Chapter I
Cancer is a group of neoplastic diseases that occur in man in all age groups and in all races, as well as in animal species. The incidence, geographical distribution and behaviour of specific types of cancer are related to multiple factors including sex, age, race, genetic predisposition and exposure to environmental carcinogenic factors. Irrespective of etiology, cancer is basically a disease of cells characterized by the reduction or loss of effectiveness of normal cellular control and maturation mechanism which regulate multiplication and other functions required for homeostasis in a complex multicellular organism.

Attempt to overcome cancer date back to civilization's earlier period. Earlier arsenic and vinegar were applied to lesions with hot iron directly or after cautery. Therefore the present approach to cancer chemotherapy is not unrelated to the ancient physicians (Selkirk, 1980). Chemotherapy interrupts programmed pattern tumour cell development and can no longer divide. But due to the inadequate knowledge about cellular biochemistry attempts to arrest cancer growth are often ineffective. For e.g. clinical data show that one patient may readily respond to a particular drug, while another may not respond at all (Selkirk, 1980). It is therefore pertinent to understand the biochemical schemes within the growing cell that lead to malignancy.

There are now several families of carcinogenic chemicals, that have become important probes into the cellular biochemistry of
which 20 Methyl-cholanthrene (MCA) needs special call. This was first synthesized by Weiland and Dane in 1933 by the selenium dehydrogenation of dehydronorchole. The molecular formula of methyl-cholanthrene was shown to be $\text{C}_{21}\text{H}_{16}$ by analysis of the pure hydrocarbon and its picrate. Methyl-Cholanthrene is variously known as 20-methyl-cholanthrene or 3-methyl-cholanthrene, depending upon the method of nomenclature.

20 methyl-cholanthrene can induce tumour remote from the site of application and has been shown to be active in hepatocellular carcinogenesis when administered orally to suckling mice (Kelly and O'gara, 1961). Suckling mice injected with this carcinogen developed hepatomas, pulmonary adenomas and forestomach papillomas, lymphocytic neoplasm and adenocarcinomas of the large intestine (Klein, 1963).

The role of metals in biological processes are enormous. There are a number of metals which are known for carcinogenicity and toxicity (Fishman, 1976), while some others are known for anticarcinogenesis (Rosenberg, 1965, Patil et al., 1989). That the platinum compound inhibits cell division and its antitumour potentiality was demonstrated by Rosenberg (1969). The recognition that variation in the element profile of cells or tissues can produce pathological states has been gathering momentum with a renewed understanding of the role of elements in cell physiology (Ranade et al., 1984). The various stages of information transfer, DNA replication, RNA synthesis and protein synthesis are related with the level of trace elements present in
the cell. Many of the trace elements are associated with the enzymes of vital physiological processes are also component of metallo-enzyme. Hence, it seems reasonable to believe that a relationship exists between malignancy and the level of different trace elements.

Earlier Carruthers et al. (1946) noted progressive fall of certain elements like Ca, Fe and Zn in the MCA induced skin carcinogenesis of mice, while Greenstein (1954) observed elevated value of K and depletion of Ca level in malignancy.

Cis dichlorodiamine platinum - II (cisplatin), which has an emperic formula \( N_2Cl_2PtH_6 \) is a planer inorganic Dalton compound with a molecular weight of 300 K; soluble in water at a concentration of 1 mg/ml. cisplatin is formed by an atom of platinum (II) surrounded by chloride and ammonia atoms in the cis position of the horizontal plane.

Cis dichlorodiamine platinum (Cis Pt\(_{11}\)) has been found as a most effective antitumour agent that interacts with the double helix of DNA and inhibits the replication (Howell, 1971; Harder, 1970). It has been proposed that cisplatin regresses the tumour and cures the animals by enhancing the host immune response (Sodhi and Agarwal, 1974, Sodhi, 1972, Rosenberg, 1971). Baldew et al.,
(1991) also explained the antitumour activity of cisplatin through an interaction with DNA, which results in the formation of bidentate adducts. The exact mechanism of action of cisplatin remains obscure but its reactivity towards the nucleophilic site of DNA appears to be responsible for its antitumour effects (Pai and Sodhi, 1991).

Electrical neutrality of the metal is apparently required for activity, probably to facilitate passage through biological membranes. Robert and Pascoe (1972) have suggested that because of a 30 fold difference in intracellular chloride content the compound may be "intracellularly activated" to a bifunctional alkylating agent. This would occur intracellularly as the two chlorides leave the platinum molecule. An alternative mechanism proposed that platinum antigenetically alters or "unmasks" neoplastic cells, which are then immunologically susceptible to destruction (Rosenberg, 1975).

Currently cisplatin is one of the most valuable antineoplastic agents as broadspectrum of neoplasm in the treatment of human cancer (Fram et al., 1990). Complete or partial responses were observed with cisplatin treatment in advanced mesodermal sarcoma with side effects of leukopenia, nausea, vomiting and mild azotemia (Thigpen et al., 1991). In chemotherapy of cisplatin, cyclophosphomide and doxorubicin results a complete remission and long term survival, free of disease among patients with unresectable urothelial tumors (Logothetis et al., 1989) and oesophageal adenoid carcinoma (Petursson, 1986). Cisplatin has
also been shown to be effective in the treatment of lymphoma (Rossoff et al., 1972); ovarian carcinoma (Willshaw and Kroner, 1976); squamous cell carcinoma of the head and neck (Hill et al., 1975; Higby et al., 1974; Jacob et al., 1978); sarcoma 180 and leukemia L1210 tumour of mice (Rosenberg, 1970).

Application of cisplatin was seriously limited due to its cytotoxicity. It has been shown to be clastogenic, mutagenic and capable of inducing sister chromatid exchanges and morphological transformation (Nagy, 1986). The other toxic effects were severe nausea and vomiting, nephrotoxicity, mild and moderate myelosuppression and loss of hearing. Renal and gastrointestinal toxicities were dose limiting and constituted major obstacles to prolonged therapy. Although nephrotoxicity was partially ameliorated by fluid and mannitol diuresis, it remains a major impediment to long term treatment (Salem et al., 1984). Piccarat et al., (1988) reported that cisplatin chemotherapy in combination is much more effective than the lone cisplatin.

An alternative approach to reduce the side effects of cisplatin is the introduction of chemoprotectors. Several agents like sodium thiosulphate (Pfeifle, 1987), diethyldithiocarbonate (Bodenner et al., 1986), sodium selinite (Allan and Smith, 1986) have been shown to reduce the cisplatin induced toxicity. But the major problem encountered in this field is the intrinsic toxicity of this chemoprotectors. Sodium thiosulphate has been shown to reduce the cisplatin antitumour potential as a result of reduced cisplatin concentration in plasma. Further studies are necessary
to establish the clinical value of Na-Selinite as chemoprotector against cisplatin's toxicity (Allan and Smith, 1986).

Vitamin C and E are among the nutrients being considered as potentially protective against cancer (Bright, 1984) by blocking the formation of nitrosamines (Newmark, 1981; Tannenbaum and Mergens, 1980). Oral administration of vitamin C,E and K prevents the formation of carcinogenic nitrosamines (Greenwald et al., 1990; Boone et al., 1990). Calheren et al., (1989) reported that co-treatment of vitamins C and E delayed tumour development in oral carcinogenesis.

Vitamin E is known to act as peroxyl radial trapping, chain breaking antioxidant and also act as a scavenger of free radicals. It is specially active in inhibiting lipid peroxidation (Oski, 1977). Vitamin E increases the influx of cisplatin into the tumour cells and acts after incorporation of cisplatin through the plasma membrane. It should be considered as a co-agent of cisplatin for the treatment of neuroblastoma (Jue et al., 1988; Sue et al., 1988).

Prasad and Rama (1980) reported that Vitamin E in combination with cisplatin produced an additive effect on the growth inhibition of melanoma cells. Vitamin E supplemented diet decrease MCA induced carcinogenesis of mice (Harber and Wisler, 1962; Newberne et al., 1984). Alpha-tocopherol inhibits the lipid peroxidation and reduces the doxorubicin induced toxicity (Geetha and Shyamala, 1993). Shklar (1982) described inhibition of oral
carcinogenesis in hamster by vitamin E. In human, low level of vitamin E has been correlated with increased risk of breast (wald et al., 1984); lung Menkes et al., 1984) and intestinal (Grey et al., 1987).

Vitamin E may influence the management of tumours through several modes of action. It can directly inhibit growth by causing cell death and inhibiting cell division or by stimulating the host immune system. Vitamin E can increase the effectiveness of tumour therapeutic agent by directly enhancing their lethal effect on cancer cells and reducing their adverse effect on normal cells (Prasad and Rama, 1980).

The antioxidant properties of Vitamin C have been known since its discovery (harper, 1975). Vitamin C inhibits hyaluronidase and influences humoral and cellular immune function (Anderson, 1980; Panush, 1979) and metabolic processes associated with cell repair. An increase in Vitamin C intake might have beneficial effects in the treatment of cancer (De Cosse et al., 1975).

Ascorbic acid was found to potentiate the antioxidant nature of alpha-tocopherol (Geetha et al., 1989). Vitamin C and E prevent chromosomal breakage and are used as antioxidant (Shamberger, 1971), Bright (1984) described both the vitamins as the nutrient, being considered as potentially protective against cancer. Vitamin C and E can block the formation of carcinogenic nitrosamines and nitrosamides (Weisberger et al., 1980; Mirvish, 1980; Edgar, 1974) by reacting with nitrite before it can react
with amines. Therefore, uses of both the vitamins E and C together in the prevention of nitrosation in human neoplasm formation would be much more effective. Although segregating effects of these two vitamins are different, yet Prasad and Rama (1984) proposed that a combination of vitamin C and E together may be more effective in the treatment of cancer. Paganelli et al., (1992) observed that Vitamin C, e and A have chemopreventive efficiency against colon cancer in animal models.

The role of element profile in biological activities has been widely attended. Also the element profile has been considered as biomarker in malignancy. The reduced Ca content in malignant tissues is a factor involved in decreased mutual adhesiveness. Calcium is also implicated as a controlling factor of glycolysis (Bygrave, 1966). A decrease in Fe and variation of Zn concentration have been demonstrated in leukemia (Tessemer et al., 1972; Andronikashvili and Mozulishvili, 1980). The Cu and Zn interact with Fe absorption and metabolism (Treuthardt, 1992). Mn is usually the most effective ions for activation of enzyme glycotransferase which are important in polysaccharide and glycoprotien synthesis. Jha et al., (1986) observed the Cu/Zn ratio as a potent marker in the oesophageal carcinogenesis.

The role of cisplatin and the vitamins in the element profile is least known. However, Dufour et al., (1990) after CDDP treatment observed further reduction of Fe level in the target tissues. Magill et al., (1986) observed hypomagnesia after cisplatin treatment in a case control study of human patients.
Tumour cells have higher rate of glycolysis and suffered from glucose hunger. To meet the glucose hunger, tumour received the glucose from non carbohydrate source (Dufour et al., 1990) and the major part of the glucose has been converted into lactic acid. That the vigorous glycolytic activity of tumour cells lead to the production of greater amount of lactic acid, when incubated with glucose resulting in decrease in local extra cellular pH of tumour cells (Von Ardenine and Reitnauer, 1971). The invasive growth of cancer cells, depends on the high synthetic capacity of the cell. The main source of this high energy requirement is oxidation of carbohydrates and fats. The high aerobic and anaerobic glycolysis, an outstanding characteristic of neoplastic cells remain unexplained (Warburg, 1956). In a search for the explanation of the high glycolysis of tumor cells, the possibility that this type of tissues differs from the non neoplastic, in its capacities for synthesis and breakdown of phosphate bond deserve consideration. The participation of phosphorelative intermediate as well as nucleotide such as ADP and ATP in many steps of glucose catabolism implies that the balance between phosphorylative and dephosphorylative ratios might strongly influence the rate of glycolysis (Wenner et al., 1955).

Cisplatin was found to be very effective in presence of repeated injection of glucose in mice bearing Dalton's lymphoma (Sarna and Bhola, 1987), hence and correlation is expected between the enzymes of the carbohydrate metabolism and cisplatin's antitumour
Lactic dehydrogenase (LDH) in malignancy altered significantly and can be employed as a tool to differentiate the metastatic tissues from the non-metastatic tissues (Khan et al., 1984). The activity of the enzyme LDH both in normal and neoplastic tissues is responsible for reversible oxidation of lactate to pyruvate. High level of LDH activity has been reported in hepatic metastasis (Khan et al., 1984); buccal mucosal; nasal and laryngeal cancer (Hariharan et al., 1977), leukemia and malignant effusion (Schwartz et al., 1971). The measurement of serum LDH for the diagnosis of cancer is gradually becoming a general practice. Due to consistent increase in the level of serum LDH in malignancy, it receives clinical attention (Bardawill and Chang, 1963). The enzymes has also been useful in following the effects of treatment. Studies with growing tumour (Riley et al., 1960) or with cultured tumour cells (Holmberg, 1961) have led to the emphasis that tumour cells elaborate and release LDH.

The higher LDH activity along with its isoenzymes act as a biochemical marker in the diagnosis of head and neck cancer (Singh et al., 1993). Heterogeneity of the LDH isoenzymes was reported (Elliot et al., 1961). In addition to their differences in electrophoretic mobility the LDH isoenzymes vary in their kinetic behaviour (Elliot et al., 1961). Therefore the LDH isoenzymes may have specific role in monitoring disease state and assessing its progress (Abdulla, 1971; Massey, 1971).
The glucose-6-phosphatase, an enzyme of gluconeogenesis, displays potent synthetic (Nordlie and Arion, 1964; Stetten, 1964) as well as hydrolytic activity and can catalyse glucose-6-phosphate ester formation from any of several hexoses (Stetten, 1965; Lygre and Nordlie, 1969); notably from glucose and a variety of phosphoryl donor and can again hydrolyse the glucose-6-phosphate to glucose and inorganic phosphate. The activity of another phenotypic marker, the liver G-6-pase (Sirica and Pitot, 1982), has been reduced to 25-40% in the liver of mice with rapidly growing hepatoma (Shapot, 1980). A sharply diminished activity of the key enzymes of gluconeogenesis, fructose 1-6 diphosphatase along with glucose-6-phosphatase has been observed in hepatoma (Weber, 1973). Blinov et al., (1974) observed the development of profound hypoglycemia as a result of reduced gluconeogenesis in mice with Ehrlich ascitic carcinoma and MCA induced sarcoma. Decrease glucose-6-phosphatase activity was reported in the liver of butter yellow fed rats (Spain, 1956) and diethylnitrosamine induced carcinoma of rats (Kil’dema et al., 1977). However, nothing is known about the cisplatin's influence on this phenotypic marker.

The change in the plasma membrane are probably an unique importance in the clinical manifestation of cancer. The scanning electron microscope (SEM) gives an excellent three dimensional image of the cell surface with the differentiation between normal, reactive and neoplastic cells (Carr et al., 1980). Presence of pleomorphic irregular microvilli, ruffles and exotopic blebs on the cell surface with wider intercellular space
was reported (Mickey et al., 1977; Trump et al., 1980; Kenemas et al., 1981) and the pleomorphic microvilli are considered as irreversible neoplastic marker (Shiral et al., 1977). Many structural and functional properties of malignant cells are related to the changes on the cell surface membrane (Takenaga et al., 1977).

The process of gluconeogenesis, may also become enhanced, when the liver glycogen breakdown is arrested. Nisselbaum (1972) showed that in the liver of fasting rats with Moris hepatoma 5123 D the glycogen reserves were not depleted and glycogen mobilisation was impaired, possibly because of the inability of phosphorylase "b" to be converted to phorylase "a" (Sato and Tsukis, 1972). Hers (1963) stated that in pathological or in diseased condition glucose is liberated directly from the glycogen by gamma-amylase activity, where phosphorylase pathway is blocked as an alternative pathway of carbohydrate metabolism (Wheelan and Cameron, 1964).

Neoplastic tissues often have increased concentration of sialic acid on tumour cell surfaces and sialoglycoprotein are shed and secreted by tumour cells which increases their concentration (Kloppel et al., 1977). Baxi et al., (1990) has reported that the sialic acid might be useful as marker for differentiating oral cancer from pre-oral cancer. Yogeeswaran (1983) examined the relationship between cell surface, sialic acid and the metastatic potential of tumour cells. Petal et al., (1991) suggested significantly elevated levels of TSA; LSA, FSA and LDH in
leukemic conditions compared to anemic conditions could be useful biochemical index, Silver et al., (1981) recorded the depleted level of sialic acid after adriamycin treatment with subsequent tumour reduction. The clear relationship between sialic acid and tumour burden indicates that sialic acid analysis could prove clinically important as a monitor of tumour burden in individual patients (Silver et al., 1981).

The cell surface as well as the ultrastructure of cancer cells demonstrated the presence of pleomorphic microvilli (Jeng, 1990) and blebs and bulbous outgrowth (Prasad and Arjun 1991) and their subsequent reduction after the introduction of vitamin E and C with cisplatin in the cancer of the oral region (Sharma et al., 1991, Sharma et al., 1994). Yasunaga et al., (1982) reported that the administration of vitamin E in mice enhanced both lympho proliferative reactions and the antitumour effects of adriamycin also reported by Sue et al., (1988).

The mechanism that vitamin E may reduce the incidence of chemically induced carcinogenesis in animals are not fully understood. Vitamin E (McCay and King, 1980) and vitamin C (Counsell and Horning, 1981) is known to exhibit both antioxidant and immunocompetent effects in vivo and in vitro, depending upon the type of carcinogen and species. The vitamin E action may in part be useful in the treatment of cancer.

The present studies were undertaken to evaluate the combined effect of cisplatin with or without vitamin C and E on the
element profile, LDH, G-6-pase, glucoamylase, sialic acid concentration and cell surface structure of the MCA induced oral carcinogenesis of rat.