiation is proposed here, not on the assumption that it will stand unchanged for a long time to come; rather it is hoped that the model will presently help explain a limited range of

phenomena, that it may lead us to proving invariance of certain quantities and thus to more or less general biological properties. The model proposed in this paper is presented because some of the constants in the model are supposed to be invariant and they are accessible to experimental determination. This should stimulate experimenters to check the validity for the prediction of the model in a wide range of environmental conditions. In order to stimulate progress, it is helpful if deviations from the theoretical predictions of the model are experimentally detected. If this is the case and such deviations are quantitatively measured, one is usually able to evaluate a better model with greater range of validity and more detailed explanations. In the present paper the phenomenon of inhibition of cell division is examined theoretically and experimentally in a set of varied conditions. Some of the statements below are in agreement with the general scheme presented by Zirkle in the previous paper. In effect the simple assumptions underlying the theory correspond to some of the "relevant" processes in Zirkle's scheme. Some processes classified "irrelevant" are omitted. One should bear in mind, however, that for different types of biological effects different processes may prove to be relevant. Further, the irrelevant processes will also modify quantitatively the predictions of the theory; usually there is a necessity to add small corrections to account for these.

For the past twenty years those interested in the mechanism of biological effects of radiation have realized that any satisfactory theory of such effects must allow for the variation in the magnitude of radiation effects depending on the rate of energy loss (*REL*) * of the ionizing particles in the medium. By and large it was found that the quality of the effects did not vary appreciably in a wide range of *REL*, but that the sensitivity of most organisms for various types of effects did vary. The effectiveness of radiations with different *REL* is often called the relative biological effect (*RBE*). Because of difficulties in producing homogeneous radiations and uncertainties of dose measurement in the past, data were obtained only after long and tedious effort. An excellent review of the work up to 1943 was written by Zirkle (1). During the past few years experimental facilities have vastly improved, and it is now possible to obtain reliable data in a fraction of the time it would have previously taken. The author's own interest in this field was originally in the physical properties and measurement of radiations. Some of the work

* The term "rate of energy loss" refers to the quantity of energy expended in an absorbing medium as ionization or excitation by a single particle during its passage through unit thickness of an absorber.

to be described here was done in association with Zirkle. The author is greatly indebted to Zirkle for many ideas and stimulating discussions.

All charged particles transfer energy to substances in essentially the same way, by ionization and excitation. This has already been explained by Morrison. The rate of energy loss has been calculated by Bohr as a function of particle charge and velocity. Neutral radiations exert their effect mostly through their charged secondaries; thus all radiations of

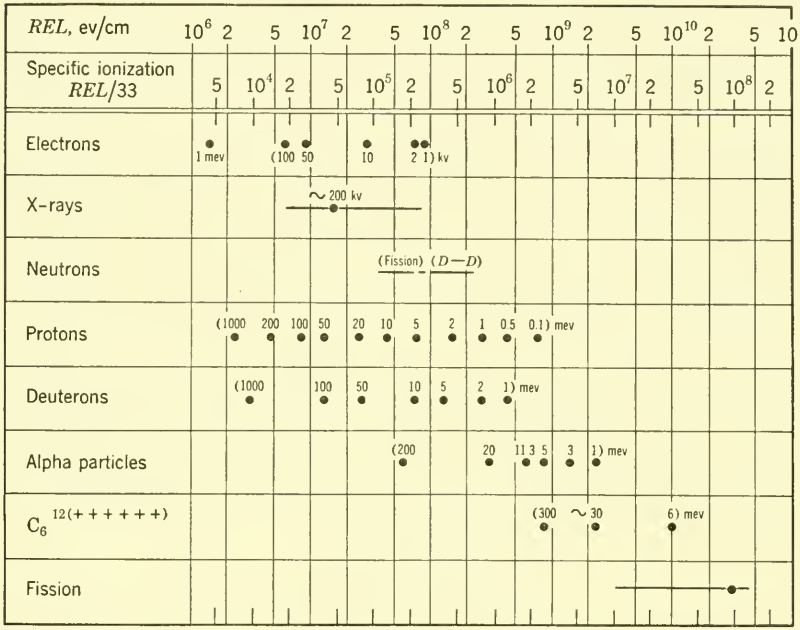

FIG. 1. Rate of energy loss from the primaries or secondaries of different types of radiations. Note that in these x-rays or neutron beams one usually obtains a wide distribution in the rate of energy loss; the data apply for water.

concern to us will be characterized by a definite rate of energy loss or by a more or less calculable range of energy losses. Since experiments have been reported with a wide variety of radiations, a nomogram correlating different radiations with their rate of energy loss in water is presented here (Fig. 1). In substances other than water the rate of energy loss has to be corrected, using the relative stopping power and the knowledge of the mode of formation of secondaries from neutral radiations. A glance at the nomogram reveals that several kinds of radiations are necessary to cover the entire range of *REL*.

Experimental Facilities

During the past two years the Berkeley 184-in. cyclotron (2) was available part of the time for biological investigations, through the courtesy of E. O. Lawrence and R. L. Thornton. The successful production of an ion beam deflected away from the magnet (3) made it possible to use this instrument for biological treatments on animals in a manner suggested by Wilson (4). In the paper by Wilson at this symposium the properties of high-energy ion beams were presented. The initial biological application of the cyclotron beam to mammals and the demonstration of effective depth doses in mouse-tumor therapy have been described (5, 6, 7). The same ion beam of protons, deuterons, and alphas may be used to advantage for biological tests in the region of *REL* 3 mev/cm tissue to 300 mev/cm tissue. Pollard (8) has recently used the deuteron beam of a small cyclotron. The Berkeley linear accelerator (9) has also become available for biological experimentation. It delivers protons at 32 mev or, if the attached Van de Graaf generator is used by itself, monoenergetic protons up to 4 mev. In the high specific ionization range, it is convenient to use polonium alpha particles, as was done in some of the experiments described below. With microorganisms of small dimensions, data may be secured up to 2 bev/cm *REL*. There are radiations available even at higher rates of energy loss. High-energy multiple ionized beams have been produced in cyclotrons (10). Slow neutron induced fission recoils (11) are also available, having a mean *REL* of 3×10^4 mev/cm tissue.

Method of Irradiation

The method of irradiation as used in the present work will now briefly be described. At the cyclotron a collimated beam of 190-mev deuterons, 380-mev helium ions, or 340-mev protons was brought out to air. The deuteron beam had intensities up to 2000 rep/min, and extended as a beam of parallel particles over a cross-sectional area somewhat larger than 1 in. diameter. This beam could be monitored in various ways. The total beam intensity (number of ions per second) was measured in a Faraday cage. The total ionization and the ionization per unit volume in air or other gases were measured in specially constructed ionization chambers. To evaluate energy absorption in tissue, experimentally measured stopping-power determinations were used (12). Several other methods are available for beam monitoring, for example measurement of the neutron flux close to the beam, fluorescent counters, and measurement of the formation of radioactive isotopes with known cross sections.

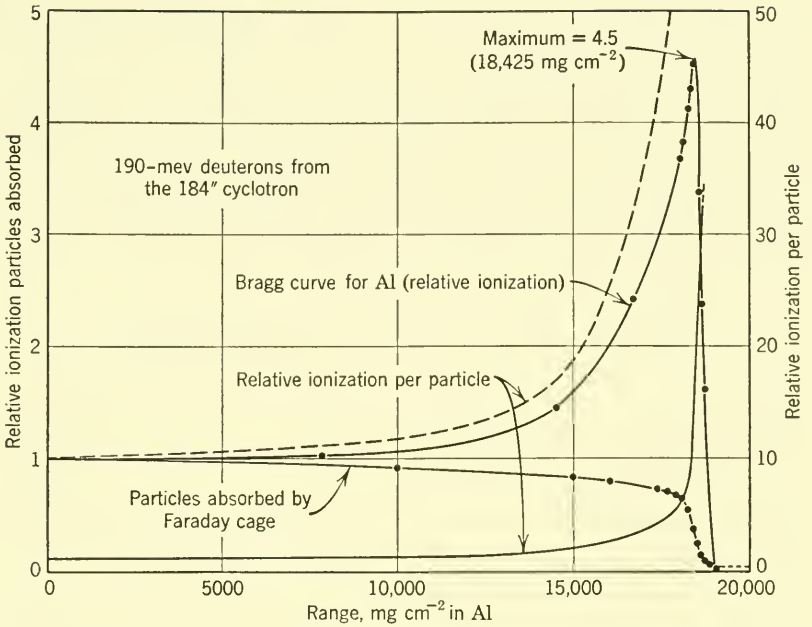

FIG. 2. Bragg curve in Al of 190-mev deuterons. The total ionization increases as the particles slow down, reaching a maximum about 4½ times the value at 190 mev. The number of particles in the beam decreases simultaneously as indicated on the Faraday curve. The mean relative ionization per particle is the ratio of the total ionization divided by the number of particles present.

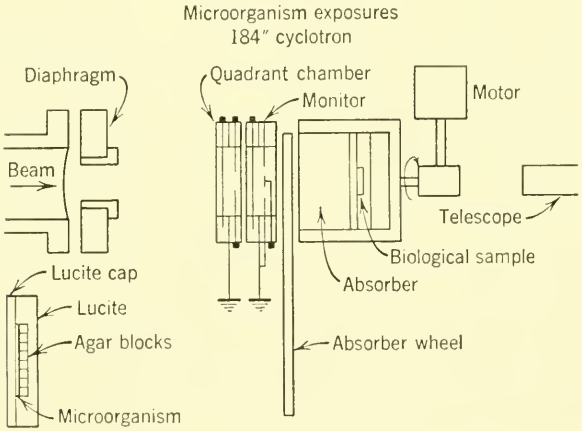

FIG. 3. The experimental arrangement for exposure of microorganisms at the 184-in. cyclotron. The beam first penetrates two monitoring ionization chambers and then passes through absorbers of variable thicknesses. The microorganisms are mounted in a Lucite chamber on agar blocks and are rotated in the beam so that all receive uniform exposure.

By placing absorbers in the beam, one obtains particles of lower energy emerging from the absorber. The mean *REL* of these will increase in measurable fashion.

Figure 2 shows the Bragg ionization curve and the range curve for deuterons and alpha particles. The mean specific ionization per particle is the ratio of these two quantities. The particular points used for experiments are indicated on the graphs. A typical schematic layout of the experiments is indicated in Fig. 3.

Biological Experiments in Progress Regarding the Effect of the Rate of Energy Loss

With the fine equipment available a number of experiments were recently performed or are being performed to provide new information or to verify old data. Among these are studies of inactivation of enzymes (Barron, Dale, Gray, Pollard), lethal effects on bacteria (Pollard, Dobson), sex-linked lethal mutations on *Drosophila* (Stern), chromosome breakages in *Tradescantia* (Giles), and skin effects on mice (Tobias). Fairly extensive information was obtained on the inhibition of cell division by radiations in yeast cells (*Saccharomyces cerevisiae*) by Zirkle and Tobias (17). These cells were particularly interesting because much earlier work was done with them by Holweck and Lacassagne (13) and Latarjet (14), who in 1940 conclusively showed that at least one strain, *S. ellipsoideus*, had multiple-hit ultraviolet survival curves. Henshaw and Turkowitz (15) showed the formation of microcolonies in *S. cervisiae*, the same strain used in the present experiments. *Saccharomyces cerevisiae* was chosen by Zirkle for a series of tests concerning the *RBE* of various radiations because, through the courtesy of Lindegren, two haploid (SC7 and SC8) and a diploid (SC6) vegetative strains were available. It was thought that a comparative study of the radiation effect would be a good test for the premises of the hit theory of the biological effect. Latarjet and Euphrussi, working along similar lines, have irradiated independently similar yeast colonies (16). Much of the discussion that follows was obtained in the course of the yeast studies and thus applies chiefly to the inhibition of cell division on these organisms. It is hoped, however, that some of the reasoning may be applied to other types of biological effects and to other organisms when sufficient data are available.

The Hit Theory

As is well known, the hit theory of biological effect of ionizing radiations is based on the idea that there exists a sensitive volume somewhere

within the cell and that one or more "events" have to occur within this volume as a result of irradiation to produce a lethal effect, a mutation, or some other measurable change. A number of authors, particularly Lea (18), have associated the "event" with the production of a single ion pair. A recent review of the target theory has been published by Atwood and others (19, 20, 21). Although recently doubts were raised by some workers, for example Opatowski (22), as to the validity of the single-event hypothesis in radiogenetics, others, for example Wijsman (23), have strengthened the mathematical basis of the single-event theory. Early success of the hit theory was due to finding survival curves for various organisms which were exponentially decaying functions of the dose (one ion pair in sensitive volume) and others which followed the multihit curves (14). However, closer examination a number of years ago revealed some discrepancies, not easily explainable in the original form of the theory. For example, the theory predicted a sharp decrease in biological effects of radiations when radiations of high specific ionization were used. Many experiments were available, however (1, 24), which showed opposite dependence on *REL* from the one expected.

Some of the proponents of the target theory have identified the target with the size of a gene (25, 18). Calculations based on dose-effect relationships have come within a small factor of the size of objects which resemble genes. However, as early as 1936 Timoféeff-Ressovsky (26) and Delbrück questioned this hypothesis. Sommermeyer (27) has postulated that ionization within a gene may produce mutations with a probability considerably less than unity. Lea and Catcheside (28) assumed that an ionization outside a gene may also produce mutation. Fano (29) reasoned that, if the dose-effect relationship is a single exponential, the passage of a single ionizing particle must be sufficient to cause mutation; therefore, a single ionization, or at most two, or a cluster of ions, is sufficient to cause mutation. The question to be resolved, then, remains the probability with which this process may occur. At this symposium Fano gave a lucid presentation of the present status of the theory of ion-cluster formation.

Intermediate Action Theory

Independent of the hit theory, certain biochemical effects on enzymes may be explained on the basis of the "intermediate action" theory of Dale (30). He and others, notably Barron and his associates, have conclusively shown that the biochemical action of radiations in aqueous solutions depends greatly on the interaction of radiations with water. Physical chemistry of the radiation effects on solutions has

taught us that water ions themselves last for very short time intervals after their production. Dissociation occurs, giving rise to H and OH radicals; these in turn may combine with each other and with cellular constituents. Dale attempted to explain the general shapes of dose-effect curves as well. He showed that monomolecular reactions lead to exponential dose-effect relationships, curves similar to those one obtains in the "one-hit hypothesis"; further, he has shown that, if there are two substances competing for ionization products, the dose-effect relationship becomes very similar to the multiple-hit-type functions of the target theory.

Comparison of Two Theories in the Light of Experiments on Yeast Cells

In the experiments described here the dose dependence of the survival of haploid colonies was compared to that of diploid colonies. If the indirect-action theory were true, one would expect the two survival

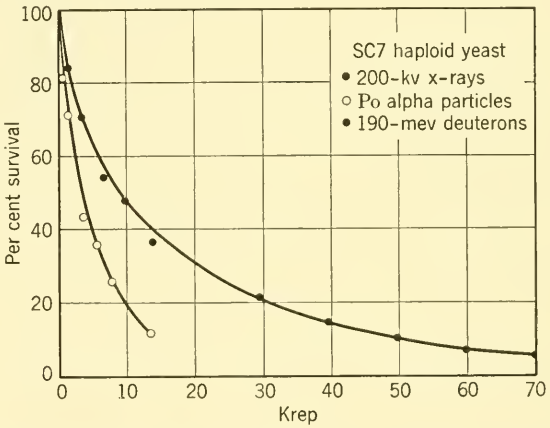

Fig. 4. Survival of haploid yeast cells as a function of dose when x-rays and alpha particles are used. The curves indicate the percentage of cells that divide more than once after irradiation. Single-hit-type curves.

curves to be qualitatively the same. Since the biochemical constitutions of the haploid and diploid cells are assumed to be similar, the survival curve of both colonies might show monomolecular reactions, or both might exhibit the presence of some protective substances. Actually the haploid cells showed the simple exponential type of survival vs. dose relationship (monomolecular reaction) in a wide range of *REL*. The survival curve of diploid cells showed some kind of "protection" effect

over the whole range of ionization. For typical survival curves see Figs. 4 and 5. For the moment one would be inclined to prefer the hit theory for simultaneous explanation of haploid- and diploid-cell-survival curves if it were not for the fact that the comparison of haploid and diploid survival repeated at different *REL* yielded data which were not in good agreement with the classical target idea. The radiations at high *REL* were more effective than those at low *REL*. The shape of the survival

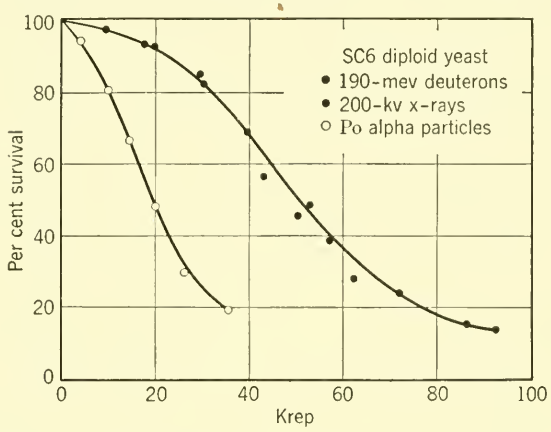

FIG. 5. Survival of diploid yeast cells exposed to deuterons, x-rays, and alpha particles. Cells are taken as survivals if they divide more than once after exposure to radiation. Multiple-hit curves are obtained.

curve for both haploid and diploid cells, however, remained approximately the same irrespective of the *REL* within the entire ionization region tested. In the course of this investigation it became clear that the two different theories may both apply to certain aspects of the same irradiation problem. In fact it seemed desirable to attempt the simultaneous use of both models of the radiation effect. In some respects this view is similar to some of the concepts advanced by others, particularly by Barron and Zirkle.

DIFFUSION MODEL

The physicist in radiobiology should be interested in the dynamics of all processes involved, even beyond the mechanism of formation of ion pairs. He should be able to make contributions to our knowledge of the formation, distribution, and absorption of ion products, and the fate of excited molecules. In a complex system such as the cell, one has to be satisfied with the crudest of approximations. The inhibition of cell

division is almost certainly a chain reaction initiated by changes in a few molecules. How such chains of events develop in the cell we do not know. All one may do is to trace out a few of the initial steps that occur between irradiation and inactivation of essential molecules. In organisms having high percentage of water one may draw an analogy between radiation effects on water and on the cell.

The physical basis of the diffusion model is very simple. In the process of ionization, the lifetime of the ion pairs and excited atoms formed is very short. Through mechanisms discussed by the radiation chemistry section, the primary initial positive ion and negative electron and some of the excited molecules may within a very short time interval give rise to radicals and by chemical combination of these to more or less stable molecules such as peroxides, which because of their ability to interact further, act as "intermediates" in the production of the biological effects. The exact chemical properties of these ionization products are of no significance at the moment, except that it is important to find out how many ion pairs are needed to form each intermediate ionization product. This depends on the spacing of individual ion pairs of the positive and negative ion columns, and on the chemical composition of the medium.

The intermediate ionization products are usually longer lived than the ion pairs themselves, and this is one reason why they are conceivably of importance. Because of their thermal Brownian motion, they will migrate through the medium until they find suitable molecules for interaction, molecules from the cell medium or other ionization products. Even if it is accepted that free radicals and other intermediates are the most frequent immediate result of ionization, one immediately gets into serious difficulties when one attempts to predict their physical and chemical properties. The intermediates can obviously migrate, but what are the laws of such migration? Even in pure water prediction of the behavior of the radicals is a serious problem. Dissolved substances are in a "solvent cage." When they migrate through the medium they carry some water molecules along. When they find another solute molecule in their migration, the two solute molecules may stay together in the solvent cage for a reasonably long time, which they would be unlikely to do if the approach of the molecules were in the gas phase. In living protein, the laws of migration may be much more complex than those for water. If one accepts some of the views of Delbrück (39), one is forced to admit that protoplasmic structure and behavior may be very different in live cells and *in vitro*. Not knowing the mechanism of migration and of interaction of solutes in cellular matter, one might proceed by assuming the simplest admissible relationships and attempt to make predictions based on these. Thus one might assume that in cells the

intermediate products of ionization are much like those in water; that they migrate and diffuse freely through the cellular medium; and that, when they interact, such interactions are of the oxidation-reduction type. These interactions may give rise to biochemical effects and some grossly observable physical cytoplasmic effects of radiation. When intermediates migrate close to nuclear material, genetic and in some cases lethal effects can occur. These effects are thus intermediate effects of radiation because they do not result from direct action of ionization but rather are due to chemical action of the intermediates. While such intermediate chemical reactions may occur quite frequently, in some cases biological effects may occur as a primary result of ionization produced in the immediate vicinity of the same cellular components. Such effects are the "direct-hit" type.

Table 1 indicates the parallelism of the mechanism of effect for extragenic and genic components of living cells. The two sets of effects may

TABLE 1

MECHANISM OF EXTRAGENIC AND GENIC EFFECTS OF RADIATION

Extragenic effects are the result of:	*Genic effects are the result of:*
1. Direct ionization or excitation of molecules. The chance for this is usually small.	1. Direct hit. Ionization or excitation of essential sites. The chance for this is usually small.
2. Indirect mechanism; chemical interaction with ionization products. This occurs frequently, but many molecules have to be inactivated before measurable biochemical deficiency occurs. Recovery may follow if essential genes are still intact. Lethal effect may result if too many molecules become inactivated.	2. Indirect action. Some of the ionization products may diffuse to the proper site through extragenic medium and interact, leading to inactivation of genes. The inactivation of a single gene may lead to lethal or inheritable changes. Ionization products causing genic effects may have originated from ion pairs located at considerable distance from the essential sites.

develop simultaneously, and with a given organism either type may dominate the other. However, the comparative importance of these effects is indicated by the following postulates:

A. In genes essential to cell division, often only a single inactivation needs to occur for inhibition of cell division.

B. The mean dose at which a single genetic effect will occur depends on extragenic composition of tissue.

Having outlined the plausibility of similarity between radiation action on genes and that on extragenic factors, we may now proceed to formulate the diffusion model of radiation effects in simple mathematical terms.

Assume a given cell with a single, genic "site" to be affected by radiation, mediated by a known intermediate ionization product P (for example, OH, HO_2, H_2O_2). Figure 6 will give a description of its path through the cell medium. P will migrate through the fluid medium of the cell along an irregular Brownian path until it has a chance to interact with some cytoplasmic constituent R, forming a compound to be designated PR; this will happen at a radial distance r from the point where the ion

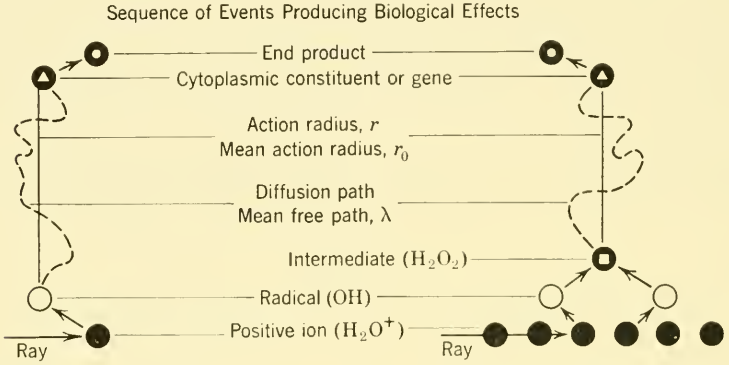

FIG. 6. Graphic presentation of the sequence of events in the diffusion model. *Left side:* The positive ions, after their production by the ionizing rays, decompose into radicals and other ions. The radicals chemically responsible for the radiation effect diffuse through the medium of the cell along an irregular path. Their mean free path for chemical interaction is λ. Most of the time these radicals are annihilated in a reaction with some extragenic molecule. Occasionally they interact with a genic molecule. *Right side:* When densely ionizing beams are available, or in ion clusters, different types of intermediates may be produced; for example, H_2O_2 is formed in water. The intermediate molecules may then diffuse and act similarly to the radicals.

pair and shortly thereafter P were formed. According to procedures of statistical mechanics the probability that P(r), the intermediate, will diffuse at least to a distance r will be

$$P(r) = e^{-r^2/\rho^2} \tag{1}$$

where ρ is a constant $\rho^2 = 4\delta\tau$; τ is the mean life of the radical, and δ is its diffusion constant through the medium.

Assuming a stationary sensitive site, a gene for example, it is easy to calculate the mean number of intermediate ionization products to diffuse to the site of the gene when ionization was produced at random in the medium. If a dose of D ev per cm^3 is given, and the energy per ion pair is w ev, and if β fraction of the ion pairs produces the intermediate P,

then the mean number of radicals $M(D)$ diffusing to the sensitive site of cross section σ becomes

$$M(D) = \frac{D\beta\sigma\rho}{w} \tag{2}$$

If a single molecule of the intermediate P is sufficient to inactivate a site essential to cell division, then the number N of surviving cells is expressed by the equation:

$$N = N_0 e^{-D\beta\sigma\rho/w} \tag{3}$$

The right side of Eq. 3 is a simple decaying exponential function of the dose, resembling the single-hit survival curve of the target theory and the monomolecular reactions in the intermediate theory. If there are two kinds of intermediates with different ionic yields β_1, β_2 and mean action radii ρ_1, ρ_2, the survival curve may be expressed as

$$N = N_0 e^{-D/(\beta_1\sigma_1\rho_1+\beta_2\sigma_2\rho_2)/w} \tag{4}$$

Multiple-hit forms of the survival formula are also readily obtained. Examination of Eq. 3 is worth while because this simple equation gives precise and possibly measurable definition to certain quantities often mentioned or described in radiobiology.

The expression $D\beta/w$ is the number of ionization products formed per unit volume in the medium, when exposed to D/w ion pairs; β is then the "ionic" efficiency of formation of intermediates such as radicals H, OH, O_2H, molecules H_2O_2, H_2S_2, or other compounds capable of migration. If the ions themselves are direct causes of the radiation effects, β may be taken equal to 1. The ionic efficiency obviously depends on the chemical composition of the medium and on the mean rate of energy loss of the ionizing particles; $\beta = \beta(\epsilon)$.

The product $\sigma \cdot \rho$ is analogous but not identical to the sensitive volume of the target theory. In the diffusion model σ is the "cross section" of the sensitive site. If inactivation occurs every time an intermediate finds itself at the sensitive site, then σ corresponds to the true, geometrical cross section. In other cases σ will be smaller than the geometrical cross section. The concept of σ is analogous to the term cross section as it is used in nuclear physics. σ may include the "cage factor," or even conceivably a delayed recovery effect. ρ has the dimension of a distance; it expresses the mean radius to which the radicals diffuse from the site of their formation before they disappear because of chemical interaction. We have called ρ the "mean action radius" of an intermediate. Equation 1 shows the connection between the mean lifetime, the diffusion constant, and ρ. As a rule, in wet tissues $\sigma \cdot \rho$ is greater than V, the volume of the "sensitive" site of the target theory.

Both the ionic efficiency β of an intermediate P and its mean action radius ρ are functions of the composition of the medium. In the limiting case of dry organisms, or viruses, or crystalline enzymes, etc., very little migration should take place; the mean action radius will then for all practical purposes define the size of the target volume in the sense of the classical hit theory. As water is added, the mean action radius may increase, so that the slope of the survival curve will not immediately correspond to the true "sensitive volume." One expects that the same organism would be more sensitive to radiation in its hydrated, wet form than in its dry state. The sensitive volumes obtained in some earlier work for various organisms should be re-examined in terms of the diffusion model. There are some cells, among them yeasts, that may be dried and irradiated in that form. Some work along this line was done by Failla (35), and suggestive data were given by Dale at this symposium for enzymes with different water dilution. In the diffusion model most ion pairs or their products, the intermediates, end their life by extragenic interaction. As long as the intermediates originate reasonably close to a sensitive site of the cell, they have some chance to reach this site in the course of their migration.

It seems possible that the diffusion model can be intelligently used for evaluating changes in the radiosensitivity of cells due to their state of division or to changes in the external medium. For example, the introduction of dissolved oxygen into an oxygen-free medium may change the ionic yield and mean action radius of H_2O_2, OH, and O_2H. Using radiations with low *REL* in the presence of oxygen increases the ionic yield of OH and HO_2; most tested organisms, indeed, have shown greater sensitivity to radiation in oxygenated media than in oxygen-free media. At high *REL*, however, the presence of oxygen does not alter the tendency to form peroxides and the yield of OH and O_2H will be small. Presence of oxygen should have less effect on radiosensitivity at high *REL*. The intelligent use of approximate knowledge of ionic yields should be helpful, even in the absence of detailed chemical knowledge, in predicting radiation protection of added substrates on living organisms.

There is a continuity of procedure between evaluating the magnitude of radiation effects on isolated sensitive sites and extragenic effects on cytoplasmic constituents, for example enzymes. In the latter case a fairly large number of individual enzyme molecules may be present in the cell, and a known or measurable fraction of these may be inactivated by the radiation.

Calculations based on the diffusion model can provide varying shapes of survival curves, usually single- or multiple-hit curves.

Recently much emphasis was placed on the findings that some externally applied chemical agents have biological effects qualitatively similar to those of radiation, particularly as far as inhibition of cell division and production of genetic effects are concerned. Such situations may be also handled similarly to the above treatment, using the diffusion model. The chief difference is in the boundary values of the diffusion

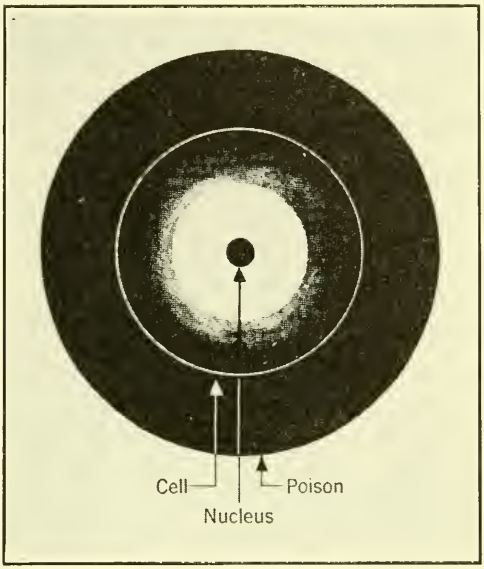

FIG. 7. Diffusion model of the action of a poison on the cell. When the cell is placed in a medium containing a uniform concentration of poison, migration of the poison through the cell wall and cytoplasm begins toward the nucleus. This figure is a graphic representation of the concentration gradient of the poison in the cell. Whether or not the effect is genic, dose-effect relationships may be calculated using the diffusion model with the proper boundary conditions.

problem. Figure 7 shows a crude representation of this problem. When a cell is placed in a medium containing the external agent, diffusion through the cell membrane into the interior of the cell begins; the density of the blackening in Fig. 7 shows a plausible concentration distribution of the poison in the process of diffusing to the nucleus of the cell. Consideration of the figure shows that, because of the larger volume of the cytoplasm to be crossed by the poison, the shape of the "survival" curve as a function of the amount and duration of poisoning might be different from the radiation survivals, and also that in some cases the cell wall and cytoplasmic constituents might effectively protect the nucleus even at the expense of their own destruction. Some German

workers (40) have applied the target hypothesis to this problem. More quantitative experiments are needed to uncover the mechanism of action of poisonous agents applied externally to living cells.

Diffusion Model and Dependence of Biological Effects on Specific Ionization

The above considerations introduce two additional constants in the radiation survival formulas. One reason for allowing these additional constants in the formulas was to allow variation of the biological effectiveness of radiations at different environmental conditions and at

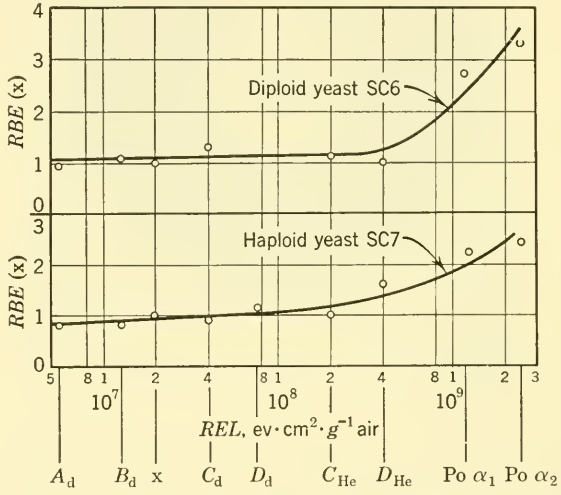

Fig. 8. Experimental data on the relative biological effect of various radiations on haploid and diploid yeast cells. Along the horizontal axis the rate of energy loss is plotted, and along the vertical axis the relative biological effect refers to 200-kev x-rays. Four experimental points were obtained with high-energy deuterons, two with high-energy helium ions, and two with polonium alpha particles. [From (17).]

different mean rates of energy loss. From data obtained under widely varying conditions, one may possibly determine the constants β, ρ, σ. As an example of the type of data one obtains, consider the comparison of the relative effectiveness of deuteron ions, x-rays, helium ions, and low-energy alpha particles on yeast cells, as shown in Fig. 8. The curves show an increase in relative biological effectiveness paralleling an increase in rate of energy loss of the radiations used. These data themselves are not striking, since the majority of the experiments available show such an effect.

We should now define relative biological effectiveness Φ of a radiation with *REL* ε in terms of that of a standard radiation, for example 200-kv x-rays with *REL* ε_0. The same biological effect is caused by dose D of ε, and dose D_0 of ε_0.

$$\Phi = \frac{D_0}{D} \tag{5}$$

In the simplest case, and neglecting the distribution of ions in tracks, the criterion might be the survival of a given fraction of cells having a single sensitive site. For this case from Eq. 3

$$\Phi = \frac{\beta\rho\sigma}{\beta_0\rho_0\sigma_0}$$

or, with $\sigma = \sigma_0$,

$$\Phi = \frac{\beta \cdot \rho}{\beta_0\rho_0} \tag{6}$$

The mathematical considerations involved in predicting the *RBE* with radiations of different *REL* become involved, since one should remember at this point that ionizing radiations produce entire tracks of ions simultaneously, not single ion pairs as it was assumed up to the present. It is necessary to calculate the probability of diffusion of intermediates to a certain distance from the center of the track. This can be done, however. It turns out that, if a sensitive site is close to densely ionizing tracks, then there is a high probability of its becoming inactivated. However, if the ion density becomes very high, some of the ions in the track will be wasted, since a single track cannot be responsible for more than the inactivation of a single cell.

It is possible to obtain approximate mathematical expressions for the relative biological effectiveness, using the diffusion model and assuming values for the ionic yield of different intermediates that are likely to be present; further, one has to consider the heterogeneous distribution of the ionization along the tracks.

If intermediates due to formation of single ion pairs only are considered, the diffusion model does not explain a rise in biological effectiveness of radiations when the rate of energy loss is increased. In order to account for the numerous older and for the present experimental findings, one is forced to assume the action of intermediates, the yield of which increases with specific ionization. In the example under discussion at present, since the water content of the cell is important one might draw a cautious analogy with the example of decomposition of water. At low *REL* the ionic yield of peroxide in pure unoxygenated water is low. At high *REL* the ionic yield of peroxide is high.

For the purpose of quantitative comparisons it seems worth while to assume a simple mathematical expression for the ionic yields of the intermediates requiring ion pairs. Exact derivation of the yield is not an easy matter when ion clusters and diffusion theory are taken into account. Progress is being made in this field by Landau (31) and Wijsman (32).

Lind (33) has shown that the ionic yield of H_2O_2 in oxygen-free pure water is close to unity in a range of energies where alpha rays are used. Toulis (34) has studied the yield as a function of proton energy in the absence of dissolved material. Although more extensive studies are desirable, the data available indicate that H_2O_2 yield remains low at proton energies above $\sim$6 mev; below this it rapidly rises until a plateau is reached with ionic yield of $\sim$½ with low-energy alpha particles. A formula was obtained for the H_2O_2 yield, β_2, by assuming that the probability of peroxide formation in water is 1 if the distance between two ion pairs formed is less than a critical distance r_0, and that the probability is zero if the distance between ion pairs is greater than r_0.

$$\beta_2 = \frac{1 - e^{-\epsilon r_0/w}\left(1 + \dfrac{\epsilon r_0}{w}\right)}{2} \tag{7}$$

where ϵ is the rate of energy loss, and w the energy required per ion pair. This formula is admittedly poor, and there is no doubt that further refinement is needed when more exact knowledge of ionic spacing (clusters), cage factors, and back reactions is available. However, until something better comes along, this formula may be helpful in qualitative studies of the mechanism of radiation effects. When the ion spacing is large, a more exact formula, derived by Wijsman and based on diffusion considerations, may be used. This yields the probability for H_2O_2 formation in oxygen-free water as

$$\beta_2 = \frac{1}{2}\frac{r_0^2\epsilon^2}{w^2}\left(1 - \epsilon rf\left\{\frac{\dfrac{w}{\epsilon} - r_0}{2\sqrt{\delta\tau}}\right\}\right) \tag{8}$$

where δ is the diffusion constant and τ the mean life of OH radicals.

To account for the observations of the change of *RBE* as a function of *REL* in yeast cells, one assumes now that OH and HO_2 radicals can cause inhibition, as well as intermediates resulting from closely spaced ion pairs.

Both the organisms tested indicate that the mean action radius of an H_2O_2-like product in the cells is much greater than that of the ions or

radicals themselves. Assuming for the OH radicals a lifetime akin to that in pure water of 10^{-7} sec, one may calculate that the cross section of a sensitive site might be only of the order of 10^{-16} cm^2 in the yeast cell. The radius computed from this reminds one more of the size of a bond than the size of a molecule! However, the cross section may include some unknown factors. There is a possibility that when an intermediate arrives at a sensitive site the probability of its reacting is less than unity. In any case, our hypothetical model of a sensitive site in the yeast cell also gives some information on the mean action radius of H_2O_2-like radicals, which should be of the order of 10^{-4} cm. Dimensions of the order of 10^{-6} cm result for the mean action radius of OH radicals.

Although the above-outlined ideas represent an attempt to put biological observations on a semiquantitative basis, it is clear that with the availability of better data more detailed and informative physical models could be constructed. The insight into the mechanisms would be materially helped if survival data were available in detail over a wide range of energy loss on several organisms, in particular (*a*) with certain dissolved substrates, for example oxygen, nitrogen, hydrogen; (*b*) at different temperatures; (*c*) with different amounts of water in the cells; (*d*) at different dose rates; (*e*) at rates of energy loss higher than those commonly used. The mechanism of biological effects will not be clear until enough of these experiments have been done. Preferably all these data should be available for a number of different organisms.

Number and Meaning of Sensitive Sites in Cells

In all the above discussion the existence of a single sensitive site * was assumed without clearly stating the recent evidence for such sites. The advantage of using the diffusion model for fitting survival curves of different kinds is that formally it can be used much like the target theory when interactions of single sites are involved. Since the observed curves were exponential type for the haploid cells and multiple-action type for the diploid cells, the curves were next fitted assuming that one sensitive site existed in the haploid cells (nuclear material damaged anywhere) and two in the diploids. It may be stated definitely that this idea did not fit the data. Next a model, originally proposed by Luria and

* The name "site" was chosen to distinguish the present form of theory from previous forms. Words like "target" and "hit" were avoided, since the radiation according to the concepts outlined does not often produce "hits" in "targets." Since we do not know the exact nature of "sites," they are not further specified. A site may correspond to one of several bonds, to a molecule, a gene, a chromosome arm, or some cell component as yet unknown.

Dulbecco (36) for ultraviolet-ray-induced effects, was tried. This model was used when the lethality of ultraviolet rays was tested on bacteriophage, attached singly or doubly to bacteria. Applying the model to the haploid yeast cell, we may calculate the number of cells N which survive and form large colonies, when initially N_0 are irradiated by dose D.

$$N = N_0 e^{-n\alpha D} \tag{9}$$

where n = number of independent sites essential to cell division and inactivated by a single intermediate molecule or radical.
$\alpha = \beta\sigma\rho/w$ = constant (see Eq. 3).
D = dose.

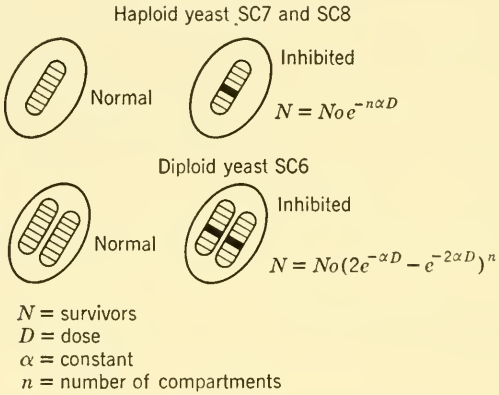

Fig. 9. Schematic presentation of model for inhibition of haploid and diploid yeast cells by radiation. It is assumed that the haploid cell has n independent sites essential to cell division. If any one of these sites is inhibited as shown by the black spot in the haploid cell in the upper right part of the figure, the cells will stop dividing. In the diploid yeast cells the number of essential sites is doubled; here a pair of essential sites has to be inhibited before cell division stops.

For similar cells of a ploidy m

$$N = N_0[1 - (1 - e^{-\alpha D})^m]^n \tag{10}$$

which for the diploid cell becomes

$$N = N_0(2e^{-\alpha D} - e^{-2\alpha D})^n \tag{11}$$

For graphic explanation see Fig. 9. With the survival data (Figs. 4 and 5) it was possible to plot the theoretical haploid-diploid survival curves as shown in Fig. 10 for different values of n. One may notice that the shape of the curve and the D_{50} are sensitive functions of n. The theoretical ratio of Diploid D_{50}/Haploid D_{50} is plotted in Fig. 11. Even if

the survival curves are not very accurate, as in the present experiment, the value n may be obtained. At the present time we believe that n is about 20 for *S. cerevisiae*. Thus, if the model used were true, one might

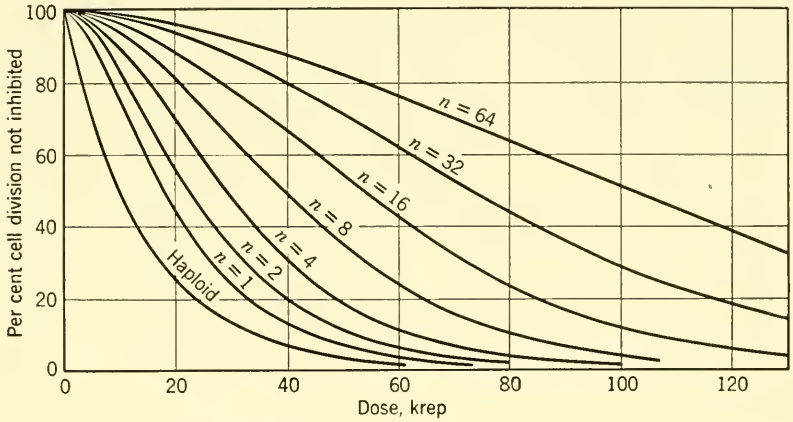

FIG. 10. Theoretical survival curves for haploid and diploid yeast cells adjusted to fit the experimental curve for haploids. The possible diploid survival curves are plotted for $n = 1$, 2, etc., up to $n = 64$.

make the following statement: "Haploid yeast cells under the experimental conditions applied exhibited approximately 20 radiation-sensitive sites essential to cell division. If any one of the sites is inacti-

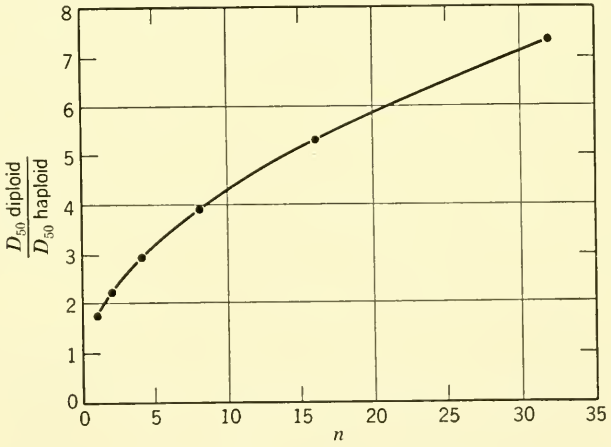

FIG. 11. The ratio of doses needed for 50 per cent inhibition of cell division, D_{50} diploid/D_{50} haploid, is plotted here as a function of the number of essential sites n. As it may be seen from the curve, this ratio is quite sensitive for the number of essential sites.

vated, the cell will not form macrocolonies." Notice that "site" has very little to do with target; ions do not have to hit the site.

A pair of corresponding sites has to be inactivated out of about 20 pairs of sites before inhibition of colony growth occurs in diploid yeast cells. When development of the yeast cells is observed under the microscope, it is found that inhibition of cell division occurs only after a number of cell divisions have taken place. Microcolonies develop; inhibited haploid cells divide usually only once after irradiation; inhibited diploids may divide several times before growth of the microcolony ceases. The rate of growth of inhibited cells and also of a few cells which will eventually grow into macrocolonies is slowed down considerably. It is thought that there are important cytoplasmic effects along with the damage to the essential sites. In addition there may be non-lethal damage to a number of genes in the cell. With only the haploid and diploid strains available, the above-advanced theory of inhibition of cell division seemed plausible but not conclusive. More evidence was added in favor of this theory by Mortimer (37), who, following a method given by Subramaniom (38), isolated some apparently multiploid yeast cells. Such cells have been obtained so far by two different methods: treatment with acenaphthene and isolation from preirradiated diploid yeasts. These appeared to be morphologically like the tetraploid yeasts of Subramaniom, although to date we have not succeeded in observing the chromosomes in the microscope or in obtaining genetic proof of ploidy. The inhibition of cell division in these cells of higher ploidy follows reasonably well what is expected for tetraploid yeasts on the basis of Eq. 10. In fact, the experimental data seem rather amazing (Fig. 12): the tetraploid yeast cells need 140,000 rep units of x-rays for 50 per cent inhibition of cell division, which is 4 times the dose required by the diploid cells and 20 times the dose required by the haploid cells. Morphologically, and as far as general viability is concerned, there seems very little difference in the yeast cells of different ploidy. These experiments indicate that at least in yeast cells radiation injury of the genetic mechanism, although mediated by the cytoplasm, is a much more important factor than extragenic radiation injury.

What is the nature of the n essential sites of radiation injury? Although no direct evidence is available, it is plausible to assume that the sites are part of the chromosomes or genes. The damage might well be chromosome break or gene mutation. Further work is needed to identify the nature of the sites and to determine whether or not there is the same probability for inactivating each of the sites. One of the requirements for a satisfactory theory of radiation effects is that the explanation offered should hold when the experiments are repeated under different

environmental conditions. Already data are available which indicate at least limited invariance of the relative shape of survival curves. There is a set of experiments available at different rates of energy loss. The relative shapes of haploid and diploid survival curves in all these experiments are substantially the same even though the relative biological effectiveness, that is the amount of dose required, changes as a function of *REL*. Further, these organisms were tested with x-rays

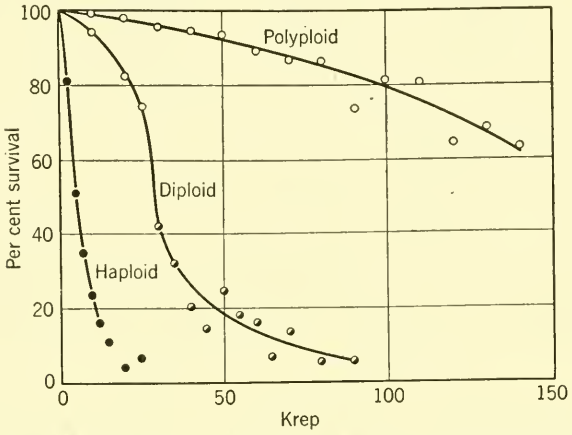

FIG. 12. Recently a tetraploid yeast colony was isolated and tested for survival with x-rays. The respective haploid, diploid, and polyploid survival curves are shown for cells which divided more than once. The tetraploid curve comes reasonably near the theoretical relationship based on the assumption that a quadruplet of essential sites out of about 16 quadruplets of sites has to be inactivated before the cell division of tetraploid cells is inhibited. The dose as given in this graph is slightly different from those in Figs. 4 and 5. Data in this graph were counted after 2 days' incubation; Figs. 4 and 5 refer to data counted after 3 days' incubation.

when they had dissolved helium, air, or oxygen gas present in their cytoplasm. Much less dose was required in oxygen to produce inhibition than in helium, but the relative shapes of survival curves, thus the apparent number of essential sites, remained the same in each experiment.

The shape of the survival curves having been established, further methods were sought in an effort to test the theory of inactivation. The study of cells which were damaged but which survived the radiation offers a convenient step in this process. In order to explain the mechanism of action of radiation it was assumed that diploid cells with unpaired defects survive. These cells are damaged but viable. If the damage is genetic, as assumed above, it is likely to be inherited in a vegetative colony as a recessive defect. Colonies grown from preirradi-

ated single cells with unpaired recessive defects may be reirradiated. The sites of pairs where unpaired defects occur should be more sensitive to radiation than undamaged pairs of sites. Figure 13 graphically illustrates the presence of weak spots in the damaged cells. The survival

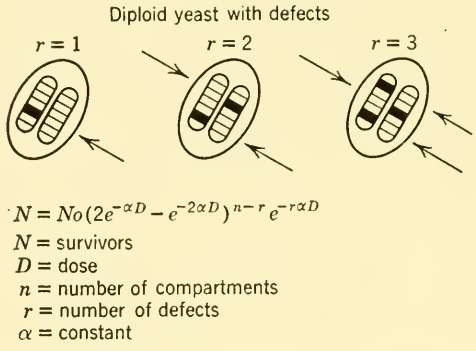

Fig. 13. In the diploid yeast cells which survive, the radiation produces some unpaired defects but no paired ones. The graph shows, in a schematic fashion, different types of diploid cells having unpaired defects. The weak spots in the defective cells are indicated by arrows.

curve of a diploid cell with n essential sites and r unpaired recessive defects can be described by the equation:

$$N = N_0(2e^{-\alpha D} - e^{-2\alpha D})^{n-r} \cdot e^{-r\alpha D} \tag{12}$$

A family of such survival curves is shown in Fig. 14. Further, if our hypothesis is true, the frequency with which defective cells occur in a

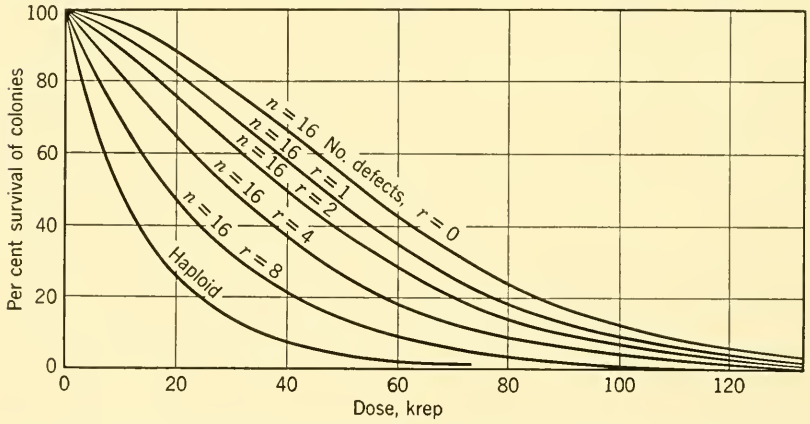

Fig. 14. Theoretical survival curves for diploid yeast cells with unpaired defects and normal diploid and haploid survival curves.

preirradiated diploid colony may be also calculated (Fig. 15). For 6 months experiments were carried out on diploid colonies grown from preirradiated single diploid cells. A number of colonies were found to show defective survival curves. These colonies are demonstrated easily because the initial slope of their radiation-survival curves differs from zero, which is the slope of normal diploid colonies. An example is shown

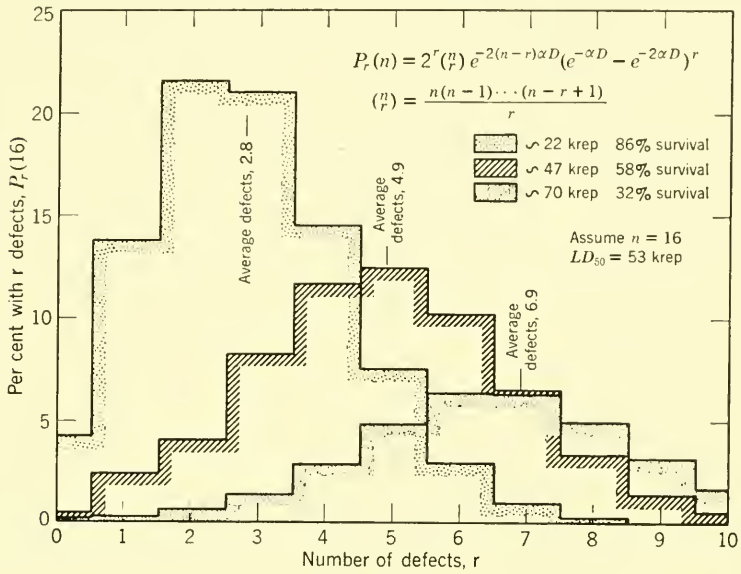

FIG. 15. Theoretical distribution of diploid yeast cells with unpaired defects produced after single irradiation of normal diploids.

in Fig. 16. However, none of these colonies retained their defective properties for more than 5 weeks. Some mechanism appears to be present which allows regeneration of normal diploid cells, which then outgrow in numbers their recessive sisters. Nevertheless, there is hope that stable and recessively defective diploid yeast colonies will be available for future study. It is interesting that preirradiated haploid cells show the same survival curves when irradiated again as normal haploids, a finding that strengthens the explanation given. In other words, for haploid cells, inactivation of a single site leads to complete inhibition of cell division. At the present time it is not known whether the probability of inactivating each of the n essential sites is the same or not. It is likely that the probability will be different for each site, the overall effect being a small deviation in the shape of the diploid survival curve. The deviations in the shape will also show up in the cells with

recessive defects. Detailed and statistical studies are needed to obtain the exact shapes of the survival curves. One might ask what the survival curves would look like if the medium on which the cells grow were changed in composition. There is some preliminary evidence that the shapes of the survival curves change for a medium different from the one used in this study. Such a change is expected because it is assumed that a cell may have more than one mechanism available to produce the

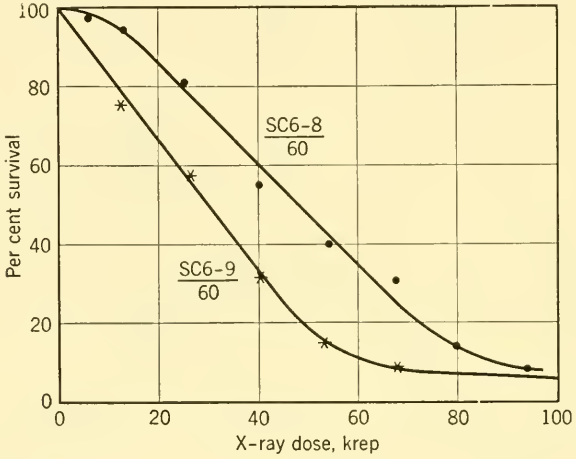

FIG. 16. Experimental survival curves of a diploid colony with about three unpaired defects. This colony was obtained by preirradiation subsequent to isolation of a single cell and its growth into a vegetative colony. The survival of a normal diploid colony is also shown.

essential components for division. One would expect the maximum number of essential sites on a minimum medium for survival, whereas on a more complex medium it would be anticipated that the number of essential sites might be decreased. There is hope that the study of such cells will furnish important clues for the biochemical defects present in each cell. This information should be of value in the interpretation of the basic mechanism of cell division.

CONCLUSIONS

1. A parallelism is demonstrated between the so-called indirect mechanism of radiation effect and the hit theory.

2. The apparent contradiction in the two theories may be eliminated by using the "diffusion model" of radiation effects. This model assumes the formation of intermediates by the ions of the primary radiation. These may migrate in the cell and chemically interact with genic and extragenic components.

3. Evaluation of the ionic efficiency in a given example allows approximate calculation of the magnitude of biological effects as a function of specific ionization.

4. Experimental tests of the diffusion model are expected to clarify some radiation mechanisms in detail. The formulation of the diffusion model allows for variation in radiation sensitivity with substrates, state of cell division, water content, etc.; it furnishes clues for study of the quantitative effects of externally applied cell poisons.

5. In the modified theory the size of the target loses significance. Individual sensitive "sites" may exist in the cell. They are important because of their biological role, not because they form "targets."

6. By means of the diffusion model the mechanism of inhibition of cell division by radiation may be quantitatively accounted for in haploid, diploid, and possibly in multiploid yeast cells.

7. The production of yeast cells with unpaired radiation-induced genetic defects should stimulate biochemical and biophysical study of the basic mechanisms involved in cell division.

Acknowledgments

Much of the work reported here concerning relative biological effects as functions of rate of energy loss was done in collaboration with Professor R. E. Zirkle, to whom the author is much indebted. Many of the ideas presented were stimulated as a result of this collaboration. The author wishes also to thank Professor J. H. Lawrence for his interest, Professors Ernest Lawrence and Robert Thornton for the use of the 184-in. cyclotron, and R. Mortimer, R. Wijsman, B. Stepka, and H. Anger for their collaboration. The paper is partially based on work done under the auspices of the Atomic Energy Commission. Some of the material in this paper has been previously reported in *Am. J. Roentgen.*, **67**: 1, 1952.

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DISCUSSION OF ZIRKLE'S AND TOBIAS' PAPERS

PUCK:

I should like to comment on three different concepts which have been employed in the course of these discussions, namely, target theory, "hit" multiplicity, and direct vs. indirect action. The theoretical implications of these

three terms should be kept distinct for maxium clarity in dealing with radiobiological phenomena. The target concept assumes that, in a series of radiation experiments, it is possible to calculate, from a knowledge of the amounts of energy absorbed and the numbers of biological events of a particular kind which result, the actual volume of the structures in which the molecular changes responsible for the given effects have taken place. The "multiplicity-of-hit" concept presupposes that, because of the fundamentally discontinuous nature of radiation absorption, it is possible to determine from the shape of the dose-effect curve the number of such elementary processes which are necessary for a single biological result of the given kind to be produced. Finally, the idea of direct vs. indirect action implies that it is possible to determine whether the primary acts of energy absorption take place within molecules of the medium which may then interact with the cellular components in a secondary reaction. It is important to note that these concepts are, to a considerable extent, independent, and that in a particular experiment any or all of these conditions may be inoperative. For example, the theoretical formulation given by Tobias for his extremely interesting experiments actually depends only on the validity of his determination of the difference in number of hits required to inactivate the haploid and polyphoid forms.

Consider first the target theory. The data from a series of radiation experiments enable one only to estimate the probability for a certain biological event as a result of a given radiation experience. If this probability is uniform for all the members of the biological population, it will be completely determined by three factors: the average radius of migration of any disturbance which is created by the energy absorption and which may produce the final biological action if it reaches a susceptible site before its energy has been dissipated; the average volume of all the sites in which the specified change can occur; and an efficiency factor which tells how often success attends the absorption of energy within the zone just defined. This efficiency factor may vary over very wide limits, assuming values either greater or smaller than 1. As an extreme example, in the reaction between H_2 and C^{12} induced by irradiation with alpha particles, this factor has a value of more than 1000. Thus, the volume of a "target" calculated in this very simple system would have no relationship whatever to the actual space occupied by any of the atoms or molecules taking part in the reaction. The conventional calculation of a sensitive volume in a radiobiological change can be meaningful only if it can be known that the efficiency coefficient is close to unity, and even in that case the value obtained will represent an average volume equal to the sum of the actual "targets" plus the diffusion space of any reactive intermediates.

In this connection, too, it is necessary to consider that the same biological end results may be achieved by several different mechanisms, particularly when the action involved is something as complex as cell death or reproductive failure. Whenever the biological effect which is observed can result from a number of different atomic and molecular events, the overall probability is a composite value of geometrical and chemical parameters of each pathway, and its expression in terms of a physical volume can be most misleading.

The hit theory or multiplicity concept is a method for estimating the minimum number of energy-absorption acts which are required, on the average, to effect the biological change under study. Its physical significance is analogous to that attached to the determination of the order of a chemical reaction. Such a determination is used to obtain information about the number of molecules, or, in radiochemical reactions, the number of separate acts of energy exchange which are involved in the given process. However, caution must be exercised in drawing conclusions from such kinetic data about actual reaction mechanisms. The order of a chemical reaction can definitely determine the number of molecules of each species involved, or the number of energy quanta which are required, only when a single, definite reaction occurs. If several consecutive steps take place, or if chains are set up, deductions concerning the actual reaction mechanism may prove difficult. A complex reaction may have several radiochemical and thermal steps. In this case, the observed dose dependence may only reflect the effect of the slowest step of the process. If the given end product can be achieved by more than one path, the slope of the dose-dependence curve will be a complex function of the various mechanisms involved. If chain reactions are initiated, a single act of energy absorption may eventually produce multiple biological effects.

Finally, the differentiation between direct and indirect action is often a very difficult matter. Ionizing radiations can transfer energy to any molecule they encounter. The extent to which a given effect proceeds more extensively through a direct rather than an indirect mechanism will depend on the relative concentrations of the various molecular species, the probability of energy absorption in each type, and the probability that such absorption will produce the specific end results under study. Reactive intermediates doubtless appear in both kinds of energy absorption. The separation of direct and indirect components of complex reactions can be accomplished with certainty only when the intermediates of the indirect action have fairly long half lives, so that unequivocal demonstration of their action is possible. Dilution studies, demonstration of protective action by various substances, and similar procedures afford only inferential evidence whose interpretation may be open to question.

These remarks should emphazise the great care which must attend an attempt to interpret radiobiological phenomena in terms of atomic and molecular transformations. Careful and detailed analysis of radiochemical kinetics in the simpler biological systems is urgently needed. Such studies doubtless will furnish useful models which should clarify the nature of the interactions of high-energy radiations with more complex biological structures.

TOBIAS:

I agree with Puck's discussion. When a single action occurs in these calculations, it cannot be assumed that the receiving molecule cannot receive from more than one source.

LATARJET:

I was very much interested in Tobias' analysis of his experimental results and by the attempt to interpret them through a single genetic hypothesis. In Paris,

Ephrussi and I, with the collaboration of L'Heritier, have tried the same sort of analysis, but it seemed to us that a simple hypothesis could not account for the results and that many more experimental facts were actually needed.

TAHMISIAN:

The chemists and physicists have ably shown that the effect of an ionizing ray lasts 1×10^{-13} to 1×10^{-6} sec. To the biologists this has been very discouraging because the effect of irradiation dosage could be demonstrated several days after the original damage occurred.

With diaphase grasshopper eggs the irradiated materials could be subjected to 0° for 6 months, after which the irradiation damage could be demonstrated when the eggs were returned to metabolic temperatures. Although 25,000 r destroyed every nucleus in the grasshopper embryo, the utilization of oxygen was not affected; it even increased. We stated that anabolic processes were more susceptible to irradiation than catabolic processes. It was demonstrated that the ability of a resting cell to maintain the *status quo* was dependent on the presence of a functioning nucleus.

Clark demonstrated that the maintenance of a dynamic equilibrium in the ameba was dependent on the nucleus. An enucleated ameba could not meet its nitrogen requirements from a medium containing urea and glucose, whereas the control could do this.

At the symposium on nucleoproteins in Holland the necessity of nucleoproteins in the cell for the formation and maintenance of enzymes and proteins was clearly brought out.

Tobias showed that a haploid yeast cell was more susceptible to ionizing radiations than a diploid cell, and the latter than a polyploid. He mentioned that the cytoplasm in these cells must also be injured. Probably the cytoplasm was injured to a much greater extent than the contents of the nucleus, since much more cytoplasm is present.

In a haploid organism the portion of the cytoplasm which was injured and was under the influence of a destroyed gene could not be repaired. In a diploid or polyploid cell, however, if one gene was destroyed the allele could take over, repair the injury to the cytoplasm, and in turn be able to maintain itself.

I believe the paper presented by Tobias has finally brought light to the mode of radiation damage to biological material. It also explains why it takes so long to demonstrate injury and why all cells in the proximity of the injured one are differentially affected. Tobias has made a great contribution to the advancement of radiobiology.

GIERLACH:

Throughout the symposium, there has been an evident need for more indicators of biological change produced by radiation. With the kind permission of Dr. Zirkle and also of Dr. Sax, I should like to present briefly our results with vital staining techniques. This work has been performed at the Medical Department Field Research Laboratory located at Fort Knox, Kentucky, by A. T. Krebs, S. Strugger, and myself. Fluorochromes, as well as diachromes,

were used in these studies of the earliest demonstrable biological effects of x-rays.* The results to be reported were obtained with the fluorochrome acridine orange (3,6-tetramethyl diamino acridine).† This dye is most unusual in that it exhibits a fluorescence concentration effect; that is, its characteristic fluorescence color depends on its concentration. If the concentration is high (*ca.* 1:500), then the fluorescence color is red, and if the concentration is low, then the fluorescence color is green. Fortunately, living protoplasm can accumulate only a small amount of dye and will fluoresce green; when damaged or destroyed its dye-binding capacity is increased, and it will fluoresce red.

Soft x-rays, unfiltered, from a beryllium-window x-ray tube were used. The tube was always operated at 50 kvp and at 10 ma. Dosage was varied by the time of exposure. Intensity measurements were made with a 250-r nylon and a 100-r standard Victoreen chamber at 10 cm. The dose was then calculated for 3.8 cm, the distance at which the sections were irradiated. The calculated dose was of the order of 150,000 r per min in air. In this study the upper epidermis of the onion, *Allium cepa*, was used. The sections of about 1 sq cm were floated in a tap-water dye bath of 1:10,000 dilution immediately after irradiation. After staining for 10 min, the sections were momentarily rinsed in tap water and placed on a slide for examination in the fluorescence microscope. Figures 1–4 show the characteristic appearance of control and irradiated specimens.

Ordinary diachromes lend themselves to biological radiation-effect studies also. One that received the most attention was neutral red. It was desirable to see how control and irradiated onion sections would appear with neutral red staining. Therefore, a neutral red dye bath of 1:5000 concentration was prepared, and in this solution sections were stained for 5 min and then examined in an ordinary bright microscope. A section of irradiated epidermis that had been stained with neutral red tap-water solution of *p*H 7.4 demonstrated the damaged "spots" as bright cherry-red areas. While this preparation was still on the microscope stage, $\frac{1}{50}$ *N* NaOH was slowly added to one edge and the excess fluid absorbed on the opposite edge with bits of filter paper. The base penetrated first into the injured cells and eventually into the others. As the solution penetrated, the neutral red was precipitated out, and what were earlier the cherry-red spots now became the foci of needle-like crystal growths. In the "spotless" cells the neutral red was thrown out of solution into a diffuse aggregate pattern.

The same section was then reacidified with a phosphate buffer of *p*H 5. The crystals redissolved, and the section returned to its original appearance with the cherry-red spots. Figures 5–7 demonstrate graphically the above-described phenomena. These spots are taken to represent local *p*H changes in the altered cytoplasm.

* Krebs, A. T., and Z. S. Gierlach, vital staining with the fluorochrome acridine orange and its application to radiobiology. I. Alpha-ray effects, *Am. J. Roentgenol. Radium Therapy*, **65**: 93, 1951.

† Strugger, S., *Fluoreszenzmikroskopie und Mikrobiologie*, M. and H. Schaper, Hannover.

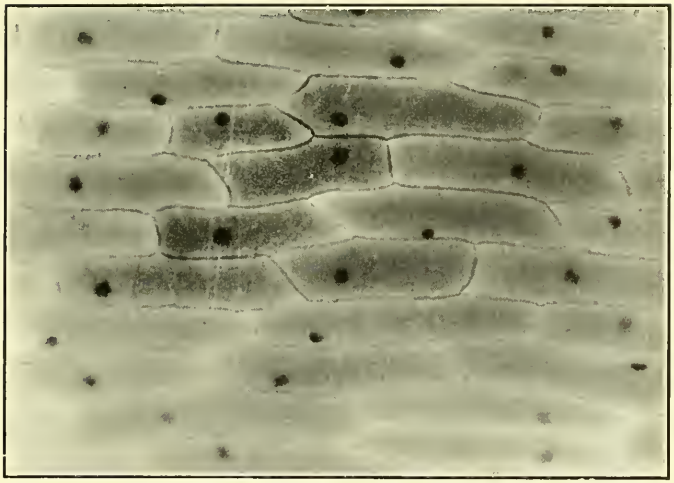

FIG. 1. Control preparation. There is rather uniform storing of acridine orange in the cell vacuole with fluorescence of various shades of yellow-orange. The cytoplasm along the cell walls fluoresces green; the nuclei are yellowish green.

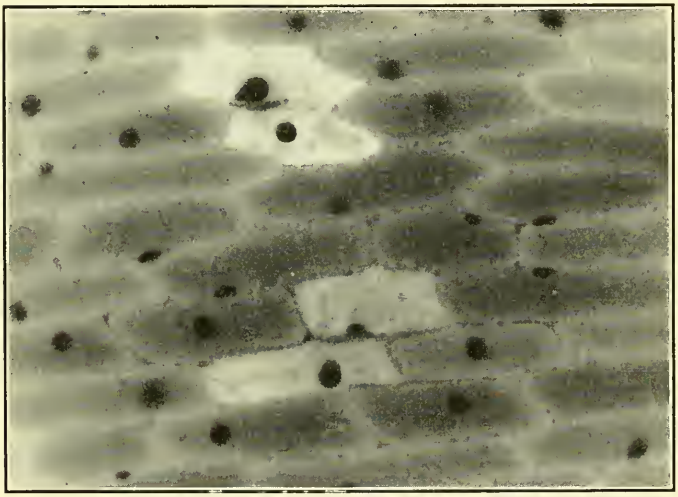

FIG. 2. Section has been irradiated for 4 min. Two "spot" areas have an intense concentration of dye (brick-red fluorescence). This has migrated from adjacent cells to the damaged cytoplasm. The remaining cells have the appearance of the control, except for some clumping of dye along the cell walls in the cytoplasm. Adjacent cells are frequently affected, and this is the cause for the double cell appearance.

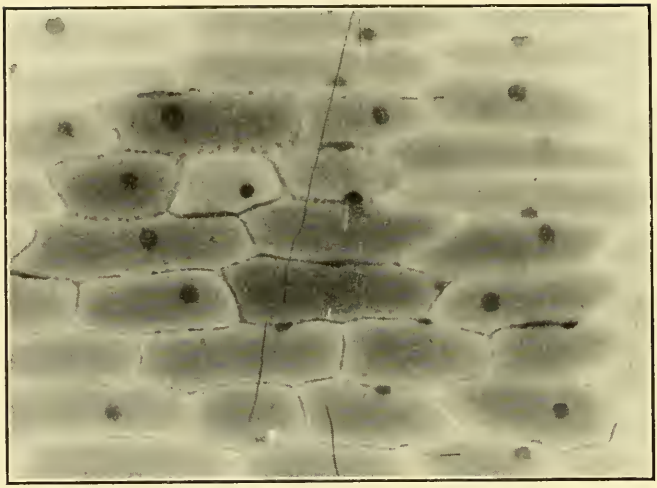

FIG. 3. Section irradiated for 6 min. Many more cytoplasmic "spot" areas appear. In this section the darker cells fluoresce green because of a lower concentration of dye. The dye has migrated to the affected cytoplasm along the length membranes. The affected areas are the darkened segments between cells. The original fluorescence here was deep brick-red.

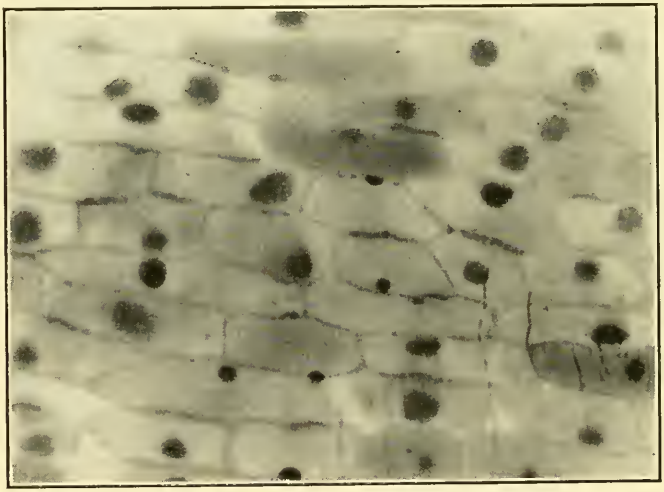

FIG. 4. Irradiated for 10 min. Only two or three cells show the "control" appearance. These are the cells that are uniformly dark, and which in the original had an overall orange tint. Most cells fluoresce green and are depicted as light gray. There are many spots along the cell membranes in the cytoplasm. The nuclei have here also been affected and are swollen.

FIG. 5. Section of 6-min-irradiated epidermis, stained with neutral red in tap water with a *p*H of 7.4. There is uniform storing of dye in the unaffected cells, while the damaged cells have marked concentration of dye in the areas of disturbed cytoplasm. These "spots" under the ordinary microscope are cherry-red.

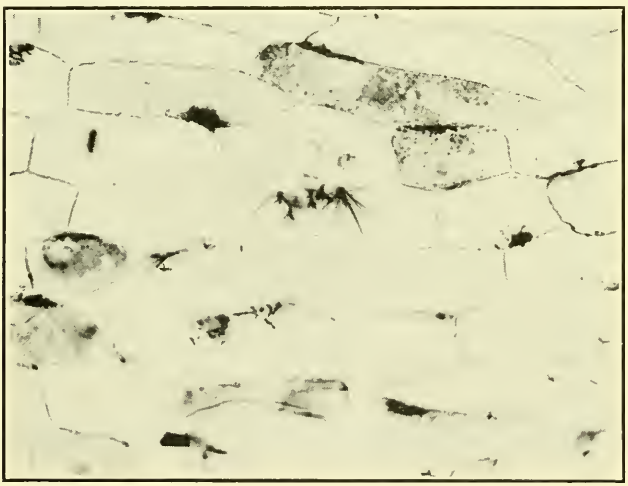

FIG. 6. Same section as above, but it now has been titrated with 1/50 *N* NaOH solution. The neutral red is precipitated out of solution and migrates to the injured areas to form needle-like crystals.

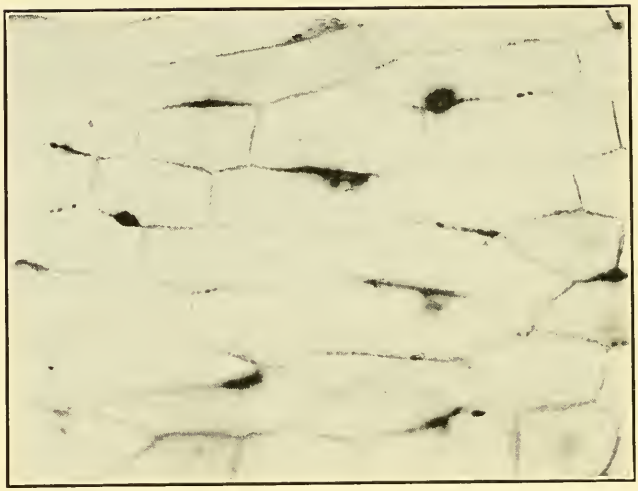

FIG. 7. Same section as above. It has now been acidified with a phosphate buffer of *p*H 5. The crystals disappear and the bright cherry spots reappear as in the original.

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The Influence of Quantity and Quality of Radiation on the Biologic Effect

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Influence of Quantity on Time and Degree of Effect per Individual

Within the usual exposure range for mice (100–1000 r, to determine lethal dose), effects appear more rapidly, become more generalized, and persist for longer periods of time as the amount of irradiation is increased. However, if time of death following irradiation is employed as the criterion and the exposure range is extended to include thousands of roentgens, it will be found that the decrease in latent period is proportional to the amount of the exposure only in certain parts of the entire curve. This is illustrated in the diagram in Fig. 1, which has been drawn from the data and figures of Krebs (38), Lamarque (39), Lawrence (42), Quastler (52), Evans (13), and Ellinger (9), with liberal translation. The values given are approximations and are included for purposes of illustration.

As shown in that part of Fig. 1 marked "late death," little or no effect is observed after exposures below 100 r. As the quantity of irradiation is increased, a few individuals will suffer a reduction in survival time. In this low-dose range the findings are not always the same, in that some exhibit aplasia, a few develop neoplasms, some die of acute infection, and others show no evident cause of death. A reduction in leukocytes follows the radiation exposure in some of the individuals suffering the "late death," but this and other moderate changes are usually only temporary.

After exposures indicated in the part of the curve labeled "early death," the survival time becomes progressively shorter as the amount of irradiation is increased. Only especially sensitive tissue such as in lymphoid organs shows damage at lower doses, but as the exposures increase the autopsies indicate more and more aplasia of hematopoietic tissue and widespread sloughing of epithelium. In addition to the

pathologic findings, several types of experiments have yielded results which implicate the intestine and lymphoid organs in this "early death" effect. Quastler (personal communication) has found that the effect of whole-body exposure in this dose range (and above) can be duplicated almost completely by irradiation of the intestine alone. The intestine

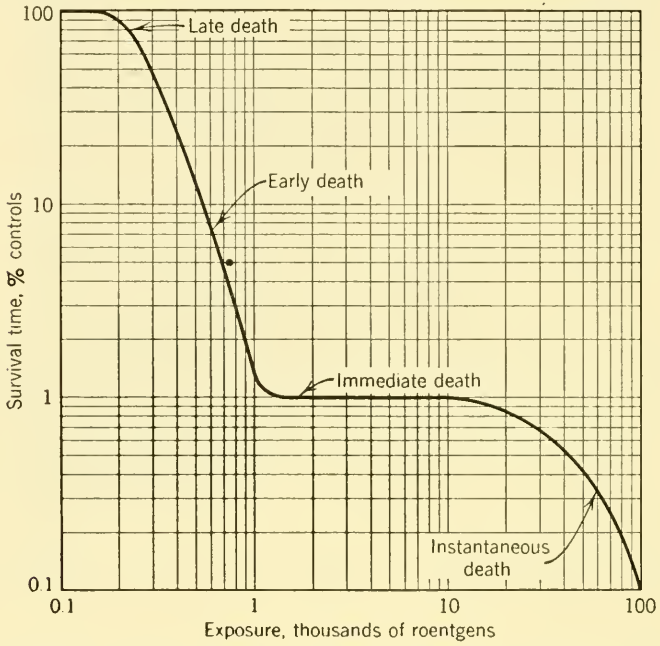

FIG. 1. Diagram of effects of a wide range of exposures (whole-body, x-irradiation) on survival time. "Late death": animals die only a few weeks before controls; "early death": survival, after irradiation, of only 1 month or less; "immediate death": survival of only 2–5 days after irradiation; "instantaneous death": animals die during or within a few minutes after massive high-intensity irradiation. Data based primarily on experiments with mice (see text for references and discussion).

was exposed through a surgical incision of the abdominal wall with the rest of the body protected by lead shielding. Jacobson *et al.* (33, 34) have irradiated mice with exposures from 600 r through 1200 r of total-body irradiation with and without lead protection of the surgically mobilized spleen. The dose required to kill half the animals with the spleen protected is almost twice as great as the dose necessary to kill half the mice with the spleen included in the irradiation. The development of anemia is obviated, and the severity of the leukopenia, etc., is significantly lessened in the spleen-protected animals.

The next part of the curve, marked "immediate death," indicates that

increases in exposure within this range do not necessarily produce death more rapidly. The animals survive 3 days, and during this time they refuse to eat, lose weight, and become progressively weaker. Pathologic damage is produced in practically all vital organs. It is as if all further growth and nutrition have been stopped but the animal is able to survive for a limited time on material already provided.

At extremely high exposures, still another time curve is demonstrated. This "instantaneous death" may occur during the exposure itself or a few minutes afterward. The changes in the animal leading to death vary in different mammals and range from convulsion and shock to general necrosis.

Quastler (51) has studied survival time and dosage in mice and has given some mathematical formulae for the time-dose relations. The study has been extended to mice of different weights and ages, and he finds these to be factors in determining the radiosensitivity as measured by reduction in survival time (52). For further details concerning animal deaths at different times after single exposures see Brues (3), Ellinger (10), Evans (13), Henshaw (28, 29, 30), Krebs (38), and Rajewsky (54).

Influence of Quantity on Percentage of Population Affected

For several years lower organisms have been employed to yield quantitative data regarding the relative effectiveness of different dosages of different kinds of ionizing radiation. Such material has the advantage of permitting the use of large numbers of individuals per exposure, and, because of small size, of being irradiated more homogeneously throughout the organism. More recently, the need for quantitative data regarding the effect of different kinds of radiation exposure on the mammal has encouraged the use of such animals as the mouse, the rat, and the guinea pig. On a smaller scale, larger animals such as the rabbit and dog have been employed to investigate radiosensitivity under various conditions of irradiation. When it has been possible to use sufficient numbers of individuals of the same strain, sex, and weight, rather symmetrical and satisfactory dose-effect curves have been obtained. Such curves are sigmoid in character (see Fig. 2), and the slope appears to vary according to the general sensitivity, the species, and certain exposure factors. A frequency-distribution diagram is included in Fig. 2 to illustrate an explanation of the sigmoid character of the dose-survival curve. This interpretation would indicate that even in a carefully selected population a few individuals are especially sensitive, a few are un-

usually resistant, but more have a sensitivity approaching the mean value. Dose increments in the latter group have a greater effect in reducing the percentage surviving. The test period is usually about 30 days. The part of the dose-survival curve that changes rapidly with increase in dose is widely used for comparing effectiveness of different conditions of irradiation and is usually expressed as the LD_{50} (lethal dose for

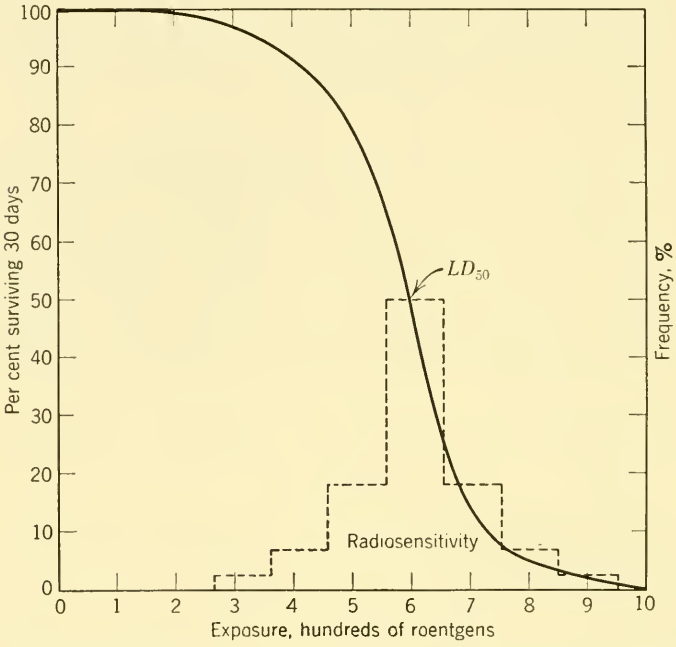

FIG. 2. Schematic dose-survival curve for whole-body exposures to x-rays, based primarily on data obtained with mice (10, 13, 27, 29, 49, 52, 53, and 54). The dose-effect curve may be seen to be sigmoid. It exhibits a threshold at about 300 r and a median effect (LD_{50}) at approximately 600 r. The shape of the curve may be interpreted as representing the frequency of individuals with a certain degree of radiosensitivity as shown in the block diagram.

50 per cent of the population). See Ellinger (10), Evans (13), Henshaw (31), Lamarque (39), and Quastler (51, 53) for dose-effect curves of different mammals after single exposures.

The LD_{50} for whole-body irradiation is lower than that for local irradiation or for general but superficial exposures. Both physical and biological factors are involved. In the first place, more energy is absorbed, and in the second, tissue destruction is more generalized. Organs which would otherwise aid in recovery (either by removing noxious materials or by replacing destroyed cells) are themselves impaired. The

volume of tissue irradiated in a given exposure is reduced if the radiation is not capable of penetrating through to the deeper parts of the body, and higher dosages are required to produce the lethal effect. For example, Raper (55) has concluded from total beta/total gamma ratios for killing in mammalian species that beta radiation brings about lethal action through a total mass or volume effect, whereas gamma radiation (which irradiates all elements of the body more or less uniformly) does not. Using various portions of the total dose as beta radiation, and the remainder as gamma radiation, gives only partial additivity. Where absorption is equivalent (in small organisms such as *Drosophila* eggs), complete additivity of the beta and gamma radiation has been reported (72).

In animal experiments, Jolles (35) found that the severity of skin reaction to x-irradiation was related to the size of the field. Evidence was found in addition, through use of a grid or sieve of lead strips, that the local effect became more pronounced the greater the total energy absorbed in the entire field. Two fields far apart were injured less (erythema of human skin) than if the irradiated areas were close together (36). The enhancement of the reaction in each field, if two were irradiated close together, has been interpreted as indicating the formation of a diffusible toxic substance with its concentration proportional to (Dose $\times$ cm^2)/Distance (37). It should be borne in mind that at least a part of the above findings might be expected on physical grounds, such as overlapping of scattered radiation at the edge of fields, and this has possibly not been evaluated completely.

Influence of Protraction

As the intensity of the radiation (roentgens per minute) is reduced, the biologic effect (that is, lethal action) for the same total dose becomes less pronounced. There is abundant evidence from studies of organisms other than mammals and from erythema studies in man (9, 18, 39, 47) that protraction decreases the effect. There are exceptions such as effect on mutation rate, inhibition of growth in tissue culture, and lethal effects in amphibia. The published data regarding this factor in whole-body irradiation of the mammal are woefully incomplete. No doubt one of the reasons for the variation in LD_{50} found by different investigators is the difference in exposure rate employed. Henshaw, Riley, and Stapleton (31) reported that increasing the period of exposure 10 times reduced the lethal action (in whole-body irradiation of mice) for a given dose to approximately 70 per cent. In a discussion by Glasser *et al.* (18), it is pointed out that the effect of protraction varies for different organ-

isms, and that the total duration of the irradiation is significant. An example of the latter factor was found to be that an exposure of 660 r produced a threshold erythema whether delivered at 75 r per min or 20 r per min. However, when given at 4 r per min, 750 r were required to produce the same reaction. Similar findings for whole-body irradiation of mice have been noted in a progress report.*. Gamma-ray exposures were given at intensities of 2300, 896, 240, and 67 r per hr. The LD_{50} values were found to be 817, 805, 796, and 1006 r. Thus, when the overall time was less than 3 hr, intensity was not a factor. However, when the exposure was extended to 11 hr, the amount of exposure required to kill 50 per cent was definitely increased.

Influence of Fractionation

In general, if a certain dose is given in several fractions the effect will be less than if it were all delivered at one time. In other words, some recovery takes place between the different exposures. However, in certain tissue-culture, amphibia, and *in vitro* tumor studies, fractionation has not been found to reduce the effect of a certain total dose. There are many variables and factors involved. These have been discussed by Glasser *et al.* (18) and by Paterson.† Two important factors are (1) the time between exposures, and (2) the amount of each exposure. Another point noted by Quimby (18) is that recovery from the first exposure may be considerably greater than that from succeeding exposures. Many attempts have been made to generalize regarding recovery rates, but it is apparent that the most reliable information comes from experimental data for each type of biologic material and condition of exposure.

It has been reported recently that little difference in mean accumulated doses occurs among groups of rats exposed to daily doses varying from 50 to 200 r per day.‡ At 25 r per day the accumulated dose rises to twice that at 50 r per day.

Thomson *et al.*§ find a greater accumulated dose in rats at 60 r per day than at 120 r per day of gamma radiation. Earlier data indicate an equivalence of 120 r per day of gamma irradiation (continuous) and 50 r per day of 200-kvp x-radiation delivered in a few minutes. It

* Thomson, John F., W. W. Tourtellotte, M. S. Carttar, and John Neff, The toxicity of gamma radiation to mice exposed at varying dose rates, UCTL, *Quart. Progress Rept.* 4, Jan. 15, 1950, pp. 9–12.

† Chapter by Edith Paterson in *The Treatment of Malignant Disease by Radium and X-rays*, Ralston Paterson, Edward Arnold Co., London, 1948.

‡ Hagen, C. W., and E. L. Simmons, CH-3815, June 17, 1947.

§ Thomson, J. F., W. W. Tourtellotte, and M. S. Carttar, Continuous exposure of animals to gamma radiation, UCTL, *Quart. Progress Rept.* 4, Jan. 15, 1950, pp. 1–8.

is suggested that daily doses, each of which is high enough to reduce survival time, produce death by so-called acute mechanisms. In rats 60 r per day (of the gamma or 25 r per day of daily short x-ray exposures) would permit a certain measure of recovery from the acute effects, and death would require a large complement of subacute or chronic radiation damage. In guinea pigs, on the other hand, both 60 and 120 r per day (gamma radiation) would be expected to produce death by acute mechanisms, as the guinea pig is more radiosensitive than the rat.

Although early effects are markedly reduced by fractionation, it is possible to obtain definite effects eventually by continued fractions, each one of which is too low to produce a noticeable effect by itself (30). The influence of dose fractionation on the lethal x-ray effect produced by total-body irradiation in the mouse has been studied by Ellinger (11, 12). Simple dose fractionation (daily exposures of 100 r) decreased the mortality rates produced by the same dose when given in one exposure. The influence of fractionation on the lethal effect of x-rays was studied in a different way by Henshaw several years ago (30). In these experiments, the fractionated exposures (at different daily levels) were continued until death. From the maximum survival time (36 weeks) in the mice exposed to 5 r per day, to the shortest (20 weeks) in mice receiving 25 r per day, the decrease in survival with increase in amount of the daily exposure approximated a straight line. It was also observed that, although animals receiving the smaller daily exposures lived longer, the total accumulated exposure at the time of death was less than that of the mice exposed to the heavier treatments. Henshaw (30) observed that the overall picture of the radiation effect was one of aging (loss of parenchymatous cells together with poor regenerative ability), and that "if radiation acts to hasten the normal process of aging, normal aging would be expected to exert a relatively greater effect in those animals that lived longest, as was observed." It is concluded by Lorenz *et al.* (46) from studies of mice that the ovarian-tumor-inducing dose is cumulative and independent of dose level. These authors have also reported that death following daily exposures is uniformly caused by anemia and thrombocytopenia in guinea pigs, but not in mice or rabbits.

It should be borne in mind that in some tissues and organs with high reproductive capacity fractionated exposures are more effective. This is apparently true for the testis (14). Although a single massive exposure is more likely to produce an immediate and pronounced depopulation of the spermatogenic elements, the mitotically inactive spermatogonia are more resistant and eventually repopulate the tubules. Continued or fractionated exposures are more likely to result in a gradual but steady depopulation of the tubules with eventual permanent sterility.

This may be due to the possibility that fractionation is more likely to expose every cell at least once in division, at which time it is susceptible to the radiation.

Influence of Quality (Change in Energy Absorbed per Gram of Tissue)

If the effective wave length of photons is shorter the penetrability of the incident beam of radiation is increased. This results in an increase in the depth dose and in the volume of tissue irradiated. It is important in comparing, quantitatively, the effect of two radiations which are widely different in quality to so arrange the exposure and the measuring instruments that the dosage can be expressed in rep (roentgen equivalent physical) rather than in "roentgens in air." If this measurement cannot be done satisfactorily one may approach in the following manner the problem of whether two radiations (differing in quality) produce similar effects. A ratio of effectiveness of the two may be determined in arbitrary units for an effect A. If the effects are similar, the same ratio should then hold for effects B, C, etc. Likewise $\frac{1}{2}\ LD_{50}$ exposure of one type of radiation plus $\frac{1}{2}\ LD_{50}$ of the other kind should be equal to the effect of LD_{50} exposure of either type of radiation (72). One should recognize the possibility of recovery between exposures when animals are first irradiated with one and later with another type of radiation.

Influence of Quality (Change in Specific Ionization)

A change in quality also produces a change in the ionization density, per micron of path, of the ionizing particle, in tissue (Fig. 3). It may be said that for certain effects (killing of larger microorganisms, chromosome breaks, etc.) which are apparently due to injury to a small volume of protoplasm the efficiency of the radiation increases with increase in the specific ionization it produces. On the other hand, for effects apparently related to changes in a still smaller volume of perhaps only one or a few molecules, cells are irradiated more efficiently by radiations producing a more sparse ionization. For these effects (killing of very small microorganisms and production of a gene mutation) apparently only one or very few ions are required, and thus any additional ones are "wasted."

Since x-rays and gamma rays produce ionization through the formation of photoelectrons, it is understandable that their effects should be somewhat similar in character and degree to those of beta-particle radi-

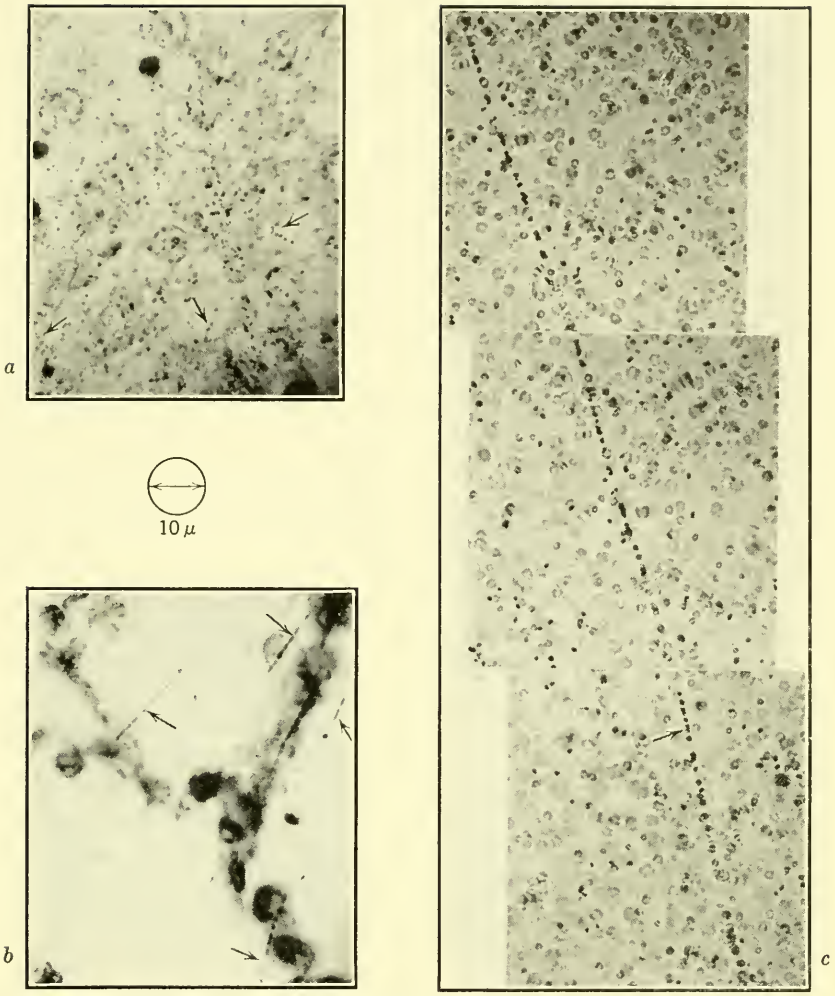

FIG. 3. Photomicrographs of particle tracks in photographic emulsion. Magnification ×750.

a. Short curved tracks (arrows) of photoelectrons produced by low-energy photons simulated by placing thin liver slice containing radioactive carbon on NTB emulsion. The nuclei of the liver cells may be used as a rough scale by which to gauge the range and ion density of the beta particles.

b. The dense, short, and straight line of ionization produced by alpha radiation is demonstrated by the affected photographic grains (arrows). The tissue cells shown (necessarily out of plane of focus of the emulsion) are of lung alveoli, and the nuclei (dark oval bodies) give some idea of dimensions involved.

c. This plate was exposed to a mixture of fast neutrons and gamma rays. The photo micrograph is of the same magnification as *a* and *b*. The dark grains scattered at random are due to ionizations of photoelectrons produced by gamma rays. A relatively long ionization track of an energetic proton (produced by a neutron) is indicated by the arrow.

ation. Although it has been difficult in the past to demonstrate a difference in biologic effectiveness of different wave lengths of x-rays in the usual therapeutic range, evidence is mounting to indicate that (for tissue effects) low-energy x-radiation is more effective than extremely high-energy x-rays and gamma rays.

When the incident beams consist of ionizing particles a difference in effectiveness related to specific ionization is very evident. With particles of large size (such as protons) and with a double positive charge (such as alpha particles) the ion track is very dense as compared to that of beta particles. The penetrability of alpha particles is very low, and thus a comparison of their effectiveness in tissue with those of beta particles and photons presents technical difficulties. Such studies are usually done by injecting, or by having the animals inhale, some alpha-emitting radioactive material (38, 54). Fast neutrons, on the other hand, have a penetrability in tissue somewhat comparable to that of 200-kv x-rays and gamma rays. They produce ionization in tissue primarily through the production of recoil hydrogen nuclei. These protons, although not ionizing as densely as alpha particles, have a specific ionization much greater than that of beta particles or photoelectrons.

It may be said that in general, for tissue effects, alpha particles and neutrons are more effective than x-rays or gamma rays per roentgen equivalent physical (rep). This holds for a single exposure and is even more marked for protracted and fractionated exposures (7, 2, 13, 18, 26, 31, 38, 49, 54).

Certain organs and tissues have been found to be especially sensitive to neutrons as compared to x-rays and gamma rays. Complete destruction of germinal tissue in the testis of the rabbit without irreparable damage to the overlying skin has been reported for neutrons (40). This is different from the findings with x-rays in a similar single exposure.

Effect of Protraction and Fractionation on the r/n Dose Ratio

Protraction and fractionation (13, 31, 49) have been found to increase the r/n dose ratio. In other words, animals recover from effects of whole-body x-irradiation more rapidly than they do after exposure to alpha or neutron radiation. Examples are shown in Table 1 (for mice). The gonads appear to be especially sensitive to small daily exposures of fast neutrons. The selective action of neutrons in producing cataracts is apparently even more marked. In several experiments, exposures to 1.4 n/day produced cataracts in about 50 per cent of the eyes in the animals surviving the LT_{50} (time required for 50 per cent of ir-

radiated animals to have died). On the other hand, similar animals receiving 11 r/day did not exhibit any definite cataracts.

TABLE 1

CHANGE IN RATIO OF DOSE IN r TO DOSE IN n UNDER DIFFERENT CONDITIONS IN MICE (13)

Condition	Dose Ratio	
Single exposure	8	
80 r/day vs. 10 n/day	8	(Accumulated exposure at LT_{50})
11 r/day vs. 1.4 n/day	12	(Accumulated exposure at LT_{50})
11 r/day vs. 1.4 n/day	14	(Accumulated exposure—anestrus)
11 r/day vs. 1.4 n/day	34	(Accumulated exposure—sterility in males)
11 r/day vs. 1.4 n/day	34	(Frequency of cataract after LT_{50})

DISCUSSION OF POSSIBLE MECHANISMS OF THE SPECIFIC IONIZATION EFFECTS

The final injury produced by either type of radiation (when this injury is of the same degree) does not disclose any characteristic difference which would allow one to determine whether the effect had been produced by neutrons or by x-rays. The changes taking place in the interaction of radiation and matter are of the same general type for the two kinds of radiation. The first step is the production of ionizing particles; the second is the production of ion pairs. Then follow the excitation of atoms and activation of molecules with production of active radicals, and finally absorption of energy in producing thermal changes. It is possible because of the great difference in the specific ionization of the proton and the photoelectron that the greater portion of energy loss of neutrons may be expended in production of active radicals, whereas some other radiation might expend a greater proportion of energy in ionization. There seem to be two lines of evidence at the present time to indicate that x-rays preferentially injure cells either beginning division or preparing for mitosis; whereas neutrons appear to damage mitotically inactive as well as dividing cells. One line of evidence comes from studies of cells irradiated in different stages of mitotic activity. Marshak (48) obtained evidence which he interpreted as indicating that cells in early prophase were more severely damaged by x-irradiation. Neutrons, on the other hand, had a more general effect on all stages, including mitotically inactive cells. Gray, Read, and others (19, 21, 22, 23, 24, 25, 26, 56, 61, 62, 65) have made extensive studies of cellular damage after irradiation with neutrons and with x-rays. The x-ray effects were primarily inhibition of prophase with later resumption of cell

division, at which time injured cells appeared. With neutron irradiation, degenerating cells appeared in large numbers even before definite recovery of the mitotic process had taken place. This generalized necrotic effect was even more evident with alpha-particle radiation.

The other line of evidence comes from the experiments using protraction and fractionation, where it appeared that organisms recovered from x-ray effects much more rapidly than they did from those of neutrons. If cells not active in mitosis are more selectively resistant to x-radiation, it is understandable that such cells will then permit rapid replacement of injured and destroyed cells. It appears paradoxical at first that the two systems in the mouse that are most sensitive to chronic exposures of neutrons represent, on one hand, rapidly growing tissue such as the gonad, and, on the other hand, slowly growing tissue, the lens. Also, the ovary is deep in the body and the lens is superficial in location. Again, the bone marrow, actively proliferative, does not appear to be as relatively sensitive to neutrons as is the testis or ovary. The changes in the skin are not different at first, but later (63, 64) the injury, especially in deeper portions, is more pronounced and irreversible after the neutron exposure.

The situation may not be quite as confused as it seems. It may be that all tissues are more sensitive to neutrons, but effects of small daily exposures are more evident in the lens because this organ does not have the ability to discharge dead cells from its system (as does the skin, for example) and the accumulated effect becomes visible. Although the testis and ovary are able to discharge dead cells, they are unable to replace them from other parts of the body. The lack of special sensitivity to neutrons of hematopoietic and bone tissue may be more apparent than real. The regenerative stimuli for blood cells are probably so regulated by body requirements that the population following small daily exposures may not reflect the complete damage to this system. The more complete story might come from a study of depletion time, and in fact it might be just some factor of aplasia that brings on death more early in the neutron-treated animals. Neither the pronounced neutron effect on the lens nor that on the gonads is particularly hazardous to the life of the organism. However, they may reflect the degree of damage produced, but not apparent, in other organs of the body. Organisms surviving neutron exposures may be, therefore, more susceptible to trauma and infection than one would expect from exposures to x-rays that had a similar early effect on chances for survival. If the lethal action of radiation on mammalian cells is like that on certain plant cells, then an explanation of the differential recovery rates (that is, of photon irradiations over those of neutrons and alpha particles) may be at hand.

Gray and his associates (23, 24, 25, 26) have several lines of evidence that cell death in the broad bean (*Vicia faba*) is related to the production of chromosome abnormalities and not to inhibition of cell division (at 3 hr). Certain of the chromosome aberrations produced by x-rays and gamma radiation are likely to recover in time. Some of the effects are irreversible and are probably produced by ionizations from several particles acting near the same region. The effectiveness (in producing chromosome damage) of gamma radiation, neutron radiation, and alpha radiation was found to be in the order, 1:9:9. The x-ray yield of the more permanent changes was found to be proportional to the square of the dose and was affected by changes in the intensity of the radiation. The majority of the chromosomal exchanges resulting from alpha or neutron irradiation were between pairs of breaks produced simultaneously by the same ionizing particle. The yield was proportional to the dose and was independent of the dose rate. The results of Catcheside and Lea (4, 43) in a study of irradiated microspores of *Tradescantia* demonstrate these findings and support the conclusions of Gray *et al.*

Possible Lines of Investigation in the Future

Although we are far from having a clear picture of the sequence of events taking place in a mammal exposed to x-radiation, techniques have been developed and progress has been made. It would be well worth while to repeat many of these studies with parallel experiments, using neutrons. The results not only may give evidence as to the cause of greater effectiveness of neutrons but may yield information concerning the fundamental effects of ionizing radiation in general.

The LD_{50} for gamma rays and for x-rays has been determined for a number of animals. It would be well to extend these studies to include the LD_{50} for neutrons as well. Care should be taken that the animals are comparable as to age, size, homogeneity, etc., in the x-ray, neutron, and control groups. Care should also be exercised that different radiations are used so as to give similar conditions of intensity and penetrability. Table 2 shows the many gaps in our information concerning the r/n ratio for dose necessary to kill half the irradiated animals. Different kinds of mammals vary tremendously in size, in relative size of different organs, in metabolic rate, and in life span. A comparison of the r/n ratio of the LD_{50} for the different kinds of mammals may help to determine whether any of the following are important factors: body size, metabolic rate, sensitivity to toxic agents, long life span, trauma.

It would also be of interest to determine more definitely whether additivity between neutrons and x-rays is complete under some conditions

and not under others. In one study (exposure of about 1 hr) complete additivity was found (72). In another investigation (exposure of 24-48 hr) additivity was incomplete (72, 49) (see Table 3).

The studies comparing x-radiation and neutron exposures on the mitotic index, the relative numbers and types of chromosomal aberrations,

TABLE 2

Comparison of Lethal Effects of X-Rays and Neutrons (Single Exposures)

Animal	LD_{50} X-Rays, r	LD_{50} Neutrons,* n	References
Mouse	400		10
	450		27, 31
	600		27
	630	86	13
	540	54	†
Rat	590		7 ‡
	650		7 §
		60–120	44
Guinea pig	200		10, 27, 31
Hamster	700		7 §
Rabbit	790		‖
		66–160	44
	825	145	†
Dog	300		7 ‖
	335		7 ‖
Monkey	500		7 ‖

* These are arbitrary units, measured with either a 25-r or 100-r Victoreen chamber. Although these chambers may vary considerably in response to neutron radiation, an approximation of the rep is generally made by multiplying these values of n by 2–2.5.

† Zirkle *et al.*, Comparison of biological action of cyclotron fast neutrons and X-rays. I. Lethal action on mice and rabbits, Con. W-7401-ENG.-37, C.H. 3903, November 1950.

‡ Report by K. S. Cole *et al.*, Metallurgical Laboratory, University of Chicago, 1944.

§ *Rochester Report* 5980.

‖ Hagen, Jacobson, Murray, and Lear, Metallurgical Laboratory, University of Chicago, 1944.

the mitotic phase ratio, and the percentage of degenerating cells should be extended to mammalian tissue.

TABLE 3

Radiations Tested	Degree of Additivity	Reference
Gamma; fast neutron (exposure of about 1 hr)	Complete	Zirkle (72)
Gamma; fast neutron (exposure of 24–48 hr)	Incomplete	Mitchell (49)
Beta; gamma	Incomplete	Raper and Barnes (57)

It has been found that sensitivity of the mammal to x-rays can be modified in various ways (increased resistance of newborn at low temperature, under anoxia, fortified with cysteine, etc., and decreased resistance of adults at low temperature). Such modification of radiosensitivity should be attempted for neutron exposures. The response may be similar under some conditions and may differ under other conditions (as, for example, protraction or fractionation).

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DISCUSSION

KAPLAN:

I should like to add a footnote to Evans' remarks on fractionation. I was particularly interested in the reference to Sax's work on the effect of rest periods and recovery on chromosome aberrations. We have had a rather large group of mice under observation for two different biological end points, acute radiation mortality and the incidence of induced leukemias, or lymphomas, in relation to three dose variables: increments of total, number of treatments, and interval between treatments. These experiments are not yet complete, but it appears that the interposition of a rest period of 4 days or more results in a striking reduction in mortality over a wide range of dosage, whereas the incidence of leukemia is at least as high as, or perhaps higher than, that in mice subjected to the same dose without a rest period. Thus the recovery factor may be of great importance for some biological processes and not for others.

Second, Evans has shown slides giving LD_{50} data for mice, dogs, etc. I think that we oversimplify matters in referring to mice, for example, because such factors as age, sex, and strain are all known to influence the response of mice to irradiation, and a greater degree of specificity is required in dealing with such data.

SMITH:

The wide variability encountered in radiation dosage-mortality studies seems to be due in large part to metabolic differences in stock animals. A total of 739 mice in nine experiments, each divided into two treatment groups approximately balanced as to litter and irradiation animals, were irradiated at one of four dosage levels between 325 and 470 r. To the diet of one treatment group 0.3 per cent of desiccated thyroid was added immediately after irradiation. The χ^2 of the responses about the dosage-mortality line was 14 for the thyroid-treated animals and 44 for the controls, each showing evidence of significant variation. The probability that the difference between the two χ^2 values is due to chance alone is about 7.5 per cent. Thyroid administration seemed to reduce the heterogeneity of response, possibly by reducing the metabolic differences between the experimental groups.

BOND:

I should like to add a footnote to this paper by relating certain observations on the effects of partial body irradiation that Marguerite Swift and I have made in collaboration with Tobias.

We have worked with rats and have used clinical signs and the dosage necessary to produce mortality as end points of biological damage. In addition, we have, with each type of partial body irradiation, determined the volume of tissue irradiated, as well as the particular organs exposed; hence dosages in gram roentgens could be approximated.

If the abdomen only is exposed, the mid-lethal dosage, expressed in roentgens measured in air, is approximately 1100 r, about 400 r above the total-body LD_{50} dose. The clinical effects are very similar to those found after total-body irradiation, and it is of interest to note that it is possible to produce this syndrome and death in this manner with considerably fewer gram roentgens than are required with total-body irradiation.

If the abdomen is shielded, however, and the remainder of the body is irradiated, the mid-lethal dose is about 2000 r, and the animals die with clinical signs quite different from those seen after total-body irradiation. If only the head is exposed and the remainder of the body is shielded, death occurs at approximately the same dose level with weight loss compatible with that seen in starvation.

It was of interest to see whether the syndrome of acute total-body radiation illness could be approximated by means of irradiation confined to small portions of the abdomen. If one half of the abdomen was exposed, the dose necessary to kill was about 1600 r and the clinical picture was not greatly different from that of total-body irradiation.

To obtain highly selective irradiation, we were fortunate in having access to the 190-mev deuteron beam produced by the 184-in. cyclotron in Berkeley. The beam traverses the entire width of a rat with essentially no lateral scatter, and the *RBE* of the ray, as determined by total-body lethality studies in mice, is approximately 1. A $\frac{5}{8}$-in.-diameter beam was used, which will irradiate approximately 12–14 grams of tissue as it passes laterally through a rat.

It was found that irradiation confined in this manner to the abdomen produced acute deaths at dosages of the order of 2000 rep, and the clinical picture showed definite variations from that seen after total-body irradiation. In addition, no particulary "sensitive" region was found; irradiation of large portions of the liver, spleen, or stomach at comparable doses did not produce a syndrome analogous to that following total-body irradiation.

Lateral irradiation to include both adrenals (plus a portion of the spine) produced no visible acute effects at 3500 rep; however, some of the animals developed paralysis of the hind limbs about 2 months after irradiation. It is of interest also that, while localized gut irradiation produced adrenal hypertrophy and thymic atrophy, depression of the red-cell elements of the bone marrow did not occur. Depression of the red-cell elements can be demonstrated 24 hr after total-body irradiation by observation of the rate of uptake of radioactive iron.

HAHN:

It may be pertinent at this time to point out that the pathological human organism may be used as a test tube in the study of irradiation effects on cells. As Tobias mentioned yesterday, there are often relatively simple relationships existing, as for instance in the equation he gave for cell survival, $N = N_0 e^{-dD}$.

In the treatment of chronic leukemia in human beings by intravenous administration of single doses of colloidal metallic gold^{198}, the isotope is deposited in the reticulo-endothelial system, largely in the liver. The resultant drop in white-

blood-cell count in the peripheral circulation when plotted on multicycle semilogarithmic paper follows a straight line even over ranges of 5×10^5 to 1×10^4. This relationship is then simply

$$\frac{dw}{dt} = aw$$

and integrating,

$$W = Ke^{at}$$

where w is white-cell count per mm^3, t is time, and a is a constant.

Similar relationships exist for the white cell vs. time following x-irradiation to the spleen. Thus, the results of beta radiation to the liver and of x-radiation to the spleen in myelogenous leukemia have a striking similarity in effect on this indicator. The most obvious common feature in the two conditions is the extremely high degree of vascularity of the organs irradiated.

FRIEDELL:

At our laboratory in Cleveland we have been conducting some work on partitioning radiation by utilizing radioactive isotopes having selective localization in the body. For example, colloidal Au^{198} will be deposited in the reticuloendothelial system and will therefore irradiate the liver and spleen primarily. Sr^{90} or P^{32} will be bone-seekers primarily. It is thus possible to irradiate these two systems singly or in combination. We have found that the combination of Au^{198} and P^{32} results in marked synergism in the killing of albino rats. For example, half the definitive dose of radiogold which will kill 20 per cent of the animals, plus half a definitive dose of radiophosphorus which kills 20 per cent of the animals, would, theoretically, result in a 20 per cent death rate. Instead, a 60 or 70 per cent death rate is obtained. Analysis of these results is not easy, but a relationship between separately irradiated systems has been suggested previously. Jacobson's work on change in death rate by protection of the spleen appears relevant to this problem.

21 ·

Some Physiological Factors Related to the Effects of Radiation in Mammals

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Initial Hyperemia and Blood Volume

Irradiation of the mammalian organism induces a number of changes. Some of these changes involve the circulatory system. An easily recognized vascular response is the radiation erythema which is apparent in man during the first day following irradiation of the skin by a few hundred roentgens of x-rays. Local irradiation with 800–1200 r produces some hyperemia in rabbit skin, and at such sites there is increased spread of intradermally injected Evans blue dye; also there is an accelerated appearance of this dye in the hyperemic area when it is given intravenously (1). These observations suggest a complicated vascular response involving perhaps changes in tissue perfusion rate as well as capillary leakage and increased movement of tissue fluid. There is no indication of a change in blood volume (Evans blue method) in the rabbit during the first 5 hr after irradiation (1). The initial hyperemia of irradiation may be due to a local effect of the radiation on the small vessels (1, 2). With severe local exposure there is often a bluish hue in the hyperemic area which may be indicative of narrowing of parts of the vessels as well as dilation and filling of other parts of the vessels (2). With increasing radiation dosage to the skin there is more and more permanent deformation of the vascular bed; the aftermath of accumulation of a few thousand roentgens is marked by the existence of telangiectasis, which has been described as composed of capillaries of the diameter of veins (3). The state is indicative of a severe disturbance of the tissue vascular bed, and in spite of the ruddy appearance of these areas the flow of blood through such tissue is probably inadequate for proper function.

There is evidence that the perfusion rate of blood through the liver * may be slightly decreased after acute specific irradiation of the organ (use of Y^{90} colloids). (See Fig. 1.) However, the irradiation dosage must be great in order to measure an effect. After an accumulation of 20,000 rep in 3 days in the liver due to uptake of Y^{90} colloid, mice showed a 30 per cent reduction in rate of uptake of chromic phosphate colloid

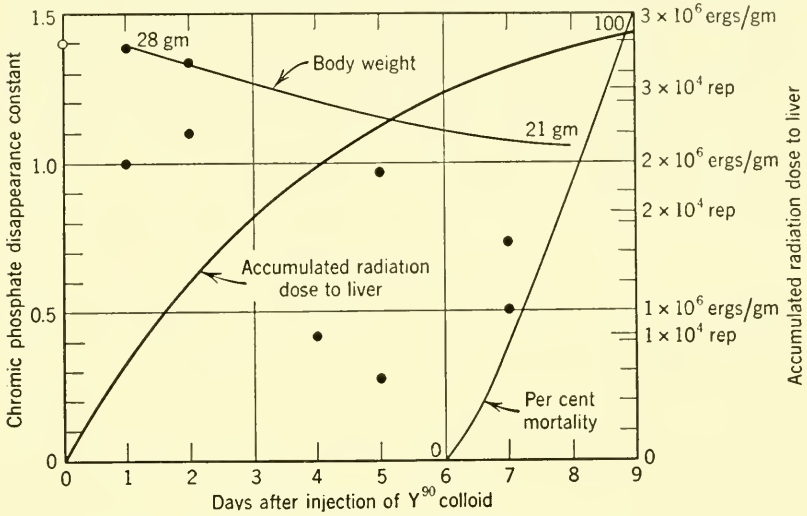


FIG. 1. The circles represent the chromic phosphate disappearance constant (obtained in mice) plotted as a function of time after the injection of a liver-localizing colloid containing Y^{90}. The open circle at zero time represents the average disappearance constant as measured on normal non-anesthetized mice. Also included on the graph are the body weight, mortality, and accumulated irradiation dose to the liver. The ordinate at the right represents the radiation dose in two sets of units, ergs per gram of liver tissue and roentgens equivalent physical. The units for the body-weight curve and for the per cent mortality curve are given at the beginning and end of the curves. [Graph by Dobson (4).]

by the liver, and this was divided between a reduction in phagocytic efficiency and decreased liver perfusion rate. After 6–9 days these animals had accumulated 30,000 rep, and the combined decrease in phagocytic efficiency and circulation amounted to a 60 per cent decrease in chromic phosphate disappearance. Thus for moderate irradiation dosages very little effect would be expected in the effective liver perfusion

* As measured by chromic phosphate disappearance. Under most circumstances the clearance of chromic phosphate through the liver is greater than 90 per cent. It is estimated that about half the irradiation effect noted here is a decrease in the phagocytic efficiency of the liver in the removal of chromic phosphate from the blood, and about half is a true decrease in the perfusion of blood through the liver (4).

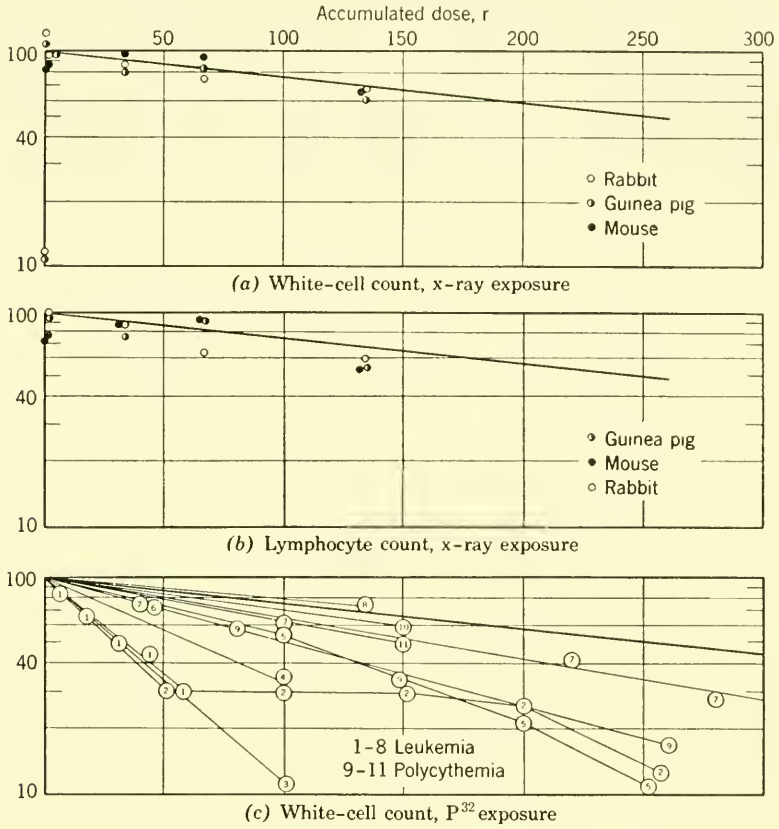
rates, and none has been observed (1). Late changes of 90 per cent reduction in perfusion are apparent in mice that have been subjected to comparable amounts of specific liver irradiation, but these are associated with cirrhotic changes in the liver such that there have been obvious degeneration and regeneration of the tissue.

With whole-body irradiation in the lethal range, there are disturbances in coagulability of blood (5). Another disturbance in the composition of the blood at the lethal level of whole-body irradiation is the development (in the rabbit) of an opalescence of the serum which is apparently due to lipids smaller than chylomicrons and larger than the usual lipid macromolecule. Such a lipemia is never present in the normal rabbit. Its development during the first 24 hr after irradiation seems to be prognostic of subsequent lethality (6).

Hemoconcentration

In contrast to the above changes which are noticeable in the severely irradiated animal, the concentrations of the cellular elements of the blood are especially sensitive and their rate of formation rapidly reflects accumulated irradiation. Changes in the level of red and white cells have long been associated with radiation effects. The production process of either of these cell types is very sensitive to irradiation. However, immediate response to radiation is usually noted in the concentration of the white blood cells rather than the red for the reason that the duration of life of the circulating erythrocyte is many days, whereas that of the white cell is a few minutes (7). Hence, the concentration of white cells will reflect early changes in their production. In Fig. 2 there is plotted on a relative scale the concentration of white cells and lymphocytes as a function of accumulated radiation. The values are for chronic exposure rates of 0.1–15 r per day, and the data for mice, guinea pigs, and rabbits are calculated from results given by Jacobson and Marks (8). A slope of best fit has been drawn for both the total white-cell and the lymphocyte count. The slope of the line indicates a 0.25 per cent change in the white-cell or lymphocyte count per roentgen in these animals. In the same figure there are plotted a number of therapeutic responses of leukemic-cell concentration as a function of accumulated radiation from radioactive phosphorus. The assumptions in this calculation are that radioactive phosphorus has a combined biological and radioactive half life of 7 days, and that the average concentration of the radioactive phosphorus in the leukemic tissues is twice the average concentration by the soft tissues. The least response to radiation by the leukemias

approximates the response of the normal leukocytes of the mouse, guinea pig, and rabbit. The reduction of white count in polycythemia is also close to a value of 0.3–0.6 per cent change per roentgen. Some of the



(a) White-cell count, x-ray exposure

(b) Lymphocyte count, x-ray exposure

(c) White-cell count, P^{32} exposure

FIG. 2*a*, *b*. The depression of leukocyte formation by x-irradiation, calculated from Jacobson and Marks (8). These values are averaged for leukocyte count and accumulated dosage for chronic exposures for the interval 20–40 days. Part *c* is the relative response of leukemia and polycythemia leukocyte count to radiation therapy with radioactive phosphate. Half the leukemias and the polycythemias approach the average sensitivity curve of the normal leukocytes as measured in the small animals.

leukemias seem to be as responsive to irradiation as a 2.5 per cent change per accumulated roentgen equivalent physical (rep).

Recently it has been possible to measure the rate of formation of red blood cells from the quantitative disappearance of labeled iron from the plasma. This method has been used to determine the relative rates of

incorporation of iron into the circulating red blood cells in the normal and the irradiated rat. Two criteria of red-cell formation can be used: (1) the relative uptake of labeled iron by the marrow-red-cell system compared with deposition in the inactive pool of iron in the liver; (2) the rate of appearance of labeled iron in the circulating blood. The data of Hennesy and Huff (9) have been recalculated on this basis, and it can be seen (Fig. 3) that the uptake of iron by the marrow decreases by 50 per cent at an irradiation dose of 150 r. The general level of the decrease in red-cell formation is 0.3 per cent per roentgen, which is comparable to the depression of leukocyte formation. It is worth noting,

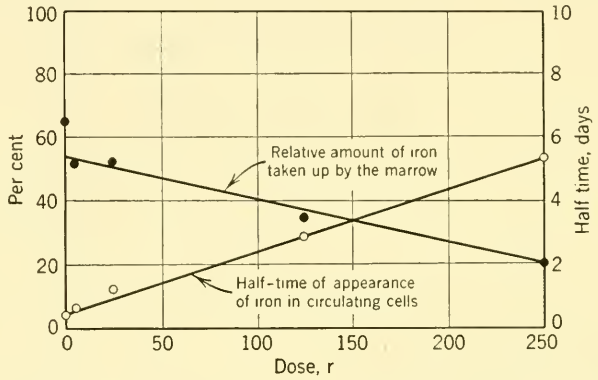


Fig. 3. The half time of the appearance of a tracer dose of radioactive iron in the circulating of blood and the fraction of the dose that enters the marrow during the first 25 days. Both are functions of the rate of red-cell formation. The lower the half time of entry into the blood and the greater the amount of radioiron reaching the circulating blood, the more rapidly new cells are being formed. [Calculated from Hennesy and Huff (9).]

however, that the erythrocyte study (9) deals with acutely irradiated rats, whereas the leukocyte response is based upon a less intense dosage rate spread over several days. Both criteria of red-cell formation indicate an irradiation effect in the range of 5–25 r.

Effects Relative to the Desoxypentose Nucleic Acid Molecule

Significant differences have been observed in the turnover of desoxyribose nucleic acid. These studies follow the general method of Hevesy (10). Whole-body irradiation depresses formation of nucleic acid as measured by the rate of incorporation of radioactive phosphate into

desoxypentose nucleic acid molecule (10). The following summarizes some of our findings (11): 320 mice gave an average value of $2.50 \pm 0.045 \times 10^{-3}$ specific activity of P^{32} in tumor * desoxyribose nucleic acid; 92 animals receiving 60-r whole-body irradiation gave an average nucleic acid specific activity of $2.23 \pm 0.041 \times 10^{-3}$. The difference is significant at the 1 per cent level, and the magnitude of the effect is 0.18 per cent depression per roentgen, which is of the same order as the depressive effect of radiation on red- and white-cell formation. Presumably the synthesis of desoxypentose nucleic acid is a function of the mitotic activity of the tissue, so that this also represents the effect of radiation on a proliferative process. In the same group of animals the specific activities of liver desoxypentose nucleic acid were examined; they are:

Controls (188 animals)	$3.83 \pm 0.19 \times 10^{-4}$
60-r total-body x-irradiation equivalent to 1.4×10^5 ergs (84 animals)	$3.16 \pm 0.26 \times 10^{-4}$
Muscles irradiated with 5.25×10^5 ergs (148 animals, radioyttrium colloid)	$2.83 \pm 0.12 \times 10^{-4}$

The difference of liver specific activities of desoxypentose nucleic acid between unirradiated controls and the 60-r whole-body irradiated animals is in the expected direction and magnitude, but it is of doubtful significance statistically; the depression of desoxypentose nucleic acid turnover is 0.28 per cent per roentgen. When (see above data) muscles are irradiated by an infiltration of radioactive yttrium colloid, a significant depression of the desoxypentose nucleic acid specific activity is observed. The effect obviously must have been indirect, as in this case the liver was unirradiated. If the ionization energy had been expended as average whole-body irradiation, the observed effect would have been a 0.16 per cent depression of specific activity per rep.

In the same animals (irradiation localized to muscle) the specific activity of the tumors was $2.10 \pm 0.042 \times 10^{-3}$ (that in the control animals, above, was 2.50 ± 0.045); the difference is significant. The effect, had the same ionization been expended over the whole of the animal, would have been 0.07 per cent depression per rep. The liver is apparently relatively more sensitive than the tumor to the indirect effects of radiation on the formation of desoxypentose nucleic acid, but this might relate to such facts as that the liver has a much greater blood supply than the tumor and that the tumor has 10 times more relative turnover of this nucleic acid than the liver. When livers are specifically irradiated with radioactive colloid (4.25×10^5 ergs), the specific activity of

* Mammary carcinoma transplants in A strain mice.

P^{32} in tumor desoxypentose nucleic acid is $1.65 \pm 0.52 \times 10^{-3}$. Thus, irradiation of liver gave a greater depressive effect on tumor desoxypentose nucleic acid turnover than muscle irradiation. This has been calculated as equivalent to 0.15 per cent per rep if the radiation had been expended over the whole body.

The above results of depression of desoxypentose nucleic acid turnover (11) confirm Hevesy's finding of an indirect effect of radiation on nucleic acid turnover (10). The quantitative effects of irradiation on the proliferative process are summarized as follows:

Whole-body x-irradiation	Depression of white-cell count Depression of lymphocyte count	0.23% per r
Whole-body x-irradiation	Depression of leukemic cells	0.23–2.5% per r
Whole-body x-irradiation	Depression of white cells in polycythemia	0.3–0.7% per r
Whole-body x-irradiation	Depression of red-cell formation	0.3% per r

Depression of Formation of Desoxypentose Nucleic Acid in Tumor

Whole-body x-irradiation	0.18% per r	
Specific-muscle β-irradiation	0.07% per rep	Reps calculated as though ionization had been expended on the whole body
Specific-liver β-irradiation	0.15% per rep	

Depression of Formation of Desoxypentose Nucleic Acid in Liver

Whole-body x-irradiation	0.28% per r	
Specific-muscle β-irradiation	0.16% per rep	Reps calculated as though ionization had been expended on the whole body

Measurements of the turnover of nucleic acid in the specifically irradiated liver are suggestive of a greater depression than the equivalent irradiation of whole body or muscle by the same total ionization, but we have not been able to quantify these data because of difficulties of separating the enormous quantity of beta-radiating colloid contained in the livers from the extracted P^{32}-labeled desoxypentose nucleic acids. Gofman (12), in a preliminary summary of leukemia therapy with radioactive phosphate as contrasted to a colloid of radioactive yttrium that localizes specifically in the bone marrow, indicates that the depression of the leukemia process is essentially related to the ionization directly expended in the marrow. Thus, much smaller radiation dosage of yttrium colloid is required than in therapy with radioactive phosphate. Probably direct irradiation of tissue is 1.5–2 times as effective in depressing the proliferative process as the indirect effects described above.

Circulation Studies

In estimation of the indirect effects of irradiation between the various tissues the circulation must be considered a factor of basic importance. If substances are produced which are removed by either the liver or the kidney with high efficiency, their effective time of detectable concentration in the blood will be very low. For example, if a material is distributed in the blood alone and removed by the liver with 90 per cent efficiency, it will fall to 10 per cent of the initial concentration in 10 min (since the liver circulation is greater than one volume of blood per minute per volume of tissue), and if the substance is produced at a steady state, the level of circulating material in the blood at any time will be only 3 per cent of the quantity produced per hour. The organs and tissues with the greatest circulation will necessarily be exposed to the greatest amount of all labile material related to radiation effects that are carried by the blood.

Animals joined together in parabiosis have been used to study indirect effects of radiation. The common bridge of tissue between such animals is a site of exchange of materials and, of more importance, of the intermingling of blood. Because of the fact that the average blood perfusion rate of skin and connective tissue is low (13), approximately 0.02 volume of blood per volume of tissue per minute, the relative exchange across the tissue bridge of parabionts must be insignificant compared to the total cardiac output. From the fact that 15 per cent of the cardiac output is used by the non-visceral tissues and from the volume of the tissue concerned in the tissue bridge, the maximum exchange between parabionts can be placed at 1.5 per cent of the cardiac output per minute, assuming that all the blood from one animal that reaches the tissue bridge is ultimately collected in the venous drainage of the other. This small exchange means that the parabiont cannot be used to detect materials which are of short biological duration in the body, since obviously most of such material will be consumed in the animal in which it is produced before it can pass the tissue bridge, and ineffective amounts will be carried to the partner. Since these circulatory exchange factors are basic to interpretation of induced effects in the parabiotic states, a study has been made to quantify the above principle. The measurement of physical exchange of blood between rats in parabiosis indicates 0.66 per cent of the blood volume per minute, which would be approximately the same value if it were present as percentage of the cardiac output (14). Exchange of an electrolyte is much slower because of the greater space occupied in either animal relative to the quantity of blood

exchanged and the tissue bridge. In the measured exchange of radioactive sodium (14) it was found that the sodium equilibration between parabiotic animals was 3.5–4.5 times slower than the exchange of labeled red cells. Thus, the distribution of material in the body is another factor which must be considered when using the parabiont as a test animal. Indeed, it must be said that negative results found in the parabiotic state should never be used to indicate an absence of indirect effect of radiation, since only relatively stable, relatively unexcreted, relatively slowly metabolized substances have a chance of crossing the parabiotic bridge in concentrations approaching the level in the irradiated member of the pair. Van Dyke and Huff (15) have shown that, when a parabiont is irradiated (900 r) with one member shielded, both become epilated and the non-irradiated member is relatively more epilated than the other. The effect occurs late, 24 days after irradiation, and reaches a maximum at 30–60 days. If this observation is evidence for a specific substance producing epilation, the substance must have a long latent period or continue to be produced in considerable quantity long after exposure to radiation. The irradiated members of the pairs are obviously stunted, and the observed effects, as the authors (15) point out, may be a "manifestation of loss of critical metabolic material from the non-irradiated to the irradiated member."

Changes in the Environment

The effect of work and fatigue upon ability to survive irradiation has been evaluated by Stapleton and Curtis (16) and Kimeldorf *et al.* (17). Both indicate an increase in the lethality of irradiation when experimental animals are worked to exhaustion after irradiation. Probably moderate physical activity does not lessen chance of survival from whole-body irradiation.

Stapleton and Curtis have evaluated physical state after a median lethal dose (neutron radiation exposure) and find a marked decrease in ability of rats to do forced work (16). Kimeldorf has measured the survival of rats that have been swum to exhaustion once a day after radiation exposure. In 860- and 700-r whole-body-irradiation groups, the rats which were swum daily died 2–4 times faster than the non-swum animals. At 600 r half the swum group died but all the non-swum lived (Fig. 4). The approximate influence of this severe exercise seems to have been to produce effects that closely simulate, as seen in survival curves, those of radiation dosages 150–200 r greater. These results are for very severe exercise carried to exhaustion. Probably moderate physical activity would not lessen chance of survival significantly.

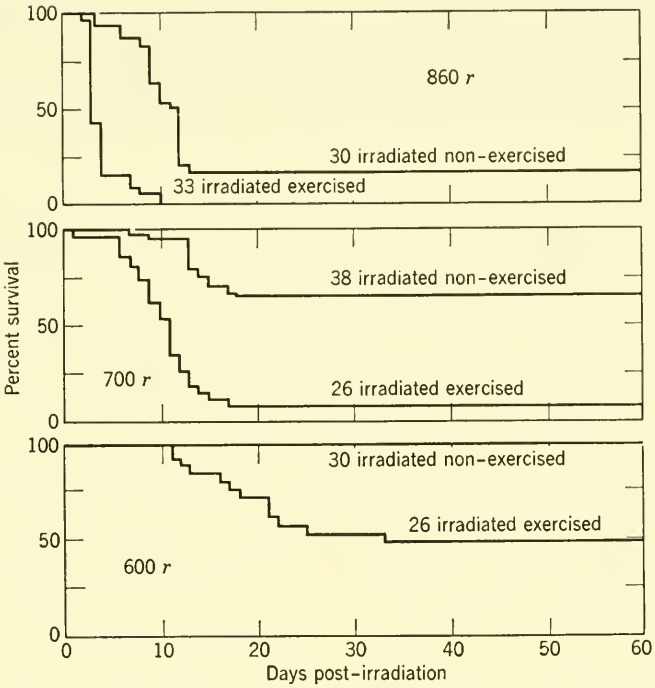


FIG. 4. Effect of exhaustive swimming once a day on the survival of rats irradiated in the lethal range. [These data were lent by Dr. Kimeldorf and Dr. Fishler of the Naval Radiological Defense Laboratory, San Francisco; see (15).]

REDUCTION OF IRRADIATION EFFECT

A vast store of information has accumulated on the mechanism of ionization and the reactions of ionization products related to biological effects of radiation. Two applications of this knowledge have given promise of methods for reducing radiation effects. (*a*) Cysteine has been shown by Patt *et al.* (18, 19, 20) to impart increased tolerance to lethal levels of radiation if given just before irradiation. The effect is roughly proportional to the quantity of cysteine administered when 50–1500 milligrams per kilo body weight are administered 5 min before irradiation. The response level of 50 per cent deaths (MLD_{50}) at 30 days shifts from 725 to 1100 r. Cysteine probably reacts with ionization products much more rapidly than other critical substances in the tissues and thereby imparts a partial protection when it is present in significant amounts. (*b*) Recognition has been made of the potentiality of dissolved oxygen to be converted into toxic substances by the energy

of the ionization process (21), and tissue oxygen depletion at the time of irradiation has been shown to increase radiation tolerance (22). Other physiological conditions that qualify the response to radiation will probably be found as our research on the problem continues.

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DISCUSSION

INGRAM:

Regarding the occurrence of lymphocytes with bilobed nuclei, I should like to remark that our observations, which were first reported quite some time ago, are somewhat different from those reported by Jones. Our results, based on

studies of human beings and dogs, showed (1) a marked increase in the incidence of the abnormal lymphocytes following exposure to extremely small doses of radiation from the cyclotron (doses difficult or impossible to measure), and (2) a peak incidence of these lymphocytes during an approximately 2-week post-exposure period. [Ingram, H., Barnes, S. W., *Science*, **113**: 32, 1951.]

ALTMAN:

Our group at the University of Rochester, including K. Salomon, G. W. Casarett, T. R. Noonan, and J. B. Hursh, has investigated the effect of total-body x-radiation on the biosynthesis of hemoglobin in rats with the use of a different precursor, namely alpha-C^{14}-glycine. We have failed to observe any depression of hemoglobin synthesis 24 hr after radiation with 300 r and 600 r, but rather have observed an increased C^{14} incorporation in hemoglobin, even as late as 48 hr after radiation. Whether this increase is due to stimulation of hemoglobin synthesis or increased availability of precursor cannot be stated at this time. Administration of alpha-C^{14}-glycine 7 days after radiation with 600 r showed marked depression of hemin synthesis and significant depression of globin synthesis. The apparent discrepancy between our findings and those reported by Jones cannot be completely evaluated, since in our experiments chemically pure hemin and globin were isolated, whereas in the experiments reported by Jones radioactive Fe was determined in the red cell without previous isolation of hemin.

FISHLER:

In relation to Jones's remarks regarding Hennesy's data on Fe^{59} uptake into the red cells of the irradiated rat, I should like to add that Hennesy has recently found that cysteine intravenously administered does not seem to protect against the x-ray-induced depression of Fe^{59} uptake into the red cells.

HEVESY:

Jones has obtained beautiful results. It looks as though the effect of radiation on tumors is proportional to the dose. Jones mentioned the figure 0.18 per cent per roentgen diminution of the rate of the formation of desoxyribonucleic acid, and with 300 r the diminution then is about 50 per cent. In other words, there is proportionality between the dose and the depression of the rate of formation of desoxyribonucleic acid, up to this level of radiation. If the dose is further increased, proportionality no longer follows. With 2000 r only 75 per cent diminution or less in desoxyribonucleic acid formation is found.

If a rat is inoculated with two tumors which are separated from each other as far as possible, and then one tumor is irradiated with 1000 r, the other tumor receiving no more than 2–3 r, the rate of incorporation of P^{32} into the desoxyribonucleic acid of the protected tumor can be shown to be affected almost as strongly as is the rate of incorporation into the directly irradiated tumor. If, however, the volume increase of the tumor in the postirradiation state is determined, the protected tumor is to a minor extent prevented from growing, whereas this is not true for the directly irradiated tumor.

JONES:

Our experiments indicate that an indirect effect on desoxyribonucleic acid metabolism is seen. We agree with Hevesy's experiments on the linearity of response in depression of desoxyribonucleic acid metabolism with doses up to 300 r.

KOHN:

In connection with the so-called "indirect effects" of irradiation upon cell division, it is of interest to recall an experiment by Friedenwald published some years ago, in which it was shown that the mitotic index of the cornea (mitotic figures per unit area) fell from about 100 to almost zero during the hour following excitement of the animals. This effect could be blocked by atropine (parenterally), and could be mimicked by instilling epinephrine into the conjunctival sac. Direct irradiation of the cornea, of course, can produce a similar fall in mitotic index. This example is not an isolated one, and it seems that many of the effects observed in radiation experiments have pertinent parallels elsewhere, both with regard to the setting up of controls and to general interpretation. It seems to me that very few of these have been considered during the course of these meetings.

EDELMAN:

1. In regard to Smith's remarks [see discussion following paper by Evans, p. 411] about the LD_{50}, may I state that the LD_{50} in our laboratory has risen in 3 years from 650 to 850 r. Critical examination of the records makes it appear that the shift in the lethal-dose values is due to better facilities for survival in handling and care of the animals. The LD_{50} is variable, and suitable controls must be run with each experiment.

2. I also want to echo Kohn's sentiments about the exciting of animals. Rats placed in a tight cage and x-rayed show an 80–90 per cent drop in leukocyte count 3 hr after 600 r. Controls, similarly restrained and placed under the machine with the tube on but with the lead cover in place so that the animal receives no radiation, show a leukocyte count drop of 70–80 per cent. This is transient, the count returning to normal after several hours, but these facts must be known and taken into consideration in radiation work.

3. Margaret Holt, in our laboratory, could not observe any bilobed white cells in smears made at intervals for 96 hr after exposure to 600 r of x-radiation. This, however, may be due to their infrequent occurrence and to the few (100–150) cells counted per animal.

22.

Mammalian Radiation Genetics

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Introduction

This symposium is concerned with the basic aspects of radiation effects. When we turn to the genetic effects of radiation in mammals, there are so few aspects on which there is any information that the problem of sorting out the fundamental findings has hardly arisen. In this paper it will, therefore, be possible to survey most of what is known and pass on to a consideration of what is needed next. Since one of the purposes of this symposium is an interchange of views between investigators in different fields, an attempt will be made to avoid technical details.

Among the practical needs in mammalian radiation genetics is a pressing one for more data on which to base estimates of the genetic hazards of radiation in man. The present paper will be concerned largely with this problem. Our own work is directed primarily in this direction, our objective being to uncover some of the basic facts in at least one mammal—the mouse. Before discussing the experimental work, however, it seems desirable to consider some of the general features of the genetic hazard of radiation.

Nature of the Genetic Hazard of Radiation

Among the hazards of exposure to radiation, the genetic effects are of importance because of several unique features.

1. There is usually no healing of the damage. Some types of damage to the genetic material, for example the breaking of a chromosome, may, under certain conditions, heal. Others, for example "cell lethal" mutations, are by their nature prevented from passing on to any descendants. However, from effects that are actually hereditary, in the sense of becoming manifest in the next or subsequent generations, the only chance of healing lies in the remote possibility of reverse mutation.

2. The damage is transmitted to descendants. This is, of course, implicit in the term "genetic." Nevertheless, it is sometimes forgotten. Arguments to the effect that we should be phlegmatic about the effects of small doses of radiation, because the total damage is probably no worse than that resulting from various other insults to the organism which man tolerates or even enjoys, ignore the fact that among commonly tolerated insults radiation is the only one known to affect descendant generations.

3. The damage is hidden for a long time before it becomes manifest. Hereditary effects obviously require at least one generation to express themselves. For the large class of recessive mutations, many generations would, on the average, be required in a large, more or less random-breeding population. This class of mutations is particularly insidious in the sense that, even when a particular recessive mutation is finally revealed, it is usually only a very small fraction of the total effect that has become manifest, the rest still being hidden in individuals heterozygous for the mutation.

4. There is no threshold dose. In other words, genetic changes may be expected at any dose, no matter how small. There is a large body of experimental evidence of various kinds which supports this contention and some actual confirming data from doses as low as 25 r in *Drosophila* [Spencer and Stern (21)]. It is true that Caspari and Stern (2) have presented data which could be interpreted as indicating that the linear relationship between gene mutations and dose does not hold at low doses, but, as the authors themselves point out, other interpretations are possible. It should also be mentioned that, although the difference in mutation rate between controls and experimentals (given 52.5 r chronic exposure) is not statistically significant in these data, this should not be allowed to obscure the fact that the rate observed in the experimentals is higher.

In drawing up safety measures against genetic effects of radiation in human populations, serious attention must be given to point 4. If there is no threshold dose, then a so-called "tolerance" dose cannot be one which produces no genetic effect, but only one which does not add a "serious" increase to the effects that already occur as a result of natural radiation and other causes. The problem of estimating the genetic hazard of radiation, therefore, resolves into two main questions: (i) What is the increase in mutation rate per dose? (ii) How great an increase can be tolerated?

The first question is relatively simple, although the designing of practical experiments to answer it may not be easy.

The second question is highly complex. In the first place, it involves

human values. Even if we postpone a consideration of these by changing the question to "What are the effects of a given increase in mutation rate?", the problem is still a tremendous one. Muller has, in his paper (p. 322), already pointed out some of the many unknowns: the relative numbers of the various types of genes, their degrees of dominance, and so forth. Other aspects, particularly effects at the population level, present a major challenge to statistical geneticists. The problem is not entirely novel to them. The same complex of variables—mutation and selection pressures, population size, coefficients of inbreeding, etc.—has been considered in dealing with the problems of evolution and improvement in domestic plants and animals. In the present state of knowledge of these variables, the diversity of theoretical possibilities that can be derived from a consideration of them is almost overwhelming. An excellent example of this diversity is provided by Wright (23) in his speculations on the effects which an increased mutation rate might have on just one single character of a population, namely, its reproductive rate.

Wright calculated that, with regard to completely recessive mutations that affect reproduction in the population solely by depressing the reproductive value of the mutants themselves, a somewhat optimistic view can be taken on the chances of survival of the human population. He points out, however, that this is not the only way in which the reproductive value of the population could be affected. If the mutants were of inferior quality (for example, with lowered intelligence or character), they could constitute a drag on the population as a whole in its ability to utilize its resources and thus bring about a further reduction in the reproductive value which, with high mutation rates, could conceivably lead to collapse rather than to a new equilibrium. If the mutants were inferior and *not* less fertile than the non-mutants, then the population would tend towards collapse with even the slightest mutation rate. A systematic increase in mutation rate would speed up the process. Finally, if the mutants were inferior but *more* fertile than the non-mutants, such a runaway process would be enormously enhanced.

Wright suggests still another line of possibilities. For example, it could be imagined that with social and economic changes resulting from the increased number of mutants, the relative reproductive values of mutants and non-mutants might change too, and even reverse their order, thus, in the last case given above, leading to a new equilibrium instead of collapse.

It is apparent that the question of how great an increase in mutation rate can be tolerated in the human population is exceedingly difficult to answer even when consideration is limited to a single, quantitatively

measurable end effect: the reproductive rate. The difficulty of estimating theoretically the combined effects of the complex of variables emphasizes the need for experimental work in population genetics, such as that being done on *Drosophila* by Wallace (22), in order to collect empirical data. In mammals there is still much to be done, in fact almost everything, on the preliminary problem of measuring the radiation-induced mutation rate. In the next section, some of our present experiments and the results of earlier investigations are outlined and discussed.

Experimental Work

Gene mutations

Mutations produced by x-rays have been reported in mice by Snell (15) and Hertwig (7, 9, 10), but the data are not adequate for a reliable estimate of mutation rate. The difficulties encountered in trying to measure mutation rates in mice, as compared with *Drosophila*, are very great and not, as is often supposed, due solely to the slower reproductive rate of mice and to the greater space and labor required to raise them. In *Drosophila*, the most reliable and most easily determinable mutation rates are those based on lethal mutations on the X-chromosome. These rates are obtained by a well-known method, devised by Muller, which makes use of sex-linked genes, acting as markers, and chromosome inversions which suppress crossing over. Neither of these being, as yet, available in the mouse, Muller's method cannot be used. Sex-linked lethals could be detected by observing a disturbed sex ratio in the offspring of daughters of irradiated males, but this would be far more tedious and relatively uncertain unless further extensive tests were made.

It would be out of place here to consider in detail the possible ways of measuring mutation rates in other whole groups of genes. For our present research at Oak Ridge they have been rejected in favor of a method for obtaining mutation rates at specific loci. This consists essentially of mating irradiated wild-type mice, and non-irradiated controls, to a strain homozygous for as many known recessive genes as can be managed practicably. A mutation at any of the loci represented by the recessives will then be detected in the first generation. The method has not been suggested before, for mice, presumably because of the relatively large number of animals needed. It was calculated, however, that, with the facilities offered by the Atomic Energy Commission, reliable mutation rates might be obtained in a reasonable time if they were not lower, or much lower, than the *Drosophila* rates.

The method has several advantages. All mutations are detected in the first generation, whereas methods for obtaining autosomal recessive

visible mutations as a group require at least three generations and then recover only a portion of the total. The mutants can be recognized at a glance, in contrast to the detailed examination, by highly trained observers, necessary when searching for mutations at all loci.

Another advantage of the method lies in the fact that the type of information obtained by it should be suitable for a meaningful comparison with results from *Drosophila*. Data on the rate of mutation of visibles at all loci would, for example, be less favorable because the number of mutants detected is dependent on the minuteness of examination of the anatomy and physiology of the organism, and it would be hard to determine comparable levels for this in species as widely different as fruit flies and mice. It is important to have data that can be compared because the total information on mutation in mice is bound to lag behind that for *Drosophila*. The estimating of human hazards will, for a long time, be dependent on some extrapolation from fruit flies to men, and this will be less conjectural if at least one meaningful cross-reference point is established between *Drosophila* and a mammal.

It is encouraging to know that, as work on specific loci in the mouse proceeds, Muller and his collaborators are making large contributions to data of this kind in *Drosophila*. In addition to increasing the reliability of the *Drosophila* side of the comparison, these data will be of help in evaluating the results obtained in the mouse. Thus, the extent of variation found in the induced rates of the many loci being tested in *Drosophila* will have a bearing on the confidence with which general conclusions can be drawn from the average rate obtained for the seven loci under examination in the mouse.

Muller's data are also likely to prove of great aid in the planning of further experiments on mice. The *Drosophila* results will include induced mutation rates at the specific loci in eggs as well as in sperm, and in immature as well as mature gametes. Observations are also being made to determine what proportion of the mutations is associated with detectable chromosomal aberrations. Information on some of these factors may be obtained in the mouse, but, as it is a major task to collect enough data for a reliable over-all rate, there can be only limited subdivision of the data or repetition of the experiment under other conditions. The *Drosophila* results on these and other factors should show which are likely to be the important ones to test.

It is too early yet to draw conclusions from the results being obtained at Oak Ridge.* A pilot experiment was undertaken while the stocks of mice were being built up to the size necessary for the large-scale program. The findings from this experiment have settled many of the problems,

* Russell, W. L., X-ray-induced mutations in mice, *Cold Spring Harbor Symposia Quant. Biol.*, **XVI**, 1951 (in press).

particularly effects on fertility, involved in an economical operation of the large project. On the basis of mutation rates in *Drosophila*, few, if any, mutations at the chosen loci were expected in the pilot experiment. Some possible ones were, however, observed, and two of these have already been confirmed by breeding tests. Since more data should be available before long, it would be premature to report the mutation rate calculated from the present figures. The results do indicate that the large-scale program should yield enough mutations to give a reliable figure on the induced rate. The anxiety involved in having to depend primarily on *Drosophila* data in the planning of this large project with mice is thus somewhat reduced.

CHROMOSOME ABERRATIONS

Much is already known about the induction by x-rays of chromosomal aberrations in the mouse. The pioneer work was done by Snell (14, 15), Snell and Ames (20), and Hertwig (5, 6, 8). Their results, which have been published in detail and have been reviewed by others [for example, Lea (11)], will be presented briefly here in order to provide a background for a few comments about their bearing on the genetic hazards of radiation in man.

When mice are exposed to a heavy dose (for example, in the neighborhood of the median lethal dose) of x-rays and then mated to non-irradiated animals, it can be shown that the offspring conceived shortly after irradiation fall into at least four distinct groups. One group dies in early embryonic stages. The death of this group is presumably due to chromosomal aberrations ("dominant lethals") some of which, as shown by Brenneke (1), result in cytologically visible abnormalities in the nuclei of cells in the early cleavage stages. The remaining three groups are born alive and all appear normal, but only one group proves to have normal fertility, the other two being sterile and semisterile, respectively. It is probable that there are other groups, including stillborn and living abnormal animals, but these seem to be relatively rare.

Since the fertile and the semisterile groups breed, they can be tested genetically. The fertile animals, when outcrossed to normal animals, produce normal, fertile offspring. The semisteriles, when outcrossed, yield three main types of progeny. The first type is comprised of embryos that die at various stages in development. The loss of these accounts for the reduced litter size, about one-half normal, by which the semisteriles were first recognized. The other two types, which occur in equal numbers, prove to be semisterile and normal fertile. On further testing of these, they turn out to be like the semisteriles and fertiles obtained in the first generation following irradiation. Thus, the semiste-

rility is passed on as a "dominant" to one-half of the viable offspring of semisterile animals.

Snell suggested that the semisterile animals carried a reciprocal translocation which was inherited by their semisterile offspring. He thus accounted for the dying embryos as having unbalanced chromosome complements and for the normal fertile offspring as being balanced and not carrying the translocation. In one case, Snell (17, 18) was able to confirm this interpretation by linkage tests.

With the above brief historical outline as a background, we can turn to some later findings and a discussion of the results.

The results of Lorenz *et al.* (12), who detected no semisterility in the offspring of mice exposed to gamma rays from a radium source, have been widely quoted by authors concerned with human hazards, with the implication that they are at variance with the findings of Snell and Hertwig and that the explanation may lie in a difference between the effects of chronic and of acute radiation. Hertwig (6), however, had shown that the incidence of semisterility is high only in offspring conceived within a short period (about 4 weeks) following irradiation, that is, as a result of irradiating mature sex cells. If the experimental procedure of Lorenz *et al.*, as given in the earlier report of Deringer *et al.* (3), is examined with this in mind, it is found that the exposed females were not bred until a month after removal from the radiation field, and that although exposed males were bred immediately after removal it is not stated whether the offspring tested for fertility came from the first or subsequent litters. In all, 42 offspring of exposed males were tested. Even if it is assumed that all of these were conceived shortly after removal from the radiation field, the total dose received in postspermatogonial stages was still not very high. From the rates obtained by Snell and Hertwig, it could have been predicted that perhaps 1 of the 42 offspring would have been expected to show semisterility. There is, therefore, no conflict between the two sets of results. The question of dependence of effect on intensity of radiation is not yet answered, but since the effect with which we are concerned applies only, or mainly, to mature gametes, it seems probable that, as in *Drosophila*, intensity may be less important than total dose.

Additional data on the incidence of sterility and semisterility in the offspring of irradiated males have been obtained by us. * They will be presented in a separate paper for publication elsewhere, but they should perhaps be given in summary here to show that they agree closely with the results of Snell and Hertwig. The animals tested, 22 males and 15

* Work performed under Contract No. W-7405-Eng-26 for the Atomic Energy Commission.

females, were offspring of males exposed to 250-kvp x-rays at an intensity of approximately 80 r per min and for doses of 500, 750, or 1000 r. They were conceived within from 2 to 30 days after irradiation of the father. Their fertility was adequately tested, and the status of those classified as semisteriles has been further checked in descendant generations. The results obtained are compared with those of Snell (15) and Hertwig (8), for similar dose ranges, in Table 1. Averaging the effects

TABLE 1

INCIDENCE OF STERILITY AND SEMISTERILITY IN OFFSPRING (MALE AND FEMALE COMBINED) OF IRRADIATED MALES

Investigator	Dose to ♂ Parent, r	Offspring			
		Percentage			Total Number
		Sterile	Semi-sterile	Fertile	
Snell	400–1200 (mostly 600–800)	5.8	31.4	62.8	121
Hertwig	500–1000 (mean 764)	11.5	25.3	63.2	182
Russell	500–1000 (mean 669)	8.1	27.0	64.9	37

over wide dose ranges is, of course, a poor procedure, but over the ranges given here is presumably justifiable for the purpose of a rough comparison; and, in any case, Snell does not tabulate his results in a way that can be used to calculate the incidence for each dose.

The high rate of induction of reciprocal translocations by radiation in the mouse is, therefore, supported by three independent investigations and is not refuted by the results of Lorenz *et al.*

It is noteworthy that this rate is far higher than in the fruit fly. The yield from an acute dose of about 600 r in the mouse is comparable to that from 5000 r in *Drosophila melanogaster*. The difference between the two species is presumably due to the larger number of chromosomes or amount of chromatin in the mouse. It is, therefore, reasonable to guess that the rate in man would be as high as that in the mouse or even higher. If the hazard from this were not controllable, it would be serious. Fortunately, as has already been pointed out, Hertwig's work

shows that the incidence of semisterility is high only in offspring sired shortly after irradiation, namely, before the beginning of the sterile period that follows within a few weeks after the giving of high acute doses. In the progeny conceived after the end of the sterile period, the incidence of sterility and semisterility did not differ significantly from that in the controls. Applying these results to man, the important practical conclusion can, therefore, be drawn that the probability of passing on certain types of chromosomal aberrations to the next generation will be greatly reduced if individuals exposed to high doses of radiation refrain from begetting offspring for a period of perhaps 2 or 3 months after exposure.

Another problem concerning translocations that is of practical importance in man is the determination of the exact times of death of the aneuploids, or unbalanced chromosomal types, which result from the matings of semisterile with normal individuals and which apparently perish while still in embryonic stages. The death of an advanced fetus would obviously constitute more of a hazard to the mother than the loss of an early embryo. In the mouse, there seems to be considerable variation in the time of death, but from the work done so far, including the study by Otis (13), who also made a careful comparison of normal mouse and human developmental stages, it may be tentatively concluded that most deaths would occur before the seventh week of human pregnancy. It must be remembered, however, that the number of semisterile lines in the mouse that has been examined for this characteristic is not yet large.

There are many other aspects to the problem of induced chromosomal aberrations in mice which require further study. For example, in two out of eleven semisterile lines being tested at Oak Ridge, the viable progeny of outcrosses of semisterile with normal do not fit the simple ratio of one-half semisterile and one-half fertile. Both these lines are producing some completely sterile descendants, and one is yielding individuals whose fertility is apparently well below that of characteristic semisteriles. It is not yet clear what the pattern of inheritance is in these lines.

The possible effects of translocations in individuals homozygous for them also need to be investigated further. The few cases obtained so far are apparently completely normal phenotypically. This indicates, as Hertwig (8) pointed out, that position effect may be relatively unimportant in the mouse as compared with *Drosophila*.

Very little is known about the induction of chromosomal aberrations in mammals by radiations other than x-rays. Snell and Aebersold (19) and Snell (16) made a few experiments with neutrons. Dosage compari-

sons are difficult, but it appears that neutrons may be more effective than x-rays in the production of both dominant lethals and reciprocal translocations.

Estimates of the Genetic Hazard of Radiation

In spite of the lack of knowledge regarding radiation-induced mutation rates in mammals, several estimates of the genetic hazards of radiation in man have been published. These are based primarily on the induced rates in *Drosophila*. A common scale for measuring the hazard is the dose of roentgens that would produce a rate as high as the spontaneous rate. For this, a knowledge of the spontaneous rate in man is required also. Here, again, little information is available. For these reasons, most, if not all, geneticists would agree that current estimates of even the basic factor in the human genetic hazard, namely, the increase in mutation rate, are little better than guesses. Nevertheless, such guesses have to be made, whether explicitly or implicitly, because of the necessity for practical decisions, involving perhaps serious consequences, about the protection of people against exposure to radiation.

Several groups concerned with the safeguarding of personnel have, for some time, assumed that the mutation rate in man would be doubled by a dose of about 30–50 r. Two estimates differing widely from this figure in opposite directions have been suggested by Evans (4) and Wright (23). A brief consideration of these estimates will serve to bring out a few more of the problems involved in calculating the genetic hazard.

Evans takes $10^{-5}/3 \times 10^{-8} = 300$, in round figures, as the dose of roentgens required to double the natural rate of gene mutations per generation in man. The denominator in this expression is the induced rate for sex-linked lethal mutations in *Drosophila*, per roentgen per locus, averaged over all loci in the X-chromosome. Evans accepts this as applying to all chromosomes in man. The numerator, 10^{-5}, is assumed by Evans to be the rate for comparable spontaneous mutations per locus and generation in man. He bases this figure on the rates for hemophilia and epiloia.

Wright criticizes Evans on the following grounds. If the spontaneous rate of lethal mutation per locus per generation in *Drosophila* is 10^{-6}, and if *Drosophila* has only one-tenth as many loci as man, both of which figures are accepted by Evans, then the spontaneous rate per *gamete* per generation in *Drosophila* is only one-hundredth of what is assumed by Evans for man. Wright argues that it is likely that *Drosophila* and man would have acquired about the same spontaneous mu-

tation rate per gamete per generation, in so far as this is adjustable in the course of evolution, or that the rate would be lower in man, where there is less wastage of offspring between fertilization and maturity. Even if the spontaneous rate per gamete and generation in man is taken to be as high as that in *Drosophila*, this figure is still only one-hundredth of that assumed by Evans, or, holding the other factors constant, 10^{-7} per locus per generation. Wright points out that there is no incompatibility between rates of the order of 10^{-5} for hemophilia and epiloia and a rate of 10^{-7} for the average of all loci.

If, as Wright claims is possible, the average spontaneous mutation rate per locus in man is only one-hundredth of that assumed by Evans, and if the other factors assumed by Evans are correct, then the dose required to double the rate of mutation in man is also only one-hundredth of that calculated by Evans, namely, 3 r. Both this figure and the 300 r obtained by Evans are based on the rate of induced mutation in irradiated sperm. Irradiation of early germ cell stages would, on the basis of results in *Drosophila*, be perhaps only from one-half to one-fifth as effective.

Regardless of the evolutionary considerations raised by Wright, it seems to the present author that the product of the figures assumed by Evans for number of loci and spontaneous recessive lethal mutation rate in man is too high. A consideration of the effect it would have on the offspring of first-cousin matings, for example, will show that it can hardly agree with the facts. The value taken by Evans for the average spontaneous rate of mutation to recessive lethals per locus and generation in man has already been given and is $a = 10^{-5}$. He accepts the view that N, the number of loci in man, probably lies between 10^4 and 10^5. His figure for a minimum estimate of the accumulation factor is $m = 50$. Accordingly, the average number of recessive lethals per gamete would be aNm = between 5 and 50. Individuals would, therefore, on the average, be heterozygous for from 10 to 100 lethals, and the two common grandparents of first cousins would together be heterozygous for from 20 to 200. The probability that a particular gene heterozygous in one of the common grandparents will not become homozygous in an offspring of first-cousin mating is 63/64. Ignoring linkage, and lethals common to the two grandparents, the probability that the child of a first-cousin mating will not be homozygous for any of the 20–200 lethals heterozygous in the grandparents is, sufficiently accurate for our purpose, $(63/64)^{20}$ to $(63/64)^{200}$, or 0.73–0.043. Thus, according to the limiting values assumed by Evans, the percentage of death as a result of consanguinity in the offspring of matings of first cousins who have average grandparents would be from 27 to 96 per cent.

There are very few data available on the results of first-cousin matings in human populations, but 96 per cent death as a result of the consanguinity must surely be too high. Even 27 per cent death from this cause seems too large, particularly if consideration is given to the fact that, in *Drosophila*, the rate of occurrence of mutations with markedly deleterious effects is at least twice that of lethals. Applying this ratio to man would mean that, calculating from the lower limit given by Evans, there would be a total of 61 per cent death or markedly depressed viability.

The effect of consanguinity would, furthermore, be even greater if, as seems probable, the accumulation factor is higher than the estimate of 50, which was, in fact, taken by Evans as a minimum value.

If a significant proportion of the lethals, assumed to be recessive in the above discussion, had a selective disadvantage in the heterozygote which was sufficient to lower the accumulation factor below 50, then the above calculations would no longer hold. In this case, however, the effect of these genes in heterozygous condition would, on the basis of Evans' values for spontaneous mutation rate and number of loci, depress viability to such an extent that it would be doubtful whether the population could survive.

Thus, irrespective of whether the lethal mutations are completely recessive or have adverse effects in the heterozygotes, it would seem that the product of the values for spontaneous mutation rate and number of loci, as assumed by Evans, is too large. As there is no reason to reject Evans' range of values for the number of loci, it appears that his figure for the spontaneous mutation rate must be too high. This agrees with the view expressed by Wright, at least as far as indicating that the number of roentgens required to double the natural rate of mutation in man is lower than that calculated by Evans.

In the above treatment, as well as in that of Wright, it has been assumed that the value of 10^{-5} was meant by Evans to apply to the spontaneous rate of mutation to recessive *lethals* per locus and generation in man. Evans certainly attaches this meaning to it, for in his calculation of the ratio of spontaneous to induced mutations he divides it by the induced rate for recessive *lethals* per locus per roentgen. In other parts of his paper, however, he mentions it as if it referred to the mutation rate to *all* recessives. Most authors have taken this to be about 6 times the rate to lethals. If Evans did mean the figure of 10^{-5} to apply to all recessives, then his value for the ratio of spontaneous to induced mutations, or the number of roentgens required to double the natural rate of mutation, should be divided by 6. This would bring it in line with the commonly accepted figure mentioned at the beginning of this section.

There are, then, various reasons for thinking that the number of roent-

gens required to double the natural rate of mutation per generation in man is not as high as the 300 r estimated by Evans, and there is at least one argument, that advanced by Wright, for thinking that it may be as low as 3 r.

It would be out of place here to attempt to discuss all the published calculations of the genetic hazards of radiation. The above examples were chosen to illustrate some of the difficulties encountered and the wide range of current estimates. It should be noted that the difficult problem, mentioned earlier in this paper, of evaluating the effect of a given increase in mutation rate was not considered here. The sole objective under discussion was an estimate of the increase in rate following a given exposure to radiation. It has been shown that a wide range of answers to this limited question is possible according to the interpretations placed on the available data which, at the present time, are comprised mainly of the induced and spontaneous mutation rates in *Drosophila* together with the spontaneous rates of a few genes in man. It is apparent that one of the pressing needs in the estimation of human hazards is for basic information on mutation rates in mammals.

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DISCUSSION

MULLER:

I should like to re-emphasize Russell's remarks on translocation effects, produced when mature sperm are radiated. If the sperm used in the fertility studies are obtained after the sterile period, no translocation effects are seen. It is evident that the translocation effects have disappeared because of death of the involved sperm, and it should be re-emphasized that genic mutations almost certainly exist but were not capable of demonstration under the circumstances of the experiments done to date.

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Analysis of Mammalian Radiation Injury and Lethality

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The first man who asked some authority for an authoritative answer to the question, "How much radiation is safe?" started, quite unwittingly, a trend in mammalian biological thinking which is of great interest and significance. It has forced the development of a primarily theoretical and holistic approach in mammalian toxicology which, like its theoretical analog in the physical sciences, can become a powerful directive influence for research in certain baffling (and generally avoided) problems such as, in this instance, senescence. This trend has obvious similarities to cybernetics, population genetics, and some of the newer tendencies in the social sciences, and, indeed, has a social motivation; it leaves us in the present position of having to explore constantly for new techniques of research and thought which will serve to fill certain vacua in our minds regarding the nature of growth, longevity, and cancer.

The question is, at first sight, an irritating one to the biologist, since it implies an authoritarian answer such as one customarily asks of physicians and safety engineers. When the physician retires to his laboratory, it is not always clear whether he does so to answer such questions or to avoid them. When a physician is asked whether he would recommend a certain drug for treatment of some common pathological state (implying a judgment of safety), he may study the effects of the drug in experimental animals, but he must still extrapolate his findings to human beings. This extrapolation takes place through a statistical observation of the occasional drug reactions occurring in human beings, and through other clinical data.

What makes the comparable radiobiological question different is the quite proper general determination to solve the human problem without recourse to significant observations on detrimental human effects, together with the fact that the effects which concern us may be observable only after a period corresponding to the human life span. Although

we can say that essentially nothing we do is without hazard and that life is a succession of naïve and qualitative risk calculations, it is in radiobiology that man has elected to attack such a problem squarely and is finding it necessary to evolve and put under scrutiny some new or neglected concepts. Whether this has had an irrational or a rational origin is beside the point; if one is willing to be more inquisitive than irritable it is bound to become scientifically fruitful.

Let us follow the development of mammalian radiobiology from the purely descriptive phase, through that of quantitative physiology, and into the beginning of the theoretical phase just mentioned. We will limit ourselves, for the most part, to the effects of penetrating total-body irradiation, understanding that partial-body effects can often be deduced therefrom. Studies of partial-body responses by experimental techniques are, of course, of great importance in elucidating the total physiological picture. A case in point is the remarkable series of findings of Jacobson and associates where a very large part of the body (all but the spleen) has been irradiated (1). In certain cases paradoxical findings of the greatest potential significance have turned up. As examples of the latter we can mention the epilation of a non-irradiated parabiotic partner (2) and the ineffectiveness of partial-body irradiation in the induction of mouse lymphoma (3).

The initial radiation response, or the radiation sickness of radiological practice, is known as a benign but unpleasant state occurring during the first few hours following irradiation, and such evidence as is available suggests that the autonomic nervous system plays an important role in this syndrome. Certain animals (rabbits and birds) may succumb during this initial period without the overwhelming dosage (100 times the LD_{50}) necessary to cause immediate death in most species (4, 5). The occurrence of immediate death in the chick has been shown to be remarkably dependent upon dosage rate (6) and is associated with rapid accumulation of uric acid in blood and tissues (7), which may account for the special susceptibility of birds to early death. In the rabbit, hypotension is characteristically seen after total-body irradiation (8). It may be that the well-known lability of its peripheral circulation lies behind the early susceptibility of the rabbit.

One of the most remarkable things about irradiation death in the higher animals is the length of time between the physicochemical events which must be primarily responsible for death and the appearance of symptoms. Another is the relatively very large dosage necessary to kill most animals (with the exceptions noted) in a short period, dosages which are comparable to those which denature proteins. Thus the bulk of the pathological effects are "secondary," at least in time.

Such "secondary" effects are not unusual in toxicology, of course, and their period of latency may depend on the length of time the organism can get along without some essential function. Cyanide kills very rapidly because it inhibits oxidative processes which are immediately necessary for life; removal of the kidneys, or some poison which has the same physiological effect, has a latency of lethal effect while metabolic products are accumulating to a lethal point. Interference with enzyme function, if irreversible, may allow similar accumulation of undesirable matter or prevent the formation of a necessary substrate; the toxic level of the first or the reserve supply of the second will determine the latency.

Previous panels of this symposium have dealt with radiation biochemistry and cytology. It is therefore proper now to consider the question of latency in cytological terms. Here it is much easier to contrive a fit between the known loss of specific tissue elements and the clinical syndrome of acute and subacute radiation sickness—between, perhaps, tertiary and quaternary radiation effects. The damage to the red-cell-forming tissues has obvious end results; that to the granulopoietic function reduces the threshold of resistance to infectious invasion; the result is invasion by organisms which may have been latent in tissues or may have gained entrance through cytological discontinuities in the digestive tract or elsewhere (9, 10). It has long been known that many of the features of the acute radiation syndrome resemble responses to infection, including fever and associated signs, death in a shock-like state, and even the improved kidney function in the middle stages (11). It may be significant that the responses of certain species having different susceptibilities correlate well with degrees of damage to the granulopoietic system (12, 13).

The bleeding tendency is related in part to a disappearance of blood platelets (again on a cytological basis) and in part to the appearance in blood of substances which, like heparin, interfere with coagulation, and it is not certain that these two parts make up the whole (14). The relation of the lymphocyte to the immune processes brings in another cytological mediation which deserves further study, since the immune functions are interfered with in irradiation (15–17).

It is frequently suggested that a part of the toxic picture is due to insult by materials discharged from injured cells at a rate which the organism is unable to handle in a normal fashion. This is, for several reasons, one of the most difficult problems to solve in the physiological laboratory. For the sake of completeness, we may mention that sterility and falling of hair would appear to have a cytological basis.

Whether the cell destruction we have been observing occurs through a mechanism of chromosome damage remains an open question. We

can say only that the dosage range for acute mammalian death is one in which the frequency of chromosome breaks and dicentrics becomes noteworthy (18); that there is good reason to believe that such lesions result in cell death; and that the cells susceptible to irradiation are, generally speaking, those which show rapid division for replacement and which do not have a large nucleic acid reserve. Marshak's observation (19) that the lymphocyte is susceptible to chromosome damage over a remarkably large part of its mitotic cycle may be pertinent here.

Although it is not the primary purpose of this conference to discuss practical matters, it is not out of order to mention therapy briefly, since our present position in therapy points up the discussion. Those forms of therapy which appear to act primarily on a chemical basis, anoxia (20) and cysteine (21), are prophylactic and must be present with irradiation. Other prophylaxes are on the cytological level, like spleen protection (1) or preirradiation stimulation of the blood-forming elements by induced anemias (22) or by estrogens (23, 24). Our position in therapy after the fact is in the realm of supportive treatment: antibiotic treatment of bacterial invasion, replacement of lost blood, etc.

Thus, most of the sequence in acute irradiation sickness can be explained on a cellular hypothesis with, of course, antecedents in biochemistry, chemistry, and physics. The striking low-level irradiation effects on enzymes as described by Barron, however, make it clear that supplementary information may lead in quite a different direction; at the present time, our thinking should be pointed both ways. The entire realm of basic information discussed here will be very pertinent to this question, and it is likewise important to note that the quest for practical knowledge is being pursued by a group of individuals alive to the basic aspects of the sciences here represented.

When we turn to the chronic, lifetime effects of irradiation, we find the situation more obscure. The main elements here are cancer and shortening of the life span. Here we see an enhancement of natural processes about which we know altogether too little. The remaining discussion will deal with some efforts in the direction of developing means of handling this problem. Although we may appear to be drawing conclusions regarding lifetime human effects, this is not the case, and we are merely trying to define the parameters of the problem as a guide to future investigation.

Let us first mention cancer. We have a wealth of information on local cancer development following local irradiation. It would appear that most tissues are susceptible to spontaneous and to radiation-induced cancer. Total-body irradiation appears to enhance the normal incidence (25, 26). One is tempted to suggest that cancer is the consequence

of a "somatic mutation" causing a single cell to behave in a special manner. The unicentric origin of cancer is obvious, and under heavy stimulation of mice with radiostrontium the victim animals show multiple bone tumors which are distributed in the treated population in good correspondence with the Poisson theorem. If the center is a single cell, then carcinogenesis is in all cases a rare event, for with an incidence of 0.1–1.0 per animal lifetime and 10^8 susceptible cells the incidence per cell is on the order of 10^{-9} per lifetime or perhaps 10^{-7} per cell generation, which places it in the statistical range of many genetic mutations.

Here we at once strike a difficult problem in extrapolation. Since man may have 10^3 times as many cells of a given type as the mouse, one would expect, other things being equal, a corresponding increment in frequency, which is obviously not true for either spontaneous or induced cancer. Other things are clearly not equal, and we may rest on the thought that the cells of a man must have a correspondingly lower susceptibility in order to survive. Possibly this is a useful concept in cancer research which has not been utilized.

Besides the size factor there is also the time factor. Since man lives 30 or 40 times as long, cancer seems in this and other ways to be tied in some way to the aging process, which leads us to the next section of our discussion.

The characteristics of the survival of groups of individuals are described by means of the actuarial functions (27). These are related mathematically; the mean survival time is a single point on one of these functions. The most useful for our purposes was described by Gompertz about a century ago (28). It is a well-established fact that the logarithm of the rate of mortality, which we call the Gompertz function, has an approximately linear dependence upon age in adult life for all species of mammals investigated and for many other metazoan forms (29). The Gompertzian plot of cancer mortality rates follows a similar pattern (30) but appears in most cases to saturate in old age. This saturation might appear to be due to relatively faulty diagnosis in the senile human being, but appears also in our studies in spontaneous mouse lymphoma in two strains (31).

Data on the incidence of cancer in human beings subject to carcinogenic chemical insults have been collected by Kennaway (32), and it is possible to infer that the Gompertz function for these groups is quite parallel to the function observed in the general population.

If we assume that the linear dependence for log of mortality rate on age holds strictly for two species of different life span, each of the actuarial functions has the same analytical form, and an origin and a time-scaling factor may be chosen such that all the functions become identi-

cal for the two species. The ensuing discussion implies that the transformation has been made straightforwardly between mouse data and man, and is to be regarded only as a working hypothesis whose implications are such that it is very important to seek ways and means of testing its validity.

By way of introducing a simplified picture of the effect of radiations on the Gompertz function, we present in Fig. 1 the effect of fractionated exposure in early life and constant exposure throughout life in mice. The effect of single exposure is an upward displacement of the function without change in slope, whereas that of constant exposure is to increase the slope of the subsequent function (Fig. 1). A further property of the Gompertz functions is that they summate, both superimposing exposures and superimposing an exposure pattern on normal aging (Fig. 2).

It may be said that the long-term effect of a schematized single dose received at age x is to decrease the after-expectation to that for an age $x + q$, where q is a function of dose and other variables. The dependence of q on dose is not simple, especially in the region of acute fractionally lethal doses or where we are concerned with fractionation of dosage. The physiological factors here involved will be discussed later; we assume as a further working hypothesis that at sufficiently low dosages the upward displacement of the Gompertz functions becomes proportional to dose.

Approaching the question of species comparison on this basis, we will consider the case that two species have equal sensitivity as measured by the Gompertz function. Then, if the normal life expectations of the unknown and known species are in the ratio $1/K$, the reduction of life expectation by a single exposure at the actuarially equivalent age in the unknown species will also be changed by the factor $1/K$; hence the per cent reduction of life expectation by a single dose will be equal for the two species.

Since the effect of continuous exposure at low dose rates is to increase the slope of the Gompertz function, we can derive this relationship: to induce equivalent per cent reduction of the life span in the two species by duration-of-life exposure we must expose the unknown species at dose rate KI. For example, if the respective expectations are 2 and 40 years, equivalent shortening of life in the two species would be accomplished by 5 and 0.25 r per day, respectively.

We have made four assumptions here, for all of which there is experimental evidence:

1. The Gompertz function for natural aging increases linearly with age.

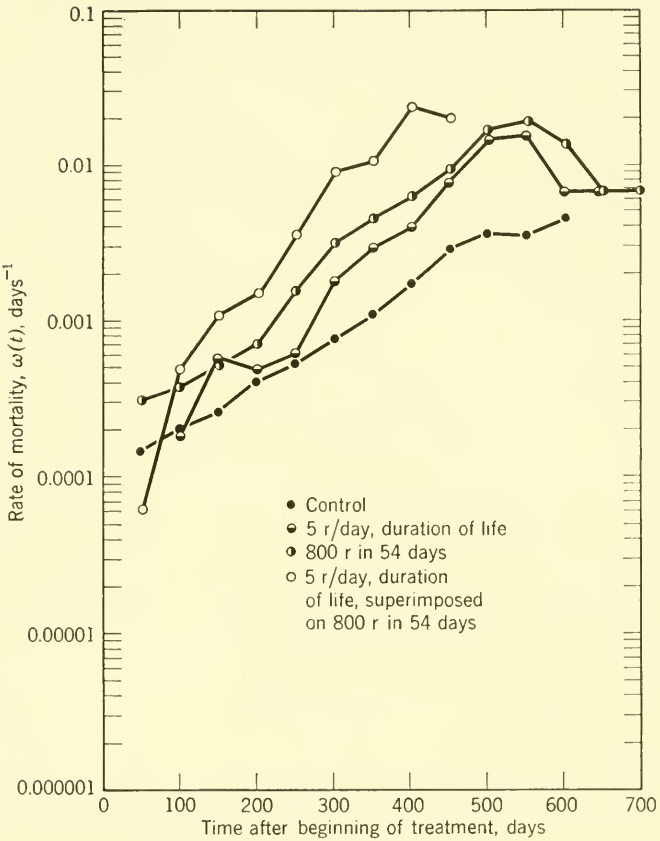

FIG. 1. Gompertz diagram (logarithm of rate of mortality vs. time) for all causes of death except lymphoma. Carworth female mice. Exposure begun at 90 days of age. Note that, after a dose of 800 r delivered early in life, the Gompertzian is displaced upward in about 200 days and thereafter runs parallel to the control line, whereas the Gompertzian line for the 5-r/day group diverges progressively from the control line (the falling off in the last 100 days of each curve is due to a small percentage of mice and is tentatively attributed to selection). These patterns have been verified in several studies (42). The two trends intersect at an accumulated daily dose of about 1750 r, as against the theoretical value of 800 r for the rectangular assumption made here. The discrepancy is due to deviations from the simple rectangular hypothesis and to non-linearity in the relation between dosage and displacement of the Gompertz function for massive dosages. The upper curve (open circles) is described more fully in the legend of Fig. 2.

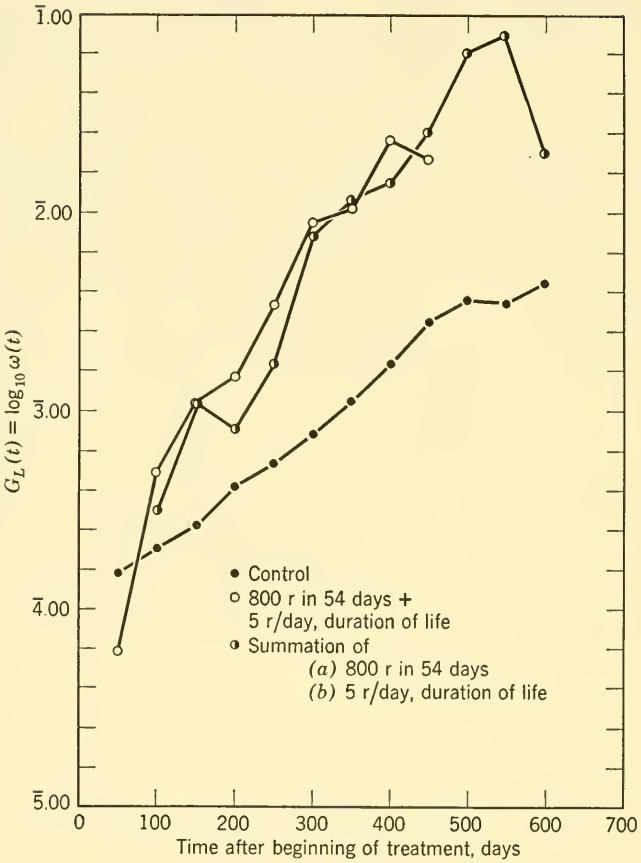

FIG. 2. The summability of radiation lethality when expressed in units of the Gompertz function. The open circles were experimental values obtained from a single group of mice which received a fractionated dose of 800 r delivered in 54 days, and also a daily exposure of 5 r/day for the remainder of life, both patterns beginning at 90 days of age. The half-filled circles were obtained by summing the following Gompertzian components from three separate groups of mice (see Fig. 1):

1. The component in excess of control for a group which received 800 r in 54 days.
2. The component in excess of control for a group which received 5 r/day for duration of life.
3. The Gompertzian curve of the control group.

There is good agreement except for a slight deficit of the summation from the experimental values in the first 200 days.

2. Under exposure to radiation the resultant Gompertz function is the sum of contributions due to aging and injury.

3. A dose delivered in a short time causes a displacement upward without change in slope (after an initial accumulation period which we neglect here).

4. The Gompertz function for a succession of doses is given by the sum of the effects of the separate doses involved.

The third hypothesis, that of indefinite accumulation of injury and lethality, is not a necessary consequence of the empirical findings. Recovery constants on the order of thousands of days cannot at present be discovered. Should they exist, there would be a profound divergence from present expectation in a favorable direction, but they are not justifiable on the basis of any present data. Even if there are such slow recovery constants, it is reasonable to admit the necessary existence of a residue of non-recovering injury having analogs in genetic injury and in degradation of order in tissues—a sort of increasing entropy such as probably accompanies the natural aging process.

It must be understood that "aging" functions discussed here as consequences of irradiation may or may not represent processes identical qualitatively with normal aging processes. In the case of cataracts, for example, the correspondence may be a partial one. Incidentally, it is worth recalling that the crystalline lens is a transparent tissue, and that changes of a similar nature may occur in other tissues but be invisible, and in fact may contribute to the actuarial picture of aging.

Calculations based on the above, using empirical constants deduced from mouse and dog survival data (33, 34) (see Fig. 9), indicate that a continuously accumulated tolerance dose might decrease the human life expectation by 10 per cent (less if we assume an unexplored late recovery function) and that even the background exposure due to cosmic rays and natural radioactivity may be responsible for the loss of a year or so of our "ideal" life expectation (Figs. 3, 4). It must, of course, not be forgotten that ionizing radiation is but one of many possible insults, and that the literature of vital statistics gives us numerous examples of comparable degrees of harm, although the experimental approach has been generally neglected except in radiobiology.

We now consider the remainder of the lethality picture, with reference to the physiological situation. If we are to hope to complete our understanding of these matters, it is urgently important that physiology and pathology be practiced, preferably on the same experimental material as is used for actuarial study.

The relation between dosage rate and survival of two strains of mice (33) shows a roughly hyperbolic form characteristic for all species and

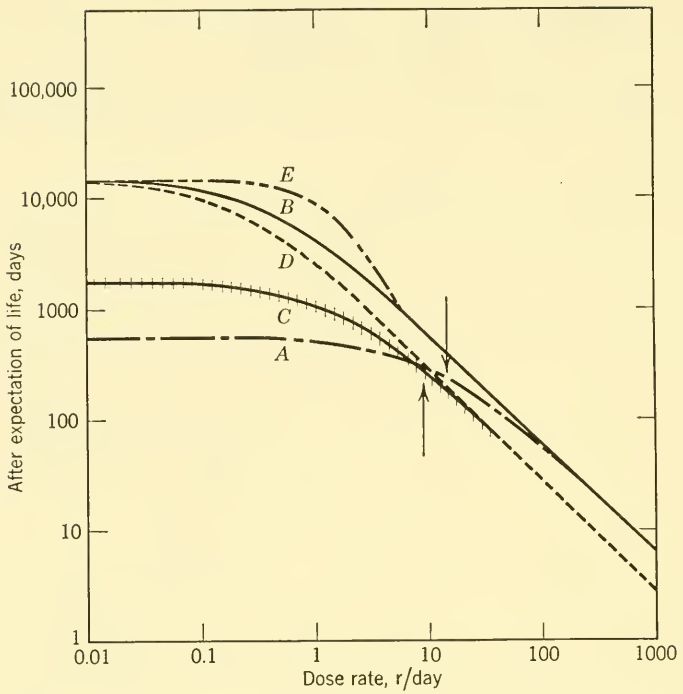

FIG. 3. Relation between dose rate and after-expectation of life in the chronic (low dose rate) range, on a log-log grid. These curves illustrate the way in which survival at low dose rates depends on both sensitivity *per se* and normal expectation of life. Curve A is for ABC mice with normal after-expectation of 540 days and lethality coefficient $k = 0.00017\ r^{-1}$ (34). Curve C is for mongrel dogs with an estimated after-expectation of 5 years and lethality coefficient $k = 0.00035\ r^{-1}$. The curves are calculated from the hyperbolic formula

$$M_I = \frac{M_0}{1 - M_0 k I}$$

using the values of M_0 and k for each curve as given. Curve B is calculated from the above formula for a hypothetical population with $M_0 = 14{,}600$ days (40 years) and $k = 0.00017\ r^{-1}$. Curve D is calculated for $M_0 = 14{,}600$ days and $k = 0.00035\ r^{-1}$. The arrows indicate the highest dose rates for which the relation holds experimentally. Curve E is calculated on the assumption that injury accumulates for 1000 days, using the constants $M_0 = 14{,}600$, $k = 0.00017$. It is to be compared with curve B, which is calculated for the same values of M_0 and k, but on the assumption that injury accumulates indefinitely. All constants employed here are deduced for the case of short daily exposure to x-rays. For continuous exposure to Ra or Co gamma rays the lethality coefficients are smaller by a factor of approximately 2, independent of species or dose rate (34).

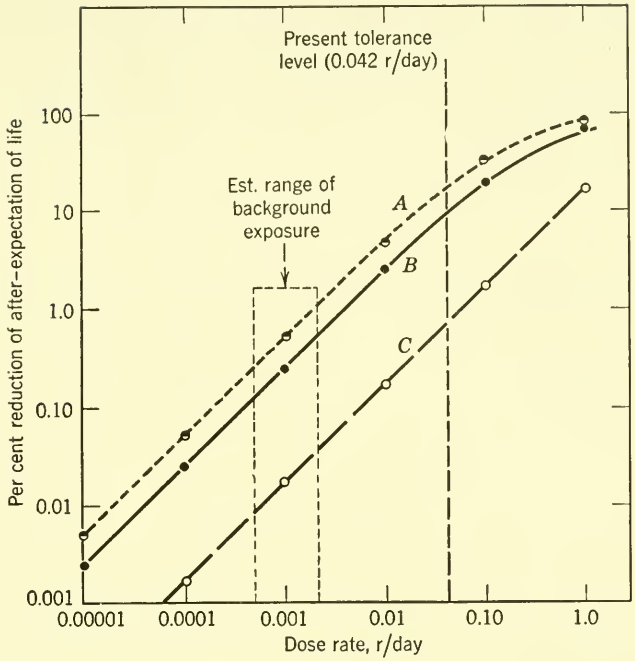

FIG. 4. Per cent reduction of after-expectation of life under continuous exposure as a function of dose rate, for a hypothetical population with a normal expectation of 40 years, under various assumptions about the accumulation of radiation injury: *A*, Chronic lethality coefficient $k = 0.00035\ r^{-1}$ (experimental dog value) and injury accumulates throughout life; *B*, chronic lethality coefficient $k = 0.00017\ r^{-1}$ (experimental mouse value) and injury accumulates throughout life; *C*, chronic lethality coefficient $k = 0.00017\ r^{-1}$, and there is a recovery constant of 1000 days.

for various qualities of radiations (Fig. 5). The mean accumulated dose to death plotted against dose rate (Fig. 6) shows a shallow minimum centering at 86 r per day, which corresponds to a mean survival time of 18 days and a minimum mean accumulated dose of about 1500 r. A similar maximum of lethality is manifested by the rat (35), and we have sufficient supporting evidence to conclude that this is a general characteristic of the survival of mammals under duration-of-life exposure. Maximum daily-dose lethality occurs a little later than the peak of single-dose killing, and the clinical and pathological features of the two patterns of exposure are almost identical in this time period (36).

These and similar observations quite early suggested that the effects of continuous exposure could be interpreted as arising from a summation of the effects of single dosages (35). This has been demonstrated on some suitable mouse data by an extension of the actuarial approach

described above (37), but for our present purposes we will use a simpler and more restricted development which permits us to make use of the

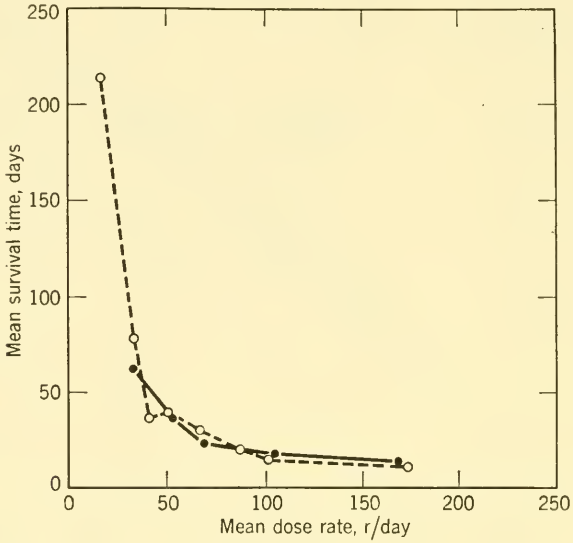

FIG. 5. Relation between mean survival time and dose rate for two strains of mice. Open circles: ABC males, mean after-survival of controls, 540 days; solid circles: CF-1 females, mean after-survival of controls, 450 days.

many studies carried out on small groups of animals, for which the actuarial methods are not suited.

In this alternative approach, which we call the kinematic method (33), the behavior of a population is described by a single representative

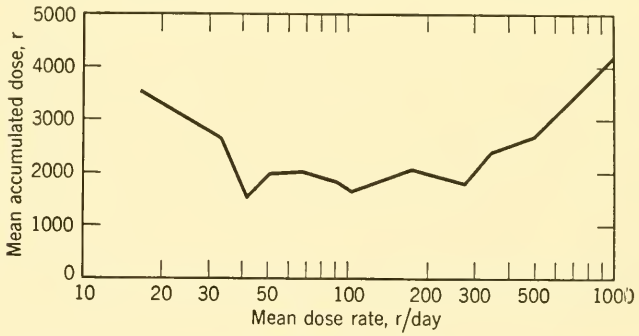

FIG. 6. Relation between mean accumulated dose and logarithm of dose rate for ABC male mice. The function has a shallow minimum in the neighborhood of 90 r/day, rises to a maximum in the neighborhood of 15 r/day, and then decreases to zero as dose rate decreases further (not shown here).

point, which may be the mean or median animal of the population. We postulate that there exists a single-dose injury function, or impulse injury function, which has the value $\phi(t - \tau)$ at time t after a unit impulse dose delivered at the earlier time τ. If exposure is administered as a function of time, $I(\tau)$, the course of injury will be described by the integral equation

$$X(t) = \beta t - \int_0^t I(\tau)\phi(t - \tau)\, d\tau \tag{1}$$

where the term βt describes approximately the accumulation of injury due to natural aging (see above, p. 446). We further postulate the exist-

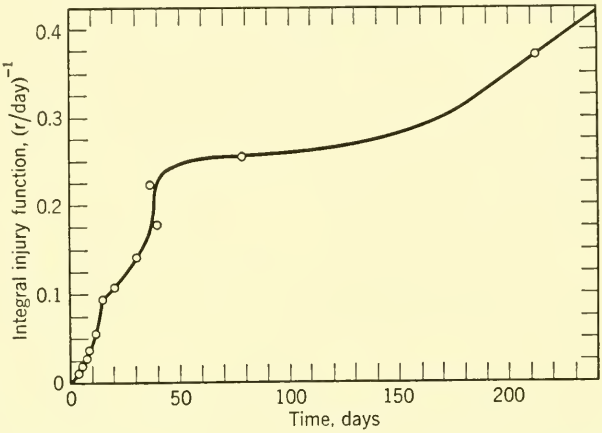

FIG. 7. Cumulant lethality function for ABC male mice, exposed at dose rates ranging from 20 to 1000 r/day. For exposure at a constant rate for the duration of life, the cumulant lethality function is given by (33):

$$C_L = \frac{1}{I}\left(1 - \frac{t_I}{t_0}\right)$$

where t_I and t_0 are mean or median survival times for the exposed and the control group, respectively. The major characteristics of the C_L as determined by the mean survival times of treatment groups may be observed in the mortality rates within single populations exposed at constant dose rates (37).

ence of a lethal bound of injury M, such that $X(t) = M$ at the time when the representative animal succumbs. For the special case that $I(t)$ = constant, this equation can be solved simply, giving

$$\frac{1}{M}\int_0^{t_I} \phi(t - \tau)\, d\tau = \frac{1}{I}\left(1 - \frac{t_I}{t_0}\right) \tag{2}$$

where the term on the left is the integral of the impulse lethality func-

tion, called the cumulant lethality function, C_L, and t_I and t_0 are the survival times of the representative exposed and control animals, respectively. The C_L for ABC mice is presented in Fig. 7. It shows three distinct branches. The impulse lethality function, S_L, is obtained by differentiating C_L (Fig. 8) and shows two pronounced peaks of phasic injury and a final constant branch. This final branch can be shown

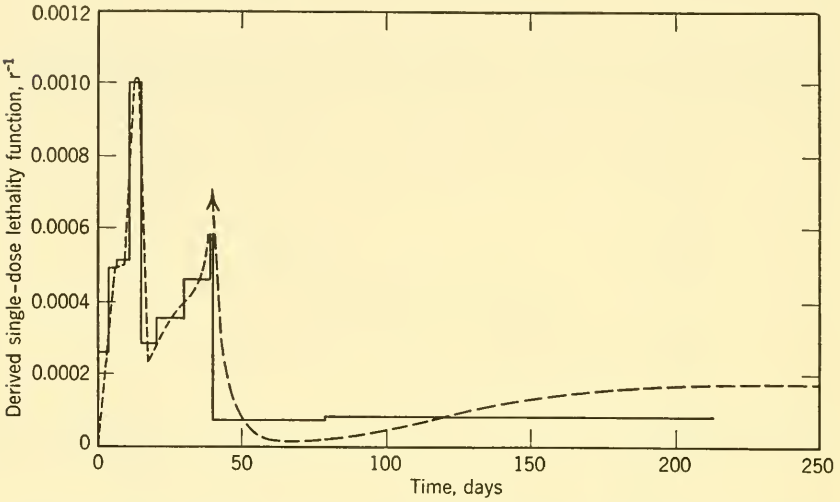

FIG. 8. Impulse lethality function, S_L, for ABC male mice. Obtained by numerical differentiation of C_L (Fig. 7). The horizontal lines give the mean derivative values estimated between adjacent dose groups, and the continuous curve indicates the nature of the function. The peaks of lethality at 10 and 40 days correspond to observed peaks of killing by single doses of x-rays.

to persist as late as 600 days (data of Lorenz; see Fig. 9). The two peaks of injury at 10 and 40 days correspond to the observed peaks of killing following single massive doses of x-rays.

Survival under daily exposure has been studied by several investigators for several species. The cumulant lethality functions for these species, with correction for normal aging, are shown on logarithmic scales in Fig. 9 (34). We see that the initial rise is nearly identical for all species and has a slope of 1.8. This initial rise is presumably relatively uninfluenced by recovery, and, since all species appear to be described by a common line, there is a suggestion that their differences in sensitivity are due almost entirely to differences in the capacity to recover, which appears in the time order in which they fall away from the branch of unretarded accumulation. Following the plateau appears the final, late branch, which appears to have unit slope and which may be thought

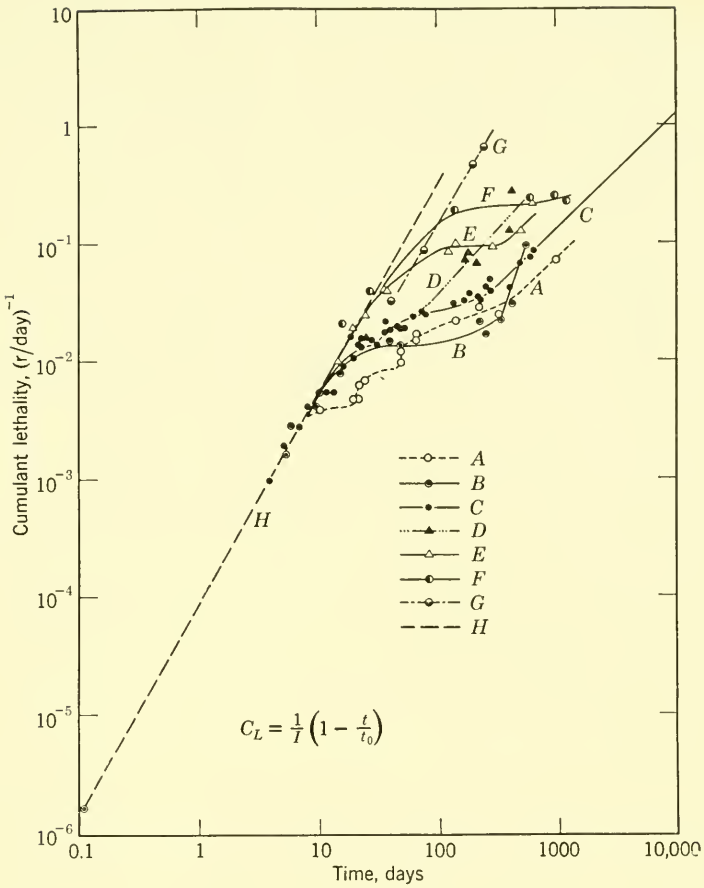

FIG. 9. Cumulant lethality functions for six species of mammals, plotted against time on a log-log grid. All x-ray-treated groups were given short exposures 5, 6, or 7 days per week. The gamma-ray series was exposed for 8 or 24 hr per day 7 days per week. The C_L for all gamma-ray groups was increased by a factor of 2 to compensate for differences in radiation quality and protraction. Neutrons equated to x-rays, using equivalent biological effectiveness for acute effects (45). Curves fitted visually. *A*, Rabbit, x-ray (43–45); *B*, rat, x-ray (35, 43, 49); *C*, mouse, x- and γ-ray (33, 46–49); *D*, mouse, neutron (46); *E*, dog, x-ray (36, 43, 50); *F*, guinea pig, x- and γ-ray (44, 48, 49); *G*, monkey, x-ray (43); *H*, common asymptote to all high dose-rate points.

to represent the non-recovering component. This presumption is based on the following:

1. The law of accumulation for this late branch is similar to that for normal aging.

2. The pathological findings are only quantitatively different from those of age.

3. Few physiological recovery mechanisms are known with time constants of thousands of days, which might flatten it further (but see above, p. 449).

4. On genetic and cytological grounds, a non-recovering component is expected to occur, and there is clinical evidence for it.

The fact that injury appears to be non-recovering is, of course, no reason for assuming that it is essentially irreversible, or that we cannot influence it. On the contrary, the production of an analog of aging in the laboratory enables us better to study the basic process of senescence and the factors determining its apparent irreversibility.

We inquire next whether there is experimental evidence to support the hypothesis that radiation injury to physiological systems is a linear process or, in other words, one which can be summated. There is some evidence, but considerably more work in this line is needed. First, response curves of certain physiological variables (as the responses of the various types of leukocytes) are linear in the sense that by a proper transformation they can be brought into a form in which the responses constitute a linear family of curves, that is, curves of the same form and with amplitudes in the ratio of the doses (38).

As to additivity of physiological responses we may consider the response of a convenient variable (weight of growing rats) to single and daily x-ray exposure (39). In Fig. 10 we show the response expressed as the difference of logarithms of weights of control and treated groups. This measure of effect can be rationalized in terms of a theory of cellular growth. The single-dose growth response is similar to the derivative of the daily-dose response, in so far as the time relations of phasic responses are concerned, but the single-dose response shows additional components of effect which may be attributed to non-linear components which arise when massive single doses are administered. One such component, which can be shown to account for a considerable part of the specific single-dose effect, is the transient anorexia which follows large exposures. A second component, for which no quantitative data exist under the experimental conditions employed here, but which has been amply demonstrated in other situations, is that of cyto- and histo-architectonic disorder resulting from the rapid recovery following massive injury. We may hope eventually to account for the observed irreversible

loss of growth capacity following massive exposures in terms of quantitative measurements of this component. Another dose-dependent component of injury is that of chromosome damage, which is considered elsewhere in this volume (18).

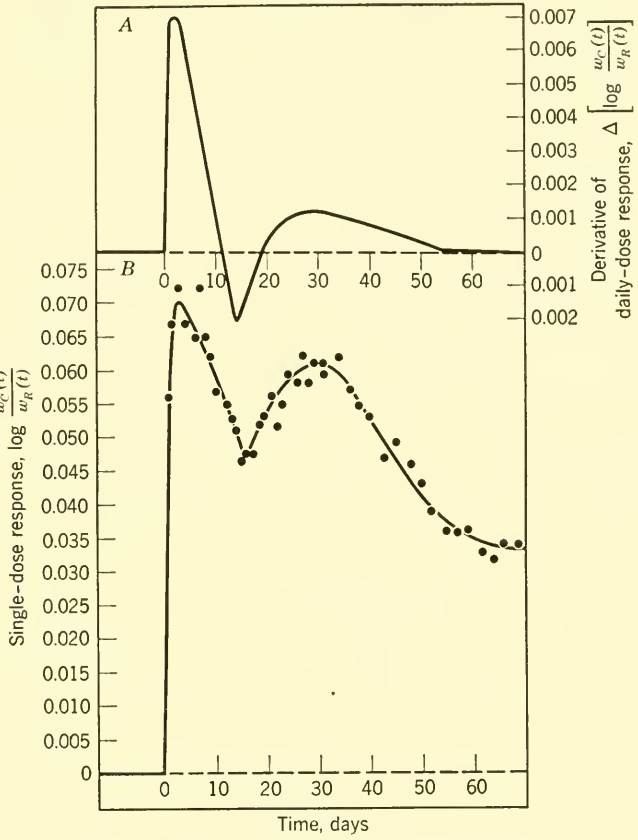

FIG. 10. Comparison of directly measured growth response of male rats which received a single dose of 200 r with the derivative of the response of male rats which received daily exposure at a constant average rate of 13.7 r/day. The two curves show similar phases in about the same time relations, but the single dose response is always positive and does not return to the pretreatment origin. This must be attributed to irreversible effects of large single doses of x-rays. Male Sprague-Dawley rats, age 53 days and weight 150 gm at beginning of treatment.

The conclusion we can reach on the basis of the above observations is that the responses of physiological systems will in general be linear only for disturbances which produce relatively small displacements from the physiological steady state.

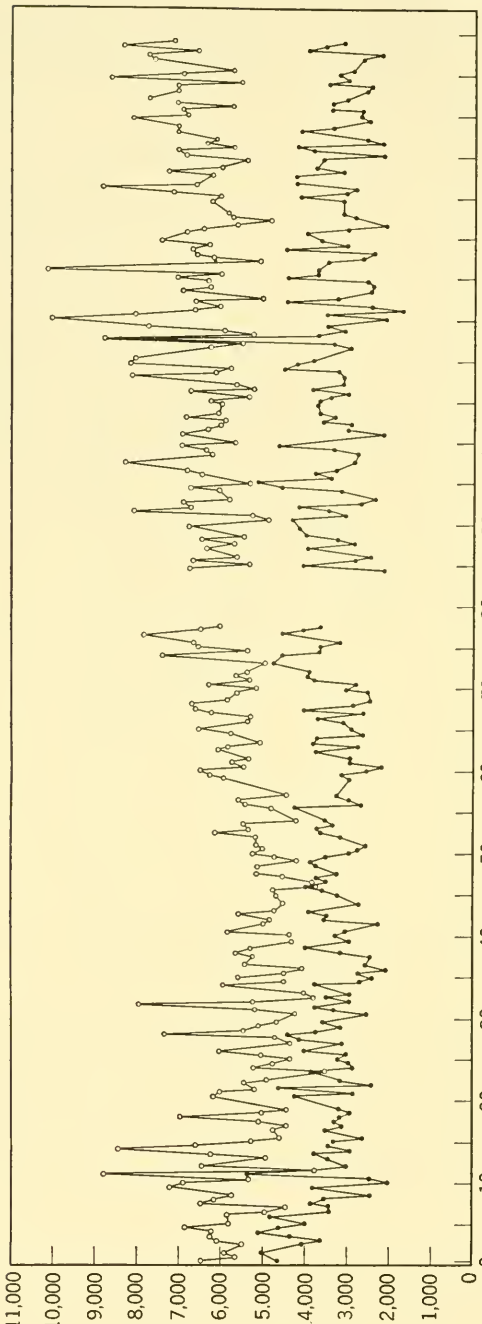

FIG. 11. Daily absolute neutrophil and lymphocyte counts on a normal male human subject for a period of 150 days. This is a sample from a study by L. O. Jacobson (51) in which four subjects were counted daily for periods up to 2 years. It is by the analysis of time series such as these that the parameters of physiological random processes must be determined.

In addition to the quasi-empirical actuarial and kinematic approaches to the problem of radiation lethality, a theoretical statistical approach is being developed (37) based on the fundamental observation that random fluctuations in physiological state bring an animal into quantita-

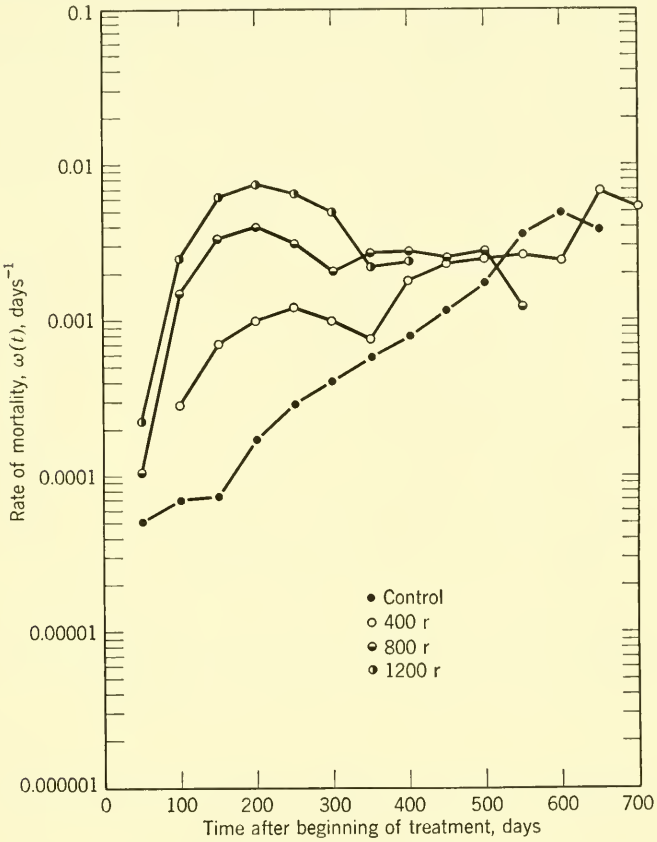

FIG. 12. Gompertz diagrams for lymphoma mortality in Carworth female mice; control and 3 fractionated doses beginning at 88 days of age. The response rises to a peak and then subsides to control levels at about 500 days.

tively different states from moment to moment, relative to the lethal limit (Fig. 11). In terms of this conception, lethality can be given a mathematicophysical formulation as arising from a rate process. The Gompertz function can be derived theoretically on this basis, and it offers another opportunity to correlate physiological variables with the total response.

Finally, a further note on carcinogenic responses is in order. Two

apparently different types are shown here. The first is the lymphoma responses in susceptible mice given x-ray dosages (40), expressed first as the directly observed Gompertz function (Fig. 12), then as the logarithmic increment above the normal Gompertzian incidence (Fig. 13).

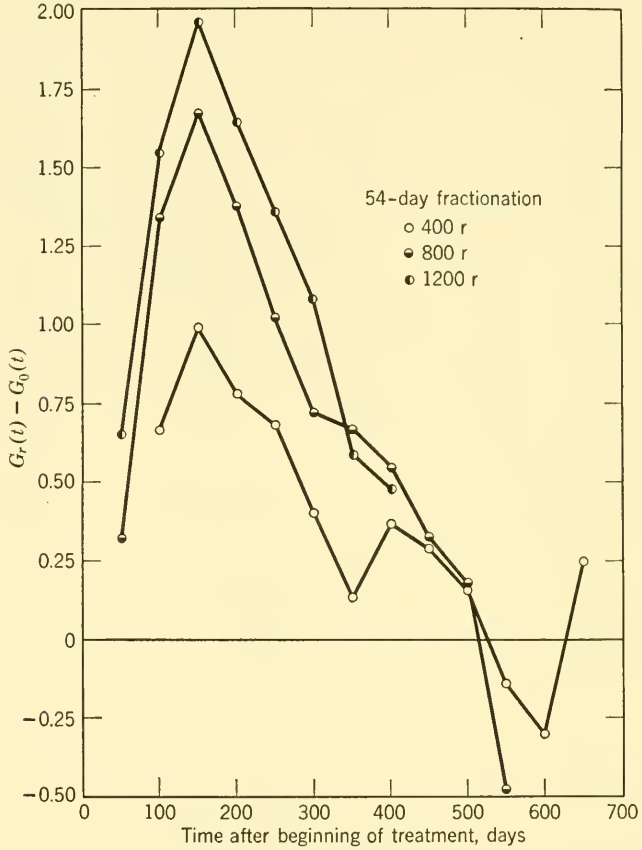

FIG. 13. The data of Fig. 12, plotted here as the difference of Gompertzian values of treatment groups and controls. When so expressed, the radiation specific lymphoma response is seen to be monophasic, rising to a maximum at 150 days and then subsiding to control level. The form of the response is the same at all dosage levels.

When expressed in this latter form, the lymphoma response appears to be a monophasic linear process, since the responses to the three doses have the same time pattern and differ only in amplitude. This suggests that the incidence of lymphoma may be a rate process, governed by a fundamental injury process consequent to irradiation.

The second type is the bone-tumor response in Sr^{89}-treated mice,

where dosage was maintained (41). These curves can be described as showing mortality rates constantly increasing with time. They fail to follow a logarithmic trend as well as the data discussed before and are plotted in linear fashion (Fig. 14). Single-dose data, not shown here, also do not show any evidence of a subsiding tumor lethality rate up to the life span of the experiments. This may be explained as a cumulative acquisition of a tendency to tumor development not limited in time,

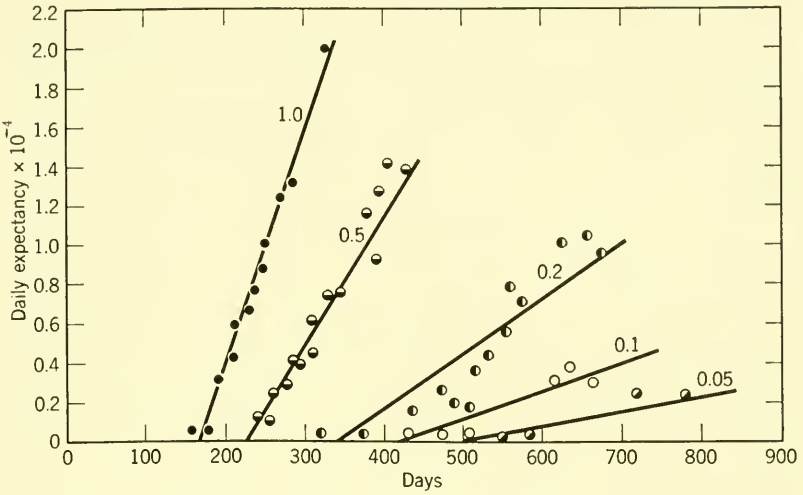

FIG. 14. Rate of morbidity from bone tumors in Carworth female mice injected with Sr^{89}. Retained dose was maintained constant on the average throughout life by repeated injection. The points for each treatment group labelled by the size of the monthly injected dose in microcuries per gram.

with a resultant accumulation of tumors as the square of time. Evans has pointed out, however, that this may be the early part of a Gaussian distribution with time dimensions greatly exceeding the period of observation (52) and the virtual disappearance of the human radium sarcoma trend in the past few years indicates that in this case also an actual limit in time (but on the order of several years) may exist.

CONCLUSIONS

The conclusions to this analysis will be stated in terms of a series of suggestions as to where it appears that maximum effort is desirable in order to answer the most important questions.

1. We should examine most carefully the characteristic parts of the lethality function (Fig. 7): (*a*) the plateaus, where the more marked dif-

ferences between species have shown up; (*b*) the relation between the early and late slopes; and (*c*) the possibility of later recovery functions than those described here.

2. It is important to correlate measurable physiological and pathological states with vital statistics data.

3. Using life-table characteristics of large populations, we should re-evaluate the chronic lethal pattern in relation to the widely differing life spans of different species.

4. Studies similar to those described here, relating to a variety of other insults (for example, chemical carcinogenic agents and industrial poisons), have been seriously neglected.

5. The relation of dosage rate or duration of administration to effect, where the dosage is delivered within a few hours, demands further inquiry.

6. The carcinogenic pattern needs further investigation, especially as to whether there is a time limitation on the development of tumors after the primary insult.

7. By the analysis of mammalian radiation injury and lethality it should be possible to gain much further information as to the underlying mechanisms by the proper use of various qualities of radiation (as neutrons and gamma rays). We are now in a position to begin to apply the elegant methods currently employed in cytological and other basic work to the analysis of the mammalian mechanisms.

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