REVIEW OF LITERATURE
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The discovery of some palaeopathological remains bearing the "evil signature" of cancer (See Bett, 1957) indicates that Cancer is older than the literature of medicine. Amongst the earliest documents in which one finds the mention of the disease "cancer" is the writings of Hippocrates (460-357 B.C.). In fact the word "carcinoma" has been inherited from him. The Greek word "Karkinos" means crab and the great veins that sometimes surrounded the malady were compared to the claws of the crab. The word "tumor" which comes from Greek "tymbos" (a mound) and the Latin "tumere" (to swell) was introduced during Galen's period (130-200 A.D.).

One of the theories propounded by the ancient Greek philosophers to explain the causation of diseases, for that matter cancer, was the four humoral theory. According to this the occurrence of cancer was attributed to an imbalance of four humors with an excess of black bile. This doctrine of four humors with which the name of Galen would forever be linked, remained unchallenged for over a millenium till Renaissance. The only laudable aspect of this doctrine was that it contradicted the dogmatic belief about the diabolical or divine interference with human health. Thus the history of cancer research till Renaissance is the history of obscurantism, errors and disappointment.
It was in the latter part of the eighteenth century when for the first time, a few scientific reports were available correlating the incidence of cancer to extrinsic factors as against the notion of a constitutional defect. Hill in 1761 traced the incidence of nasal polyps and cancer to prolonged and excessive use of tobacco snuff (Redmond, 1970). In 1775, Percival Pott, a British physician, attributed the high incidence of scrotal cancer among the chimney sweeps to their continual contact with coal tar and their poor hygienic living conditions. Even at that time, the possibility of cancer prevention was recognized, since, according to Clemmesen (1951), three years after Pott's observation the Danish chimney sweepers' guild urged its members to take daily bath. The apparent success of this preventive measure was recognised about 100 years latter by Butlin (1892) who reported that lower incidence of scrotal cancer among the chimney sweeps in northern Europe as compared with the incidence of English chimney sweeps was related to the better personal hygiene and the protective clothing of the former group. The next convincing report regarding occupational cancer in man (Von Volkmann, 1875) was of skin cancer exposed to coal tar arising as a by-product in the industrial production of coal gas.

Isolation and Identification of PAH:

It is quite surprising that the obvious sequel to the
observations (correlating coal tar with skin cancer) the experimental reproduction of the disease took nearly 150 years. In 1915, Yamagiwa and Itchikawa succeeded in inducing skin tumors in the rabbit’s ear by long continued application of coal tar. There followed a spate of tar-painting experiments and Woglam (1926) published the first comprehensive review on the subject.

In the 1930's the stage was set for isolation and identification of the particular component responsible for the carcinogenic action of soot, tar and mineral oils. The pioneering task to fractionate the coal tar and ultimately the isolation of the active material was undertaken by a group of English investigators led by Cook. Cook et al (1933) for the first time isolated from several tons of coal tar a few milligrams of 3, 4-benzopyrene. This constituted one of the major landmarks in the field of experimental carcinogenesis and heralded the era for the testing of a great variety of polycyclic hydrocarbons. Mice served as the test animals in most cases, rats and other species being used occasionally. The "Leit motif" of cancer research in the 1930's, was Polycyclic Aromatic Hydrocarbons in particular. Yosida (1933) reported the hepatocarcinogenicity of a pure aminoazo dye in the rat. Lacassagne (1932) showed the ability of estrone to elicit the formation of mammary tumors in mice. Hueper et al (1938) induced urinary tumors in dog by administration of 2-naphthylamine. Thus, by the end of 1930's there were identified
a good number of chemicals as carcinogens - having very low in common with Polycyclic Aromatic Hydrocarbons, or indeed with one another. The following four decades, i.e. up to the end of 1970's, cancer research went through several different themes or paradigms.

Two-stage concept:

The availability of chemicals that could induce cancer at various target tissues in experimental animals was very exciting. It offered the experimentalist the necessary tools for dissection of the carcinogenic process. Rous & Kid (1941), Friedwald & Rous (1944) were among the first to provide experimental evidence suggesting a two-stage mechanism for carcinogenesis in skin. They demonstrated that local application of coal tar to the ears of rabbit for a period of time, followed by wounding with the cork borer resulted in the appearance of tumors. Rous & his colleagues used the term "initiation" for the process resulting from the tar application and "promotion" for the function of wounding. Friedwald & Rous (1944) postulated that carcinogenesis was composed of an "initiating process" which involved the conversion of normal cells into latent tumor cells as a result of previous insult with carcinogen (tar) and a "promoting process" - whereby these latent tumor cells were made to develop into actual tumors by some non-specific stimuli. The two stages depended on different kinds of stimuli - therefore two independent mechanisms were
involved. In subsequent studies Berenblum & Shubik (1947, 1949) clearly demonstrated the existence of two independent stages in the skin carcinogenesis of mice and referred to them as "Pre-carcinogenic" and "epicarcinogenic". Nevertheless in the interest of uniformity and in order to avoid confusion, one single terminology of "initiation" and "promotion" as proposed by Rous & his colleagues was accepted by Berenblum & Shubik (1947). Extensive discussions on the concept of the sequential nature of carcinogenesis have been published by Boutwell (1964, 1974), Berenblum (1974), Yuspa et al (1976), Pitot & Sirica (1980).

Mottram (1944) simplified the technique and proved that a single application of carcinogen (instead of repeated applications) followed by repeated applications of promoter (croton oil) was adequate to elicit tumor formation. His simplification of procedure had some important significance, enabling the two phases to be analysed in more accurate fashion without overlapping. Thus, through the efforts of various workers such as Shimkin & Andervont (1940), Cramer & Stowell (1942) Shubik (1950), Salaman (1952) and Roe (1959, 1962) a valuable model system "The skin carcinogenesis" was made available for analysing in detail the biological and biochemical events relevant to chemical carcinogenesis and the following generalizations emerged.
(a) Carcinogenesis i.e. the neoplastic transformation is not a single-step process. The two-stage phenomenon in the natural history of neoplastic development is not limited to skin carcinogenesis, but it is a much more general phenomenon extending to other organ systems (Goerttler et al, 1979; Pitot & Sirica, 1980).

(b) A single action of carcinogen followed by repeated application of croton oil was adequate to induce tumor formation (Mottram, 1944; Klein, 1956). Initiator and promoter have qualitatively different roles in tumor formation.

(c) Initiation is an instantaneous reaction.

(d) Initiated cells are irreversibly altered - they do not lose this induced property with time, since they respond to croton oil promotion equally well at long or short intervals after initiation.

(e) Promotion is not an instantaneous process - it is a gradual change since repeated treatment with croton oil is required.

(f) Promoting agents are non-carcinogenic.

(g) Promotion is not irreversible, at least in early stage.

(h) Latent period i.e. the time lag between initiation and the appearance of the identifiable neoplastic cells is considered to be universal in the natural history of chemically induced tumor.
Latent period is dependent on the efficacy of the promoting action.

In 1950's with the emergence of molecular biology, advanced method of biochemistry, electron-microscopy, immunology and virology, cancer research acquired a new impetus and became a cross-road of various disciplines. With a battery of advance tools, experiments were designed by various schools of thought to gain better insights into the complexities of the process of carcinogenesis. However, in the 50's and 60's, the predominant approaches were chemotherapy, viral oncology, and immunology. The most popular theme for the 1970's was again the chemical carcinogenesis. Cancer was proposed by the enthusiasts to be caused due to exposure to the pollutants in the environment in as many as 90% of the cases. Thus keeping track of all the developments of cancer research from 1950's onwards is a formidable problem in view of the explosion of literature on cancer research, covering wide range of topics, each one of which deserves a special review and would run into several pages.

The present review is not at all purported to make itself all inclusive. Considerable selectivity has been exercised, so as to bring into focus the information and interpretations that form the basis for the present investigation.

Covalent binding of PAH to Cellular Macromolecules:

An important step towards the understanding of the
mechanism of carcinogenic action was the discovery of covalent binding of carcinogens with cell constituents. This finding really came as no surprise, since biological effect of this nature (induction of neoplasia) could hardly be conceived without some chemical interaction in the cell. However, it posed two important questions.

(a) Was it the carcinogen itself or one of its metabolites?
(b) Which particular receptor in the cell was the target for such a chemical interaction?

Upto 1950, before the unique role of DNA as the bank of genetic information in the cell was established, it was speculated that specific protein-bound derivatives of carcinogens might be critical intermediates in the initiation of carcinogenesis (Miller & Miller, 1947, 1952). Protein binding by carcinogenic hydrocarbon in mouse skin following topical application of Benzo(a)pyrene was demonstrated by Miller (1951), Miller & Miller (1952), Brookes & Lawley (1964), Grover & Sims (1968) and Warwick (1969). This observation received ready attention because it could readily account for the irreversible nature of neoplastic transformation. It was but a natural step to reach the generalization that all carcinogens, either as such or via their metabolites, might act by causing a specific chemical mutation in the information macromolecule(s).

Bioactivation:

By the latter part of 1960's and early 1970's it was
almost generalized that most, if not all, chemical carcinogens are converted in vivo, to reactive electrophilic or electron deficient derivatives. An electrophilic reactant is a positively charged molecule that can react with potential negatively charged atom. There is evidence for the formation of such reactive metabolite for most of the known types of chemical carcinogens including aromatic azo compounds, aromatic amines, polycyclic aromatic hydrocarbons, aflatoxins, safrole and N-Nitroso compounds (Miller & Miller, 1974, 1976; Magee et al, 1975). Alkylating agents like β-propiolactone (BPL) do not need any prior bioactivation to combine with DNA and other cell molecules (wheeler, 1962; Lawley, 1964). It was further established that the electrophilic derivatives combine with nucleophilic macromolecule(s) such as nucleic acid and proteins (Gelboin, 1964; Grover & Sims, 1968). It was presumed that some of these reactions with DNAs, RNAs and/or proteins were critical to the initiation of carcinogenic process. The existence of the three major targets for reaction with reactive electrophilic metabolite, does not preclude the possible occurrence of other macromolecules, such as polysaccharide component of the plasma membrane, since it was becoming evident that altered cell membrane function was an important property of neoplastic cells. Rubin (1980) and Miller & Miller (1981) reported the possibility of non-genetic changes as the primary events in the initiation of carcinogenesis in some cases. Mintz (1975) had shown that in appropriate in vivo environments
teratocarcinoma cells could differentiate to normal, non-neoplastic tissues. Scribner & Boutwell (1972) and Berenblum (1974) suggested that nucleic acid binding and protein binding constitute separate metabolic stages in carcinogenesis, rather than alternative pathways. Nucleic acid binding brings about initiation only and protein-binding is involved with promotion i.e. with the phenotypic expression of the change(s) in the genome leading to further growth.

Activation Enzymes:

It is now abundantly clear (Jerina & Daly, 1974; Sims & Grover, 1974) that PAH are metabolically activated by the complicated microsomal cytochrome-450 containing monooxygenase systems otherwise known as Aryl Hydrocarbon Hydroxylase (AHH). This enzyme system is localized in the membranes of the endoplasmic reticulum of the liver and extra hepatic tissues such as lung, kidney, gastrointestinal tract and skin (Kinoshita & Gelboin, 1972; Weibel et al, 1975). It is not yet clear how many individual enzymes are involved in the make-up of this system. This enzyme system is characterized by its dualistic nature: it detoxicates the PAH into dihydrodiols and different conjugates which are non-active and at the same time potentiates PAH to active intermediate metabolites like epoxide— which readily react with tissue nucleophiles or undergoes further conversion to less reactive products including Dihydrodiols, phenols and various conjugates (Fig.1). Recent investigators like Dipple et al (1979), Levin et al (1979), and Slaga et al
Fig. 1 - Metabolic transformation of PAH mediated through mixed function Oxidase system.
(1979) have identified Diol-epoxides in the bay-region as the principal determinants of PAH carcinogenicity (Fig. 2). The major enzymatic detoxication pathways include microsomal epoxide hydrase - which converts epoxide to (a) dihydrodiols or (b) to Glutathione conjugates (GSH) by soluble Glutathione - s-transferases. The non-enzymatic detoxication reaction includes isomerization to yield phenols. Some of these less reactive metabolites like dihydrodiols, possibly phenols may recycle through the AHH system to produce secondary metabolites such as dihydrodiol epoxide (Simp et al., 1974). Thus it appears that the fate of a particular chemical resides in a delicate balance between 'activating' and 'detoxicating' pathways and the sensitivity of the target tissue or cell. Kinoshita & Gelboin (1972) suggested that the metabolism of carcinogen like Dimethyl benz(a) anthracene gets completed within 12 hr of its application. In terms of environmental health these enzymes are of great importance, especially in tissues that serve as portals of entry for xenobiotics into the organism. Animal studies have shown that the age, sex, strain, nutritional and hormonal status of the animal and its exposure to various drugs and environmental chemicals can markedly influence the metabolism of the foreign chemicals (Conney, 1967; Gillette et al., 1972). Individual differences exist among people in their ability to oxidise drugs, chemical carcinogens and other foreign compounds, (Conney & Levin, 1974). This invididuality
Fig. 2- Structure of 7,12-Dimethyl benz (α)anthracene.
may play a major role in determining the degree of
danger, different people face when exposed to chemical
carcinogens.

Promotion phase:

Mottram's (1944) remarkable finding that single
application of carcinogen followed by multiple applications
of croton oil for the formation of tumors clearly estab-
lished croton oil as a initiation-promoter of plant origin
and the different qualitative role it plays in carcinogenesis.
Extensive works have been carried out to find out the
biochemical mechanism of tumor formation. These have been
reviewed by Boutwell (1974). Although the exact chemical
nature of the process is not yet clear, a lot of information
have been generated by various workers. Some of the high-
lights of these findings are detailed below.

(a) Promotion of the initiated cell involves the
expression of a new phenotype, with the ability to grow in
a disorganised relationship to its neighbours.

(b) Among the active tumor-promoting principles from
croton oil, 12-0-tetradecanoyl phorbol-13-acetate (TPA) is
the one exhibiting promoting activity on mouse skin (Hecker
1968).

(c) As reactive electrophiles are the critical metabolic
entities responsible for key event(s) in initiation, similarly
Hecker (1978) concluded that TPA (sometimes called PMA) is the critical entity to interact with possible macromolecular receptor in or on epidermal cells in vivo.

(d) Elevated Ornithine decarboxylase (ODC) activity is an essential component of skin tumor formation (O'Brien, 1976; Verma & Boutwell, 1977; Boutwell 1978).

(e) There is much evidence that primary action of the promoter with epidermal cells is at the plasma-membrane (Wenner et al 1974; Van Duuren et al, 1976).

(f) TPA interferes with normal tissue differentiation.

(g) In addition to croton oil, some more initiation promoters have been identified, namely: Synthetic phorbol esters, Anthralin, Certain euphorbia latices, Citrus oil and Fatty acid methyl esters.

Modulating factors:

In all disease processes which owe their origin to extrinsic factors, the interplay between causative agents and the responsiveness of the body to their action determines the final outcome. Apart from primary causative factors, there are many ancilliary factors - both intrinsic (genetic, hormonal) and extrinsic (dietic and a variety of artificial influences) which affect the course of events. Such "modulating factors" influence the course of carcinogenesis in two ways - it may be augmented or negatively it
may be inhibited. The agent of group of agents that brings about augmentation are referred to as "cocarcinogen" whereas the inhibitory factors are known as "inhibitors". The designation "cocarcinogen" should not be considered synonymous with promoter, because in respect of the two-stage skin tumorigenesis in mice, promotion inherently denotes a specific step in the sequence of events leading to the skin tumor formation. In other words, a promoter brings about the completion of an inadequate process - the encouraging of 'dormant' tumor cells (induced by prior initiating action) to develop into progressively growing tumors. Conversely cocarcinogenesis describes a situation in which response to a carcinogen is increased by a second factor introduced concurrently with the carcinogen with no implication of impact at a specific step in tumorigenesis (Boutwell, 1964). Procedurally in designed experiments, they are generally administered concurrently with the carcinogen (Barenblum 1978). The cocarcinogen is usually non-carcinogenic when applied alone. Hence there is an operational difference between a promoter and a co-carcinogen. The difference lies in its sequential or concomitant application.

Sivak (1979) made a comparative study of activities of some potent promoters like phorbol ester, anthralin in the cocarcinogenic protocol and found all these promoters to be
active carcinogens. Sivak concluded that difference between cocarcinogen and promoter appeared to be more methodologic than mechanistic. Thus, there was some confusion with regard to the meaning of the term "cocarcinogenesis". Hecker (1976) redefined co-carcinogenesis as "one of the prototype process of syncarcinogenesis resulting from simultaneous or sequential exposure of the host or target tissue to at first one solitary (or one incomplete) carcinogen and then to an unequivocally identified causative agent non-carcinogenic and non-initiating per se. The latter factor is called a cocarcinogen and may exhibit tissue specificity. Again, Hecker (1978) subdivided the cocarcinogenic agents into (a) initiation (or tumor) promoter and (b) Initiation modifiers, possibly on the basis of either sequential or concomitant application respectively.

Inhibitors:

Much before the concept of initiation and promotion was formulated, inhibitory effect of Sulphur mustard (\(\beta\beta'\)-dichlorehylsulfide) on mouse skin carcinogenesis was demonstrated by Berenblum (1929). Another interesting early example of systemic anticarcinogenic action on mouse skin carcinogenesis resulting from caloric restriction of diet during the promotion phase was reported by Tannenbaum (1959). Caloric restriction during initiation phase had no influence, was reported by Boutwell (1964).
The effect of actinomycin D, on the two-stage system of skin carcinogenesis as originally studied by Gelboin et al. (1965) pointed to the specific inhibition of the initiating phase. Inhibition was pronounced when the actinomycin D was applied on day 0 or 1 relative to the application of initiator, weak when applied 7 days before or 4 days after, and absent when applied on day 7 or later. When administered systemically instead of being applied locally on the skin, actinomycin had no effect at all. The conclusion drawn from these results were questioned by others (See Hennings & Boutwell, 1967; VanDuuren, 1969). Their results under different conditions of dosage etc pointed rather to an effect on the promoting phase. Inhibition occurred when actinomycin D was applied as late as 30 days after initiation (Van Duuren, 1969).

In recent years, the anticarcinogenic studies have engaged the attention of a large number of workers like, Gelboin et al. (1970), Wattenberg et al. (1976), Slaga & Bracken (1977), Verma et al. (1979), Weeks et al. (1980), Chitnis et al. (1980) and Lesca (1981, 1983). Exhaustive reviews on Inhibition of chemical carcinogenesis have been made by Van Duuren and Melchionne (1969), Falk (1971) and Wattenberg (1979', 1983). An interesting aspect of these studies is that many natural compounds present in wide variety of vegetables and fruits consumed by man have been
identified to have the capacity to inhibit neoplastic events at various target tissues. These natural compounds include Coumarins, phenols, organic isothiocynates, indole and flavones, plant sterols, selenium salts, ascorbic acid and carotenes (See Wattenberg, 1983). The chemical diversity of these inhibitors indicate that the capacity to inhibit neoplastic growth is not restricted to limited chemical structure (Wattenberg, 1979, 1983).

The mechanism of action of most inhibitors are poorly understood. This lack of information makes it difficult to organize them into a cohesive pattern. One means of providing an organizational framework is to classify inhibitors according to the time in the carcinogenic process at which they are effective. Utilizing this framework Wattenberg (1983) divided the inhibitors into 3 categories. The first category consists of compounds that prevent the formation of carcinogen from precursor substances. Ascorbic acid and tocopherol have this mechanism of action. The inhibitors of the second category inhibit carcinogenesis by preventing carcinogenic agents from reaching or reacting with critical target sites in the tissues. These inhibitors have been designated "blocking agents". The third category of inhibitors acts subsequent to exposure to carcinogenic agents. These inhibitors are termed "Suppressive agents" since they act
by suppressing the expression of neoplasia in cells previously exposed to doses of a carcinogenic agent. The most extensively investigated inhibitors of this class are the retinoids.