Basic Molecular and Cell Biology

Effects of radiations on cells

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Evidence of the biological effects of ionising radiations on cells has been with us throughout this century and for much longer for ultraviolet light. Yet, for obvious reasons, there has never been more interest in the damaging effects of radiations on living cells than there is today. Study of the actions of radiations is not only helping to bring about a better understanding of cell biology but is of considerable practical importance for developments in radiotherapy and in protection against accidental exposure. Radiations may be divided into two main types: ionising and non-ionising. These reflect the differences in their physical interactions after absorption in matter. Ionising radiations, which include *x* rays, cause ionisation of atoms by ejection of electrons; hence their name. This article is concerned mainly with these radiations. Non-ionising radiations, of which most wavelengths of ultraviolet light from the sun are examples, are insufficiently energetic to eject electrons but can raise electrons to a higher than normal energy level in atoms.

Relation between dose and response

The most obvious biological effects of radiations on cells are killing, induction of mutations, and conversion to a precancerous state. The causation of cancer by an agent which is well known as a means of treating cancer may at first sight seem paradoxical, but it aptly illustrates the dependence of effect on the dose of radiation applied. It is likely that only a relatively small dose is required to induce a cell to become cancerous, but for it to initiate a tumour it must be capable of continual division over many generations of cells. The high doses used in radiotherapy are designed to prevent that division by killing malignant cells. To the radiation biologist killing usually means that the cell suffers "loss of reproductive capacity" and is incapable of supporting more than a few divisions. The "dead" cell, however, may still be able to sustain virus replication, for example, or synthesise particular proteins; it may therefore retain some biological functions. Of course, if the dose of radiation is high enough all activity is extinguished, and the cell is stopped virtually in its tracks. In other words, an issue of major concern is the relation between the dose of radiation received and the response of the cell, or more usually a population of cells, to that exposure. This aspect is not always fully appreciated by the lay public, who often think only in terms of an all or none effect.

Much effort has been devoted to the measurement of the dose-response relations for the biological effects mentioned. The induction of so called point mutations—that is, changes in the molecular structure of the deoxyribonucleic acid (DNA) genetic material—appears to occur at a rate that is directly proportional to the dose. This means that even the smallest dose of radiation has a finite probability of causing such a mutation. In germ cells, however, the expression of that mutation in offspring depends on a range of factors in addition to the molecular lesion initiated by the original damaging event. The rate of induction of carcinogenic change in cells exposed to low doses of radiation has until now been almost impossible to measure, but recently developed methods for examining neoplastic transformation in rodent cells in vitro suggest that very small doses may be sufficient to cause malignant change. Furthermore, it is beginning to emerge that there may not be a dose of radiation below which carcinogenic change cannot occur in cells. On the other hand, small doses appear to be relatively less effective than large ones in killing cells. Much debate has been centred on why this should be. It may be that accumulation of damage is required before substantial killing is induced. Alternatively, as now seems more likely, it may be a manifestation of the capacity of cells to repair, within limits, some of the radiation lesions that they suffer. Whatever the explanation, it means that non-lethal damage caused by small doses that may lead to mutation and induction of cancer, for example, may be readily propagated in sublethally damaged cells. Even for killing, the dose of ionising radiation in terms of energy required is remarkably small. As Dr L H Gray, an eminent British radiation biologist, pointed out, the *x* ray energy needed to kill a mammalian cell is roughly equivalent to the heat energy in a cup of tea. The hot brew is not harmful because its energy is not deposited in the same way as ionising radiation, which is deposited in discrete quanta, or packets, of energy capable of disrupting chemical bonds in molecules.

How do ionising radiations cause their effects?

After the deposition of energy, how do ionising radiations cause their biological effects in cells? The answer to that question is not clear. Characteristically, the ionisation events are not only discrete but are distributed randomly so that reactions occur in molecules roughly in proportion to their concentration in the cell. The art in answering the question lies in being able to recognise relevant damage in essential molecules, defined as sensitive or critical radiation targets, in a background of "noise" comprising less important or even unimportant biochemical lesions. Professor J A V Butler, a distinguished physical chemist, once suggested that the problem faced by the radiation biologist, intent on unravelling the nature of critical damage in cells, was analogous to that of deciphering why telephones did not work after lobbing bricks through the windows of a telephone exchange. He might have added that the exercise is made even more difficult if knowledge of the way in which the exchange works normally is meagre in the first place. We are facing a tall order, and perhaps we may never understand fully how ionising radiations kill cells.

We need not be too gloomy, however, because much pertinent information has been unearthed, especially in recent years. Firstly, there is no doubt that the critical target(s) are located in the nucleus and not in the cytoplasm of the cell. Again, the most important target in the nucleus is DNA. This must be accepted not only from the unique and central role of DNA or chromatin in the cell but also

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from the demonstration, before our eyes under the microscope, of chromosomal alterations. Of course, there are molecules other than DNA present in chromosomes, mainly basic proteins, so that we must not forget that potentially there are additional target molecules which, if damaged, may contribute to the final biological response. By means of low energy electron beams of limited but well defined penetration, an area of particularly high sensitivity has been identified immediately within the nuclear membrane. As there is now clear evidence of attachment of chromatin to regions of the nuclear membrane, it may be that sites at which DNA and membrane are complexed are peculiarly vulnerable.

Free radicals

Simple chemical experiments in the test tube suggest that target molecules in the cell are damaged by two routes. One is by direct absorption of radiation energy in the molecule, so that after ejection of an electron the target, which can be represented by the symbol RH, is converted into a free radical. The radical is a chemical species containing an unpaired electron that renders it highly reactive. The ionised target can be shown as $R^{\bullet}$; the dot delineates the unpaired electron. The second, but indirect route to the same $R^{\bullet}$, is by reaction of the target with free radicals formed by the action of radiation on molecules that cannot be defined as important targets themselves. Because of its preponderance in cells, water is the main source of these radicals, and one that appears to be particularly potent is the hydroxyl radical ($OH^{\bullet}$). From time to time the contribution of each of these routes to the production of $R^{\bullet}$ is hotly debated, but the view of the majority appears to be that after exposure to *x* rays about three quarters of the initial damage to target(s) arises from indirect interactions with water radicals.

The subsequent chemical and biochemical history of the $R^{\bullet}$ species determines the biological fate of the irradiated cell. Within a few milliseconds of its formation $R^{\bullet}$ will react with other available chemical species, which will compete either to fix permanently the damage that has been initiated or to repair the lesion and restore it to its original configuration. For example, if oxygen is present it is capable of rapid reaction with $R^{\bullet}$ through the unpaired electrons that it possesses normally, and the result is the formation of a peroxidised derivative of the target. The damage is now unmodifiably fixed. This mechanism suggests one way in which the well known radiosensitising effect of oxygen can be explained. The other side of the picture is that if hydrogen atoms ($H^{\bullet}$) are available they can also react with $R^{\bullet}$ and restore it to its original structure.

Chemical compounds that are good donors of hydrogen atoms may be given to cells so that, to some but limited extent, they can protect them against the effects of radiation by helping to restore $R^{\bullet}$ to RH. Within the cell itself the tripeptide glutathione, a normal constituent, can also fulfil the role of hydrogen donor. The extent of protection, however, depends on the nature of the chemical environment near the target. For instance, if even small amounts of oxygen are present the competition for $R^{\bullet}$ is strongly in favour of damage fixation and sensitisation because of the high affinity of oxygen for unpaired electron sites. Attempts to make tumour cells more radiosensitive or conversely to render normal tissue cells less sensitive, and so enhance the effectiveness of radiotherapy, often revolve round devising ways of modifying the initial chemical reactions of ionised targets.

Nature of damage caused by radiations

Having identified DNA as a major target, what is the nature of the damage caused in it? Also, of the chemical lesions that are known to occur can any of these be implicated in the biological damage that is expressed? With the development of new and more sensitive techniques some lesions can now be detected after exposure to doses of radiation that are almost as small as those required to kill cells. These include a range of changes induced in the bases of the nucleic acid, breakage in the continuity of the strands in the double helix, and abnormal cross links formed in the DNA or between it and cellular proteins. It is proving difficult to link these changes conclusively with events leading to killing, but a major lesion is the so called double strand break, in which the structure of the DNA is interrupted at about the same position in both strands. There is the suspicion, however, that ease of detection may result in concentration on damage that may be less critical than that which is more difficult to recognise and measure. Albeit, with the advances in molecular biology, the molecular basis of mutation induction and possibly also of radiation induced carcinogenesis is rapidly becoming better understood. It is now possible to insert pieces of "custom built" DNA, containing known molecular changes caused by irradiation, into the genome of cells and then examine the host for the expression of mutations. In this way the link between chemical and biological events may be elucidated.

Repair enzymes

Even if the damage becomes fixed in DNA, all is not lost as far as the cell is concerned. Enzymes capable of eliminating lesions and restoring the integrity of the DNA can be brought into action by the cell. The discovery of repair enzymes almost 25 years ago was a jewel in the crown of radiobiological research and is perhaps the field's greatest contribution to progress in general cell biology. These enzymes, with a range of activities, can locate and eliminate lesions, trim and reconstitute the structure of the DNA, and enable cells to resist radiation and other types of cell injury. Of course, if excessive exposure occurs these defences will be overcome, but within the limits of their effectiveness repair enzymes fulfil an essential role in cells. This point is made particularly well by the fact that certain genetically determined diseases appear to be associated with patients whose cells are in some way defective in repair enzyme functions. We are probably still only at the beginning of appreciating the complexities of enzymatically controlled repair in cells. Many fascinating problems await solution; none more than the realisation that repair enzymes exist, for which no obvious lesions as substrates have yet been discovered.

Further reading

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Should elderly people entering nursing or residential homes be routinely immunised against tetanus?

Spores of *Clostridium tetani* may be found in human faeces and in areas heavily populated by domestic animals. They have also been found in the atmosphere, in clothing, in homes and hospitals, and in surgical dressings. Spores entering damaged mucosal membranes or skin may germinate but the bacteria grow only when the environment is strictly anaerobic. Bacterial lysis in the affected wound releases tetanus toxin.

In 1984 there were only six notified cases[1] affecting all ages (of which three were fatal[2]). Housebound elderly people at risk from tetanus include those with chronic leg ulcers and incipient gangrene of the limbs who are also faecally incontinent. At present the risk of contracting tetanus seems to be small and not sufficient to justify mass immunisation programmes for the housebound elderly and aged, particularly since tetanus toxoid carries its own small risk of hypersensitivity reactions.[3]—BRIAN LIVESLEY, professor elect, The Care of the Elderly (Geriatrics), London.

1 Office of Population Censuses and Surveys. *Registrar general's communicable disease statistics, 1984. England and Wales*. London: HMSO, 1986:6. (Series MB2, No 11.)
2 Office of Population Censuses and Surveys. *Registrar general's mortality statistics, 1984. England and Wales*. London: HMSO, 1985:2-3. (Series DH2, No 11.)
3 Associated British Pharmaceutical Industry. *Data sheet compendium 1986-87*. London: Datapharm Publications, 1986:1663.