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ENDPOINTS AND TARGETS IN RADIOBIOLOGY

by

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What to Study

From a programmatic viewpoint, broad categories of end effects of principal concern can be identified as: cell killing, mutagenesis, carcinogenesis, and teratogenesis. Cell killing is of basic importance in connection with the treatment of cancer with radiation. Radiation mutagenesis and carcinogenesis involve the production of heritable changes as a result of changes in the genome of a cell or changes in the expression of the genome, and ^{and} radiation teratogenesis concerns the production of developmental anomalies. The latter three end effects are of concern in the diagnostic uses of radiation in medicine, and the possible consequences of the large-scale use of atomic energy on populations of people.

A factor common to mutagenesis, carcinogenesis, and teratogenesis is that in each case, cell survival is usually required for the development and/or expression of a radiation induced change. At the least, this is because to express a heritable or developmental change, a viable cell(s) is required. But it may also be true that the molecular alterations involved in mutagenesis, carcinogenesis, or teratogenesis have significant elements in common with those which lead to cell death. Thus, while the relevance of studies of cell killing to radiotherapy is immediately evident, such studies are basic to the other end effects as well.

Cell killing is also worth considerable attention for a second reason. To perform the cyclic changes involved in repeated cell growth and division, many chemical subsystems and organelles must function in an integrated and coordinated way. Cell survival might appear to be a narrowly defined endpoint since it involves essentially only "yes" or "no"

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answers. In fact, this endpoint is quite broad because it reflects the functioning of a number of biologically inter-related systems.

A third reason for considering cell killing, when concerned with mechanisms in radiation biology, derives from technical factors. The long standing techniques of microbial genetics, plus those of more recent date for cultured mammalian cells, permit the rapid, quantitative assessment of cell survival and, by difference, of cell killing. Insofar as concerns proliferating cells, a surviving cell is one which can give rise to a large number of offspring. Under appropriate circumstances, this permits the formation of a colony of cells to be taken as an indication that initially at least one viable or surviving cell existed. This, plus the ability to assess survival on a logarithmic scale through the use of dilution procedures, permits accurate dose-effect relationships to be constructed with facility. A degree of resolution results not available in many other biological endpoints.

Mammalian versus Microbial Cells

Past work has made clear that microbes -- particularly bacteria and their viruses -- have afforded very considerable insights into the molecular biology and genetics of radiation action. No doubt, this will continue to be true in the future. But because mammalian cells differ in essential ways from bacteria, and because current-day techniques permit quantitative studies with cultured mammalian cells, and at moderate expense, it is highly desirable to encourage their exploitation. While organ and tissue interactions are at best difficult to reproduce

in the dish, in other respects mammalian cells in culture are significantly closer to man than are bacteria.

Where and What are the Targets?

In respect to cell killing, as explained in Chapter I, in eukaryotes there are good reasons to believe that the nucleus plays an essential role. These reasons rest on the observation that dose-for-dose, energy deposited in the nucleus has an appreciably greater effect than in the cytoplasm. (Mitotic cells, a special case, need not be considered ^{here} since they usually constitute a small percentage of a population.) In spite of this, it is instructive to consider the radio-energetics of damage registration.

For every picogram (1×10^{-12} gms) of matter in a mammalian cell -- which, ordinarily, consists of some thousands of picograms -- on average, one bond-breaking event (i.e., one ~60 ev event) will be registered per rad. A "mean lethal dose" -- that is, about 100 rads -- deposits: about 1000 bond-breaking events in the DNA; about 3-5000 in the RNA; and about 20,000 in the protein. About 100,000 events are scored in the water in a cell per 100 rads. Clearly, this lack of specificity implies that after doses sufficient to kill modest proportions of cells, not only is the number of molecules inactivated important, but also their function and the ability a cell might have to repair or bypass damage in them. The lack of specificity in the absorption process, therefore, must be understood to be complemented by a special kind of specificity in target properties. With ionizing radiation, we measure the folding together of three sorts of things: 1) the physical size of molecules; 2) their importance relative to function

(e.g., cell division); and 3) the ability cells have to repair or bypass damage deposited in them.

DNA-Related Targets

In respect to mammalian cells, evidence cited in Chapter I supports the idea that nuclear DNA, possibly in relation to the nuclear envelope, is a principal target in cell killing (see refs. 7 and 8, Chapter I). In terms of molecular size, nuclear DNA molecules could conceivably be as large as 1×10^{11} daltons. They have very considerable biological amplification in cells. The antibiotic actinomycin D, which binds selectively to duplex DNA, sensitizes cells by reducing their capacity for sublethal damage and interfering with its repair (see refs. 8 & 9, Chapter I). These observations led to the schematic representation of a cell following irradiation shown in the left half of Fig. 1. (1).

In analogy with energy level diagrams of atoms and molecules, we imagine a cell relative to a scale of damage increasing in a downward direction. An acute dose of radiation shifts a cell downward. For example, if we take 250 rads to be a 50% survival dose, immediately after exposure the DNA of all the cells exposed contains about 2500 single-strand breaks (2), and about 50-125 double-strand breaks (see Friefelder, these Proceedings). Therefore, whether or not a cell survives probably depends upon whether or not it can raise itself from the "lethal domain", into which it was rendered, to the "sublethal domain", and it must do this before the damage becomes fixed. If a cell reaches the sublethal domain soon enough, in time thereafter it will

probably fully repair its damage and return to the ground state (3). Actinomycin D can be imagined to act by: raising upward the demarcation between lethal and sublethal damage, thus decreasing a cell's capacity for sublethal damage; slowing the rate of repair; or both.

Also shown in Fig. 1 is a schematic representation of the results of studies of the sedimentation of DNA from irradiated mammalian cells. Since the introduction of the alkaline cell lysis innovation for bacteria (4), many investigators have extended and adapted the method to mammalian cells. The main advantage of the approach is that cells are lysed on top of the solution through which they will be sedimented. This eliminates the need for further manipulation of the DNA from these cells and permits thereby, large molecules of DNA to be examined. Prior to the use of this approach, chemical extraction invariably resulted in damage to the DNA and hence, radiation damage had to be examined on top of a high, often unreproducible, background level.

Although DNA release on top of a gradient minimizes mechanical damage, the high alkalinity leads to the separation of complementary strands. Hence, velocity sedimentation under alkaline conditions is generally considered as a means of examining damage in single DNA strands. In spite of this, and the relatively low ratio of information-to-effort, considerable work has been performed using this method because of the severe limitations of other techniques (for review, see 5).

From studies in which the lipid, or membraneous, material of a cell is radioactively labelled as well as its DNA, it has been shown that DNA and lipid cosediment(6).

Therefore, the "ground state" of DNA in a cell is visualized as being in association with membrane. Radiation registers lesions in both materials, and if cells are incubated after radiation, the lesions disappear. With respect to single-strand breaks, complete and rapid repair occurs (within the resolution of the method) even after supralethal doses (e.g., 2-5000 rads) -- doses which kill essentially all the cells. In view of this, one must consider the possibility that such changes represent "misrepair" and consequently lesion fixation. Incubation after irradiation resulting in a return of sedimentation properties to those of unirradiated cells by no means assures that the DNA has returned to its unperturbed state since other forms of damage may still exist (e.g., small deletions and/or damaged bases). Still, the fact that repair appears to be complete raises an interesting radio-chemical question.

Enzymatic single-strand repair is generally attributed to the action of a polynucleotide ligase. This enzyme has been shown to have the substrate requirements of 5'-phosphate and an adjacent 3'-hydroxyl group. If absorption events are registered at random in the DNA, how are these substrate requirements met? There are several possibilities but not to be overlooked is the possibility that energy transfer mechanisms cause random absorptions in DNA to result in discrete damage, damage which directly satisfies, or is converted to, the substrate requirements for enzymatic repair by polynucleotide ligase.

Damage to DNA-containing material released from mammalian cells has also been detected after small doses, doses in the 5-80% survival range (2, 6-8). This damage results in the

disassociation of DNA and lipid, and cells effectively repair such damage. As a result, the lipid and DNA become reassociated (6). Actinomycin D interferes with this repair process but not with the repair of radiation induced single-strand DNA breaks (9). While these observations support a relationship between damage to the DNA-lipid complex and cell killing, more evidence is needed before a cause-effect association can be identified.

To understand the relevance of the DNA-lipid complex, in the context of radiation effects, probably it will be necessary to know what it represents in the cell. This points up the relationship between: programmatic problems; the research to which they lead; and the intimate connection between the latter and basic, general questions in biology. To advance our understanding of radiation-induced abnormal states in cells, we must confront the gaps in our knowledge of the normal biology of cells.

Radiobiology and biology are locked together -- both must advance if programmatic questions are to be answered.

Perspective

As one would expect, the areas of inquiry being pursued in radiation research reflect the backgrounds and interests of the individuals involved. As typified by these Proceedings, growing points which concern primarily physics, chemistry, and biology are easy to identify. Frequently, they seem to be connected only by an "electromagnetic thread". As ephemeral as such may be, I view it as real. Progress in understanding radiation effects perforce must be concerned with the physical, the chemical, and the biological. While the insights developed thus far may not appear to be connected, this should

change in time provided that communication between these disciplines is maintained and improved. Enlarging areas of knowledge, from the beachheads already established, in time will make contact. When they do, we can expect more rapid advances in our overall understanding of radiation effects.

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Fig. 1. Schematic representations of the effect of radiation on cells (left panel), and on a DNA-membrane complex in the nucleus of cells (right panel). Also sketched are the effects of repair processes which set in after exposure.