Chapter 2

Theoretical Aspects

This chapter deals with theoretical aspects. It contains Cancer-cellular oscillations, theoretical models on cancer, radiation dose and dosimetry, and target theory. The principles and procedures involved in the theoretical models on cancer, the radiation dose and dosimetry, various experimental procedures and calculations of target theory on cancer are explained in detail.
2.1. **Cancer – Cellular oscillations**

A phenomenon of cell rotation during mild dielectrophoresis (DEP) was observed to occur at selected frequencies of the applied field. In this case yeast cells were held by the gentle application of an a.c field against platinum electrodes. The cells generally stack up upon one another due to mutual DEP, especially if a moderate to high concentration of cells is used. Some of the cells busily spin about an axis normal to the field lines and at a rate of several revolutions per second.

This spinning of each particular cell was observed to occur at a sharp frequency. As the frequency is changed cells can be seen to start then stop spinning here and there in the field of view. Spinning can be seen to occur among the cells over a range of frequencies anywhere in the field, whether the cells were ones resting against an electrode, against another cell or floating freely in the medium.

It is not unusual to see several cells in a long pearl chain of cells to be spinning individually in their places. As the frequency is changed, the spinning rate of a particular cell would vary, becoming a minimum in a particular and narrow frequency range. A cell which had stopped spinning as a result of a frequency change is often observed to resume spinning if the frequency is returned to its original value. By and large, as the frequency is changed, some cells stop spinning and others start
up. The speed of rotation depends directly upon the square of applied voltage. The spinning response of the various cells examined is relatively sharp in frequency suggests that there is a resonant response; hence the term cellular spin resonance (CSR) is suggested to describe it.

To help confirm the mechanism of CSR, the spin response in the presence of normal a.c sine wave and pulsed d.c of the same frequency was experimentally examined.

Murine ascites tumour fibroblasts (sarcoma) were obtained from the peritoneal fluid of Swiss mice grown by Prof. E.M. Hodnett. The cells were separated from the fluid by centrifugation where upon the supernatant was discarded. After rinsing thoroughly many times, the cellular suspension of high resistivity was prepared and then diluted with sucrose to enable to place one drop into the CSR chamber for examination.

In the same way yeast cells were prepared with a cell suspension that was diluted in deionizer water for use in the CSR studies. The CSR technique consists of placing a suspension of cells into a chamber provided with two parallel platinum electrodes set at 1 mm apart in a flat capillary, all fixed onto a glass microscopic slide.

The two parallel wires pass in parallel through a micro slide. The micro slides provide a tubular hollow of highly flattened cross section
and satisfactory optical quality. The wires can be held away from the edges of the micro slide by fine glass tubing and bonded to the supporting ordinary microscope slide by epoxy polymers. A fine platinum wire of 27 μm diameter was used.

The cells in the above described CSR chamber were observed under a microscope at about 440x using kohler illumination. To the wire electrodes were attached leads from signal generator in parallel with an A.C.voltmeter. In case where the spin rate of the cells was determined, illumination was provided by strobotac. The flash rate of the strobotac lamp could then be synchronized to the rotation of the cell under observation and the spin rate can be measured with precision, for the cell would appear to stand still at the matching rate of illumination.

In a typical experiment, the cells were introduced into the CSR chamber and the desired voltage applied to the electrodes at the selected frequency. The cells would collect at both wire electrodes, usually in short strings or pearl chains running at right angles to the wire surface. In the case of yeast cells, it would be usual to observe some cells in various pearl chains to be spinning at 14 Vrms and 1 MHz.

As the frequency was changed, a particular cell could be seen to stop spinning, but another could begin and as the frequency was brought back to the original value, the first cell could usually be seen to
resume spinning. It is typical to observe that a particular cell would have a rather sharp range of frequency response within a factor of 2 or less of a central value. A yeast cell would begin to spin as the frequency reached 0.6 MHz and keep on spinning until the frequency reached 1.5 MHz then stop spinning at higher frequencies. To account for the various possible mechanisms for the observed CSR, a circuit for supplying either sine wave a.c or pulsed d.c was used. The output from the signal generator was fed into a switching circuit that preceded the CSR chamber. The output of the signal generator passed through a protective load of 330 ohms, then to the high terminal of the CSR chamber.

The other terminal of the signal generator and between the 330 ohm resistor and the connection to the CSR chamber was connected a bypass. It consisted of a variable 10000 ohms potentiometer in series with a silicon diode. The 10k ohm potentiometer controls the wave shape delivered to the CSR chamber. The signals to the CSR chamber were monitored with an oscilloscope.

With the potentiometer set in the high range the wave shape applied across the CSR chamber was effectively normal sine wave. With the potentiometer put in the shunted mode, the wave shape across the CSR chamber was effectively rectified to half wave, or pulsed DC of the same frequency as the full sine wave.
Direct observation of the spinning rate of mouse ascites cells suspended in sucrose of specific resistivity 180 to 200 k ohms cm was made with the aid of the strobotac lighting. The cell was placed between the platinum wires of the CSR chamber and subject to mild DEP so as to gather them onto the wires at 14 Vrms and 200 kHz.

The spin rate was then examined for particular cells as the applied voltage was varied. The spin rate was observed to vary linearly with the square of the applied voltage. A similar result was obtained for yeast cells. Yeast cells provide a useful model for the study of CSR for their morphological changes with their phase in the life cycle. Yeast cells from the early phase were used to examine the way in which the conductivity affected the position and amplitude of the response spectrum.

The small single cells (3.3 to 4.5 μm) are observed to spin readily in the range of about 1500 kHz, whereas the larger (diameter 6 to 8 μm) spun most readily at about 100 kHz. The peak positions shift downward in frequency as the resistivity is increased. This is reminiscent of the dielectrophoretic spectrum of cells and other particles in water. As the conductivity of the medium is increased, the DEP response peaks move towards higher frequencies.

The spin spectra of yeast cells are quite sharp. The starting and stopping frequencies are narrowly spaced pairs. The small unbudded
cells (3.5 to 4.5 μm diameter) as a group spin at frequencies in the range 0.5 to 11 MHz, with a Centrum at about 2 MHz. The larger unbudded cells (6 to 8 μm diameter) spin when driven by frequencies in the range 0.6 to 200 kHz, with a Centrum at about 30 kHz.

The spectrum of the small single cells shows the major response to lie at about 100 Hz to 1.5 MHz. The large single cells show responses at about 100 Hz and at 30 kHz. The small budded yeast cells spin actively at about 80 Hz and at about 1 MHz.

The large double cells respond down in the 100 Hz range. The loss factor maximum for cell-free serum shows no appreciable dielectric loss until at very high frequency (about 10 MHz and above), while for cell-rich flesh shows two maxima, one at about 100 Hz, the other at about 300 kHz. These facts may have relevance to our understanding of electrical factors in cellular reproduction and its control.

2.1.1. Origins of cellular spin resonance (CSR)

There are four main reasons why an applied electrical field could evoke cell spinning.

1) Ambipolar rotational conduction: The deposition of both the cations and the anions of the conducting medium onto a particle having a conductivity and dielectric constant differing
from that of the medium can produce a dipole on the particle surface. This can evoke cellular rotation.

2) Asymmetric (tensorial) polarization: In this case, the particle is considered to have only the dipole induced by the applied external field.

3) Asymmetric cell to cell polarization: The cell to cell interaction may be a contributor to the observed spinning, but it cannot be the sole cause, if we observe the lone cells to be actively spinning when they are removed from others.

4) Natural, intrinsic oscillating cellular dipoles: The natural rf oscillating dipoles arise from oscillating chemical reactions coupling in the cells to physically mobile regions of ions so as to produce charge density waves.

   The several known oscillating reactions are considered to oscillate between reaction phases which are rich in ionic and free radical reactions. During the ionic phase, the outward speed of travel of the positive ions does not exactly match that of the negative ions, then a charge wave will develop.

   As these developing charge waves encounter structures within the cell that effect a parallelization of the wave development, a collimation of the charge waves will ensue. The various charge waves travelling within these parallel regions will then develop a cooperative correlation to form a
coherent set of charge waves involving large regions of the cell in a cooperative oscillating dipole.

There are a number of structures in cells to effect parallelization of such charge waves. These are mitotic spindle apparatus, the walls of the endoplasmic reticulae etc. Oscillating systems in biology are well-known. The kinetic data based upon the speed of the assumed charge waves implies that it is more reasonable to expect that the charge waves proceed across in the case of the high frequency oscillations.

The presence of the ionic double layers at the numerous interfaces within the cell is well recognized. These ionic double layers would be expected to play a significant role in the natural oscillations of cells. The phenomenon of the mechanically stimulated electromagnetic radiation from vibrated systems having ionic double layers may play a role.

For both murine and yeast cells, the spin rate of cells is observed to be proportional to the square of the applied field. There exists a considerable electrical conductivity of the cell as it floats in the medium, so that the induced polarization and its resulting torque is expected to be field dependent. From this observation it can be concluded that the CSR is due to natural rf oscillations as modified by the polarisability of the cell and medium and acting in response to the applied field.
**AC and DC responses:**

If natural and internally driven dipoles are present, they can be expected to evoke a torque and a spinning of the cells if the dipolar frequency, \( f_D \), matches that of the external field, \( f_E \), as seen during the cellular spinning at frequency \( f_R \), i.e.,

\[
f_D = f_E \pm f_R
\]

It is expected that the frequency \( f_D \) will be slightly modifiable by the presence of external field \( f_E \) and by the presence of its internal polarization \( \alpha \). Such a response of a natural oscillating dipole dressed in the internal polarization of the cell, can be expected to result in spinning whether the applied field is sinusoidal a.c or pulsed d.c of the same basal frequency.

Accordingly yeast cells were subjected to a.c, then pulsed d.c fields over a wide frequency range so as to ascertain their behavior. If the cell spins while in the presence of a high frequency ac field and continues to spin when this field is smoothly altered to that of a pulsed d.c field of the same frequency, then we may be assured that it has a natural rf dipole. The typical results show the fraction of some 500-800 yeast cells in the field of view which are actively spinning at each of a number of frequencies and while the applied field is either ac or pulsed dc.
The results show that the ac and the pulsed dc spectra are quite similar and show that the cells continue to spin whether in an a.c or a pulsed d.c field of the same basal frequency and applied field strength (14.14 V\text{rms}). This finding assures us that the cells are possessed of natural, intrinsic oscillating electric fields. Cells killed by heat or phenol do not spin when subjected to high frequency electric fields, a.c or pulsed d.c.

The evidence that living cells naturally produce rf oscillating electrical fields proceeds from a number of experimental evidences such as

1) by CSR: CSR is observable with sharp resonances in living cells but not in dead cells.

2) Living cells continue to exhibit CSR in either ac or pulsed dc fields.

3) By Dielectrophoresis: The motion of tiny polarisable neutral particles towards a region of high electric field intensity, confirms the presence of natural rf electrical oscillations in cells in a number of ways. This is observable in a wide range of organisms namely bacteria, yeasts, avian, and for normal, fetal and cancer mammalian cells and vary with physiological state of the organism.

4) The extreme simplicity of the experimental means available for observing these natural rf oscillations encourages us to believe that their study will be attractive and useful to life scientists.
2.1.2. Electrical oscillation and contact inhibition of reproduction in cells

By observing the attraction of cells for polarisable powders, it is evident that certain cells in the reproductive state emit radio frequency electric fields. Electrical oscillatory phenomena must precede or accompany the cellular reproductive act. Without the presence of electrical oscillatory phenomena, cellular reproduction cannot succeed. A normal cell exhibits contact inhibition of reproduction, a cancer cell does not. A cell or any cell must oscillate to reproduce, especially during mitosis.

If so, then one aspect of the contact inhibition of reproduction would be that the requisite electrical oscillations associated with mitosis is now energetically damped out by the presence of other neighboring cells, but could proceed in the presence of normal non-cellular media.

It is possible that one or more types of cancer cell use this aspect of the reproductive process. Cancer cells are characterized by the pathologist by their ability to invade and metastasize. This is because cancer cells have their oscillators rarely damped by the presence of neighboring cells and would therefore rarely exhibit contact inhibition of their reproduction.
The term contact inhibition has a number of meanings as applied to cell biology. When cultures of fibroblasts are examined with time-lapse photography, it is found that the cells are in continual motion.

This motion is greatest at those parts of the cell which are not in contact with other cells. Contact between these cells appears to inhibit movement at the contacting surfaces. Cellular contact can also bring about cellular adhesion. Some strains of cells which are derived from malignant tumors of connective tissue show neither adhesion nor contact inhibition of motion. The role of protein molecules in evoking adhesion and the role of the lowered degrees of cohesive forces probably present in cancer cells has drawn much attention. Laki and Ladik, upon using quantum theoretical arguments indicate that the cohesive forces depend strongly upon electronic desaturation in proteins.

Here, the aspect of contact inhibition relative to reproduction, connected with electromagnetic oscillations is emphasized. Here we are directly concerned with the two aspects recognized by the pathologist as characteristic of malignant cancer, invasive growth and metastasis. The evidence for the presence of electrical oscillations in reproducing cells is reasonably direct and based on the observation of the dielectrophoretic attraction of polarisable particles to such cells.
The tests were done mostly on mammalian (murine) cells from either normal or ascites (cancer) stock. The first biological oscillator system to come to notice was the heart. The number of biological systems for which oscillatory behavior has been observed is quite large.

It ranges for systems which consist of whole organisms with periods of months and even years, to those of smooth muscle ($10$ to $10^4$ s); to peristalsis ($1$ to $10$ s), respiration ($0.3$ to $3$ s); heart ($0.03$ to $10$ s); nervous action ($10^{-3}$ to $1$ s). The shortest period so far observed in such systems is about one millisecond. It is well known that living cells exhibit both a higher dielectric constant and at times a higher dielectric loss than that of the surrounding fluid. With this in mind it is evident that a cell which is ready in all other respects to pass along into the reproductive state could be inhibited from oscillating and hence reproducing, because its oscillation would be damped by unfavorable surroundings.

There are three ways in which a cell may override the normal inhibition due to the presence of other cells. They are power level increase, insulation from its neighbors and the oscillation frequency shift. One mechanism by which fetal or cancer cells show less contact inhibition of growth than normal cells, may well involve one of these three possible mechanisms.
In particular, the invasive character of cancer cells could, in some cases, involve such behavior. This model predicts that cells bearing a thickish coat of low loss material would show reduced contact inhibition due to electrical dissipative means. Further evidence for this in the case of cancer cells was presented by Dvorak and his collaborators at the Massachusetts General hospital in Boston.

They showed that in the guinea pig some tumours can insulate themselves from the host animal’s defenses by building a ‘cocoon’ of gel like fibrin about them. While the presence of fibrin is known to stimulate blood vessel development, crucial to the tumour support, it is also possible that the cocoon affords the necessary electrical insulation for the cancer cell so that electrical oscillation necessary for the continued reproduction (or invasion) can continue. The cocoon probably also affords some protection from antibody attacks.

2.2. Theoretical models on cancer

The progression of cancer is a multi-step process. Over 80% of malignant tumors are carcinomas that originate in epithelial tissues from where they invade the connective tissue. At some point, subpopulations of cells may detach from the primary tumor and spread via the bloodstream and the lymphatic system.
Some of them give rise to metastases in distant organs. The metastatic cascade is a very inefficient process; as only one in about a thousand cells that leave the primary tumor goes on to form a macroscopic secondary tumor.

The main contribution to metastatic inefficiency arises from the failure of cancerous cells to grow inside invaded organs. Metastatic tumors also show preferential growth in different organs. Hence, the efficiency of the metastatic process depends on specific interactions between the invading cancer cells and the local organ tissues.

Risler, Prost and Joanny from Curie Institute in Paris suggest that this is due to a difference of pressure between tumor cells and the host tissue. Combining the laws of mechanics and the biological state of homeostasis, the authors propose that every biological tissue regulates to a preferred pressure called homeostatic pressure, and that an increased homeostatic pressure is a generic trait of neoplastic tissues.

This property can drive tumour growth at the expense of the host tissue. Metastases account for the majority of patients’ deaths due to cancer, and thus understanding the metastatic process is of critical importance.
The mathematical models were established since 1950 to account for angiogenesis and molecular mechanism to explain the tumour growth and generation of specific data. A number of mathematical models have been developed to study the details of specific immune cell activities within a tumour and the immune response for tumour growth and progression.

Multicellular spheroids (MCS) are clusters of cancer cells, used in the laboratory to study the early stages of avascular tumour growth. Mature MCS possess a well-defined structure, comprising a central core of necrotic, or dead, cells, surrounded by a layer of non-proliferating, quiescent cells, with proliferating cells restricted to the outer, nutrient-rich layer of the tumour.

Angiogenesis is the process which enables a solid tumour to make the transition from the relatively harmless, and localised, avascular state to the more dangerous vascular state, wherein the tumour possesses the ability to invade surrounding tissue and metastasize to distant parts of the body.

Tumour cells secrete several chemicals (angiogenic cytokines) which induce blood vessels from the neighbouring host tissue to sprout capillary tips which migrate towards and ultimately penetrate the tumour, providing it with a circulating blood supply and, therefore, an
almost limitless source of nutrients. Preventing the capillary network from forming or supplying chemotherapy drugs to the tumour via the capillary network offer potential strategies for the treatment of cancer.

The recent discovery of many molecular mechanisms responsible for cancer invasion makes this a prime area for mathematical models to act as a link between microscopic and macroscopic data. The final aim of this work is a single, verified model for the invasive cascade, but an essential precursor to this is the separate study of the contributing factors.

Thus, the mechanism such as imbalance between proteolytic enzymes and their inhibitors, or changes in cell–cell adhesion, can initially be modeled separately before being combined into a single model framework. Similarly, the role of a pH gradient at the tumour–host interface has been studied mathematically, predicting a relationship between morphology and growth rate.

An understanding of the spatial and temporal processes underlying metastasis (formation of secondary tumours) is crucial for key issues such as reliable markers for the success of particular therapies, and nonlinear mathematical models are the natural vehicle for this understanding. Within a single tumour mass, nonlinearities are
manifested in the irregular shape of the boundary between the tumour and the surrounding tissue.

Detailed data on the fractal nature of this boundary is now emerging for a range of human tumours, and is a crucial yardstick for theoretical models; moreover, local and global fractal dimensions can be a valuable prognostic indicator of invasion.

The progression of malignant cancer typically involves an unquestionably complex chain of events, including initial tumourigenesis, onset of hypoxia and acidosis, angiogenesis, tumour vascularization and growth, and migration and metastasis.

While such processes have historically been studied via experimental and clinical observations, the use of mathematical modeling to develop an effective tumour growth model could prove an invaluable complement.

Mathematical modeling and simulation can potentially provide insight into the underlying causes of tumour invasion and metastasis, help understand clinical observations, and be of use in designing targeted experiments and assessing treatment strategies. The ultimate goal is to develop individualized therapeutic protocols based on patient-specific tumour characteristics.
Mathematical models of cancer growth tend to fall into two categories: discrete cell-based models and continuum models. In discrete modelling, individual cells are tracked and updated according to a specific set of biophysical rules.

While this method can address detailed biological processes, it is computationally demanding for larger-scale systems. For example, a full simulation of a small 1 mm$^3$ tumour, which may contain several hundred thousand cells, is not currently feasible.

Continuum models treat tumours as a collection of tissue, describing densities or volume fractions of cells and other elements. Lowengrub and co-authors – from the University of Texas Health Science Center at Houston and the University of Tennessee Knoxville – begin by examining tumour growth in homogeneous tissue, using a basic model that represents the tumour as a sphere-like structure without direct access to the vasculature.

This evaluates the relevance of theoretical modelling to patients suffering from brain and breast cancers. Excellent agreement was found when comparing the tumour “virtual histopathology” to clinical histopathology.
Lowengrub points out that such encouraging results support the idea that sophisticated multiphase tumour simulators, capable of simulating vascularized tumour growth in three spatial dimensions, and calibrated by in vitro and in vivo data, have the potential to predict cancer behaviour in patients.

While continuum modelling is appropriate at the tissue scale where gross tumour behaviour can be quantified, it cannot model individual cells and discrete events. Hybrid-modelling frameworks offer a promising alternative. Here, the tumour tissue is described using both discrete (cell-scale) and continuum (tumour-scale) elements. With the potential to combine the best features of both approaches, such models may provide more realistic coupling of biophysical processes across a wide range of length and time scales. Mathematical modelling has revealed that parameters controlling the tumour shape may also control its ability to invade, suggesting that tumour morphology could serve as a predictor of invasiveness and treatment prognosis.

2.3. Radiation dose and dosimetry

Like most physical quantities, the radiation quantities are also defined, refined and updated depending on the actual need, in the field of radiation dosimetry. Radiation dosimetry requires various quantities for continuous and orderly advancement in applied radiation technologies,
inclusive of nuclear power, medical & industrial applications, health physics and so on.

When x or gamma radiation passes through matter it ionizes and or excites atoms or molecules of the matter, leading to energy transfer to the matter from the radiation. Through primary ionizations in matter, energetic electrons are produced which in turn cause further excitations and ionizations in the constituent entities in matter.

High energy x or gamma radiation also interact with the atomic nuclei giving raise to nuclear excitations and or emission of nucleons/photons and some radiation products. In dosimetry we are interested in the energy transferred to the medium in the form of ionization and excitations to the entities of the medium.

Whenever ionizing radiation either in the form of x-ray photons or gamma ray photons or electrons are made use of in medicine, it is inevitable to measure the quantity and quality of the radiation delivered from the telecobalt units as well as from high energy linear accelerators. In diagnostic x-ray, nuclear medicine, CT scans, PET-CT etc., this measurement is useful to optimize the quality of the image and for the purpose of radiation protection of the staff and the general public. But in radiotherapy, radiation dosimetry i.e., the measurement of radiation that
comes out of the machine is very much important since large doses of radiation are delivered to the tumour in cancer treatment.

And the efficacy of the treatment depends on the dose delivery with an expected accuracy of ± 5% to ensure maximum dose to the tumour with minimal damage to the healthy normal tissues.

When an ionising radiation passes through an absorbing medium such as body tissues, some of the energy carried by the beam is transferred to the medium where it produces biological damage. The energy deposited per unit mass of the medium is known as the absorbed dose that estimates the biological effects.

The interaction process involves the collision between a photon and some electron in the body which results the scattering of some radiation and the setting in motion of a high speed electron. The high speed electron produces a track along which ionization occur, excitation of atoms takes place and molecular bonds are broken. All these events result in the expected biological damage.

Most of the energy is converted to heat. Some of the high speed electrons collide with nucleus and bremsstrahlung takes place. The scattered photon and bremsstrahlung will again undergo the same type of interactions as the original photon.
The use of dosimetry based on biological response in clinical dosimetry is based on the quantity absorbed dose. Clinical dosimetry plays a critical role in the dosimetry chain and hence it is based on dosimetry protocols developed and approved by scientific societies such as the American Association of Physicists in Medicine (AAPM).

**Exposure:** It is a measure of radiation in terms of its ionization in air. It was defined by the ICRU (International Commission on Radiological units and Measurements) to quantify the radiation beams. Its measurement was done in air with the help of standard air chamber. Exposure is denoted as $X$ and is defined as:

$$X = \frac{dQ}{dm}$$

Where $dQ$ is the absolute value of the total charge of the ions of one sign produced in air when all of the electrons liberated by photons in a volume of air having a mass $dm$ are completely stopped in air. Exposure is measured in coulombs per kg. Its unit is the roentgen, $R$.

$$1 \text{ R} = 2.58 \times 10^{-4} \text{ C/kg}$$

If the radiation beam is monoenergetic, then the beam can be described as the number of photons, $dN$ that would cross an area, $da$, taken at right angles to the beam.

Fluency or photon fluence; $\Phi = \frac{dN}{da}$ (number of photon/area)
Energy transfer is a two stage process – KERMA and ABSORBED DOSE

**Kerma:** The transfer of energy from a photon beam to the medium takes place in two stages. The first one involves the interaction of the photon with an atom that causes the electrons in motion.

The second stage involves the transfer of energy from the high energy electron to the medium through excitation and ionization which is illustrated in **Fig. 1** ICRU introduced a quantity called KERMA which stands for Kinetic Energy Released in the Medium

**Fig. 1** Schematic representation of the transfer of energy from a photon (hv) to the medium

Kerma; \( K = \frac{d\mathcal{E}_{tr}}{dm} \) (energy/mass)

Where \( d\mathcal{E}_{tr} \) is the kinetic energy transferred from photons to electrons in a volume element whose mass is \( dm \). This quantity describes the
radiation beam with its effects. For a beam of photons with energy $h\nu$ and photon fluence $\Phi$, the kerma is given as

$$K = \Phi \left( \frac{\mu}{\rho} \right) \bar{E}_{tr}$$

Where $(\mu/\rho)$ is the mass attenuation coefficient for the medium and $\bar{E}_{tr}$ is the average amount of energy transferred to electrons in every interaction. The product $\Phi \left( \frac{\mu}{\rho} \right)$ gives the number of photon interactions per unit mass of material irradiated by a photon fluence $\Phi$ and $\bar{E}_{tr}$ is the average energy transferred to electrons by these interactions. The units of kerma are joules per kg. Kerma are a useful quantity in the radiation dosimetry.

**Absorbed dose:** This quantity is frequently used in radiotherapy and radiobiology. The absorbed dose is the energy retained in the medium by the ionizations and excitations that takes place along the track which equals the range of electron. The ICRU has defined Absorbed dose $D$, as

$$D = \frac{d\bar{E}_{ab}}{dm}$$

Where $d\bar{E}$ is the mean energy imparted by the ionizing radiation to a mass $dm$ of matter. The old unit for absorbed dose was rad. The new unit is gray (Gy) which is defined as

$$1 \text{ Gy (gray)} = 1 \text{ J/kg} = 100 \text{ rads}$$
Ion chambers are used to measure the absorbed dose in beams of ionizing radiation. Therefore the necessity of ion chambers to be regularly calibrated against NRC’s national measurement standards is mandatory. A calibration factor will be provided by the standards laboratory for a quantity, exposure that is different from absorbed dose by making use of the Co-60 as the calibration field.

At present the radiotherapy is done with mostly with high-energy photon or electron beams from accelerators (5 to 25 MeV), there is a major change in the performance of clinical dosimetry. The goal is to make clinical dosimetry much simpler and to improve its accuracy. Absorbed dose of radiation by the biological systems can be measured in several ways. The most primitive method is by the use of calorimeter.

2.3.1. CALORIMETER

When a medium is bombarded with radiation, most of the energy absorbed gives rise to heat and a little appears as chemical change. The rise in temperature produced by the absorption of 1 Gy in water is $2.39 \times 10^{-4} \, ^\circ\text{C}$. This small change in temperature can be measured using a thermistor. These are semiconductors that exhibit a large change in resistance for a 1 degree change in temperature.
The change in resistance of a $10^5 \, \Omega$ resistor produced by one Gy in water will be 1.2 $\Omega$. By using a carefully designed Wheatstone bridge, this change in resistance can be measured precisely.

**Fig. 2**

a) Schematic diagram of calorimeter designed to measure energy locally absorbed

b) Arrangement of components in a wheat stone bridge to measure the rise in Temperature

c) Arrangement of components to measure the rise in temperature of the core when heat is injected electrically.
A calorimeter is designed to measure energy locally absorbed. The absorber or core of the device is placed inside a jacket and a shield. Which is in turn placed in a large thermally controlled mass of buffer. The core, jacket, shield and buffer are made of the same material and are thermally insulated from each other with a vacuum between all of its components that takes care of heat losses by conduction and convection. Radiation losses can be minimized by covering the components with a thin layer of aluminium.

All the components are fitted with thermistors and small electric heaters to enable to measure and control the temperature. Initially the core, jacket, shield and buffer are brought to the same temperature and then the radiation beam will be made on.

When the radiation gets absorbed, the core will rise in temperature as well as the jacket and shield. Heat transfer from core to the jacket is minimized. The rise in temperature, $\Delta T_r$ is measured by a Wheatstone bridge and the change in temperature is measured by connecting the bridge output to an amplifier recorder system.

If the mass and specific heat of each component of the core were known, the energy input from the measured rise in temperature ($\Delta T_r$) can be calculated. It is the practice to dissipate a known amount of electrical
energy, $E_h$ in the core and measure the rise in temperature, $\Delta T_r$, produced.

The energy absorbed from the radiation beam is given by

$$E_{ab} = E_h \frac{\Delta T_r}{\Delta T_h}$$

**Determination of Dose in water:**

Place the core of the calorimeter in the radiation field. For a given irradiation, the calorimeter gives the absorbed dose, $D_0$, in the calorimeter material which may be tissue equivalent plastic or graphite. Remove the calorimeter from the beam and put a water phantom with an ion chamber which is connected to its electrometer.

Expose the chamber to the same quantity of radiation. Note down the meter reading as $M_0$. Calibration of ion chamber can be done for any beam with similar properties. Let the meter reading be $M$ when the chamber is placed in a similar beam in water phantom, then the dose $D$ is

$$D = \left( \frac{M}{M_0} \right) D_0 K = M N_D$$

Where

$$K = \frac{\text{Energy absorbed by calorimeter material at } P}{\text{Energy absorbed by sample water at } P_1}$$

and $N_D$ is the absorbed dose calibration factor.
2.3.2. Determination of Absorbed dose using an absolute ion chamber:

An ionization chamber made of a known material and known volume is called an absolute ionization chamber. This chamber is made use of to determine the absorbed dose in the medium. When the radiation falls on this chamber the charge, $Q$, will be liberated in the cavity. Since the volume of the cavity is known, one can calculate the mass of the gas, $m$.

It is desired to determine the dose to the medium at $P$, when the medium is placed in a radiation field. (Fig. 3)

![Diagram](image)

**Fig. 3** Determination of absorbed dose using an absolute ion chamber

At $P$ we place the Bragg-Gray cavity (Fig. 4) filled with gas traversed by electron tracks. Ionisations will be produced in the gas in
the cavity by the electrons, giving rise to absorbed energy in the gas. The collection of charge liberated in the gas can be measured. The average energy required to cause one ionization in the gas is constant. It is denoted by $W$,

$$
\frac{eV}{\text{ion pair}} = \frac{\text{joule}}{\text{coulomb}}
$$

The dose absorbed in the gas can be related to the ionization produced in the gas by the equation

If $Q$ is in coulombs and $m_{\text{gas}}$ in kg, then the absorbed dose $D$ is in joules/kg or grays. The commonly used gas is air.

The above equation gives only the energy absorbed in the gas. We want the net energy to unit mass of the wall surrounding the gas and exposed to the same electron fluence. Since the air cavity assumed is so small, the gas in the cavity will have the same electron fluence as does the wall.

Therefore

$$
\frac{D_{\text{wall}}}{D_{\text{gas}}} = \frac{S_{\text{Wall}}}{\text{gas}}
$$

$S_{\text{Wall}}$ is the ratio of averaged stopping powers in the medium.
Combining the above two equations we get

\[
D_{\text{wall}} = \frac{Q}{W \cdot S_{\text{wall}}} \quad m_{\text{gas}} \quad g_{\text{gas}}
\]

This is the important Bragg-Gray formula which relates the ionization in a cavity to the absorbed dose in the wall surrounding the cavity. The above relation tells us to calculate the dose to the medium from a measurement of the ionization produced in an air filled cavity within the medium. But the medium which is of interest will be water.

Therefore one is forced to place the practical ion chamber with its wall in the water phantom and make measurements and calculate the dose to the wall and then from the properties of the wall relative to water, calculate the dose to the water.

The wall thickness of the Bragg-cavity must be greater than the range of electrons to ensure that the electrons arise in the wall only and not in the medium. A correction factor is to be introduced due to the insertion of the ion chamber with its wall and air cavity has disturbed the dose to point P in the homogeneous phantom. (**Fig. 3**)
For determining the absorbed dose, three materials are involved, the gas, the chamber wall and the medium in which the chamber is placed. The Bragg-Gray formula gives the dose to the walls of the chamber. But the dose to the medium is needed.

The composition of the walls of the cavity or chamber and the surrounding medium are assumed to be nearly same. Hence no appreciable change in the photon spectrum due to the wall material. The absorbed dose in the wall material will be different from its value in the medium. The ratio of doses in the two materials is nearly equal to the ratios of collision kermas in the two materials.

\[
\frac{D_{\text{med}}}{K_{\text{med}}} = \frac{D_{\text{wall}}}{K_{\text{wall}}} = \left( \frac{\mu_{\text{ab}}}{\rho} \right)_{\text{med}}^{\text{wall}}
\]

\[
D_{\text{med}} = D_{\text{wall}} \left( \frac{\mu_{\text{ab}}}{\rho} \right)_{\text{med}}^{\text{wall}}
\]
The above expression helps to calculate the dose to the medium from a measurement of $Q/m$ in the gas inside the chamber wall material and $(\mu_{ab}/\rho)_{med/wall}$, the ratio of averaged mass energy absorption coefficients including the correction $K_c$, for the finite size of the ion chamber to take care of perturbing effect of the chamber.

\[
D_{med} = (33.85 \text{ J/C}) (Q/m) S_{wall} (\mu_{ab}/\rho)^{med}_{wall} K_c
\]

\[
D_{med} = (33.85 \text{ J/C}) (Q/m) S_{wall} (\mu_{ab}/\rho)^{med}_{med/wall} K_c
\]

2.4. **Target theory**

Radiation is classified as directly ionizing or indirectly ionizing. All the charged particles such as electrons, protons, alpha particles, heavy charged ions are directly ionizing. They can directly disrupt the atomic structure of the absorber during their passage and produce chemical and biological changes. Neutrons and Electromagnetic radiations such as x-rays and gamma rays are indirectly ionizing.

They do not produce chemical and biological damage themselves. When they pass through matter, they give up their energy to produce fast moving charged particles. These charged particles interact with biological matter and cause the damage.
The biological effects of radiation result mainly from damage to DNA, which is the critical target in the cell. Any form of radiation, either x or gamma rays, charged or uncharged particles, is absorbed in biological material, they will directly interact with critical targets in the cells.

The atoms of the target itself gets ionized or excited which ends up with biological change. This is called direct action of radiation. This is possible with high LET (Linear Energy Transfer) radiations such as neutrons or alpha particles. (Fig. 5)

**Fig. 5**  a) Schematic representation of DNA and the tracks of an electron and α particle through it. The first electron is depicted as depositing energy at 0.25 keV/μm, while the α particle 5 MeV is shown depositing the energy at the rate of 100 keV/μm.  

b) Schematic drawing of a charged particle track illustrating the track, ion cluster and α ray spurs.
The radiation may interact with other atoms or molecules in the cell, particularly water to produce free radicals and damage the critical targets. This is called the indirect action of radiation.

Since 80% of a cell is composed of water, the water molecules may become ionized upon interaction of a photon or a charged particle. This may be expressed as $\text{H}_2\text{O} \rightarrow \text{H}_2\text{O}^+ + e^-$

$\text{H}_2\text{O}^+$ is an ion radical and it has short lifetime and decays to form free radicals. These are not charged but have an unpaired electron. In water, the ion radical reacts with another molecule to form the highly reactive hydroxyl radical (OH$^-$).

These free radicals possess nine electrons and one of the electrons is unpaired. It is highly reactive free radical and can diffuse to reach the critical targets of the cell. Two thirds of the x-ray damage to DNA in mammalian cells is due to the hydroxyl radical.\textbf{(Fig 6)}
Fig. 6 Direct and indirect actions of radiation

Fig. 7 Diagrams of single and double strand DNA breaks caused by radiation
DNA is the principal target for the biological effects of radiation, including cell killing, mutation and carcinogenesis. DNA consists of two strands that form double helix. Each strand is composed of a series of deoxynucleotides, the sequence of which contains the genetic code.

Sugar molecules and phosphate groups form the backbone of the double helix. The bases on opposite strands are complementary. Adenine pairs with thymine, while Guanine pairs with Cytosine.

When cells are irradiated with x-rays or gamma rays, many breaks of single strand occur. These can be observed and scored as a function of dose. Single strand breaks are of little biological consequence as far as a cell killing is concerned since they are readily repaired using the opposite strand. If the repair is incorrect, mutation occurs. If both strands of the DNA are broken and the breaks are well separated, repair again occurs since the two breaks are handled separately.

If the breaks on the two strands are opposite one another, or separated by a few base pairs, this may lead to double strand break and it is the most important lesion produced in chromosomes by radiation. The interaction of two double strand breaks may result in cell killing, mutation or carcinogenesis.

If cell killing is the result, the biological effect may be expressed hours to days later, when the damaged cell attempts to divide. Biological
systems are extremely sensitive to radiation. When a charged particle passes through any material, it leaves a track of excited and ionized atoms and molecules. For fast electrons, the energy releases are widely spaced and even if the track passes through the DNA, there is a chance that no energy gets deposited on the track and damage may not occur.

The track left by an alpha particle is so dense that when the track passes through DNA, there will be a lot of energy depositions and much damage can occur. Therefore alpha particles have greater LETs whereas electrons have lower LETs. Greater the charge, greater the LETs of the particles and greater biological damage can occur leading to cell death. Damage to DNA is critical for cell survival. Such damage may lead to bases in the DNA being altered or to the sugar phosphate backbone of the molecule being broken. The break can be in one strand or in both.

It is noted that there is a direct relationship between the number of microorganisms which are killed and the radiation exposure they receive. To express this in mathematical terms, the target theory was proposed by Growther and expanded by Lea.

The target theory is strictly a model which is considered to be applicable when the biological effect meets certain criteria in its relation to dose. The target may be a whole cell, part of a cell, or a critical molecule. And the measured effect in a system studied may be cell death.
or inability to grow and divide. In the simplest form of target theory it is assumed that a single hit is directly proportional to the dose.

Simple target theory assumes that the degree of effect is not influenced by the dose rate. Other assumption is that the experimental conditions during irradiation or immediately after are not of importance. These assumptions are not justified in most of the circumstances encountered in present day investigations.

The target theory is of great significance in explaining the kinetics of dose response of many organisms and the target theory has been modified to explain the mechanism of action of radiations in various cell systems.

Drastic biological effects such as killing of large animals have been observed with doses of 5 Gy. Due to this dose, the number of ion pairs that are produced in each cell due to ionizations will be of the order of $10^6$. Hence the number of possible chemical changes is of the order of $10^6$.

If it is assumed that the entire cell participates in the interaction, only a small fraction ($10^{-5}$) of the atoms will be affected (since the total number of atoms present in a cell is of the order of $10^{11}$). Thus it was assumed that only a limited volume is vital and all the biological effects
could be explained on the basis of the production of ionization in or in the vicinity of this critical volume which was given the name target.

The production of ionization within such a target is termed a hit. It was also assumed that in the cell or any other biological material which is being irradiated, there are one or more sensitive volumes within which either one (called a single hit) or several (multiple hit) ionizations have to occur to bring about the observed effect. From experimental data, it is possible to calculate $n$, the number of hits required and the size of the sensitive volume.

### 2.4.1. Single target theory

One of the most radiosensitive functions of a cell is its ability to retain indefinite proliferative capacity after irradiation. Cells that retain this capacity sufficiently to provide evidence of macroscopic growth are said to have survived the irradiation, while those that do not exhibit the same, are regarded as having been killed even though they may undergo a few divisions and continue to be present in the cell population.
The typical survival curves are shown in the Fig. 8 where the surviving fraction of cells is plotted on a log scale as a function of dose on a linear scale. At large doses these curves become straight showing that in this region the survival decreases exponentially with dose, while at small doses the curves are flattened giving a shoulder region.

The curves are based on the concept of the random nature of energy deposition by radiation. In a single hit process the survival curve is exponential. At very low doses, the probability for producing more than one ionization in one organism is very small. At such levels there is a linear relationship. As the process continues, larger doses needed to be given to inactivate same number of organisms.

This is because of the fact that the physical uptake of energy occurs to the same extent on organisms which have not been so far
affected as in those already affected. Ionisations occurring in already inactivated organisms will be wasted since one hit only is necessary.

If the initial linear relation had continued, each organism will be hit once. Thus the dose $D_0$ will be theoretically able to destroy each and every organism. Since radiation is wasted on organisms already inactivated, this dose $D_0$ does not destroy all the organisms in effect. The fraction surviving a dose of $D_0$ is seen to be 37%. Hence $D_0$ is called $D_{37}$ or the mean lethal dose (MLD).

The exponential nature of the survival curve and the significance of $D_0$ can be mathematically shown. Let the initial number of organisms be $N_0$, the number of organisms surviving a dose $D$ be $N$. An additional dose $dD$ will produce a further reduction of $dN$ in the number of organisms surviving.

\[-dN \propto N \text{ (number surviving)}\]
\[-dN \propto dD \text{(added dose)}\]
\[-dN \propto N \times dD \text{ (negative sign shows decrease in number surviving)}\]

Therefore \[-dN = k \cdot N \cdot dD\] or \[-dN / N = k \cdot dD\]

Where $k$ is the constant of proportionality.

Integrating,
\[- \log N = kD + c \text{ where } c \text{ is constant}\]

Applying the boundary condition, at \( D = 0, N = N_0 \) (initial number of Cells)

\[- \log N_0 = c\]

\[- \log N + \log N_0 = kD\]

\[\log \left(\frac{N}{N_0}\right) = -kD\]

\[\frac{N}{N_0} = e^{-kD} \text{ (single hit survival curve)}\]

Where \( \frac{N}{N_0} \) is termed as surviving fraction and \( k \) the inactivation constant

This equation signifies that the number of organisms surviving should decrease exponentially. When \( D = D_0, \frac{N}{N_0} = 37/100 \)

\[0.37 = e^{-kD_0} \quad \text{but} \quad 0.37 = 1/2.7183 = 1/e = e^{-1}\]

Therefore \( kD_0 = 1 \) or \( k = 1/D_0 \)

\[dN = -\left(\frac{1}{D_0}\right) N \, dD\]

If we place \( D = D_0 \), we get \( N = 0.37 \) N0. The mean lethal dose \( (D_0) \) is the dose required to reduce the population of entities from any value \( N \) to 0.37 \( N \) on parts of the survival curve that are exponential. Due to the random nature of the energy deposits, some energy will go to cells that have already been destroyed and will be wasted, while some cells will
escape altogether so that instead of destroying all the cells, the mean lethal dose ($D_0$) destroys only 63% of them.

Limitations of the simple target (single hit) theory: Target volume calculations based on this theory appears to be true only to some biologic targets such as enzymes, viruses and simple cells like bacteria. The multitarget, single hit model applies to more complicated biologic systems such as human cells.

2.4.2. Multi target single hit model

Complex biologic specimens such as human cells are thought to have more than a single critical target. Suppose that the human cell has two targets, each of which has to be inactivated for the cell to die. At very low radiation doses there will be nearly 100% cell survival.

As the radiation dose increases, fewer cells will survive because more will have sustained a hit in both target molecules. At some higher radiation dose all cells will have sustained at least one hit in one of its two targets. All cells that survive this dose will have one target hit, and therefore at all higher doses the dose-response relationship would appear as the single target, single hit model. The model of cell survival is multitarget, single hit model.
Fig. 9 The multitarget, single hit model of cell survival is characteristic of human cells containing two targets.

Fig. 10 Survival response curve for organisms, which has multi targets. Survival is plotted against dose on semi-logarithmic plot.
Suppose the cell has \( n \) targets, each of which must be inactivated to cause cell death. The probability of a single target not being hit is \( e^{-kD} \). Therefore the probability that any individual target will be hit is \( (1 - e^{-kD}) \). Hence the probability of all of the \( n \) targets within one cell being hit is \( (1 - e^{-kD})^n \). Thus the probability of survival of a cell that contains \( n \) targets is \( 1 - (1 - e^{-kD})^n \). This leads us to the multi-target single hit equation.

\[
N = N_0 (1 - (1 - e^{-kD})^n)
\] (multi target single hit)

For doses large compared with \( D_0 \), the above expression reduces to

\[
N = N_0 n e^{-kD}
\] valid for \( D >> D_0 \)

Or \( S = n e^{-kD} \)

When we express this in logarithmic form

\[
\log S = \log n - Kd
\]

Survival curves corresponding to multitarget type (Fig. 9) start with a less sensitive region at low doses and then tend to become exponential at larger doses. Due to this reason they present a picture of shoulder at smaller doses and hence termed as sigmoid or shoulder type survival curves.
Plotting log S against dose D we get a straight line. The slope is ‘−k’ for the straight line portion and when the straight line portion is extrapolated it forms an intercept ‘n’ referred to as, extrapolation number. ‘n’ represents number of targets. *(Fig. 10)*

**2.4.3. Limitations of multitarget single hit theory**

The multi target has two limitations. The first problem is the mathematical approach, which indicates zero slope at zero dose for any biological system showing number of targets n greater than 1. Hence the plot of survival versus dose must have zero dose. But experimental observations failed to meet this requirement.

This problem is overcome by assuming that some fraction of cells is killed through direct interaction of radiation. The corrected model describes the total damage by single target single hit in addition to the usual multi target single hit model.

The revised model is \[ S = e^{-k_1D} (1 - (1 - e^{-k_nD})^n) \] where \( k_1 \) refers to the portion of cells that are killed by single hit and \( k_n \) refers to multitarget portion as described by multi target single hit theory.

The second problem is a constantly increasing slope with decreasing survival fraction. This problem is addressed in linear quadratic model of cell survival *(Fig. 11 & Fig. 12)* which has two
components to cell killing by radiation; one is that proportional to dose and another proportional to the square of the dose.

**Fig. 11** Relationship between chromosomes aberrations and cell survival. Cells that suffer exchange type chromosome aberrations are unable to survive and continue to divide indefinitely. At low doses, the two chromosome breaks are the consequence of a single electron set in the motion by the absorption of x – or γ rays. The probability of an interaction between the breaks is proportional to dose. This is the linear portion of the survival curve. At higher doses, the two chromosomes breaks may result also from two separate electrons. The probability of an interaction is proportional to (dose)². The survival curve bends when the quadratic component dominates.

The notion of a component of cell inactivation that varies with the square of the dose introduces the concept of dual radiation action. This idea dates back to the early work with chromosomes in which many chromosome aberrations are clearly the result of two separate breaks.

By this model the expression for the cell survival curve is
$S = e^{-\alpha D - \beta D^2}$

Where $S$ is the fraction of cells surviving a dose $D$ and $\alpha$ and $\beta$ are constants. The components of cell killing that are proportional to dose and to the square of the dose are equal when

$$\alpha D = \beta D^2 \quad \text{or} \quad D = \frac{\alpha}{\beta}$$

**Fig. 12** Shape of survival curve for mammalian cells exposed to radiation

a) The experimental data fitted to linear quadratic function. There are 2 components of cell killing; one is proportional to dose ($\frac{\alpha}{D}$), while the other is proportional to the square of the dose ($\beta D^2$). The dose at which the linear and quadratic components are equal is the ratio $\frac{\alpha}{\beta}$. The linear quadratic curve bends continuously but good fit to experimental data for the first few decades of survival.

b) The curve is described by the initial slope ($D_1$), the final slope ($D_0$), and parameter that represent the width of the shoulder, either $n$ or $D_q$. 
A characteristic of the L-Q formulation is that the resultant cell survival curve is continuously bending. There is no final straight portion. The linear and quadratic components $\alpha D$ and $\beta D^2$ vary with the type of cells. For early responding tissues with a large fraction of fast dividing cells, $\alpha/\beta$ values are larger (10 Gy), and for late responding tissues the $\alpha/\beta$ values tend to be much smaller (1-2 Gy). $\alpha/\beta$ dose corresponds to the dose where the linear and quadratic components become equal. L-Q model can also be used to predict the cell killing or radiation damage following fractionated radiation.