CHAPTER - 1

INTRODUCTION
“Oxidative stress”, resulting from the deleterious reductive-oxidative imbalance emerges in recent years as a suspected component in the pathogenesis of cancer in man and animals. The complex and intriguing field of the relation of the oxidative damage to carcinogenesis is a multi-stage project characterized and validated by numbers of laboratory methods for assessing oxidative stress in human beings supported by a cascade of research evidence, investigations and interpretations.

Aerobic organisms deriving their energy from the reduction of oxygen are susceptible to the damaging actions of the small amount of $O_2^-$, $OH^-$ and $H_2O_2$ that inevitably formed during the metabolism of oxygen, especially in the reduction of oxygen in the electron transfer system of mitochondria. These three species, together with unpaired intermediates in the peroxidation of lipids, referred to as Reactive Oxygen Species (ROS) have been recognized as possible “triggers” in the initiation of an imbalance between radical generating and radical-scavenging systems - a condition “oxidative stress” (Spring, 1997).

Increased damage causing reactive oxygen intermediates - the free radicals are generated at the same time that stores of naturally occurring antioxidant reducing agents are depleted resulting the uncontrolled presence of oxygen containing molecules which may cause damage to cell membranes, proteins and nucleic acids and alterations in the intra- and inter-cellular environments. The net effect of this damage is oxidative stress (Lemens and Sterrit, 1994).
A free radical is a cluster of atoms one of which contains an unpaired electron in its outermost shell of electrons. This is an extremely unstable configuration and radicals quickly react with other molecules or radicals to achieve the stable configuration of 4 pairs of electrons in their outermost shell. Produced from the interaction of ionizing radiation with biological molecules, leaked away of electrons from the main path while passing "down" the respiratory chain resulting the reduction of oxygen molecules to the superoxide anion and by dedicated enzymes NADPH oxidase and myeloperoxidase in phagocytic cells like neutrophils and macrophages - these strong oxidants like the various reactive oxygen species can damage other molecules and the cell structures of which they are a part (Barber and Harris, 1994).

The most important of these are the actions of free radicals on the fatty acid side chains of lipids in the various membranes of the cell, especially mitochondrial membranes (Sies, 1991; Barber and Harris, 1994). Abstraction of a hydrogen atom from a polyunsaturated fatty acid initiates the process of lipid peroxidation. Metabolism of toxins in the human body is also associated with production of free radicals (Slater, 1984).

Much of the high reactivity of ROS is due to their generation of such molecular chain reactions, effectively amplifying their effects manifold (Halliwell and Gutteridge, 1989). The changes brought on adjacent molecular targets can vary in magnitude, because many of the components of the living cell are particularly susceptible to free radical injury. The molecular chain reactions can have substantial
effects on the structure and function of living tissue (Betteridge, 2000). Natural selection has driven the evolution of a number of intracellular defence mechanisms to neutralize or control the potentially destructive reactivity of ROS. Mammalian cells possess elaborate defence mechanisms to detoxify radicals. The key metabolic step is Super Oxide Dismutase (SOD), catalysis of the dismutation of superoxide (Spring, 1997). The potential significance of these ROS defence mechanisms is apparent from considerations of the whole body and subcellular distribution of the different components. Vitamin E, the enzymes - SOD, catalase and glutathione peroxidase and substrates (GSH) tend to be in higher concentration in more highly oxygenated locations. (Marx, 1985; Halliwell and Gutteridge, 1989; Ames et al., 1993; Mckerise, 1996; Betteridge, 2000).

Endogenous sources such as energy generation from mitochondria, the detoxification reaction involving the liver cytochrome P - 450 system, phagocytic cells and even specific neurotransmitter release leads to increased generation of free radicals. Reiter (1995) reported the formation of an estimated 10¹¹ free radicals/cell/day including perhaps up to 10⁵ oxidized DNA residues formed/cell/day. Exogenous sources which significantly increase the endogenous oxidant load includes - oxides of nitrogen from cigarette smoke, iron and copper salts etc. - the resultant oxidative stress are - lipid peroxidation, oxidative attack on proteins and lesion in DNA.

Lipid peroxidation have profound effects on cellular function by altering membrane function - increasing fluidity, compromising
permeability and inactivation of membrane bound receptors and enzymes. Oxidative attack on proteins results in site specific amino acid modification, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis (Videla and Fernandez, 1988; Ames et al., 1993).

Lesion in DNA cause deletions, mutations and other lethal genetic effects. Both the sugar and the base moieties of DNA are susceptible to oxidation, causing base degradation, single strand breakage and cross linking to proteins. These oxidative stress effects manifested in cell death and production of leading factors to many pathophysiological conditions including aging, cancer, AIDS (Marx, 1985; Halliwell and Gutteridge, 1989; Ames et al., 1993; Mckerise, 1996; Betteridge, 2000).

Oxidative DNA damage participates in ROS induced carcinogenesis (Breimer, 1990; Guyton and Kensler, 1993). The common form of damage is the formation of hydroxylated bases of DNA considered as an important event in chemical carcinogenesis (Breimer, 1990; Choudhary et al., 1994). The adduct formation interferes with normal cell growth by causing genetic mutations and altering normal gene transcription. Several different pathways by which oxidative DNA damage leads to mutations have been proposed including chemical modification of nucleotide moieties in DNA causing alteration in their hydrogen bonding, exacerbation of polymerase specific hot-spots, conformational changes in the DNA templates and the induction
of a DNA polymerase conformation that is error prone (Lissi et al., 1992; Feig et al., 1994).

An increasing amount of experiments and epidemiological evidences implicates the involvement of oxygen derived radicals in the pathogenesis of cancer development (Kawanishi et al., 2001). Scientist are of opinion that activated or mutated version of key regulatory genes, or oncogenes play an important role in the development of cancer and the mechanism of this has direct or indirect but strong link with oxidative stress. C-myc is one such oncogene which appears to be unusually powerful in terms of its ability to induce the cellular changes associated with cancer and link them to oxidative stress (Ying et al., 2003). Short term activation of C-myc induce oxidative stress and ultimately cause DNA damage. C-myc increased DNA damage and simultaneously compromised the damage-sensing mechanism which enhance the probability that damaged DNA would be copied and inherited by daughter cells. This trend finally triggers the generation of preneoplastic cell formation. C-myc enhanced E2F1 activation - another factor that drives cell proliferation and is also often observed to be mutated in tumours results in an accumulation of ROS (Cerutti, 1985; Guyton and Kensler, 1993; Cooke et al., 2003). The role of oxidative stress in the later stages of carcinogenesis is reported by Feig et al. (1994). However, direct evidences are fragmentary. The tumour promoters have been reported to create an environment of oxidative stress and free radicals play a very prominent and major role in malignant conversion (Trush and Kensler, 1991). By selective modification
of gene expression in initiated cell populations, tumour promoters can elicit the production of clonally-derived benign growth. These tumours can be converted into rapidly growing malignant neoplasms through further DNA damage (Birnboim, 1983). Treatment of papillomas with either an initiator or a free radical generating agent that can elicit DNA damage, such as benzoyl peroxide could produce cancers from benign tumours. This may result in further direct DNA base modification or the transposition of genetic material (Guyton and Kensler, 1993).

There is convincing evidence from animal model system that prolonged exposure of cells to the products of oxidative stress can result in cell injury and play a role in several stages of carcinogenesis (Vuillaume, 1987). The accumulation of oxidative damage has been implicated in both acute and chronic cell injury including possible participation in the formation of cancer. Acute oxidative injury may produce selective cell death and a compensatory increase in cell proliferation. This stimulus may result in the formation of newly initiated preneoplastic cell and enhance the selective clonal expansion of latent initiated preneoplastic cells. Sublethal acute oxidative injury may produce unrepaired DNA damage and result in the formation of new mutation and potentially, new initiated cells (Klaunig et al., 1998). In contrast, sustained chronic oxidative injury may lead to a nonlethal modification of normal cellular growth control mechanisms. Cellular oxidative stress can modify intercellular communication, protein kinase activity, membrane structure and...
function, and gene expression and result in modulation of cell growth (Klaunig et al., 1998). Oxidative damage act as a possible mechanism by which non-genotoxic carcinogens may function. In studies with the selective mouse liver carcinogen dieldrin, a species-specific and dose dependent decrease in liver antioxidant concentrations with a concomitant increase in ROS formation and oxidative damage was observed. Increase in oxidative stress is correlated with an increase in hepatocyte DNA synthesis (Vuillaume, 1987; Klaunig et al., 1998). It is reported by various investigators that the effect of nongenotoxic carcinogen which function through oxidative mechanism may be amplified in rodents but not in primates because of rodent’s greater sensitivity to ROS (Trush and Kensler, 1991). Damage to cellular fatty acid may result in several possible sequelae including modification of the structure and function of the membrane and resulting in a loss of cell homeostasis. Protein oxidative damage can result in the modification in structure, enzyme-activity and signalling pathways. The mediation of ROS on gene transcription may inhibit normal cell apoptosis by modulation of myc, bel-2 and p53 expression and result in an increase in cell number (Ames, 1983).

Oxidative stress interacts with all three stages of the cancer process - initiation, promotion and progression. During the initiation stage oxidative DNA damage may produce gene mutation and structural alteration of the DNA, resulting in a heritable mutation. During the promotion stage ROS and oxidative damage can contribute to abnormal gene expression, blockage of cell to cell communication
and modification of second messenger system, resulting in an increase in cell proliferation or a decrease in apoptosis in the initiated cell population. This results in the clonal expansion of the initiated cells to preneoplastic focal lesion (Toyokuni et al., 1995; Klaunig et al., 1998). Oxidative stress may also participate in the progressive stage of the cancer process by imparting further DNA alteration to the initiated cell population. These changes may result in changes in enzyme activity and make the lesion resistant to normal growth control (Sies, 1991; Toyokuni et al., 1995; Brawn et al., 1995; Kolaja et al., 1996).

The involvement of reactive oxygen species particularly $H_2O_2$, in the tumour promotion process is supported by both in vivo and in vitro studies (Huang et al., 1997). $H_2O_2$ is capable of promoting neoplastic transformation in several two-stage transformation systems, including rat urothelial cells (Okamoto et al., 1996), murine myeloid progenitor cells (Ruch et al., 1989), mouse epidermal cells (Muehlematter et al., 1989) and mouse embryo fibroblasts (Zimmerman and Cerutti, 1984). In vivo studies also suggest that $H_2O_2$ is a mouse skin tumour promoter (Mitchel et al., 1987). The production of ROS and $H_2O_2$ is a common feature of tumour promoters such as 12-0-tetradecanoylphorbol-13-acetate (TCA), Okadaic acid (OA), thapsigargin, 2,3,7,8-tetrachlorodibenzofuran (TCDD), peroxisome proliferators etc. ROS, including $H_2O_2$, may play a critical role in the tumour promotion process (Mitchel et al., 1987). Recent findings of cancer research has focussed on oxidative stress as potent factor which may have effects on the growth and metastatic potential of breast carcinoma (Brown
and Bicknell, 2001). The role of ROS in breast carcinoma may not be limited to early mutagenic events, however, carcinoma cells are frequently under persistant oxidative stress. Human tumour cell lines in vitro produce ROS at a far greater rate than do non-transformed cell lines (Szatrowski and Nathan, 1991) and markers of constitutive oxidative stress have been detected in samples from in vivo breast carcinomas (Toyokuni et al., 1995; Portakal et al., 2000). 8-hydroxy-2-deoxyguanosine, one of the major oxidatively modified DNA base products, is almost 10 times more prevalent in invasive ductal breast carcinoma cells than in normal control samples from the same patient.

Age related increase in the proportion of free radical like OH-induced mutagenic base lesions is likely a significant factor in prostatic cancer development (Donald et al., 2001; HU, 2004). There is higher rate of generation of oxidative damage in cellular DNA of lung cancer patients (Gackowski et al., 2003; Van der Kemp et al., 2004).

Experimental works and clinical observation suggested that thyroid secretion plays an important role in the evolution of cancer, particularly of the genitalia and the breast (Sicher and Waterhouse, 1961). Sommers (1955) reported histological evidence of thyroid atrophy in patients with advanced breast carcinoma. Hyperthyroidism has been described in various carcinomas as chorion carcinoma, embryonal cell carcinoma, urogenital carcinoma and laryngeal carcinoma (Vorobev et al., 1978; Dunzendorfer et al., 1979; Mahanta, 1996). Spencer (1954) suggested that thyroid function is associated with
susceptibility or immunity to cancer. Thyroid deficiency creates changes in the hypophyses which in turn causes conditions favourable to cancer evolution.

The molecular mechanism of thyroid hormone action is the latest line of research in present research world scenario. Thyroid hormone 3,3',5-triido-L-thyronine (T₃), exhibits diverse biological activities. The mechanism by which T₃ induces these various effects remain unclear, although most appear to involve the interaction of thyroid hormone nuclear receptors (TRs) with specific response elements in the promoter region of T₃ target genes (Oppenheimer et al., 1996). Four TR subtypes, alpha-1 and alpha-2, derived from the alpha gene and beta-1 and beta-2 derived from beta gene have been identified. Mutated or truncated forms of certain members of the thyroid receptor superfamily have oncogenic potential. The aberrant forms compete with the normal receptors for binding to responsive elements on the DNA and thus interfere negatively with the normal transcriptional control mechanism. When thyroid hormone level increases, thyroid receptors in the cell nucleus increase DNA transcription which increases the synthesis of specific mitochondrial proteins. Increased synthesis of these mitochondrial proteins up-regulates mitochondrial energy production (Kadenbach et al., 1995; Luft, 1995; Nelson et al., 1995) which ultimately acts as a leading source of reactive oxygen species generation.

High frequencies of mutation of TR-alpha or TR-beta genes have
been identified in human hepatocellular carcinoma, renal clear cell carcinoma, and papillary thyroid carcinoma. Cheng (2003) has developed knock-in mice harbouring mutant TR-alpha or TR-beta genes to test the hypothesis that mutant TRs act as modifiers in the development of human cancer. It is reported by several investigators that during carcinogenesis, the thyroid hormone action is abnormally regulated (Burgos and Koenig, 1999; Cheng, 2003) and aberrant expression of TR α and TR β- mRNAs in renal clear cell carcinoma (RCCC), suggesting possible involvement of TRs in the carcinogenesis of RCCC (Kamiya et al., 2002). Together with aberrant expression pattern, these mutated TRs could contribute to the carcinogenesis of RCCC (Ying et al., 2003; Nygard et al., 2003). Recent investigations on thyroid-carcinoma relationship have demonstrated that a novel steroid thyroid hormone receptor-related gene inappropriately expressed in human hepatocellular carcinoma. The hap gene product may be a novel ligand-responsive regulatory protein of which inappropriate expression in liver may relate to the hepatocellular carcinoma (Hugues, et al., 1987). The prevalence of TR mutations found in the tumours of patients with hepatocellular carcinoma suggests that mutant TRs could play an important role in the liver carcinogenesis (Li et al., 1999).

Thyroid hormones play an important roles in normal brain maturation and normal brain function (Griggs, 1992; Bernal and Nunez, 1995). It has also been suggested that thyroid hormones may have pathophysiological roles in the development of brain tumours.
Thyroid hormone receptors have been identified in brain tumours (Magrassi et al., 1993) and are suggested to be involved in the proliferation of brain tumour cells (Toms et al., 1998). T₄, which is a major secretory product of the thyroid gland, needs to be converted to T₃ by iodothyronine deiodinase to exert its biological activity (Leonard and Koehrle, 1996; Guadano-Ferraz et al., 1997; Calvo et al., 1998). Two different isozymes have been demonstrated for the iodothyronine deiodinase to catalyze T₄ activation. Recent researches suggest that type II isozyme DII (iodothyronine deiodinase) is expressed in brain tumours (Murakami et al., 1988). Thyroid hormones are involved in tumourigenesis and tumour growth and thus these hormones are required for malignant transformation of cultured cells by ionizing irradiation or chemical induction (Guernsey et al., 1980; Borek et al., 1983; Hercbergs, 1996; Rodriguez-Pena, 1999; Bianco et al., 2002).

Furthermore, thyroid hormone interacts with the tumour suppressor gene p53, resulting in a modulation of its own transcriptional activity (Yap et al., 1996). Thyroid hormone depletion inhibits the proliferation of astrocytoma, indicating the role of thyroid hormones in the proliferation of brain tumours (Leonard and Larsen, 1985; Toms et al., 1998). Local T₃ production of DII in brain tumours may be involved in tumourigenesis and tumour growth.

Thyroid hormone induced calorogenesis contributes to liver oxidative stress and promotes an increased respiratory burst activity in kupffer cells (Videla and Fernandez, 1994) which may triggers the process of pre-neoplastic cell formation. The hyperthyroid state in the
rat increases the circulating levels of tumour necrosis factor alpha by actions exerted at the Kupffer cell level and these are related to the oxidative stress status established in the liver by thyroid calorigenesis (Fernandez et al., 2002; Ying et al., 2003; Venditti et al., 2004). One of the major effects of thyroid hormone is to increase mitochondrial respiration by many complex changes in the number and activity of mitochondrial respiratory chain components (Nishiki et al., 1978; Roodyn et al., 1965). Accelerated mitochondrial electron transport brought about by a thyroid hormone-induced hypermetabolic state, results in the increase generation of superoxide at the site of ubiquinone (Turrens et al., 1985). Superoxide radical can lead to the formation of many other reactive species, including hydroxyl radicals, which can readily start the free radical process of lipid peroxidation (Change et al., 1979; Asayama et al., 1987).

In vitro studies of response to oxidative stress suggest that the susceptibility to oxidative challenge is increased in all tissues of hyperthyroid rats and in heart and muscles of hypothyroid animals. Thyroid hormone acts on the mitochondria to "uncouple" the process of oxidation from the generation of ATP. When thyroid hormone is present in excess, more oxygen must be processed to produce the same amount of useful energy for the cell. This leads to increase heat generation and to greater production, hence more leakage of oxygen free radicals from the mitochondria and thus ultimately open the path for serious pathophysiological events. The liver oxidative stress damages observed in experimental and functional hyperthyroidism is mediated by thyroid hormone (Tapia et al., 1997; Venditti et al., 2004).
The role of thyroid hormone in carcinogenesis and tumour metastasis and the mechanisms responsible for arising these critical conditions are studied by different workers at different times but the information regarding the interrelationship between thyroid hormone, oxidative stress and chemical carcinogenesis is still fragmentary. Therefore, the present study is aimed to investigate the “Role of thyroid hormone on oxidative stress during cholanthrene carcinogenesis” in the following plan —

(1) To study the effect of thyroxine on some oxidative stress markers during cholanthrene carcinogenesis

(2) To study the effect of thyroxine on some metabolic markers during cholanthrene carcinogenesis

(3) To study the effect of thyroxine on the thyroid hormone status during cholanthrene carcinogenesis.

(4) To study the effect of thyroxine on some cancer markers during cholanthrene carcinogenesis.


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