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HANDBOOK of RADIOBIOLOGY

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TABLE OF CONTENTS

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.

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[24] 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

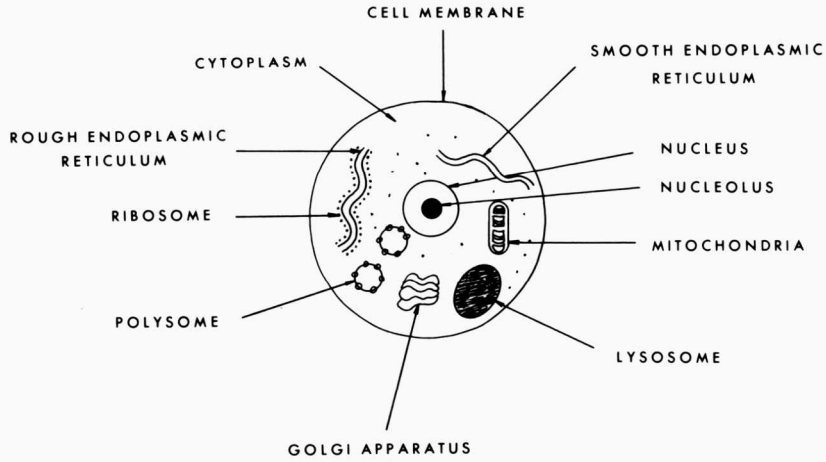

FIGURE 1. Diagrammatic representation of the ultrastructures of a cell. The following structures are seen: nucleus, nucleolus, nuclear membrane, chromatin materials, mitochondria, Golgi apparatus, rough endoplasmic reticulum, ribosomes, polysomes, and lysosome.

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.

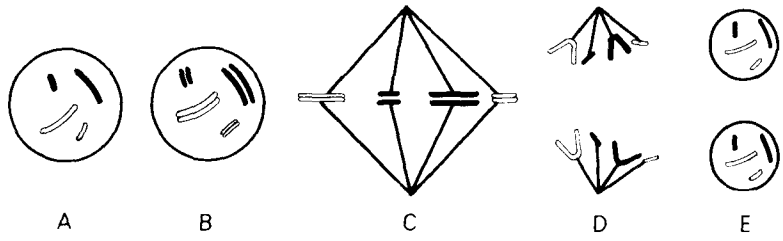

FIGURE 2. In mitosis each chromosome duplicates itself. The duplicates separate as the nucleus divides, so that the daughter nuclei are identical in chromosomal consitution. Prophase: A,D; metaphase: C; anaphase: D; and telophase: E. (From Sharp, L. W., *Fundamentals of Cytology*, McGraw-Hill, New York,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

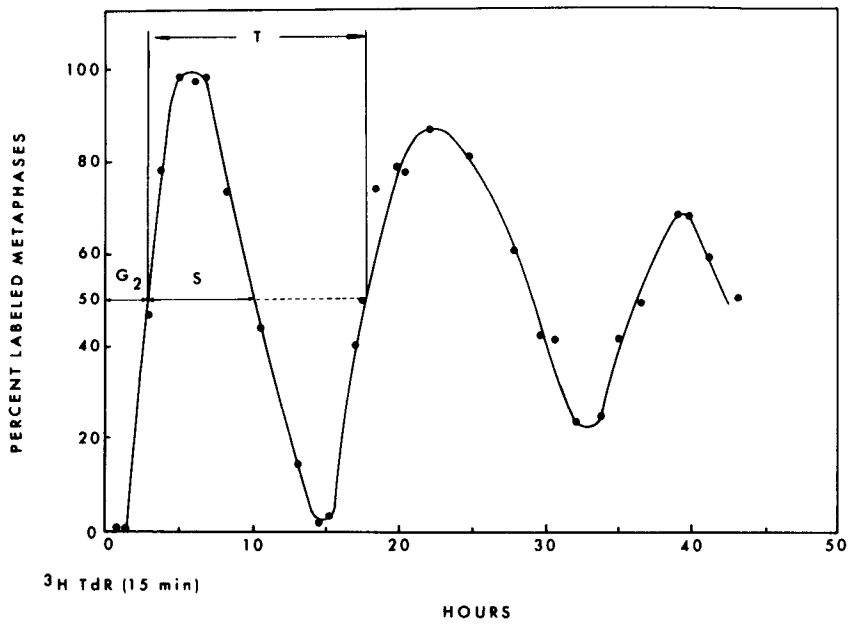

FIGURE 6. The percent of labeled metaphase as a function of time in a mouse L cell culture exposed to ^{3}H-thymidine for 15 min at time zero. T is the average generation time. (From Till, J. E., Whitmore, G., and Gulyas, S., *Biochem. Biophys. Acta*, 72, 277, 1963. With permission.)

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

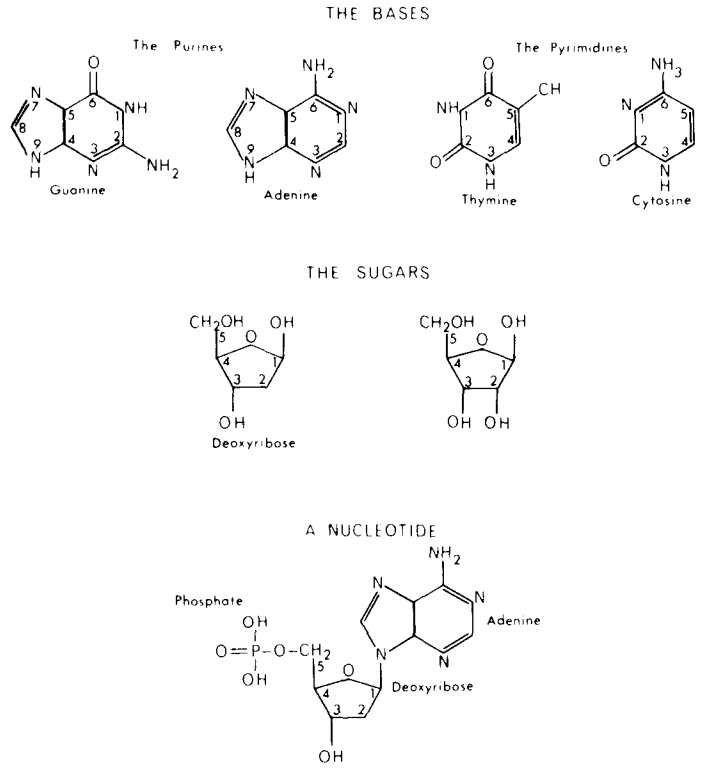

FIGURE 7. The chemical composition of nucleic acid. The bases are linked to a sugar and a phosphate to form a nucleotide. Nucleotides are linked together to form a nucleic acid or polynucleotide chain.

and can form a DNA/RNA hybrid in vitro. This species of RNA is called a "messenger"-RNA because of its intermediary role in relaying the genetic code for protein synthesis to the functional site in the cytoplasm. mRNA brings the genetic code from DNA in the form of a triplet. For example, UUU dictates the incorporation of phenylalanine and CCC of proline and no other amino acid.

tRNA and rRNA

Transfer RNA picks up an activated amino acid in the cytoplasm. Each tRNA is specific for an amino acid, and tRNA carrying an amino acid attaches to a ribosome (30S) which moves along the mRNA strand and picks up a 50S ribosome along the way; the tRNA-ribosome complex recognizes a particular code on the mRNA strand and transfers the amino acid to the growing polypeptide chain. tRNA and the ribosome come to lie in the cytoplasm and again repeat the sequence. Ribosomes can exist in the cytopasm as a free form (70S) or in the form of subunits (50S and 30S).

Stability of mRNA

The concept of the stability of mRNA has changed markedly since it was originally proposed from studies in bacteria where the turnover of mRNA is very rapid, the half-life being 3 to 4 min. However, in mammalian cells, the half-life of mRNA is relatively long and often varies from one species to another. The half-life of mRNA in the mammalian liver is in the range 4 to 40 hr.[1] In the amphibian liver *(Amphiuma tridactylum)*, protein synthesis continues to occur at a normal rate for 96 hr after the production of new RNA is blocked by actinomycin D.[16]