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RAYMOND ELLIOT ZIRKLE
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A Biographical Memoir by

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BY ROBERT P. PERRY

RAYMOND ZIRKLE WAS A pioneer in the field of radiation biology. He made seminal contributions to our knowledge of the effects of high-energy radiations on cells and devised ingenious means for using radiation to ablate small regions and individual structures of cells. His early studies of alpha-particle irradiation of fern spores—which identified the cell nucleus as the major target for radiation lethality and demonstrated the importance of linear energy transfer—were classical investigations of this type. In later studies he and his coworkers developed microbeam technology, which they used to dissect the functions of particular cell structures for chromosome movements during mitosis.

Ray Zirkle was born in Springfield, Illinois, and spent his early years on a farm in northern Oklahoma. His primary education was gained in one-room country schoolhouses in Oklahoma and later in southern Missouri. Both parents taught in country schools. In these rural settings Ray's main link to the outside world was through books, of which he was an avid reader. The first stimulus toward a scientific career might have been provided by his reading of *The Lost World* by Sir Arthur Conan Doyle. This adventure novel, an early example of science fiction, was serialized in a small weekly newspaper in the Missouri Ozarks. Nine-year-old Ray waited eagerly for each issue and was enthralled by the fantastic creatures and their exotic habitat.

After several years the Zirkles decided that farming in the Ozarks was not profitable, so they moved into the town of West Plains and bought a grocery store. From 1915 to 1919 Ray attended West Plains High School, where he showed an aptitude for mathematics and took several engineering courses. After graduation he joined the Missouri National Guard and served for several years. In 1924 he married Mary Evelyn Ramsey, who spent her early years in a rural area of western Kansas and, similarly to Ray, received her primary education in a one-room country schoolhouse. They had two children, Raymond Jr. in 1927 and Thomas in 1929.

In 1928 Zirkle received an A.B. from the University of Missouri, where he was elected to Phi Beta Kappa and to the honorary scientific society Sigma Xi. From 1928 to 1932 he carried out graduate studies in botany at the University of Missouri, earning his Ph.D. in 1932. During this time he served as an assistant (1928 to 1930) and later as an instructor (1930 to 1932) in the Botany Department.

Zirkle's thesis research was the forerunner of experiments and ideas that occupied him for the rest of his scientific career. In this research he irradiated spores of the fern *Pteris longiflora* with alpha particles emitted from a polonium source and studied the effects on their subsequent germination. The choice of alpha radiation with its relatively simple dosimetry and low penetrating power and the fern spores, which can be irradiated in a dry or slightly moistened state and have a size commensurate with the alpha-particle range, was well considered. In these premiere studies he observed that the alpha rays inhibited three distinct processes of germination: cracking of the spore wall, development of chlorophyll, and cell division. The effect on cell division was achieved at a substantially lower dose than those needed to inhibit the other processes. Most importantly, he noted that when the spores were oriented so that their nuclei were included in the radiation field, the effects were substantially greater than when the nuclei escaped irradiation. These results were developed and extended in his later work.

After receiving his doctorate Zirkle joined the Johnson Foundation for Medical Physics at the University of Pennsylvania in Philadelphia. Initially he had a fellowship from the National Research Council, which had also supported his graduate studies. He remained at Penn as a Johnson Foundation fellow and a lecturer in biophysics until 1938. During this time he investigated the quantitative relationship between ionization per unit path of alpha particles and their biological effectiveness, which was not clear-cut from the data obtained up to that time. By placing fern spore nuclei either near the beginning of the path (where the ionization density was low) or near the end of the path (where it was high) or in intermediate positions, he was able to calculate the number of alpha particles per nucleus that was necessary to produce a given effect, such as the inhibition of cell division. He found that the biological effectiveness is not only a function of the total number of ions formed in the nucleus but is also dependent on the variable concentration of ions formed in different portions of the path of the alpha particle. His data suggested the relationship $B = kI^{2.5}$, where B is the biological effectiveness per alpha particle, k a proportionality constant, and I the ionization per unit path. The quantitative aspect of this work was unusual for such studies at that time and established Ray Zirkle as a leader in the field of radiation biology. A generalization of these results to other types of radiation by Zirkle and others led to his later formulation of the concept of linear energy transfer.

Additional studies carried out at the Johnson Foundation, which enjoyed the interest and encouragement of its director, Detlev W. Bronk, dealt with the relative effectiveness of alpha particles, X rays, and fast neutrons on various biological materials and also explored environmental modifiers of radiosensitivity. In an incisive theoretical analysis he used existing knowledge of the specific ionization properties of different types of radiation to interpret experimental results with alpha particles, neutrons, and X rays. At one end of the spectrum are X or gamma rays, which set electrons in motion with the lowest specific ionization (ions per unit path traversed), and at the other end are neutrons, which accelerate mainly carbon, oxygen, and nitrogen nuclei with the highest specific ionization. Intermediate are protons and alpha particles. From this analysis he concluded that "the greater the specific ionization of a radiation, the greater the ionic effectiveness, that is, the less absorbed energy needed to produce a given degree of injury."

Zirkle developed a strong friendship with another Johnson Foundation fellow, the crystallographer A. Lindo Patterson, who became an assistant professor of physics at Bryn Mawr College in suburban Philadelphia when he completed his fellowship. A little later, in 1938, Zirkle was recruited to Bryn Mawr as an assistant professor of biology. Patterson and his wife, Betty, became lifelong friends of Ray and Mary despite both couples' moving to other academic locations.

In 1940 Zirkle was appointed professor of biology at the University of Indiana; however his academic career was interrupted during World War II when he became one of the principal investigators in the biological program of the Manhattan District. His research in this project was chiefly concerned with the comparative effects on living systems of fast and slow neutrons, beta rays, and gamma rays. A substantial part of the wartime research carried out under his direction was reported in several volumes of the National Nuclear Energy Series, of which he was the health editor.

Much of the biological research in the Manhattan Project was carried out at sites where particular radiation sources were located, such as the Clinton Laboratories near Oak Ridge, Tennessee; the Radiation Laboratory at the University of California; the National Cancer Institute in Bethesda, Maryland; and the Metallurgical Laboratory at the University of Chicago, which later became the Argonne National Laboratory. Research at the latter site brought Zirkle into contact with many faculty members from the University of Chicago who shared common interests with him. Thus, it is perhaps not surprising that in 1944 he was offered and accepted a professorship there and that in 1945 he became director of the newly founded Institute of Radiobiology and Biophysics. This institute, like the Johnson Foundation, became a focal point for scientists and students with a penchant for physics and an interest in biological problems. The Zirkles purchased a home in Olympia Fields, south of Chicago, which had space for a large flower garden. William Doyle, a faculty colleague of Ray's, recalled to me the many pleasant Saturday afternoons when he and his wife visited the Zirkles and enjoyed games of bridge with them.

The era of nuclear energy was spawned by the first chain reaction, produced by Enrico Fermi and colleagues under the West Stands of the athletic stadium at the University of Chicago. With this era came the Plutonium Project, which was assigned the task of purifying the artificial element plutonium for use in atomic bombs. The chain reaction used for making plutonium emitted huge amounts of gamma rays and neutrons. Moreover, diverse forms of radiation were present in the fission products produced in the purification process. Since these radiations posed serious hazards to personnel who would be involved in this project, it was important to set up stringent control measures for exposure and to carry out intensive radiobiological research in order to evaluate the nature and severity of the hazards. Zirkle was a major participant in the radiobiological studies, particularly in defining the acute lethal action of slow neutrons produced in the atomic piles. At the annual meeting of the Radiological Society of North America, held in Chicago in 1946, he hosted a symposium that brought together many of the other participants and summarized their major findings.

In the late 1940s and early 1950s Zirkle continued his theoretical and experimental studies of the effects of radiation on living cells. As knowledge of the chemical composition of biological material began to accumulate, he attempted to relate the chemical effects caused by the absorption of radiant energy to the ultimate biological effects. He fully appreciated that an understanding of the multitude of diverse radiobiological effects—such as gene mutations, chromosome breaks, increased membrane permeability, inhibition of cell division, induction of neoplasms, and lethality of cells and organisms—would require a detailed knowledge of the intervening chemical modifications. Yet, the level of knowledge of the molecular composition and dynamics of cellular constituents was still very primitive. The relationship of DNA and proteins to genes was still uncertain. Nothing was known about the existence of DNA repair mechanisms or the molecular basis of mitosis or the mechanisms responsible for cell proliferation and cell death. At this time one had to be content with discriminating direct from indirect effects of the radiation and for establishing criteria that could sort out the relevant chemical consequences of the ionization or excitation of molecules. Zirkle's analyses provided a rational conceptual framework for dealing with this complex problem.

Zirkle had a special interest in the quantitative aspects of dose-effect curves. Using methods based on the target theory of Timoféeff-Ressovsky and Zimmer, he developed mathematical formalisms for interpreting the experimental survival curves obtained when simple organisms were exposed to ionizing radiation. In a study with Cornelius Tobias the radiobiological influence of linear energy transfer was investigated with respect to the survival of haploid and diploid strains of budding yeast. At all values of linear energy transfer, the survival curves of the haploid strain were exponential, while those of the diploid strain were strongly sigmoid and of a shape essentially independent of linear energy transfer. They interpreted these results as being consistent with a theory in which cell division in haploid cells can be inhibited by inactivating any one of multiple chromosomal sites with a single ionizing particle, whereas in diploids it is necessary to inactivate both members of an allelic pair of corresponding sites. Although the observed variations of relative biological effectiveness with linear energy transfer were not consistent with simple target theory, they could be explained in terms of chemical intermediates that diffuse from their places of origin in the ionization tracks to the sensitive chromosomal sites. Further elaborations of yeast survival curve analysis were carried out by two of Zirkle's biophysics graduate students, Thomas H. Wood and Robert B. Uretz, as the subjects of their dissertation research.

During 1951-1952 Zirkle devised a new experimental approach to his long-standing interest in partial-cell irradiation. Together with William Bloom, a professor of anatomy at the University of Chicago, he developed the methodology for irradiating living cells with a microbeam of ionizing radiation and observing the consequences with time-lapse photography. Bloom, a coauthor with Alexander Maximov of a classical textbook of histology, brought to this project his knowledge of cell morphology, microscopy, and microphotography, as well as his expertise with the newly emerging technique of cell culture. Zirkle contributed his keen knowledge of the physics of radiation and of the instrumentation necessary to generate and control it. Their initial account of this project, published in a 1953 *Science* paper, was considered a tour de force.

The microbeam project necessitated careful consideration of a myriad of details. In order to produce a sufficiently intense collimated microbeam with minimal scattering, it was decided to use 2-Mev protons produced by a Van de Graaff electrostatic generator. An emergent macroscopic beam was allowed to impinge on a metallic shield pierced by a microaperture that produced microscopic beams as small as 2.5 microns in diameter. For biological material Zirkle and Bloom concentrated on actively dividing mitotic cells in cultures derived from newt heart. These large, relatively flat cells were excellent specimens for this type of experiment because of their favorable dimensions and their ability to proliferate at ambient temperature. The cells were grown on mica coverslips, 5 microns or less in thickness, in order to avoid serious energy attenuation of the protons. An effective locator system was devised for directing the microbeam to selected regions of the dividing cells and to ensure the observation of the same cell before and after irradiation. The cells were observed with customized phase-contrast microscopes and photographed with rigidly mounted 16-mm Bolex movie cameras. The highly skilled instrument makers and the superb machine shop facility at the institute were critical components of this endeavor.

The initial results of these experiments indicated the potential power of this approach for understanding the basic principles of chromosome movement and cell division. Irradiation of chromosomes in prophase or metaphase with a few dozen protons regularly produced fused chromosome bridges that interfered with the subsequent anaphase movement, or chromosome fragments that became detached from the spindle and formed small micronuclei after the completion of anaphase.

I vividly remember these experiments because I actually participated in them. In 1951 I entered the graduate program in biophysics, which was administered by a committee rather than a department. The committee was composed of a prestigious group of University of Chicago faculty members from various disciplines, many of whom had research laboratories in the building at 57th Street and Ellis Avenue, where the Zirkle and Bloom laboratory was located. On the first floor there was the laboratory of the physicist James Franck, who with Hans Gaffron was studying electronic transitions in the photosynthetic pigment chlorophyll. On the second floor, the physicist Leo Szilard and his colleague, Aaron Novick, were studying enzyme induction and feedback inhibition in the bacterium *E. coli*. The laboratory of Zirkle and Bloom was on the third floor. On the fourth floor, directly above the microbeam apparatus, was the temperamental Van de Graaff generator, its collimated proton beam protruding through a hole in the floor.

After I passed the requisite qualifying examinations in physics and physical chemistry, I joined the Zirkle-Bloom laboratory as a neophytic graduate student of Ray Zirkle. Before beginning my own thesis research I was engaged in the proton microbeam experiments in a variety of ways, the most interesting of which was keeping the mitotic cells in sharp focus during the pre- and post-irradiation filming. I soon learned that experiments of this complexity often do not go as smoothly as anticipated. Most trying on Zirkle's patience were problems with the generator's vacuum system or the operation of its belt, which seemed to happen at inopportune times when carefully staged mitotic cells were awaiting irradiation. He sometimes suffered from intense migraine headaches that seemed to be exacerbated by these annoying situations.

The technical difficulties with the microbeam experiments were eliminated when the radiation source was switched from protons to ultraviolet light. Zirkle's student, Robert Uretz, whose thesis research dealt with the additive effects of X rays and ultraviolet light on yeast cell survival, designed and built a relatively simple optical instrument that could focus an intense microspot of ultraviolet light on the mitotic cells. The ease with which this instrument could be used for perturbing the mitotic process by chromosome ablation or spindle destruction led to a large number of experiments, many with interesting, informative outcomes. Localization of the irradiated regions of chromosomes could readily be verified because the intense ultraviolet energy absorbed by the chromosomes caused a dramatic decrease in refractive index at the irradiated site and a concomitant loss of DNA from the site, a phenomenon that was termed "paling."

Zirkle and his coworkers selectively irradiated isolated centrophilic chromosomes that had not yet become aligned on the metaphase plate either in the kinetochore region or in a distal part of the chromosome. They observed that the normal movement of the chromosomes to the metaphase plate was inhibited when the kinetochores were irradiated, but not when the distal regions were irradiated. The chromosomes with ablated kinetochores drifted around until anaphase occurred and were squeezed into one of the two daughter cells after cytokinesis. This was the earliest observation of the importance of the kinetochore for what is now known as mitotic checkpoint control.

An especially striking effect termed "false anaphase" was observed when the mitotic spindle was destroyed by microbeam irradiation. In cells with irradiated spindles the orderly metaphase configuration of chromosomes became transiently deranged and then formed a quasirosette arrangement in which the kinetochores of whole chromosomes rather than sister chromatids were attracted to the centrosomes. Later the quasirosettes dissociated into two rosettes, followed by cytokinesis and nuclear reconstitution. In this case the chromosome complement was randomly distributed to the two daughter nuclei. These experiments clearly demonstrated a distinction between the molecular mechanisms responsible for moving chromatids to the spindle poles during normal anaphase and those responsible for moving the poles apart prior to cytokinesis.

Zirkle and Bloom amassed a huge collection of 16-mm movie films that documented the orderly progression through mitosis of normal cells and the abnormalities that occur in microbeam-irradiated cells. Zirkle continued to analyze this vast repository of data for more than a decade after the actual microbeam experiments were completed. In 1970 he published an extremely detailed account of his observations, some of which continue to be quoted until the present day. In the mid-1970s he retired from the university. The Zirkles moved to a home next door to their son Tom in the foothills of the Rocky Mountains, west of Castle Rock, Colorado. Zirkle died in a nursing home in Castle Rock in 1988.

Zirkle was a member of several scientific societies. He was president of the Radiation Research Society in 1952-1953 and a councilor from 1954 to 1956. He was a founding member of the Biophysical Society, in which he served as a councilor from 1957 to 1961 and again from 1964 to 1966. He served on the editorial boards of seven journals and on committees and study sections concerned with research in radiobiology and training in biophysics and medical science. He was honored by election to the National

Academy of Sciences in 1959 and to the American Philosophical Society in 1960.

Zirkle's research was carefully carried out, meticulously described, and cautiously interpreted. His experiments with alpha-particle-irradiated fern spores—which showed especially high sensitivity of nuclei compared with cytoplasm and demonstrated the importance of ionization density—were seminal discoveries, made prior to an understanding of the molecular basis of gene expression. Similarly his microbeam experiments provided the first evidence for the importance of kinetochores in mitotic checkpoint control. A colleague of mine who is presently studying the regulation of the mitotic process said, "Zirkle was ahead of his time. He doesn't get referenced as much as he should in the current literature." Nevertheless it is gratifying to know that his contributions are still appreciated. It is also noteworthy that these achievements were made by someone whose education began in a one-room country schoolhouse.

I AM VERY GRATEFUL for the personal information about Raymond Zirkle that was communicated to me several years ago by Mary E. Zirkle, Elizabeth Patterson, and William L. Doyle. I am also deeply indebted to Robert B. Uretz for critically reviewing this memoir and for helping me obtain reprints of Zirkle's publications, which were collected by the late Robert H. Haynes. A review of this memoir by Thomas H. Wood is also greatly appreciated.

HONORS AND DISTINCTIONS

Hitchcock Professor, University of California, Berkeley (1951).

Member of the editorial boards of the following journals: *Progress in Biophysics and Biophysical Chemistry* (1958-1962); *Biophysical Journal* (1960-1965); *Journal of Photochemistry and Photobiology* (1961-1964); *Radiation Research* (1953-1956); *Review of Scientific Instruments* (1948-1951); *Annual Review of Nuclear Science* (1953-1959); and the *Journal of Cellular and Comparative Physiology* (1951-1954).

Member of the following organizations: American Association for the Advancement of Science; Biophysical Society (councilor 1957-1961 and 1964-1966); American Physiological Society; American Society of Zoologists; Botanical Society of America; Radiation Research Society (president 1952-1953; councilor 1954-1956); American Society of Naturalists; American Society of Plant Physiologists; American Roentgen Ray Society; National Academy of Sciences; and the American Philosophical Society.

Member of the following committees: Biophysical Sciences Training Committee, National Institutes of Health (1958-1962); Radiobiology Study Section, NIH (1947-1949); Radiation Study Section, NIH (1955-1957); Subcommittee on Radiobiology, National Research Council (1947-1960; chair, 1953-1956); Committee on Nuclear Science, NRC (1953-1956); Medical Scientist Training Committee, NIH (1963-1967); U.S. National Committee for Pure and Applied Biophysics (1964-1968).

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