REVIEW OF LITERATURE
A. CARCINOGENESIS
Carcinogenesis

Cancer is considered to be genetic disorder since it is caused by changes in DNA that are stably inherited by progeny cells. Over a period of time accumulation of genetic mutations in particular classes of genes in a cell results in cancer. The process of induction of cancer is called carcinogenesis. Carcinogenesis is induced by carcinogens which results in genetic and epigenetic damae in susceptible cells. These cells gain selective growth advantage and undergo clonal expansion as a result of activation of proto oncogenes and/or inactivation of tumor suppressor genes. This leads to the disruption of normal cellular functions thus enable a clonal expansion of abnormal cell to form a tumor.

Aetiological Factors :

Epidemiological and experimental evidence show that cancers can be induced by various factors. 1. physical, 2. biological and 3. chemical.

1. Physical :

The most insidious physical cause of cancer is radiation. Human tissues are susceptible to ionizing radiation and non-ionizing ultraviolet induced carcinogenesis (IARC. 1992). Ionizing radiation are known to produce somatic mutations, activate oncogenic viruses or other proto oncogenes and causes misrepairs in nucleic acid sequences which result in neoplastic changes (UNSCEAR. 1994). Demple and Linn (1982) reported the induction of pyrimidine dimers in DNA by ultraviolet
radiations. The expression of various oncogenes e.g. Myc, K-ras, N-ras and H-ras have been reported in radiation induced tumors or transformed cells (Kaminsky et al., 1985). Electric and magnetic field exposure also have been reported for their carcinogenic action (Pitot 1986).

2. Biological factors:

Living organisms such as viruses, which can get integrated into host cellular DNA are important biological factors for inducing cancer. In the last 25 years, major steps have been taken to identify viruses that cause cancer or act as co-factors in the promotion of cancer in humans.

The types of cancer in human being which are attributable to a virus, are liver cancer (due to chronic infection with hepatitis B virus), Epstein barr virus and Burkits lymphoma in children. human papilloma virus causes cancer of the uterine Cervix and human immune deficiency virus (HIV) is known to produce Kaposis sarcoma (Bishop, 1983, Pitot, 1986) parasitic infections such as schistosomiasis have also been identified as risk factors for bladder cancer.

3. Chemical factors:

Evaluation of occupational exposure to chemicals has given an insight into the fact that foreign chemicals could result in cancer (Weinstein, 1985). Chemical carcinogenesis was discovered in the end of eighteenth century by Sir Percival Pott (1775), an English surgeon who correlated the incidence of scrotal skin cancer in some of his patients who worked as chimney sweeper during childhood. They were exposed to coal tar.

One hundred and fifty years later an experimental basis for pott's clinical observation was reported. In 1918 Japanese pathologist Yamagiwa
and Ichikawa successfully induced skin tumor in the rabbit's ear by long continued application of coal tar. Since then, several studies have shown that the skin of mice is also susceptible to the carcinogenic action of coal tars.

In 1930's for the first time particular component responsible for the carcinogenic action of Crude Tar were isolated and synthesized by Kennaway and Hieger as 1, 2, 5, 6 - dibenz(a)anthracene.

Later on 1933, Cook et al. for the first time isolated few milligram of 3, 4 benzo(a)pyrene from several tons of coal tar, now called or is known as benzo(a)pyrene. This constituted one of the major land marks in the field of experimental carcinogenesis and heralded the era for the testing of a great variety of polycyclic aromatic hydrocarbons for carcinogenecity.

Today, several industrial chemicals have been identified as risk factors for cancer e.g., arsenic, nickel, chromium, vinyl chloride, benzene, naphthalamine and polycyclic aromatic hydrocarbons, etc. (table II). Laboratory investigations carried out in experimental animals further reinforced the findings on the effects of foreign chemicals in the causation of cancer. The role of life style factors such as tobacco and alcohol, in the development of cancer is evident from numerous epidemiological and experimental studies (Weinstein, 1991). Cigarette smoking, a major cause of cancer of lung, larynx, oral cavity and oesophagus, has been reported to contribute the development of bladder, pancreas and kidney cancers. In fact, 30% of all cancer deaths have been related to tobacco smoking and chewing (Doll and Peto, 1981).
Table II: Common cancers that have been associated with certain occupation (Costanza, 1990)

<table>
<thead>
<tr>
<th>Industry of material</th>
<th>Carcinogen</th>
<th>Site of associated cancer</th>
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<tbody>
<tr>
<td>Asbestos</td>
<td>Asbestos</td>
<td>Lung</td>
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<tr>
<td>Brewing</td>
<td>Alcohol</td>
<td>Liver</td>
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<tr>
<td>Commercial fishing</td>
<td>Ultraviolet light</td>
<td>Skin</td>
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<tr>
<td>Demolition</td>
<td>Asbestos</td>
<td>Lung</td>
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<tr>
<td>Furniture manufacturing</td>
<td>Wood dusts</td>
<td>Nasal</td>
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<tr>
<td>Glue factories</td>
<td>Benzene</td>
<td>Leukemia</td>
</tr>
<tr>
<td>Insulation</td>
<td>Asbestos</td>
<td>Lung</td>
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<tr>
<td>Ion exchange resin production</td>
<td>Bos (chloromethyl) ether</td>
<td>Lung</td>
</tr>
<tr>
<td>Isopropyl alcohol manufacturing</td>
<td>Isopropyl alcohol</td>
<td>Lung</td>
</tr>
<tr>
<td>Mineral oil</td>
<td>Polycyclic hydrocarbons</td>
<td>Lung</td>
</tr>
<tr>
<td>Nickel refining</td>
<td>Nickel</td>
<td>Respiratory</td>
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<tr>
<td>Ore manufacturing</td>
<td>Chromium</td>
<td>Lung</td>
</tr>
<tr>
<td>Outdoor occupations</td>
<td>Ultraviolet light</td>
<td>Skin</td>
</tr>
<tr>
<td>Pesticides-arsenic</td>
<td>Arsenic</td>
<td>Lung, skin</td>
</tr>
<tr>
<td>Petroleum production</td>
<td>Polycyclic hydrocarbons</td>
<td>Lung</td>
</tr>
<tr>
<td>Pigment manufacturing</td>
<td>Chromium</td>
<td>Lung</td>
</tr>
<tr>
<td>Rubber manufacturing</td>
<td>Aromatic amines</td>
<td>Bladder</td>
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<td>Shipyards</td>
<td>Asbestos</td>
<td>Lung</td>
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<tr>
<td>Smelters</td>
<td>Arsenic</td>
<td>Lung, skin</td>
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<tr>
<td>Uranium mining</td>
<td>Ionizing radiation</td>
<td>Lung, others</td>
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<tr>
<td>Varnish</td>
<td>Benzene</td>
<td>Leukemia</td>
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<tr>
<td>Vinyl chloride</td>
<td>Vinyl chloride</td>
<td>Liver</td>
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Mechanism of Chemical Carcinogenesis:

During the last 80 years a multiple process in carcinogenesis have been evolved. In 1941 Rous and Kidel termed a two stage carcinogenesis model as initiation and promotion. However, it has been proposed that many factors are required for a tumor to progress. It is now an established fact that carcinogenesis is a multistep process.

Skin carcinogenesis is one of the best model to understand various steps. The initiation and promotion stages in skin carcinogenesis was demonstrated with tar and 3, 4 benzopyrene (Berenblum, 1941, Mackenzie and Rous, 1941).

Berenblum (1941) and Mackenzie and Rous (1941) showed that the administration of a limited dose of a chemical carcinogen to mouse or rabbit skin causes a change in some cells that are imperceptible in the absence of further treatment and do not by themselves result in tumors. The initiation is generally considered to be completed rapidly and irreversible.

The second stage promotion, occurs over a period of weeks and months and is, at least in its early phase largely reversible.

The classical promoting agent for mouse skin is croton oil which was first used by Berenblum as a co-carcinogen (Berenblum, 1941).

From the accumulated information about the complete process of chemical carcinogenesis (Areos et al., 1968; Miller, 1970; Heidelberger, 1973; Marks and Frustenberger, 1988) certain general principles about tumor development could be derived.
1. Tumor is focal in origin. It is commonly held that each tumor is descended from a single altered cell, referred to as monoclonal in origin (Fialkow, 1972).

2. Chemical carcinogens are capable of acting on normal cell during mitotic cycle (Iversen, 1973; Berenblum and Armuth, 1977) converting it first into a latent or dormant tumor cell (Berenblum, 1941, 1975).

3. The entire process of carcinogenesis i.e. the neoplastic transformation of a normal cell is not a single step process, but comprise at least 2 components.
   a. An initiating phase responsible for the conversion of the normal cell into a dormant tumor cell and
   b. A promoting phase, responsible for the awakening of the dormant tumor cells, or its progeny into a progressively growing tumor (Boutwell, 1964; Van Duuren, 1969).

4. Initiation is an instantaneous, irreversible reaction, presumably, and involves the covalent binding of an ultimate carcinogen to DNA. Initiating cells do not lose this induced property with time. Although the precise mechanism underlying tumor initiation and it's maintenance over indefinite periods of time are not known. DNA replication resulting in adduct induced miscoding appears to be essential for tumor initiation (Farber, 1984 a and b).

5. Promotion is a gradual change since repeated treatment with a promoter is required and is reversible in early stages.

At present initiation and promotion systems using DMBA/croton oil and a mouse epidermis as the target organ is one of the most developed investigational models of tumorigenesis.
Miller and Miller (1971) has proposed the possible genetic and epigenetic mechanisms of chemical carcinogenesis (Figure VI).

The first genetic mechanism is the somatic mutation concept in which the copying of a chemically changed DNA could lead to alteration, deletions, or rearrangement of the DNA nucleolide sequence that would be perpetuated with permanent changes in growth control. The second mechanism is fixation altered DNA which occurs during reverse transcription of RNA. The third mechanism is based on the observation of Speyer and associates (Speyer et al., 1966) that poor accuracy with which their DNA polymerase copies the phage DNA. Chemical alteration of only a few DNA polymerase molecules might be sufficient to introduce critical mutations into cellular DNA favouring the eventual emergence of neoplastic cells.

The reactions of chemical carcinogens with proteins and RNA's lead to consideration of epigenetic mechanisms.

The first epigenetic mechanism is based on the popular model of cellular differentiation. The variety of expressions of embryonic antigens and enzymes in chemically induced tumors, which have been reported in the past years could be examples of effect, of chemically carcinogens on gene read out. These changes in transcription could also include derepression of past or all of integrated tumor virus genomes or of the oncogenes (Huebner and Todaro, 1969).

Epigenetic mechanisms can also be envisaged that would permit the preferential proliferation of previously existing neoplastic or pre neoplastic cells.
Figure VI: Mechanism of action of chemical carcinogens.

Genotoxic carcinogens induce mutation and epigenetic carcinogens modulate the expression of DNA lesions.
Further, Miller and Miller (1976) have classified DNA-reactive carcinogens (genotoxic) to two major classes according to their metabolism. The first class, those direct acting carcinogens which do not need metabolic activation and bind as such with DNA and act as mutagens.

The second class is procarcinogens that must be converted metabolically by different metabolizing enzymes in the host to yield reactive ultimate derivatives or metabolites, known as ultimate carcinogens, without which they are noncarcinogenic and non mutagenic. The ultimate carcinogens are highly reactive electrophiles (positive charge metabolites) that react with nucleophilic macromolecules in the cell. The critical target most probably being DNA (Miller, 1978).

The direct acting or activation independent carcinogens possess the necessary chemical structure for their reactivity as electrophilic reactants.

Thus, direct acting and indirect acting (ultimate carcinogens) brings about transformation of cells and the formation of tumors through binding to and causing changes in the DNA.

In both cases genetic change induced by the chemical carcinogens could include gene amplification, gene mutation, chromosomal rearrangement and a neuploidy providing the initiated cells with an altered responsiveness to their micro environment and also a selective advantage of clonal expansion compared to surrounding cells (Yuspa and Harris, 1982. Cerutti, 1985).

Direct acting carcinogens include alkylating agents such as some anti cancer therapeutic chemicals (e.g. cyclophosphamide, chlorambucil and nitroso ureas etc.) examples of indirect acting carcinogens or procarcinogens are polycyclic aromatic hydrocarbon, azo dyes and natural metabolites (such as aflatoxin B1 produced from fungal contamination of food) and nitros amines (produced by nitrite in food).
B. A CHEMOPREVENTIVE APPROACH OF CANCER
Chemoprevention

Inspite of various attempts have been made to cures of tumors at the advanced stages, overall mortality for most forms of cancer in the past 25 years has not declined (WHO, 1997). More recently attention has been focused on chemoprevention to reduce the cancer incidence and mortality.

The term, chemoprevention was used for the first time by Sporn in 1979 who had introduced the concept of chemoprevention of cancer at the National Cancer Institute in the United States. At present cancer chemoprevention is one of the important subjects in cancer research and a new medical strategy for cancer prevention.

Carcinogenesis could be prevented by blocking any stage in the pathway between environmental agent and the interaction of its activated form with critical cellular macromolecules which are ultimate target for carcinogenesis.

It is fortunate that the progression from initiation to invasive epithelial cancer may often take twenty years or more in man (Saccomanno et al., 1971; Farrow et al., 1976), which is known as latent period. There is thus long interval available to prevent progression of pre malignant epithelial disease to an invasive malignant state if the appropriate pharmacological agents could be developed and used during this latent period.

Thus, chemoprevention is the use of pharmacological or natural agents that inhibit the development of invasive cancer by either blocking the DNA damage that initiated carcinogenesis or by arresting the progression of premalignant cells by which the damage has occurred (Wattenberg, 1997; Kelloff, 1997).
The goals of chemopvention are:

1. inhibition of carcinogens
2. logical intervention for persons at genetic risk for cancer
3. treatment of pre cancerous lesions, and
4. confirmation and translation of leads from dietary epidemiology into intervention strategies.

There are two forms of subject categories for chemopvention one. in general population chemopvention entails efforts of providing measures applicable to large population groups, and the other targeted chemopvention is directed towards individuals increased risk of cancer in specific tissues (Wattenberg, 1992, 1997).

Chemopreventive Strategies:

1. **Molecular Target for Chemopvention**:

   Many enzymes, genetic lesions and other cellular constituents associated with initiation and progression of precancerous to invasive disease have been identified. Systematic evaluation of classes of agents which interfere with the expression and / or activity of these molecules have chemopreventive potential.

   Examples of chemopreventive agents develop on the basis of this approach e.g. selective estrogen receptor modulator for breast cancer, androgen receptor modulating for prostate cancer and retinoids for nuclear receptor (retinoid receptors) as modulator agents and cyclo-oxygenase (Cox-2) inhibitors for colon carcinogenesis (Hong and Sporn, 1997; Kelloff et al., 1997; )
2. Mutagenesis and Mitogenesis: Empirical Targets for Chemoprevention

Experimental and epidemiological carcinogenesis study have shown that more than 90% of cancers are associated with mutagens and mitogens. Thus there is a need for the identification and characterization of chemopreventive agents.

Thus the empirical approach is used in conjunction with the molecular targeting (Hong and Sporn, 1997, Kelloff et al., 1997) that inhibit or reverse cellular processes associate with carcinogenesis.

A normal functioning cell has three possible fates:
(1) programmed cell death (from senescence, or in response to damage or environmental conditions such as over population or hormone withdrawal);
(2) maturation or differentiation and
(3) proliferation

Mutagenesis can damage the cell and disrupt normal growth controls resulting in loss of programmed cell death and maturation pathways and increased (hyper) proliferation.

So, this second strategy is to look for agents, that block cell disruption, inhibits mutagenesis and proliferation and are inducers of apoptosis and differentiation (Kelloff et al., 1997).

Wattenberg (1985) has classified chemopreventive agents in animal models into three major classes based on the time of action process of carcinogenesis (see fig. VII).
(1) Inhibitors of carcinogen formation  
(2) Blocking Agents of carcinogen  
(3) Suppressing Agents  

![Diagram](image)

**Fig. VII**: Classification of chemopreventive agent on the basis of the time at which they exert their protective effects. This scheme is based on one developed by Wattenberg (1985)

Inhibitors of carcinogen formation, inhibit the formation of active carcinogen species from their precursors eg. vitamin C which prevent formation of nitrosoamines from their precursors the nitrates and secondary amines.

Blocking Agents of carcinogen, prevent the initiation state of carcinogenesis by preventing the carcinogens from reaching or reacting with the cellular targets eg. Isothiocyanates, flavones etc.

Suppressing agents acts mainly on the promotional stage of carcinogenesis. They prevent the expression of neoplasia in cells previously exposed to carcinogen etc. vit. A, selenium salts, protease inhibitors etc.
However, the ideal chemopreventive agent should have the following qualities (Morse and Stoner. 1993):

(i) little or no untoward or toxic effects;
(ii) high efficacy;
(iii) capability of oral administration;
(iv) a known mechanism of action; and
(v) low cost.

Mechanisms of Chemoprevention

The possible potential mechanisms of chemoprevention have been summarized (Kelloff et al., 1996b).

(1) Carcinogen : blocking activities:

(i) Inhibition of carcinogen uptake: Agent that inhibit carcinogen uptake appear to react directly with putative carcinogens - both initiators and promoters. For example, calcium inhibition of colon tumor promotion and colonic hyperproliferation in rats has been attributed partially to this mechanism. Calcium binds to excess bile and free fatty acids that irritate the colon lumen and promote of tumors.

(ii) Inhibition of carcinogen formation / activation: Inhibition of the formation of carcinogenic N-Nitroso compounds by vitamin C is probably the best known representative of this mechanism. Vitamin C inhibits the induction of lung tumors induced by the combination of methyl urea and nitrite. Vitamin E prevents the formation of nitrosamines from their precursors by scavenging nitrite.
Table III: Possible mechanisms of chemoprevention and associated agents (Kelloff et al., 1996)

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<tbody>
<tr>
<td><strong>I. Inhibition of carcinogen uptake</strong></td>
<td><strong>I. Scavenging of reactive electrophiles</strong></td>
<td><strong>I. Modulation of signal transduction</strong></td>
</tr>
<tr>
<td>Calcium</td>
<td>GSH-enhancing agents</td>
<td>Glycyrrhetinic acid, NSAIDs, polyphenols, retinoids, tamoxifen</td>
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<tr>
<td><strong>II. Inhibition of carcinogen formation activation</strong></td>
<td><strong>II. Scavenging of oxygen radicals</strong></td>
<td><strong>II. Modulation of hormonal growth factor activity</strong></td>
</tr>
<tr>
<td>Arylalkyl isothiocyanates, DHEA, NSAIDs, polyphenols, (+)-vorozole and other aromatase inhibitors</td>
<td>Polyphenols, vitamin E</td>
<td>DHEA, NSAIDs, retinoids, tamoxifen</td>
</tr>
<tr>
<td><strong>III. Deactivation detoxification of carcinogens</strong></td>
<td><strong>III. Inhibition of arachidonic acid metabolism</strong></td>
<td><strong>III. Inhibition of oncogene activity</strong></td>
</tr>
<tr>
<td>Indole-3-carbinol, olitipraz, other GSH-enhancing agents</td>
<td>Curcumin, glycyrrhetinic acid, NAC, NSAIDs, polyphenols, tamoxifen</td>
<td>DHEA, genistein, NSAIDs, monoterpenes</td>
</tr>
<tr>
<td><strong>IV. Prevention of carcinogen binding to DNA</strong></td>
<td><strong>IV. Inhibition of polyamine metabolism</strong></td>
<td><strong>IV. Inhibition of polyamine metabolism</strong></td>
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<tr>
<td>Olitipraz, polyphenols</td>
<td>Polyamines, vitamin E</td>
<td>Polyamines, vitamin E</td>
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<tr>
<td><strong>V. Enhancement of level or fidelity of DNA repair</strong></td>
<td><strong>V. Induction of terminal differentiation</strong></td>
<td><strong>V. Induction of terminal differentiation</strong></td>
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<tr>
<td>NAC, protease inhibitors</td>
<td>Calcium, retinoids, vitamin D3</td>
<td>Calcium, retinoids, vitamin D3</td>
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<td><strong>VI. Restoration of immune response</strong></td>
<td><strong>VI. Restoration of intercellular communication</strong></td>
<td><strong>VI. Restoration of immune response</strong></td>
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<tr>
<td>NSAIDs, selenium, vitamin E</td>
<td>Carotenoids, retinoids</td>
<td>NSAIDs, selenium, vitamin E</td>
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<tr>
<td><strong>VII. Enhancement of intercellular communication</strong></td>
<td><strong>VII. Restoration of tumour suppressor function</strong></td>
<td><strong>VII. Enhancement of intercellular communication</strong></td>
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<td><strong>IX. Induction of programmed cell death (apoptosis)</strong></td>
<td><strong>IX. Inhibition of telomerase</strong></td>
<td><strong>IX. Induction of programmed cell death (apoptosis)</strong></td>
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<tr>
<td>Butyric acid, genistein, retinoids, sulindac sulphone, tamoxifen</td>
<td>Folic acid</td>
<td>Butyric acid, genistein, retinoids, sulindac sulphone, tamoxifen</td>
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<tr>
<td><strong>X. Inhibition of telomerase</strong></td>
<td><strong>X. Correction of DNA methylation imbalances</strong></td>
<td><strong>X. Inhibition of telomerase</strong></td>
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<tr>
<td><strong>XI. Inhibition of angiogenesis</strong></td>
<td><strong>XI. Correction of DNA methylation imbalances</strong></td>
<td><strong>XI. Inhibition of angiogenesis</strong></td>
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<tr>
<td>Genistein, retinoids, tamoxifen</td>
<td>Folic acid</td>
<td>Genistein, retinoids, tamoxifen</td>
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<td><strong>XIII. Inhibition of basement membrane degradation</strong></td>
<td><strong>XIII. Inhibition of basement membrane degradation</strong></td>
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<tr>
<td>Protease inhibitors</td>
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<td>Protease inhibitors</td>
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<td><strong>XIV. Activation of antimetastasis genes</strong></td>
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The carcinogen formation can also be inhibited by preventing metabolic activation of aprocarcinogen. Many chemopreventives have this type of activity, e.g. allylic sulphides, arylalkyl isothiocyanate.
carbomates, and falvonoids and other polyphenols. Steroid aromatase, a cytochrome P-450 enzyme, catalyses the first step in estrogen biosynthesis from androgens in humans. Several compounds that inhibit aromatase also inhibit chemical carcinogenesis in estrogen-sensitive tissues e.g. 4-hydroxyandrost-4-ene-3,17-dione suicide inhibitor of aromatase, inhibits, 7, 12-dimethyl-benz (a) anthracene (DMBA) induced tumours in rat mammary glands (Lubet et al., 1994).

(iii) **Deactivation / detoxification of carcinogens**: Carcinogen deactivation and detoxification is generally regarded as a very important mechanism of cancer recurrence formation. Its enhancement may prove to be an important strategy for chemoprevention. Two metabolic pathways are crucial to deactivation / detoxification. The first is the introduction or exposure of polar groups (e.g. hydroxyl groups) on xenobiotic compounds via the phase metabolic enzymes, which are primarily the microsomal mixed function oxidases. In many cases, the polar groups become substrates for conjugation. The second pathway is via the phase II metabolic enzymes responsible for conjugations and the formation of glucuronides, glutathione (GSH) conjugates, and sulphates. Chemopreventive agents that act primarily by the second pathway are generally considered more promising than those that modulate the phase I enzymes, since the microsomal mixed function oxidase system is also involved in carcinogenesis activation. GSH is a prototype carcinogen scavenger. It reacts spontaneously or catalyses by GSH S-Transferases (GSTs) with numerous activated carcinogens including N-methyl-N-nitrosoguanidine (MNNG), aflatoxin B₁ (AFB₁), and benzo(a)pyrene (B[a]P) diol epoxide and other activated polycyclic aromatic hydrocarbons.
Similarly, GSH protect against mouse skin tumors induced by DMBA/12-O-Tetra decanoyl phorbol 13-acetate (TPA). rat fore stomach tumors induced by MNNG, and rat liver tumors induced by AFB₁.

A number of promising chemopreventive agents are potent inducers of GSH and GST. Prominent among these chemopreventive agents allylic sulphides, natural products found in onion, garlic and other members of the allium genus. Oltipraz is a potent GST inducer with a wide spectrum of chemopreventive activity.

(iv) **Inhibition of DNA Adduct Formation**: DNA carcinogen adduct formation is a measure of carcinogen exposure. In most cases it is probably secondary to other mechanism of carcinogenesis such as carcinogen activation and formation. Similarly inhibition of DNA adduct formation is typically an indirect measure of other mechanisms of chemoprevention, particularly inhibition of carcinogen formation and activation, and enhancement of carcinogen detoxification. For example, oltipraz prevents the formation of AFB₁-DNA adducts, an effect that has been attributed to increased rates of AFB₁ detoxification by GST. Nonetheless inhibition of DNA adduct formation is a convenient assay for screening potential chemopreventive agents that are expected to modulate carcinogen metabolism. There is also limited evidence of chemopreventive agents directly obstructing adduct formation for example, the inhibition of nitroso-compound carcinogenesis by the polyphenol ellagic acid has been attributed to blocking of methylation of guanine of the O⁶ position. Presumably, this effect is due to ellagic duplex form of DNA.

(v) **Enhancement of level or fidelity of DNA repair**: Three possible chemopreventive mechanisms involve DNA repair. First, the over all
level of DNA repair may be increased. Second the level of the DNA damage modulating enzyme poly (ADP-ribosyl) Transferase (ADPRT), which is decreased by carcinogens, may be stabilized eg. NAC, prevents the decrease in ADPRT caused by the carcinogen 2-acetylaminofluorene. The third mechanism is suppression of error-prone DNA repair. it has been suggested that protease inhibitors such as Bowman-Birk Soybean Trypsin inhibitor (BBI) might prevents carcinogenesis by inhibiting an error-prone repair system activated by proteases that, in turn, are induced by tumor promotors.

(2) Anti oxidant / anti inflammatory activities

Scavenging of reactive electrophiles, potent nucleophiles that react directly with carcinogens and other electrophiles may be chemopreventive.

(i) Scavenging of oxygen radicals: Activated oxygen species (i.e. singlet oxygen, peroxyradicals, superoxide anion, and the hydroxyl radical) have been shown to be involved in carcinogensis, acting both in initiation and in promotion and progression. For example, oxygen radicals are mutagenic by oxidizing DNA bases, and radicals also cause DNA strand breaks and chromosome deletions and rearrangements.

Many compounds that scavenge activated oxygen species are chemopreventing agents. For example, NAC and other chemopreventive thiols are known to react with hydroxyl radicals. The reaction of B-carotene with singlet oxygen and its participation in other free-radical-trapping reactions is well documented. Phenolic antioxidants are known to scavenge peroxy radicals, particularly, vitamin E, (α-tocopherol) is known to scavenge peroxy radicals,
singlet oxygen and super oxide radicals. Non phenolic antioxidants also scavenge oxygen free radicals for example, GSH reacts with alkyl peroxyradicals. Other potential chemopreventive agents - e.g. the protein bound polysacchride psk - I. and bismuth nitrate - appear to work by inducing oxygen - radical - scavenging proteins such as Mn-superoxide dismutase, catalase and GSH-Px.

Anti oxidants that are potent inhibitors of arachidonic acid (AA) metabolism perform perhaps the most prominent scavenging of oxygen free radicals associated with inhibition of carcinogenesis.

(ii) **Inhibition of arachidonic acid (AA) metabolism** : The role of AA metabolism in carcinogenesis has been reviewed by Marnett (1992) and by Zenser and Davis (1992). AA metabolism is increased during inflammation. Two aspects of metabolism are associated strongly with carcinogenecity both are inhibited by antioxidants and anti inflammatory agents.

The first, is the PG synthetic pathway, involving the enzyme prostaglandin H synthase (PHS). This enzyme has two activities, cyclo-oxygenase, which catalyses the formation of prostaglandin G2 (PGG2) from AA, and hydroperoxidase, which catalyses the reduction of PGG2 to PGH2. To return to its native state, the hydroperoxidase requires a reducing co-substrate, procarcinogens, for example amylarnino and arylnitro compounds, are, such substrates. According to the model proposed, the procarcinogens are activated (oxidized) during catalysis to free radicals and electrophiles that can form adduct with DNA and initiate carcinogenesis. This process can be stopped in four ways:
(1) at the formation of PGG$_2$ via inhibition of cyclo-oxygenase.

(2) by inhibition of peroxidase activity,

(3) by prevention of formation of reactive intermediates, and

(4) by scavenging reactive intermediates (e.g. by GSH conjugation).

Relevant to these potential mechanisms, cyclo-oxygenase inhibitors such as non steroid anti-inflammatory drugs (NSAIDs e.g. aspirin, ibuprofen, Indomethacin, and piroxicam) and certain antioxidants (e.g. flavonoids) are effective inhibitors of carcinogenesis. Additionally, PGH$_2$ itself break down to form a known direct acting mutagen malondialdehyde (marnett, 1992). Thus inhibition of cyclo-oxygenase may directly prevent the formation of a potential carcinogen.

The second aspect of AA metabolism associated with carcinogenesis is the burst of PHS and lipoxygenase activity that is seen during inflammation and is stimulated by the tumor promoter TPA compounds that inhibit lipoxygenase such as vitamin E, inhibit tumor promotion in mouse skin, like wise lipoxygenase inhibitors that are stable. The electron donors that competitively inhibit the production of unstable free radicals and electrophiles by PHS (e.g. curcumin flavonoids and tea polyphenols) also inhibit tumor promotion in mouse skin.

The release of AA from membrane phospholipids is another control point in the AA metabolic path way.

AA can be released from phospholipids by some mechanisms e.g. via phospholipase C (PLC) and diaetylglycerol (DAG) lipase resulting in higher levels of phospholipase activity (Zenser and Davis, 1992). Interestingly, the NSAID aspirin may be an inhibitor of PLC (Powis and Workman 1994).
The control of AA release by DAG lipase and phospholipases may be mediated via signal transduction pathways, compounds that block signal transduction at the membrane level, including tamoxifen, flavonoids and glycyrrhetinic acid, may inhibit AA metabolism and be chemopreventive against cancer by this mechanism.

(3) **Antiproliferation / anti progression activities modulation of signal transduction.**

Weinstein (1988) described carcinogenesis as a progressive series or disorders in the function of signal transduction pathways. Signal transduction being the means by which the hormones and growth factors that regulate cell growth, proliferation and differentiation communicate across cell membranes via receptors and receptor-associated enzymes, then through the cytoplasm and into the nucleus via a net work of intermediary molecules known as second messengers. Second messenger include cyclic AMP. Inositol- Tri phosphate. DAG, PGS and various regulatory proteins such as the mitogen - activated protein (MAP) kinases. Whitfield (1992), Brunton and Workman (1993) and Powis (1994), have provided a comprehensive reviews of signal transduction pathways and their potential as targets of chemotherapeutic and chemopreventive drugs. The components of the signal transduction pathways provide many possible sites for chemopreventive activity by restoring normal cellular controls or inhibiting activities that are out of control. In fact most of the anti proliferation / anti progression activities important to chemoprevention, affects some part of the signal transduction path ways. For example, one of the steps in signal transduction involves activation of proteins kinase C (PKC) by DAG. There is evidence that carcinogenesis may be suppressed by inhibiting this enzyme. The phorbol tumor promoter such as TPA can replace DGA in activating PKC. Chemicals that inhibit PKC, such as the flavonoids, also inhibit TPA-induced tumor promotion in mouse skin.
(i) **Modulation of hormonal/growth factor activity**

Chemicals may inhibit carcinogenesis associated cell growth and proliferation by directly regulating the induction and activity of specific hormones and growth factors that initiate steps in signal transduction. This regulation may occur at the levels of the hormones and growth factors themselves, membrane receptors (receptors for growth factors, peptide hormones, and neurotransmitters) or cytoplasm and nuclear receptors (the steroid receptor super family consisting of estrogen, progesterone, retinoid, glucocorticoid, vitamin D, and thyroid receptors).

Transforming growth factors B (TGF-B) has anti proliferative activity in both normal and neoplastic cells in vitro and in mammary glands and liver in vivo. There is evidence from studies in rat intestinal cryptepithelial cells that TGF$_b$ may also promote differentiation. These observation suggest that chemicals that activate TGF-B could also control proliferation in carcinogenesis. The chemopreventive agents tamoxifen and retinoids induce various isoforms of TGF-B in epithelial cells (Roberts and Sporn, 1988, Glick et al., 1991, Sporn et al., 1992).

Modulation of receptor activity by chemopreventive agents is illustrated by anti estrogens such as tamoxifen and toremifene. These bind to nuclear estrogen receptors, preventing the binding and activity of estrogens. Phytoestrogens such as the Isoflavone genistein, have antiestrogenic activity and are potential chemopreventive agents. Studies on human breast and cancer MCF-7 cells indicate the antiestrogenic effect. This may effect from slow translocation of genistein bound receptor from the cytoplasm to the nucleus compared with that estradiol-bound receptor.
(ii) **Inhibition of oncogene activity**

During cell proliferation in carcinogenesis numerous oncogenes are expressed abnormally and their products may act aberrantly as intermediates in the signal transduction pathways. The evidence for oncogene activity in signal transduction in carcinogenesis is based on the similarity of some of the oncogene products (e.g. protein kinases) to known intermediates (Weinstein, 1988). There are several points during activation at which the function of oncogene product Ras can be inhibited. There are data relating this inhibition to chemoprevention. For example, a membrane receptor linked enzyme Tyrosine kinase is involved in Ras activation and inhibitors of this kinase would be expected to prevent Ras activation. Particularly interesting are compounds such as genistein that inhibit tyrosine kinases, and thus may not interfere with normal cellular processes mediated by other types of kinases.

Ras oncogenes are involved in mammary gland carcinogenesis induced by MNU and, to lesser extent by DMBA. Gould and Colleagues (Haag et al., 1992) showed that D-limonene inhibits the progression of mammary tumors induced in rats by MNU or DMBA. They also showed that D-limonene inhibits the farnesylation of small GTP-binding proteins (21-26 KDa) which is necessary for Ras activation. These data suggest that D-limonene could be preventing oncogene activation by inhibiting post-translational farnesylation of P21 Ras (Crowell et al., 1991). It has been also reported that protease inhibitors and retinoids may also inhibit oncogene expression.

(iii) **Inhibition of polyamine metabolism**:

Polyamines play a significant role in cell proliferation differentiation, and malignant transformation (Pegg, 1988; Verma, 1992).
A critical step in polyamine biosynthesis is the ODC-catalysed formation of putrescine from ornithine. There is ample evidence that ODC participate in carcinogenesis - e.g. the enzyme is induced during cell transformation by chemical carcinogens, viruses and oncogenes. The blocking of ODC inhibits transformation.

TPA and other Tumor promoters increase ODC actively in skin, colon, bladder and liver. Chemicals that inhibit induction or deactivate ODC are also chemopreventives. The chemopreventive agent DFMO is a specific, mechanism-based irreversible inhibitor of ODC that inhibits carcinogen-induced tumors in mouse and rat colon, rat mammary gland, mouse skin and mouse and rat urinary bladder.

Chemicals that inhibits PKC and AA metabolism and those that can scavenge free radicals may also inhibit the induction of ODC, hence they may be chemopreventives. For example, several of the PKC inhibitors including glychrrhetinic acid, inhibit ODC induction and tumor promotion in mouse skin. AA metabolism inhibitors also inhibit both ODC induction and TPA-promoted mouse skin tumorigenesis as do free-radical scavengers such as GSH, flavonoids and green tea polyphenols, and retinoids such as 13-Cis retinoic acid and all Trans-N-(4-hydroxy) phenyl-retinamide (4-HPR).

(iv) Induction of Terminal differentiation:

Terminal differentiation is a step in normal, regulated cell proliferation in epithelial tissues. Proliferating cancer cells often lose the ability to differentiate. Abundant evidence demonstrate that restoring the ability of abnormally proliferating cells to differentiate suppresses carcinogenesis. Several classes of chemopreventive agents also induce differentiation eg. retinoids. Evidence indicate that retinoids control
differentiation via intra cellular binding proteins (cellular retinol-binding protein and cellular retinoic-acid-binding protein) and nuclear receptors.

Calcium and vitamin D3 are well-known differentiation inducing agents that also inhibit carcinogenesis. Calcium induces differentiation in epithelial tissues including rat oesophagus, mouse skin and human mammary gland and colon. Vitamin D3 induces differentiation in human colon, human and mouse myeloid leukemia cells, mouse skin cells - mouse melanoma cells, and other cells. It has been suggested that the effects of the two chemicals on differentiation may be mediated by the same signal transduction pathway, involving the vitamin D3 nuclear receptor with calcium as the messenger.

(v) Restoration of immune response:

The role of the immune response in chemoprevention is significant study of antibodies to oncogene products or oncoproteins are important in the inhibition of cell transformation and tumour growth (De Flora and Ramel. 1988).

Chemopreventive retinoids are well known immuno stimulant. Retinoid acid increases cell-mediated and natural killer (NK) cell cytotoxicity. retinoids also cause some leukemia cells to differentiate to mature granulocytes comparable to mature neutrophiles.

Pharmacological doses of vitamin E fed with normal, well-balanced animal diets increase humoral antibody production, especially, IgG production. The effect has been observed repeatedly in chickens, mice, turkeys, guineapigs, and rabbits. Vitamin E also stimulates cell-mediated immunity and induced tumor necrosis factor.
Perhaps the most important action of selenium in inhibiting chemical carcinogenesis is its effect on the cytotoxicity of immune system cells. Compared with normal cells, both T and NK lymphocytes from selenium-deficient mice have a decreased ability to destroy tumor cells in vitro. Selenium supplementation enhances the ability of rat NK cells to kill tumor cells.

(vi) **Enhancement of intercellular communication**

Gap junctions are the cell component that coordinate intercellular communication. They are composed of pores or channels in the cell membranes that join channels of adjacent cells; these pores are regulated and, when open, allow passage of molecules up to about 1000 Da.

Bertram et al. (1992) have proposed that gap junctions allow growth-regulatory signals to move between cells. In vitro studies have shown that inhibition of gap-junctional intercellular communication occurs in the proliferative phase of carcinogenesis. For example TPA, other phorbol ester tumor promoters and mezerein inhibit gap junctional communications in animal and human fibroblasts and epithelial cells. The ability to inhibit intercellular communication correlates with tumor-promoting activity in vivo.

Research carried out by Bertram and colleagues on C3H10T1/2 cells (Zhang et al., 1991) strengthens the concept that carcinogenesis may be inhibited by enhancing intercellular communication carotenoids. Such as β-carotene and canthaxanthine, and retinoids such as [E]-4(2,5, 6, 7, 8-tetrahydro - 5, 5, 8, 8 - Tetramethyl - 2 - naphthalenyl -1-propenyl) benzoic acid and vitamin A, enhanced communication in C3H10T1/2 cells initiated with MCA. The enhancement of
communication correlated with inhibition of transformation in these cells.

(vii) **Restoration of Tumour Suppressor function**

Several so called tumor suppressor genes involved in controlling proliferation and differentiation in cells have been found. They are associated with control of abnormal growth in carcinogenesis. Several of these genes have been identified and implicated in tumor suppression by the presence of mutated or other wise dysfunctional forms in specific cancers eg., Rb in retinoblastoma, osteosarcoma and tumors in lung, bladder, prostate and breast; P53 in adenocarcinomas in colon and breast; human T-cell leukemias. Sarcomas and Tumors in lung and Liver; WT in Wilm's Tumor and DCC in colon tumors. Friedmann (1992) has reviewed research demonstrating the potential for treating cancer patient, with exogenous functional tumor suppressor genes to inhibit tumor growth. Possibly, chemicals will be found that can modulate the expression and activity of tumor suppressor and inhibit carcinogenesis by this mechanism.

(viii) **Induction of programmed cell death (apoptosis)**

Apoptosis is a well-regulated function of the normal cell cycle, it requires gene transcription and translation. Tumor suppressors, such as wild-type P53 and growth factors, particularly TGF-B1, have been implicated as apoptosis inducers. Apoptosis has been described as the complement to mitosis in the maintenance, growth and involution of tissues: it is the process by which damaged and excessive cells are eliminated (Bursch et al., 1992). Apoptosis is inhibited by tumor promoters such as TPA and phenobarbital, and other chemicals that stimulate cell proliferation such as hormones.
For example chemopreventive retinoids (Delia et al., 1993) and sulindac sulphone (Piazza et al. 1995; Thompson et al., 1995) inhibit tumorgenesis, and induce apoptosis.

(ix) **Inhibition of telomerase**

The possible role of Telomeres and telomerase in carcinogenesis has been reviewed recently (Rhyu, 1995; Sommerfeld et al., 1996).

Telomeres are repeating base-pair sequences that form chromosome ends and are essential to maintain chromosome integrity. In humans the repeating sequences is TTAGGG, which may extend upto 15 Kb in length. As cells progress through mitotic cycles, their Telomeres gradually shorten until they reach crisis when chromosome integrity is compromised (e.g. by chromosome fusion). The cells then exit the mitotic cycle, and senescence ensues.

Generally, the activity of the reverse transcriptase telomerase which catalyses the telomere synthesis, is low in normal cells. However, telomerase activity that stabilizes the shortened telomeres and extends proliferative capacity has been observed in cancers and other immortalized cells.

For example Harley, Shay and Colleagues (Kim et al., 1990) found Telomerase activity in 90/101 Tumors Tested (12 different cancers) and 98/100 immortalized cell lines.

Alzcoffey and Colleagues (Sommerfeld et al., 1996) have described telomerase activity as a marker of prostate cancer, based on the analysis of matched samples of carcinoma, benign hyperplasia, and normal tissue, from 25 prostatectomy patients (21/25 cancers have telomerase activity).
These studies suggest that Tolemerase inhibitors may have chemopreventive potential by inhibiting abnormal proliferation and immortalization.

(x) Correction of DNA methylation imbalances

Wainfan and Poirier (1992) indicated that changes in DNA methylation patterns involved in carcinogenesis. Methyl-deficient diets cause fatty livers, increased cell turnover, and promote the development of carcinogen-induced liver tumor in rats and mice. Conversely, methyl-rich diets (fortified with Choline and Methionine) prevent or reduce these effects.

Changes in gene expression-increased expression of proto-oncogenes and decreased expression of growth factors and growth factor receptors in liver appear in animals on methyl deficient diets. These effects are similar to those seen in rodents given tumor-promoting chemicals, and they are reversible on methyl-deficient diets for one week or longer.

Essentially these observations support the hypothesis that hypomethylation of DNA results in alterations in the expression of genes involved in cell growth and regulation. This observation, in turn supports the more general concept that changes in DNA methylation including hypomethylation as well as mutation and steric blocking can affect carcinogenesis. There is only very limited evidence associating chemicals that affect DNA methylation with inhibition of carcinogenesis. Methionine, which is involved with choline, folic acid and vitamin B12 in regulating intracellular methyl metabolism, inhibits DMBA-and MNU-induced mammary gland cancers in rats. Also, folic acid inhibits Isoniazd induced lung tumors in mice.
(xi) **Inhibition of angiogenesis**

Angiogenesis is the process leading to formation of new blood vessels. In normal tissue, it is a highly regulated mechanism essential to reproduction development and wound repair. In carcinogenesis. It is required in tumor growth and involved in metastasis.

There is indirect evidence that, certain chemicals that inhibit carcinogenesis may inhibit angiogenesis. For example, \( \text{PGE}_1 \) and \( \text{PGE}_2 \) are angiogenic, therefore compounds that inhibit PG synthesis may block carcinogenesis by inhibiting angiogenesis.

(xii) **Inhibition of basement membrane degradation**

Tumor cells produce various enzymes that destroy this basement membrane that acts as a barrier against malignant cancer cells and prevents cancer spread. These enzyme include the proteases collagenase cathepsin B, and plasminogen activator. Protease inhibitors are known to act against thrombin and type IV collagenase, which are among the proteases hypothesized to participate in the destruction of basement membranes during cancer invasion. Thus chemopreventive protease inhibitors may derive this effects. by inhibiting basement membrane degradation.

(xiii) **Activation of anti metastasis genes**

Evidence has accumulated that tumour invasion and metastasis like tumor cells proliferation are controlled by effector genes (Liotta et al., 1991). Several genes have been identified that are involved in the suppression of metastasis, including nm23. which apparently encodes nucleoside diphosphate kinase. Levels of nm23 expression correlate inversely with the prognosis and metastasis state in human breast cancer. Although, at this time, there is no substantial evidence that
chemicals that inhibit carcinogenesis induce antimetastasis genes. It is possible that chemicals that increase the expression of the genes or enhance levels of their products (e.g. nucleoside diphosphate kinase) may inhibit the progression of cancers by these mechanisms.

**CHEMOPREVENTIVE AGENTS**

The field of cancer chemoprevention has experienced a rapid growth in identification and characterization of vast number of anti carcinogenic substances that are present naturally in many of our food (lp et al., 1994).

**Table IV : Nutrients and food components that influence carcinogenesis in laboratory animals (Rogers et al., 1993)**

<table>
<thead>
<tr>
<th>Nutrient, food components</th>
<th>Organ site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A, retinoids</td>
<td>Bladder, skin, mammary gland, respiratory tract, colon</td>
</tr>
<tr>
<td>Methionine, choline</td>
<td>Liver, mammary gland, pancreas, colon</td>
</tr>
<tr>
<td>Selenium</td>
<td>Colon, liver, skin, stomach, mammary glands</td>
</tr>
<tr>
<td>Fiber (wheat bran. cellulose)</td>
<td>Colon</td>
</tr>
<tr>
<td>Zinc</td>
<td>Esophagus</td>
</tr>
<tr>
<td>Calcium</td>
<td>Colon</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Skin, stomach</td>
</tr>
<tr>
<td>Whole foods</td>
<td>Many sites</td>
</tr>
<tr>
<td>Fat</td>
<td>Mammary gland, colon, pancrease, skin</td>
</tr>
<tr>
<td>Calories</td>
<td>Many sites</td>
</tr>
</tbody>
</table>

So far more than 1000 potential chemopreventive agents have been identified from over 20 classes of components.

The components of the diet such as macro / micro / non-nutrients that have gained wide recognition in aetiology and chemoprevention of cancer are discussed below:
1. **Micro nutrients**:

Several micronutrients common in the diet of humans have been suggested to be associated with a reduction in the incidence of cancer or inhibition of carcinogens induced neoplasms. These micronutrients are vitamin A, E, D and C.

Vitamin A has been shown to inhibit chemical carcinogenesis in skin lung, urinary bladder, breast, oesophagus pancreas, liver, oral cavity, prostate and stomach of experimental animals (Moon et al., 1994).

The biological functions of retinoids, which are considered to be responsible for their inhibitory effects on carcinogenesis are: induction of cell differentiation, suppression of malignant transformation, counteracting of tumor promoters, inhibition of cell proliferation influenced by growth factor, maintenance of intercellular cell communication through gap junction, alteration of humoral and cell-mediated immunity.

Vitamin E acts as a major free radical scavenger in lipid membranes and is known to potentiate the inhibitory effects of selenium on the promotion or proliferative phases of carcinogenesis (IP and Horvath, 1983). In vitro studies have shown that vitamin E reduces the expression of C-myc and H-ras (Prasad. 1990).

Vitamin C is considered efficient in preventing formation of carcinogenic nitrosamines in stomach (Mirvish. 1986, Wattenberg. 1985) and also in scavenging free radicals in the metabolic process of body (Sauberlich and Machnin, 1992). Interesting functions of vitamin C may be important in inhibiting tumor spread and micro metastases, basement membrane integrity and hyaluronidase inhibition. There are clinical trials (Phase II) for vitamin C currently under way.
Vitamin D has been reported to inhibit the proliferation of myeloid leukaemia cells in vitro and methycholanthene (MCA) induced skin papillomas in mice (Abe et al., 1983; Wood et al., 1981).

Beta carotene is known as a potent antioxidant, inhibiting several types of cancers (Bendich and Olson, 1989).

2. Minerals

Minerals like calcium, copper, iron, zinc and selenium have been found to be effective chemopreventive agents.

Calcium intake has been reported to reduce colon cancer. An inhibiting effect of increased dietary Ca$^2+$ on colon carcinogenesis in rats has been reported and attributed to reduction of crypt epithelial cell hyper proliferation induced by carcinogens or irritants such as bile or fatty acids (Wargovich et al., 1984, Reshef et al., 1990). Iron deficiency has been associated with cancer of upper alimentary tract.

A lowering of latent period for liver tumors is seen in iron deficiency (Bird et al., 1986; Vitale et al., 1978; Rogers et al., 1993).

High dose of copper is associated with decreased chemically induced tumor incidence (Brade and Altman, 1978).

Zinc deficiency has been shown to modify growth of neoplasms (Fenton et al., 1980).

The inhibitory effect of selenium on chemically induced tumors is well documented (Willet, 1986, Ip, 1986, Griffin, 1979). Selenium has been shown to have a protective role in animal models against certain types of tumors : skin, liver, lung, breast and colon cancers (Birt, 1986). The best defined function of selenium in its role as a cofactor for glutathione peroxidase, an enzyme that protect against oxidative tissue damage. It also
supresses cell proliferation, enhances immune responses or alter the metabolism of carcinogens towards production of less toxic compounds via its role in the mixed function oxidase system in the liver. Mc Connel et al., (1980) found significantly lower serum selenium levels in 35 women with breast cancer, compared to women with non-malignant diseases.

3. Dietary Fiber:

Dietary Fiber (the indigestible carbohydrates like cellulose and pectin) provide bulk to the diet. The major categories of foods which provide fiber are whole grain cereals, pulses, vegetables and fruit.

A number of epidemiological studies have indicated direct relationship between dietary fiber and cancers (Hockman, 1989). Colon cancers and breast cancers have been reported to be lower in population consuming high fiber diets (Lubin et al., 1986). Experimental evidence have supported this evidence in MNU induced mammary carcinogenesis.

Components of dietary fiber or substances eg. phytic acid associated with it have been tested for anti carcinogenic activity in rodents.

Several mechanism have been proposed by which dietary fiber might act to reduce colon cancer risk are as follow: Altered bile acid concentration; physical dilution of fecal contents; decreased fermentation; microbial fermentation; production of short chain fatty acids and butyrate; lower pH; lower ammonia levels; decrease in mutagenicity of intestinal contents; alterations in mucins; alterations in mucosal cytokinetics; decrease in ornithine decarboxylase or aryl hydrocarbon, hydroxylase; altered transit time, reduced gut hormones or other peptide growth factors; enteric circulation of hormones, and decreased availability of total dietary energy (Lanza et al., 1992; Klurfed, 1992).
It has also been suggested that dietary fiber protect against breast cancer because of its influence on estrogen metabolism or its association with lignans (Rose, 1990).

4. Fat:

Experimental findings suggest that with low intake of fat the incidence of tumors at different sites particularly of breast and colon is low but it dramatically increases when the fat intake is high (Howe, 1990, Willet and Stampfer, 1990, Prentice and Sheppard, 1991). A high dietary fat content has been shown to increase the DMBA and MNU - induced mammary gland tumors in rats (Kritchevsky et al., 1984).

Amount and type of fat consumed is known to affect the process of carcinogenesis e.g. saturated fat increases the risk for colorectal cancer more than poly unsaturated fat. Diets with polyunsaturated omega 6 fatty acids (PUFA) have strong positive association with breast cancer, and poly unsaturated 3 omega fatty acids, derived from fish oil protects against breast cancer and so is true for olive oil consumption (Welsch, 1992). The mechanisms by which dietary fats act on carcinogenesis process have been intensively sought but are not known.

However the effect of dietary fat found to be organ site specific (Ip et al., 1985; Roebuck et al., 1985).

5. Vegetables and Fruits

High vegetable and fruit consumption is associated with a striking reduction in susceptibility to cancer. (Steinmetz and Potter, 1991a) reviewed the relationship between the cancer risk and quantity of fruit and vegetables consumed, by showing that with increased consumption, a strong and consistent reduction in relative risk for many types of cancer in 128 of 156 studies analysed.
Many studies showing a protective role for vegetables and fruits indicate almost 50% reduction in cancer risk when highest v/s lowest intake levels were compared (Steinmetz and Potter, 1991b).

The inverse association between fruit and vegetables consumption and risks for cancer at several sites may be linked to folate, beta-carotene, other carotenoids, vitamin C, fiber, nutrient or non-nutrient antioxidant available in them (Garewal et al., 1992; Steinmetz et al., 1993, Rogers et al., 1993).

Reduction in cancer risk has been shown with a variety of vegetables and fruits particularly green vegetables, cruciferous vegetables, broccoli, cabbage, lettuce, citrus fruits, grapes (Weinstein, 1991; Wattenberg, 1992)

6. Phyto chemicals :

Major efforts in the cancer prevention are focused heavily on the antioxidant defence systems provided by phyto chemicals, which comprise hundreds of nutrient and non-nutrients constituents found in plant-foods.

Dietary ellagic acid, flavone and coumarin which are constituent of normally consumed vegetables and fruits eg. barriesnut and grapes, show strong chemopreventive effects by enhancing glutathione and glutathione S-transferase enzymes in rats (Nijhoff and Peters, 1994).

Phytic acids. is an antioxidant that composes 1-6% by weight of cereals, nuts, oil seeds and legumes, reduces chemical carcinogenesis in the Azoxymethan and dimethyl hydrazine models of carcinogenesis in the rodent colon (Rogers et al., 1993).

Limonene, a major constituent of orange peel oil has been shown to have anticarcinogenic activity when present in the diet during the process of 7, 12-dimethyl benz(a)anthracene (DMBA) induced rat mammary carcinogenesis and NNK induced lung carcinogenesis (Bon et al., 1988).
An extract of Ocimum sanctum showed chemopreventive activity on mouse skin papillomagenesis and enhanced skin GST and acid soluble sulfhydryl level (Prashar et al., 1994). There was also an increase in the GST activity of hepatic and extra-hepatic tissues and also an induction in reduced glutathione level (Prashar and Kumar, 1995; Banerjee et al., 1996).

Live 52 (an ayurvedic drug containing several plant products) has been proven to show chemopreventive activity on DMBA induced skin papillomagenesis in mice (Prashar and Kumar, 1994).

Oral and topical treatment of mice with green tea polyphenols offered chemoprotective activity against skin carcinogenesis (Wang et al., 1989) curcumin an active principal phenolic constituent of tumeric showed strong inhibitory properties towards Cyt P450 and GST, in addition to its antioxidant property and may help explain its anti carcinogenicity and anti mutagenicity towards environmental mutagens, carcinogens, including BP and DMBA (Oetari et al., 1996).

Brassini, a phytoalexin from cabbage, has been proven to possess anticarcinogenic activity during both the initiation and promotion phases of DMBA induced skin carcinogenesis (Mehta et al., 1995).

Organosulfur compounds found in allium species, including garlic and onion inhibits N-nitroso diethylamine-induced carcinogenesis of the forestomach and lung and enhanced GST activity in the mice (Wattenberg et al., 1989).

Benzyl isothiocyanate, a constituent of cruciferous vegetables inhibited DMBA - induced mammary carcinogenesis and increased GST activity (Wattenberg, 1985). Naturally occurring indoles in edible cruciferous vegetables have been shown to inhibit polycyclic aromatic hydrocarbon (PAH) induced neoplasia in rats (Wattenberg, 1982).
Naturally occurring coumarins inhibit DMBA-DNA adducts in mouse epidermis (Cai et al., 1997).

Silymarin a polyphenolic flavonoid isolated from milk thistle. has been reported to afford high protection against tumor promotion in SENCAR mouse skin carcinogenesis model (Chatterjee et al., 1999).

It has been also shown that silymarin inhibits the formation of transformed rat tracheal epithelial cell colonies induced by exposure to benzo (a) pyrene in culture (Steele et al., 1990).

**ANIMAL MODELS USED BY THE CHEMOPREVENTION PROGRAM**

The chemoprevention branch presented the following three general concepts for testing possible preventive agents in animal tumor models (Boone et al., 1992).

(i) the animal tumor model should provide the results within 6 months, to avoid excess cost and time.

(ii) a possible preventive agent should be tested in a battery of different organ tumor models, because a compound is often effective in one organ systemic but not in another; and

(iii) inhibition of a compound should be tested as target organs of animal. Tumor models that have a similarly high incidence of human cancers.

The study on preventing agents in the battery of animal tumor models provides promising and useful results for further investigation. In a consideration of the above concepts the Chemoprevention Branch of the National Cancer Institute (Boone et al., 1990) has developed the following battery of in vivo tumor model systems for screening the efficacy of candidate chemopreventive compounds.
Table - V : Animal tumor models for screening of cancer chemopreventive agents

<table>
<thead>
<tr>
<th>Organ</th>
<th>Models</th>
<th>Animals</th>
<th>Carcinogens</th>
<th>Endpoints : Inhibition of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Hamster</td>
<td>DEN</td>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Trachea</td>
<td>Hamster</td>
<td>MNU</td>
<td>Squamous cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>Mouse</td>
<td>MAM</td>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>AOM</td>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Mammary</td>
<td>Rat</td>
<td>MNU</td>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>DMBA</td>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>Mouse</td>
<td>OIH-BBN</td>
<td>Transitional cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Mouse</td>
<td>DMBA/TPA</td>
<td>Papilloma/carcinoma</td>
<td></td>
</tr>
</tbody>
</table>

Source: Boone et al., 1990

Skin (Mouse)

This has become the classic model of the experimental study of mechanisms of carcinogenesis. The two-stage protocol involves the sequential application of DMBA to the dorsal side of skin, followed 1 week later by twice weekly applications to the same area of the tumor promoter TPA. Papillomas begin to appear after 6-7 weeks, and the number of mice with papillomas, as well as the number of papillomas per mouse, continue to increase with time as long as TPA treatment continues. Squamous cell carcinomas also appear.

Breast (Rat)

Two well-known models are in use involving either the induction of mammary adenocarcinomas in virgin rats by a single intragastric dose of DMBA or by a single intravenous dose of MNU (methyl nitroso urca).

Lung (Hamster)

Two hamster models are in use. One consists of the induction of invasive squamous cell carcinomas in the trachea by the application of
MNU directly to the tracheal mucosal through the use of specially designed catheters. The other hamster model consists of multiple subcutaneous injections for 20 weeks to produce tracheal tumors in 90-100% and lung tumors in 60-70% of animals.

**Bladder (Mouse)**

In this model, O11-BBN given by intragastric instillation to (C57BL/6 x DBS/2)F1 mice produces invasive transitional cell carcinomas of the bladder that morphologically resemble their human counterparts.

**Colon (Rat and Mouse)**

1. 2-Dimethylhydrazine will produce adenocarcinomas of the colon in both rats and mice when given intraperitoneally.

**CHEMOPREVENTIVE AGENTS IN CLINICAL DEVELOPMENT**

Clinical trials include three phases (Kelloff et al., 1992). Phase I is concerned primarily with safety and toxicity of a possible chemopreventive agent: pharmacokinetic properties including absorption, distribution, metabolism and excretion of the compound are also assessed in this phase. Phase II is a small scale efficacy study for evaluating the modulation of an intermediate marker as an end point; it does not mean the reduction of cancer incidence in this phase. Phase III a large efficacy study for evaluating the reduction of cancer as the usual end point.

Table VI lists agents in the early stages of clinical drug development.

They are currently completing preclinical toxicology evaluation before initiating clinical safety and pharmacokinetics (Phase I) trials.
<table>
<thead>
<tr>
<th>Agent</th>
<th>Toxicology/ Phase I</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Allocysteine</td>
<td>Tox</td>
<td>Lung</td>
</tr>
<tr>
<td>Curcumin</td>
<td>I</td>
<td>Colon, breast</td>
</tr>
<tr>
<td>Fluasterone</td>
<td>Tox</td>
<td>Breast</td>
</tr>
<tr>
<td>Genistein</td>
<td>Tox</td>
<td>Breast, prostate, colon</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>I</td>
<td>Colon, bladder</td>
</tr>
<tr>
<td>Indole-3-carbinol</td>
<td>I</td>
<td>Prostate</td>
</tr>
<tr>
<td>Perilly alcohol</td>
<td>I</td>
<td>Breast, colon</td>
</tr>
<tr>
<td>PEITC</td>
<td>I</td>
<td>Lung</td>
</tr>
<tr>
<td>9-cis-Retinoic acid</td>
<td>I</td>
<td>Breast, cervix</td>
</tr>
<tr>
<td>Sulindac Sulfone</td>
<td>I</td>
<td>Colon, breast</td>
</tr>
<tr>
<td>Tea/EGCG</td>
<td>Tox</td>
<td>Colon</td>
</tr>
<tr>
<td>Ursodiol</td>
<td>I</td>
<td>Colon</td>
</tr>
<tr>
<td>Vitamin D3 analogs</td>
<td>Tox</td>
<td>Breast, colon</td>
</tr>
<tr>
<td>p-XSC</td>
<td>Tox</td>
<td>breast, colon</td>
</tr>
<tr>
<td>NAC + DFMO</td>
<td>Tox</td>
<td>Breast</td>
</tr>
<tr>
<td>DFMO + 4-HPR</td>
<td>Tox</td>
<td>Breast</td>
</tr>
<tr>
<td>DFMO + Oltipraz</td>
<td>Tox</td>
<td>Bladder, colon</td>
</tr>
<tr>
<td>DFMO + Piroxicam</td>
<td>I</td>
<td>Colon, bladder</td>
</tr>
<tr>
<td>4-HPR + Oltipraz</td>
<td>Tox</td>
<td>Breast, bladder</td>
</tr>
<tr>
<td>NAC + Oltipraz</td>
<td>Tox</td>
<td>Lung</td>
</tr>
</tbody>
</table>
**Table VII: Promising Cancer Chemopreventive Agents in Phase II/III Clinical Trials (Kelloff et al., 1997)**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Phase</th>
<th>Target(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>III</td>
<td>Lung</td>
</tr>
<tr>
<td>all-trans-Retinoic Acid</td>
<td>II</td>
<td>Cervix</td>
</tr>
<tr>
<td>t3-cis-Retinoic Acid</td>
<td>II/III</td>
<td>Head and neck, lung, oral cavity</td>
</tr>
<tr>
<td>4-HPR</td>
<td>II/III</td>
<td>Bladder, breast, cervix, lung, oral cavity</td>
</tr>
<tr>
<td>Calcium</td>
<td>II/III</td>
<td>Colon</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>II/III</td>
<td>Cervix, lung, oral cavity</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>II/III</td>
<td>Breast</td>
</tr>
<tr>
<td>Finasteride</td>
<td>III</td>
<td>Prostate</td>
</tr>
<tr>
<td>DFMO</td>
<td>II</td>
<td>Bladder, breast, cervix, colon, esophagus, oral cavity, prostate</td>
</tr>
<tr>
<td>NSAIDs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>II/III</td>
<td>Colon</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>II</td>
<td>Colon</td>
</tr>
<tr>
<td>Sulindac</td>
<td>II/III</td>
<td>Colon</td>
</tr>
<tr>
<td>Oltipraz</td>
<td>II</td>
<td>Liver (DNA adducts), lung, prostate</td>
</tr>
<tr>
<td>DHEA</td>
<td>II</td>
<td>Breast, prostate</td>
</tr>
<tr>
<td>NAC</td>
<td>III</td>
<td>Lung</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>II</td>
<td>Colon</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>II/III</td>
<td>Colon, lung, prostate</td>
</tr>
<tr>
<td>Selenium (l-Selenome-thionine)</td>
<td>II/III</td>
<td>Prostate, skin</td>
</tr>
<tr>
<td>Folic acid</td>
<td>II/III</td>
<td>Cervix, colon</td>
</tr>
<tr>
<td>Aromatase inhibitor</td>
<td>II</td>
<td>Breast, prostate</td>
</tr>
<tr>
<td>Antiestrogen</td>
<td>II</td>
<td>Breast</td>
</tr>
<tr>
<td>Antiadrogen</td>
<td>II</td>
<td>Prostate</td>
</tr>
<tr>
<td>Steroid 5α-reductase inhibitor</td>
<td>II</td>
<td>Prostate</td>
</tr>
<tr>
<td>Aspirin + Calcium</td>
<td>II/III</td>
<td>Colon</td>
</tr>
<tr>
<td>Aspirin + folic acid</td>
<td>III</td>
<td>Colon</td>
</tr>
<tr>
<td>4-HPR + tamoxifen</td>
<td>II</td>
<td>Breast</td>
</tr>
<tr>
<td>l-Selenomethionine + Vitamin E</td>
<td>II</td>
<td>Breast, prostate</td>
</tr>
</tbody>
</table>
More than 20 agents have advanced to early phase II (and a few to larger phase II and phase III) chemoprevention efficacy trials: these agents are listed in table VII. The current primary focus of clinical chemoprevention studies is the early phase II trials to identify and characterise surrogate end point biomarkers (Kelloff et al., 1997).

Primary intermediate biomarkers that validated as surrogate end point for cancer and targets of chemoprevention are intra epithelial neoplasia (IEN), which are almost always cancer precursors, in the causal path way to cancer or both phase II and small phase III clinical chemoprevention trials initially may be conducted in patients with current or previous intraepithelial neoplasia (IEN).

A primary goal is characterization and standardization of quantitative measurements of chemopreventive agent induced morphometric and cytometric changes in these lesions. Results showing reversion, slowed progression, or inhibition of recurrence of the target lesions can be obtained within 3 to 24 months in such Cohorts.

Further, an important component of clinical (and pre clinical) studies in chemoprevention is identification of earlier intermediate biomarkers in IEN that reflect carcinogenesis/chemoprevention mechanisms: proliferation (e.g., proliferating cell nuclear antigen, MIB-1), differentiation signals (e.g. actins, vimentin, blood group antigens), and genetic/regulatory changes eg. (apoptosis. DNA methylation, oncogene and tumor suppressor expression) (Kelloff et al., 1994).
C. CARCINOGEN METABOLIZING ENZYMES AND ANTIOXIDANT DEFENCE SYSTEM
PHASE I REACTIONS

There are two major oxidative enzyme systems: (1) Cytochrome p-450 system (mixed function oxygenase, MFO system) and (2) mixed function amine oxidase (aflavin-monoxygenase MFAO system).

1. Cytochrome p-450 dependent mixed function oxidation systems (MFO)

The most important enzyme system involved in phase I reactions are the cytochrome p-450 containing mono oxygenases. The cytochrome p-450 system is a group of enzymes (EC 1.14.14.1) primarily responsible for the biotransformation of xenobiotics. Cytochrome p-450 gets its designation from the fact that in the reduced form [P-450 (Fe2+)] it binds carbon monoxide and then absorbs light most intensely at a wave length of 450 nano meters. The amplitude of the absorbance peak is the basis of quantitative studies of the enzyme.

Cyt P-450, the carbon monoxide binding pigment of microsomes is a haemoprotein of the b cytochrome type (protohaem as the prosthetic group, not covalently bound to the protein). Unlike most cytochromes, it is not named based on the absorption maximum of the reduced form in the visible region, but rather on the unique wave length of absorption maximum of the carbon monoxide derivative of the reduced form i.e. 450 nm. Various forms of cyt p-450 probably exist in all living organisms and have been demonstrated in bacteria, yeast, plants, fish and mammals
(Ioannides and Parke, 1990). The molecular weights of all cytochrome-P-450 enzymes characterised to date fall in the range of 45,000-60,000 dalton (Guengerich, 1990). The numerous isoenzymes of the p-450 system have arisen from one common ancestral gene (Wolf, 1986) and now more than 150 enzymes have been characterised which vary from about 10 to over 90% in sequence homology (Coon et al., 1992). In addition, the types and amounts of cytochrome p-450 vary with species, organ, age, health, sex, stress and chemical exposure.

The enzyme system has been found in all mammalian tissues, but they are predominant in endoplasmic reticulum, mitochondria and nuclear envelope of the liver, although they are present in the brain, adrenal, kidney, gonads and skin (Sipes and Gandolfi, 1986).

The significance and existence of multiple forms of cytochrome P-450 in part, rationalize the substantial differences in metabolism that are observed as a function of sex, species, age, nutritional status etc. Secondly, the multiple forms provide an explanation of why only certain tissues are susceptible to a chemical carcinogen. Thirdly, drug-drug-interactions (in case of combination therapy) are to be viewed in terms of their combined effects on these phase I pathway enzymes in order to obtain a fair idea of dose fixation of drugs and to maintain effective therapeutic levels (Gibson and Skett, 1986; Sipes and Gandolf, 1986)

These enzymes are well known to play major role in metabolism of chemicals including carcinogens, drugs, alcohol, pesticide, petroleum products and natural products. They are also involved in the metabolism of various endogenous, physiologically important substances e.g. cholesterol, fatty acid, acids, steroids, bile acids, and metabolism of vitamin D3 (Ioannides and Parke, 1990). (See fig. VIII)
Fig. VIII: Functions of mammalian cytochrome p-450
(from Wolf, 1986)

- BILE ACID BIOSYNTHESIS
- HORMONE BIOSYNTHESIS AND METABOLISM (Steroids, Corticosteroids, Vitamin D)
- CYTOCHROME P-450
- THROMBOXANE SYNTHESIS
- DRUG OXIDATION (Foreign compounds, Drugs, Steroid Catabolism)
- FATTY ACID METABOLISM (Fatty acids, Prostaglandins, Leukotrienes)
- EXCRETION
- MUTAGENIC METABOLITE
- TOXICITY, CANCER
Due to their dual functions of bioactivation and detoxification, cytochrome P-450, enzyme have extensively studied in chemical carcinogenesis and cancer chemotherapy (Coon et al., 1992; Guengrich, 1992).

The recent awareness of using diet, nutrient and phytochemicals as modes of cancer chemoprevention led to the study of dietary effects on cytochrome-P-450 enzyme system, as these enzymes are concerned with the biotransformation of exogenous substrates (Guengrich, 1995).

The general reactions which they catalyse are mixed function oxidations:

\[ \text{NADPH} + \text{H}^+ + \text{O}_2 + \text{SH} \rightarrow \text{NADP}^+ + \text{H}_2\text{O} + \text{S-OH} \]

where the substrate (SH) may represent the drug or another chemical that has an alkane, alkene, aromatic ring or hetero cyclic ring substituent serve as a site for oxidation. This reaction is called a mono-oxygenation, and P-450 isoenzymes are therefore mono-oxygenase because only one of the two oxygen atoms is incorporated into the substrate (Spatznerger and Jaeger, 1995).

In the endoplasmic reticulum the cytochrome P-450 is made up of the following enzyme components:

i) **Aflavoprotein - NADPH cytochrome P-450 reductase**: It is aflavin-containing enzyme consisting of one mole of flavin adenine dinucleotide (FAD) and one mole of flavin mononucleotide (FMN) per mole of apoprotein. This made NADPH cytochrome P-450 reduction relatively unique, as most other flavoprotein have only FAD or FMN as their prosthetic group.

This enzyme is viewed as intermediary electron transfer between the NADPH+H⁺ (a 2 - electron donor) and cytochrome P450 (a 1-electron acceptor), thus acting as a transducer of reducing equivalents.
This enzyme has a molecular weight ranging from 74,000 to 80,000 dalton depending on the terminal species (Guengerich and Liebler, 1985).

ii) **Aheme - cotingaining protein - cytochrome P-450**: Cytochrome P-450 is the terminal oxidase or oxygenase component of an electron transfer system and is classified as ahaem - containing enzyme with iron protoporphyrin IX non covalently bound to a single polypeptide chain (Murray and Reidy, 1990). The name cytochrome P-450 is derived from the fact that the cytochrome (pigment) exhibit a spectral absorbance maximum at 450 nm when reduced and complexed with carbon mono oxide.

The haemoprotein serves both as the oxygen and the substrate binding locus for the MFO reaction and in conjunction with the associated flavoprotein reductase, NADPH-cytochrome P-450 reductase. It undergoes a cyclic oxidation / reduction of the haem iron that is mandatory for its catalytic function.

The catalytic cycle of cytochrome P-450 showing the electron transport system and the oxidation of a xenobiotic is depicted in fig. IX.

iii) **Phospho lipid component**: The phospholipid matrix of the endoplasmic reticulum in which the P-450 enzyme system is embedded plays a crucial role in cytochrome P-450 reactions, as it facilitates the interaction of cytochrome P-450 and NADPH-cytochrome P-450 reductase molecules. The lipid component by itself, is not an electron carrier nor its presence is obligatory for substrate binding.
Fig. IX: Cytochrome P-450 electron transport system and oxidation of a xenobiotic
One of the 3-methylcholanthrene inducible forms of cytochrome P-450 has a high catalytic turnover for benzo (a) pyrene. This isoenzyme gives the characteristic absorption peak at 448 nm and not at 450 nm. This form of cytochrome P-450 has long been called cytochrome P-448, cytochrome P-450 or aryl hydrocarbon hydroxylase because of its substrate specificity towards polycyclic aromatic hydrocarbons (PAH's). Thus, this enzyme is involved in the converting many PAH compounds that are chemically inert towards water soluble forms via the formation of transient reactive intermediates that may be cytotoxic (Gelboin et al., 1969) mutagenic (Ames et al., 1973) and / or carcinogenic (Gelboin et al., 1970).

2. Mixed function Amino oxidase (MFAO) system / flavin containing mono oxygenase (FMO)

This is another oxidative enzyme involved in phase I biotransformation. This FAD - flavoprotein is present in the endoplasmic reticulum and is capable of oxidizing nucleophilic nitrogen and sulfur compounds.

It is found to be the only known flavoprotein hydroxylase among mammals. This enzyme activity is exceptionally high in humans and pigs, while low in rats. It has molecular weight of approximately 56,000 and contains one molecule of FAD (Guengerich and Liebler. 1985) Flavin-containing mono oxygenase competes with the cytochrome P-450 system in the oxidation of amines. The endogenous substrate of this enzyme is cysteamine, which is oxidized to cystamine. FMO is not under the same regulatory control as cytochrome P-450.

The enzyme concentration appears to be under hormonal regulation as it’s level can be modulated by steroid sex hormones (Sipes and Gandolphi. 1986; Zeigler. 1990).
Cytochrome b5:

Cytochrome b5 is another microsomal enzyme whose function is not clearly established due to lack of uniform observations.

It is found in conjunction with its reductase - NADH cytochrome b5 reductase. Both of them together are implicated in desaturation of fatty acids (Schenkman et al., 1976).

The cytochrome b5 dependent reactions may probably occur by the following electron flow:

\[
\text{NADH} \rightarrow \text{NADH - cytochrome b5 reductase} \rightarrow \text{cytochrome b5} \rightarrow \text{cytochrome p-450}
\]

The NADH - dependent reduction of cytochrome p-450 has been reported by Ichikawa and Loehr (19720. It has also been reported, cytochrome b5 is reduced by the NADPH - cytochrome p-450 reductase, thereby placing another drain on the reducing agents of this flavoprotein. The interrelation between cytochrome p-450 and b5 has been reviewed by Schenkman, Jansson and Robie - Suh (1976). Cytochrome b5 has been shown to form a complex with cytochrome p-450 which the substrate turnover rate of cytochrome p-450 (Jansson et al., 1987).

PHASE II REACTIONS

Glutathione-S-Transferase

Glutathione-S-Transferases (EC 2.5.1.18) are a family of enzymes that catalyse the initial step in the formation of N-cetylsteine (mercapturic acid) derivatives of a diverse group of foreign compounds. The cofactor
for reactions catalysed by these enzymes is the Tripeptide glutathione (GSH), which is composed of glycine, glutamic acid and cysteine.

The glutathione-S-transferase catalyses the formation of thioether bond between glutathione (GSH) and a large number of lipophilic compounds that possess an electrophilic centre. This conjugation reaction is considered as the first step in the synthesis of mercapturic acid (Jakoby, 1978; Chasseaud, 1979).

These enzymes are localized in both the cytoplasm and the endoplasmic reticulum. However, the cytosolic glutathione S-transferases activities usually 5 to 40 times greater than the microsomal activity (Sipes and Gandolfi, 1993).

The enzyme activity resides in almost all organs of the body with maximum activity in the liver, testis, intestine, kidney and adrenal gland (Sipes and Gandolfi, 1993).

The glutathione-S-transferases (GST) play a central role in the cellular metabolism of cytotoxic and carcinogenic compounds (Chasseaud, 1979; Mannervik, 1985). This role is fulfilled either by catalyzing the conjugation of glutathione with electrophilic species (Mannervik and Danielson, 1988) or by reducing reactive organic peroxides (Kramer et al., 1988, Tan et al., 1988).

GST acts as a marker of preneoplasia in animal models (Sato, 1989) and has been implicated in cellular inactivation of anti-cancer drugs and in some cases the acquisition of enhanced resistance of cells to these drugs is related to their GST expression (Mannervik and Danielson, 1988, Coles and Ketterer, 1990: Arm Strong, 1991). The cytosolic GST's are dimeric protein existing as homodimers or heterodimers.
Fig. X: The glutathione S-transferases are a family of enzymes that catalyse the initial step in the formation of N-acetyl cysteine (mercapturic acid) derivatives of a diverse group of foreign compounds. The glutathione S-transferase catalyse the reaction of the nucleophilic sulfhydryl group of glutathione with compounds containing electrophilic carbon atoms. The reaction of the glutathione thiolate anion (GS) results in the formation of a thioether bond between the carbon atom and the sulfhydryl group of glutathione.
At least 7 different forms of subunits and 8 isoenzymes have been identified in humans (Sun, 1990). These have been designated into three families, α, μ and π based on their primary structure. The combination of identical subunits gives rise to homodimers and different subunits of the same family to heterodimers. The GST isoenzymes have overlapping substrate specificities.

It has been found that different isoenzymes of GST are characteristic of a particular tissue and occurrence of different forms of GST change in an organ specific manner during the change from fetal to adult state (Mannervik and Danielson, 1988). Some characteristic forms of GST’s are expressed in tumor tissue, thus making these good biochemical makers (Coles and Ketterer, 1990).

The peroxidase activity of some forms of glutathione-S-transferase is very important in dealing with peroxidised lipids and peroxidised DNA (Ketterer et al., 1987; Tan et al., 1988). These enzymes are called selenium independent glutathione peroxidases. These enzymes have relatively low activity towards organic hydroperoxides but not at all towards $\text{H}_2\text{O}_2$ (Sun, 1990).

Thus, GSTs along with glutathione peroxidase are believed to be involved in GSH-dependent defence against lipid peroxidation.

In addition to the catalysis of these detoxification reactions, GST’s have the ability to bind to various endogenous compounds which are not substrates for their enzymatic reactions (Ketley et al., 1975).

The GST family plays a profound role as binding proteins that serve as a storage function for endogenous toxic compound like bilirubin in liver.
The GSH conjugation (thioethers) or their N-acetyl cysteines are mostly excreted in the bile. These conjugates may also be converted into mercapturic acids, excreted via the urine, through a number of enzymatic steps occurring mainly in the kidney.

**NAD(P)H : (Quinone Acceptor) oxidoreductase / DT Diaphorase (DTD)**

The NADH and NADPH linked quinone oxidoreductase (EC 1.6.99.2) is a flavoenzyme, predominantly present in the cytosolic fraction (95% of the total activity) of liver. Ernster first characterised and termed this enzyme, as DT-Diaphorase (DTD) for its catalyzing property of the oxidation of NADH and NADPH (at that time known as DPNH and TPNH) at equal rates.

Subsequently, the same enzyme has also been designated variously as quinone reductase, menadione reductase NAD(P)H dehydrogenase and vitamin K reductase depending on the substrate used for reduction (Benson et al., 1980). DTD is shown to have abroad specificity for a variety of hydrophobic quinones, including benzoquinones, naphthoquinones, ubiquinones and vitamin K derivatives (Ernster et al., 1962). Human exposure to quinones is extensive as they are widely distributed in air and in our food. They serve as substrates for many flavoenzymes during xenobiotics metabolism including NADPH-cytochrome P-450 reductase, NADH cytochrome b5 reductase, NADH-ubiquinone oxido reductase as well as DTD. There are two alternatives pathways in the quinone reduction. A one-electron reduction (catalysed by MFO systems) yielding a semiquinone radical. In the presence of oxygen most semiquinones rapidly autoxidize to form the superoxide anion radical (O$_2^-$) and has been invoked to explain the cytotoxic and anti tumor properties of quinonoid drugs via binding to nucleic acids (Benson et al., 1980). Alternatively, a 2-electron reduction
Fig. XI : PATHWAYS INVOLVED IN QUINONE METABOLISM

A and C lead to formation of toxic products.
B and D lead to detoxified metabolites
catalyzed by DTD converts the quinone into non-toxic hydroquinones. These are subsequently converted into sulfate / glucuronyl conjugates in presence of UDP glucuronic acid or other phase II enzymes (Fig. XI). Therefore this enzyme is considered as an important component of phase II system for its role in quinone detoxification.

**Antioxidant enzymes in the cellular Defense Systems**

The antioxidant enzyme system consists of copper and zinc containing super oxid dismutase (Cu, ZnSOD), manganese - containing super oxide dismutase (MnSOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR). These enzymes function to protect against toxic oxygen radicals produced during normal metabolism and after exposure to radiation, carcinogens and xenobiotics. Thus cells exhibit various inherent protective features collectively termed as “Anti oxidant defence mechanisms” (Pulgia and Powell, 1984). The components of cellular defence are present in higher concentration in tissues with high $O_2$ utilization.

These enzymes as follows:

**Super oxide dismutase (SOD)**

This enzyme was first discovered by Mc Cord and Fridovich in 1969. The enzyme has dismutase two molecules of $O_2$ to form $H_2O_2$ and $O_2$. The SOD family consists of four metallo forms: two containing copper and zinc, one manganese and one iron Cu. ZnSOD is found in the cytosol of most eukaryotic cells (Fridovich, 1975). A different form of Cu, ZnSOD is found in extracellular fluids (EC), where it is called EC-SOD (Marklund et al., 1982, Marklund, 1984).
MnSOD is located in the mitochondrial matrix as well as in bacteria, while FeSOD is present in many aerobic bacteria (Fridovich, 1974) while Fe-SOD and Mn-SOD share considerable amino acid homology, they are quite different from Cu-SOD and Zn-SOD (Bannister et al., 1987).

In eukaryotic cells, three forms of SOD are known to exist. Cu, Zn-SOD, EC-SOD and Mn-SOD. Cu-SOD and Zn-SOD found in cytosol has a MW of 32,000 daltons with two identical subunits. The EC-SOD also contains copper and zinc, however, it has a MW of 135,000. EC-SOD is composed of four equal non-covalently bound sub-units. Mn-SOD is found in mitochondrial matrix and has a molecular weight of around 88,000 with four equal sub-units.

**Catalase (CAT):**

Catalase (EC 1.11.1.6) is one of the oldest known enzymes. It was named by Loew in 1901 (Percy, 1984).

Most aerobic cells contain this enzyme. In animals, CAT is present in all major body organs, being especially concentrated in the liver and erythrocytes.

At the subcellular level CAT is found mostly in peroxisomes (80%) and cytosol (20%), and deals with a large amount of hydrogen peroxides (H₂O₂) present in haem. Some Fe-binding macromolecule like transferrin and ferritin are prevented from forming free radicals by this system.

\[ 2H_2O_2 \rightarrow 2H_2O + O_2 \]

The usual form of CAT has a MW of about 240,000 and consists of four protein subunits, each containing aneme [Fe (III)-protoporphyrin] group bound to its active site. Dissociation of the molecule into its subunits causes loss of activity (Halliwell and Gutteridge, 1985).
Glutathione peroxidase (GPX)

Glutathione peroxidase (EC 1.11.1.9) as first described in 1957 by Mills. This enzyme is capable of catalysing the conversion of hydrogen peroxides and or lipid hydroperoxides to water and lipid. In this reaction GSH gets oxidized to GSSG.

\[ \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GSSG} + \text{H}_2\text{O} \]

Depending on the glutathione peroxidase's selenium dependency, GPX can be divided in two forms (Sun, 1990).

These are:

1. Selenium-dependent GPX: Se-dependent GPX is a tetramer of Molecular weight 84,000 with very high activity towards both \( \text{H}_2\text{O}_2 \) and organic hydroperoxides. It contains one residue of selenocysteine per mole at each of the active sites and is found in both cytosol (70%) and mitochondria (30%).

2. Selenium-independent GPX: The Se-independent GPXs are the GSII-S-transferases (GST, EC 2.5.1.18). The enzymes are dimers of molecular weight (MW) approximately 50,000. They have low activity toward organic hydroperoxides but not at all toward \( \text{H}_2\text{O}_2 \).

Glutathione peroxidase is found in mitochondria and cytosol and act as an antioxidants as they are directly involved in the elimination of reactive oxygen species which play important role in all stages of tumorigenesis (Cerutti, 1985; Kosower and Kosower, 1978).
Glutathione reductase (GR):

Glutathione reductase (EC 1.6.4.2) was initially observed in liver from various animals by Hopkins and Elliott in 1931, and later isolated from Ox, sheep and rabbit liver by Mann in 1932.

It is a flavo protein catalyzing the conversion of the oxidized form of glutathione (GSSG) to the reduced form (GSH). It uses NADPH which is mainly taken from the pentose phosphate shunt.

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow \text{GSH} + \text{NADP}
\]

The reaction is essential for maintenance of glutathione levels which is playing important role for several cellular functions including detoxification (Calberg and Mannervik, 1985). Glutathione reductase is known as secondary antioxidant as it helps in regenerating of reduced glutathione which is consumed by GPX to protect cell from \( \text{H}_2\text{O}_2 \) and hydroperoxides mediated damage.

The enzyme has a molecular weight of approximately 120,000. It has two subunits each containing FAD at its active site (Sun, 1990). It has been show to decrease during vitamin B6 deficiency (Dubick et al., 1995). The enzyme has a similar distribution as GPX i.e. in the mitochondria and cytosol.

Glutathione (non-enzymatic anti oxidant defense system)

Glutathione (\( \gamma \)-glutamyl-cysteinyl-glycine) is the most important non protein thiol present in animal cells as well as in most plants and bacteria. GSH is characterised by is reactive thiol group and its \( \gamma \)-glutamyl bond which makes its resistant to normal peplidase activity. Most of its biological functions depends on the thiol group of its cysteinyl residue
(Chasseud, 1979). The intracellular concentration of this tripeptide plays an important role in determining the sensitivity of cells to radiation and drug induced toxicity (Mitchell et al., 1989; Weiss et al., 1990).

GSH performs many diverse functions. It functions in catalysis, metabolism, and transport as well as protection. It is involved in the synthesis of proteins and DNA. Transport of amino acids, ions and/or sugars, regulation of enzyme activity, protection of cells against reactive oxygen compounds and free radicals and maintenance of membrane integrity as well as cytoskeletal organization (Meister and Anderson, 1983; Mitchell et al., 1989; Meister, 1988).

The maintenance of high GSH/GSSG ratio has been shown to be vital for the functional integrity of cell membranes (Lotscher et al., 1979). Unless membrane sulphhydrils were kept in a highly reduced state, the membrane could become leaky, resulting in uncontrolled release of calcium into the cytoplasm from the mitochondria as well as influx from the extracellular spaces (Lotscher et al., 1979). This had been postulated as a common path way of cytotoxicity for chemicals that induce oxidative stress (Schanne et al., 1979).

Depletion of GSH in vivo and in vitro is known to cause inhibition of GSH peroxidase leading to lipid peroxidation (Younes and Siegers, 1980, 1984, Halliwell and Gutteridge, 1985).

The GSH redox cycle is the major pathway for providing cells with reducing equivalent for bio reduction of drug or radiation induced superoxides and peroxides (Reed 1986). The peroxide mediate oxidation of glutathione occurs nonenzymatically or enzymatically catalized by selenium dependent glutathione peroxidase and selenium independent
Fig. XII: Structure of glutathione (from Kretzschmar and Klinger, 1990)
glutathione-S-transferases (Flohe et al., 1973; Flohe, 1982). The induction of glutathione deficiency in tumors has been postulated as potentially useful in cancer therapy (Meister, 1991).

GSH is used as a co-factor in the enzyme catalysed conversion of hydrogen peroxides \((H_2O_2)\) or lipid hydroperoxides to water and lipid. The enzyme catalysing reaction is glutathione peroxidase (Reed, 1986, 1990).

GSH participate in detoxification reactions of electrophilic substances such as carcinogen epoxide metabolites and certain drugs by conjugation between nucleophilic thiol of GSH and an electrophilic site on another molecule. Hydrogen peroxide and lipid peroxides are also detoxified by reducing glutathione, generating oxidized glutathione which can be enzymatically reduced again by glutathione reductase and NADPH (Kosower and Kosower, 1978).

The thiolate anion of GSH act as an alternative or a completing nucleophilic site to the nucleophilic portion of DNA (Coles and Ketertere, 1990) and this offers protection against genotoxic products.

It has been reported that GSH depletion may occur in response to starvation, excess electrophilic burden on the body, oxidative stress, hormonal imbalance, growth and development (Kretzschmar and Klinger, 1990) reduced GSH levels.

GSH also serve as storage form of cysteine as it is required for resynthesis of GSH in other tissues.

GSH is synthesized intracellularly and is exported from cells. its break down is initiated by \(\gamma\)-glutamyl transpeptidase, an enzyme attached to the external surface of cell membranes. The broken down products are then transported and utilized (Meister, 1994).
Regulation and maintenance of regularly supply of GSH is maintained by glutathione cycle-which occurs in two enzyme catalysed steps:

(1) The formation of γ-glutamyl bond between glutamic acid and cysteins. This is catalysed by the enzyme γ-glutamyl-cysteine synthetase (GCS)

\[ \text{L-Glu} + \text{L-Cys} + \text{ATP} \rightarrow \text{L-γ-Glu-L-Cys} + \text{ADP} + \text{Pi} \]

This is a rate limiting step.

(2) The formation of the peptide bond between γ-glutamyl cysteine and L-glycine which is catalysed by glutathione synthetase (GSHS)

\[ \text{L-γ-Glu-L-Cys-L-Gly} + \text{ATP} \rightarrow \text{GSH} + \text{ADP} + \text{Pi} \]

**Mechanism of carcinogen metabolizing enzymes**

Two main categories of blocking agents that enhance carcinogen detoxification systems have been identified in inhibition of carcinogenesis by Wattenberg (1983). They are known designated as type A and type B inhibitors.

Type A (bifunctional inducers). These compounds which elevated the activities of certain cytochrome P-450 (Phase I) as well as phase II enzymes thereby enhancing total detoxification eg. TC DD, azo dyes, PAH and B-naphthoflavone. and (ii) type B (Monofunctional inducers) are these compounds which increases the activities of phase II enzymes without significantly affecting the activities of cytochrome P-450's eg. phenolic antioxidants, 1, 2-dithiol-3-thiones, aromatic isothiocyanates and thiocarbamates (Prochaska and Talalay, 1988).

Regulation of P-450 induction has been found primarily at the
transcriptional level (Okey et al., 1986). The cytochrome P-450's are primary determinants of the rate and specificity of xenobiotic biotransformation.

Modulation or alteration in the levels of various species of cytochrome P-450 can have profound effects on the response of an organism to xenobiotics.

The highly reactive metabolites produced by phase I metabolism, if not conjugated in phase II reactions may attack plasma membrane components, protein or nucleic acid (DNA). Thus the induction of phase II enzymes or the co-induction of phase-I and phase II enzymes are more promising and safer methods for chemoprevention action, against cancer reducing the risk of P-450 induction alone.

It has been well known that Ahh is genetically regulated. Variable levels of Ahh are found in different inbred strain of mice. This observation led to discovery that there are strains which are responsive to Ahh inducibility by many agents and other strain that are non-responsive depending on the presence or absence of Aryl hydrocarbon (Ah) locus.

It has also been observed that Ah-responsive strains are to be more susceptible to the polycyclic aromatic hydrocarbons (PAHs) induced cancers (Nebert, 1987).

The molecular mechanism whereby bifunctional inducers (large planer aromatic) elevate AHA and related activities appears to be well established. Using bifunctional inducer 2, 3, 7, 8 Tetra chlorodibenzo-p-dioxin (TCDD), Poland et al. (1976) identified a high affinity binding site for TCDD (which was named as Ah locus) in cytosol of liver and other tissues. The inducing agent TCDD interacts with the receptor to form a receptor ligand complex
which then translocates to nucleus and interacts with cytochrome P-450 genes to yield an elevated AHH activity (Eisen et al., 1983; Nebert et al., 1984).

![Diagram](image)

**Fig. XIII: Regulatory Mechanism of Enzyme Induction**
*(Prochaska and Talalay, 1988)*

Bi: Bifunctional Inducers  
Mo: Monofunctional inducers  
A, B and C: Represent the three mechanisms of enzyme induction

The PAH class of inducers seems to be operating by this mechanism. However such cytosolic receptor for other class of inducers has not been identified so far. Since it was found that induction of phase II enzymes by planar aromatic generally occurs only in the mouse strains that have a functional Ah. locus. The regulation of phase II system has been assumed
to occur via the same mechanism as that of AHH. However experiments attempting to demonstrate the direct participation of Ah locus with phase II enzymes regulation have not been convincing (Kumaki et al., 1977; Felton et al., 1980). On the contrary monofunctional inducers exhibit no apparent structural similarity and their mechanism does not seem to involve a conventional receptor for functional Ah receptor (Delong et al., 1987). A mode for the phase II enzymes regulation by mono and bifunctional inducers has been proposed by Prochaska and Talalay (1988) which appear somewhat convincing.

The model suggested by them represents basically 3 mechanisms by which enzymes may be induced (See fig. xiii).

**Mechanism A**: Monofunctional inducers activate phase II enzymes via an electrophilic signal that is independent of Ah receptors or AH induction.

**Mechanism B**: Bifunctional inducers form complexes with Ah receptor that translocate to nucleus and enhances transcription of AHH as well as phase II enzymes system.

**Mechanism C**: Bifunctional inducers susceptible to metabolism by Ah (which is enhanced by mechanism B) are converted to electrophilic products that elevate phase II enzymes via mechanism A.
D. SKIN CARCINOGENESIS
Skin Carcinogenesis

The two most common kinds of human skin cancers are based on cell carcinomas and squamous cell carcinomas which are grouped together as non-melanoma skin cancer. Non-melanoma skin cancer is the most common malignancy affecting the human population it accounts for most one million new cases diagnosed each year in the United States (Miller and Weinstock, 1994). It is clear that solar ultra violet (UV) radiation is the major cause of these cancers (Matsui and Deleo 1995). Neoplasms arising because of exposure to UV. radiation include tumors that develop primarily on the head, neck, hands and fore arms. The majority of squamous cell carcinomas, and about two thirds of basal cell carcinomas occur at such sites, some squamous cell carcinomas originate from precancerous lesions such a clinic keratoses.

Carcinogenesis in mouse skin and most likely in human skin and other tissues is a step wise process comprising initiation, promotion, and progression stages (Agarwal and Mukhtar, 1991, Di Giovanni, 1992).

Yamagiwa and Ichikawa (1918) reported the first production of skin tumors in rabbit and mice by the application of coal tar to the skin. The investigator repeatedly applied crude Coal tar to the ears of rabbit for a number of months, finally producing both benign and later, malignant epidermal neoplasms. 1, 2, 5, 6 - diabenz anthracene was the first pure chemical carcinogen used to induce tumors (Kennaway and Hieger, 1930). In 1933 Cook et al. isolated the carcinogen 3, 4 benzopyrene from coal tar.
Mackenzie and Rous (1941) and Berenblum (1941) in their animal experiments showed that there are two stages in the development of skin cancer and these were termed as initiation and promotion.

In 1941, Berenblum reported that alternate application of croton with 3, 4 - benzopyrene had remarkable ability to cause tumors in the skin of mice.

Berenblum showed that a single dose of carcinogen benzopyrene - result in genetic material (DNA) alteration or damage of the cell and this is known as the initiation phase whereas croton oil causes epigenetic changes (that is, a change in the pattern of gene expression without a change in the DNA sequence) and results in promotion of already initiated cells (previously exposed to a genotoxic carcinogens).

These experiments showed the persistence of initiated cells. An initiator can thus be defined as an agent capable of causing an irreversible and heritable change in cells (Pitot, 1990). As a result, the cells some how escape the normal control of cell division and acquire relative autonomy with regard to cell division.

A critical aspect of the two-stage carcinogenesis system in mouse skin is the irreversibility of tumor initiation. A delay up to one year between the application of the initiator and the beginning of the promoter treatment invokes a tumor response similar to that observed when the promoter is given only one week after initiation (Slaga et al., 1982; Slaga, 1984). Unlike the initiation stage the promotion stage is reversible and requires repeated application of the promoter to induce tumors (Slaga, 1984). The progression stage is defined as that events occurring after the initial appearance of a skin tumor and it represents the transition from a benign lesion (papilloma) to a malignant lesion (Squamous cell carcinoma) and finally to metastatic tumors.
The property of causing cancer is highly stereospecific and depends very strongly on the structure of the chemicals. Carcinogeneity studies of 239 aromatic compounds and hetero aromatic compounds in skin of mice show that carcinogeneity depends heavily on the hydrophobicity of chemicals, energy of the highest occupied orbitals and the presence of substituents on the I. and K regions of the carcinogen (Sontag, 1991).

One of the most potent chemical carcinogens are the polycyclic aromatic hydrocarbons (PAH's). They are often referred because they contain multiple benzene ring (i.e. three or more aromatic rings that share a pair of carbon atoms).

Polycyclic aromatic hydrocarbons are widely dispersed in nature and occur in a variety of environmental products such as coal soot, tar, tobacco-smoke, petroleum and cutting oils. They are produced during incomplete combustion or pyrolysis of organic materials and high temperature processing of crude oil, coke, leak, or other industrial carbon compounds. In the meanwhile, many different PAHs have been reported in foods, including benz(a)anthracene, benzo(a)pyrene, benzolfluoranthene, chrysene, dibenz(a, h) anthracene, 7, 12-dimethyl benz(a)anthracene and methyl cholangthrene in boiled, barecued or smoked meats and fish (Fazio and Howard, 1983; Strickland and Groopman, 1995).

Many of the carcinogenic polycyclic aromatic hydrocarbons are derived from an angular benz (a) anthracene skeleton. Anthracene per se is not carcinogenic but benz (a) anthracene shows to have weak carcinogeneity. Addition of another benzene ring in certain positions results in agents with powerful carcinogeneity such as dibenz(a, h)anthracene or benzo (a) pyrene, which are "natural product" resulting from incomplete combustion processes of carbonaceous materials. In
addition, substitution of methyl groups on specific carbons of the ring also enhances carcinogenicity. Thus 7, 12 dimethyl benz(a)anthracene (DMBA) is one of the most potent synthetic, polycyclic aromatic hydrocarbon carcinogen known.

Studies with several polycyclic aromatic related to the electronic structure of the hydrocarbons. certain area of the molecule called the K region, was related specifically to the carcinogenic potential of a given compound. On the other hand, substitution of another area of the molecule, the I region such as 7 and 12 carbons in benz (a) anthracene, of the compound increased carcinogenicity potency, if these parts were free there was marked a decrease in its carcinogenicity action. The reactive sites of 7, 12 dimethyl benz (a) anthracene (DMBA) were termed as the “bay region”.

The most essential step in the process of skin tumor initiation is the metabolic activation of DMBA by the complicated microsomal cytochrome. P-450 dependent mixed function oxidases and NADPH in the epidermis to a high reactive electrophilic metabolites (carcinogenic forms), which are capable of forming bonds with DNA leading to irreversible alterations in the genome of target cells causing initiation.

Kinoshita and Gelboin (1972) have suggested that the metabolism of a carcinogens like dimethyl benz(a)anthracene gets completed within 12 hours of its application.

The major DNA adduct, formed from DMBA in fetal mouse cells in culture (Sawicki et al., 1983; Dipple et al., 1983) and in mouse skin after topical administration of 7, 12 DMBA (Bigger et al., 1983) arise from the derivatives of dihydrodiol epoxides of bay region.
Fig. XIV: Chemical Line Structure of 7, 12 - Dimethyl Benz(a)anthracene (DMBA)

It has been reported that, the ultimate carcinogenic derivatives of DMBA, both syn and anti stereo isomers of 3, 4-dihydrodiol 1, 2 epoxide are involved in the binding to DNA in mouse skin (Bigger et al., 1983).

DNA adducts produced by the ultimate carcinogenic forms results primarily in the modification of the exocyclic aminogroups of deoxyadenosine and deoxyguanosine residues (Dipple, 1995).
Perchellet and Perchellet in 1989 showed that 90% of mouse skin tumors initiated with DMBA have a point mutation (specific $A \rightarrow T$ transversion) at the 2nd nucleotide in codon 61 of c-rasH and also added that the skin papilloma becomes visible when the clonal expansion of the initiated epidermal cell reaches a size of $10^5$-$10^6$ cells.

The next phase of development of skin cancer is called promotion. The main feature of promotion phase which distinguishes it from the initiation stage is just its reversibility especially at early stages (Boutwell. 1974; Slaga et al., 1982; Slaga. 1984; Tatematsu et al., 1983; Pilot 1991) and requires repeated application of the promoter to induce tumors.

Promoting agents by definition are neither mutagenic nor carcinogenic and therefore incapable of initiation of complete carcinogenesis by themselves. Pure compounds active as promoting agents were isolated from croton oil by Hecker (1971) and Vandenuren and Orris (1965). The most active constituent in croton oil was identified as 12-0 tetra-decanoyl phorbol 13 acetate (TPA) or phorbol myristate acetate by Hoppe et al. (1967).

TPA is required in vivo in mouse skin carcinogenesis to generate tumors from initiated cells (cells that have acquired a growth stimulating mutation) which would otherwise be dormant. Boutwell (1978) also demonstrated that TPA stimulates the expression of the abnormal genetic information within the initiated cells which because of their altered programme of differentiation acquire a neoplastic phenotype and a proliferative advantage over their normal neighbours.
Fig. XV: The structure of 12-O-tetradecanoyl phorbol-13-acetate or phorbol-12-myristate-13-acetate, a very potent promoter for tumorigenesis in mouse skin (Miller, 1978)

The skin-tumor promotion stage is characterised by selective and sustained hyperplasia leading to the specific expression of the initiated cells into papillomas (Slaga et al., 1987; Digiovanni, 1992).

Tumor promoters induce a number of other epigenetic changes in skin, including membrane and differentiation alterations increase in protease activity, increase in cAMP - independent protein kinase activity, increase in glucocorticoid receptors (Yuspa and Pourier, 1988 and Digiovanni, 1992; Troll et al., 1984).

In addition to causing inflammation and epidermal hyperplasia, skin tumor promoters produce many others morphological biochemical and molecular changes in skin (Slaga, 1989). Of these, the induction of epidermal cell proliferation ornithine decarboxylase and subsequent polyamines, and prostaglandin have the best connection with promoting activity (Slaga, 1989; Digiovanni, 1992).
Fig. XVI: The operational Stages in Experimental Skin Carcinogenesis

Stage is defined by the biologic consequences of a specific experimental protocol. This scheme as a framework for molecular analysis of multistep carcinogenesis (Yuspa, 1994).

Many reports have show that cellular effects of TPA are probably the result of stimulation of activity of calcium and phospholipid dependent protein kinase C (PKC) that phosphorylate serine threonine residues (Blumberg, 1988). Treatment with TPA can alter the state of phosphorylation of receptor for epidermal growth factor, insulin and somatomedin C with concomitant inhibition of receptor binding. Such an alteration is dependent on interaction of the promoter with specific receptors in target cells (Weinstein et al., 1984; Shoyab et al., 1981).

Phorbol ester receptors have been purified both from brain of rat and (Kikkawa et al., 1983; Lepeuch et al., 1983) mouse (Ashendal et al., 1983 a, b) and have shown to copurify with PKC. Purified PKC is activated directly by TPA in the presence of phospholipid (Castanga et al., 1982).

TPA application has been shown to decrease the level of total glutathione in the cells and this increases with ontogeny of the tumor (Solanki et al., 1981; Kensler et al., 1983) and has been shown to decrease
the activities of free radical scavenging enzymes such as superoxide dismutase and catalase in the cells (Riner et al., 1991).

Glutathione peroxidase activity also decreases in response to TPA application (Perchellet et al., 1985).

Several investigations have shown that treatment with TPA inhibit cell to cell communication which provides an important clue about the process of tumor promotion, because cell to cell communication is thought to play a crucial role in the control of cell proliferation and differentiation (Trosko and Chang, 1984).

Hennings (1983) proved that the tumor promoter TPA is ineffective in the conversion of papillomas to carcinomas, as the malignant conversion requires further genetic damage in papilloma cells. The ineffectiveness of TPA may be due to lacking of mutagenic activity.
E. MUSTARD SEEDS
Mustard Seed

*Brassica campestris* (var sarason) is one of the most popular species of mustard of the family cruciferae. Mustard seed is widely utilized in the preparation of varieties of edible sauces, pastes and pickles.

The medicinal properties of the volatile, pungent oils obtained from seeds of *Brassica* species belonging to cruciferous plant family have been known for many centuries and are part of folklore in many societies. A number of these plants were subsequently grown for their culinary uses being valued as vegetables, relishes, salads and condiments.

Scientific investigation into the nature, origin and composition of the oils (termed mustard oils) have been described for over 300 years and by the end of the last century it was known that the volatile oils were isothiocyanates which were not present in the plant as such but as volatile precursors and that they were only obtained after the plant or seed was crushed in water (Mc Danell and McLean. 1988). Organic isothiocyanates, which are known as mustard oil, are widely consumed by humans and are responsible for pungent and acrid flavour and odour of condiments such as mustard, horse radish and biting taste that develops after consumption of cruciferous vegetables.

In mustard seed, allyl isothiocyanate are present accompanied by large quantities of their cognate glucosinolates. Isothiocyanates have been implicated in various pharmacological and toxic activities anti bacterial, antifungal, anti protozoal, ability to attract and repel insects, cytotoxicity
chromosomal abnormalities and neoplasia and blocking of carcinogenesis (Zhang and Talalay, 1994; Hecht, 1995).

Isothiocyanates arise in plants as a result of enzymatic cleavage of glucosinolates by myrosinase which is released upon injury to the plant. Myrosinase promotes the hydrolysis of glucosinolates and intramolecular rearrangement of intermediate to yield isothiocyanates. hydrogen sulfate and glucose as end product (Hecht, 1995) (See fig. XVII).

There has been growing interest in recent years in the potential of Brassica vegetables (cabbage, cauliflower, broccoli) as vectors for the introduction of anti carcinogenesis compounds into the diet.

Anti carcinogenic properties of cruciferous vegetables in isolated compounds have been studied in several investigations where animals were first-fed a diet rich in cruciferous vegetables and then exposed to various carcinogens (Stoewsand et al., 1978; Wattenberg and Loub. 1983).

Epidemiological evidence suggests that consumption of cruciferous vegetables is associated with decreased incidence of cancer in the human population (Graham. 1983; Hirayama, 1986).

The compounds involved in the inhibition of carcinogenic activity were indole-3-carbinol and 3-3 diiododiy1-methane (Loub and Wattenberg: 1975). Other active phytochemical in Brassicaceae family involved in cancer chemoprevention are indole glucosinolates, aromatic isothiocyanates, dithioliithiones and phenols (Nugon-Baudon and Rabot, 1994).

Many experimental studies on commonly consumed varieties of Brassica vegetables and chemoprevention of cancer have focused on purified indolyl glucosinolates and their derivatives omitting the presence
Fig. Hydrolysis of glucosinolates by myrosinase and formation of isothiocyanates and other products
of other potentially confounding substances (Wattenberg, 1992; Wattenberg and Loub, 1978; Zhang and Talalay, 1994). Isothiocyanates have been shown to block chemical carcinogenesis of DMBA induced mammary tumors in female Sprague Dawley rats (Wattenberg, 1977, 1981). Inhibition of fore stomach tumors by isothiocyanates has also been observed in mice (Wattenberg and Loub, 1978).

The chemoprevention action of Indole glucosinolate in DMBA induced mammary carcinogenesis in rats has also been demonstrated by Wattenberg and Loub (1978) in aflatoxin B1-induced hepatocarcinogenesis in fish by Nixon et al. (1984). Tumor blocking effects of glucosinolate has been attributed to Quinone reductase (QR) induction in murine hepatic C7 cell line (Tawfiq et al., 1995).

Administration of Isothiocyanates to rodents has been shown to produce either increase or decrease of microsomal cytochrome P-450 but is known to induced phase II enzymes like glutathione-S-Transferase (GST) and Quinone reductase (QR) (Wattenberg, 1992; Tawfiq et al., 1995). It has also been shown to decrease DMBA induce tumor incidence in progeny of mice exposed mustard seed oil during gestation and lactation (Hashim et al., 1998).