CRC Handbook of Radiobiology

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Dr. Kedar N. Prasad, Professor of Radiology and Director of Center for Vitamins and Cancer Research, at the School of Medicine, University of Colorado Health Science Center, Denver, has done pioneering work on the modification of the effect of ionizing radiation by physiological substances such as adenosine 3′,5′-cyclic monophosphate (cAMP), butyric acid, vitamin C and vitamin E on tumor cells in culture. In addition, he has published original work on the control mechanisms of differentiation and malignancy, using neuroblastoma cells in culture as an experimental model. This work has led to the addition of cyclic AMP stimulating agents in the treatment of metastatic human neuroblastomas. His recent studies on the effect of vitamin E on tumor cells has led to the phase I trial of vitamin E in the treatment of several human neoplasms. Dr. Prasad has published over 100 full publications, several reviews, and books in radiation biology and cell biology. He has edited several books and has estabished an annual series on "Advances in Vitamin, Nutrition and Cancer Research."

Dr. Prasad is a frequent speaker at a major national and international conference on cancer. He is a member of several national and international scientific organizations. He has participated in the establishment of a new society called "International Association of Vitamins and Nutritional Oncology", and is serving as acting President of the society.

Dr. Prasad received his M.Sc. in Zoology from Bihar University, India, and Ph.D. in Radiation Biology from University of Iowa, Iowa City. He did his postdoctoral work at the Brookhaven National Laboratory, Upton, N.Y.

PREFACE

The purpose of this handbook is to provide the most recent information on the effects of ionizing radiation on mammalian cells, with emphasis on human tissues. The dose-effect relationship will be emphasized in a quantitative manner. The book contains recent data on the late effects of low levels of radiation on human subjects. This handbook also contains some of the late consequences of radiation therapy which are being detected among survivors of patients with various neoplasms. Recent studies on the interaction of radiation with hyperthermia, electronaffinic compounds, and other new radiomodifying agents have been analyzed and discussed. This would serve as a reference book for radiobiologists, residents in diagnostic, nuclear medicine, radiation therapists, and graduate students in radiation biology.

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DEVELOPMENT OF RADIOBIOLOGY: A REVIEW

INTRODUCTION

The development of radiation biology began immediately after the discovery of the X-ray by Roentgen in 1895. One year later, Becquérel and Curie observed that certain substances (compounds of uranium, radium, and polonium) were naturally radioactive. Since then, the development of radiation biology has been linked with the advancement of nuclear physics and basic cell biology on the one hand and with the growing awareness of the hazards and usefulness of ionizing radiation on the other. Some of the major discoveries in nuclear physics[9,10,17] and biology[4,13,16,21-24] which have influenced the growth of radiation biology are briefly described.

MAJOR DISCOVERIES IN NUCLEAR PHYSICS

Soon after the discovery of the X-ray and naturally occuring radioactive substances, Thomson defined the physical properties of electrons and protons.[10] In 1911, Rutherford, at the University of Cambridge, discovered alpha particles and in 1932 Chadwick made the discovery of neutrons.[10] The availability of neutrons made possible the production of several radioisotopes of biological and medical interest. Also, the relative biologic effectiveness of neutrons with respect to the X-ray was investigated. In 1932, the invention of particle accelerators (the cyclotron) by Lawrence at the University of California, Berkeley, was of great significance.[10] Since then, the cyclotron has been used as a means of production of several radioisotopes of biological and medical interest. Also, the relative biologic effectiveness of neutrons with respect to the X-ray was investigated. On December 2, 1942, Fermi and associates at the University of Chicago accomplished a chain reaction from the fission of uranium atoms in a pile of graphite blocks.[10] This remarkable discovery became the basis for manufacturing the atom bomb and nuclear reactor. Today, most of the radioisotopes of biological and medical interest are produced in the nuclear reactor. In addition to this, the nuclear reactor serves as a source of neutrons of different energies which are being utilized for the study of radiation injuries as well as for medical purposes.

Recent advances in accelerator technology make possible the attainment of very high-intensity proton beams. Such proton beams are adequate for providing pure, high-intensity beams of negative pions (π^-). The accelerator which produces π is referred to as a "meson factory"[17] and is now in use at the Los Alamos Scientific Laboratory, New Mexico. Theoretically, it appears that such a beam could deposit, at essentially any depth in animals and humans, more energy than could be deposited by other particles such as protons, neutrons, and alpha particles. This is due to the fact that when a negative pion is captured by an oxygen nucleus, the mass of the pion is converted into energy with a consequent violent disruption of the oxygen nucleus. From the nucleus emerge neutrons, protons, alpha particles, Li, Be, B, and C ions; however, the dominant mode involves alpha particles, which have short range. Negative pions are being used in radiobiological studies and in the treatment of local neoplastic lesions.

The availability of a variety of radioisotopes has served both as a source of radiation for evaluating the biological hazards of ionizing radiation and as a tracer for the study of the function of various organs and cells.[15] It has also helped in providing a better knowledge of the mechanisms of radiation injuries.

During the last decade our dosimetry has markedly improved;[19] therefore, at present we can establish a more accurate dose-effect relationship than before.

MAJOR DISCOVERIES IN BIOLOGY

Several major advancements in cellular and molecular biology have markedly influenced the development of radiation biology. For example, the establishment of the mammalian cell line in vitro and the identification of various phases in the life cycle of a cell have increased our understanding of cellular radiosensitivity.[4] The study of ultrastructures of a cell by an electron microscope has been very useful in investigating radiation injuries on a subcellular level. Although radiation-induced changes in the ultrastructures of a cell appear nonspecific, these cellular alterations, in combination with biochemical ones, have increased our understanding of radiation injuries. Radiobiologists have not yet taken advantage of the scanning electron microscope, which shows the surface structure of entire cells in great detail.

In 1953, the discovery of the double-helix model of DNA structure had a big impact[23] on the development of radiation biology. The structure of DNA and the mechanism of its replication have contributed to our understanding of the mechanisms of radiation damage and repair. The elucidation of protein biosynthesis[23] has also increased our knowledge of the mechanisms of radiation damage on the molecular level. The effects of irradiation on biosynthesis and kinetics of nucleic acid and protein synthesis have continued to be studied.

It is now established[12,13] that mitochondria contain DNA which is capable of coding at least certain mitochondrial proteins. This is substantiated by the fact that mitochondria synthesize RNA and protein in vitro. The radiosensitivity of mitochondria has been studied primarily on the basis of morphologic changes, oxidative phosphorylation, and ion transportation, but the effcts of irradiation on the biosynthesis of mitochondrial nucleic acid and protein have not been investigated.

Many studies have been performed on the effect of hormones in the regulation of cellular RNA and protein synthesis.[21,22,24] Several hormones increase enzyme synthesis, which is related to an increased nuclear RNA synthesis. The radiosensitivity of newly formed RNA and protein has not been adequately investigated. Since hormones play an important role in the regulation of the metabolic function of the cell, such a study would increase our understanding of cellular radiation damage and repair. Recent studies have established that cyclic nucleotides, adenosine 3′, 5′-cyclic monophosphate (cAMP) and guanosine 3′, 5′-cyclic monophosphate (cGMP) are important for several cellular functions. The importance of cyclic nucleotides in the modification of radiation response has not been adequately studied. These cyclic nucleotides affect the growth, morphology, and differentiation of mammalian cells in culture. The role played by cAMP and cGMP in the radiosensitivity of cells would be important to investigate in order to understand more about the mechanism of radiation damage. In addition, several new growth factors and transformation factors have been identified and isolated. The significance of these factors in modifying the radiation injury remains to be defined.

The technique of somatic cell hybridization[16] of two different cell types may prove a very useful tool in obtaining some new insights regarding the radiosensitivity of mammalian cells. Hybrids are produced by fusing two cell types in the presence of an inactivated Sednai virus.

Radiation has contributed directly to the understanding of several aspects of cell biology which would have been difficult to understand by other means. Since radiation is efficient in killing only certain types of cells, the importance of such cells can be more easily evaluated by radiation rather than by other agents such as chemicals, which kill cells nonspecifically. On the basis of this principle, a map of organogenesis has been prepared by irradiating the embryo at different stages of development. Radiation also induces a high incidence of mutation; therefore, it has contributed considerably to our knowledge of mutagenic processes. For example, today we know that mice are much more sensitive to radiation-induced mutation than *Drosophila*. In addition, the repair of premutational changes occurs in mice.

The use of ^{3}H-thymidine has helped in identifying various phases of the cell cycle and in estimating the period of each phase. The use of other radioactive labeled compounds as a tracer has increased our understanding of the physiological, biochemical, and metabolical functions of various organs.

AWARENESS OF HAZARDS AND USEFULNESS OF RADIATION

Isolated cases of radiation lesions were observed soon after the discovery of the X-ray. Both Becquérel and Curie suffered from acute *radiation dermatitis* or so called *radium burn*. These lesions appeared on areas continuously exposed to radiation. The first case of radiation sickness was described 6 years after the discovery of the X-ray. The incidence of radiation injury increased considerably following Rutherford's discovery of artificial nuclear fission and Frederick and Irene Joliot Curie's discovery of how to obtain radioactivity artificially.

The earliest known case of radiation-induced cancer was reported in 1902. Curie herself died of aplastic anemia which was probably due to a prolonged exposure to radiation. Nine deaths due to bone cancer were recorded between 1922 and 1924 among watch industry workers who painted dials with radium. Constant licking of the radium brush during painting procedures led to the accumulation of large amounts of radium in the bones over a long period of time. Irradiation of bones induced bone cancer. Jacob Furth induced leukemia for the first time, in mice, by a single whole-body exposure of 400 R or by a closely spaced fractionated dose of 800 R. In the early days, a high incidence of skin cancer and leukemia were observed among radiologists who were exposed to chronic doses of X-rays during the course of their work. Today, growing awareness of the hazards of radiation and improved safety devices for radiation sources have eliminated the possibility of radiation-induced neoplastic disease among radiation workers. Schwarz[19] has published an excellent review, *Radiation Hazards to the Human Fetus in Present-Day Society*, in which he discusses the hazards of diagnostic X-rays in pregnant women, concluding that the diagnostic X-ray is very harmful for the fetus and therefore such women should not receive diagnostic X-rays unless it is absolutely essential for their health. A most recent BEIR (Biological Effect of Ionizing Radiation) Committee report also has extensively discussed the effects of low levels of radiation on humans. This is the most authoritative source of estimation of low-dose radiation injuries in humans.

The discovery that radiation induced gene mutation in *Drosophila* further dramatized the hazards of radiation. When animals were used to study radiation-induced mutation some new concepts emerged.

The great tragedy caused by the atomic bombardment of Hiroshima and Nagasaki aroused serious concern among physicists, biologists, and the public. This led to the rapid expansion of radiation biology, the primary purpose of which was to evaluate the possible hazards of radiation and to understand the mechanisms of radiation injury. Human data on the biologic effects of single whole-body radiation exposure came primarily from the people of Hiroshima and Nagasaki who were exposed at the time of atomic explosion.

It should be emphasized, however, that radiation has been useful in biology and medicine. Henri Coutard was the first to develop the "fractionation dose technique", which involves the administration of daily fractional doses of X-rays. This allows the delivery of large radiation doses in "the most effective period of time." The purpose of such a radiation regimen was to destory the tumor while inflicting minimal permanent damage to the skin and other normal tissues. This type of therapy would be more effective if the radiation therapist had some knowledge of cell kinetics. Unfortunately, the estimation of cell kinetics in human tumors in vivo is extremely difficult. Radiation is being used extensively in the diagnosis of several diseases. Early diagnosis of many diseases has cured patients and prolonged their lives.

AGRICULTURE AND FOOD PRESERVATION

Radiation induces mutations in both plants and animals. Although most mutations are deleterious, careful selection and breeding of beneficial mutants have led to the production of mutant strains which produce a greater yield of crops than the wild type. Several studies have shown the possibility of using a massive dose of radiation for food preservation.

SOME MAJOR DEVELOPMENTS IN RADIATION BIOLOGY

Law of Bergonié and Trebondeau

As early as 1906 the French scientists, Bergonié and Tribondeau, working with rat testes, proposed a new hypothesis on the radiosensitivity of cells which in broad terms is as follows: (1) less differentiated cells are more radiosensitive than highly differentiated ones, and (2) proliferating tissues are more radiosensitive than nonproliferating ones. The generality of this law is still true, with the exception of lymphocytes and oocytes which are very radiosensitive in spite of the fact that they are highly differentiated and are not dividing.

Target Theory

In order to explain the biologic effects of ionizing radiation, several ideas were introduced. Among these, the concept of target theory originally proposed by Dessaur in 1922 and later expanded by Lea[11] proved useful in the study of radiation biology. This theory in its simplest terms predicts that inactivation of biological molecules increases exponentially as a function of dose. This theory assumes that the inactivation of the molecules is caused by a direct hit and, therefore, is also referred to as "direct action" or "direct effect".

Indirect Effect

The target theory was found inadequate to explain cellular radiation injuries. Dale, Evans, and Gray developed the concept of indirect effect or indirect action of radiation,[1,3,6,8,18] according to which biologic molecules in aqueous solution are inactivated by free radicals which are formed when radiation interacts with water.

Relationship Between Chromosome Volume and Radiosensitivity

On the basis of target theory, Sparrow[20] proposed a new hypothesis to explain some of the discrepancies in the radiosensitivity of various species. According to his hypothesis, the radiosensitivity of a cell is directly proportional to its interphase chromosomal volume. This hypothesis is consistent with his observations on several plant species. He further speculated that if one expresses the dose as energy absorption per chromosome, an apparent difference in the radiation response of various animal species may largely disappear. The data obtained from several plant species are consistent with Sparrow's hypothesis; however, the validity of this hypothesis for mammalian species remains to be established.

Oxygen Effect

Oxygenated tissues were more sensitive to irradiation than hypoxic ones.[4] This finding has become a theoretical basis for hyperbaric radiation therapy of those tumors which have hypoxic cells.

Concept of Relative Biological Effectiveness (RBE)

The concept of RBE evolved because of the availability of several types of radiation which produce different degrees of damage with the same dose. This is due to the fact that the linear energy transfer (LET) for each type of radiation is different. For the same total dose, the radiation of high LET (α-particles, protons) produces greater damage than that of

low LET radiation (X- and γ-ray). In addition, the oxygen effect, which is so marked with the radiation of low LET, is negligible with radiation of high LET.

Modification of Radiation Damage

The discovery of several radioprotective[2-4] and radiosensitizing agents[1,6,18] has increased our knowledge of radiation injuries. Extensive work has been done on radiation injuries of the small intestine and bone marrow.[2] Bond et al.[2] have recommended an excellent therapeutic regime for accidentally exposed individuals. This involves a "functional replacement therapy" which requires transfusions of fresh platelets, whole blood, and antibiotics whenever needed. Spleen, spleen cells, and bone marrow transplantation protect animals after exposure.[6] Cell-free spleen extract as a radiation therapeutic agent was first shown by Ellinger[5] and recently confirmed by Ford et al.[7] Several new modifying agents have been identified. Electronaffinic compounds are in clinical trials.[26]

Quantitative Radiation Biology

The development of quantitative radiation biology owes much to the discovery of the colony technique.[4] This technique measures the reproductive integrity of irradiated cells and is very precise and reproducible. Recently, the technique of counting the number of colony-forming units (CFU) in the spleen of lethally irradiated mice was also developed.[4] This method provided a very useful tool in the assaying of radiation injuries of the spleen and bone marrow in vivo. In addition to these biologic parameters, electron paramagnetic resonance (EPR) is being used to measure the free radicals produced in irradiated materials.

Cellular Radiosensitivity and Cellular Repair

Success in identifying various phases of the cell cycle and in culturing synchronized mammalian cell in vitro has provided new information regarding the radiosensitivity of cells in relation to the cell cycle.[4] On the criterion of cell death, the mitosis phase of the cell cycle is considered to be the most radiosensitive; however, on other criteria such as reduction of DNA synthesis or chromosomal damage, this may not be true. Like bacteria, mammalian cells repair radiation damage.[4,14] Mammalian cells in vitro repair sublethal and potentially lethal damage.

SUMMARY AND COMMENTS

In our nuclear era, radiation biology will continue to grow as an important field in modern biology. The extensive use of atomic energy in various branches of national economy, technology, science, biology, and medicine has made the study of radiation injury and radiation protection an important subject. It is for this reason that biologists, physicians, physicists, and chemists are working together in the area of radiation research to obtain a better understanding of radiation injuries and their modifications. Close collaboration between radiation biologists and radiation therapists has become necessary for the most effective treatment of neoplastic diseases, but one has to constantly remember that while radiation treats cancer, at the same time it has the potential to induce cancer. Therefore, radiation should be used only when necessary, and all measures must be taken to minimize the exposure of normal tissues. To increase the efficiency of radiation therapy, investigators are studying in three major areas: (1) radioprotective agents, (2) radiosensitizing agents, and (3) the effect of high LET radiation. With the growing use of nuclear energy in industry, technology, and the sciences radiation biology will continue to grow. The author believes that the current emphasis on the modification of radiation injury of tumor and normal cells will eventually increase the management of tumors by radiation therapy. Based on our present knowledge of radiation effect, the maximum permissible dose (MPD) is recommended as an "acceptable

risk''. Therefore, the benefit from radiation must be overwhelming before an individual or the public is exposed to the MPD. Since there is no radiation dose known as ''safe'', continuous effort must be made to minimize the exposure level as much as possible. the extent to which these efforts are successful may well affect the whole future of nuclear energy.

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BASIC CELL BIOLOGY

INTRODUCTION

In order to understand the radiation injury of cells, one must be thoroughly aware of their structures, kinetics, and functions. Therefore, a brief description of basic cell biology in its simplest form is presented in this chapter.

ULTRASTRUCTURE OF A CELL

The electron microscope has provided a powerful tool in the study of subcellular structures of a cell. Figure 1 shows the diagrammatic representation of the ultrastructure of a cell. The most common cytoplasmic organelles such as mitochondria, smooth and rough endoplasmic reticulum, ribosomes, polysomes, and golgi apparatus are seen. These structures may differ in quantity from one cell to another, but qualitatively they are common to all cell types. However, in certain highly specialized cells such as melanocytes, new subcellular structures (premelanosomes, melanosomes, and melanin granules) are present in addition to the usual cytoplasmic organelles.

Mitochondria

The structures and functions of mitochondria have been studied extensively.[10] Each mitochondrion has a double membrane. The outer membrane is continuous to form a sac, whereas the inner membrane has many infoldings, some of which have formed septa. These infoldings have been referred to as "cristae". The matrix in the most mitochondria is continuous throughout the lumen.

The mitochondria contain the complete machinery for orderly oxidation of pyruvate and fatty acid via the Krebs cycle, including enzymes, coenzymes, and essential metals. During oxidation processes, adenosine triphosphate (ATP) is generated and is utilized in the metabolic function of cells. In addition, the mitochondria accumulate certain ions such as K^+, Ca^{2+}, Mg^{2+}, and HPO_4^{2-}, by active transport mechanisms; they thereby participate in the homeostatic regulation of ions in cells.

The mitochondria contain DNA which, like bacteria, is circular in shape. The mitochondrial DNA is capable of coding the information for the biosynthesis of some mitochondrial proteins. Indeed, it has been shown that mitochondria synthesize RNA and protein in vitro. These studies indicate that mitochondria occupy semiautonomous status within the cell.[11] Further work is being done to elucidate the role of mitochondrial DNA in cellular metabolism.

Ribosomes and Polysomes

Ribosomes (also referred to as ribonucleoprotein or microsomes) from widely different organisms are remarkably uniform in their general properties.[9] These particles are composed of 40% protein and 60% RNA and constitute 80 to 90% of cellular RNA. Ribosomes are metabolically stable and occur in the cell either as a free form (70S) or as subunits (50S and 30S). During the process of protein synthesis, more than one ribosome is present on the messenger mRNA strand, and ribosome-mRNA complexes are referred to as polysomes.

Endoplasmic Reticulum

The endoplasmic reticulum is an elongated membrane structure and occurs with or without the association of ribosomes; the former is called rough endoplasmic reticulum, whereas the latter is referred to as smooth endoplasmic reticulum. These structures, at least in the rat liver, have been suggested to participate in the transport of synthesized protein.[12]

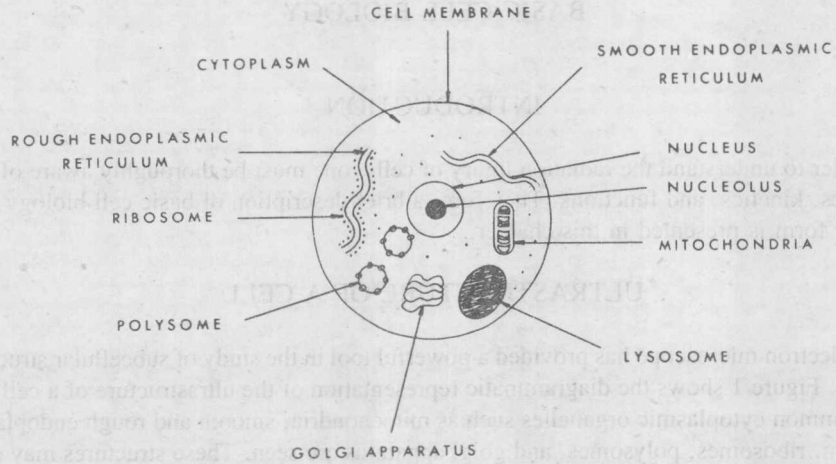

FIGURE 1. Diagrammatic representation of the ultrastructures of a cell. The following structures are seen: nucleus, nucleolus, nuclear membrane, chromatin materials, mitochondria, Golgi apparatus, rough endoplasmic reticulum, ribosomes, polysomes, and lysosome.

Lysosome

Lysosomes contain a number of hydrolytic enzymes, particularly acid phosphatase.[2] They are found in a wide variety of tissue and participate in the removal of unwanted cellular materials. Rupture of a lysosome releases the hydrolytic enzymes which may cause cell lethality.

Golgi Apparatus

In an electron micrograph, the Golgi apparatus exhibits a variable appearance. It consists of a collection of double membranes, large vacuoles, small vesicles and granules. These structures participate in the secretory activity and increase in size during the elaboration of secretory substances by the thyroid. These organelles may also serve as a condensation center for materials being absorbed by the cells.

Structure of a Nucleus

The nucleus consists of a nuclear membrane, nuclear sap, one or more nucleoli, and small granular elements called chromatins. The basic proteins of the nucleus appear homogeneously electron dense when stained with osmic acid. The nuclear membrane is generally thicker than the plasma membrane surrounding the cytoplasm. The nuclear sap is usually more viscous than the cytoplasm. The nucleoli are round, dense, and well-defined bodies which are composed of RNA and associated proteins. The chromatin granules are composed of DNA and associated basic proteins. The nucleus contains the genetic material DNA and is essential for metabolic function of the cell. The nucleus is also necessary for cell division.

MITOSIS

The nucleus of a cell has a chromosome set which differs from one species to another. For example, man has 46 chromosomes, whereas the mouse contains 40 and the golden pea only 14. Chemically, chromosomes contain not only DNA and histones, but also RNA and other proteins. It is well known that DNA is the genetic material which is responsible for the heredity of characters. Therefore, any change in the structure of DNA of the germinal cell (spermatozoa or ova) would be manifested in the offspring. However, if DNA of the somatic cells such as skin, liver, intestine, etc. has changed, such alterations would not be transferred to the offspring.

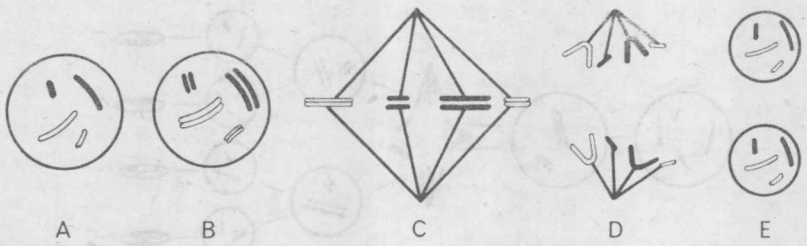

FIGURE 2. In mitosis each chromosome duplicates itself. The duplicates separate as the nucleus divides, so that the daughter nuclei are identical in chromosomal consitution. Prophase: A,D; metaphase: C; anaphase: D; and telophase: E. (From Sharp, L. W., *Fundamentals of Cytology*, McGraw-Hill, New York, 1943, 64. With permission.)

In mitosis, each chromosome duplicates itself. The duplicated strands separate as the nucleus divides, so that the daughter nuclei have the same set of chromosomes as their parent cell. Figure 2 shows a diagrammatic representation of the process of mitosis in a cell. During mitosis, a cell passes through four stages: prophase, metaphase, anaphase, and telophase.

During prophase, each chromosome doubles itself and the nuclear membrane and nucleus disappear. During metaphase, spindles form and chromosomes lie on the equatorial plate. During anaphase, chromosomes separate and each half moves towards a pole. During telophase, the nucleus appears and the cell divides into two daughter cells, each having an identical set of diploid chromosomes. The process of mitosis is so precise that any change in the chromosomes or DNA would definitely reflect in daughter cells after completion of cell division.

MEIOSIS

This kind of nuclear division occurs only in the germinal cells (ovary and testis). In the testis, during meiosis, each member of a paired chromosome duplicates and the duplicated members come to lie side by side in a four-strand configuration. The successive nuclear divisions result in the formation of four sperm, each with a haploid set of chromosomes (half of the parent cell). During meiosis, the first nuclear division is a mitotic one in which each daughter cell receives an identical set of diploid chromosomes. The second nuclear division is a reduction division in which each daughter cell contains only the haploid set of chromosomes. Diagrammatic representations of meiosis in the testis and ovary are shown in Figures 3 and 4. In the testis, spermatogonia divide by mitosis to form primary spermatocytes which undergo reduction division to form spermatids. Spermatids have a haploid set of chromosomes. The spermatids undergo a maturation process to form spermatozoa. The entire process of the formation of spematozoa is called spermatogenesis. The basic process of meiosis in the female is the same except that each oocyte gives rise to only one functional egg, whereas each spermatocyte produces four functional spermatozoa. The process of forming the functional egg is called oogenesis.

CELL CYCLE

The life cycle of a cell is divided into four phases.[5] These include DNA synthetic phase (S), pre-DNA synthetic phase (G_1), post-DNA synthetic phase (G_2), and mitosis (M). A diagrammatic representation of the four phases of the cell cycle is shown in Figure 5.

Recently, another phase (G_0) has been identified in the cell population. This period is referred to as the "no-growth" period and signifies a time after mitosis, but before the onset of G_1. The G_0 period is not part of the cell cycle, therefore it should not be included in the

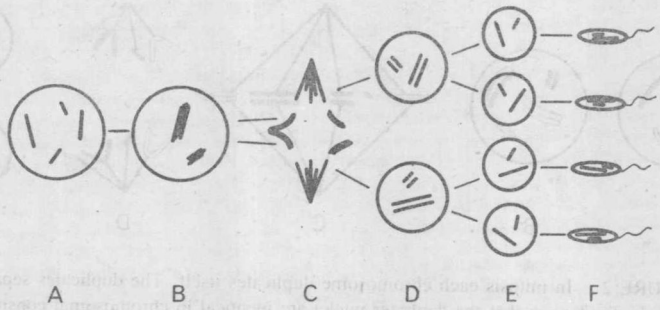

FIGURE 3. In the formation of sperm, duplicated members of each pair of chromosomes come to lie side by side in four-strand configuations (B). Two successive nuclear divisions result in the formation of four sperm (E), each with one member of each pair of chromosomes. The first division (C,D) is mitotic, whereas the second division is reduction (E). (From Sharp, L. W., *Introduction to Cytology*, McGraw-Hill, New York, 1934, 251. With permission.)

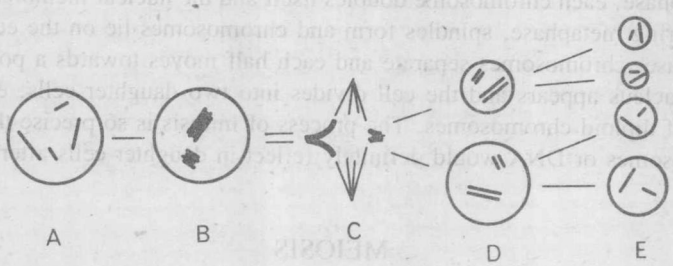

FIGURE 4. Meiosis in a female animal gives rise to only one functional egg from each primary oocyte and three polar bodies (E). Duplicated members of each chromosome lie side by side in four-strand configurations (B). The first division is mitotic (C,D), whereas the second division is reduction (E). (From Sharp, L. W., *Introduction to Cytology*, McGraw-Hill, New York, 1934, 251. With permission.)

generation time, which represents the growth cycle only. The concept of G_0 is described in detail in a recent review.[3] In brief, G_0 cells are those which are not dividing, but are capable of dividing or entering the cell cycle after a proper stimulus. A significant portion of tumor cells in a tumor mass is in the G_0 phase of the cell cycle. These cells are considered very resistant to X-rays and γ-rays.

There is a growing bulk of evidence that cell cycle time for a given cell type remains substantially constant. More recent works have shown that the G_1 phase is most sensitive to change. A large variation in the G_1 phase of transplanted tumor cells occurs, whereas the S, G_2, and M phases are fairly constant. It has been shown[8] that a hydrocortisone-induced increase in doubling time of HeLa Chessen cells is primarily due to an extension of the late G_1 phase of the cycle.

The doubling time refers to the average time taken for the cell number in a population to double. The generation time refers to the average time taken for cells to complete one growth cycle (G_1-S-G_2-M). Doubling time equals generation time if the following criteria are met in a given cell population: (1) growth is strictly exponential and devoid of fluctuation, (2) all cells are viable, and (3) all cells have the same generation time. The generation time is usually shorter than the doubling time.

The most common method of determining the generation time and the period of each phase of the cell cycle is the labeled mitosis method (Figure 6). Cells are pulse labeled with

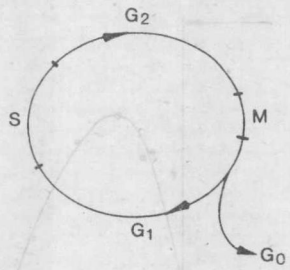

FIGURE 5. Diagrammatic representation of a cell cycle. The end of mitosis (M) marks the beginning of the cycle. A gap (G_1) occurs before the onset of DNA synthesis (S). DNA synthesis is followed by another gap, (G_2) (post-DNA synthesis phase).

^{3}H-thymidine, and radioautographs are prepared as a function of time after ^{3}H-thymidine injection. The labeled mitoses are plotted as a function of time after treatment with ^{3}H-thymidine. From this cyclic curve generation time (T), G_2, and S are estimated as indicated in Figure 6. For an exponential distribution of cells, the mitosis period (M) is calculated as follows: $M = (T/0.693) \times$ mitotic index. After obtaining these values, one can determine $G_1 = T - (G_2 + S + M)$. Table 1 compares the generation time and the period of each phase of the cycle in various cell types.

NUCLEIC ACID AND PROTEIN SYNTHESIS

In order to understand the mechanisms of radiation damage, a basic knowledge of nucleic acid and protein synthesis is necessary.[22] Therefore, the mechanisms of biosynthesis of DNA, RNA, and protein are presented here briefly. A diagrammatic representation of the molecular events in the cell is given below:

$$\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein}$$

DNA Synthesis

DNA is a long chain of nucleotides and therefore is referred to as a polynucleotide. Each nucleotide is composed of three compounds linked together: (1) phosphoric acid; (2) a sugar, in the form of deoxyribose; and (3) a base (Figure 7). Bases are divided into two classes, purine and pyrimidine. In DNA, purines are adenine (A) and guanine (G) and pyrimidines are cytosine (C) and thymine (T). Thymine is very specific for DNA structure; therefore, ^{3}H-thymidine has been used extensively in the study of DNA synthesis. In 1953, Watson and Crick proposed a double-helix structure for DNA in which two bases are joined together by hydrogen bonds.[24] A diagrammatic structure of DNA is shown in Figure 8. It should be noted that adenine pairs with thymine, whereas cytosine pairs with guanine. Figure 9 shows a schematic representation of DNA replication. During replication of each strand, the newly formed strand attaches with the proper base of the old one to form a double-stranded DNA. A new DNA strand was synthesized in vitro by using a specific enzyme polynucleotide pyrophosphorylase, precursor of DNA, and DNA from other sources. The newly formed DNA was identical to DNA which was added to the reaction mixture. This showed conclusively that DNA acts as a template for the synthesis of another strand of DNA.

Several enzymes are required for DNA synthesis. The enzymes thymidine kinase and DNA polymerase have been studied in relation to radiation damage in some detail. DNA is degraded by a specific enzyme referred to as DNase (deoxyribonuclease).

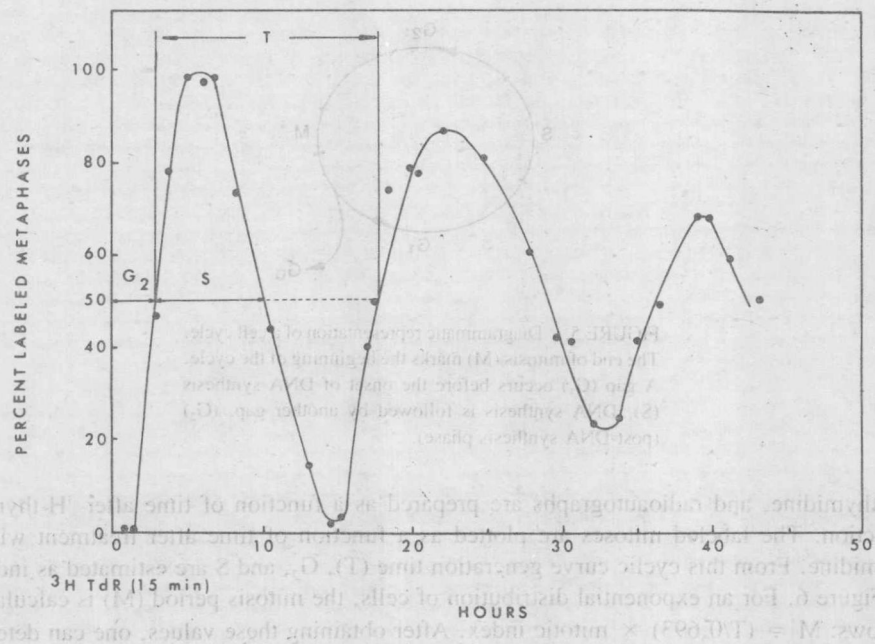

FIGURE 6. The percent of labeled metaphase as a function of time in a mouse L cell culture exposed to ^{3}H-thymidine for 15 min at time zero. T is the average generation time. (From Till, J. E., Whitmore, G., and Gulyas, S., *Biochem. Biophys. Acta*, 72, 277, 1963. With permission.)

Table 1
CELL CYCLE PARAMETERS

Cell Type	G1	S	G2	M	T
Mouse cells	9.5	7	3	0.5	20
In culture	8.2	6.2	4.6	0.6	19.6
HeLa	3	7	1.5	0.5	12
Mouse hair follicle	3	7	1.5	0.5	12
Ehrlich ascites tumor	3	8.5	1.5	1	18

Data were summarized from Elkind, et al. 1967.

The amount of DNA per nucleus within a given species is fairly constant; however, it varies markedly from one species to another.

RNA and Protein Synthesis

Like DNA, RNA is also a polynucleotide chain and consists of four bases, sugar, and phosphoric acid. RNA differs from DNA in the following respects: (1) it has sugar in the form of ribose, rather than deoxyribose, and (2) it has pyrimidine base uracil in place of thymine. The enzyme RNA polymerase is required for RNA synthesis, and the enzyme RNase degrades RNA. There are several classes of mammalian RNA, three of which are most important: "messenger" (m) RNA, ribosomal (r) RNA, and "transfer" (t) RNA. All these types of RNA participate in protein biosynthesis.

"Messenger"-RNA (mRNA)

Investigators now believe that all information for protein synthesis is coded in DNA in the form of triplets (any combination of three bases). mRNA is synthesized on a DNA strand