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Biology Department

EXCELLENT SUMMARY OF THE HISTORICAL ACCOMPLISHMENTS OF OHER SPONSORED RESEARCH.

January 14, 1983

Dr. David A. Smith
Health Effects Research
Office of Health and Environmental Research
Room F-209, ER-72, GTN
Department of Energy
Washington, D. C. 20545

Dear Dave:

Enclosed is a copy of our OHER risk assessment report and summary entitled "Impact of Radiation Biology on Fundamental Insights in Biology" which was sent to Dr. Robert E. Rosenthal, Office of Program Analysis, on July 8, 1982.

Sincerely yours,

Richard B. Setlow
Chairman

is
Enc.

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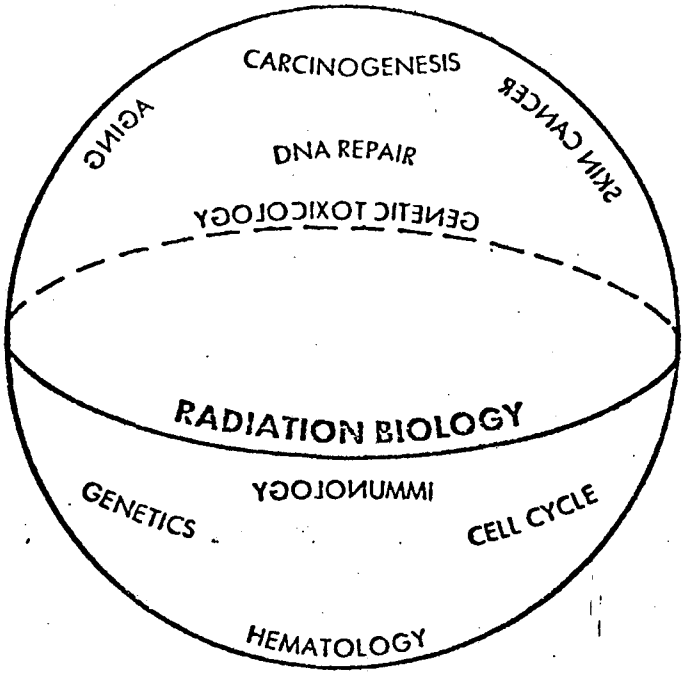
7/27/82

Impact of Radiation Biology on Fundamental Insights in Biology: Summary

Research supported by OHER and its predecessors has as one of its major goals an understanding of the effects of radiation at low doses and dose rates on biological systems, so as to predict their effects on humans. It is not possible to measure such effects directly. They must be predicted from basic knowledge on how radiation affects cellular components such as DNA and membranes and how cells react to such changes. What is the probability of radiation producing human mutations and what are the probabilities of radiation producing cancer? The end results of such studies are radiation exposure standards for workers and for the general population. An extension of these goals is setting standards for exposure to chemicals involved in various energy technologies. This latter problem is much more difficult, because chemical dosimetry is in a primitive state compared to radiation dosimetry.

The problems facing OHER are general biological ones. They can only be solved by knowing how biological systems work. In reaching for such a solution, OHER sponsored research, beginning in 1947, has had a major impact on our approach to and our understanding of biological systems. The various areas of biology cannot be viewed as separate entities, but are part of an interacting whole indicated in the Figure. Without Radiation Biology as a guiding force, the scientific fields in the Figure would have developed, if at all, at very slow rates.




Some research areas that interact strongly with Radiation Biology. The spherical surface is designed to show that there are strong interactions among many fields of biology and that no one field in isolation could profitably develop by itself.

Radiation Biology with its quantitative dosimetry has led the way in determining dose response relations that are so essential for setting exposure standards. The methodologies devised for doing quantitative work on mutagenesis, chromosomal aberrations and DNA repair were instrumental in starting the new field of genetic toxicology that is so important now in evaluating the effects of chemical hazards in the environment. DNA repair itself is a product of Radiation Biology. We now know that repair mechanisms are able to ameliorate a large fraction of the deleterious effects of

environmental agents on DNA and as a result reduce the probability of initiating the carcinogenic process by anywhere from 10 to $10^4$-fold.

Simple discoveries, such as the use of $^3$H-labeled thymidine as a DNA precursor, were instrumental in helping elucidate the characteristics of DNA synthesis in mammalian cells and the properties of the different phases of the cell cycle. A knowledge of such phases is important not only for estimations of mutagenesis and carcinogenic risk, but for fields such as radiation therapy.

An important part of the reactions of animals to radiation and to various chemicals is their immunological response, and immunological and hematological studies have played important roles in Radiation Biology and are fields that only advanced rapidly as a result of the input from Radiation Biology. The fields are now preeminent in the areas of tissue transplantation and the numerous blood diseases.

An essential part of quantitative biology is the determination of the relations between the structure and function of biological systems because it is only through such knowledge that one can correlate a change in structure with a change in function--the end result of the action of radiations or energy related chemicals.

The future benefits to be gained by OHER support in Radiation Biology and in quantitative biology include: 1) a detailed understanding of the relations between structure and function resulting from the use of powerful national facilities devoted to such problems; 2) a new set of exposure standards for neutron irradiation (because it seems as if the present atomic bomb data are useless for this purpose); 3) an estimate, based on basic biological knowledge and theories of radiation and chemical action, of induced mutation rates in humans exposed to both chronic and acute doses; 4) measurements of the

variations in the ability of humans to repair damages to their DNA and so provide estimates not only of the average response in the population but of sensitive subsets in the population. The outcome of even this limited number of specific goals is bound to be much greater than answers to the questions asked. For example it might be that DNA repair mechanisms are under some sort of metabolic or lifestyle control.

Radiation Biology is not only molecular biology but it is quantitative biology. It is only by its application to radiation and other environmental problems that there will be reasonable assessments of hazards and the setting of standards.

**Impact of Radiation Biology on Fundamental Insights in Biology**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Footnote: In this presentation, we refer to the Office of Health and Environmental Research and its predecessors in the Atomic Energy Commission and the Energy Research and Development Administration as OHER.

7-8-82

## INTRODUCTION

The emergence of Radiation Biology as a major area of scientific research resulted from the establishment of the Atomic Energy Commission and its Division of Biology and Medicine (later called the Office of Health and Environmental Research or OHER) after World War II. The theme was The Peaceful Uses of Atomic Energy. Large-scale programs designed to examine the biological effects of atomic radiation were initiated at a number of the OHER-supported Laboratories including Oak Ridge, Brookhaven, Argonne, Los Alamos and Hanford (Battelle-Pacific Northwest Laboratory), as well as within several nongovernment institutions, notably the Universities of California, Rochester and Utah, and the Lovelace Foundation (Inhalation Toxicology Research Institute). A smaller program of university-based, contract-supported research was also instituted.

The farsighted and continued support from OHER throughout three decades allowed Radiation Biology to expand and make outstanding contributions to the growth of biology as a whole, particularly during the 1950s and 1960s when monumental changes were taking place and new fields emerging. What evolved has been many years of dedicated and unencumbered research, encompassing every field of basic science. There were some doubts voiced at first as to whether government-sponsored research would produce meaningful solutions to the many questions which concerned scientists at that point. What ensued has long since laid those doubts to rest.

The discoveries within the field of Radiation Biology have had a major impact in many areas - in genetics, biophysics, immunology, DNA repair and carcinogenesis, to mention only a few. But Radiation Biology does not stand in isolation. Basic and applied knowledge in other fields has changed the course of Radiation Biology itself. As a result, the various areas cannot be viewed as separate entities but as parts of an interacting whole (Fig. 1).

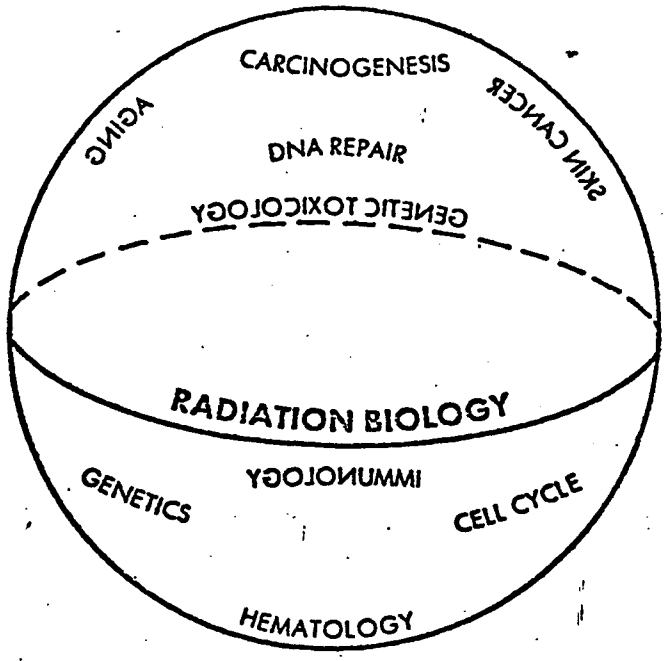


Fig. 1: Some of the research areas that have interacted strongly with Radiation Biology are depicted on this spherical surface. The diagram is designed to show that there exist interactions among many fields of biology and that no one field in isolation could profitably develop by itself.

The interaction between various scientific fields is well exemplified by our understanding of the role of DNA in Radiation Biology and in chemical carcinogenesis. Thus, although early quantitative work on the action of monochromatic ultraviolet light (UV) on micro-organisms indicated that effects on nucleic acids were responsible for killing and mutagenesis by this

radiation, the emphasis on DNA in Radiation Biology came finally with the biochemical appreciation of the central role this polymer had in the replication of genetic material and in its transcription to yield messenger RNA. The ability of cells to repair damaged DNA—another discovery of Radiation Biology—led the way to an appreciation of the existence of radiation-sensitive subsets in the population, which is of great importance to epidemiologic studies in carcinogenesis.

Another important contribution from the field of Radiation Biology was the development of new techniques and instruments which extended previous limits of observation. Three examples are the preparation of $^{3}H$-labeled thymidine, the development of the fluoromicrometer, and the invention of the scanning transmission electron microscope, all of which provided the shortcuts needed for study of the mammalian cell cycle and the structure of molecules.

Undoubtedly one of the major impacts on the expansion of biology in the '50s and '60s resulted from the incursion of physicists and biophysicists into the realm of Biology. They brought with them new modes of thought, new theories, and new approaches into a traditional field. This conceptual impact arose from an emphasis on quantitative dosimetry—the energy absorbed per unit mass—and on the tremendous background in the physics of energy loss and absorption in tissues, cells and molecules. The magnitude of the initial energy absorption per cell, its distribution about the mean, and estimates of its effect on cellular macromolecules can be calculated, and functions of both dose and type of radiation (X rays, neutrons, alpha particles, and UV, for example) can be predicted.

The importance and value of the quantitative approach provided by Radiation Biology is perhaps best illustrated in the field of chemical carcinogenesis. The dose-response relationships for chemical carcinogens are poorly understood, and the wealth of quantitative information from radiation research currently provides the basis and standard for the evaluation of other environmental hazards. The big difference between chemicals and radiation lies in the many steps between exposure to a chemical and reaction with crucial molecules, such as DNA (Fig. 2). Radiation can react directly with this important substance, but the action of chemicals is an indirect one. Chemicals may enter the body by a variety of routes, and many chemicals require metabolic activation to transform them into reactive forms. Metabolic activation varies from tissue to tissue, and, hence, the reactions between macromolecules, such as DNA, and environmental carcinogens will vary quantitatively from tissue to tissue. In other words, radiation has good dosimetry, and chemicals have very poor dosimetry. An extensive effort is now underway to improve the dosimetry of chemicals by using internal indicators, such as damage to DNA measured by immunological techniques, or changes in chromosomes measured by cytological techniques.

We invited a number of researchers, all of whom have been active investigators in Radiation Biology and are recognized experts in their fields, to give their views on the impact that radiobiology has had on basic insights in biology and medicine. All of the contributors were most enthusiastic about being given this opportunity to recognize the enormous contribution that OHER-supported radiation research has made in their respective areas. Since the sections were individually written, they vary in style and content, and there is overlap in subject matter; no attempt has been made to alter this redundancy as it again emphasizes the relevance of Radiation Biology to many

disciplines. The contributions have been immense. For many years, some of the best research in quantitative genetics and molecular biology was carried out in OHER-supported laboratories and many young scientists, both American and foreign, were trained in these facilities. The stimulus for the current revolution in biology and medicine is at least in part, a product of this research and these scientists.

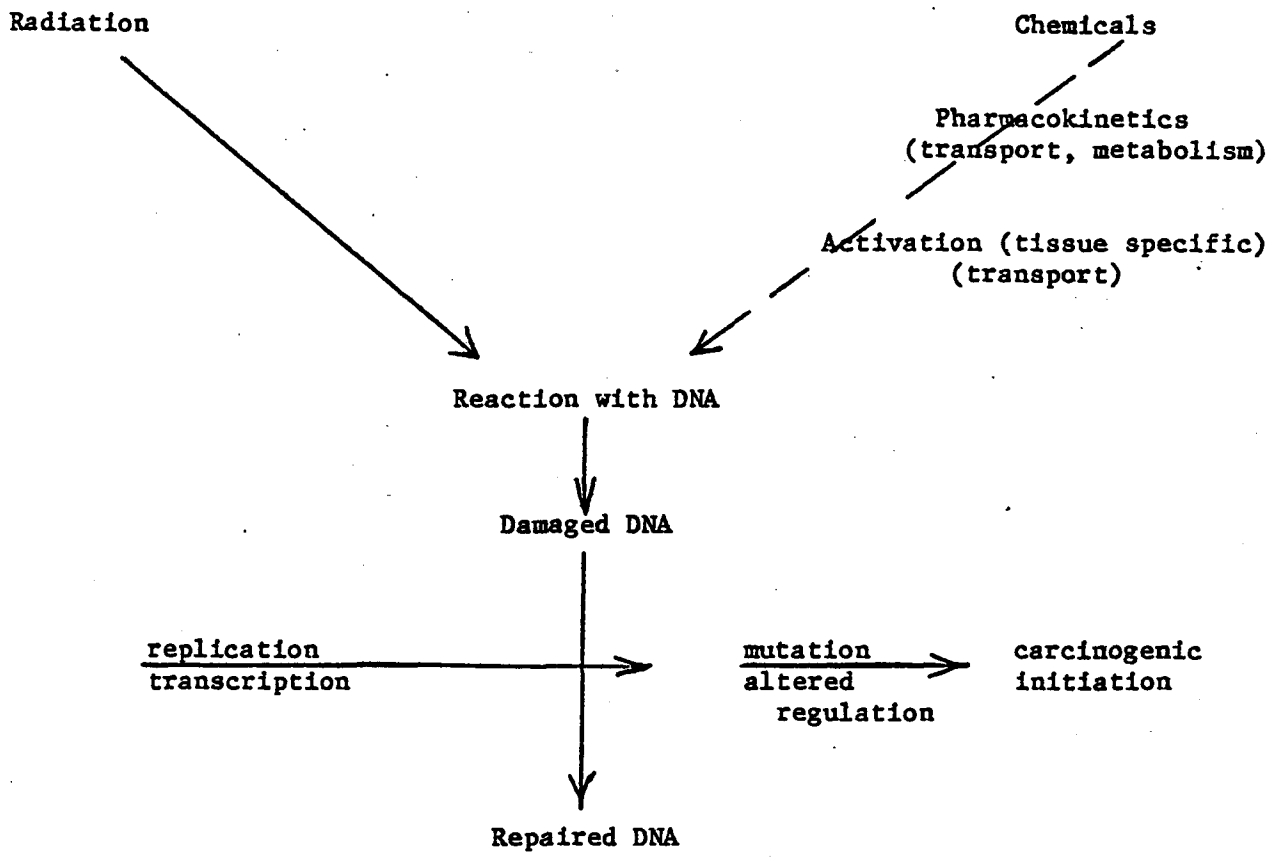


Fig. 2: Steps involved in the damage of DNA by exogenous chemicals or by radiation. Whether damaged DNA results in biological effects or not depends on the relative rates of repair, replication and transcription. If the latter are rapid, one may expect mutation or altered gene regulation resulting in the initiation of the carcinogenic process.

## Chromosomal Effects

When cells are exposed to mutagenic agents such as ionizing radiation, the genetic material within the nucleus may be damaged. This damage can be manifested as either gene mutations or gross chromosomal mutations that result from the breakage and rejoining of the chromosomes in abnormal ways. If this damage affects vital genes, it can lead to cell death, a phenomenon that has been exploited in the use of radiation and chemicals to treat cancer. Nonlethal mutational damage, however, also can have a profound effect. For instance, if the damage is in the germ cells, it can lead to detrimental mutations that could affect future generations, whereas, if it is in somatic cells, it could lead to effects on the immediate generation such as cancer in the exposed individuals.

The production of aberrant chromosomes by ionizing radiation beginning in the mid-1930s was invaluable in the development of two fields, genetics and biophysics. It was important in genetics because various types of recombinant chromosomes could be induced at will, thereby facilitating the study of genetic and cytogenetic mechanisms. In the field of biophysics, the induction of aberrant chromosomes was very important for the development of the target theory, which interprets radiation effects in terms of a simple direct interaction between radiation and certain specific loci, or targets, within the cell. In the early target theory experiments, a population of cells was irradiated with lethal doses of radiation and then inferences were made about the biological targets being hit from the surviving cells. If chromosome aberrations are studied, however, inferences no longer have to be made from cells that were not hit, for the damaged targets themselves can be observed directly under the microscope.

The importance of this work, both from a fundamental and a practical point of view, was early recognized by the OHER, and the major advances made in this area in the United States came from work that the agency supported. The names of the people funded reads like a Who's Who of early genetics and biophysics. Names that come to mind are Muller, Sax, Giles, Swanson, Conger, Wolff, Chu, Bender, Brewen, Preston, Heddle, Carrano, Sparrow, Steffensen, Atwood, and Taylor.

Biophysical studies on the kinetics of the induction of chromosome aberrations by sparsely ionizing radiations indicated that one class of aberrations, deletions resulting from unrepaired breaks, was the product of single hits on the chromosome. The occurrence of deletions was linearly related to dose, indicating that radiation breaks chromosomes with a simple probability which is directly proportional to the dose. Another class of chromosome aberrations, however, came from the interaction of two broken chromosomes. These interchanges increased approximately as the square of the dose, since the probability of producing both breaks is the product of the probability of each occurring separately. With densely ionizing radiation, such as neutron radiation, both breaks are produced by the same ionizing particle, so that the yield of two-break aberrations increases linearly with dose. Biophysical studies further showed that, with sparsely ionizing radiation such as X rays, the portion of the dose found in the tails of the radiation tracks is densely ionizing. Thus, two-break aberrations are induced with a one-hit component from such tails as well as with a two-hit component from the rest of the ionizing tracks. The induction of two-break aberrations with sparsely ionizing radiation can thus be expressed by a linear-quadratic model.

Studies of chromosomal aberrations indicated that, although some breaks remained open (deletions) and others united (two-hit aberrations), most of them restituted to reform the original chromosome. This restitutional repair was later shown to be under metabolic control and, in fact, constituted some of the first evidence that radiation-induced genetic damage was subject to metabolic repair, a concept that has had far-reaching consequences.

In a more practical vein, scientists within the OHER-supported laboratories were the first to realize that the induction of chromosome aberrations could be used as a biological dosimeter. The first demonstration made use of the classic cytological tool, Tradescantia, inflorescences of which were flown through radioactive clouds during the atomic bomb test, Operation Greenhouse in 1951. Later, it was found that chromosomal aberrations in the lymphocytes of the peripheral blood of humans exposed to radiation could be used as a measure of the dose. This method now comprises the standard test to detect and measure exposure of people to radiation and other genotoxic agents either by accident, or in the work place, or from some environmental source.

In recent years, the OHER-supported laboratories, with their extensive expertise in chromosome aberration studies and cytogenetics, have been instrumental in developing a new cytological technique which allows sister chromatid exchanges (SCE) to be seen readily through the microscope. The ease of scoring the SCEs, their exceedingly high sensitivity, and the fact that mammalian, including human, cells can be used have made this technique a method of choice for screening of genotoxic compounds.

It may be anticipated that, as more specific reagents become available, biochemical and biophysical studies on induced chromosome breakage and repair will lead to further insights regarding chromosome structure and function. With increased awareness of environmental hazards, the cytogenetic tests of chromosome aberration and SCE induction will be used ever more frequently not only to determine if various agents are potentially hazardous, but also to monitor exposed human populations.

## Mammalian Genetics and the Setting of Radiation Exposure Standards

The Mammalian Genetics Section of the Biology Division of Oak Ridge National Laboratory was started in 1947 with William L. Russell as Scientific Director. At that time, estimates of genetic hazards in humans were based on results from the fruit fly, *Drosophila*. It was obviously desirable to obtain information on an organism more closely related to humans, and the goal of the Mammalian Genetics Section was to develop methods for measuring the genetic effects of radiation in the mouse.

Many of the methods used for detecting radiation-induced mutations in mammals were developed in the Mammalian Genetics Section. One of these, the specific-locus method is currently the only practical, reliable method for detecting whether a mutagen causes heritable forward mutations in mammals; in this test, mutations are scored by changes in coat color, such as the appearance of a tan spot on an otherwise black coat. This test was used extensively to determine the effect on mutation rate of a large array of radiation variables including dose, dose rate, dose fractionation, radiation quality, and biological factors such as sex, cell stage, and age at exposure.

The early work in the mid- to late '60s showed that the mouse was more sensitive than *Drosophila* to the induction of mutations by high doses of acute irradiation. This finding resulted in a lowering of recommended permissible levels of radiation, although other considerations were also involved. Since then, two major findings from the mouse work have changed the picture. The first was the discovery that, unlike *Drosophila*, the mouse shows a marked effect of dose rate on mutation induction. A given dose administered at a low dose rate produces only one-third as many mutations in spermatogonia as are induced by the same dose at a high dose rate. The second observation was that the arrested oocyte (the germ-cell stage in the female that is primarily at

risk), is so resistant to mutation induction by radiation that no significant increase over spontaneous mutation frequency has been detected.

These two findings taken together indicate that the genetic risk is only about one-sixth of what it was originally estimated to be when the permissible levels of radiation were established on the basis of results from high dose rates and the assumption that oocytes would have the same sensitivity as spermatogonia. Although the new data did not result in a raising of permissible levels of radiation, they showed that the permissible levels had a good margin of safety. We can give an estimate of what this decision may have had in monetary terms. If these findings played a role in the decision not to lower permissible levels by a factor of 10, then there was a saving of 3 billion dollars in the initial cost of retrofitting and a saving of 430 million dollars in annual manpower expenditures (DOE Report No. DOE/EV-0045, 1978). This estimate does not include the savings to public utilities, which might be at least equal in amount to the above figures.

The specific-locus method has been used primarily for comparative purposes, such as comparisons of species sensitivity, of the effects of various factors affecting mutation rate, and of induced and spontaneous mutation rates. This last evaluation yields the "doubling dose," which can be used in the so-called indirect method of estimating total genetic damage. The other, or direct, method requires a measurement of actual physical disabilities resulting from the mutational events. The Mammalian Genetics Section developed a system of scoring skeletal malformations in mice which provided data for the reports by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) and the advisory committee on the Biological Effects of Ionizing Radiation (BEIR) in their direct assessment of genetic risk. The specific-locus findings are also used in extrapolating the

risk of exposure to low doses and at low dose rates; it is also used in estimating the risk to females.

The data and conclusions provided by the Mammalian Genetics Section have thus formed the foundation of genetic risk estimations made by both the UNSCEAR and BEIR committees. At about the same time as the Mammalian Genetics Section was established, genetic studies on the populations of Hiroshima and Nagasaki were initiated. Despite long-continued and intensive work, these studies have not been able to measure radiation-induced genetic damage in the offspring of the exposed survivors, other than to deduce an upper limit of risk based on the negative findings. Thus, results of the mouse studies still provide the basis for national and international estimations of human genetic risk.

## Genetic Toxicology

In the past ten years there has been a considerable increase in research in the general area of genetic toxicology. From a wide variety of studies designed to elucidate the mechanisms of induction of genetic endpoints by chemical and physical agents and to develop assay systems for the effective measurement of these endpoints, it has been possible to arrive at a series of tests that can be applied to determine the potential mutagenicity of an agent. It has further been suggested that there is a relationship between mutagenicity and carcinogenicity, such that studies of mutagenicity may eventually be used to predict carcinogenicity.

Although these assays are most commonly used for predicting the response of exposure to chemical agents, the point of relevance for this discussion is that the majority of them were developed specifically from radiation studies sponsored by OHER. A general resume of five of the most commonly used ones will serve to emphasize this point.

1) Unscheduled DNA synthesis (UDS)

The ability of cells to repair damage to their DNA was initially demonstrated by Richard Setlow at the Oak Ridge National Laboratory. These studies showed that ultraviolet-induced DNA damage could be excised from the DNA, and new DNA synthesized to fill the resulting gap. Rasmusson and Painter, in studies supported by OHER, further demonstrated that if mammalian cells were exposed to radiation and subsequently incubated with high specific activity $^{3}$H-thymidine, the radioactive label was incorporated into the DNA as the result of unscheduled DNA (repair) synthesis; this UDS is quite separate from the normal semiconservative replication of DNA. These observations form the basis of the commonly used UDS assay. If mammalian cells are exposed to a wide variety of different agents that can cause damage or alterations to DNA,

then unscheduled DNA synthesis, associated with the repair of this damage, takes place. Thus, an observation of unscheduled DNA synthesis is indicative of DNA damage and repair. Agents that induce UDS are almost all mutagenic.

2) Sister chromatid exchange (SCE)

The method for demonstrating exchanges between sister chromatids, using $^{3}$H-thymidine autoradiography, was developed by Taylor, Woods and Hughes at Brookhaven National Laboratory. Their observations and analyses were utilized to provide important insights into chromosome structure and replication. However, it was some twenty years later before the utility of the analysis of SCE was really appreciated, which was due very largely to the fact that a completely new non-autoradiographic technique was developed. This technique makes use of the differential staining of chromatids when they incorporate bromodeoxyuridine into their DNA. Much of the initial development of this particular assay is attributable to Sheldon Wolff of the OHER-supported laboratory at the University of California, San Francisco. The analysis of SCE is widely used as a sensitive assay for estimating DNA damage measurable at the chromosome level. It has been validated for a wide range of chemical compounds, and has been applied to monitoring human populations for genotoxic exposures. It has been shown that the majority of agents that induce SCE are mutagenic, and also that a large proportion of carcinogens also induce SCE; however, there are exceptions to this general principle.

3) Somatic cell mutagenesis

Another frequently used assay is that for measuring mutations in somatic cells grown *in vitro*. Several systems for selecting mutant phenotypes are available, using a variety of cell lines of Chinese hamster, mouse or human origin. The techniques for performing quantitative studies using *in vitro* cultures were developed by Theodore Puck, initially for the study of cell

survival after radiation exposure, while the development of a somatic mutagenesis assay is largely attributable to Chu and Malling at the Oak Ridge National Laboratory. These early studies have led to the development of a very sensitive assay that can be used for the detection of the mutagenic potential of an agent, and also, equally importantly, to an understanding of the mechanisms of mutagenesis in mammalian cells.

4) Human lymphocyte assay

Cultures of human lymphocytes can be used to measure the frequencies of chromosome abnormalities, sister chromatid exchanges, and mutations following an exposure *in vitro* or *in vivo* to clastogenic (chromosome-breaking) or mutagenic agents. The lymphocyte assay represents one of the most feasible ways by which a direct estimate of the exposure of human populations or groups of people can be made. In the past 20 years there have been many examples of its use. The application to the estimation of accidental or occupational chemical exposures is much more recent, and is less quantifiable at this time. The development of the lymphocyte assay for use as a biological dosimeter is largely attributable to the research of Michael Bender, at the Oak Ridge National Laboratory.

5) Salmonella assay (Ames assay)

The most widely used assay for determining the mutagenic potential of physical and chemical agents is the one developed by Bruce Ames whereby histidine reversion is studied in a large number of strains of *Salmonella typhimurium* that can detect specific kinds of mutation. The original studies were made using exposures to radiation. The assay has been subsequently modified and expanded for use with agents that cause mutations by a range of different mechanisms. Its simplicity and utility has, with little doubt, made it the most commonly used test system.

The five systems discussed above are representative of a series of assays that have been widely used, or are proposed for use, for estimating genotoxic exposure either *in vivo* or *in vitro*; they provide the basis for the proposed tier system of testing. It is noteworthy that these five assays, together with many others, were developed with OHER funds, as a means of aiding in the understanding of the mechanism of induction of mutations by radiation, or as a means of estimating exposure. The significance of this reseach is far-reaching, as it has direct relevance to industry in terms of mutagenicity testing, to regulatory agencies, and to the research community. Of course, there is a more indirect impact upon society, because of the necessity to provide information on the potential mutagenicity (and, by possible correlation, carcinogenicity) of agents to which populations or groups could be occupationally or environmentally exposed.

DNA Synthesis and the Mammalian Cell Cycle

The history of support by OHER for research on the cell cycle and on DNA synthesis extends back to the very beginnings of the establishment of the AEC. A most important and far-reaching breakthrough was made in 1955, when Walter Hughes working in the Medical Department at Brookhaven National Laboratory prepared $^{3}H$-labeled thymidine for the first time. Reichard and Estborn in Sweden had previously shown that $^{15}N$-thymidine was a specific precursor of DNA in animals and was either incorporated into DNA or catabolized into smaller molecules such as water, carbon dioxide, and other abundant products.

The research in the Medical Department at Brookhaven was designed to develop $^{3}H$-thymidine as an agent to irradiate nuclear DNA, not as a tracer compound. In the summer of 1956, Herbert Taylor (Columbia University, New York) was a visiting scientist in the laboratory of Phillip Woods in the Biology Department. Taylor wanted to try the newly synthesized tritiated thymidine for autoradiographic studies, because although $^{14}C$-thymidine was available, its poor resolution made it unsuitable for distinguishing between the chromatids of a single chromosome. The three investigators began a productive collaborative study which culminated in 1957 in the publication of a paper showing that the DNA in chromosomes of *Vicia faba* replicated semiconservatively. This demonstration is a milestone in the field of genetics and cell biology, for it preceded by a year the work of Meselson and Stahl using $^{15}N$ which showed that DNA molecules in bacteria also replicate semiconservatively.

The experiments of Taylor, Woods, and Hughes were quickly followed by many others using $^{3}H$-thymidine as a precursor for DNA. Highly significant among these was the study of Quastler and Sherman at Brookhaven National

Laboratory who used $^{3}H$-thymidine to measure the parameters of the cell cycle --G1, S (DNA synthesis), G2 and M (mitosis) in which G1 and G2 refer to the two intervals between S and M. This work was the forerunner of a huge amount of current investigation on the kinetics of mammalian cell cycle.

The preparation of $^{3}H$-thymidine was also important for the study of DNA repair. Without the high specific activity it afforded DNA repair would not have been detected, because the amount of DNA synthesis during repair is extremely small (< 0.1%) compared to that occurring normally during S. The studies on DNA repair have had many important consequences, outstanding of which was the demonstration that individuals with the genetic disease xeroderma pigmentosum are very inefficient in repairing DNA after sunlight-induced damage; this defect is undoubtedly the basis for the multiple skin cancers that often metastasize and finally result in death.

Another important contribution to the field of cell cycle studies was accomplished at Los Alamos Laboratory in 1969 when Van Dilla and his collaborators produced the first flow ~~film~~ microfluorometer (FMF). This device can measure the DNA content in as many as one million cells per minute so that the percentage of cells in various parts of the cell cycle can be measured directly. With the FMF, the effects of agents on the kinetics of cell proliferation can be monitored without the use of radioactive tracers. The FMF has subsequently been used for monitoring the proliferation of suitably stained cell macromolecules other than DNA.

The development of $^{3}H$-thymidine and the FMF are outstanding examples of the impact of OHER support in the fields of cell kinetics and DNA synthesis. But other examples are easily cited. For instance, Hughes and his collaborators developed $^{125}I$-deoxyuridine as a tracer for DNA, which has the great advantage that it can be monitored from outside the intact animal. The

work using $^{3}H$-thymidine, $^{125}I$, and the FMF in the past 25 years probably amounts to tens of thousands of papers. It has led to a detailed understanding of DNA replication, DNA repair, and cell cycle parameters, of how they vary in different pathological states, and the way they are affected by various endogenous and exogenous agents. Thus, a more rational and scientific use of chemotherapeutic agents for the management of cancer and other proliferative diseases has been adopted.

The development of $^{3}H$-thymidine and other radioactive tracers has had tremendous financial impact. In the first year of production of $^{3}H$-thymidine, the Schwartz Biochemical Company made profits in the order of several million dollars. In subsequent years, production of $^{3}H$-labeled compounds (which were considered unsuitable for biological studies before 1957) became a multimillion dollar a year business throughout the world. The development of liquid scintillation counters was a direct consequence of the development of $^{3}H$ as a biological tracer. Before 1956, $^{3}H$ had to be counted either by a Geiger counter with a very thin window (at an efficiency of only about 1%) or by proportional counters, and sample preparation was so unwieldy that only a few samples per day could be prepared. Because of the need to count accurately large numbers of $^{3}H$-labeled samples, the then primitive concept of scintillation counting was vigorously developed so that highly sophisticated scintillation counters with high counting efficiency (30-40%) for $^{3}H$ are now available. Each instrument costs $15,000 or more but is a necessity for most biological and chemical laboratories; this investment is another example of a multimillion dollar industry (mostly American) founded upon OHER-sponsored research. The development of the FMF also led to its commercial production. Although these instruments are very expensive, in the order of $100,000 or more, they are found in laboratories throughout the world.

The ability to perform the procedures in molecular biology now common in laboratories throughout the world clearly results from the earlier work on radioactive precursors of DNA. For DNA sequencing studies, $^{32}P$-labeled deoxynucleotide triphosphates are used to label DNAs by *in vitro* nick translation techniques. These techniques are being put into practical use in genetic engineering for the production of insulin and growth hormones. Another consequence of the early studies on DNA synthesis is the current interest in human genetic diseases associated with chromosome fragility that have defects in DNA metabolism, or in the proteins that, along with DNA, form chromatin. Diseases of this type are xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome and Franconi's anaemia. The armamentarium of molecular biology, including many radioisotopic procedures, makes it certain that the chemical basis of these genetic diseases will be understood in the next decade or two. Then the goal will be to use advanced genetic engineering procedures to prevent or correct these diseases.

DNA Repair

The continuity of life as we know it depends upon the three Rs: replication, duplication of genetic material; recombination, the exchange of genetic material to yield genetic diversity; and repair, the removal of damage to DNA resulting from environmental agents, or from mistakes in replication. As a result of repair, cell survival increases, the mutation rate and the probability of neoplastic transformation decreases. The discovery of DNA repair was a direct outcome of OHER-supported investigations into nucleic acid chemistry, photochemistry and photobiology, radiation chemistry and radiation biology and genetics.

As pointed out in the Introduction, no one field of endeavor is independent of others but all are closely interconnected (Fig. 3).

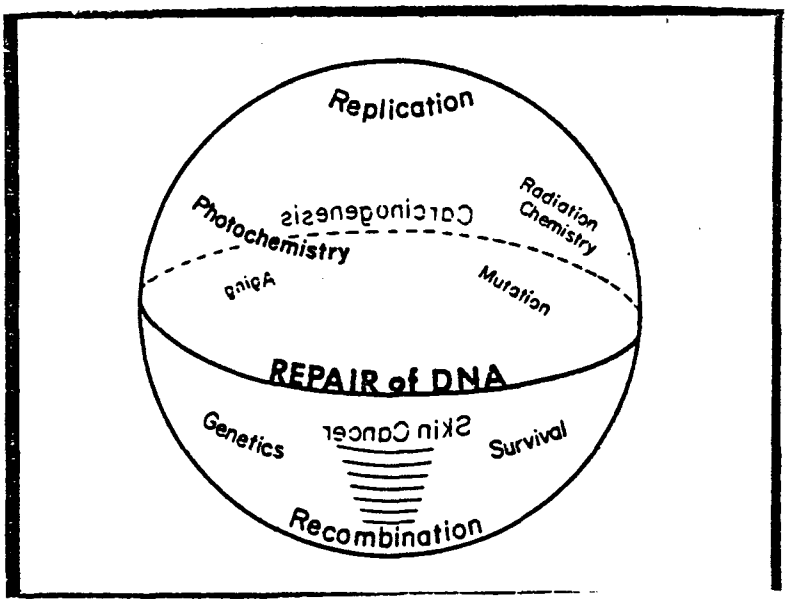


Fig. 3: A schematic picture of some of the fields that have contributed to our understanding of the repair of DNA, and some of the other areas that have also profited from this knowledge.

Knowledge of DNA repair came from the desire to know how cells recover from radiation damage and why some bacterial mutants are more radiation sensitive than normal or wild type strains. The answers to these questions at the molecular level had to wait until the damages affecting DNA were identified. The first damages identified were pyrimidine dimers resulting from ultraviolet irradiation. Such dimers, representing the joining together of two pyrimidines in a single strand of a DNA duplex, were shown to inhibit DNA replication, to kill and mutate bacterial cells, and to destroy the ability of DNA to transform bacteria genetically from one type to another. The identification was first made at the Oak Ridge National Laboratory by R. Setlow and his collaborators.

Once the lethal lesions had been identified (in E. coli, pyrimidine dimers accounted for over 80% of the lethal effects of UV), it was possible to ask a simple question: Are UV-resistant bacterial cells resistant because they contain fewer damages, or because they have developed ways of coping with the damage that are absent in sensitive bacterial strains? In 1964, Setlow and Carrier, Boyce and Howard-Flanders demonstrated that the latter hypothesis was correct. Resistant strains are able to cut out pyrimidine dimers from one strand of a double-stranded DNA by a series of enzymes and the resulting hole is patched by using the opposite good strand as a template. The process is analogous to plastic surgery at the molecular level and is called "excision repair." The discovery of excision repair was closely followed by the work of Hanawalt and his collaborators and by Painter and his coworkers who measured repair by observation of the patching mechanism.

The discovery of the excision repair of UV damage in bacteria quickly led to three other significant findings. One, the observation that normal human cells were able to excise UV damage to their DNA and, from this fundamental discovery, the significant finding in 1968 by Cleaver that individuals with the light-sensitive, cancer-prone disease xeroderma pigmentosum were deficient in some aspects of DNA repair. Two, a technique was developed in 1966 by McGrath and Williams for the measurement of single-strand breaks in DNA irradiated *in vivo* by ionizing radiation. Their method opened the gates to a flood of similar experiments with bacterial and mammalian cells. Three, enzymes were isolated that were able to do the many steps in the various repair pathways. Such repair enzymes are now used as reagents to measure both ultraviolet and radiation damage to the DNA of mammalian cells irradiated both *in vitro* and *in vivo*.

The concepts of DNA repair arose from attempts to explain radiobiological phenomena. The concepts and methodologies of DNA repair have now been extended to three important research areas--chemical carcinogenesis, genetic repair-deficient human diseases, and aging. The connection with carcinogenesis arises from the important discoveries that the modes of DNA repair of chemical damages to human cells either mimic ultraviolet damage (that is, the repair involves long patches and is defective in cells from people with xeroderma pigmentosum) or mimic the repair of damage of ionizing radiation (the repair patches are short). Hence, DNA repair is a general phenomenon, not one peculiar to radiation. Its presence in bacterial or mammalian cells treated with potential mutagens or carcinogens is taken as evidence for DNA damage and is the basis for several short-term tests for genetic toxicology. The use of repair-deficient bacterial mutants in such tests as the Ames test enhances their sensitivity by orders of magnitude.

Subsequent to the discovery of xeroderma pigmentosum as a UV-repair deficient disease, other diseases have been identified as repair deficient. One of these is ataxia telangiectasia. Individuals with the disease are unusually sensitive to ionizing radiation. The disease is genetically inherited; the homozygotes are extremely sensitive, and there are indications that the heterozygotes, who form approximately 1% of the population, have an enhanced cancer risk. A number of other putative repair-deficient human diseases are known in which cells seem to be extraordinarily sensitive to treatment with various chemical agents such as cross-linking agents or alkylating agents.

The difference in cancer susceptibility between individuals with xeroderma pigmentosum and the normal white population in the United States is approximately $10^4$-fold. Since, on the average, xeroderma pigmentosum cells are approximately 70% deficient in DNA repair, these observations led to the hypothesis that smaller differences in repair, such as 10-20%, as might be observed in the normal population, would result in enhanced cancer risk of several to ten-fold. If indeed this idea turns out to be correct, it would imply that whether or not a person gets cancer depends not only on random exposure to carcinogens but also on the deterministic nature of an individual's repair process. Future research will show the extent to which repair processes are genetically or environmentally determined.

In 1974 Setlow and Hart made the intriguing finding that the ability of cultured cells from different mammalian species to do the ultraviolet type of excision repair increased as the life span of the species increased. Human cells are very good at excision repair; mice cells are poor. These observations do not demonstrate a causal connection between DNA repair and aging. But they do reinforce the notion that the two events are related, an

idea derived from early experiments at Oak Ridge on the accumulation of single-strand breaks in the DNA of tissues of old mice. This area of new and fruitful research was opened up by OHER support for Radiation Biology and DNA repair.

The Office of Health and Environmental Research and its predecessors, have supported the research of a number of people in the field of DNA repair. The list that follows is only a partial one; others have made valuable contributions to this field.

1. C. S. Rupert, Johns Hopkins University: photoreactivation
2. P. C. Hanawalt, Stanford University: repair replication
3. J. E. Cleaver, Radiobiological Laboratory, University of California at San Francisco: xeroderma pigmentosum
4. R. B. Painter, Radiobiological Laboratory, University of California at San Francisco: unscheduled DNA synthesis and ataxia telangiectasia
5. Ruth Hill, Columbia University (with H. H. Rossi): discovery of sensitive bacterial strains
6. R. B. Setlow, J. K. Setlow, J. D. Regan, F. J. Bollum, R. W. Hart, Oak Ridge National Laboratory and Brookhaven National Laboratory: identification of dimers as lesions, repair in prokaryotes and eukaryotes, identification of dimers as tumorigenic changes in DNA, relation of DNA repair with life span, long and short modes of repair
7. B. M. Sutherland, Brookhaven National Laboratory: purification of photoreactivating enzyme, existence of such enzymes in human skin, and transformation of human cells by UV
8. M. M. Elkind, Brookhaven National Laboratory and Argonne National Laboratory: split-dose effects and transformation of cells in culture

## Quantitative Biology

There has been a marked increase in the use of quantitative methods in Biology and Medicine over the last several decades. The recognition of the importance of a quantitative approach in these sciences, which had previously been largely qualitative, has been the result of the productive interaction of biologists and physicians with physicists, chemists and engineers. One important origin of such interaction was the multidisciplinary research in radiobiology and nuclear medicine that has been supported by OHER.

It is generally accepted that the advanced radiotherapy techniques used in the treatment of cancer or for radiosurgery require a quantitative approach to treatment planning. A very large number of hospital radiotherapy units provide treatment planning through clinical physics groups that apply quantitative techniques to determine tumor position, target volume and irradiation dose. The basic conviction that a quantitative evaluation of these parameters would be vital to the clinician in providing the best patient care grew to a large extent from the need to quantitate the biological effects of ionizing radiation. Before World War II physicians tended to estimate radiation therapy dose in terms of exposure units (R). The necessity for a more precise description of the biomedical effect of radiation led to measurements of the dose being expressed in increments of energy absorbed per unit mass of tissue (rad), and to clinical applications of such physical properties of radiation as linear energy transfer (LET). A summary of the current attempts to further quantitate the radiation dose and its biomedical effects can be found in the National Council of Radiation Protection Report on dose-response for low LET radiations (NCRP-Report No. 64, April 1, 1980). The

input from OHER-supported investigators to this report demonstrates the early recognition of the essential importance of the quantitative approach to radiobiological and radiotheraputic research.

A second strong connection between OHER-supported research and the growth of quantitative biology came about through the application of radioactive isotopes for the precise measurement of biological parameters. Metabolite concentrations, pool size and, perhaps most importantly, metabolite movement and turnover could be measured with an accuracy never before attainable with the advent of radioisotope-labeled compounds. The importance of this ability to quantitate basic biomedical parameters was seen almost immediately as a major break-through and the pioneering work of George Hevesy in this field of isotopic tracers was recognized with the Nobel Prize in 1943.

Hevesy completed his survey book on radioactive indicators in 1948 in the OHER-supported Donner Laboratory of the University of California. A review of the references cited in that book shows the major contribution of principal investigators working with the OHER programs (for example, J. H. Lawrence, J. G. Hamilton and H. B. Jones). The power of radioactive isotopes as a quantitative tool in biology continued to be developed by OHER-supported investigators and the technique played an important role in many fundamental discoveries. Notable among these achievements was the establishment of the chemical steps occurring during photosynthesis by the Nobel Prizewinner Melvin Calvin. The continuing contribution of OHER researchers to quantitative diagnostic techniques of nuclear medicine, such as the Anger Camera and PET Scanners, is documented in Gottshalk and Potchen's "Diagnostic Nuclear Medicine" (Williams and Wilkins, Baltimore, 1976).

The general trend towards quantitative biology can be seen in the increased emphasis placed on physico-chemical content in biology courses taught in American universities. For example, a comparison of the General Catalog of the University of California for the academic year 1935-36 with the current 1981-82 catalog shows there are two new departments, Biophysics and Medical Physics, and Molecular Biology. The largest of the Graduate Groups, is now in Biophysics and Medical Physics and Bioradiology. The 1935-36 catalog also shows that a common route for the introduction of physico-chemical biology was through the study of radiobiology. In that early catalog, the advanced course in physico-chemical biology was described as "dealing especially with the biological effects of radiant energy". The study of such effects, so closely associated with the programs of OHER provided the essential combination of disciplines of physics, chemistry, biology and medicine that could foster new academic areas such as biophysics. The value of a solid background in physical science to the biological or medical student is now well accepted.

The basic insights discussed here are that a quantitative evaluation of biological and medical questions leads to a more productive and accurate result than the traditional qualitative approach alone and that a close interaction between physicists, engineers, biologists and physicians such as took place in radiobiological research is essential to further this quantitative approach. Such interdisciplinary research and training are very effective in bringing about rapid advances in both basic and applied aspects of quantitative biology and medicine.

The research supported by OHER played a major role in demonstrating the worth of this approach and continues to make very valuable and unique contributions to quantitative biomedical studies.

## Carcinogenesis

The fundamental insights into biologic processes gained from research carried out in OHER-supported programs of Radiation Biology has had a major impact in three areas of carcinogenesis. The first of these areas is in molecular biology and genetics, especially through fundamental studies of nucleic acid biochemistry and DNA repair processes. The second involves the formulation of the concept of risk assessment based on dose-response relationships for carcinogenesis derived from experimental studies and from large-scale human epidemiologic investigations. The third is in the realm of quantitative cellular biology and includes the development of techniques and models for studying cellular responses to DNA-damaging agents. The basic knowledge and experimental models derived from radiobiologic studies are now being applied to the study of the effects of chemical carcinogens and mutagens.

A major milestone in the early post-World War II days was the establishment of the new Biology Division at the Oak Ridge National Laboratory under the leadership of Alexander Hollaender, with the overall goal of seeking biochemical explanations for the genetic and carcinogenic consequences of radiation exposure. Strong programs were set up in bacterial, *Drosophila* and mammalian radiation genetics, and in mammalian cytogenetics. Specific studies on carcinogenesis at Oak Ridge were initiated by Jacob Furth, based largely on the somatic mutation theory. This particular area of investigation was further developed by Arthur Upton who was especially interested in the phenomenology of cancer and in the derivation of dose-response relationships.

Basic studies of nucleic acid biochemistry were an important feature of the Oak Ridge program and formed the framework for the development of the field of DNA damage and repair by Richard and Jane Setlow. Measurements of the production and rejoining of breaks in DNA strands were pioneered by McGrath and Williams also at Oak Ridge. Major research programs also were based in several OHER-supported university laboratories such as that of Cleaver at the University of California where the disease xeroderma pigmentosum was first described as a human repair defect, and the research of Philip Hanawalt at Stanford on repair-replication of DNA. The production of DNA damage by chemical and physical carcinogens and its subsequent modulation by molecular repair processes has become a critical concept in the initiation of cancer and is today one of the major subjects of research. The foundations for this central theory in current carcinogenesis undoubtedly were laid in such OHER-supported laboratories.

The impact of Radiation Biology on risk assessment and the development of dose-response models for carcinogenesis came from several different areas of research. Most of the basic animal studies designed to determine dose-response relationships were carried out with radiation; these results constitute the major dose-response data available, as few experiments have been carried out as yet with chemical carcinogens. Earlier studies included the development of mammary and ovarian cancer models in which the interactions between hormonal effects and radiation were studied. The animal carcinogenesis program is being continued by Fry, Ullrich and their coworkers at Oak Ridge with unique studies designed to elucidate the dose-response relationships for the induction of cancer at various sites following low doses of radiation.

In a number of OHER-supported laboratories, parallel experiments were initiated to evaluate the carcinogenic potential of internally emitting radionuclides. These studies include the large-scale rodent and dog experiments at the Argonne, Lovelace and Pacific Northwest laboratories and at the Universities of Utah and California at Davis, and the intensive epidemiological follow-up at the Argonne laboratories of dial painters who had been occupationally exposed to radium. These results may have had less significance than those from the external radiation studies in terms of the general field of physical and chemical carcinogenesis, but they are nevertheless unique in their quantitative elucidation of the carcinogenic potential of radioactive isotopes emitted from man-made and natural sources.

Another major contribution of the OHER to risk assessment and the determination of dose-response relationships for radiation-induced cancer in humans derives from their major support of the Atom Bomb Casualty Commission. The careful and responsible epidemiologic follow-up of the survivors in Hiroshima and Nagasaki has yielded clear and significant dose-response data for the induction of cancer at five different sites in the body; this information has formed the basic foundation for risk estimations from radiation exposure which will serve for years to come as a model for the exposure of human populations to physical and chemical carcinogens in the environment.

In relation to risk assessment, it should be pointed out that the most widely used assay at the present time for the carcinogenicity of chemical agents was developed by Bruce Ames through support from the OHER at the University of California. This assay is based currently upon the mutagenic potential of chemical agents in bacteria.

The final area of cancer research in which Radiation Biology has had a major influence is that of quantitative cellular biology; more specifically, the elucidation of the cellular effects of DNA-damaging agents. The first colony-forming assay for the survival of mammalian cells exposed in cytotoxic agents was developed by Puck and Marcus at the University of Colorado Medical School at Denver utilizing ionizing radiation. This technique was exploited over the ensuing 15 years almost entirely by radiobiologists, but it has now become a fundamental and widely applied technique in cellular carcinogenesis research.

The development of methods for synchronizing mammalian cells and the study of cell cycle-related sensitivity were also largely developed in radiobiology laboratories, as is also true of the elucidation of cellular recovery processes and of dose-rate effects at the cellular level.

Radiation-Induced Carcinogenesis and Exposure Standards

Ionizing radiation is probably the most thoroughly studied of all environmental hazards and the quantitative risk of human health effects, particularly cancer, from radiation exposure is probably better known than that for any other agent. This state of affairs results in large part from a number of epidemiological studies on irradiated human populations, many of which have been or are presently supported by OHER. The single most important epidemiological study has been the one that has been conducted on the survivors of the atomic bombings in Japan. This study is of unusual value because the radiation was not given for medical reasons so that interpretation is not complicated by pre-existing disease, the sample size is large, all ages were included, and the radiation doses were sufficiently high that positive associations between cancer incidence and radiation exposure could be established unambiguously for many cancers. Until agreement was reached with the government of Japan to share the costs more equitably by establishing the Radiation Effects Research Foundation, the principal support for these clinical, epidemiological, and dosimetric evaluations was provided by OHER.

Despite the significant amount of data from human populations exposed at high doses and dose rates, the estimation of risks in the dose range of interest (that is, low doses and/or low dose rates) depends on the use of models in order to extrapolate from the effects of high doses and dose rates to low doses and dose rates. The reason is that direct studies of human populations exposed to low doses and/or low dose rates have yielded negative or equivocal results. Recent improved models have been heavily influenced by developments in biophysical theory and microdosimetry and by experimental results obtained in a variety of biological assays. In 1972, the BEIR Committee (BEIR-I) used a linear, no threshold, no dose-rate effect model to

extrapolate to cancer risks at low total doses and dose rates. Subsequently, both UNSCEAR (1977) and the ICRP (1977) implicitly adopted the assumption that at low doses and dose rates the risk is less than that predicted by the simple linear model. In 1980, BEIR-III explicitly adopted a second degree polynomial equation to extrapolate to low doses. The NCRP (1980) concluded that at low doses or low dose rates, low LET radiation is 2 to 10 times less effective than would be predicted by the BEIR-I model. The consensus of these expert committees, then, is that risks from low doses and dose rates (where the effects have not been seen) are overestimated by a linear extrapolation from effects seen in the high dose range. These modifications to the extrapolation models could not have been adopted had it not been for recent developments in theory and in experimental studies supported byOHER. For example, Harold Rossi at Columbia University, has been in the forefront in developing theories of radiation action.

It is of interest to note that throughout the 1970s there was pressure on the part of some scientists and organizations to reduce the radiation exposure standards by about a factor of 10. The arguments for change were based heavily on the use of linear or "supralinear" models. The more recent consensus by the UNSCEAR, ICRP, BEIR-III, and NCRP committees that effects of low doses are overestimated by linear models seems to have lessened this concern.

Another area of debate which appears to have been settled recently is whether the death rate from diseases other than cancer, such as cardiovascular or cerebrovascular disease, is increased in irradiated populations. The Japanese studies have consistently indicated that there is no such effect although the results from other population studies yielded somewhat conflicting evidence. Extensive experimental studies, principally in mice, in

which careful autopsies were done indicate that life-shortening can be accounted for by the increased incidence or earlier onset of malignant neoplasms. This work, which was supported by OHER, together with the most recent report on the Japanese survivors, clearly indicates that there is no excess mortality from causes other than cancer. Most experts now agree that the only significant hazard to death in irradiated subjects is from cancer. As more insight on mechanisms of radiation carcinogenesis and on factors influencing cancer expression is obtained from experimental studies, there will be improvements in extrapolation models and refinements in risk estimates.

We can identify a specific problem which OHER will face in the future and which appears solvable only by experimental study. If, as seems possible or perhaps even likely, the most recent dosimetry studies show that the neutron component of the radiation dose in Hiroshima was negligible, then we are faced with the prospect of having essentially no data on neutron-induced carcinogenesis for humans. Neutron exposure standards would necessarily have to be based on the results of new experimental studies. Otherwise risk estimates will be very uncertain, with some experts arguing that the risk is high and others arguing that it is low, with neither side having sufficient information to reach a reasonably supportable judgment. Accurate risk estimates are needed both by OHER contractors and the utility industry. If the risk is high, their workers need additional protection. If the risk is low but the lack of data results in unrealistically stringent standards, then both operating and retrofitting costs could run into substantial sums of money unnecessarily.

There is no way of accurately estimating the indirect benefits of OHER-supported programs in radiation carcinogenesis. For example, to what extent did these programs lead to reduction of unnecessary radiation exposure with a subsequent sparing of people from genetic defects and cancers? It does seem clear that the research fundings have had a major impact on the setting of radiation standards for the general population and for these occupationally exposed. These standards seem to have struck a reasonable balance between protecting people from undue risk on the one hand, and from prohibitively expensive costs of construction and operations on the other.

According to an OHER-sponsored study on the impact of reductions in the external radiation exposure limits (DOE report No. DOE/EV-0045, 1978), there have been savings of well over 3 billion dollars by not reducing the limits. The extent to which OHER-sponsored research influenced this decision is not known. It seems plausible to assume that the cognizant authorities were willing to accept existing standards (based on estimates of risk) as being reasonable and as not posing an undue hazard. These estimates of risk, in turn, were heavily dependent on OHER-supported research. If OHER research contributed as little as 10% to the decisions, it follows that the savings effected by OHER's programs amounted to between 43 and 86 million dollars annually and a savings of 300 to 600 million in construction costs. We do not have precise figures on the cost of OHER's radiation carcinogenesis programs, but it appears to us that they represent a real bargain.

Immunology and Hematology

Radiation Biology has had a massive impact on human medicine since the late 1940s. We can get a glimpse of its significance from two recent summaries of the use of bone marrow transplantation in contemporary medical treatment. R. A. Good writing in The New England Journal of Medicine this year says, "Indeed more than 20 previously fatal diseases can now be successfully treated with bone marrow transplantation. These disorders range from genetically determined severe combined immuno-deficiency diseases to acute leukemias and include a number of life-threatening aplastic and aregenerative anemias, immunohemopoietic disorders, genetically determined congenital hematologic abnormalities, and even the awful marble-bone disease, osteopetrosis." Similarly, E. Beutler in The Journal of the American Medical Association in 1981 points out, "Until recently, our mind-set was that leukemia was not curable. Anyone who claimed otherwise was clearly either a fool or a charlatan." In selected leukemia patients and under appropriate circumstances, "the vast majority (well over 90%) survive the transplantation procedure, and the extent of morbidity does not differ greatly from that associated with ordinary induction chemotherapy..[D]espite the occurrence of graft-vs-host disease and occasional relapses, the overall cure rate of this selected group of patients seems to be between 70% and 80%." This remarkable state of affairs for treatment of lethal diseases of blood-forming tissues is almost entirely the result of basic research in mammals sponsored by OHER.

The impact of research on radiation effects on the defense mechanisms (immunology) and the blood-forming tissues (hematology) is so great and so diffuse that most experimental immunologists and hematologists owe a great deal of their contemporary research methods to the far-sighted support of OHER. The spleen colony assay is a noteable example. The use of radioactive isotopes in immunology and hematology as well as the rest of biomedicine is so extensive it would cover many volumes.

This basic research started in the late 1940s and early 1950s when Leon Jacobson and his colleagues at Argonne National Laboratory in collaborative research with Egon Lorenz and his colleagues at the National Cancer Institute established the field of bone marrow transplantation. The primary goal in those early years was the treatment of total-body irradiation injury and the prevention of death from hemorrhage, infection and anemia. Early attempts were also made to cure cancer by these techniques; this research has continued to the present time. The dynamic atmosphere created by the radiation injury issues and the attempts to modify the injury through tissue and organ transplants soon influenced the fields of immunology, pathology, clinical medicine, and genetics.

An example of the significance to human medicine of many years of research in Radiation Biology was seen in 1981 when E. D. Thomas was awarded the Kettering Prize for his work on bone marrow transplantation which depended in a great measure on sponsored basic mammalian radiation research in the National Laboratories. In accepting the award, Thomas paid tribute to the earlier work of Jacobson and Lorenz. In the same way, the development of radioisotopes, in particular, that of tritiated thymidine at Brookhaven National Laboratory, provided the tools necessary for basic kinetic studies of the progenitors and the production of blood cells.

The use of marrow transplants and the spleen colony assay in animals is the basis for our current understanding of the interrelationships of cells in the blood, bone marrow, and blood-forming tissues. The identification and isolation of the erythropoietin, a controlling hormone of the red blood cell system, resulted from the work at the Argonne Hospital (now the Franklin McLean Memorial Research Institute). Our understanding of cellular immunology and of the relationship of lymphatic tissues to bone marrow also stems from

these early radiation studies. Clinical transplantation techniques have been strongly dependent upon development of methods of cross matching and immune suppression. At present in terms of patients treated, renal transplants are the most important, but now marrow and other organ transplantation are increasingly performed. Basic studies of cell kinetics of the bone marrow and lymphoid tissue have also had a large impact on the design of clinical protocols for the chemotherapy of cancer.

As in the past, so in the future, Radiation Biology can be relied upon to provide new insights and methods in many fields. These advances are likely to come from research on:

1) radiation effects on sensitive tissues; the working out of the intermediate events in the injury process as a model for many kinds of injury to cells—but particularly chemical injury.

2) the differences between effects on radiation-sensitive versus radiation-insensitive tissues; comparisons with chemical sensitivity and chemical insensitivity of tissues.

3) processes that modify radiation effects; particularly the working out of intermediate events and their analogies in modifying chemical injury.

4) Radiation Biology as a model for studying chemical effects.

The social correlate of this research, the objective of keeping people alive by marrow transplantation, cannot be reasonably considered in terms of cost effectiveness. But, for the sake of argument, if we can theorize a thousand patients are cured each year by marrow transplantation and if a human life is worth 100,000 dollars and if OHER programs can claim half the credit for this form of treatment, then the annual dollar savings that can be credited to the Agency is $50 million. Additionally, if 5000 kidney transplants are done annually and in this case we consider the OHER programs

are 10% responsible for their success, we can add another $50 million. As Alex Comfort points out, medicine and our humanity has always accepted the prolongation of an active and healthy human life as one of its self-evident objects--a statement which can bear aggressive restatement.

## Hematology

Under the sponsorship of OHER and the Department of the Navy, a number of careful studies were started in 1945 to analyze the cause of death in the Japanese people exposed to the nuclear bombs. Continued OHER-supported studies in the field and laboratory lead to the demonstration that the two predominant causes of death from irradiation in the lethal dose range are bacterial infection and hemorrhage, due to loss of granulocytes and platelets induced by bone marrow hypoplasia. The techniques of platelet and granulocyte transfusions to minimize these effects emerged directly from the studies on the nature of mammalian radiation injury. Working in the Medical Department at Brookhaven National Laboratory, Cronkite and Bond established the practical management of radiation injury in the midlethal dose range in dogs by utilizing fresh blood and platelet transfusions as necessary and sequential antibiotics. This treatment reduced the mortality of dogs within the lethal dose range to near zero; the regimen is ineffective in the supralethal dose range where bone marrow transfusions are effective. This study in turn led to the present treatment of radiation- or chemical-induced aplasia of the bone marrow in man.

The line of research that finally led to successful bone marrow transplantation has been nearly totally supported by OHER. The notion of bone marrow transplantation as a treatment for bone marrow failure goes back many decades, but early transplantation experiments invariably ended in failure. However, a series of studies by Leon Jacobson and his associates at the Argonne Cancer Research Hospital, some of which were initiated during the days of the Manhattan District, resulted in successful bone marrow transplantation in the mouse. This study started with the observation that mice given 2 μCi/g strontium did not develop anemia, although their bone marrow became nearly

aplastic. The lymph nodes and spleen became sites of intensive erythropoietic activity. The next step was the removal of the spleen either before or after the adminstration of strontium which resulted in severe anemia. If the spleen was shielded with lead and the rest of the body irradiated, mortality was strikingly reduced. The bone marrow regenerated if the spleen were left in place only for a few hours. Brecher and Cronkite showed that parabiotic rats would survive when one animal had been given a lethal irradiation while the other was shielded with lead. These studies unequivocally demonstrated that some factor is being transmitted from nonirradiated spleen or whole body to the irradiated animal, initiating and accelerating bone marrow regeneration. Jacobson and his associates logically concluded that the effect could be explained by one of three mechanisms: by detoxification by the nonirradiated tissue; of substances produced by irradiation; by cellular seeding and tissue reconstitution; or by the production of a humoral substance that initiates functional reconstitution of cells.

Since the murine spleen normally contains elements ordinarily seen only in the bone marrow in mammals such as erythrocytic precursors, megakaryocytes, and granulocytic foci, it was natural to test the efficacy of bone marrow injections in increasing the survival of fatally irradiated mice. In a series of experiments supported jointly by OHER and the National Cancer Institute, Lorenz, Congdon and Urso quantitatively examined the effectiveness of bone marrow transplantation in guinea pigs, rats and later in dogs. It was soon realized that the closer the genetic relationship of donor and recipient, the greater the success in initiating recovery with fewer complications. Through the work of many laboratories and particularly Oak Ridge National Laboratory, it has been shown that in the disease known as graft vs. host disease (GVH) the immunologically competent cells from the donor repopulate lymphoid tissues

in the recipient and initiate immunological attacks against the recipients' tissues. GVH disease still remains the major stumbling block in the successful application of bone marrow transplantation in humans.

At Argonne National Laboratory, Brookhaven National Laboratory, and Oak Ridge National Laboratory, a systematic study of the influence of radiation upon immunity also was pursued. Taliaferro and associates at Argonne National Laboratory, Stoner and associates at Brookhaven, and Makinodan and associates at Oak Ridge demonstrated dose-effect relationships between exposure to radiation and the depression of primary and secondary immune responses. The immune suppression was ameliorated by the shielding of lymphoid tissues such as the appendix in the rabbit and the spleen in the mouse, and by the transplantation of syngeneic lymph node, bone marrow, or spleen cells. These studies led to application of this treatment in immune-deficient children with some success, and also to the application of whole-body irradiation to suppress immunity to prepare patients for kidney transplantation with modest success. More effective chemical methods of suppressing immunity are now used. These studies did, however, suggest a method of managing acute leukemias of childhood in which there was no response to chemotherapy or in which the children had relapsed. The best protocols were established in which the children were given first massive combined chemotherapy and then lethal whole-body irradiation, followed by bone marrow transplantation from a sibling in which the HLA antigens were identical. In the few cases where there have been identical twin donors, the results have been striking. In the case of siblings with perfect HLA compatibility, there have been some triumphs but GVH disease remains the major problem.

Another aspect of radiation and immunity was developed at the Brookhaven National Laboratory Medical Department by Cronkite and associates. This approach utilized extracorporeal irradiation of the blood (ECIB) so that predominantly the radiosensitive lymphocytes circulating through the blood were killed rather than irradiating the entire animal and destroying other radiosensitive cells necessary for survival. To the extent that ECIB has been applied in chronic lymphocytic leukemia, several patients have responded dramatically, with suppression of the disease process and at times a diminution in transfusion requirements.

Extracorporeal irradiation of the blood has also been used to deplete animals of preformed antibodies against allogeneic tissues along with suppression of their cellular immunity. This research has been conducted in collaboration with the Transplantation Center at State University of New York, Stony Brook. To date, this treatment has been quite beneficial in getting dogs to accept heterorenal transplants. Presently, plans are under way to develop a program on extracorporeal irradiation of the blood in candidates for kidney transplantation, who are unsuitable as they have circulating antibodies which would cause hyperacute rejection of the transplanted kidney. In the event that these studies are successful, this would have a major impact on the cost of medical practice. Currently in the New York area, there are about 900 individuals who would otherwise be candidates for kidney transplantation but must be managed by tri-weekly dialysis, a very costly procedure. In the U.S., there are probably about 10,000 such patients.

Radiation Biology has had a significant impact on our understanding of erythropoietic proliferation. Hennessey, Van Dyke, Huff and others at Donner, Lawrence Berkeley Laboratory, early demonstrated that the uptake of radio-iron in circulating red cells was inversely proportionate to the dose of

radiation. This led to the use of the uptake of radio-iron in red cells and to determine such functions as its turnover rate as a means of studying clinical states of hemopoiesis with considerable accuracy and increasing insight into aberrations of erythropoiesis. One of the most significant breakthroughs in expanding our understanding of the anemias of humans was the development of a biological assay for the hormone erythropoietin, which is the major regulator of red blood cell production. This assay is dependent on the incorporation of radio-iron into red cells. Schooley and Garcia under the sponsorship of the OHER were the first to produce an antibody which was capable of neutralizing the biologic activity of erythropoietin. Since that time various investigators have attempted to develop a radioimmunoassay (RIA) for the hormone. Garcia, working at the Donner Laboratory, has now succeeded.

This work together with earlier work sponsored by the OHER by Jacobsen and his associates, who were the first to demonstrate that the kidney was the major site of production of erythropoietin, has made possible the very recent attempts to treat the anemia of end-stage renal disease with erythropoietin injections, and so eliminate the need for transfusions of red cells in these patients. One of the major reasons for rejection of renal transplants is the large amounts of these transfusions prior to the surgery, with subsequent sensitization of the patients to red cell antigens. If, indeed, the use of erythropoietin in the anemia of renal disease is successful, a highly significant increase in the number of successful renal transplants will occur thereby lessening the need for costly dialysis procedures.

Finally, it is through the extensive studies on the beneficial effect of shielding bone marrow that the current highly successful radiation therapy of Hodgkinson's disease has been established. The crucial experiments were made at Argonne National Laboratory, Brookhaven National Laboratory, and Oak Ridge National Laboratory. These studies were used as the basis of setting up shielding studies to shield bone marrow while the unshielded parts of the body were given very heavy irradiation in an attempt to kill the lymphoma cells. This has resulted in a major improvement in the survival of patients.