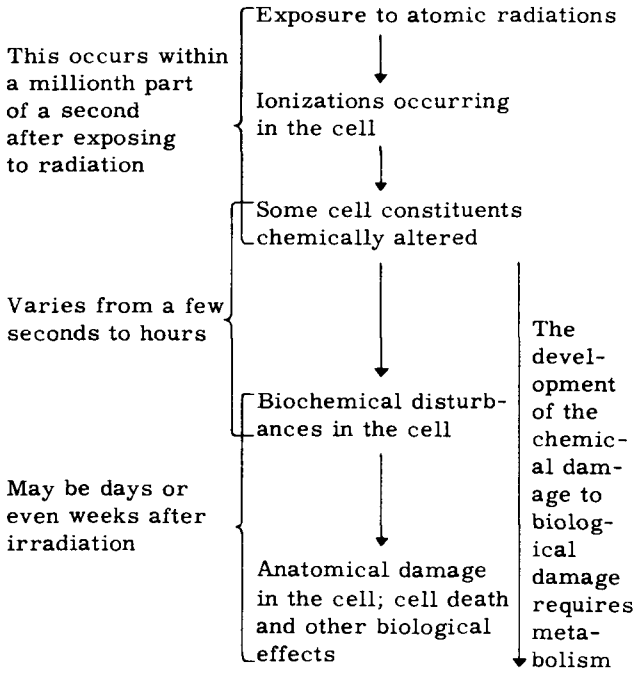
RADIOSENSITIVITY OF CELLS

by Peter Alexander

(Dr. Peter Alexander, who is Head of the Radiation Biology Section of the Chester Beatty Research Institute of the Royal Cancer Hospital, London, is conducting an investigation into the reasons for variations in the radiosensitivity of different micro-organisms under a research contract awarded by IAEA)

Atomic radiations are a very diverse group of physical agents all of which, however, produce damage in living cells. The common property which links them is the ability to knock out electrons from the atoms and molecules through which they pass. This process, known as ionization, is the starting point of radiation injury. Mammalian cells are amongst the most sensitive of all living matter, and this is the reason why such stringent safety precautions are necessary in dealing with radioactive materials or with machines that produce atomic radiations like X-rays. Yet, paradoxically, it is the high radioresistance of some micro-organisms that has so far prevented sterilization by irradiation from being a useful method for general food preservation. If we knew the mechanism by which radiation kills cells it might be possible to devise means to alter the radiosensitivity of cells and thereby make possible new applications of atomic energy to serve man.

To kill a human cell growing freely in tissue culture with a dose of X-rays the amount of energy that would have to be expended would be sufficient only to raise the temperature of the cell by $1/2000^{o}C$. How such a minute dose of radiation (in radiation units, 200 rads) is able to kill cells is not known, but we do know that all the energy is not converted into heat and that a part of it (something of the order of 25 per cent) is used to bring about chemical reactions within the cell. Most of these reactions are trivial but a few affect some key points in the cell and initiate the long series of events shown in the chart below that lead to cell death.



The photograph in Fig. 1 shows one way in which irradiated cells can die. The photograph is of cells in tissue culture, but a similar situation exists in some of the particularly radiosensitive organs such as the bone marrow where the rate of cell division is high. After irradiation, the volume of the cell continues to increase, i.e. the cell constituents continue to be made, but the cell fails to divide

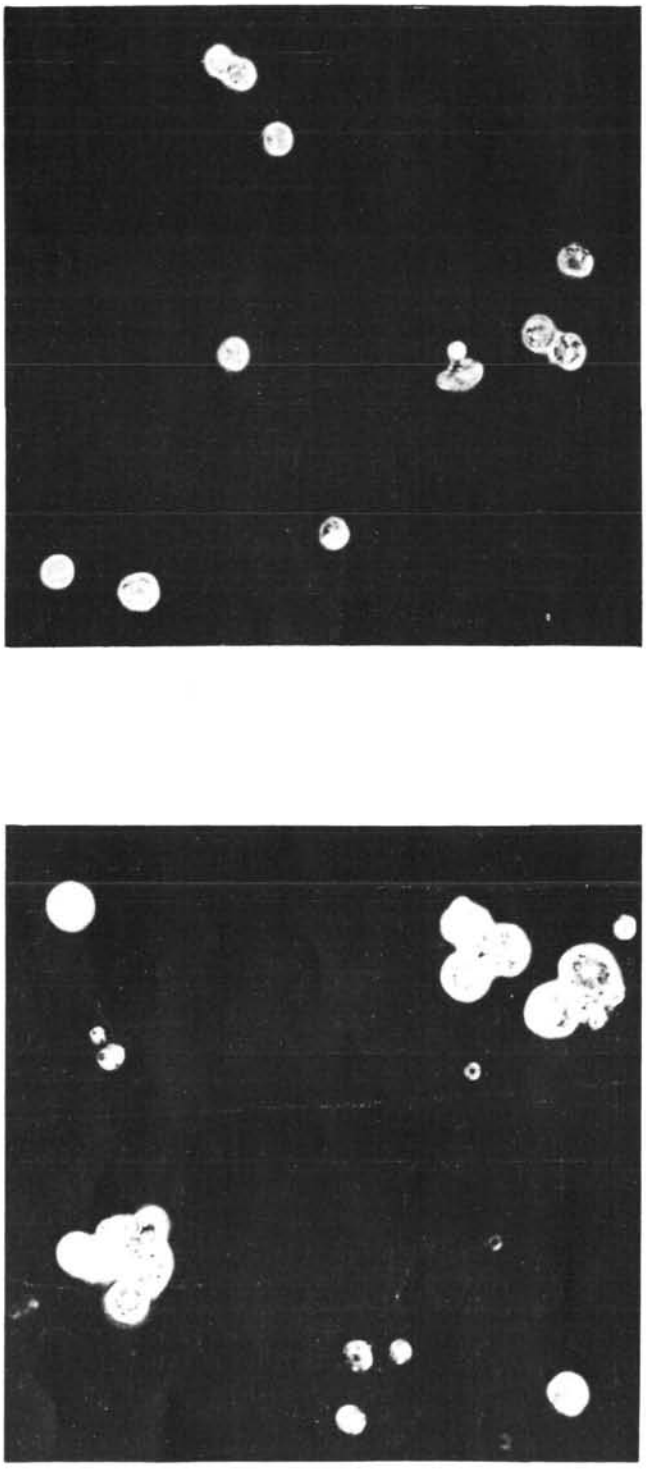


Fig. 1. Mouse leukaemia cells grown in tissue culture. Picture on top shows the normal unirradiated state, while the one below shows the condition two days after receiving a radiation dose of 300 r of X-rays

and becomes larger and larger. These so-called giant cells are fragile and do not survive for long.

This is only one of several biological end effects by which radiation kills cells and there are other forms of damage which do not involve the formation of giant cells, but which still results in the death of the cell. The same principle underlies the development of all these cell injuries. A very few chemical reactions initiated by the minute amount of energy needed to kill a cell start a chain of reaction that leads to death.

Doses for Different Organisms

Under conditions where the cells grow rapidly such as in tissue culture, most mammalian cells have similar radiosensitivities. With micro-organisms this is not the case, and the dose of X-and gamma rays* needed to kill (or sterilize by preventing cell division) varies from some 1900 rads for the bacillus pseudomonas fluorescens to 40 000 rads for certain micrococci. The dose for some spores is even higher. This is the average dose; to ensure an acceptable degree of sterility, the amount of radiation that has to be given will be some 10 - 100 times greater. Doses of the order of a million rads, however, produce unfavourable side effects in the irradiated food; not only do they frequently spoil the flavour, but there is the real risk that some of the radiation induced chemical changes may result in the formation within the food of substances that constitute a possible health hazard. The type of harmful effect which is most to be feared - and most difficult to detect - is not one that reveals itself in immediate toxicity, but one that gives rise to delayed illness that may only be seen many years after eating irradiated food. For these and other reasons it is not practical to irradiate food with the doses needed to kill a high proportion of radioresistant organisms that might be present. The object of our investigation which is supported by IAEA is to try and find the reasons for the variation in sensitivity between different cells.

In principle, the differences in behaviour between sensitive and resistant bacteria could be due to one of two factors. Either the resistant organisms have some built-in protective mechanism which reduces the amount of chemical damage done by the irradiation, or the processes which occur between the initial chemical damage and the final biological end effect (i. e. killing) may be different. Many years of research in radiobiology have taught us a great deal about the end effects of radiation and also about the intermediate stages. But we have no

* *These figures refer to the so-called LD 63 dose, i.e. amount of radiation sufficient to kill 63 per cent of the organism. This value is chosen since statistically the average dose needed to kill one cell is equal to the LD 63 when the percentage in activation versus dose relationship is exponential as is frequently the case with micro-organisms. For food sterilization, essentially all the organisms must be wiped out and so the dose that has to be given is correspondingly much greater. If there is an exponential killing response then the dose of radiation to reduce the number of organisms to one-millionth is sixteen times the LD 63.*

knowledge about the nature of the initial chemical reaction produced immediately after exposure to radiation which is responsible for starting off the whole chain of events. An analogy with photography is quite appropriate here. When we expose the plate for a fraction of a second, we know there is a latent image in the emulsion, but we cannot detect this until the changes produced by the light on the plate are multiplied many hundreds of times by the developer. The amount of change on the plate before developing is too small to be detected by analytical means. The same situation applies to radiation injury.

We know from experiments with pure chemicals that radiation is capable of changing most cell constituents in such a way that they become biologically useless. But the amount of radiation needed to kill a cell is so small that the total fraction of any one type of cell constituent affected by the radiation is minute. For this reason most of the chemical reactions that occur are wasted since the cell can survive the loss. At the moment there are no clear-cut experiments to indicate which of the many reactions that occur is biologically important.

Tremendous Complexity

One possibility which we have envisaged is that radiation induced chemical changes result in damage to barriers within the cell. In this way substances that are normally confined by intracellular structures to certain parts of the cell may be released and diffuse to points within the cell where they can do damage. Perhaps the most characteristic feature of a living cell is its tremendous complexity. The myriad of processes which occur within the cell all require their own compartments. There are many incompatible components and for effective functioning of the cell it is essential that these are kept apart by a complex network of internal membranes which have been revealed by the electron microscope (see photograph). For example, the cells of the potato contain the basic building material for a brown pigment known as melanin as well as the catalyst or enzyme that links these molecules together to produce melanin. However, the brown pigment is not normally seen because the two cell constituents needed for its formation are kept apart. When the potato is cut with a knife some of the internal barriers are broken, the two constituents get together and the potato discolours due to the formation of melanin. Differences in radiosensitivity could, if this hypothesis proved to be true, be due to protection of cell barriers against damage from ionization or to the presence of different quantities of the harmful substance or substances that are released.

Damage to cellular fine structures is by no means the only mechanism that has been considered for cell death. The suggestion has frequently been made that it is the damage to the essential cell constituents, the deoxyribonucleic acids - referred to as DNA - which initiates the chain of events leading to cell death. Molecules of DNA are extremely

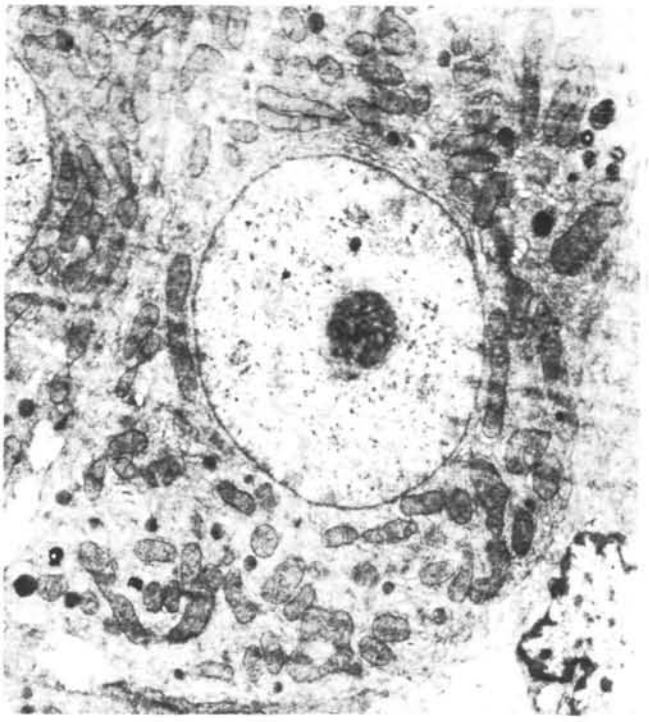


Fig. 2. Electron-micrograph of a cross section through a liver cell to illustrate the complex network of membranes that exist within the cell

large and their function is to convey the genetic code which tells the cell what to do and make. An ionization occurring in a DNA molecule may destroy a "blue print" for an essential constituent which will then no longer be made. It may be that the DNA in radioresistant cells is more difficult to damage or that the more resistant cell is capable of withstanding the loss of DNA better. These possibilities are all open to test.

Ways of Increasing the Response

Once we know the reason for the differences in radiosensitivity of different micro-organisms we can begin to look rationally for ways of enhancing the radiation response of the more sensitive organisms. An investigation of this type has implications far beyond food sterilization, as it cannot fail to provide fundamental facts about radiation injury to cells in general. Cancer researchers have looked for many years for means of sensitizing cancer cells to radiation. In general, it can be said that cancer cells are not inherently more radiosensitive than normal tissue cells and it is this factor which limits the use of radiation therapy. Often it is not possible to give a dose of radiation sufficient to ensure complete sterilization (i.e. killing of all tumour cells) because the radiation would produce too much damage to the tissue surrounding the cancer. If one knew more

about sensitizing cells to radiation, it might be possible to produce a differential effect between normal and malignant cells and thereby greatly increase the scope of radiotherapy.

Our ignorance about the initial chemical lesion is also the principal reason why so far no post-irradiation treatments have been developed for preventing radiation damage from manifesting itself. Many substances are known which, if taken before irradiation, reduce the harm produced by subsequent exposure. None of these agents are effective if taken immediately after irradiation (even within seconds) and they probably work by reducing the extent of the chemical damage that occurs within a micro-second of exposure. Until we know the nature of the initial lesions, there is no rational way of looking for post-irradiation treatments, and health and safety measures are greatly hampered by the fact that there is no antidote to radiation. The protective agent may be very useful for personnel having to enter heavily contaminated areas, but they are no use to victims of accidents. An antidote can only be designed when the sites and nature of the initial damage have been discovered. A good example is the case of British anti-lewisite, a substance which was developed to treat injury from the war gas lewisite. Once the chemical reactions by which this poison damaged cells was known, an antidote could be designed which reversed the initial chemical reaction and prevented the occurrence of the biological damage. In radiation injury of man, the only thing which we can do after irradiation is to take measures to help the body replace the damaged cells. One of these procedures which holds out some promise is the introduction of bone marrow cells to help restore the severely damaged bone marrow, but as yet cannot be recommended as a general procedure. This technique of grafting new cells is bound to be difficult. How much better it would be to have a true antidote that restored damage within irradiated cells and then prevent them from dying.