CHAPTER 1

CANCER BIOLOGY

The present thesis is an attempt to make a systematic study of treatment of cancer employing lasers, at cellular level. This chapter gives a brief outline of how cancer cells differ from normal cells, causes of cancer and different conventional modalities of cancer treatment.

1.1 STRUCTURE OF CELLS

The basic unit of a living organism, including human, is the cell. When the cell was studied with the optical microscope it was observed that, although their individual shapes varied considerably, most had two main compartments - an inner nucleus and an outer region known as the cytoplasm. It was also recognized that the complete cell was enclosed by a limiting boundary called membrane, which served as a protective layer between the cell and its environment. After the advent of electron microscopy and the recent research in biochemistry and molecular biology, the existence of different constituents inside and outside the nucleus could be distinguished.

The nucleus carries a vast amount of coded information. In fact, each nucleus carries sufficient information to carry out all functions of any cell in the body. Among the two nucleic acids, deoxyribonucleic acid (DNA) plays a highly important role in cellular inheritance, in growth and development, in cell division and in the synthesis of protein molecules and other cell constituents.
Long strands of DNA are coiled up to form the chromosomes. The genetic information is conveyed from the nucleus to the cytoplasm by the intervention of a particular type of ribonucleic acid (RNA). In the cytoplasm the information is translated with the co-operation of other RNA molecules, when protein synthesis occurs.

The protein synthesis takes place at the site of units known as ribosomes. Ribosomes may be freely dispersed or particularly in cells which secrete protein to the surrounding medium, they may be attached to an assembly of interconnected membrane known as the endoplasmic reticulum.

Glucose, a simple sugar molecule is the source of energy for most cells. Mitochondria are the sites of energy production in cells and certain enzymes which are necessary for the extraction of energy from the breakdown products of glucose and other food stuffs. They then make it available for cellular use.

Golgi apparatus is a system of interlocking fibrils, or in some cells just scattered threads, usually localized around the centriole. The apparatus consists of lipids and proteins. The function of the Golgi body has been something of a mystery. Functionally the Golgi body has a close relationship with the secretory activity of the cell and also associated with the formation of the spindle in cell division.

Centrosome is a clear area of the cytoplasm attached to the outer side of the nuclear membrane and contains the two centrioles which are important components of the spindle in cell division. Under optical microscope the centrosome is only visible when the cell begins to undergo division.
The brief outline of the main components of animal cell is summarized diagrammatically in Figure 1.1. The thread like strands of DNA are not in fact visible in the so called resting cells (cells not actively engaged in division). Resting nuclei in both plant and animal cells have the much more uniform appearance shown in Figure 1.1, the only clearly detectable organized structure being the nucleolus, which contains most of the nuclear RNA [1,2].

1.2 CELL GROWTH AND MULTIPLICATION

Growth of the organism begins with the fertilized ovum and increase in size is achieved by cell division, known as mitosis. The period between the end of one cell division and the end of the next is called the cell cycle. The various stages of the cell cycle are shown in Figure 1.2.

In the cell cycle, the period of DNA synthesis is commonly designated as S phase. The interval between the previous mitosis and the S phase is called G₁ phase, the presynthesis phase. RNA and the cytoplasmic synthesis occur in the G₁ phase. The gap between S phase and mitosis is called postsynthesis phase (gap) called G₂. Mitosis, the fourth period of the cycle, is designated by the letter M. The function of mitosis is to ensure that the DNA contained in the chromosomes is divided equally between the two daughter cells after replication. Provided that the coded sequences in the DNA are identical in the two daughter cells, these cells will be genetically identical with the parent cell, a condition which is essential for the preservation of the character of the organism. Mitosis also depends on a coordinated interaction between nucleus and cytoplasm as the cell approaches division.
Figure 1.1 Generalized diagram of an animal cell
(from J. Brachet (ed.), 1961 by courtesy of The Living Cell)
Figure 1.2 The various stages of the cell cycle
The duration of the cell or mitotic cycle, i.e. the time between two consecutive divisions of a cell, is the sum of the durations of the separate phases. The latter vary relative to one another in magnitude in various tissues in the following sequences: $M < G_2 < S < G_1$ [3].

It has been suggested that the term growth is not very precise because it could mean only the enlargement of a cell and therefore many biologist prefer the term proliferation. Proliferation rates vary widely between different tissues and are influenced by the rate and extent of cell loss. There is a very rapid rate of proliferation during early embryonic life which slows down with the maturity and development of the organism. By adult life there will be some tissues with no proliferative activity, such as certain types of nerve and muscle cells. Others will maintain a high level of proliferation throughout the life, such as bone marrow and the epithelial lining of the gastrointestinal tract. Others, such as the skin, have proliferation rate that can be increased considerably when necessary, as in wound healing.

The processes which cause a cell to duplicate the chromosomes and subsequently divide, remain to be established. It has been proposed that in some tissues, if not all, the cells principally concerned with the growth of the organ are constantly in a state in which they are prepared to divide. The cell division is, however, prevented from doing so by chemical inhibitors called chalones. When the level of these inhibitors in the surrounding fluids falls, then the cells divide. For example, the outer layer of the skin cells which are continuously shed, are normally replaced from the basal layer of dividing cells. These basal cells are to some extent inhibited by a chemical produced by
upper, more mature, cells. If the upper cells of an area of skin are destroyed, the inhibitor they produce will no longer be available to inhibit the basal cells, which therefore divide to produce new cells to repair the damage.

Any tissue in which a large number of cells show duplication of chromosomes and subsequent cell division, is said to have high mitotic activity. The mitotic index, a measurement of mitotic activity, is the percentage of cells in an area which are undergoing mitosis.

Also it is suggested that at any one time not all cells are proceeding through the cycle since they may leave the cycle to rest, entering a state called $G_0$. This distribution of cells between resting and cycling states are also important, since if cycling cells are destroyed by any means, for example by drugs, radiation, more resting cells will be drawn back into cycle to restore the original population. In addition to the cycling and resting cells, there is another group of cells which are non dividing which have permanently left the cycle and are destined to die without carrying on any further cell division.

1.3 DIFFERENTIATION

To develop into various organs with widely different structure and function, cells must specialize. The process by which cell differences arises during development so that cells of the various tissues take on definite characteristics, is known as differentiation. Starting from one cell, the fertilizer ovum, a whole series of different cell populations arise during embryonic development. This is a result of a combination of proliferation and
differentiation. Differentiation may be defined as progression along the pathway of specialization in the activity of a cell. Such specialized activity is thought to be regulated by the DNA; various parts of the DNA code being repressed or de-repressed may disallow or allow the expression of the messenger in that part of the code.

1.4 CONTACT INHIBITION

The nature and mechanism of contact inhibition is not fully understood. It probably depends on the property of the cell's surface, the cell membrane. It is also thought to be involved in the normal recognition of self since if two different types of normal cells are brought into contact they may show inhibition on meeting each other. For example, following a fracture of the femur, growth will occur if a space is allowed between the two ends of the broken bone (traction). On meeting, the cells recognize the self, growth ceases and union takes place. If, however, a different tissue is placed between the two ends of bone, growth will cease and there is a recognition of non self and union will not take place. The factors which initiate and terminate the whole process are as yet not fully understood.

1.5 MALIGNANT CELLS

Cancer is the second most common cause of death in the world after cardiovascular disease. Humans of all ages and sex develop cancer, and a wide variety of organs are affected. The incidence of many cancers increases with age, so that as people live longer, they are more prone to the disease.
Cancer is a new growth which has gone out of control of regulating mechanisms. Cancerous growth, often synonymously called tumour or neoplasm is manifest as abnormal swelling or enlargement in any part of the body. Of course, there are many pathological swellings which are not truly neoplastic. Inflammatory lesions, cysts and congenital malformations are some of the examples of non-cancerous growth. Abnormal swellings occur very frequently in the female breast; some of these are malignant tumours and some are benign tumours, but many are not neoplasms at all but simple tissue proliferation probably resulting from hormonal stimulation.

A very neat definition of cancer according to Professor R.A. Willis is: "A tumour is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues, and persists in the same excessive manner after cessation of the stimuli which evoked the change"[4].

From this definition it is seen that true neoplasms differ in certain very important respects from other tumefactions such as inflammatory swellings. In its early stages the swelling of an inflammatory lesion (such as that following invasion of the body by bacteria) results from dilation of blood vessels and the passage of fluid and cells from the blood into the tissue spaces. If the bacteria (the stimuli which evoked the change) are eliminated, the excessive cellular proliferation rapidly subsides. In case complete return to normal is not possible, a permanent swelling composed of fibrous scar tissue remains. However, such a residual lesion displays no tendency for continued growth.
It is the popular belief that cancer cells grow faster than normal cells; this is not always true. In reality the cell division is the same but tumours constantly divide, whereas normal cells divide depending upon the requirement.

The next of the essential feature of cancer cell is that it loses its normal differentiated state. Both contact inhibition of cell movements and density dependence of mitosis are generally deficient to varying degrees in tumour cell types in monolayer culture. The cells show reduced cell-cell adhesion and changes in surface properties like cell surface negative charge. It was found that cells from a line of hamster kidney fibroblasts, transformed in vitro by polyoma virus to malignant cells, produce clones (colonies of cells grown from a single cell), about half of which consists of cells with a higher negative charge than the original fibroblasts. It was also shown that, in human leukaemias, myeloid cells of characteristic mobility in normal persons are replaced by cells of higher mobility. This may be a general characteristic of tumour cells, along with increased sialic acid of the cell surface, lack of cellular adhesiveness, and inability to form normal contacts with other cells [1].

In general, loss of contact inhibition and loss of the characteristic to recognize self from nonself enables malignant cells to infiltrate surrounding tissue and eventually spread to distant organs of the body (metastasize). These secondary growths are termed metastases. Table 1.1 shows the differences between normal and malignant cell systems.
Table 1.1 The differences between normal and malignant cell systems

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth</strong></td>
<td>1. Controlled and coordinated</td>
<td>Loss of control and coordination allowing tumour formation</td>
</tr>
<tr>
<td></td>
<td>2. Control mechanisms regulate development and maintenance of cell populations.</td>
<td></td>
</tr>
<tr>
<td><strong>Differentiation</strong></td>
<td>Cells well differentiated with specialist functions</td>
<td>Loss of differentiation with loss of function and adoption of inappropriate function.</td>
</tr>
<tr>
<td><strong>Contact Inhibition</strong></td>
<td>Cells display contact inhibition and recognize other cells as self or non self. They do not infiltrate adjacent tissues.</td>
<td>Loss of contact inhibition and loss of ability to recognize self from non self resulting in infiltration and dissemination</td>
</tr>
</tbody>
</table>
1.6 CAUSES OF CANCER

Agents causing cancer falls into three broad groups: radiant energy, chemical carcinogens and viruses.

1.6.1 Radiant Energy

Ultraviolet rays, X-rays and gamma rays are mutagenic and carcinogenic. These rays damage DNA in several ways. Apart from direct effects on DNA, X-rays and gamma rays cause the formation of free radicals in tissues. Free radicals being highly reactive can attack several sites in the DNA molecule. Two very vulnerable sites are the pyrimidine bases (particularly thymine) and the sugar-phosphate back bone. Free radicals may a) abstract hydrogen from the methyl group of thymine, b) add OH to the 5-6 double bond of thymine, and c) open up the ring structure. Such damages may result in loss of chemical identity of the base or alteration in their base-pairing properties. The damaged bases may also link with one another leading to inter and intra strand cross links. Free radicals may also attack the covalent bond between the phosphate and the sugar of the back bone and lead to single and double strand breaks. Such damages may also be formed by the direct ionization of DNA. These damages may lead to DNA degradation, interference with DNA replication and alteration in genetic information content. A schematic representation of DNA molecule and the site of damages are shown in Figure 1.3.

DNA degradation and interference with its replication results in cell death. However, cells have a capacity to repair much of the base damages and single and double strand breaks. Most often repair of these damages
Figure 1.3 Radiation induced damages to DNA
take place without any error and cell is restored. In some instances repair will be accompanied by errors in the reconstituted DNA. These errors may be in the form of chromosomal aberrations or point mutations. Such cells are viable but have altered genetic information. If such error-prone repair occurred in somatic cells, it may lead to cancer in the exposed individual. If it occurred in the germ cells, the consequence may appear as hereditary defects in the offspring [5].

1.6.2 Chemical Carcinogens

It is estimated that up to 80% of human cancers are caused by environmental factors, principally chemicals. Exposure to such compounds can occur because of a person's occupation (e.g., continual exposure to benzene, asbestos); diet (e.g., aflatoxin B₁, which is produced by the mold Aspergillus flavus, sometimes found as a contaminant of peanuts and other food stuffs); life-style (e.g., cigarette smoking); or in other ways (e.g., certain therapeutic drugs can be carcinogenic).

Both organic and inorganic molecules may be carcinogenic. The organic carcinogens have been most thoroughly studied. Some, such as nitrogen mustard and β-propiolactone, have been found to interact directly with target molecules (direct carcinogens), but others require prior metabolism to become carcinogenic (procarcinogens). The process whereby one or more enzyme-catalyzed reactions convert procarcinogens to active carcinogens is called metabolic activation. Any intermediate compounds formed are proximate carcinogens, and the final compound that reacts with cellular components (e.g., DNA) is the ultimate carcinogen. The sequence is thus represented as:
The procarcinogen itself is not a chemically reactive species, whereas the ultimate carcinogen is often highly reactive. At least two reactions are required to convert the procarcinogen, e.g., 2-acetylaminofluorene (2-AAF) to the ultimate carcinogen, the sulfate ester of N-hydroxy-AAF. An important generalization is that ultimate carcinogen are usually electrophiles (i.e., molecules deficient in electrons), which readily attack nucleophilic (electron-rich) groups in DNA, RNA and proteins.

When chemical carcinogens are administered to animals or placed in cultured cells, it can be shown that they (direct carcinogen) or their derivatives (ultimate carcinogen) generally bind covalently to cellular macromolecules, including DNA, RNA and proteins. The chemical nature of the adducts formed by interaction of certain ultimate carcinogens with their target molecules have been determined. Much interest has been focused on products formed with DNA. Carcinogens have been found to interact with purine, pyrimidine, or phosphodiester groups of DNA. The most common site of attack is guanine, and the addition of various carcinogens to the \( N_2, N_3, N_7, O_6 \) and \( O_8 \) atoms of this base has been observed [6]. This covalent interaction of direct or ultimate carcinogens can result in several types of damage; this damage can be repaired. Despite the existence of repair systems, certain modifications of DNA of chemical carcinogens persist for relatively long periods of time. It is possible that these persistent unrepaired lesions are of special importance in generating mutations critical to carcinogenesis.
In certain organs such as skin and liver, it has been shown that carcinogenesis can be divided into at least two stages. The classic example is skin. Typically, identical areas of the skin of a group of mice are painted once with benzo(a)pyrene. If no other subsequent treatment is used, no skin tumours develop (Figure 1.4). However, if the applications of benzo(a)pyrene is followed by several applications of croton oil, many tumours subsequently develop. Applications of croton oil alone (i.e., no pretreatment with benzo(a)pyrene) do not result in skin tumours. Many other variants of this basic protocol have been carried out, permitting the following conclusions:

i. The stage of carcinogenesis caused by application of benzo(a)pyrene is called initiation and this state appears to be rapid and irreversible. It is presumed to involve an irreversible modification of DNA, perhaps resulting in one or more mutations. Benzo(a)pyrene is thus called an initiating agent.

ii. Much slower (i.e., months or years) state of carcinogenesis, resulting from application of croton oil is called promotion. Croton oil is thus a promoting agent, or promoter. Promoters are incapable of causing initiation.

iii. Most carcinogens are capable of acting as both initiating and promoting agents.

Many tumour promoters appear to act by causing alterations of gene expression, but the precise mechanisms by which promoters influence the initiated cell to become a tumour cell remain to be determined [6].
Figure 1.4 Diagrammatic representation of the stages of initiation and promotion of chemical carcinogenesis in skin.

A: One application of the initiator
B: The application of the initiator followed by a number of promoter
C: The promoter applied first, and then the initiator applied.
1.6.3 Cancerous viruses

The role that viruses play in causing human cancer remains ambiguous. The viruses that have been strongly associated epidemiologically with human cancers are listed in Table 1.2. No straightforward and direct proof of a casual role has been established for any of these associations to date. However, many viruses can cause tumours in animals, either as a consequence of natural infection or after experimental inoculation. In vitro studies show that tumour viruses can cause transformation. Transformation is a stable, heritable change in cell properties that can be caused by a single viral gene. The most prominent changes associated with transformed cells include the following:

i. Altered Morphology: More rounded cell shape.

ii. Altered Pattern of Growth: Increased rate of growth, decreased requirement for serum growth factors, decreased cell adhesion to a substrate, enhanced ability to grow in semisolid medium, and loss of contact inhibition so that transformed cells are no longer inhibited by contact with other cells, as normal cells are, but tend to pile up to form a focus. Induction of foci can provide the basis for a quantitative assay for certain tumour viruses.

iii. Biochemical Changes: Increased metabolic rate, increased glycolysis, and changes in the composition and properties of the cell membrane with the production of new proteins and new antigens in the cell.
<table>
<thead>
<tr>
<th>Virus Family</th>
<th>Virus Genus</th>
<th>Human Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpesviridae</td>
<td>Herpes type 2 virus</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>Papovaviridae</td>
<td>Papiliomavirus</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Laryngeal papiloma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(carcinoma?)</td>
</tr>
<tr>
<td>Hepadnaviridae</td>
<td>Hepatitis B virus</td>
<td>Primary hepatocellular carcinoma</td>
</tr>
<tr>
<td>Retrovirus</td>
<td>HTL virus</td>
<td>Adult T-cell leukemia</td>
</tr>
</tbody>
</table>
iv. **Tumourigenicity**: Production of tumours when transformed cells are injected into appropriate test animals, especially immunologically deficient animals.

Tumour viruses are classified among different virus families according to the nucleic acid of their genome. DNA tumour viruses are classified into the papova-, adeno-, herpes-, hepadna-, and pox- virus groups.

RNA tumour viruses were once called oncornaviruses but now are designated as retroviruses. Retroviruses are unique in that they carry an RNA-directed polymerase that constructs a DNA copy of the RNA genome of the virus. The DNA copy becomes integrated into the DNA of the infected host cell, and it is from this integrated DNA copy (provirus) that all proteins of the virus are translated. To sum up, DNA is the critical target molecule in carcinogenesis. The following facts support this conclusion.

i. Cancer cells beget cancer cells, i.e. the essential changes responsible for cancer are transmitted from mother to daughter cells. This is consistent with the behavior of DNA.

ii. Irradiation, chemicals and viruses damage DNA and are capable of causing mutation in DNA.

iii. Many tumour cells exhibit abnormal chromosomes.

iv. Tranfection experiments indicate that purified DNA (Oncogenes) from cancer cells can transform normal cells into (potential) cancer cells [7].
1.7 DIFFERENT MODALITIES OF CANCER TREATMENT

1.7.1 Surgery

Since classical times, surgery has played a leading role in the treatment of cancer. The belief that surgery could be curative is based on the view that malignant change in a tissue remains localized at the primary site for long enough to be diagnosed and excised. Here surgery may involve taking out portions or even whole organs.

The possibilities of surgery playing an important role in the palliation of malignant disease are numerous. The distress of local ulceration can be overcome by mastectomy in patients with cancer of the breast, even if spread is known to have occurred. Obstruction of the gastrointestinal tract at almost any level causes pain and systemic disturbances from which great relief may be afforded by resection, bypass or colostomy.

Preoperatively the patients are often somewhat debilitated and anemic with low plasma proteins. It may be impossible to correct this without a period of intravenous feeding and blood transfusion. Some malignant conditions (e.g., leukemia), and some forms of treatment such as chemotherapy, affect blood clotting and may lead to hemorrhage if surgery is undertaken unless preoperative platelet infusions are given.

During surgery special care is taken to avoid touching a malignant lesion either during excision or mobilization. The vascular pedicles supplying the tumour are ligated as early as possible to minimize the chance of spread via the blood-stream. This technique is important.
during the resection of a kidney tumour when the renal pedicle can be ligated; during resection of testicular tumour when the cord can be ligated; during resection of colorectal carcinoma when the inferior mesenteric pedicle is ligated early in the operation. During the post operative period the wound should be observed for signs of hemorrhage, dehiscence and infection in view of the special circumstances which increase the dangers of these various complications. The significant problems after surgery are as follows:

i. Surgery is effective only if malignancy is localized i.e., when no metastases has reached.

ii. Even after resection of a part or whole of the afflicted organ, malignancy may not have been completely removed and

iii. The psychological blow resulting from loss of organs, especially superficial organs like breast.

Indeed surgery is not the last stop in the road to cure, but is very often followed up by radiotherapy and chemotherapy depending on suspicion of spread of disease.

1.7.2 Chemotherapy

Drugs are often given to kill cancer cells. Chemotherapy may be the sole mode of treatment or may be used in conjunction with surgery or radiotherapy to kill microscopic remnants of cancer that may persist even after surgical removal. Chemotherapy is generally given over a period of several months to a year or more.
In experimental studies it has been shown that chemotherapy is most likely to be curative when the number of malignant cells present is the smallest. Skipper et al. [8] developed a quantitative assay for determining the number of malignant cells in a mouse which could be killed by a given course of drug treatment. They were then able to demonstrate that the effect of an antitumour agent on malignant cells follows first order kinetics. This means that for a given dose of a cytotoxic drug a fixed percentage of the total tumour cell population (not a fixed number) is killed. A simplified version of this concept is shown in Table 1.3 which gives the relationship between the malignant cell number and eradication of tumour.

It is apparent that the same drug, achieving the same fraction of cells killed, given in the same manner, to animals bearing a similar type of tumor can either be completely useless when the number of malignant cells present is large or curative only when the number of malignant cells present is small. These experimental data highlight the need to detect a tumour at an early stage in its development.

Unfortunately at the present time for most solid tumours the techniques available are unable to detect in man malignant cell numbers less than about $10^9$ or 1 gm of tumour (Figure 1.5). Since the patient will die when the total number of malignant cells increases to between $10^{12}$-$10^{13}$ (i.e., tumours weighing between 1 and 10kg), it follows that at the time of detection the tumour has already passed through at least two thirds of its life-span. In biological terms, therefore, there is no such thing as an early detectable lump. The same point applies to the detection of secondary deposits also. It is likely that
Table 1.3 The relation between cure rate and tumour cell number \[8\]

<table>
<thead>
<tr>
<th>CURE</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almost all animals</td>
<td>with 10,000 malignant cells</td>
</tr>
<tr>
<td>40%</td>
<td>of animals with 1 million malignant cells</td>
</tr>
<tr>
<td>None</td>
<td>of animals with 1 billion malignant cells</td>
</tr>
</tbody>
</table>
Figure 1.5 The relationship between tumour cell number, tumour weight, clinical detection and death of the patient
many patients with apparently "local" tumours at presentation already have undetectable distant micrometastases. Therefore local treatment alone, such as surgery or radiotherapy (RT), cannot hope to eradicate a disease whose true extent is impossible to define accurately.

To improve the cure rate there is a need to integrate chemotherapy with the best methods of local treatment.

Since antitumour drugs are most likely to be curative when the number of tumour cells in an animal is smallest, it follows that adjuvant chemotherapy should be given immediately after the bulk of the primary tumour has been removed or destroyed by surgery and/or radiotherapy. The application of this principle has markedly increased the cure rate in certain experimental tumours in animals, and is outlined diagrammatically in Figure 1.6.

Over the past few years it has become obvious from certain well established experimental principles and some preliminary clinical studies that traditional role of chemotherapy in the overall management of cancer should be critically re-evaluated. In the past it was usual to reserve antitumour drugs until all other kinds of treatment had failed, i.e., they were used only as a last resort when surgery or radiotherapy had failed to cure the primary tumour. This concept is illustrated in Figure 1.7.

Besides the above, the development of successful techniques for cell synchronization in vitro has enabled to determine the phase or phases of the cell cycle where anticancer agents exert their effects on cells [9]. In
Figure 1.6 The advantages of combining surgery and chemotherapy.
Figure 1.7 Traditional approach to solid tumour therapy
general, drugs exert two main types of effects: a delay or hold up in progression of cells through the cycle; and a lethal or cell killing effect. Examples of these effects are shown in Table 1.4 and Figure 1.8.

In general the use of lower drug concentrations for short periods of time results in the blocking effect, which is often reversible, while higher drug concentrations given over longer periods cause cell death.

But the enormous morbidity and expenses together with the occasional drug induced death make chemotherapy a difficult treatment modality. Besides the side effects, like nausea, loss of appetite, weight loss and stomach discomfort, drug induced cancer again is a vexing question as in the case of radiotherapy. Hence the current state of art dictates that chemotherapy be carefully chosen in select patients to maximize its advantages.

1.7.3 Radiotherapy

Radiotherapy plays an important part in the treatment of most of the malignancies and it uses ionizing radiation like X-rays, gamma rays, electron beams and high energy charged particles. The biological effect of such ionizing radiation on tissues can be classified into direct and indirect effect.

1.7.3.1 Direct effect

Direct effect of radiation will cause ionization and excitation in tissues. For example, if we expose a tissue with gamma ray of dose 400 rads ( unit for radiation absorbed dose ), about $7.65 \times 10^{15}$ atoms per gram of tissue
Table 1.4 Effect of some anticancer agents on the progression of cells through the cycle [10].

<table>
<thead>
<tr>
<th>Phases of cycle where delay in progression or arrest occurs</th>
<th>Some Anticancer drugs</th>
</tr>
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<tbody>
<tr>
<td>$G_1$</td>
<td>Actinomycin -D, 6-meracaptoporine</td>
</tr>
<tr>
<td>$G_1$ &amp; S boundary</td>
<td>AD, Cytosine grahninoside 5-Fluorouracil, Hydroxy urea</td>
</tr>
<tr>
<td>$S$ &amp; $G_2$ boundary</td>
<td>AD, cytosine arabinotial mitomycin, Bleomysin</td>
</tr>
<tr>
<td>$S$</td>
<td>BCNU CCNU, Methy CCNO</td>
</tr>
<tr>
<td>$G_2$</td>
<td>AD,CCNU, Bleomycin Mitomysin, Nimisemusted</td>
</tr>
<tr>
<td>$G_2$ &amp; M boundary</td>
<td>ICRF/SG,Puromycin</td>
</tr>
<tr>
<td>$M$</td>
<td>ICRF/SG,Vinblshine</td>
</tr>
</tbody>
</table>
Figure 1.8 The phases of the cell cycle where antitumour agents exert their lethal effects.
are directly affected both by ionization and excitation. This direct effect of radiation is non specific and may occur on any cell in the body. When thus directly affected a protein molecule or nucleic acid get excited or ionized leading to irreparable damage like point mutation, in which there is change in a single gene locus.

1.7.3.2 Indirect effect

Since, most of the body contains water, most of the direct action of radiation therefore is on water. The result of this energy absorption by water is the production of highly reactive free radicals in the water that are chemically toxic and which may exert their toxicity on other molecules also. When pure water is irradiated we have

\[ \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}^+ + e^- \] (1.1)

and the positive ion dissociates immediately according to the equation

\[ \text{H}_2\text{O}^+ \rightarrow \text{H}^+ + \text{OH} \] (1.2)

while the electron is picked up by a neutral water molecule

\[ \text{H}_2\text{O} + e^- \rightarrow \text{H}_2\text{O}^- \] (1.3)

which dissociates immediately

\[ \text{H}_2\text{O}^- \rightarrow \text{H} + \text{OH}^- \] (1.4)

The ions \( \text{H}^+ \) and \( \text{OH}^- \) are of no consequence, since all body fluids already contain significant concentrations of both these ions. The free radicals \( \text{H} \) and \( \text{OH} \) may combine with like
radical, or they may react with other molecules in the solution. Their most probable fate is determined chiefly by the Linear Energy Transfer (LET) of the radiation. In the case of a high rate of LET, the free radical OH are formed close enough together to enable them to combine with each other before they can recombine with free H radicals which lead to the production of hydrogen peroxide

\[ \text{OH} + \text{OH} \rightarrow \text{H}_2\text{O}_2 \]  

(1.5)

and this radical, \( \text{H}_2\text{O}_2 \) is relatively a stable compound and it persists long enough to diffuse to points quite remote from their point of origin. Also hydrogen peroxide is a very powerful oxidizing agent and it can thus affect molecules or cells that did not suffer radiation damage directly.

Radiation is thus seen to produce biological effects by two mechanisms, viz. directly by dissociating molecules following their excitation and ionization; and indirectly by the production of free radicals and hydrogen peroxide in the water of the body fluid.

1.7.3.3 Physical basis of radiotherapy

When the high energy ionizing radiation is absorbed by the material (tissues), three basic physical mechanisms will take place such as Photoelectric effect, Compton effect and Pair Production. Among the three effects, the photoelectric effect is utilized for the diagnostic purpose, the compton effect for radiotherapy. Photoelectric effect is predominant at lower energies up to 0.1 MeV and it depends on cube of the atomic number of the absorber, this property is being utilized in diagnostic radiology. On the other hand, the compton effect is predominant over the energies
from 0.1 MeV to 10 MeV. At these energy range, the absorption depends upon the electron density of the materials. Since the tissues, water and bone are having almost the same electron density, one can get the uniform distribution of radiation dose due to the compton effect. This property is being used in radiotherapy.

Since the absorption of ionizing radiation at these energies depends on the electron density of the tissues and unfortunately, all tissues (normal tissue, bone tumour tissue and water) are having the same electron density, selective interaction of ionizing radiation with tumour is not possible. Figure 1.9 represents the photon energy Vs. absorbed dose for various tissues and from this it can be seen that at therapeutic energy level (0.1 to 10 MeV), all the tissues are absorbing almost equal dose. At this stage during radiotherapy it is important to protect the skin, the mucous membrane of the intestines, gonads and bone marrow for otherwise the radiation can cause non healing lesions which lead to a permanent disability. In order to achieve the correct therapeutic effect in individual case, i.e., to minimize the unwanted doses to the normal surrounding structures and to deliver maximum dose to the tumour, contour planning (treatment planning) is done as a basis for determining the physical dose distribution.

To obtain the optimal effect in each case, careful choice of the type of radiation and the radiation geometry are made. The therapy is divided into a number of individual treatments, the so called fractionation. In determining these parameters, the most important factor is the depth of the tumour in the body. The technique is dependent on the depth, and a distinction is made between brachytherapy (superficial) and teletherapy (deep). The desired radiation
Figure 1.9 Energy absorption per roentgen of various tissues
Figure 1.10 The build-up effect for different types of radiation
dose in the tumour is important; the dose varies with the type of tumour and usually lies between 4000 and 8000 rad (40-80 Gy). The type of radiation is chosen to obtain a suitable depth of penetration. Both photon and particle radiation, with different energies, can be used.

Since the use of photons (X-rays and gamma rays) attenuate exponentially with build-up effect, skin is getting more dose which results in erythema and disfiguring. This has prompted many workers to go for particle radiation which has limited range (Figure 1.10).

Besides its effectiveness of treating cancer with ionizing radiation, the problem of radiation induced cancer in the normal areas is the vexing problem. This led many investigators to go for other treatment modalities. In this context, phototherapy has its attraction due to its selective interaction with the tumour tissues without affecting the normal tissues. The present study is one such attempt wherein non-ionizing radiation (such as laser) is used in conjunction with a photosensitizer for selective, specific interaction with tumour tissues.