INTRODUCTION
PRINCIPLES OF CARCINOGENESIS

The disease "Cancer" in reality is a process, namely "Carcinogenesis", rather than a state. The actual disease is an evolving molecular and cellular process, not a static event.

The uniting phenomenon of carcinogenesis is not the cause but the similar effect of cellular damage due to different incitants, leading to the malignant transformation of cells and the invasion of those in neighboring tissues. Neoplasms are an aberration of the biological phenomenon of growth. Cancer causes are irritants of several groups of diversified factors, which are able to act in chain reactions. They are Chemical carcinogens, Physical carcinogens, Radioactive emissions, Viruses, Genes, Bacteria and Parasites (1).

TWO-STAGE MODEL OF CARCINOGENESIS

Berenblum and Shubik suggested that at least two stages are involved in chemical carcinogenesis in animals (2). Stochastic multistage models have been proposed to account for the age-dependent increase in age-specific incidence rates observed for many human carcinomas (3). Armitage and Doll have proposed a two-stage model in which cells in the first stage grow exponentially and those in the second escape from control and become malignant (4).
A two stage model is biologically reasonable because no more than two distinct stages have been experimentally demonstrated. Such a model is biologically attractive because there is considerable evidence that somatic mutation is important in carcinogenesis and two stages are consistent with the development of homozygosity at a "cancer gene" locus (5).

If two events are necessary for carcinogenesis, a cell that has sustained the first event must survive in the tissue, long enough, to sustain the second event. Thus the target cells are probably normal stem cell (SC) in a tissue and the rapidly renewing tissue that demands SC division is most susceptible to malignant transformation.

In a small time interval, a given SC may divide with a certain probability to give rise to two daughter SC or it may differentiate (or die) and thus leave the pool of susceptible cells or it may divide (with a small probability) into two cells. One is normal and the other, which has suffered the first event to become an Intermediate cell (IC) (one hit). The IC may in turn give rise to two intermediate daughters, die or differentiate or give rise (with a small probability) to one IC and one malignant cell (MC). Once an MC is generated, it gives rise to a malignant tumor. The model is schematically represented in Fig.1.
Fig. 1.

Two-stage model for carcinogenesis. S = normal stem cell, I = Intermediate cell (one hit), D = dead cell and M = Malignant cell. $\mu_1 =$ rate at which first event occurs. $\mu_2 =$ rate at which second event occurs. (5)
MULTISTAGE AND MULTIFACTORIAL NATURE OF CARCINOGENESIS

Carcinogenesis in humans and animals is a complex, multistage process. Most cancers appear to be the result of one or more heritable alterations that reside at the level of the individual tumor cell, as originally shown by Furth and Sobel (6). Nowell proposed that multistage carcinogenesis is driven by carcinogen induced genetic and epigenetic damage in susceptible cells that gain a selective growth advantage and undergo clonal expansion as a result of activation of protooncogenes and/or inactivation of tumor suppressor genes (7).

Carcinogenesis is a mature multidisciplinary field of cancer research that has a rich history of scientific accomplishments achieved by epidemiological observations (8,9); development and exploitation of animal models relevant to human cancer (10) and in vitro cell and tissue models including the use of human tissues (11); and most recently, advances in molecular genetics (12-15).

The traditional view of carcinogenesis is derived primarily from studies of animal models (Fig.2). In experimental models, such as mouse skin tumorigenesis, the process has been identified by at least three distinct steps: initiation, promotion and progression (16). From the perspective of the organism, the multistep nature of tumorigenesis is easily rationalized; each step in the process represents a physiological barrier that must be breached
Figure 2.

Carcinogenesis is a multistage process involving multiple genetic and epigenetic events in proto-oncogenes, tumor suppressor genes and antimetastasis genes. (11)
FIGURE 2.

Initiation

- Defects in Terminal Differentiation
- Defects in Growth Control
- Resistance to Cytotoxicity

Promotion

Conversion

Progression

- Activation of Proto-Oncogenes
- Inactivation of Tumor Suppressor Genes
- Inactivation of Antimetastasis Genes
in order for a cell to progress further toward the end point of malignancy. Such multiple barriers conspire to ensure that successful completion of the tumorigenic process is a rarely achieved event. Experimental studies of chemical carcinogenesis established the principle that different chemicals affect different stages in the carcinogenic process. A two-stage model involving initiation and promotion was proposed initially (2-5); but it is now recognized that multiple stages are possible within each of these stages and additional changes are required for tumor progression.

The first stage of carcinogenic process, tumor initiation, is thought to be an initial or early event that predisposes a cell to malignant transformation. The predominant view of initiation is that either normal growth control genes are mutated or their expression is altered to produce an active oncogene and/or there is loss or inactivation of a tumor suppressor gene(s). 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 in addition exerts a selective clonal expansion advantage, when compared to the surrounding normal cells (17). The initiated cells may have decreased responsiveness to the inter- and intracellular signals that maintain normal tissue architecture and regulate the homeostatic growth and maturation of cells. For example, initiated cells may be less responsive to negative growth factors, inducers of terminal cell differentiation and/or programmed cell death (18,19).
Tumor 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 subpopulation of initiated cells converting to malignancy can be substantially increased by their further exposure to DNA-damaging agents (20) that may activate protooncogenes and/or inactivate tumor suppressor genes (21).

Tumors often become more aggressive in their behaviour and more "malignant" in their characteristics during their life history, although the time course may be quite variable. This phenomenon has been termed "tumor progression". It describes the later stages of cancer development characterized by intrinsic genomic instability that is manifested by the abnormal number and structure of chromosomes, gene amplification, altered gene expression associated with increased growth rate, invasiveness and metastases (22). Foulds first pointed out that the process appears to develop in a stepwise fashion through qualitatively different stages. Malignant cells continue to exhibit progressive phenotypic changes during tumor progression. Tumor progression may also be accompanied by the elaboration of increased amounts of particular proteins by the malignant cells. Some may contribute to the capacity for invasion and metastasis (23).
It must be remembered that initiation, promotion and progression are highly dependent on the dose rate and total level delivered to the target tissue and the particular experimental model and species under study.

**METASTASIS**

Metastasis, the spread of cells from the primary neoplasm to distant sites and their growth there, is the most fearsome aspect of cancer (24,25). The process of cancer metastasis consists of a long series of sequential interrelated steps, each of which can be rate limiting since a failure or an insufficiency at any of the steps aborts the process (26). The outcome of the process is dependent on both the intrinsic properties of the tumor cells and the responses of the host; the balance of these interactions can vary among different individuals. In principle, the steps or events required for the formation of a metastasis are the same in all tumors (Fig.3).

Major steps in the formation of a metastasis are as follows: (a) after the initial transforming event, either unicellular or multicellular, growth of neoplastic cells must be progressive; (b) extensive vascularization must occur if a tumor mass is to exceed 2 mm in diameter. The synthesis and secretion of several angiogenesis factors play a key role in establishing a neocapillary network from the surrounding host tissue (27); (c) local invasion of the host stroma by some tumor cells could occur by several mechanisms that are not mutually exclusive (28). Thin walled venules, like lymphatic channels, offer very little resistance to penetration by tumor cells and provide the most
Figure 3.

The pathogenesis of a metastasis. To produce metastases, tumor cells in a primary neoplasm must complete a series of sequential and selective steps, each of which can be rate limiting since a failure or an insufficiency at any of the steps aborts the process. (25)
FIGURE 3.

Primary malignant neoplasim

Vascularisation

Invasion

Transport

Lymphatics
Venues
Capillaries

Interaction with host platelets lymphocytes and other blood elements

Extravasation

Adherence of tumor cells

 Arrest in capillary bed of organs

Establishment of microenvironment and growth into metastases

Lung
common pathways for tumor cell entry into the circulation; (d) detachment and embolization of small tumor cell aggregates occurs next; (e) tumor cells that survive the circulation must arrest in the capillary beds of organs; (f) extravasation occurs next, probably by the same mechanisms that influence initial invasion; (g) proliferation within the organ parenchyma completes the metastatic process. To produce detectable lesions, the metastases must develop a vascular network, evade the host immune system and respond to organ specific factors that influence their growth (29). Once they do so, the cells can invade host stroma, penetrate blood vessels and enter the circulation to produce secondary metastases, the so-called "metastasis of metastases". Only a few cells in a primary tumor can give rise to a metastasis.

The major restraint to the treatment of metastases is the biological heterogeneity of cancer cells in primary and secondary neoplasms. This heterogeneity is exhibited in a wide range of genetic, biochemical, immunological and biological characteristics, such as cell surface receptors, enzymes, karyotypes, cell morphologies, growth properties, sensitivities to various therapeutic agents and ability to invade and produce metastasis (30).

**Tumor Heterogeneity**

Tumors are variable in several ways. Their characteristics change with organ site and cell origin. Numerous host variables, such as age and hormonal status are implicated. Heterogeneity is a feature of neoplastic
development that can precede the tumor itself. Cellular heterogeneity must be viewed then, as a feature of both normal and precancerous tissues (31). It seems not unlikely that the mechanisms that are responsible for variability under these circumstances, could also be responsible for generating tumor heterogeneity. Besides, "normal" mechanisms, heterogeneity may arise by tumor specific mechanisms. Increased genetic instability is a case in point. This instability leads to more errors in tumor cell DNA (point mutations, genomic rearrangements, chromosome losses, gene amplification, etc.) and is reflected in increased phenotypic variability. A hypothesis that narrows the apparent differences between genetic and epigenetic mechanisms was provided by Frost and Kerbel, who suggested that DNA hypomethylation and the activation of otherwise repressed genes, may be the cause of tumor variant production (32).

Selective Clonal Expansion

The majority of human cancers and those induced by chemical and physical carcinogens in animal models are unicellular in origin (7, 33). The clonal nature of neoplasia is the cornerstone in the concept of multistage carcinogenesis and provides the basis for current strategies designated to elucidate the component genetic changes. If all of the clonally derived cells in a tumor, although heterogenous in other aspects, have in common the critical genetic lesion(s) that triggers and/or drives their selective clonal growth.
The clonal evolution events for selective growth advantage of initiated cells are likely to be numerous and involve cell-cell interactions; cell-matrix interactions; cell-growth factor interactions; intrinsic aberrations in regulation of the cell cycle; terminal differentiation and programmed cell death. A schematic model for clonal progression is depicted in Fig.4.

Mechanisms of Clonal Evolution

**Genetic Instability of Tumor Cells.** There is evidence that most neoplastic cells are more genetically unstable than comparable normal cells and that this may be a major factor contributing to the phenomenon of clonal evolution. Both *in vitro* and *in vivo*, there is evidence that neoplastic cells may be more susceptible than normal counterparts to chromosome breakage, nondisjunction and ploidy changes, sister chromatid exchange and other genetic alterations. There are even limited experimental data indicating that this enhanced mutability increases with tumor progression. Ling *et al.* have pointed out that high and changing rates of mutation play an important role in what they call the "dynamic heterogeneity" of malignant cell populations with respect to such properties as drug resistance and capability for metastasis (34).

**Host factors in clonal evolution.** In addition to the alterations within the neoplastic cells that play a role in tumor progression, one must also consider those factors in the host environment that provide the selective pressures determining, which mutant cells expand into predominant
**Figure 4.**

A schematic model of clonal evolution in neoplasia. Carcinogen induced change in progenitor normal cell (N) produces a diploid tumor cell (T₁, 46 chromosomes) with growth advantage permitting clonal expansion to begin. Genetic instability of T₁ cells leads to production of variants (illustrated by changes in chromosome number, T₂ to T₆). Most variants die, due to metabolic or immunologic disadvantage (hatched circles); occasionally one has an additional selective advantage (for example, T₂, 47 chromosomes), and its progeny become the predominant subpopulation until an even more favorable variant appears (for example, T₄). The stepwise sequence in each tumor differs and results in a different aneuploid karyotype in each fully developed malignancy (T₆). (7)
subpopulations at various times. The host immune system represents one type of selective pressure on evolving neoplasms, particularly in the early stages. In addition to aspects of immune regulation, there also remains much to be learned, concerning other substances that may influence growth at the local level (e.g., growth factors) and to which the neoplastic population may respond abnormally (35). One important aspect may be damage to the microenvironment of an early neoplasm, leading to serious disruption of local regulatory mechanisms and thus playing a significant role in subsequent tumor progression.

**Increased Cell Division as a Cause for Cancer**

Recent advances in molecular genetics of cancer have provided a molecular basis for the concept that cell division is essential in the complex process of the genesis of human cancer. Cell division *per se* increases the risk of genetic errors of various kinds (36). Cell division is necessary for conversion of adducts or other single-stranded DNA damage to gaps or mutations. Cell division also allows for mitotic recombination (e.g., nondisjunction, gene conversion) which results in more profound changes than those from a single mutation. The development of a fully malignant tumor appears to involve the activation or altered expression of protooncogenes to oncogenes and the loss or inactivation of tumor suppressor genes, the function of which is to control the normal cellular activity (37). A series of distinct genetic alterations accumulates in a cell
before it becomes malignant. The activation of oncogenes whether by mutation, translocation or amplification requires cell division (38).

Carcinogenesis research is increasingly concerned with chemicals that are not genotoxic and yet can induce cancer at high dose. Whereas genotoxic agents directly alter DNA, these non-genotoxic agents appear to have their effect by increasing cell proliferation (39). Chemicals administered at the maximum tolerated dose commonly cause cell proliferation, presumably because this cytotoxic dose causes cell death, which stimulates proliferation of surviving cells as well as stimulating phagocytosis and its associated production of oxygen radicals and inflammation.

Cell Proliferation

Cell proliferation has been linked to the carcinogenic process and is an important mechanistic aspect for both genotoxic and non-genotoxic carcinogens (40-41). For example, cigarette smoking is known to cause bladder cancer in humans, perhaps due to a hyperplastic effect on the urothelium of many cigarette smokers, in addition to the probable genotoxic damage that occurs.

Cell proliferation may act at each stage of the carcinogenic process, altering the size of the pool of cells at risk for the next event. The pool sizes are dependent on the kinetics of cell birth, cell death and cell differentiation. Carcinogens may act with different activities on those cells that comprise one or more stages of the multistage process of carcinogenesis (Fig. 5).
Figure 5.

Schematic representation of multistep carcinogenesis, including the roles of genetic damage and cell proliferation.
Normal cell proliferation

Cell proliferation is regulated primarily by the cellular environment and state of differentiation. The environment provides positive, stimulatory signals and negative, inhibitory signals. The balance between these signals regulates the growth, development and maintenance of complex tissues and indeed, of organisms. The state of differentiation dictates whether and how a cell will respond to a particular environmental signal (42).

Cell proliferation occurs only after an orderly progression of biochemical and cellular events. Many of these events are common to all cells, regardless of the state of differentiation or nature of the environmental signals. Normal and tumor cells do not differ in these basic events that are required for cell division. Rather, tumor cells are defective in one or more regulatory mechanisms that control proliferation (Fig. 6A).

Abnormal cell proliferation

Cell proliferation is regulated by both positive and negative regulatory mechanisms that control growth state transitions and progress through the cell cycle. Neoplastic growth can result from increased sensitivity to or activation of growth stimulatory mechanisms or from decreased sensitivity to or inactivation of growth inhibitory mechanisms (42,43).

The basic biochemistry of normal and tumor cell is remarkably similar. Tumor cells do not necessarily grow faster than normal cells and
they generally go through all four phases of the cell cycles, when they proliferate. Tumors cells are defective in one or more regulatory mechanisms that normally prevent inappropriate proliferation. In addition, tumor cells often modify their local environment such that it becomes more favorable for cell proliferation. These changes are generally the result of one or more hereditary mutations in the tumor cell DNA. The relaxed control shown by tumor cells can result from increased sensitivity to positive signals or decreased sensitivity to negative signals (Fig. 6B).

ONCOGENE ACTIVATION

Oncogenes are derived from normal genes that are highly conserved in evolution and code for proteins having important roles in normal cellular processes. The discovery of oncogenes has led to a major breakthrough in our understanding of carcinogenesis at the molecular level. Because cellular oncogenes are mutated forms of normal cellular genes, they provide clear indication of the genetic targets that suffer alteration at the hands of mutagens. Cell growth is obviously controlled by a complex network of pathways involving different gene families, each member of which is potentially capable of activation by chemical carcinogens. The information obtained will have relevance to studies on mutagenesis, in particular, in identifying critical adducts or mutations that contribute to the transformed phenotype, to the control of cell growth and differentiation and finally to the elucidation of the role of environmental carcinogens in the development of human cancer. Some oncogenes can be activated directly by interaction
Figure 6A.

Mechanisms that regulate the proliferation of normal cells. The proliferation of normal cells is regulated by positive and negative environmental signals that interact with the cell surface and ultimately alter gene expression. Proto-oncogenes are positive intracellular regulators of proliferation, whereas suppressor genes are negative regulators of proliferation. The balance between positive and negative signals determines whether a cell will initiate DNA synthesis and undergo cell division. R - receptor.

Figure 6B.

Mechanisms by which tumor cells escape from normal growth control. Tumor cells may acquire autonomy from or increased sensitivity to positive growth signals, they may also acquire decreased sensitivity to negative growth signals. These changes occur because of mutation that enable the tumor cells to modify their environment, that activate proto-oncogenes or that inactivate suppressor genes. R - receptor.
A. NORMAL CELL PROLIFERATION

- **POSITIVE GROWTH CONTROL**
  - MITOGENS
  - R
  - GENE EXPRESSION
    - PROTOONCOGENES
    - SUPPRESSOR GENES
  - DNA SYNTHESIS

- **NEGATIVE GROWTH CONTROL**
  - INHIBITORS
  - R

B. ABNORMAL CELL PROLIFERATION

- **POSITIVE GROWTH CONTROL**
  - MITOGENS
  - R
  - GENE EXPRESSION
    - PROTOONCOGENES
    - SUPPRESSOR GENES
  - DNA SYNTHESIS

- **NEGATIVE GROWTH CONTROL**
  - INHIBITORS
  - Degradative Activity
  - Autocrine Factors
between the target genes and chemical carcinogens. Others may be activated indirectly during the progression of tumorigenesis by non-targeted genetic events in somatic cells (14,44-47).

Critical DNA Targets: Protooncogenes and Tumor Suppressor Genes

Protooncogenes are normal cellular genes that, when inappropriately activated as oncogenes, cause dysregulation of growth and differentiation and enhance the probability of neoplastic transformation (Fig. 7). Carcinogens can cause the genetic changes that can lead to the activation of protooncogenes, including base substitution mutations, chromosomal translocations and gene amplifications (48).

In contrast to protooncogenes, tumor suppressor genes are normal cellular genes that, when inappropriately inactivated, cause dysregulation of growth and differentiation pathways and enhance the probability of neoplastic transformation (49).

Oncogenic Function

There are several ways in which normally functioning cellular oncogenes may be converted into transforming oncogenes. Firstly, the normal gene may be abnormally expressed either by interference with its control region or by transposition to an abnormal site where its expression is unregulated. Secondly, the gene may become amplified, resulting in larger
Figure 7.

Central role of cellular oncogenes in normal and abnormal growth control. (12)
Figure 7.

Normal Growth

v-onc → c-onc → cancerogens

Increased expression

Abnormal expression

CANCER
quantities of mRNA production simply because of the larger number of gene copies. Thirdly, the gene itself may be altered with the production of an abnormal product with transforming potential. These mechanisms are not mutually exclusive. Finally, there are the recessively acting genes in which abnormalities at both alleles are necessary to remove an inhibitory control on cell division (12).

Assays are available to evaluate oncogene activity in human tumors. DNA can be examined for multiple copies or abnormal forms of suspected oncogenes. mRNA can be analyzed for abnormal transcripts or for inappropriate quantities of a normal transcript. The protein products of oncogenes can be looked for, in either inappropriate forms or quantities, using suitable monoclonal antibodies. Finally, the putative oncogene can be "implanted" into suitable cell cultures to look for its transfecting potential.

Mutational Spectrum

Since mutations are largely responsible for activating protooncogenes and inactivating tumor suppressor genes, the mutation profile of chemical and physical carcinogens is of interest, to define endogenous and exogenous mutational mechanisms. Studies using prokaryotic and simple eukaryotic assays and site-specific mutagenesis assays have shown that each carcinogenic agent produces a "fingerprint" linking specific types and locations of DNA adducts with the mutational spectrum of the agent (50).
The p53 tumor suppressor gene is ideally suited for analysis of mutational spectrum: (a) p53 is well conserved in evolution (51) and 5 domains are >90% homologous in DNA sequence among humans and rodents that are frequently used in animal models of carcinogenesis; (b) p53 is mutated in diverse types of human cancer (38) and cancers in laboratory animals (11,12); (c) a wide spectrum of mutational types and codon sites have been observed that presumably define regions of the p53 protein likely to be essential for its biological activity in cell cycle control, in tumor suppression and for its interaction with other cellular and viral proteins. A recent review has concluded that the majority of the mutations in human tumors occur in the evolutionarily highly conserved domains in exons 5-8 of the p53 gene and the missense mutations are predominantly transitions at G:C base pairs and are almost all (>95%) at amino acids that are entirely conserved in mouse, rat, monkey and human (52).

**Oncogene activation in gastric carcinogenesis**

Ras oncogenes are most frequently responsible for the morphological transformation. Ras oncogenes exists in a variety of human cancers and their overall incidence is estimated to be 5-40%. A few studies have shown that foci of intestinal metaplasia of the gastric mucosa express oncogene products. Noguchi et al have reported elevated Mr 21,000 ras oncogene product in metaplastic cells (53). Amplification of oncogenes such as c-erb B or k-sam has been observed in gastric carcinoma tissues and in established gastric cancer cell lines (54,55). Furthermore, mutations of the p53 gene located at
chromosome 17p13 have been detected in primary human gastric cancer and gastric cancer cell lines (56).

Classification of carcinogens

Carcinogens can be classified according to their mode of action as genotoxicants, non-genotoxicants and cytotoxicants (Table 1).

Genotoxic carcinogens: The association of mutagenic activity with carcinogenic activity has been a valuable concept and is the basis for most current models of risk assessment and predictive assays. Genotoxic chemicals are generally DNA reactive or are metabolized to species that covalently bind to DNA and induce mutations. Other genotoxic agents interact directly with the genetic material and alter chromosomal structure or number. Examples of genotoxicants include methyl methane sulfonate and N-nitrosodimethyl-amine (57).

Nongenotoxic carcinogens: Nongenotoxic chemicals are those that lack DNA reactive or direct chromosome altering activity as a primary biological effect. There are many different types of nongenotoxic carcinogens; one class is mitogens, which induce cell proliferation directly in the target tissue without apparent cytolethality; a second class is cytotoxicants, which produce cell death in the target tissue followed by regenerative cell proliferation. Mutagenic activity may occur as an event secondary, to induce cell proliferation. Altered cell division and cell death rates produced by either
Table 1

Classification of carcinogens according to the mode of action

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotoxic</td>
<td>DNA-reactive or DNA-reactive metabolites.</td>
</tr>
<tr>
<td></td>
<td>Direct interaction to alter chromosomal structure or number.</td>
</tr>
<tr>
<td></td>
<td>May also be mitogenic or cytotoxic.</td>
</tr>
<tr>
<td>Non-genotoxic</td>
<td></td>
</tr>
<tr>
<td>Mitogens</td>
<td>Mitogenic stimulation of growth.</td>
</tr>
<tr>
<td></td>
<td>Mutations may occur secondarily to cell proliferation.</td>
</tr>
<tr>
<td></td>
<td>May cause preferential growth of preneoplastic cells.</td>
</tr>
<tr>
<td>Cytotoxicants</td>
<td>Cytolethal.</td>
</tr>
<tr>
<td></td>
<td>Induce regenerative growth.</td>
</tr>
<tr>
<td></td>
<td>Mutations may occur secondarily to cell proliferation.</td>
</tr>
<tr>
<td></td>
<td>May cause preferential growth of preneoplastic cells.</td>
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</table>
mitogens or cytotoxicants may provide a preferential growth and advantage to spontaneous or chemically induced precancerous or cancerous cells (41).

Predictive assays and risk assessments for the various classes of nongenotoxic carcinogens should be dealt with individually and require understanding of mechanism(s) of action, reasons for target organ and species specificity and quantitative dose-response relationships between endpoints such as induced cell proliferation and carcinogenic potential (58).

**Short-term assays**

The deployment of short-term assays for the detection of carcinogens must be based inevitably on the genetic alterations actually involved in carcinogenesis (Table 2). The evaluation of short-term assays for carcinogenesis involves mainly two correlations, that is, between mutation and animal cancer data on the one hand and between animal cancer data and human carcinogenicity on the other. The primary aim of short-term mutagenicity assays is to provide evidence of whether a compound can be expected to cause mutations in humans and such evidence has to be considered seriously, even against a background of negative cancer data (59,60).

**Long-term carcinogenicity assays**

Long-term experiments have become an integral part of cancer research. Carcinogenicity bioassays are conducted to identify those agents
Table 2

Short-term assays for mutagenicity and genotoxicity

\[ ^{32}\text{P} \] post-labeling detection of DNA adducts.

Ames Salmonella typhimurium plate incorporation mutagenicity assay.

Cell transformation. (Mammalian cell transformation).

Rabin's Test. (Degranulation of rough endoplasmic reticulum from rat liver).

DNA strand-break analysis.

Tetrazolium - reduction Test. (Reduction of tetrazolium red by mouse skin).

Sebaceous gland test. (Mouse sebaceous gland suppression).

Implant Test. (Tissue reaction to subcutaneous implants in mice).

Purchase et al. (60)
(chemicals, mixtures of chemicals, environmental and occupational exposures or biological and physical agents) that may induce cancer in humans. Besides human experience and epidemiological investigations, long-term studies in laboratory animals are currently the most generally accepted means for determining carcinogenic hazards to public health. Such toxicology studies are typically carried out using one or both sexes of one or more species of rodents, divided into control and exposure groups. Duration of exposure is generally 18-30 or more months. Their importance in the process of assessing cancer risks has been discussed repeatedly and even with the increasing use of short-term mutagenicity assays, animal experiments remain an indispensable component of cancer risk assessment (59, 61,62).

The primary objective of most whole-animal bioassay systems has been to identify carcinogens rather than their mechanism of action; however, even in the standard bioassay, some information can be gained on mechanisms.

**Metabolic Activation**

Many chemical carcinogens require metabolic activation generally to high energy electrophiles to exert their carcinogenic effects. This concept of metabolic activation was proposed by J. and E. Miller on the basis of their pioneering studies (63-65). Carcinogens and mutagens that require metabolic activation to manifest biological activity are referred to as "procarcinogens" and "promutagens" (Fig.8).
Figure 8.

Metabolic activation of procarcinogens to ultimate carcinogens. (64)
FIGURE 8.

Procarcinogen

Proximate carcinogen

Inactive metabolites

Ultimate carcinogen

Noncritical binding

Spontaneous decomposition

Critical covalent interaction with informational macromolecules
The metabolism of most xenobiotics involves reactions catalyzed by phase-I and phase-II enzymes (Table 3). The primary role of these enzymes is to form hydrophilic metabolites that are easily excreted; however, electrophilic intermediates may also be produced which can react with nucleophilic centres in macromolecules, such as nucleic acids and proteins. Among the phase-I enzymes, cytochrome(s) P450-dependent mixed-function oxidases have received particular attention, as they are considered to be the major catalysts of the first, very often rate-limiting, step(s) in the pathways leading to the metabolic activation of most chemical carcinogens and mutagens. In many cases, phase-I metabolites (sometimes called "proximate" carcinogens or mutagens) and certain procarcinogens require metabolism by phase-II enzymes to form "ultimate" carcinogens or mutagens - highly reactive species which readily form adducts with DNA. Phase-I and Phase-II enzymes not only play a role in the metabolic activation of various chemicals, but also catalyze detoxification reactions which either favor the excretion of xenobiotics or inactivate their active metabolites (64). The production of carcinogenic or mutagenic metabolites is thus result of a balance between activation and detoxification pathways which is often difficult to evaluate (Fig.8). Though interspecies differences have long been known in the metabolism of xenobiotics, the metabolic pathways of activation and the resultant carcinogen-DNA adducts are generally qualitatively similar among various animal species, including humans. These observations support the qualitative extrapolation of carcinogenesis data from laboratory animals to the human situation.
**Table 3**

*Phase-I and Phase-II enzymes that catalyze the metabolism of carcinogens*

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azo-nitro-reductases (M/C)</td>
<td>Acyl-transferases (C)</td>
</tr>
<tr>
<td>Cytochrome P450-dependent mono-oxygenases (M)</td>
<td>Glucuronyl transferases (M)</td>
</tr>
<tr>
<td>Cytochrome P450-independent oxidases (M)</td>
<td>Glutathione-S-transferases (C)</td>
</tr>
<tr>
<td>Expoxide hydratase (M/C)</td>
<td>Sulfotransferases (C)</td>
</tr>
<tr>
<td>Hydrolases (M)</td>
<td></td>
</tr>
<tr>
<td>Dehydrogenases (C)</td>
<td></td>
</tr>
</tbody>
</table>

M - microsomal  C - cytosolic
DNA Damage by Chemical Carcinogens

The molecular and atomic analyses of the physical interactions between chemical carcinogens and DNA represent a major achievement of cancer researchers in the last two decades. The information gleaned from these studies of carcinogen-DNA adducts has provided a mechanistic explanation of the mutagenicity of many carcinogens, including their mutational spectra and a rationale for improved carcinogen exposure assessment in molecular epidemiological studies of human cancer.

A grossly simplified model that attempts to relate damaged DNA to mutagenesis is presented in Fig. 9. Mutagenesis results from unrepaired DNA damage. During each cell division cycle, DNA polymerases copy past the damaged DNA and insert noncomplementary nucleotides opposite to the site of damage. A mutational cause of malignancy presupposes that among the dispersed mutations in the genome are mutations in key genes that alter the properties of cells, allowing them to escape homeostatic mechanisms that regulate cell division, invade and metastasize (66).

Spontaneous Mutations as a Cause of Cancer

As in the case of DNA damage by exogenous chemical agents, spontaneous damage would need to occur at a sufficiently high frequency to exceed the capacity of the cell for DNA repair. It should be noted that human cells possess an unusually high efficiency in repairing DNA damage. This repair capacity has been postulated to be responsible for the
Figure 9.

Schematic representation of the relationship of DNA damage to mutagenesis. (67)
Unrepaired DNA Damage → Mutations

DNA Damage → Quiescent Cells → Increased Cell Proliferation → DNA Replication → No change in sequence → Mutations

Repair → Error-Free Incorporation → Error-Prone (SOS) → Mutations
comparatively long lifespan of our species. Spontaneous mutations could have the same potential for inducing cancer as those caused by exogenous environmental agents (67).

**Structure-activity relationships in predicting carcinogenesis**

The application of structure-activity concepts to the elucidation of the action of chemical carcinogens may proceed by two approaches: the hypothesis and the knowledge-based approaches. The former, exemplified by the "structural alters" devised by Ashby and associates, derives from the recognition of the electrophilic nature of carcinogens that damage DNA (68). The latter approach does not assume an а priori mechanism of action but derives information from the establishment of relationships between structural features and carcinogenicity.

Several approaches have been applied to the elucidation of structural relationships among carcinogens for predictive purposes. These include the Hansch and SIMCA (Soft, Independent Modeling of Class Analogy) methods, which are based upon extrathermodynamic approaches and the ADAPT (Automatic Data Analysis using Pattern Recognition Techniques), CASE (Computer Automated Structure Evaluation) and Enslein procedures, which are based upon connectivity relationships. Altogether, the classification power of these procedures varies from 75 to 96%, depending upon the data base that is available (69).
GASTRIC CARCINOGENESIS

Gastric cancer incidence

As of 1980, gastric cancer was the most frequent neoplasm registered in the world (70,71). The American Cancer Society estimated 20,000 cases in 1989 and 23,800 in 1991. Stomach cancer is the third commonest malignancy in India particularly in the South (72). Fig. 10A & 10B shows the latest published incidence rates. Although gastric cancer incidence has been declining in recent decades, it remains one of the most serious health burdens throughout the world. The highest death rates for many decades were registered in Japan, followed by northern Europe and the Andean populations of Latin America. The latest published death rates, are the highest in Costa Rica and relatively high in eastern Europe. At present decline has been more marked in Japan and northern Europe (73). Data for China are not available in this series but gastric cancer accounts for the highest cancer mortality in that country.

Etiology of Gastric Cancer

Case control and cohort studies in a wide variety of populations have shown increased risk associated with frequent consumption of smoked, salted and fried foods and use of starchy foods. The risk is lowered with frequent intake of green leafy vegetables. As shown in Fig. 11, an etiological hypothesis has been proposed to explain the progressive tissue and cellular
**Figure 10A.**

International comparison of Age adjusted (AAR) gastric cancer (ICD-9:151) incidence rates, males and females, per 100,000 population. (70)

Country
- A. Japan (Miyagi)
- B. Costa Rica
- C. China (Shanghai)
- D. Finland
- E. United Kingdom (Oxford)
- F. United States (Connecticut) White
- G. United States (Connecticut) Black
- H. Kuwait

**Figure 10B.**

Age adjusted (AAR) gastric cancer incidence rates, males and females, in Indian population per 100,000. (72)

State
- A. Madras
- B. Bangalore
- C. Bombay
- D. Bhopal
- E. Delhi
- F. Barshi
FIGURE 11. Hypothesis of gastric cancer etiology

Diet

Irritant
(NaCl - aspirin)

Nutrition Deficits
(Animal Proteins)
(Vitamins)

Gastric Cavity

Normal

Superficial Gastritis

Antibodies

Gastrectomy

Atrophic Gastritis

Higher pH

Bacterial Growth

NO₂

NO₃

Anti-oxidant Deficit

Nitrogen Compounds
(Foods - Drugs)

Bile Acids

N O Mutagens

Small Intestine Metaplasia

Carotenoids Deficit

Irritants

Colonic Metaplasia

Mild Dysplasia

Severe Dysplasia

Carcinoma

Invasion
changes and to identify the etiological forces acting at different points in the chain of causation (74,75).

Etiological Factors

The original hypothesis published in 1975 considered 3 major etiological factors, namely excessively salted foods and low intake of ascorbic acid and carotenoids (76). High salt intake causes excessive cell replication, an event well known to increase cancer risk because of potentiation of the action of carcinogens and the possibility of increased rate of endogenous mutations (77).

Chronic atrophic gastritis, because of the loss of acid secreting parietal cells, leads to higher gastric pH and proliferation of anaerobic bacteria which reduce nitrate, abundant in many foods, to nitrite. The latter molecule has a propensity to react with other nitrogen-containing compounds to form N-nitroso derivatives that are mutagens and carcinogens. These processes may be inhibited by naturally occurring antioxidants such as ascorbic acid. The protective effect of this vitamin continues to receive strong support from epidemiological and laboratory research. All independent case-control studies which addressed the subject have reported significant reductions of gastric cancer risk (78).

The role of β-carotene has been linked to late events in the gastric precancerous process, especially in dysplasia and early invasive carcinoma. Although the mechanism of its protective action has not been elucidated, its
role as free radical scavenger and its capacity to open intercellular gap junction communications have been pointed out (79).

**Cell Proliferation in Gastric Cancer**

In human pathology predisposing to cancer and in rodents after treatment with chemical carcinogens, the earliest modifications that develop in gastrointestinal epithelial cells is, increased proliferative activity in a basal region of epithelium. Eventually, excessive numbers of proliferating cells accumulate in the epithelium, without undergoing normal maturation. In the gastrointestinal tract the epithelial lining has one of the most rapid turnover rates of any tissue in the body.

Many factors modulate the proliferation and differentiation of cells in the stomach; some are common to all cells and others are specific for a region or cell type. In the stomach, in addition to general growth stimulating factors, several polypeptides including gastrin, epidermal growth factors and bombesin act as trophic factors in cells within the mucosa, while somatostatin appears to have inhibitory effect (80).

**Classification of Gastric cancer**

**Human Etiological Model**

Morphological alterations on the human stomach have been documented in several countries for more than a century. The morphological
changes observed fall into three categories: inflammation, atrophy and loss of cellular differentiation. The inflammatory changes (chronic gastritis) are more accentuated in younger individuals and become progressively less conspicuous with age. Atrophy or gland loss, becomes more advanced and conspicuous with age; in extreme situations the gastric mucosa becomes practically devoid of its original glands. The loss of differentiation in reality appears to represent successive mutations (or similar changes in the genetic material of the cells), since the gastric epithelial cells disappear as such and are replaced by cells with intestinal phenotype; the daughters of these "mature" intestinal cells then display apparently progressive phenotypic changes, lose some of their normal cytoplasmic secretions and gain autonomy, which eventually leads to uninhibited replication and invasion of the neighboring tissues. They have, among other things, led to two major conclusions. (a) There are at least two distinct clinicopathological entities covered by the name "gastric carcinoma." One is called "intestinal" or "expansive" type, which predominates in high-risk populations ("epidemic type") and is preceded by a prolonged precancerous process. The second type, usually called "diffuse" or "infiltrative," is relatively more frequent in low-risk populations and is not preceded by well-defined precancerous lesions. (b) The precancerous stages of the intestinal type, represent a very complex process, part of which results in a transformation of the normal mucosa into an intestinal type of mucosa (Fig. 11). Observations in several populations at high gastric cancer risk have documented a series of lesions which are more severe and more extensive in older individuals, leading to the hypothesis that
they form a continuum which reflects increasingly regressive phenotypical changes (76).

**Phenotypic changes**

The first step in the process (inflammation and atrophy) do not change the normal phenotype of the gastric epithelial cells: they involve cell loss and cell regeneration, processes that are normal in the gastrointestinal epithelium and only become exaggerated when the forces responsible for the atrophic gastritis set in. Some of these forces or factors have been identified in epidemiological studies as irritants and as a suboptimal supply of micronutrients (81). After the step of atrophy, the forces involved in the process apparently require the presence of genotoxic agents, since at this stage hereditary cellular changes are implied which should represent changes in the structure or function of nuclear DNA. It has been proposed that these mutagenic agents are synthesized *in vivo* in the stomach by the action of nitrite on nitrogen-containing organic compounds (76). The synthesis of nitroso compounds and the cellular damage that they may cause are probably modulated by other compounds or as inhibitors through antioxidant or similar roles (79).

**Environmental risk assessment in gastric carcinogenesis**

Quantitative risk assessment is currently more mathematical extrapolation and scientific intuition than a rigorous science. The discovery of multiple genetic and epigenetic changes during carcinogenesis is an
example that provides opportunities for both early diagnosis of preneoplastic lesions and novel interventive methods to inhibit or reverse carcinogenesis.

Exposure to environmental nitrate and nitrosation of smoked foods has been associated with an increased risk of stomach cancer (82-84). Early studies from this laboratory demonstrated that pan fry cooking induced the formation of Polycyclic aromatic hydrocarbons (PAH) in meat (85). Nitrite is abundant in the gastric cavity of subjects with atrophic gastritis, at least partially as a result of bacterial reduction of dietary nitrate. In addition, nitrate and nitrite may be produced by macrophages, which are also present in chronic gastritis. The synthesis of nitroso compounds and the cellular damage that they may cause are probably modulated by other compounds. Although the key nitrosating agent is nitrite, the situation with regard to the formation of carcinogenic nitroso compounds is greatly complicated by the presence of other chemicals in the environment. In this respect, phenolic compounds react with nitrite under acidic conditions to form potent genotoxic substances such as diazonium derivatives (86-88).

Although our knowledge on the modulation of the gastric carcinogenesis process has advanced, the identification of the carcinogen(s) remains elusive. Understanding its causation is important for the primary and secondary prevention of the disease.
Phenolic compounds in the Environment

These phenolic compounds are ubiquitously present in our environment as contaminants in cigarette smoke as well as constituents of vegetables and fruits and similarly they might be expected to exert synergism in glandular stomach carcinogenesis. Therefore, phenolic compounds are the potential environmental promoters or carcinogen active in human gastric carcinogenesis.

Catechol (1,2-benzenediol) is a major phenolic component in both mainstream and side stream of cigarette smoke, up to 0.5 mg/cigarette and an important industrial chemical (89). It is also cocarcinogenic with benzo(a)pyrene on mouse skin (90). Catechol may therefore be involved in the tobacco-related cancers, which include cancer of the lung, oral cavity, oesophagus, pancreas and bladder. In addition, it is present in certain foods such as onion, crude beet sugar, coffee and beverages (91,92) and is formed by the metabolic degradation of many synthetic and naturally occurring organic compounds (93).

In addition, p-methylcatechol (PMC) and o-methylcatechol (OMC) are further dihydroxybenzene species of environmental significance and although the amount is small, the p-methylcatechol is shown to be excreted in human urine (90,94). o-methylcatechol and its derivatives have also found widespread application as expectorants. The chemical structures of the above dihydroxybenzene derivatives are shown in Fig. 12.
Figure 12.

Chemical structure of the phenolic compounds Catechol and its derivatives; p-methylcatechol and o-methylcatechol.
FIGURE 12.

Catechol

p-methylcatechol  o-methoxyphenol
Catechol and its derivatives in Human Urine

The presence of catechol as a constituent of human urine has been reported previously (95). The quantitative analysis of catechol and its conjugates in human urine of smokers and non-smokers on restricted and unrestricted diets have been reported (94). Levels of catechol and its conjugates in the urine of nonsmoking volunteers on an unrestricted diet are 1-30 mg per day. Smokers are known to excrete higher levels of catechol conjugates than nonsmokers. PMC (p-methylcatechol) has been shown to be excreted in human urine at levels up to 11 mg per day.

Animal model

Animal models of carcinogenesis have contributed substantially to our understanding of neoplastic and preneoplastic events. The relevance of animal experiments to human situation was demonstrated by the induction of bladder tumors in dogs exposed to aromatic amines (96). Typically, laboratory experiments seek specific information on suspected carcinogenic agents and for that reason usually focus on one agent at a time. To test the suspect agent, an adequate yield of neoplastic lesions is desirable, usually several orders of magnitude greater than the frequency observed in humans; that requires the administration of doses many times greater than those prevailing in most human situations. The high yield also requires that animal strains be selected which have a proven susceptibility to the compound, thus concentrating on genetically homogenous (often inbred)
populations of experimental animals. The reproducible induction of specific types of tumors in animals by particular chemical or physical carcinogens therefore provides an ideal opportunity to investigate the sequential molecular events associated with the different stages of carcinogenesis (Fig.13). As the mechanisms of carcinogenesis become more thoroughly understood, a rational approach can be evolved for extrapolation from high dose experimental data in animals to low dose natural exposure in human situation.

The model under study looks at the formation of genotoxic agents such as diazonium derivatives via nitrosation of catechol in the stomach and brings about successive cellular transformation. To sum up, this was achieved as evidenced from the data presented in the following chapters.
Figure 13.

Strategy for studies of molecular carcinogenesis and molecular epidemiology to improve interspecies extrapolation of carcinogenesis data and to identify high cancer risk individuals. (11)