The Biology of Cancer: Part 1, Cell Proliferation

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<Opening titles>

<Intertitle>

Dr Austin Crathorn

A general introduction of Cell Replication

<Crathorn to camera>

In this, the second in our series Cancer Research Today, we are going to consider the cell as an entity. That is to say, while not forgetting that cancer is a disease of the whole organism, we are going to consider those aspects of growth and cellular proliferation which can be more easily studied by examining the single cell or populations of cells which have some features in common.

This is of dual interest as we are concerned in cancer research as a whole in both the initiation of the carcinogenic situation as well as the cure of cancer, and agents which are used in therapy such as ionising radiation and cytotoxic drugs are also

known to be carcinogenic. So, I am merely going to set the scene for the subsequent speakers and remind you of a few of the facts which will be discussed by Drs Revell, Roberts and Steel. And we are considering the cell merely as a replicating situation. We are not considering any other function of the cell than its multiplication and we first of all see a film where we have a cell doing just this.

<Crathorn narrates briefly over film, then to camera. Then refers to diagrams and more films, narrates over them, interspersed with talk to camera>

It's growing and it's dividing. This is a eukaryotic cell and it is only with cells which have a separate nuclear structure that we shall be concerned. So, we shall remind ourselves of the cell cycle, <*refers to diagram*> the sequence of events between one division and the next through which a cell goes: this is a cell after mitosis, a period G1 – conventionally called when the cell is preparing for the synthesis of the genetic material, the replication of DNA; and followed by a G2 period during which the cell is getting ready for its next division and it then divides.

Now, this is a cell in cycle; not all cells in the body are in this condition, in fact, a number of them are in a pre-G1 period, some of these will not divide again, some of them on the receipt of the appropriate stimulus will go through at least one such cycle. From our point of view, the region of the cycle which we are most interested is the replicative period for the DNA. And DNA, as you will recall, is a twin helical structure. <*Film*> The main features we want to remember at this stage are the pairs of bases in the centre of that structure, surrounded by the backbone, the phosphate sugar backbones which hold the whole structure together.

However, from the point of view of discussion <*next diagram*> it will be easier to think of DNA first in terms of its essential helical structure – this obviously is the backbone structure of the molecule, but we will formally unwind it to make two parallel lines. And here we show the features which concern us in a lot of this discussion – the bases which give the specificity to the DNA molecule. We've also indicated the replicative fork in a formal sense.

I don't think we want to spend much more time on DNA except to remind ourselves that the other <*next diagram*> feature of DNA is also important. Here is the replication demonstrated, but we also, since it carries all the genetic information, we also have the feature of transcription, that is to say the RNA which is formed on the DNA template and which in turn acts as a template for the formation of protein.

These features are, of course, also important in the merely replicating cell so that anything which prevents the exact replication of the DNA may be regarded as potentially dangerous to the continued viability of that cell, plus, I think, we should consider at this stage the effects of agents which attack DNA <*to camera*>. These are the agents which, as I said, are used in therapy, but which are also in themselves carcinogenic. <*Next diagram*> And one way in which we can study such actions is on the survival of the cell, that is to say, the survival of the cell and its ability to grow and divide indefinitely. In this diagram we have plotted the results of an experiment in which HeLa cells, that is the human tumour cell line, were irradiated with varying doses of x-rays. And we have on this axis the percentage or fraction of cells surviving. By 'surviving' we mean able to produce colonies such as that.

<Crathorn holds up Petri dish of colonies in front of diagram>

These colonies were obtained by plating out single cells after irradiation onto this Petri dish and allowing them to grow and divide. The growth was stopped after the colonies were a visible size, stained, and then the colonies can be counted, and in that way we can obtain a graph such as this.

<Crathorn to camera>

This is probably all I need to do to remind you of the features in which we are interested and Dr Revell will now discuss what we can learn of the effects of radiation on cells at the cellular and chromosomal level. He will be followed by Dr Roberts who will discuss, at the molecular level, the ways in which a cell, or the DNA of a cell, attempts to repair damage which is introduced by such agents. Finally, we will turn to

Dr Steel who will discuss the problems of cell population kinetics and how a study of these can be used to improve tumour therapy.

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<Intertitle>

Dr Stanley Revell

Effects of radiation at Cell and Chromosomal level

<Revell to camera>

The initial damage which ionising radiation causes in cells must be in the form of minute chemical defects and this is also true of the initial damage caused by those chemical agents which imitate the effects of radiations. Now, Dr Crathorn has already indicated to you that the cell's DNA is the most likely site of this initial damage. Some of this initial damage to its DNA the cell can repair and Dr Roberts will be talking to you about this later, but the cell doesn't succeed in repairing all the initial damage to its DNA. And some of this probably goes on to develop into visible chromosome changes of the kind that we can actually see in cells at their next mitosis if we fix and stain them in suitable ways. This damage appears as actual breaks in chromosome structure and also changes in chromosome sequence. Let's have a look at two pictures which show this sort of thing.

<Revell refers to photomicrographs and diagrams and narrates over them, interspersed with talk to camera>

Here we have two Syrian hamster cells, fixed and stained, one of them has received no radiation dose; it's dividing its chromosomes quite normally into two groups, but this other cell which received 2,000 roentgens about 10 hours beforehand, as you can see, is in serious trouble. It's got chromosomal fragments stretched between the two groups of departing chromosomes and there are other bits and pieces which are not responding to the cell spindle at all. Now, the consequence of fragment formation of this sort is that if this cell had been allowed to go into division instead of being

fixed and stained, we should have two deficient daughter nuclei, deficient, of course, by the bits and pieces of chromosome that are left out.

Now, it's reasonable to wonder whether effects of this sort, how they affect cell function and particularly how they affect the cell's capacity to divide. <*Next diagram*> There is evidence that they have considerable effects on the cell's capacity to divide. First of all, we find that if we consider the relationship between fragment frequency, measured on this axis of the graph, the effect of fragment frequency induced by different doses of x-rays in this case, we find that there is a very strict relationship. The graph goes nicely back to the origin as you can see here. This is because any quantum of radiation has a certain likelihood as it goes through the cell of causing or at least contributing to a fragment loss. For the same reason as x-ray dose is raised, there is an increasing likelihood that any particular cell will suffer damage, that is to say, fragment loss.

Now, you'll appreciate, I hope, that curves of this sort have an obvious general resemblance to the survival curves of the type that Dr Crathorn showed you earlier where an increasing proportion of cells failed to form colonies as x-ray dose is raised.

So, we have a very clear correlation here, is it a real one? <*Next diagram*> Well now, there are other ways in which one can attempt to confirm this correlation. One can study the actual sequence of events in live cultures studied on a suitable incubated microscope. If we give a single cell a dose of x-rays and then follow it through its division, observe fragment losses at the first mitosis after the x-ray dose, we find at the stage when two cells are formed from the first division that the fragment or fragments which were left out of that mitosis, they form a micronucleus. We can see this in live cells.

<*Photomicrograph*> Here is such a live cell. This is the main nucleus, of course. The cell spread out again on the glass. And here is the small micronucleus with all the chromosome fragments which this nucleus is deficient by. Now, the fragments here are of no use to the cell because this will soon degenerate so this cell is going to be deficient. <*Previous diagram*> Now, under the conditions in which these experiments

were done, a micronucleus in either this daughter cell or this daughter cell indicates that both cells are deficient, and if in our live cell experiment, which we're conducting you remember on an incubated microscope, incubated to 37 °C, we can follow these cells, both of which are deficient, to see if they form colonies or not, and we find either that these cells stop dividing altogether and produce quite an insignificant colony, or at most they may struggle on dividing at infrequent intervals so that they produce only a dwarf colony. On the other hand, if the cells in our experiment which were lucky enough to escape the effect of x-rays at the particular dose one was employing, no fragments are lost at the mitosis. Therefore no micronucleus is observed here and in these cases we find that growth continues and we get a normal colony, essentially of the same type that one observes and scores when one is constructing conventional survival curves.

<*To camera*> Now, this then confirms the correlation that we thought we had observed earlier on with simply by the construction of survival curves and the observation of chromosome aberration frequency. Now, it would be wrong of me to leave you with the impression that experiments of this sort tie up this subject. There's quite a lot of controversy as to how much of the impairment of colony forming capacity is due to chromosome fragment loss, but what I think these experiments do show is that chromosome fragment loss must be at least an important contributor to colony forming impairment.

00:15:53:02

<Intertitle>

Dr John Roberts

The repair of damaged genetic material

<Roberts to camera. Then refers to diagrams and photograph and narrates over them, interspersed with talk to camera>

I will now discuss mechanisms which the cell employs to overcome damage to DNA since it is probable that it is damage to DNA which is responsible for the

chromosome aberrations, which have been discussed by Dr Revell, following ionising radiation treatment, and also which occur as a consequence of treatment by a variety of chemical agents. What has become clear in the last decade is that there are many environmental factors which can damage DNA in a variety of ways, some of which are depicted on this scheme here <*diagram*>. These will be plant products – the antibiotics; various types of radiation – ionising radiation, x-rays or ultraviolet radiation; as well as a variety of drugs – alkylating agents which are used in cancer chemotherapy; as well as products formed by the combustion of other products such as the aromatic hydrocarbons.

<*To camera*> Now, much of our understanding of repair mechanisms which have evolved to handle this sort of damage, introduced by these compounds, stems from studies using microorganisms. These have had the advantage that we have available there a number of mutant cells which might lack a particular step in the repair process. However, guided by these studies, it is clear that the mammalian cell probably possesses analogous mechanisms to those found in microorganisms.

Now, I'm going to discuss first one particular form of repair which we call excision repair, or cut and patch repair, and which is depicted in this scheme here. <*Next diagram*> The effect of damage to DNA, and here we're going to show one particular type of damage – the formation of a thymine dimer, that is the linking of two thymines in the same strand of DNA together, this is a chemical linkage formed between them, distorts the DNA structure, probably due to the weakening of hydrogen bonds between the bases. Now, this distortion is recognised by an enzyme known as an endonuclease which inserts a nick near to or adjacent to the lesion. The next step involves the action of a complex enzyme system which does two things. It peels back the damaged strand of DNA or the strand containing the damage as shown here by an exonuclease action and degrades it at the same time, breaking it up into a series of nucleotides, while at the same time it inserts bases by a polymerase action – the original sequence of bases, which were present in the original strand here, to restore the original structure. A final step in this repair process now involves the linking of this newly synthesised piece of DNA to the original parental strand by an enzyme known as a ligase. We have illustrated this process showing the excision of a few

nucleotides but, in fact, this can be up to several hundred in some situations depending on the nature of the damage or the organism which has been investigated.

<*To camera*> It is clear now, so that many other types of damage are recognised by this or similar enzyme systems. <*Next diagram*> And we've illustrated two types of chemical damage in this next picture here. Here we are illustrating the linking of two guanine molecules on opposing strands of DNA or on the same strand because it is this reaction, this linking of two molecules of DNA together, which is known to occur following treatment of cells with some of the cytotoxic alkylating agents, the difunctional compounds. And the effect of this chemical linkage is to block the action of a DNA polymerase which replicates the DNA. And it's this failure to replicate DNA which is probably responsible for the antimitotic effect of these difunctional alkylating agents and their antitumour action. And this damage also will be recognised by the same system.

<*To camera*> It now appears that repair of DNA plays a role in protecting cells from damage which can otherwise lead to cancer. This follows from observations on patients who suffer from the rare skin disease xeroderma pigmentosum, <*photograph of child*> which is characterised by extreme sensitivity to sunlight and invariably results in actinic skin cancer. And these patients have a genetic defect which is associated with a decreased ability to excise thymine dimers, the chemical lesion formed following UV radiation. And this autosomal recessive mutation has been characterised as a deficiency in the level of the enzyme which recognises the thymine dimer, the endonuclease which inserts the nick.

<*To camera*> Now, since differences in the sensitivities of cells to various agents cannot always be correlated with these various repair mechanisms or steps in this particular repair mechanism that I've discussed so far, it's suggestive that there is another mechanism present in cells for overcoming damage to their DNA. Now I'd like to discuss what we know about another mechanism which is called post-replication repair.

<*Next diagram*> Here we are depicting damage to DNA, possibly arising as a consequence of a chemical reaction with a guanine base. In this situation we envisage that the damage is not sufficient to cause distortion of the DNA twin helix so that replication of DNA does proceed, and we've shown this here. The replication on this intact strand takes place to produce a perfectly normal twin helix. However, replication on the damaged strand seems to induce further damage into the DNA because it fails to replicate a section of the DNA in order to lead to a gap in the DNA depicted here, in which a number of nucleotides have failed to be inserted. Replication now on this strand of DNA in this molecule attempts to replicate, we reproduce this sort of damage when we replicate on that strand, but replication on the strand containing the break will lead to further damage and a double-strand break if this gap is not repaired. However, we have evidence that in some cells this further damage, this post-replication damage, can be repaired, hence it is called post-replication repair. And the evidence from microorganisms suggests that this gap can be filled by the actual insertion of a piece of DNA, a piece of homologous DNA containing the same sequence of bases which should have been inserted during replication. It is why it is called recombination repair.

In mammalian cells some evidence suggests that the gap may be filled by DNA synthesis in which the bases are inserted sequentially to fill the gap. There are still a number of problems associated with this mechanism of DNA repair. Is the correct base sequence always restored or does this DNA synthesis produce errors in DNA by inserting the wrong sequence of bases?

<*To camera*> And no doubt there are many other mechanisms present in the cell for overcoming damage to DNA, but this just illustrated two that we know something about.

00:23:38:19

<Intertitle>

Dr Gordon Steel

Cell population kinetics and tumour growth

<Steel to camera>

Having discussed the proliferation of cells in culture and their response to damage, we now want to move on to consider the proliferation of cells in vivo and its relationship to therapeutic response. Many of the cytotoxic agents that are at present in use the treatment of cancer are proliferation dependent, that is to say in most cases they kill rapidly proliferating cells more effectively than slowly proliferating cells. And this is true not only of cells within the tumour but also cells within the normal tissues of the host, so the success of treatment depends on achieving a differential cell kill between the tumour and the normal tissues. And at the present time it's widely believed that the achievement of such a differential depends on the, at least in part, on whether the cells within the tumour are proliferating more rapidly or more slowly than the cells in the normal tissues and also on their relative capacity for regeneration after damage. So, what I want to do is briefly to summarise some of the things we know about the kinetic state of cells in tumours and in normal tissues and I'd like to begin with the situation in normal tissues.

<Steel refers to series of diagrams and narrates over them, interspersed with talk to camera>

The epithelium in normal tissue of the small intestine is a rapidly renewing cell population which diagrammatically consists of units like these. Cells are produced by division in the crypts of Lieberkühn. And having been produced here, they migrate as a sheet up the villi where they are lost from the tip into the lumen of the gut. Cell proliferation is normally restricted to the lower two thirds or three quarters of the crypts of Lieberkühn, and in this region they proliferate very rapidly. In laboratory animals the cells down here divide continuously twice every day. These cells are therefore very sensitive to proliferation-dependent cytotoxic agents. But it seems that what often saves the intestine when intensive chemotherapeutic treatment is given is the existence of a small proportion of cells deep in the crypt which proliferate more slowly than the rest of the cells.

A similar situation exists in the bone marrow. <*Next diagram*> If we look just at the erythroid system, we see that this consists of a sequence of morphological cell types. Within these earlier cell types, proliferation is rapid, but beyond the polychromatic erythroblast stage, the cells cease to divide, they extrude their nuclei and finally emerge into the circulation as erythrocytes. Now, the maintenance of this cell population is due to the existence of a stem cell population feeding it. And these stem cells, although they cannot be recognised morphologically, have been studied in great detail. Some of the stem cells are pluripotent in that they can produce cells of the granulocyte and megakaryocyte series as well as the cells of the erythroid system. But other stem cells seem to be committed to one of these three particular lines. The evidence is that the stem cells of the bone marrow proliferate relatively slowly under normal circumstances, but that some types of damage can stimulate them into greater proliferative activity. The significance of this for chemotherapy is that combinations of an agent which speeds up the stem cells with another agent which is proliferation dependent could be very damaging to the marrow and therefore very toxic.

<*To camera*> So one general conclusion that one can draw about cell proliferation in these two normal tissues is that their sensitivity to cytotoxic agents is partly due to the rapid proliferation of many of the cells, but the survival of the tissue during intensive chemotherapy seems to depend on regeneration from a small proportion of slowly proliferating stem cells.

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Now, what about the situation in tumours? It's often imagined that the growth of tumours is the result of accelerated cell proliferation, but this seems unlikely to be the case. It's nearer the truth to say that the initiation of tumour growth is due to a failure in the containment of the stem cell population; the stem cell population gets out of hand and begins to enlarge. And what is clear is that tumour cells often divide more slowly than cells in some of the normal tissues of the host, particularly cells in the marrow and the small intestine. It has been shown that within a tumour, one invariably has a wide range of intermitotic times. <*Next diagram*> The usual way to

measure this, measure the intermitotic times of cells in vivo, is to label those cells that are preparing for division, and in this diagram the labelled cells are represented by the areas labelled S. One labels the cells with a radioactive DNA precursor and then we follow their movement through successive mitoses, and as they move, one can generate a curve of this type, which shows often a series of peaks. The first peak is produced by cells moving to their first post-labelling mitosis. The second peak is the result of their movement to their second post-labelling mitosis. And if one sees two clear peaks of this type, the peak to peak interval is a measurement of the average intermitotic time.

Now, when this technique has been used on tumours, it has been found that the second peak is usually very poorly defined. The curve shows rapid damping. This implies that within the whole cell population of the tumour, there is a broad range of intermitotic times, some cells have a short cycle, others have a long cycle. There is no clear cycle for the whole tumour.

<*To camera*> Now, a great deal of detailed experimental work has been done on cell proliferation in normal tissues like the intestine and the gut and the success of it is largely due to our ability to define a simple architecture, either a spatial architecture as in the intestine or a morphological architecture as in the bone marrow. Unfortunately, in tumours it's very difficult to do this. Tumours are often very heterogeneous and they come in a great variety. And it's been difficult to make simple generalisations about the relationship between cell proliferation and tumour structure.

The clearest work is probably that of Tannock who used a mouse memory tumour which had what we call a corded structure. <*Next diagram*> When viewed microscopically, one could see that the tumour consisted of units like these: these are tubes running through the tumour, each one fed by an axial blood vessel. Each blood vessel is surrounded by a cuff of proliferating tumour cells. And beyond a range of about 100 microns, we have necrotic tissue, dead tissue. Now, using titrated thymidine, Tannock was able to show that this is a rapidly renewing cell population. The division of cells close to the blood vessel displaces cells that are further away,

out towards the necrotic boundary, and as cells move away from the axial blood vessel, they move into a situation where the supply of oxygen and other nutrients is diminishing. We have reasons to believe that the point at which necrosis sets in is related to the range at which the oxygen tension falls to zero. Now, cells within this tumour cord make the transition from near to the vessel out to the necrotic region in a period of about 48 hours. I want to stress that in a sense this is a dramatic observation. Under normal circumstances of growth, most of the cells in this region of tumour have a lifespan of less than 2 days before they or their descendants die in a natural process of necrosis.

<*To camera*> Now, I mention this work in order to make two main points. First, although cords of this type are not common to many tumours, the phenomenon of natural death amongst tumour cells has been widely observed, and it seems, in fact, in some tumours to be one of the main determinants of growth. Many tumours that grow slowly do so not because they're producing cells at a low rate but because they're losing cells almost as fast as they're producing them. This situation has been shown to be more characteristic of carcinomas than of sarcomas.

The second point I want to make relates to the nature of tumour stem cells. I define a stem cell to be one that has the capacity to produce a large family of descendants. <*Previous diagram*> Within this tumour cord the effective stem cells are cells that are close to the axial blood vessel. But when we treat this tumour with radiation or with cytotoxic drugs, we will probably kill the cells near to the vessel more effectively than cells that are further out, and our explanation of the problem of curing this and other tumours is that some cells that are remote from the vascular system, which may be proliferating slowly, and are therefore resistant to many cytotoxic agents and which are hypoxic and therefore resistant to radiation; these cells which would normally have died in the natural process of cell turnover may have the capacity to act as stem cells for re-growth after treatment. So we have to distinguish between effective stem cells under normal growth circumstances and potential cells, but potential stem cells in the situation that follows tumour treatment.

00:35:57:10

<Steel to camera>

Now, I want to finish by talking briefly about how we study the tumour stem cells because we believe that to a large extent the kinetic problem of tumour response to treatment lies with them. What we have to do in order to study these cells is to take them out of the tumour, separate them into a suspension of single cells and then place the cells in a test environment and say: can you grow? We have to try and estimate what proportion of the cells in the tumour have the capacity to produce a visible colony. We term this procedure an assay for tumour stem cells, and at the present time a number of different assays are in use: some of them test the growth of the cells in vitro, others test their growth in vivo, and with any one of them, one can compare a tumour after treatment with the situation before treatment and one can deduce what proportion of the stem cells have been killed or have lost their capacity to act as stem cells, which is the important thing. And so an assay allows us to produce an in vivo cell survival curve.

<Steel refers to series of diagrams and narrates over them>

This curve is similar to the one that Dr Crathorn showed earlier in the programme. The cell's grown and treated in culture and I want you to note that in this case we've treated the tumour in vivo, removed the cells and then assessed their capacity to form colonies in a test environment. The ordinate in this chart is a logarithmic scale of cell survival. I want you to note that it's a long scale covering many decades of cell survival. At the bottom end of the scale, we have a survival of one cell in a million. The reason why we have to go a long way down is because every gram of tumour may have 10^{-7} or 10^{-8} stem cells and a leukaemic patient on presentation may present with 10^{-12} leukaemic cells of which perhaps 10^{-11} may be stem cells. So, before we can be confident of cure, we have to go down many decades of cell kill.

Now, the curve you see here is an in vivo radiation survival curve. It differs from the in vitro one that you saw earlier in that it appears to consist of two components. The majority of cells may be quite sensitive to radiation and have the cell survivor curve

shown by the dashed line coming downwards, but there appears also to be a resistant component, consisting perhaps of 10 % of the cells in the tumour which give rise to this exponential more resistant component. There is good evidence that these are the cells that are hypoxic, cells that are probably remote from the vascular system.

<*Next diagram*> Now with drugs we get a range of other in vivo survival curves. Some of them give an exponential survival curve as shown here and in some cases the steepness of this curve has been shown to depend on the rate of proliferation of the tumour stem cells. <*Next diagram*> Other drugs give survival curves that look like this with a plateau produced by the existence of a proportion of cells that are very resistant to treatment. We tend to see this picture with drugs that are termed cycle-phase-specific drugs which only kill cells in one part of the mitotic cycle.

<Steel to camera>

The objective of work in this area of cancer research is to try to understand the response of tumours to therapy and the mechanisms involved in this. In part, it's clear that this depends on proliferative factors. We need now to gain more information about the survival and regenerative capacity of cells in the critical normal tissues of the host. We need also to understand mechanisms of cell kill within the tumour and to understand its capacity for re-growth. The end product we hope will be a more rational understanding of the way in which we can use cytotoxic agents in the treatment of cancer.

<**End credits**>