Chapter-I

General Introduction
CANCER AND CARCINOGENESIS

CANCER

Cancer is a popular generic term for malignant neoplasms, a great group of diseases occurring in all human and animal populations and arising in all tissues composed of potentially dividing cells. The term ‘Cancer’ literally is derived from Latin word “cancrum” which means crab, probably because of the way a cancer adheres to any part that it seizes upon in an abstinate manner like the crab. It is a popular, generic term because the actual medical term for cancer is neoplasia, which from the Greek, means new formation (Kothari and Mehta, 1972). The basic characteristic of cancer is the abnormality of cells that is manifested by reduced control over growth and function leading to serious adverse effects on the host through invasive growth and metastases.

Characteristics of Cancer Cells

Cancers are new growths of the cells in our bodies. Cells are the basic unit of life-each of us has trillions of them. Our cells help us carry out all functions of life-from the beating of the heart to the throwing of a football. Malignant neoplasms refer to the fact that the new growth has virulent or adverse properties that may display in the body. The expression of these properties can cause destruction of major organs, and in some cases, life threatening destruction of major organs, and in some cases, life threatening disturbances in body function. Cells are dynamic as they are constantly in the process of making decisions about what they want to do next. Cells grow by dividing in half, such that one cell will become two, and two become four (these new cells are called daughter cells), etc. Normally, there are very strict rules as to when a cell can grow or not. These rules are set down by a variety of players, including all of the cells around it, various hormones in the body, and various external factors to which the cell may respond. One important example is growth of our bones from infancy to early adulthood.

The cell basically is set loose to divide without its normal control. When this happens, the cell continuous to divide eventually forms a new growth that is what we know as tumor or neoplasia. When the division reaches the point where the number of daughter cells is 1,000,000,000 (one billion), it is detectable by
conventional examination methods, like a chest x-ray or a rectal examination. The basic characteristic of cancer is the abnormality cells that are manifested by reduced control over growth and function leading to serious adverse effects on the host through invasive growth and metastases. When a cell is set loose from normal control, it becomes what is known as transformed.

Basically the cell no longer looks like its neighbors in terms of its shape, size, and its internal components. This transformed property is conferred upon all of daughter cells. That is all subsequent cells that arise from that initially transformed cell will also look different and grow in an uncontrolled manner. This is the nature of cancer—once one cell becomes cancereous, all cells that arise from this abnormal cell will also take on this characteristic. They gain these properties because rapid growth causes more mistakes to be made in the DNA of the cell—the chemical in the cell that allows it to carry out is normal functions. It now may become motile, as if it were a white blood cell, or it may lose the ability to remain in contact with its neighbouring cells. When these events take place, the malignant cells may leave the local site and travel in the body fluids to a distant organ. This process is known as metastases.

**Etiology & Occurrence of cancer**

Cancer is one of the major public health problems and the largest causes of death. Currently it is the second largest cause of death and epidemiologist suggest that it will become the leading cause in near future. The scientific study of cancer classically dates from 1775 when Sir Percival Pott observed that men who had been chimney sweeps as boys had a high rate of death due to cancer of the scrotum. According to Sir Pott, tumors might arise from exposure to the chimney soot and recommended frequent washings and change in clothes to reduce exposure to the carcinogen (agents capable of producing cancer). He also demonstrated that cancer might develop many years after exposure to such factors. It is well known that three types of factors individually or in combination increase an individual’s risk of developing cancer. These three risk factors include (a) life style (b) environment and (c) heredity. Life style factors include different behaviors over which the individuals have some control e.g. use of tobacco, diet, alcohol use, excessive exposure to sunlight, different sexual behavior patterns and general personal hygiene.
Environmental factors include both occupational exposure to carcinogens and various naturally occurring contaminants in air, water and land. Genetic factors include conditions inherited at conception. About 90% of cancers in USA are estimated to be caused due to style patterns and environment conditions (Doll and Peto, 1981). It is apparent that a variety of different and seemingly unrelated factors can cause cancer. They include a large number of different chemicals, viruses and both ultraviolet and ionizing radiation (Rous, 1941). Most of these agents share the property of causing damage or alteration of the cellular deoxyribonucleic acid (DNA).

This common property suggest that DNA is probably the essential target of all carcinogenic agents and that cancer arises as a result of changes in cellular DNA (Kohn, 1979). This hypothesis has been immeasurably strengthened by the discovery of a number of genes, called oncogenes, which can transform normal cells into cancer cells (Bishop, 1985). The normal mammalian cells contain genes called proto-oncogenes, which are similar in base sequence to the transforming oncogenes that are carried by some tumor viruses (Hunter, 1984). One or more of these proto-oncogenes may be altered in malignant cells. Some types of chromosomal rearrangements, characteristics of particular tumors, have been shown to involve proto oncogenes, with resultant changes in the protein products of these genes (Rowley, 1984).

Free radicals are molecular with one or more unpaired electrons. The reactive radicals responsible for tissue damage are generally short-lived species that are generated in situ (Freeman and Crapo, 1982). Free radicals are produced in normal or pathological cell metabolism, from Xenobiotics or through ionizing radiation. An important feature of free radical reactions is that they result in new radicals, which leads to chain reactions (Halliwell and Gutteridge, 1985). Electron acceptors such as molecular oxygen react easily with free radicals to become radicals themselves the oxygen free radicals (OFR). OFR are continuously generated in cells exposed to an aerobic environment. Despite the antioxidant defenses OFR-related damage of proteins (Davies, 1993) and DNA (Lindahl, 1993) accumulates during life and has been postulated to lead age dependent disease like atherosclerosis, neuro degenerative disorders and cancer (Halliwell et al, 1992;
A number of endogenous and exogenous cancer risk factors generate OFR \textit{in vivo} (Nakayama et al, 1985). In recent years, convincing evidence has accumulate that OFR are indeed a relevant class of carcinogens (Guyton and kensler, 1993; Cerutti 1994; Feig, et al, 1994).

\textbf{Differentiation of Cancer Cells}

Cell proliferation is controlled by positive as well as negative regulatory mechanisms and influence growth state transition and progress through the cell cycle. Neoplastic growth is due to increased sensitivity to the activation of growth stimulatory mechanisms or from decreased sensitivity or inactivation of growth inhibitory mechanism (Marx, 1986). The development of growth of any complex organism requires coordinated interactions between cells of the organism. The growth of cancer demonstrates the failure of such control mechanisms. This failure may results from carcinogenic damage to the DNA of cancer cell, rendering unresponsive to normal control mechanisms or it may result from disturbance in the homeostatic control mechanisms themselves. Most normal tissues are differentiated i.e., they have developed a specialized function and appearance. During the differentiation process, cells generally lose their ability to proliferate, but many tissues retain a fraction of undifferentiated stem cells, which can divide to replace the loss of mature cells (Strauss, 1981).

Most cancers appear to originate from such precursor cells (Vogeistein, et al 1985). A large body of evidence suggests that cancers are clonal i.e. arising from a single precursor cell (Nowell, 1976). While the tumors originate from single clone of cells, the process of neoplastic development is associated with development of marked heterogeneity of biochemical, cytogenetic and antigenic features. Neoplastic development is also associated with progressive autonomy from control mechanisms and normal differentiation is impaired with the development of progressively more anaplastic morphology (Foulds, 1975).
Cell Lineages in Cancer

Most tissues that are targets for carcinogenesis either consist of renewing cell populations, or populations that can undergo renewal in the face of demand created by tissue damage, or excessive hormonal stimulation. In renewing cell populations, it is widely held that stem cells, that is, those cells capable of renewing themselves through division or giving rise to differentiated progeny, are the targets for carcinogenesis. For tissue outside of the haemopoietic or lymphoid systems there are usually no marks for stem cell populations (Hall and Watt, 1989). From a large body of work describing these neoplasms, the conclusions may be drawn (Greaves 1986). First leukemic stem cells resemble very closely normal pyrogenitor cells at various stages of the differentiation pathway, from the multipotential stem cells of chronic myeloid leukemia to the immunocompetent cells that makeup myelomas. Second, the normal counterparts of the malignant cells are difficult to underneath, because they comprise only a tiny minority of adult tissue cells. The normal counterparts of lymphoid tumor cells are present in substantial, numbers only during fetal life or tissue regeneration which is not surprising because, in general, stem cells only constitute a major population of the tissue. Finally the leukemias and lymphomas may be described as a set of ‘frozen’ states of blood cells differentiation arrested at various stages of development.

In most other types of malignancy the relation of the tumor to normal stem cells populations is more problem oriented. In mouse teratocarcinoma, biological and immunological data suggest that these cells are very similar to the multipotent stem cells of the early embryo (Martin, 1980). In the human malignancies a related family of cell types can be described, some of which are multipotent and some of which have undergone commitment to differentiate along extra-embryonic lineages to trophoblastic or yolk sac cells (Pera, et al., 1990). In the extensive experimental analysis of liver cancer, disparate opinions hold either the tumors arise from a cell that undergoes differentiation towards a fetal phenotype, or there are stem cells in the liver, which are called into, play during repair and the progenitors of the tumors (Sell and Dunsford, 1989). Despite the prevailing tendency to view neoplasms as caricatures of cell renewal and to consider stem cells targets in chemicals carcinogenesis, there are other aspects of cancer cell phenotype that are difficult to
reconcile with conventional ideas of cell lineage. Evidence for other types of lineage relations exists (Anderson, 1989). From biological standpoint two major postulates of the origin of cancer are: Cancer arises from differentiated expressive cells by ‘dedifferentiation’ or from pluripotential stem cells by aberrant differentiation.

One of the fundamental postulates of the stem cell theory of cancer is that the incidence of cancer arising in a given tissue is directly related to the rate of cell divisions in that tissue. Stem cells are defined as multipotent cells that divide to produce one daughter cell that stays as a stem while the other daughter cell expresses a differentiated phenotype. Tissue stem cells are determined i.e., they lack the biochemical and structural markers of differentiation but are determined for differentiation to specific cell type (Pierce, et al., 1978). Stem cells respond by proliferation and differentiation to replace senescent cells under normal circumstances or to restore destroyed tissues in pathological condition. The epithelium of the skin is the classic example or the former. Stem cells may participate in tissue replacement in organs that normally undergo renewal, such as haemopoietic cells. But most of the actively dividing cells in these organs are not the pluripotent stem cells but mitotically active partially differentiated expressive cells that give rise by further division and differentiated to terminally differentiated cells that make up the ‘mature’ cells of the organ (Stocum, 1984).

CARCINOGENESIS

Carcinogenesis is a general term used to denote the development of neoplasia. It may be actively induced in living organisms by a variety of different agents. Carcinogenic agents can be grouped into four relatively distinct categories. (a) Physical (b) Chemical (c) Biological and (d) Genetic (Pitot, 1987; Enomoto, et al., 1990). In experimental carcinogenesis, the action of some carcinogenic agents may play a major role in uncontrolled growth of cells (Pitot and Dragon, 1991). Table 1 represents examples of each type of carcinogenic agents and the range of their molecular masses in Daltons. In both biological and genetic carcinogenesis the carcinogenic agent consists of informational macromolecules, either DNA or RNA, of relatively high molecular weight. On the other hand, radiation carcinogenesis may not result from the action of informational macromolecules, but rather from the direct (Haseltine, 1983) or indirect (Biaglow, 1981) action of high-energy photons or
particles with existing DNA. Carcinogenesis by many small molecular weight chemicals involves either a direct action of the chemical on cellular DNA or metabolism of the parent chemical to an active or ultimate form, which can then react with cellular DNA to produce a permanent chemical change in the DNA structure (Pitot, 1986a).

Further there are a number of examples, particularly in chemical carcinogenesis, in which the induction of carcinogenesis does not directly involve structural modification of DNA (Mikol, et al., 1983; Ashby and Tennant, 1988). Examples of such carcinogenic agents are given in Table 2. Furthermore, some agents are able to induce structural changes in DNA molecule without evidence that they are carcinogenic (Shelby and Stasiewiez, 1984). Thus, it would not appear possible to develop a general mechanism of carcinogenesis that is applicable to all carcinogenic agents (Pitot and Dragon, 1991). On the other hand, a reconciliation of the disparate actions of carcinogenic agents with a general process of carcinogenesis may be viewed differently in the light of our increased understanding of natural history of pathogenesis of neoplasia.
Table 1.
General Classification of Carcinogenic Events

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples</th>
<th>Relative Molecular Mass (in Daltons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYSICAL</td>
<td>Ionising x-ray and γ- ray, particle radiation and Ultraviolet (UV) radiation (UVA &amp; UVB)</td>
<td>$5 \times 10^{-1} - 5 \times 10^{4}$</td>
</tr>
<tr>
<td>CHEMICAL</td>
<td>Ploycyclic hydrocarbon, Aromatic amines and halides, Diet, Hormones, Metals &amp; Polymer surfaces.</td>
<td>$&lt;&lt;&lt;1-1^+$</td>
</tr>
<tr>
<td>BIOLOGICAL</td>
<td>Viruses (Papova, Herpes, Retro and Hepanda virues).</td>
<td>$3 \times 10^{6} - 170 \times 10^{6}$</td>
</tr>
<tr>
<td>GENETIC</td>
<td>Transgenesis by enhancer-promoter- oncogene constituents and selective breeding.</td>
<td>$10^{6} - 10^{8}$</td>
</tr>
</tbody>
</table>

Table 2.
Carcinogenic agents or processes exhibiting no capacity for inducing direct structural DNA changes

Carcinogenic Agents
Polypeptide and steroid hormones
Inert substances (Plastic film carcinogenesis)
Alcoholic beverages (ethanol)
Specific dietary alterations (methyl deficiency, galactosamine excess)
Chemical with no DNA-reactive intermediates, calorie intake
**Multistage of Carcinogenesis**

Neoplasia, like much other disease, is preceded by a latent period from the time of the first application of cancer producing agent (carcinogen) to the development of visible neoplastic lesions. The initial understanding of biological events occurring during the latency period of neoplasia was pioneered during the early 1940s by several investigators studying epidermal carcinogenesis in mouse model (Rous and kidd, 1941; Berenblum and Shubik, 1947). These studies demonstrated that at least two distinct stages of the latent period could be identified. These were termed initiation and promotion. Foulds, (1954) modified the concept of stage of promotion to include all events after initiation of neoplastic process by using mouse mammary adenocarcinoma model. He used the term progression to describe all post-initiation events in neoplastic development. So that, it has become clear that at least two stages now termed promotion and progression, following the stage of initiation is into existence. This demarcation was partly a result of the use of carcinogenic protocols different from those of the original experiments of the 1940s and 1950s (Potter, 1981; Hennings and Yuspa, 1985).

In addition, the multistage concept of the development of neoplasia has been demonstrated during carcinogenesis in variety of different tissues in the post decades. Now cancer development is commonly recognized as a micro evolutionary process that requires the cumulative action of multiple events (Klein, 1987). These events occur in one cell clone and include in simplified three-stage model: (1) Induction of DNA mutation in a somatic cell (initiation). (2) The stimulation of tumorigenic expansion of the cell clone (promotion). (3) The malignant conversion of tumor into cancer (progression). Oxygen free radical can stimulate cancer development at all three stages, initiation (Hussain, et al., 1994), promotion (Nakamura et al., 1988) and progression (Salim, 1993). The most significant characteristic features of initiation promotion and progression are given in Table 3.
### Table 3.

**Characteristic Features of the stages of Carcinogenesis**

<table>
<thead>
<tr>
<th>Stages</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation</td>
<td>Irreversible</td>
</tr>
<tr>
<td></td>
<td>Requires Fixation</td>
</tr>
<tr>
<td></td>
<td>Additive No threshold</td>
</tr>
<tr>
<td>Promotion</td>
<td>Reversible</td>
</tr>
<tr>
<td></td>
<td>Environmentally modulated</td>
</tr>
<tr>
<td></td>
<td>Maximal response</td>
</tr>
<tr>
<td></td>
<td>Threshold</td>
</tr>
<tr>
<td>Progression</td>
<td>Irreversible</td>
</tr>
<tr>
<td></td>
<td>Somatic aneuploidy</td>
</tr>
<tr>
<td></td>
<td>Progressive karyotypic instability</td>
</tr>
</tbody>
</table>
Initiation

The stage of initiation, which occurs first in the natural history of neoplastic development, reflects a permanent and irreversible change in the initiated cell (Boutwell, 1964). It involves exposure of normal cells to chemical, physical or microbial carcinogens that cause a genetic change(s) providing the initiated cells with both, an altered responsiveness to their microenvironment and moreover exerts a selective clonal expansion advantage when compared to the surrounding normal cells (Yuspa and Harris, 1982). The initiated cells may have decreased responsiveness to inter and intracellular signals that maintain normal tissue architecture and regulate the homoeostatic growth and maturation cells. For example, initiated cells may be less responsive to negative growth factors, inducers of terminal cell differentiation and/or programmed cell death, i.e. apoptosis (Yuspa and Poirier, 1988; Moses, et al., 1990; Rotello, et al., 1991). However the efficiency of initiation is related to cellular DNA synthesis and cell division (Ishikawa, 1980). Furthermore, DNA synthesis is required for the fixation and thus the irreversibility of the initiated cells (McCormick and Bertram, 1982). The stage of initiation can be altered by exogeneous and endogeneous factors.

A variety of chemicals in several different tissues can inhibit the metabolism of chemicals to their ultimate forms, thereby blocking initiation (Wattenberg, 1978). The presence or absence of a threshold or no-effect level for initiating agents has been evaluated only by extrapolation in most studies. Inferences as to the absence of a threshold for initiating agents comes from the study of mutations that results from these agents. Because initiation cannot in all circumstances be equated with initiation, one measurement of initiation can be performed by quantitatively evaluating the number of preneoplastic focal lesions induced by the initiating agent such as enzyme-altered foci in multistage hepatocarcinogenesis (Pitot, 1990). With this system, the process of initiation appears to be linear dose-related phenomenon that does not exhibit a readily measurable threshold (Pitot, 1987). Using this method, it is possible to calculate the relative potency of agents as initiators for hepatocarcinogenesis. The calculations are used to evaluate complete carcinogens for their initiation capacity; it is critical that the dose be non-toxic and sub-carcinogenic as shown in Table 4.
Table 4
Classification of chemical carcinogens in relation to their action on one or more stages of carcinogenesis

<table>
<thead>
<tr>
<th>INITIATOR (Incomplete Carcinogen)</th>
<th>An agent capable of initiating cells only</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROMOTER</td>
<td>An agent capable of causing the reversible expansion of initiated cell clones (e.g. UVA radiation)</td>
</tr>
<tr>
<td>PROGRESSOR</td>
<td>An agent capable of converting an initiated cell or a cell in the stage of promotion to a potentially malignant cell (e.g., Hepatitis B virus)</td>
</tr>
<tr>
<td>COMPLETE CARCINOGEN</td>
<td>An agent possessing the capability of inducing cancer in normal cell, usually possessing properties of initiating, promoting and progressor agents.</td>
</tr>
</tbody>
</table>
Promotion

The principal characteristic of promotion that distinguishes it from the stage of initiation and progression is its operational reversibility. Promotion results in proliferation and/or survival of the initiated cells to a greater extent than normal cells and enhances the probability of additional genetic damage including endogenous mutations accumulating in the expanding population of these cells. The probability of a sub-population of initiated cells converting to malignancy can be substantially increased by their further exposure to DNA-damaging agents (Hennings, 1989) that may activate protooncogenes (Yuspa, et al., 1990) and/or inactivate tumor suppressor genes. This characteristic is evident in several model systems of multistage carcinogenesis (Hendrich, et al., 1986). In contrast to the stage of initiation, promotion may be continually modulated by a variety of environmental factors, including frequency with which the promoting agent is administered (Wattenberg, 1978), age of the test animal (Van Duren, et al., 1975), and composition and amount of the diet (Boutwell, 1964).

Many promoting agents exert their effect on the cell through receptor mediated mechanism (Pitot, 1986a), the dose response curve of promoting agents exhibit a threshold or no-effect level as well as maximum response (Verma and Boutwell, 1980; Goldsworthy, et al., 1984). The latter effect results from the fact that a given dose of initiating agent will give rise to a finite number of initiated cells, and no more can be promoted if the promoting agents itself possesses no initiation. Promoting agents increase the risk of cancer development by increasing the proliferation rate of normal cell, enhancing the likelihood of propagating a genetic error. Moreover, selective increase in the growth of passively initiated cells can result in tumorigenesis from exposure to promoting agents. Continuous exposure to promoting agents in the human as in the experimental animal can result in malignancy as a result of the passive (Spontaneous) occurrence of the stage of progression in one or more cells in the stage of promotion (Pitot and Dragon, 1991). Most promoting agents are tissue specific, do not required metabolic activation and are non-mutagenic (Diamond, et al., 1980) do not bind covalently to DNA, but bring about a number of important epigenetic changes (Slaga, et al., 1983). They are structurally specific, so that, minor changes in chemical structure markedly affect promoting activity (Hecker, 1978; Diamond, et al., 1980). Experimental
demonstration of the reversible stage of promotion depends to a significant degree on the dose and nature of carcinogenic agent used to induce the stage of initiation.

**Progression**

The stage of progression may be characterized primarily by its Karyotypic instability and the development of irreversible, aneuploid malignant neosomes distinguishes progression from both initiation and promotion (Pitot, 1987). Such alterations in the structure of genome of the neoplastic cell during this stage are directly related to the increased growth rate, invasiveness, and metabolic capability and biochemical changes in the malignant cells. These changes are reflections of karyotypic alterations; continue to evolve (progress) during this stage of progression in a variety of different neoplasms, such as multistage hepatocarcinogenesis (Sergent, at al., 1989). The irreversibility of progression is assumed because of obvious alterations in the cell genome that accompany this stage, distinguishing progression from the reversible, preceding the stage of promotion. However, it is clear that under certain circumstance, cells in any stage of carcinogenesis may terminally differentiate, thereby removing from the continued advancement to the malignant state (Reiss, et al., 1986). Other environmental alterations can produce change in gene expression, growth rate and functional processes with in cells during progression (Noble, 1977; Horsfall, et al., 1986). Evidence is beginning to develop that there are certain limited “windows of differentiation” in which particular genes are important in neoplastic development (“oncogenes”) and may functional certain cell lineages and stages of oncogeny (Croce and Nowell, 1985; Ford and Maizel, 1986).

Agents that can act only during progression are at least advance a cell from promotion to progression, have not yet been defiantly characterized in most systems, although the free radicals generators like benzoyl peroxide appear to act as progressor agent in experimental epidermal carcinogenesis (O’Connel, et al., 1986). Theoretically such progressor agents should be capable of inducing the genetic damage characteristic of progression: examples of such agents would be clastogens and complete carcinogens, i.e. single agent capable of inducing malignant transformation from initiation through progression. And other hand, considerable evidence supports the propoission that malignant neoplasms may all exhibit an
abnormal expression of one or more proto and cellular oncogenes, although know single such gene is involved in all malignant neoplasia (Pitot, 1986b). It is possible that transcriptional and/or mutational activation of proto and cellular oncogenes, growth factors, nuclear DNA binding proteins and other as yet unknown cellular oncogenes may occur early during the stage of progression and be mechanistically associated with its development (Pitot and Dragon, 1991).

In multi stage hepatocarcinogenesis in rat, transcriptional activation of proto and cellular oncogenes as not been consistently identified in altered hepatic foci during promotion it has been repotted that increase in C-Ha-ras gene expression in altered in hepatic foci after a necrogenic dose of DEN, which itself can induce the stage of progression (Galand, et al., 1988; Sargent, et al., 1989). Such a mechanism might be predicted from the carcinogenesis by the actually oncogenic retroviruses.

**Mitogenesis and cancer**

The study of the mechanisms of carcinogenesis is rapidly developing field that can improve regulatory policy. Both DNA damage and mitogenesis are important aspect of carcinogenesis and increasing either substantially can cause cancer (Ames, et al., 1989; Farber, 1987; Pitot, et al., 1987; Dunsford, et al., 1990). Mutagens are thought to be only exogeneous agents, but endogeneous mutagens cause massive DNA damage (oxidative and other adducts) that can be converted to mutations during cell division. It is estimated that the DNA hits per cell per day from endogeneous oxidants are normally= $10^5$ in the rat and= $10^4$ in the human (Ames, 1989, Fraga, et al., 1990). Thus, any agent causing chronic mitogenesis can be indirectly mutagenic (and consequently carcinogenic) because it increases the probability of endogeneous promutagenic DNA adducts being converted to mutations. Furthermore, endogeneous rates of DNA damage are so high that it may be difficult for exogeneous mutagens to increase the total DNA damage are so high that it may be difficult for exogeneous mutagens to increase the total DNA damage significantly by low dose that do not increase mitogenesis (Ames and Gold, 1990).
At near toxic doses some chemicals interfere with cell-cell communication in quiescent tissues (e.g., the liver, the major target site for carcinogenesis in rodents), thereby causing mitogenesis and carcinogenesis. Trosko (1989) have proposed that suppression of gap junction communication in contact-inhibited cells could lead to cell proliferation by cell death, cell removal, promoting chemicals, specific oncogenic products, growth factors and hormones. Thus, agents causing mitogenesis are proper carcinogens and are important in human cancer. The cell division induced in the rat liver by certain mitogens (without cell killing) is less potentially carcinogenic than cell division induced by toxicity (cell killing and cell replacement) (Collumbano, et al., 1990). Classical tumour”promoters” such as Phenobarbital and phorbol myristate acetate cause mitogenesis and are in fact complete carcinogens in animal (leversen, 1988).

**Chemical Carcinogens**

The biological effects of chemical carcinogens depend upon their interaction with cell constituents, which occurs via covalent reactions that lead to the formation of carcinogen adducts (Cooper, et al., 1995). When the known mechanisms of action of carcinogenic agents are placed in the context of the natural history of neoplastic development, it is possible to reconcile many if not all of the apparent discrepancies. Paramount among such considerations is the possibility of the more selective action of carcinogenic agents at one or two rather than all three stages of neoplastic development. The classification of such chemical agents is depicted in Table 5. Some of the chemical agents although exerting their primary effects at one stage of carcinogenesis, may exhibit some capacity for effects at different stage. It has been difficult to identify chemical agents that act only as inhibitors but most complete carcinogens, when administered at doses that give rise to few, if not may malignant neoplasms during the life span of the animal, may be spontaneously reactive in biological media but for most part, they are inert and require conversion to a reactive intermediate.
Table 5.
Stage of Carcinogenesis induced by Specific Agents

<table>
<thead>
<tr>
<th>Group</th>
<th>Example</th>
<th>Stage(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DiethylNitrosamine 1,2 Dimethyl hydrazine Aflatoxin B 2- Acetylaminofluorene Methylcholanthrene</td>
<td>Initiation Promotion &amp; Progression</td>
</tr>
<tr>
<td></td>
<td>Phenobarbital, Tetradecanoyl, Phorbolacetate, Dietary fat and Calories.</td>
<td>Promotion (Progression)</td>
</tr>
<tr>
<td></td>
<td>Prolactin, Estrogens and Androgens, Asbestos, Benzene, Potassium arsenite</td>
<td>Promotion Progression</td>
</tr>
<tr>
<td>II</td>
<td>Ionizing radiation (UVB and UVC) UVA radiation</td>
<td>Initiation Promotion &amp; Progression</td>
</tr>
<tr>
<td>III</td>
<td>Papova, Retro and Epstein-Barr viruses</td>
<td>Promotion &amp; Progression</td>
</tr>
<tr>
<td></td>
<td>Herpes and Hepanda Viruses</td>
<td>Progression</td>
</tr>
<tr>
<td>IV</td>
<td>Transgenesis</td>
<td>Promotion &amp; Progression</td>
</tr>
<tr>
<td></td>
<td>Selective Breeding</td>
<td>Initiation Promotion &amp; Progression</td>
</tr>
</tbody>
</table>
It is likely that promoting agents play a major role in the development of the most common human cancer in the world today, including lung, breast, G.I.tract, prostate and others. Potential human progressor agents include asbestos, benzene and arsenicals as well as diethyl-stibestrol. One commonality of these agents is that they are clastogenic or capable of inducing chromosomal abnormalities in vivo and in vitro (Pitot, 1985). Studies on the mechanism of carcinogen action have focused the role of DNA adducts and their ability to introduced heritable changes in DNA (Cooper, et al.,1995). Therefore, DNA has become universally recognized sa a critical of action of chemicals and chemical mixtures in relation to specific stage of neoplastic development makes possible reconciliation of the mechanistic theories of chemical carcinogenesis in the light of multistage carcinogenesis (Pitot and Dragon, 1991).

**COLON CARCINOGENESIS**

*Anatomy and Physiology*

The large intestine extends from the end of the ileum to the anus and the anal canal. The colon is divided into the caecum, the colon rectum and the anal canal. The colon is defined as the part of the large intestine, which extends from the caecum to the rectum. Surgically, it is considered too begin at ileocecal valve and extends to the peritoneal reflexion at the junction of the sigmoid colon and rectum. It is about 120-200m in length, not more than one fourth of the length of the small intestine with the diameter diminishing gradually from 7.5-2.5 cm at the termination. However, the colon is capable of a great increase in circumference by distention. The arrangement of the lymphatics is uniform throughout the colon. The colon can be further subdivided into 4 parts: the ascending, transverse, descending and sigmoid colon (Gray, 1962). The major function of the mammalian colon is to render the waste products of digestion fit for elimination, to a form and at a time causing least inconvenience to the organism. This is accomplished through absorption of water and electrolytes from the chyme-About 1500of chime pass into the large intestine each day. Most of the absorption of water and electrolytes occur in the proximal half of the colon.
The colon mucosa has a high capability for active absorption of the sodium ion and the electrical potential created by the absorption of sodium and chloride ions create an osmotic gradient across the colonic mucosa which in turn cause absorption of water. Storage of fecal matter until it can be expelled. Expulsion of the excreta in a clear and efficient manner by a well coordinated series of never pulses and muscular activity. In the performance of these functions the colon may be considered physiologically to comprise of two separate units; the right colon being concerned with absorption and reduction in the bulk of the excreta and the left colon with the storage of feces and their expulsion. The movement of the colon are normally sluggish as intense movements are not required for these functions. They can be characterized further into mixing movements comprising of haustrations and propulsive movements concluding both haustral contractions and mass movements. The colon secretes only mucus manufactured by mucosal membrane and hence has no digestive function. The mucus essentially acts as protection of the mucosa and lubricates fecal mass since it has an average N-content between 7-12%, large amounts of nitrogen may be lost by people having chronic diarrhea associated with a profuse discharge of mucus. Numerous bacteria, especially colonic bacilli, are present, even normally, in the absorbing colon. These are capable of digesting small amounts of cellulose in this way providing a few calories of nutrition to the body each day.

**Epidemiology**

Colorectal cancer is a dynamically changing disease entity and the reason for it are multifactorial. Worldwide the increase rates of colorectal cancer vary widely from 3.4/100000 populations in Nigeria to 35.8 cases/10900 populations in Connecticut (Schottenfeld and Winawer, 1982). North America, Australia, Newzealand and portions of Northern and Western Europe have a high incidence of the disease (Ziegler, et al., 1986). Approximately 133,500 new cases of colorectal cancer were diagnosed in U.S in 1996 and 54,000 patients died of the disease. There is also an increase in the incidence and mortality of colon cancer in Japan and China as well as in immigrants to the United States, from low risk areas (Chu, et al., 1994). It has been also observed that there is a progressive trend towards disease of the right colon and fewer in the left colon and rectum (Steele, 1994). There is much controversy regarding the reasons for the overall decline in colon cancer mortality in
the U.S (Kelsen, et al., 1994). Possible explanations include the improvements in surgical and adjuvant therapies, dietary changes such as an increase in fiber and decrease in saturated fat and cholesterol and more extensive screening programs (Giovannucci, et al., 1992). Two religious groups in the U.S have diminished risk for bowel cancer.

**Etiology**

It has long been postulated that colorectal cancer is caused or promoted by environmental factors, especially by factors that affect the dietary milieu (Weisburger and Wynder, 1987). This common malignancy results from a complex interaction between environmental and host factors. It is suspected that carcinogens are present in feces (Willett and MacMahon, 1984). Mutagens are present in stools of many people who eat a Western diet (Reddy, et al., 1989). The role of these mutagens as an etiologic factor in colorectal cancer remains undefined. Although it is not possible to identify a specific cause of colon cancer, epidemiologic studies of nutritional habits and migration patterns are revealing. Both national and international studies reveal a clear association of human colorectal cancer with certain diets, such as those rich in animal fat and meat and poor in fibre and with certain high risk populations (Palmer and Bashi, 1983). Dietary factors also have an impact on the formation of adenomas. At this time, it is still unclear at what stages dietary and nutritional factors exert their effects as well as their interaction, if any, with other variables such as genetic factors.

A number of case control studies reveal an association between increased fat intake and colorectal cancer. Studies of immigrant populations to Hawaii, populations in Nebraska (Pickle, et al., 1984). Limitation of total dietary fat and cholesterol has been often proposed as a mechanism to decrease colorectal cancer (Weisburger and Wynder, 1987). Studies with animal models of colon cancer strongly demonstrate that fat act during the promotion stage of carcinogenesis and that both the type and quantity of fat are important determinants (Greenwald and Witkin, 1991). Giovannucci and colleagues reported that daily alcohol intake was associated with an almost two fold increased risk of colon cancer (Giovannucci and Rimm, 1995). The correlation was stronger for distal more than proximal cancers. In a trial by Martinez and co-workers, current alcohol use as well as past and current
smoking each independently increased the risk of developing colorectal adenomas (Martinez and McPherson, 1995).

To identify the causative factors more precisely, a number of etiologic hypotheses have been tested preclinically, epidemiologically or with clinical intervention. They include

**Fecapentanes:** are potent mutagenic compounds found in human feces and thought to be produced by gut microflora. They were active in the Ames Salmonella assay and in mammalian cell systems (Curren, et al., 1987).

**3-Ketosteroids:** Presumed to be derived from metabolic products of cholesterol, 3-Ketosteroids are potential tumor promoters or inhibitors. They induce genetic damage in cell cultures and rodent bowel (Susuki, et al., 1986). At least two of these compounds have been identified in human feces and they may be present in high concentration in persons at higher risk for colon cancer (Bird and Bruce, 1982). Because the agent thought to be responsible for conjugation (4,6-dience-3-one), has not been found in feces, interest in these compounds has waned (Bird, 1986).

**Pyrrolysis products:** Compounds that result from the broiling or frying of meat at high temperature, such as benzo (a-pyrene), have proved carcinogenic in rodents (Wait, et al., 1985). Gerhardson and colleagues reported that the association between meat consumption and colorectal cancer risk was highest when the meat surface was heavily browned during frying.

**Normal Bile Acid:** Directly related to the intact of fat, bile acids such as deoxycholic and cholic acids are thought to induce gut lumen proliferation (Tanaka, et al., 1985). Populations that consume more fat have more bile acid secretion and an associated increased incidence of colon cancer (Hill, et al., 1975). It is the free not total bile acid concentration that is most critical (Minsky and Huant, 1995). High-risk groups for colon cancer development also include

- Family History
- Family Adenomatous Polyposis
- Personal History of Cancer of polyp
- Ulcerative Colilitis.
The theory of colon carcinogenesis suggests an orderly progression of tumor development from normal mucosa to hyperproliferative mucos to small polyps with little malignant potential, to larger polyps with dysplasia, to the transformed but non-invasive cells that constitute carcinoma in situ, to tumors with distant metastasis. “Every dogma must have its day”, wrote HG Wells and reiging dogma in colorectal carcinogenesis since the late 1980s has been the multistep model created by Fearon and Vogelstein (1990). Figure 1. premalignant changes in colonic mucosa appear to the microscopist as “ dysplasia” a term used in this setting to signify a neoplastic transformation with the potential to proceed to malignancy. Dysplasia takes the form of cells with enlarged, dark nuclei, often with decreased amounts of cytoplasmic mucin that pile up in the colonic crypt. Even small neoplasms are clonal, indicating that the mutations that cause dysplasia occur in one or a few stem cells and confers a proliferation/survival advantage that allows the neoplastic clone to replace first one crypt, then several adjacent crypts.

Eventually the Proliferating cells produced a mass visible to the naked eye, the adenoma. An adenoma, then is a mass of cells showing low-grade dysplasia. A sub-set of adenomas will develop areas of high-grade dysplasia (sometimes called carcinoma in situ) with loss of normal architecture and significant variation in nuclear size and shape. Experience has shown that adenomas are more likely to harbor high-grade dysplasia when they are larger and when they have villous architecture. “Intermediate adenoma” corresponds to an adenoma with high-grade dysplasia.
<table>
<thead>
<tr>
<th>Morphologic Changes</th>
<th>Mutated or Lost Genes (Chromosomes)</th>
<th>Gene Classification &amp; presumed function</th>
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<tr>
<td>Normal Mucosa</td>
<td>APC (5q)</td>
<td>Tumor suppressor gene, Cell adhesion</td>
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<tr>
<td>Early Adenoma</td>
<td>K-RAS (12q)</td>
<td>Oncogene; cell membrane-bound signal transducer</td>
</tr>
<tr>
<td>Intermediate Adenoma</td>
<td>DCC (18q)</td>
<td>Tumor suppressor gene; cell adhesion molecule</td>
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<tr>
<td>Late Adenoma</td>
<td>P53 (17p)</td>
<td>Tumor suppressor gene; cell cycle regulator</td>
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Figure 1. The multistep model of colorectal cancer. Progressive morphologic changes are accompanied by increasing numbers of genetic alterations. The net accumulation of mutations is more important than the order in which they are acquired.
Several asides are necessary here. First, “preadenomatus” lesions have been proposed: the aberrant crypt foci (ACF). ACF were originally described in carcinogen-treated rats and mice but have since been found in humans. They are collections of enlarged crypts with thickened epithelial lining. ACF are slightly elevated above the surrounding mucosa and can be seen grossly under a magnifying lens. The epithelial lining varies, some ACF have a serrated lining similar to that of hyperplastic polyps, others have dysplastic (adenomatous) lining and still others have a lining not unlike that of normal colon. This variety has led some to conclude that ACF represent a non-homogeneous collection of small, hyperplastic polyps, small adenomas and incidental, non-specific mucosal irregularities.

Aberrant crypt foci may all be morphologically and biologically heterogeneous—not all ACF progress to malignancy in animal models—but it is intriguing that immunohistochemical stains for CEA are consistently and diffusely positive in human ACF. CEA is an oncofetal protein highly expressed during fetal development but much less so in adult colon. It plays a role in cell-cell and cell-substrate adhesion and may help regulate cell migration from crypt base to mucosal surface. Thus abnormal CEA expression in ACF correlates with the histologic impression of abnormal crypt architecture. In addition, K-ras mutations have been found in adenoma and carcinomas but not in normal mucosa, the case for ACF as early cancer precursors is further strengthened. Many data suggest that most colorectal carcinomas develop from benign polyps (O’Brein, 1995). Familial predisposition to multiple polyps is associated with greatly increased risk for colon cancer (Haggit and Reid, 1986). It must be emphasized that the “adenoma-carcinoma sequence” is not synonymous with the “poly-carcinoma sequence”. A more accurate term for the process would be “dysplasia-carcinoma sequence”. As the model does not insist that all carcinomas must develop from grossly visible masses of dysplastic tissue, it is quite possible that some lesions acquire the mutations necessary to move from low-grade dysplasia to high-grade dysplasia to carcinoma without even forming a visible polyp. A literal interpretative of the “adenoma-carcinoma sequence” has given rise to the unsatisfactory term “de nova carcinoma” to describe small carcinomas with recognizable residual adenoma. The de nova carcinoma versus adenoma-carcinoma dichotomy is only a semantic
disagreement. In some malignancies carcinogenesis may be accelerated or achieved along alternate pathways but there is no evidence that de nova carcinoma; the general framework of the multistep model probably applies in all cases. Again it is clear that all adenomas are not created equal. One variant is the “flat adenoma”. Flat adenomas are masses of dysplastic tissue that form slightly raised plaques rather than polyps in colonic mucosa. Histologically flat adenomas have dysplastic tubules concentrated near the surface of the mucosa. The importance of flat adenomas is that they seem to have a higher prevalence of high-grade dysplasia than polypoid adenomas of similar size suggesting that they have a number of important roles as cancer precursors.

A second example of variability in the adenoma carcinoma sequences is the concept of the “aggressive adenoma”. This term was coined to explain two observations about patients with HNPCC: (1) they have a 80-90% change of developing colon cancer despite the fact that they form adenomas only about as often as the general population and (2) they commonly develop carcinomas within 2-5 years of a normal colonoscopic screening examination, a very short interval compared to the general population. Molecular analysis of these lesions has been done to determine whether progression from stage to stage is a stochastic phenomenon or is dependent on new mutations. Identification of the important genes involved in tumor progression has been aided by the characterisation of multiple karyotypic abnormalities in this disease (Fearon, 1995). Figure 2 shows the mutations most often detected in colorectal cancer. These are likely to represent important steps in the development of colon cancer. Specific chromosomal deletions are especially significant. They usually represent loss of suppressor genes (Levine, 1995). Several autosomal dominant syndromes for the cancer development, some associated with multiple polyposis (Haggit and Reid, 1986), some not (Lynch, et al., 1993), have been described. Together, they probably account for less than 10% colorectal cancers. However, these are important to study since they result from the presence of germline mutations in suppressor genes that are important in preventing the development of precursor lesions. Burt and colleagues (1985) have shown that polyp formation may also be explained in different environment.
The common genetic lesions that have been identified as important in the development of colorectal cancer are outlined:

- Genetic instability is a common feature of these tumors evidenced in most cases by aneuploidy and allele loss. Hypomethylation may contribute to this phenotype. In most tumors p53 mutations occur with full transformation and may contribute to further genetic instability.

- Colorectal cancers contain many mutations, many of which are probable required for full malignant transformation and metastasis. Mutations in the TGF-β receptor, APC, p53 and ras genes are necessary for tumorigenesis. This is probably why genomic instability is required for cancer to develop.

- APC mutations and hypomethylation tends to occur early in small adenomas. The ras mutation occurs later, in large, dysplastic adenomas. P53 mutations usually occur even later at the transition to carcinoma. However, this ordering is statistical and not invariant with the possible exception of a requirement for APC inactivation early in the sequence.

- Inactivation of the growth inhibitory pathway regulated by TGF-β is probably a key event in the development of bowel malignancies.
Inflammatory Bowel Disease

Normal Mucosa → At-Risk Mucosa → Commited Mucosa → Malignant Mucosa

(i.e. aberrant crypt foci, hyper proliferative mucosa, transitional mucosa)

Early → Intermediate → Late adenomas

Flate → Few K-ras adenomas

Less allelic loss at 5q.18q.17p

Low grade → High grade Dysplasia
(p53 mutations an early event: fewer K-ras mutations)

Carcinoma

More microsatellite (diploid DNA)

Proximal carcinoma
More allelic (aneuploid DNA)

Distal carcinoma

"DE NOVA" Carcinoma

IBD Carcinoma

Figure 2. Colon cancer is a heterogeneous disease and the lesions that precede it may be heterogeneous as well. Some possible pathways to malignancy within the overall framework of the multistep model are shown.
Experimental Colon Carcinogenesis- An overview

Animal models have contributed a great deal to our understanding of the pathogenesis of colon cancer. Studies in animals allow a systematic evaluation of the growth, morphologic, cellular and molecular features of ACF under defined experimental conditions. Although technical limitations prevent sequential measurements in the same organism, it is possible to monitor events by examining the dissected colons of randomly selected animals from a group at different time intervals as the disease progresses. This approach has been informative.

Colon tumor induction can be divided into two distinct stages, initiation and promotion and the promotion stage is reversible. Initiation of carcinogenesis alters the genetic information of the cell, whereas Promotion leads to the expression of genetic changes and malignancy, which involves loss of control over cell proliferation. The concept of tumor progression has been distinguished from tumor promotion although an experimentally absolute distinction between the two is difficult. Many factors, including diet, may enhance or suppress these processes at certain stages. A number of carcinogens such as 1,2 dimethyl hydrazine (1,2 DMH) azoxymethane (AOM), MAM-acetate, 3,2’-dimethyl- 4 – aminobipheny, methylnitrosourea and N-methyl-N’-nitroso-N-Nitroso guanidine have been used to induce benign adenomas and malignant adenocarcinomas in the colons of laboratory rats and mice and to systematically investigate the risk factors observed in human epidemiologic studies.

The histopathology and regional distribution of colon tumors induced by these chemicals have been found to be similar to colorectal tumors in humans. Thus, a number of risk factors observed in humans could not have been established without careful, deliberate investigations carried out in laboratory animals. Thus, laboratory animal model studies have provided evidence that not only the amount but also types of dietary fat differing in fatty acid composition are important factors in determining the promoting effect of this nutrient in colon tumor development.

The strength of the association between dietary fat and colon cancer risk and the experimental evidence, as well as the biological plausibility indicates that these
associations are real. In addition to types and amount of dietary fat, laboratory animal model studies support the human epidemiological evidence that caloric restriction significantly inhibits chemically induced colon tumor incidence by 20-40% as compared to those fed ad libitum. Animal models have also been used to determine the role of types and mount of dietary fibre in colon cancer. The results therefore generated, suggest that (a) the inhibitory effect depends on the type of fibre and (b) whet bran appears to inhibit colon tumor development more consistently than the other types of fibre. Possible mechanisms by which certain dietary fibres may reduce the risk of colon cancer include dilution and adsorption of carcinogens, co-carcinogens and/or promoters present in the gut. In addition, dietary fibre affects the gut microflora by modifying both its metabolic activities and its composition, which, in turn alter the breakdown products of non-starch polysaccharides, fat and protein in the gut. These breakdown products act as promoters and/or inhibitors in the colon. Thus laboratory animal model studies support the human epidemiological evidence and propose plausible mechanistic pathways for promotion and/or inhibition of colorectal carcinogenesis.

For practical reasons, experiments on carcinogens are carried out on rodent. Propper (1978) has stated that a small fraction of human population exposed to a strong carcinogen have cancer but even if only a small percentage of man exposed to carcinogen, develop carcinomas the total number in the human population becomes exorbitant. The assessment of the balance between risk and benefit is in principle not scientific but a political decision. Explanation of pathogenesis may provide the only reliable answer that science can offer.

CANCER PREVENTION

Several incidences indicate that a major fraction (50-80%) of the cancers is potentially preventable, as the factors that determine their incidence are largely exogeneous (NCI 1990; Ries at al., 1991). This comes mainly from epidemiological studies that include, (a) Time trends in cancer incidence and mortality (b) Geographic variations and effects of migration (c) Identification of specific causative factors like cigarette smoking, occupation and environmental chemicals, radiation, dietary and socio-economical factors, specific viruses, etc and (d) The fact that a vast majority of human cancers do not show simple patterns of inheritance.
In principle, majority of cancers are preventable if the external causative factors could be identified. Alternatively, early detection of certain forms of cancer could also aid in prevention through the development of effective methods of intervention, viz, chemoprevention.

**Cancer Chemoprevention**

The time span between tumor initiation and frank malignancy often exceeds a decade. Hence, there is considerable timeframe wherein the carcinogenic process could be halted or reversed (Alberts and Garcia, 1995). However, cancer chemoprevention seeks to reverse carcinogenesis in the premalignant phase. The aim of cancer chemoprevention is to circumvent the development and progression precancerous cells through the use of non-cytotoxic nutrients and/or pharmacologic agents. Recent studies in cancer chemoprevention have been directed at reversing precancerous lesions, preventing disease in populations at high risk, for recurrent or new disease and reducing the incidence of specific tumors in the general population. Compounds belong to over 20 different classes of chemicals have been shown to have chemopreventive capabilities (Wattenberg, 1985). The great chemical diversity is appositive feature in that it indicates the likelihood that a verity of approaches can be made to prevent and that the options for selecting the optimal compounds will be large. It is of great interest that some of these inhibitors are natural products present in various foods (Hong, et al., 1991; Lippman, at al, 1991; Elson and Yu, 1994; Earnest, at al, 1994; Kennedy, 1995).

The chemoprevention branch of prevention program, Division of cancer Prevention and Control, National Cancer Institute has developed a program with the following objectives.

- To identify and characterize agents with proven efficiency in preventing carcinogenesis in animal or with high probability of preventing human cancer based on epidemiological studies.
- Conduct efficacy and toxicity testing of candidate compounds in animal model systems and
- To conduct human intervention trial of potential chemopreventive compounds.
During the course of selecting candidate chemopreventive compounds for the further development, questions concerning mechanism of carcinogenesis had to be considered. This was in an attempt to obtain a balanced selection that would act at many different points in the neoplastic process (Figure 3). Three current models of carcinogenesis employed, when trying to interpret questions that arose in the process include-

The multistep model generated over the last five decades, with discrete steps name “Initiation”, “promotion”, “Conversion”, and “progression” is now adhered to by the majority of cancer scientists. It is based principally on interferences from animal experiments involving skin painting with a carcinogen, using initially the rabbit ear (Rous and Kidd, 1941) and later mouse skin (Slaga, et al., 1983).

The single –Continuum Model is another well known but less considered model of carcinogenesis, is based on the landmark observations of human cancer biology ay the clinical and histological level and of the mouse mammary tumor (Foulds, 1975) made by Lester Foulds. It may not be adequately appreciated that the term “progression” was first used by Foulds to describe the entire neoplastic process as a single continuum from its earliest inception. Only later was the term “progression” adapted for use with more restrictive meaning as the name of a “step” in the Multistep model.

The colon Evolution model of human tumors at the chromosomal level described by Nowell (1976) consists of an initial mutation and clonal overgrowth followed, possible slowly at first by repeated mutations and selective clonal ever growths leading to a multiclonal population with expanding phenotypic diversity that is increasingly able to escape growth controls and finally invade the surrounding normal tissue i.e., to behave in a malignant manner.
Figure 3. Potential sites of action of inhibitors of chemical carcinogenesis. Number represents sites where inhibitors may be effective.
Classification of chemopreventives according to the mechanism of action divides agents into 4 categories, namely

**(A) Chemopreventives that block or suppress the effect of mutation.**

At present most antimutagens act by inducing enzymes, which mediate reactions that enhance the solubilization and elimination of carcinogens, or that increase the concentration of GSH. GSH reacts with electrophilic sites produced on the carcinogen molecule the Cyy-P-450 hydroxylases, thereby blocking their nucleophilic attack on DNA. Chemical carcinogens, like other xenobiotics are eliminated from the body through the action of two types of water solubilizing enzyme reactions that form part of the xenobiotic detoxification system in the liver and other tissues:

1. Hydroxylation which is medicated by Cyt P-450 MFOs and
2. Conjugation with highly water soluble molecules such as glucuronate and sulfate.

Unfortunately the hydroxylation reaction also sometime produces epoxides, which are highly electrophilic and carcinogenic. Thus, the Cyt P-450 MFOs may activate pro-carcinogens to their ultimate carcinogenic form. Compounds such as benzyl isothiocyanate and indole-3-carbinol, which are present in cruciferous vegetables in high amounts, induce the conjugation enzymes and therefore, would be expected to be anti-initiatory because they would hasten the elimination of carcinogen. Compounds, which specifically induce only the conjugases or only GST, which mediates electrophile scavenging, are highly desirable. The dithiolthione oltipraz is such a compound.

**(B) Chemopreventives that block promoter**

Compounds, which inhibit proliferation or inflammation, can be expected to be antipromotional. Examples are selenium compounds, the antiestrogen tamoxifen, certain steroids and agents that suppress arachidonicacid metabolism such as piroxicam. Vitamin A and synthetic retinoids and possibly β-carotene may inhibit proliferation through a tendency to induce maturation of epithelia with secondary suppression of proliferation.
(C) Chemopreventives that block mutation and promotion

Agents that suppress prostaglandin synthesis, in addition to being antipromotional, may also be antimutational because of blocking the generation of mutagenic alkosy free radicals during prostaglandin hydroperoxide synthesis.

(D) Chemopreventives whose mechanism is undetermined

Agents, which act through uncertain mechanism, are grouped here. Molybdate is an example.

NSAIDs IN CHEMOPREVENTION

Nonsteroidal anti-inflammatory drugs prevent colorectal cancer (Shiff and Rigas, 1997). A combination of epidemiological, animal, and basic studies make a compelling case that regular use of these compounds lowers the risk for the development of colorectal cancer, as well as adenomas. Recently, Sulindac was also shown to eliminate aberrant crypt foci in the colorectum of patients who have had adenomatous polyps. These lesions are similar to adenomas but are not yet visible to the naked eye, and probably grow to become adenomas (Takayama et al., 1998). Overall, the weight of the evidence indicates that NSAIDs are the preeminent colorectal cancer chemopreventive agents. The cellular or molecular mechanisms influenced by NSAIDs that may contribute to their antineoplastic effects, classified in relation to their dependence on inhibition of Cyclooxygenase catalytic activity shown in Table 6.

COX dependent effects

NSAIDs inhibit the catalytic activity of cyclooxygenase (COX, more properly called prostaglandin H synthase, or PGHS), and this is thought to be the predominant mechanism by which they act as analgesic, antipyretic, and anti-inflammatory agents (figure 4). Reports indicating that Acetyl salicylic acid (ASA) and other NSAIDs inhibit colon carcinogenesis provided the impetus for a series of studies showing that the levels of both PGE₂ and COX-2 are augmented in human colon cancer (Shiff and Rigas, 1997) as a result, it generally has been assumed that the antineoplastic effects of NSAIDs were depended upon inhibition of COX activity and PG synthesis. COX-2, mRNA expression and protein were found to be
Table 6.
NSAIDs classification in relation to their dependence on inhibition of Cyclooxygenase catalytic activity

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<tr>
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<th>COX dependent</th>
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<tr>
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<td>Cell turnover- proliferation/apoptosis</td>
<td>Cell turnover- proliferation/apoptosis</td>
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increased in human colorectal adenomas and adenocarcinomas (Tsujii, et al., 1997; Kargman, et al., 1995). Conversely specific COX-2 inhibition, either by targeted knock out of the COX-2 gene or by pharmacological intervention, as been shown to effectively decrease the growth of murine intestinal adenomas (Oshima, et al., 1996; Reddy, et al., 1996; Oshima, et al., 2001).

In a rat model of chemically induced colorectal cancer, the COX-2 selective inhibitor Celecoxib suppressed the formation of aberrant crypt foci, precursors of adenomas (Yoshimi, et al., 1997). As noted above COX-2 over expression is important during colorectal carcinogenesis, however it is unclear exactly where in the multistep process COX-2 deregulation occurs (figure 5) (Janne and Mayer, 2000; Chan, 2002). Because COX-2 over expression occurs even in small adenomas, it is thought to represent an early event, promoting tumor proliferation and suppression of apoptosis. In vitro, many NSAIDs including Sulindac, indomethacin, naproxen, peroxicam, aspirin, and COX-2 specific inhibitors are known to cause apoptosis in colon cancer cells. Thus a growing body experimental data supports the hypothesis that NSAIDs exert their chemopreventive effect by restoring to the normal frequency of apoptosis in colonic mucosa (Chan, 2002).

Yet evidence is mounting that NSAIDs produce some of their clinical and experimental cancer chemopreventive effects via mechanisms that are independent of COX inhibition. It was demonstrate that NSAIDs induced inhibition of cell transformation, inhibition of cell proliferation, and initiation of apoptosis are not dependent on the expression of COX isozymes (Zhang, et al., 1999). In a study NSAIDs inhibited the proliferation and induced apoptosis in human colon cancer cell lines (Shiff and Rigas, 1996).
Membrane Phospholipids

Arachidonic Acid

COX-1 or COX-2

Inactive Carcinogen

Active

PGH₂

15- (R)- HETE

E₂, F₂₀, I₂

PG₂, TxA₂, MDA

Peroxyl radical

Figure 4. Arachidonic acid metabolism by Cox isoenzymes. Phospholipase A₂ (PLA₂) release arachidonic acid (AA) from membrane phospholipids, which is in turn converted by either COX-1 or COX-2 to PGG₂ (C, cyclooxygenase catalytic cativity of COX) and then to PGH₂ (P, peroxidase catalytic activity of COX).

PGH₂ is converted to either PGs (e.g., E₂, F₂₀, I₂, D₂); thromboxane A₂ (TxA₂); or Malondialdehyde (MDA). MDA is a direct-acting mutagen and carcinogen and can be produced without COX by direct lipid peroxidation. AA can be converted directly to 15- (R)- HETE by both COX is enzymes.

COX-1 is constitutively expressed in most tissues, whereas COX-2 is induced by cytokines, growth factor, tumor promoters, or other agents after the initiation of specific physiological events. Compounds other than PGG₂, e.g., Procarcinogenic hydroperoxides, can serve as substrates for the peroxidase activity of both COX enzymes.

Inactive carcinogen serving as electron acceptors can also become activated by this activity. The COX isoenzymes are also involved in the formation of peroxide radicals that can activate procarcinogens.
Figure 5
Colon carcinogenesis and the effects of chemopreventive agents
**COX independent**

A potential important means by which NSAIDs prevent colorectal neoplasia is to affect cell turnover in the colorectal epithelium. Cell death and renewal are critical for the regulation of the structural integrity of all tissues. The growth rate of a tissue or tumor is determined by the rate of proliferation and counter balanced by the rates of cell loss by apoptosis or necrosis of the cells that comprise them. Apoptosis is suppressed in sporadic colorectal adenomas and carcinomas and in the flat mucosa or adenomas of patients with FAP. In FAP patients Sulindac normalizes apoptosis in normal rectal mucosal colono sites while reducing the size and number of their adenomas. Thus it is possible that cell kinetic effects play a major role in the antineoplastic effects of these compounds (Shiff, and Rigas, 1997).

Salicylates inhibited phorbol ester (TPA) induced transformation of mouse epidermal JB6 cells (Dong, et al., 1997). Sulindac sulphide inhibited transformation of primary rat embryo fibro blasts by activated H-ras and SV 40 T antigen or other transformation inducing stimuli (Herrmann, et al., 1998). In both cases transformation was inhibited at drug concentrations below those required to inhibit cell proliferation or cell viability. Inhibition of ras signaling may also explain the effects of NSAIDs on proliferation and apoptosis at higher drug concentrations. ras inhibition may link the effect of NSAIDs to NF κ B and MAP kinase activity (Ljungdahl, et al., 1997). Both ASA and Non salicylate NSAIDs reduce micro satellite instability in colon cancer cell lines deficient in DNA mismatch repair (Ruschoff, et al., 1998).

**Role of COX unclear:**

Apoptosis was markedly inhibited by transfection of antisense Myc (Lu, et al., 1997). Indomethacin binds and activates Peroxisomal Proliferator-activated Receptors (PPAR) which is important for the chemoprevention of colorectal cancer by NSAIDs (Lehmann, et al., 1997). NSAIDs may boost mechanisms of tumor immune surveillance; tumors are hypothesized to escape from immune mediated destruction by thwarting mechanism that detects tumor-associated antigens. Piroxicam upregulates the expression of MHC genes in the colonic mucosa of rat treated with a carcinogen (Shiff, and Rigas, 1997).
ASPIRIN IN CANCER PREVENTION

Aspirin is a synthetic analogue structurally related to the salicylate family of compounds, salicylic acid being its principal metabolite. After absorption from the stomach and small intestine, aspirin is rapidly hydrolyzed to salicylic acid (2-hydroxybenzoic acid) in the liver and blood where it is tightly bound to plasma proteins and distributed to all tissues in the body. Consequently some of the anti-inflammatory and anti-carcinogenic effects of aspirin are potentially due to salicylic acid. Since salicylic acid is common to both aspirin and fruits and vegetables, and both are known to protect against colon pathologies, including cancer. Acetylsalicylic acid (ASA) is among the most commonly used NSAID for relieving pain, inflammatory symptoms, and fever. ASA also has an established efficacy in the prevention of myocardial infarction and ischemic stroke, as well as in the treatment of acute myocardial infraction (Awtry, et al., 2000).

Although the importance of the clinical outcomes are clear, the molecular mechanism leading to the antineoplastic effect of aspirin remains controversial. The reversible blockade of cyclooxygenase-2 (COX-2), this mechanism does not adequately explain the chemopreventive and pro-apoptotic effects of aspirin. For example, cells that do not express COX-2 also undergo apoptosis in response to NSAIDs (Piazza, et al., 1997).

ASA has been shown to reduce the risk for colorectal cancer by as much as approximately 40%, a property that is shared by other NSAIDs. Evidence for this effect comes from multiple epidemiological studies, most of which have found that ASA reduces the risk of colorectal adenoma (Garcia Rodriguez, et al., 2000) and carcinoma, (Garcia Rodriguez, et al.,2001) as well as from experimental colon cancer in animal models (Reddy, et al.,1993). The most compelling evidence that NSAIDs prevent colon cancer comes from epidemiological data indicating that people who regularly take have a 40-50% reduced risk of dying from this disease compared with matched controls (Krishnan, et al., 1997; Collet, et al., 1999; Thun, , et al., 1991). Aspirin induces activation of NF-κB, which is required for its antitumor activity and may contribute to the protective effect of aspirin that has been observed in clinical trials (Stark, et al., 2001). Studies of colorectal adenomas
provide additional support for the protective effect of Aspirin/NSAIDs on reducing the likelihood of colorectal polyp recurrence (Sandler, et al., 2003; Baron, et al., 2003).

The observed interactions for aspirin/NSAIDs and IRS1 and VDR genotypes suggest that mechanisms other than COX-2 inhibition may be contributing to the protective effect of aspirin and NSAIDs on colorectal cancer risk (Martha Slattery, et al., 2004). NSAIDs inhibit cytokine-mediated IκB kinase (IKK) activation, and consequently NF-κB activation, in colorectal cancer cells and suggested that this specific interference is responsible for the antineoplastic activity of these agents (Yamamoto, et al., 1999). Aspirin does not affect ODC allele-specific promoter activity, and we have no evidence that the ODC RNA levels or enzyme activity in HT29 cells, but it activates polyamine catabolism, specifically increasing the expression and activity of SSAT and causing polyamine content to drop. Thus, both the ODC A-allele and aspirin act to suppress tissue polyamine contents. This reduction, in turn, might reduce the risk of colorectal neoplasia (Meyskens, et al., 1999; Erdman, et al., 1999).
OBJECTIVES OF THE PRESENT STUDY

The present work embedded in the thesis is an attempt to understand the role of aspirin in experimental rat colon carcinogenesis. A careful attempt has been made in the present study to evaluate the anti carcinogenic potential of aspirin in 1,2 dimethyl hydrazine induced rat colon carcinogenesis. A brief outline of the essential objectives undertaken into consideration includes-

- First to correlate various biological events by analyzing morphological, and histological parameters in order to arrive at a meaningful explanation of the antitumorigenic potential of aspirin in chemically induced rat colon carcinogenesis.

- Second to study the effect of aspirin on the expression of metallothionein expression by immunohistochemical method in chemically induced rat colon carcinogenesis.

- Third, to study the role of aspirin on cellular proliferation during the progression of colon tumorigenesis by 5-bromo-2-deoxyuridine (BrdU) incorporation in chemically induced rat colon carcinogenesis.

- Finally, attempts have been made to justify the anticarinogenic potential of aspirin on the induction of apoptosis by TUNEL assay.