CHAPTER - II

REVIEW OF LITERATURE
Thyroid Hormone:

The isolation of thyroxine by Kendall (1919), its identification as $I_3$, $5, 3'$ and $5'$ tetraiodothyronine and its synthesis (Harrington, 1927) gave to biochemistry of thyroid hormone a fundamental impulse. Gross and Pitt-Rivers (1953) showed the presence of another amino acid $I_3$, $5, 3'$-triiodothyronine, in the gland as well as in circulating blood. It is closely related to the thyroxine and physiologically more active than thyroxine.

Nature of the Thyroid Hormones:

The true definition of hormone is very rightly applicable in case of thyroid hormone. Certain thyroid constituents of high activity present in the gland only in traces. The transformation products of the substances secreted by the gland are formed in its receptor cells and are true physiologically active substances.

Hormone iodine constitutes about one-third of the total thyroid iodine in many vertebrates. A very small parts of the hormone not over 2 percent is present in the form of low molecular weight compounds, amino acids and sometimes peptides and nearly all of it becomes part of a specific protein thyroglobulin. Using normal subjects it can be extracted almost quantitatively from frozen slices of the organ with isotonic saline solution. This protein is formed in the epithelial cells lining the vesicles and is secreted into the vesicles after having been iodinated. It remains there only temporarily before being broken down and liberates its iodinated amino acids. This process is regulated by the
anterior pituitary thyrotrophin hormone (Einhorn and Larson, 1959). The accumulation of thyroglobulin (a large glycoprotein molecule with a molecular wt. of 670,000) in the gland results in an increase in size of the colloid vesicles which is an indication of hypofunctioning of the gland. Its hyperfunction is marked by an increase of volumes of the protein producing epithelial cells and a decrease of the colloid (Shepard, 1965).

L-thyroxine contains three fourth of the hormone iodine. It could be isolated in a pure state and crystallised after alkaline or enzymatic hydrolysis of thyroglobulin. It is then found in the non-protein fraction soluble in n-butanol at pH 1—2, and can not be extracted from it by alkaline washing (Blau, 1935). Roche et al. (1963) reported that its presence in blood has been shown either by radio autochromatography or by isotopic dilution.

The presence of I₃, 5, 3'-triiodothyronine in thyroglobulin was shown by Gross and Pitt-Rivers (1952). It is liberated from thyroglobulin by enzymatic proteolysis without any deiodination of L-thyroxine so that it cannot be a degradation product of it. Its presence in the protein is constant but its concentration can vary, probably depends on the intensity of the iodination in the gland at the time.

Under favourable condition triiodothyronine iodine will not go above one-fifth of the thyroxine iodine that is upto 5 to 6 percent of the total iodine the ratio is same in blood plasma. But according to Brown and Jackson (1954) the ratio is often less and sometimes the quantity is so small that it cannot be detected. As soon as it was isolated and synthesized, it shows the same properties as thyroxine (Gross and Pitt-
Rives, 1952). It has the same direct action on basal metabolism, on heart rate, on nitrogen metabolism, on cretinuria and on survival of the mouse in anoxia. Quantitatively $I_3, 5, 3'$ - triiodothyronine is much more active than L-thyroxine from 3 to 6 times.

The thyroid gland contains an average of 8 mg. of iodine. Iodine enters the body chiefly with food and water. It can readily enter via the intact skin or lung. Some organic iodine compounds such as thyroxine are absorbed intact. Most of them are reduced to ionic iodide and absorbed from gastrointestinal tract within an hour and transported as inorganic iodide in the blood. The thyroid gland and the kidneys remove circulating iodide from the blood. One-third is taken up by the thyroid to form thyroid hormone and two-third is lost in the urine as inorganic iodide. In absence of thyroid gland, 98 percent of a dose of radioiodine is excreted in the urine. Iodide secreted by the salivary and gastric glands is reabsorbed. The normal thyroid clears all the iodide from an average of 17ml, the range is 8 to 45 ml of plasma per minute. The kidney clear only an average of 35 ml of plasma per minute, the range being 11 to 68 ml. The renal tubules reabsorb 97 percent of the filtered iodide load and return it to the blood. Approximately 75μgm of iodide must be accumulated by the thyroid each day in order to maintain normal hormonogenesis (Williams and Bakke, 1963).

**Iodide trapping:**

The first stage in the formation of thyroid hormone is transport of iodides from the extracellular fluid into the thyroid glandular cells and follicles. The basal membrane of the thyroid cell
has the specific ability to pump the iodide actively to the interior of the cell. This is called iodide trapping. The iodide concentrating mechanism of the thyroid gland is commonly referred to as the “thyroid iodide trap” or “iodide pump”. Under normal circumstances it concentrate iodide at least twenty five times the plasma level. Organic binding of iodide occurs within seconds. Under certain circumstances the gland concentrate iodide ions to as much as 500 times the plasma level. This concentrating ability is the first step in collecting iodide for subsequent thyroid hormone synthesis. Riggs (1952) using radioiodide have shown that the thyroid trap contains less than 10 μgm of exchangeable or “free” iodide in contrast to 7500 μgm of organically bound iodine. All iodide entering to the gland passes through the trap. The physical location of the thyroid iodide trap appears to be within the follicle rather than in aciner cells. (Doniach and Logothetopoules, 1955). Oxygen dependent enzymes iodases continually transport or pump iodide ions from plasma through the aciner cells into the colloid.

The trapping of iodide against an electro chemical gradient require a steady input of energy from adenosine triphosphate. This is supplied by aerobic ATP synthesis in mitochondria or by anaerobic glycolysis (Tyler et al., 1968). In a glucose free medium, in vitro ATP synthesis and I' transport depend almost entirely on aerobic metabolism in mitochondria (Tyler et al., 1968). Under anaerobic conditions I' influx is reduced and efflux increased in rat thyroid lobes (Surks, 1967). Reduction of oxygen concentration by as much as 88 percent increases I' efflux from rat thyroid lobes in vitro without any change in influx (Surke, 1967).

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Thyroid mitochondria possess the same phosphorylating respiratory chain as other mammalian mitochondria. The mitochondrial inhibitors, rotenone, antimycin and oligomycin, which act at the sites of respiratory reduce both thyroid slice respiration and T/M (I'') (thyroid to medium concentrating ratio for iodide). The lowering of T/M (I'') by oligomycin shows that high energy intermediates of oxidative phosphorylation cannot be used instead of ATP to support accumulation. Exogenous ATP cannot replace endogenous ATP, which indicates that I'' transport mechanism can accept ATP only from the inner side of the cell membrane (Tyler et al., 1968).

The iodide pump is dependent upon sodium efflux (Sodium pump) since it is impaired when the sodium pump is impaired by reduction of the potassium level. On the other hand excess K+ in the medium also depressed T/M (I'') by reducing thyroid I'' influx and accelerating efflux (Scranton and Halmi, 1965). The ability of thyroid slices to accumulate I'' is directly related to Na+ concentration in the medium over a range of 0-170 mm (Alexander and Wolff, 1964) and higher concentrations are inhibitory. Injection of TSH causes a rapid increase in thyroid Na+ and K+ in chicks. Kapitola et al. (1970) suggested the role of Na+ -K+ stimulated Mg2+ dependent ATP ase (Na+-K+ ATP ase) in the active transport of electrolytes across the biological membrane and thus its role in thyroid iodide transport. The cellular site of origin of the enzyme is uncertain. Wolff (1970) reported that beef thyroid plasma membranes are enriched with 40 to 75 fold in Na+ - K+ ATP ase. TSH given to guineapigs in vivo increases both thyroid to serum concentration ratio (T/Sr) and Na+-K+ ATP ase activity (Wolff and Halmi, 1963).
In thyroid slices, ouabain and other cardiac glycosides inhibit Na\(^+\)-K\(^+\) ATPase activity and I\(^-\) accumulation (Knopp et al., 1970).

It was demonstrated that TSH and cyclic AMP stimulates the I\(^-\) transport in dispersed thyroid slices (Bagchi and Fawcett, 1970; Knopp et al., 1970).

The calcium ion is also required for iodide accumulation in thyroid slices (Alexander and Wolff, 1964). In a calcium free medium T/M(I\(^-\)) is reduced by 50 percent. This is completely corrected by addition of Ca\(^{2+}\) to a concentration of 0.9 mM. Variations of Mg\(^{2+}\) concentration in the medium from 0 to 19 mM produce no change in T/M(I\(^-\)).

Like other carrier mediated transport systems, the trapping of iodide can be inhibited by other ions. Thiocyanate, perchlorate and nitrate ions are among the common ones which exhibit inhibitory properties. The blocking effect of thiocyanate and perchlorate on iodide uptake by the thyroid reduce the synthesis and subsequent release of thyroid hormones.

Tissue other than thyroid such as the salivary glands, intestinal mucosa, mammary gland, the ciliary body and choroid plexus can concentrate iodide to some extent. They can form small amounts of monoiodotyrosine (MIT), but these tissues are unable to synthesis thyroid hormone.

**Thyroid hormone storage:**

Thyroid hormone is stored within the gland as iodothyronines incorporated in peptide linkage within the protein thyroglobulin.
Thyroglobulin is the principal constituent of the follicular colloid. The molecular weight of thyroglobulin is 680,000. The molecule is too large to enter the capillaries unless the acinar walls are grossly disrupted by thyroiditis, surgical manipulation, radiation or tumour growth. In addition to thyroglobulin there are two other iodoproteins in the human gland. These iodoproteins differ from thyroglobulin in their low sedimentation coefficients and higher solubilities. Iodinated serum albumin seems to be the main constituent in most cases. Torresani *et al.* (1968) showed that this protein contained labelled iodotyrosines and thyrionines. Rall *et al.* (1964) reported that in congenital goiters and in other pathological thyroid glands, these soluble non thyroglobulin iodoproteins are found in large quantities. This protein contained monoiodotyrosine, diiodotyrosine, thyroxine and triiodothyronine as \( ^{125}\text{I} \) labelled constituents.

The other iodoprotein is associated with subcellular particles and normally contain 11 percent of the protein bound radioiodine. This is synthesized independently of thyroglobulin and is found in highest concentration in thyroid tumours.

Proteolytic enzymes found in thyroid tissue catalyzes the proteolysis of thyroglobulin with the liberation of thyroid hormone. The proteolytic activity of thyroid is increased by TSH and decreased by iodides. This thyroid proteases are active primarily at pH range of 3.6 to 5.7. Ahn and Rosenberg (1970) reported that the first step in digestion is the reduction of the disulfide bridges in the thyroglobulin molecule to split it into smaller fragments. This is accomplished through the action of some naturally occurring agent such as glutathione. The
breaking of the disulfide bonds make thyroglobulin much more susceptible to proteolysis by thyroid lysosomal enzymes and by proteases of extrathyroid origin.

The iodotyrosines are apparently deiodinated as soon as they are released in the free form during digestion of thyroglobulin. Roche (1963) detected specific deiodinating enzymes within the thyroid gland, which are capable of splitting iodide from free moniodotyrosine and diiodotyrosine. These enzymes are unable to deiodinate free $T_3$ or $T_4$ or globulin bound moniodotyrosine or diiodotyrosine. The iodide that is released is reused to make new hormone within the thyroid gland. A variable proportion, about 10 percent escapes into the peripheral circulation. The most important iodinated materials entering the blood stream are, the thyroid hormones, triiodothyronine and thyroxine. However, the thyroglobulin or other iodinated proteins and peptide fragments are also discharged from the gland under normal physiological conditions. For these large iodinated molecules the major pathway is lymphatic system (Ahn and Rosenberg, 1970).

**Synthesis and release of thyroid hormones**:

Iodide, which is ingested in food and water, is actively concentrated by the thyroid gland, converted to organic iodine by thyroid peroxidase, and incorporated into tyrosine in intrafollicular thyroglobulin within the colloid at the basal cell surface of the thyroid follicular cell. The tyrosines are iodinated at one (moniodotyrosine) or two (diiodotyrosine) sites and then coupled to form the active hormones - diiodotyrosine + diiodotyrosine $\rightarrow$ tetraiodothyronine [thyroxine, $T_4$];
diiodotyrosine + monoiodotyrosine → triiodothyronine [T₃]. Another source of T₃ within the thyroid gland is the result of the outer ring deiodination of T₄ by a selenoenzyme: type I 5'-deiodinase (5'-D-I). Thyroglobulin, a glycoprotein containing T₃ and T₄ within its matrix, is taken up from the follicle as colloid droplets by the thyroid cells (Robbins and Rall, 1960).

Lysosomes containing proteases cleave T₃ and T₄ from thyroglobulin, resulting in release of free T₃ and T₄. The iodotyrosines (monoiodotyrosine and diiodotyrosine) are also released from thyroglobulin, but only very small amounts reach the bloodstream. Iodine is removed from them by intracellular deiodinases, and this iodine is used by the thyroid gland (Robbins and Rall, 1960; Robbins et al., 1980).

The T₄ and T₃ released from the thyroid by proteolysis reach the bloodstream, where they are bound to thyroid hormone-binding serum proteins for transport. The major thyroid hormone-binding protein is thyroxine-binding globulin (TBG), which has high affinity but low capacity for T₄ and T₃. TBG normally accounts for about 75% of the bound hormones. Other thyroid hormone-binding proteins—primarily thyroxine-binding prealbumin, also called transthyretin, which has high affinity but low capacity for T₄ and albumin, which has low affinity but high capacity for T₄ and T₃—account for the remainder of the bound serum thyroid hormones. About 0.03% of the total serum T₄ and 0.3% of the total serum T₃ are free and in equilibrium with the bound hormones. Only free T₄ and T₃ are available to the peripheral tissues for thyroid hormone action (Freinkel et al., 1957).
All reactions necessary for the formation of $T_3$ and $T_4$ are influenced and controlled by pituitary thyroid-stimulating hormone (TSH), also called thyrotropin, which stimulates follicular cells in the thyroid gland. TSH binds to its thyroid plasma membrane receptor on the external follicular cell surface and activates the enzyme adenylate cyclase, thus increasing the formation of adenosine $3': 5'$-cyclic phosphate (cAMP), the nucleotide that mediates the intracellular effects of TSH. Pituitary TSH secretion is controlled by a negative feedback mechanism modulated by the circulating level of free $T_4$ and free $T_3$ and by conversion of $T_4$ to $T_3$ in the pituitary thyrotropic cells. $T_3$ is the metabolically active iodothyronine. Increased levels of free thyroid hormones ($T_4$ and $T_3$) inhibit TSH secretion from the pituitary, whereas decreased levels of $T_4$ and $T_3$ result in an increased TSH release from the pituitary. TSH secretion is also influenced by thyrotropin-releasing hormone (TRH), a 3-amino acid peptide synthesized in the hypothalamus. TRH, released into the portal system between the hypothalamus and pituitary, binds to a specific TRH receptor on the thyrotropic cells of the anterior pituitary and causes the subsequent release of TSH. The precise regulation of TRH synthesis and release is not clear (Braverman et al., 1991; Foster et al., 1992), although thyroid hormones do play a role.

About 20% of the circulating $T_3$ is produced by the thyroid. The remaining 80% is produced by monodeiodination of the outer ring of $T_4$ ($5'$ D-1), mainly in the liver. Monodeiodination of the inner ring of
$T_4$ (5-deiodinase [5D-III]) also occurs in hepatic and extrahepatic sites to yield $3, 3', 5'$-T$_3$ (reverse T$_3$ or rT$_3$). This iodothyronine has minimal metabolic activity but is present in normal human serum and in insignificant amounts in thyroglobulin. About 99% of the circulating rT$_3$ is generated by inner ring deiodination of $T_4$ in peripheral tissues.

rT$_3$ levels increase in many instances in which serum T$_3$ levels fall because of decreased activity of outer ring $5'$ D-1 (eg, chronic liver and renal disease, acute and chronic illness, starvation, and carbohydrate-deficient diets). This increase in rT$_3$ occurs primarily because of decreased outer ring ($5'$ D-1) activity, which markedly decreases the clearance of rT$_3$. These states of chronic illness, therefore, result in decreased production of the active hormone T$_3$ and in increased serum rT$_3$ levels due to decreased rT$_3$ clearance. The decreased production of T$_3$ might be an adaptive response to illness (Braverman et al., 1991; Foster et al., 1992).

**Distribution of Thyroid Hormone:**

The iodine content of plasma exists in a number of discrete fractions. Of the total amount of iodine in plasma, which is 6 $\mu$g/100 ml. in normal individual, 90 percent is in organic form and remainder is free iodide. About 95 percent of the organic fractions is bound to protein. These protein bound fractions collectively known as “protein bound iodine” (PBI) which is comprised of bound T$_4$ and T$_3$. Previously PBI was used extensively as a diagnostic test for hyper and hypothyroidism.

The amount of thyroid hormone fraction in body tissues is very small. Free T$_4$ and T$_3$ are estimated to be in the range of $5 \times 10^{-11}$ M.
Thyroxine binding protein:

Although circulating thyroxine is non-dialysable and is precipitated with the serum proteins, it is readily extracted with butanol, which indicates that it is loosely bound to protein. Gordon et al. (1952) reported that specific proteins are responsible for binding T4 in serum. Free thyroxine is in equilibrium with three such proteins, thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA), and serum albumin. Gordon et al. (1952) reported that albumin is a relatively weak secondary carrier.

Electrophoretic studies with human serum indicate that TBG despite its avidity for T4 has a relatively small capacity for the hormone. As the concentration of added hormone is increased, a progressively smaller proportion migrates with TBG and more becomes associated with albumin (Ingbar and Freinkel, 1960; Robbins et al., 1980). It has been estimated that the TBG in 100 ml of normal human serum binds approximately 20 μg of T4. TBG appears to be a small protein with a molecular weight of approximately 50,000 and an isoelectric point of 4.5. According to Rall et al. (1964) it is acid glycoprotein.

In 1958, Ingbar obtained evidence of a third thyroxine binding protein in serum. When human serum containing I\(^{131}\) - T4 is subjected to electrophoresis in a buffer of pH 8.6 some T4 binds to a protein fraction which migrates to the prealbumin zone. Thyroxine-binding prealbumin (TBPA) has less T4 binding affinity than TBG. But it has greater binding capacity of 200-300 μg T4 per 100 ml of serum which is due to its greater concentration in normal serum. It has a
molecular weight of 73,000 and is rich in tryptophane.

**Free thyroxine in serum:**

$T_4$ participates in a reversible binding equilibrium with proteins which has been expressed as —

$$T_4 + \text{TBP}_{\text{EC}} \rightleftharpoons T_4 \cdot \text{TBP}_{\text{EC}}.$$ 

$\text{TBP}_{\text{EC}}$ represents the sum of the extracellular $T_4$ binding components, $T_4 \cdot \text{TBP}_{\text{EC}}$ represents the $T_4$ bound to these proteins, and $T_4$ represents unbound hormone (Ingbar and Freinkel, 1960). The distribution of hormone among the three major binding proteins is determined by their relative $T_4$ binding affinities and the concentration of hormone present. A fraction of the serum $T_4$ is present in the free or unbound form. This is supported experimentally by the results of in vitro studies with systems containing extracellular binding proteins $I^{131} - T_4$ and a cellular phase such as red blood cells. During incubation, some hormone becomes bound to the cells. Under standard conditions, the percentage of the total $I^{131} - T_4$ bound to the cells is proportional to the concentration of hormone and the mass of the cellular phase present and inversely proportional to the binding activity of the extracellular proteins. The concentration of free $T_4$ in normal human serum has been found to be between $10^{-10}$ and $10^{-11} \text{M}$, or less than 0.1 percent of the total circulating hormones (Sterling and Hegedus, 1962).

The percentage of circulating $T_3$ which is present in the unbound
form is much greater than that of free $T_4$. The ratio of the two is $9:6$.

**Transport of thyroid hormones:**

Recent studies (Gharbi-Chini and Torresani, 1981; Osty et al., 1990; McGavack and Thomas, 1995; Hennemann et al., 2001) have confirmed heterogeneity in the transport of thyroid hormones between species. In human there are three main carrier proteins which reversibly bind circulating thyroid hormones, thyroxine-binding globulin being the major carrier protein for $T_4$. Transthyretin (formerly known as thyroxine-binding prealbumin) is a minor binding protein in human, accounting for about 20% of circulating $T_4$, while albumin, through nonspecific binding, carries only 10% of the circulating hormone (Nagasawa et al., 1995). Collectively these three proteins transport more than 95% of $T_3$ and $T_4$ hormones in human. In rats, a significant difference is that thyroxine-binding globulin occurs only as a postnatal surge, declining to nondetectable levels by early maturity at 8 weeks, but reappearing in senescence. Thus, through most of their lives rats lack one of the major carrier proteins for $T_4$, with transthyretin serving as the primary plasma transporter. The binding affinity of transthyretin, however, is several orders of magnitude lower than for thyroxine-binding globulin. Likewise, $T_3$ is transported in humans in a bound state to thyroxine-binding globulin and albumin, but only by albumin in rodents. A primary function of the transport proteins is believed to be extrathyroidal storage of thyroid hormones as a mechanism to control hormone release, thereby protecting target tissue from excessive hormone influence. By virtue of the absence of a major
high-affinity binding protein, this buffering action protecting targets such as the thyrotrophs of the anterior pituitary would appear to be lower in rodents. Furthermore, in species in which $T_4$ binding is limited to transthyretin and albumin, the proportion of unbound $T_4$ is greater than it is in human. Coupled with a considerably shorter half-life for $T_4$ of 12 to 24 hours in rats compared to 5-9 days in human, these various interspecies differences imply a greater predisposition of rodents to TSH perturbation by chemicals that influence thyroid status (Hennemann et al., 2001).

**Metabolism of thyroxine:**

Riggs (1952) reported the important kinetic considerations involved in thyroxine iodine or thyroid hormone metabolism. Albert et al. (1949) showed that the thyroxine is well absorbed from the gastrointestinal tract and quickly appears in the blood. Upon intravenous administration thyroxine $^{131}$ disappears from the blood at a double exponential rate, the final component having a half life of 3.8 days. The rate of disappearance of L-thyroxine from the blood is slower. D-thyroxine disappears from the blood at an even more rapid rate than L-thyroxine. Homolsky et al. (1953) reported that when plasma is removed from patients given $^1$ and infused this labelled hormone into other patients, the rate of loss of endogenously labelled thyroid hormone is more rapid than that of labelled thyroxine. Homolsky et al. (1953) showed that when labelled hormone was transfused into thyrotoxic recipients, it was turned over more rapidly than in euthyroid.
recipients.

Gross and Labond (1951) observed no difference between endogenously labelled hormone and labelled thyroxine in rat.

In a series of investigations, Albert et al. (1953) however, reported a marked difference in the rat between the metabolism of exogenously labelled thyroxine and endogenously labelled thyroid hormone. In all cases liver played a major role in the excretion of the thyroxine.

Myant, (1956) showed in patients with bile cannulas, the presence of approximately 56 μg. of degraded thyroxine in the bile daily. The principal metabolite in the bile is the inert water soluble conjugate with glucuronide. About a third of the biliary iodine reabsorbed from the intestinal tract after hydrolytic release of free thyroxine. This hydrolysis is the result of intestinal bacterial - glucuronidase activity.

The quantitative determination of the biliary secretion in the man shows that in general about 10 percent of the radioactivity administered as I\(^{131}\) -labelled L-thyroxine appeared in the bile (Johnson and Beierwaltes, 1953). Klitgaard (1953) reported that the thyroidectomized and thiouracil treated rats showed a diminished biliary excretion of radioactivity. There is a quantitative difference between rats and man in the catabolism of thyroxine.

The role of the liver in the metabolism of thyroxine has been investigated by transplantation of the thyroid into the spleen (Bondy, 1951). It has been reported that the intrasplenic injection of thyroxine results in about the same metabolic effect as subcutaneous injection. Lipner et al. (1952) reported the distribution of labelled thyroxine in the nuclei, mitochondria, and supernatant fractions of liver cells. Over
half the radioactivity was found in the supernatant fraction, about one-fourth in the mitochondria and the rest in the nuclei.

The final and important metabolic fate of the thyroid hormones is their deiodination. The deiodination is accomplished by a tissue deiodinase activity which differs from thyroid deiodinase in that it has a high specificity for \( T_4 \) and \( T_3 \). Iodide which is liberated is retrapped by the thyroid or excreted in urine.

Flock et al. (1962) reported that deiodination from the ring is depressed in hepatoectomized dog. Isolated rat liver deiodinates \( T_3 \) faster than \( T_4 \) and the deiodination of \( 3.3', 5'-T_3 \) and \( 3.3'-T_2 \) is extremely rapid. Stanbury (1960) reported the conversion of injected \( T_4 \) to \( T_3 \) in vivo. Partially deiodinated products of ring deiodination have been detected in the bile of normal dogs and in plasma and urine of hepatoectomized dogs.

Oxidative deamination and decarboxylation of the thyroid hormones also occur in vivo. The acetic acid analogues of \( T_4 \), \( T_3 \), \( 3.3', 5'-T_3 \) and \( 3.3'-T_2 \) have been detected following injection of corresponding iodothyronine (Stanbury, 1960; Ingbar and Galton, 1963). Ingbar and Galton (1963) reported that both TA\(_4\) and TA\(_3\) are derived in vivo from endogenous hormone. They are found in liver and kidney but not in plasma of mice and rats given \( ^{131}\)I. The pyruvic acid and lactic acid derivatives of \( T_4 \) and \( T_3 \) are also formed in rats following injection of \( T_4 \) or \( T_3 \) (Ingbar and Galton, 1963). Oxidative deamination is resulted from the action of non specific L-amino acid oxidase system. Yamamoto et al. (1960) and Nakano et al. (1963) identified two separate enzyme systems in mitochondria which are capable of altering the side chain of
T₄ and T₃ - an oxidase system and a transaminase. The latter requires -ketogluterate and pyridoxal phosphate for activity.

The deiodination of the thyroid hormones and their analogues in vitro has been demonstrated in a variety of tissue preparation (Ingbar and Galton, 1963; Rall et al., 1964). Roche et al. (1963) suggested that before deiodination, the hormones become linked to tissue protein, possibly an enzyme. Partial deiodination then occurs and deiodinated molecule remains in the protein complex. According to Wynn and Gibbs (1963) complexing of the T₄ with protein involves oxidation of the phenolic hydroxyl group with the formation of a free radical or quinoid intermediate.

A variety of compounds has been shown to stimulate the deiodination of T₄ in tissue preparations in vitro. These include ferrous ions (Fe²⁺), flavin compounds, and reducing agents as ascorbic acid and sulphhydryl compounds (Ingbar and Galton, 1963; Rall et al., 1964). Galton and Ingbar (1963) had shown that the deiodination of T₄ in vitro is mediated by a hydrogen peroxide-peroxidase system which is inhibited in the presence of catalase.

**Thyroid Hormone Receptors:**

Receptors for thyroid hormone are members of a large family of nuclear receptors that include those of the steroid hormones. They function as hormone-activated transcription factors and thereby act by modulating gene expression. In contrast to steroid hormone receptors, thyroid hormone receptors bind DNA in the absence of hormone, usually leading to transcriptional repression. Hormone binding is
associated with a conformational change in the receptor that causes it to function as a transcriptional activator (Brent, 1994).

**Receptor Structure:**

Mammalian thyroid hormone receptors are encoded by two genes, designated as alpha and beta. Further, the primary transcript for each gene can be alternatively spliced, generating different alpha and beta receptor isoforms. Currently, four different thyroid hormone receptors are recognized: alpha-1, alpha-2, beta-1 and beta-2 (Brent, 1994; Tsai and O'malley, 1994).

Like other members of the nuclear receptor superfamily, thyroid hormone receptors encapsulate three functional domains:

- A transactivation domain at the amino terminus that interacts with other transcription factors to form complexes that repress or activate transcription. There is considerable divergence in sequence of the transactivation domains of alpha and beta isoforms and between the two beta isoforms of the receptor.

- A DNA-binding domain that binds to sequences of promoter DNA known as hormone response elements.

- A ligand-binding and dimerization domain at the carboxy-terminus. The DNA-binding domains of the different receptor isoforms are very similar, but there is considerable divergence among transactivation and ligand-binding domains. Most notably, the alpha-2 isoform has a unique carboxy-terminus and does not bind triiodothyronine (T3) (Brent, 1994).

The different forms of thyroid receptors have patterns of
expression that vary by tissue and by developmental stage. For example, almost all tissues express the alpha-1, alpha-2 and beta-1 isoforms, but beta-2 is synthesized almost exclusively in hypothalamus, anterior pituitary and developing ear. Receptor alpha-1 is the first isoform expressed in the conceptus, and there is a profound increase in expression of beta receptors in brain shortly after birth. Interestingly, the beta receptor preferentially activates expression from several genes known to be important in brain development (e.g. myelin basic protein), and upregulation of this particular receptor may thus be critical to the well known effects of thyroid hormones on development of the foetal and neonatal brain. The presence of multiple forms of the thyroid hormone receptor, with tissue and stage dependent differences in their expression, suggests an extraordinary level of complexity in the physiologic effects of thyroid hormone (Brent, 1994).

**Interaction of thyroid hormone receptors with DNA:**

Thyroid hormone receptors bind to short, repeated sequences of DNA called thyroid or T₃ response elements (TREs), a type of hormone response element. A TRE is composed of two AGGTCA “half sites” separated by four nucleotides. The half sites of a TRE can be arranged as direct repeats, palindromes or inverted repeats.

The DNA-binding domain of the receptor contains two sets of four cysteine residues, and each set chelates a zinc ion, forming loops known as “zinc fingers”. A part of the first zinc finger interacts directly with nucleotides in the major groove of TRE DNA, while residues in the second finger interact with nucleotides in the minor groove of the
TRE. Thus, the zinc fingers mediate specificity in binding to TREs (Brent, 1994; Zhang and Lazer, 2000).

Thyroid hormone receptors can bind to a TRE as monomers as homodimers or as heterodimers with the retinoid X receptor (RXR), another member of the nuclear receptor superfamily that binds 9-cis retinoic acid. The heterodimer affords the highest affinity binding, and is thought to represent the major functional form of the receptor. Thyroid hormone receptors bind to TRE DNA regardless of whether they are occupied by T3. However, the biological effects of TRE binding by the unoccupied versus the occupied receptor are dramatically different. In general, binding of thyroid hormone receptor alone to DNA leads to repression of transcription, whereas binding of the thyroid hormone-receptor complex activates transcription.

Ligand-free state: The transactivation domain of the T3-free receptor, as a heterodimer with RXR, assumes a conformation that promotes interaction with a group of transcriptional corepressor molecules. A part of this corepressor complex has histone deacetylase activity (HDA), which is associated with formation of a compact, “turned-off” conformation of chromatin. The net effect of recruiting these types of transcription factors is to repress transcription from affected genes.

Ligand-bound state: Binding of T3 to its receptor induces a conformational change in the receptor that makes it incompetent to bind the corepressor complex, but competent to bind a group of coactivator proteins. The coactivator complex contains histone transacetylase (HAT)
activity, which imposes an open configuration on adjacent chromatin. The coactivator complex associated with the T₃-bound receptor functions to activate transcription from linked genes. A growing number of specific proteins have been identified as members of the corepressor and coactivator complexes. The alpha-2 receptor is unable to bind T₃ and acts as similarly to a dominant-negative mutant of the receptor, but its carboxy-terminus can be differentially phosphorylated, which affects DNA binding and dimerization. Also, the beta-2 isoform apparently fails to function as a repressor in the absence of T₃ (Tsai and O'Malley, 1994; Zhang and Lazer, 2000)

**Thyroid hormone and cancer:**

The thyroid provides the simplest example of tumour formation through hypofunction and feedback failure. The thyroid have profound indirect effects in the growth and development of the body. Loeser (1954) thought that it is associated with tumour formation and possibly with metastasis. Thyroid tumours may be produced experimentally by anything which induces thyroid deficiency leading to a feedback stimulation of the pituitary.

The thyreotrophin or thyroid stimulating hormone (TSH) of the anterior pituitary supports normal thyroid tissue growth and metabolism. It has been shown to affect neoplastic thyroid tissue Trunnell et al., 1949). Thyroid carcinoma presenting clinical manifestation of thyrotoxicosis indicate that TSH may play a definite role in the pathogenesis of thyroid malignancy (Herts, 1951). Dalton et al. (1948) reported that prolonged administration of thiouracil in mice causes
dearrangements of thyroid-pituitary relationships and which led to the thyroid tumour formation. In these instances it was suggested that secretion of the thyroid are inhibited. The pituitary then increases the production of thyreotrophin hormone to compensate for thyroid deficiency and thus stimulates the thyroid hypertrophy and hyperplasia. The addition of thyroxine or of thyroid active materials prevents thyroid tumourigenesis.

The relation of the thyroid to pituitary tumourigenesis has been reviewed by Gorbman (1947). Thyroid hormone was effective in suppressing the pituitary tumours resulting from $^{131}$I treatment. A partial block of thyroid hormone produces thyroid tumours and more complete block results in both thyroid and pituitary tumours and a complete destruction of the thyroid epithelium results only pituitary tumours (Dent et al., 1955).

**The Roles of thyroid hormone receptors in disease pathogenesis (Thyroid hormone receptor mutation in cancer):**

The role of thyroid hormones in carcinogenesis has been controversial. Importantly, research workers have identified high frequencies of mutation of TR-alpha and TR-beta genes in human hepatocellular carcinoma, renal clear cell carcinoma, and papillary thyroid carcinoma. Investigators (Burgos and Koenig, 1999; Wu Koenig, 2000) therefore have developed knock in mice harboring mutant TR-alpha or TR-beta genes to test the hypothesis that mutant TRs act as modifiers in the development of human cancer. Knock-in mutant TR mice are being crossed with other mouse models of human cancer.
Characterization of the phenotypes of the offspring shed new light on the role of thyroid hormone in human cancer (Cheng, 2003).

The thyroid hormone receptors (TRs) mediate the pleiotropic activities of the thyroid hormone in growth, development, and differentiation and in maintaining metabolic homeostasis. They are ligand-dependent transcription factors and are members of the steroid hormone/retinoid acid receptor superfamily. Two TR genes, $\alpha$ and $\beta$, located on human chromosomes 17 and 3, respectively have been identified. They are cellular homologs of the retroviral $v$-erb A oncogene which suggest their possible involvement in carcinogenesis.

Recent studies showed altered expression of TRs at both the mRNA and protein levels and identified somatic mutations of TRs in several human cancers. Male transgenic mice overexpressing $v$-erb A oncogene develop hepatocellular carcinoma. A targeted germline mutation of the $TR\beta$ gene leads to the occurrence of metastatic thyroid carcinoma in homozygous mutant mice. These findings provide evidence to support the critical role of TRs in human cancer (Cheng, 2003).

**Genomic changes during thyroid hormone-induced cell proliferation:**

Despite recent progress in the understanding of the transcriptional regulation of TRs by $T_3$; the molecular basis of the growth promoting effect of $T_3$ remains unknown. It is reported that the retinoblastoma protein plays a key role in $T_3$-induced cell proliferation (Wu and Koenig, 2000) for further identification of genes associated with $T_3$-induced cell proliferation, use of cDNA microarrays to profile genomic changes in a model cell line, GC cells of 358 respon-
sive genes identified, 88 percent were not previously reported to be transcriptionally or functionally modulated by T3. Analysis of these genes revealed that T3 induced cell proliferation requires activation of multiple cellular pathways including glucose metabolism, biosynthesis, transcriptional regulation, protein degradation, and detoxification in T3-induced cell proliferation. The finding of rapid repression of T3 in the expression of key regulators of the Wnt signaling pathway and suppressed the transcriptional downstream elements of the beta-catenin / TCF complex. These results indicate that thyroid hormone induced cell proliferation is accompanied by a complex coordinated transcriptional reprogramming of many genes in different pathways and that early silencing of the Wnt pathway is critical to this event. The critical role of the Wnt signaling pathway in T3-induced cell proliferation under normal and pathological conditions is currently being examined using mouse models (Cheng, 2003).

**Triiodothyronine and its nuclear receptors in tumourigenesis:**

The gene and its protein products are thought to be involved in tumourigenesis when chromosomal anomalies and/or mutation of these genes as well as markedly disturbed expression and function of the encoded protein are found in tumour tissue (Kuznicka et al., 2002). It is reported that triiodothyronine receptors (TRs) belong to the group of such proteins. This hypothesis is supported by four facts. Firstly, physiological function of triiodothyronine (T3), exerted through TRs, is the regulation of proliferation, differentiation and apoptosis, the processes that are deeply disturbed in tumour tissue. Secondly, action of TRs is
connected to the action of some protooncogenes (c-Jn, Mdm 2) and tumour suppressor (p53). Thirdly, loss of heterozygosity (LOH) of chromosome fragments containing between other TR alleles as well as disturbed expression of TR on the mRNA and protein level are observed in tumour tissue. Finally, TR α is a cellular homolog of v-erb A, a viral oncogene that behaves as a dominant negative mutant receptor. In addition, TR gene point mutations changing amino acid sequence are observed, resulting in abnormal receptor function as transcription activator. It was reported that mutant cloned from liver cancer behave as dominant negative mutants (Kuznicka et al., 2002).

Expression of mutant thyroid hormone nuclear receptors is associated with human renal clear cell carcinoma:

The action of thyroid hormone nuclear receptors have been shown to be abnormally regulated during carcinogenesis (Ying, 2003). It is already established that 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. To establish the molecular actions of TRs in RCCC, cDNAs for TR β1 and TR α1 were cloned from 22 RCCC tissues and 20 surrounding normal tissues. Mutations were found in seven TRβ1 and three TRα1 cDNAs. Two TR β1 cDNAs had a single mutation, while five TR β1 and three TRα1 had two or three mutations. Most of the mutations were localized in the hormone-binding domain. Using the TRs prepared by in vitro transcription/translation, investigators found that these mutations led to a loss of T3 binding activity and/or impairment in binding to thyroid
hormone response elements (TREs) (Kamiya et al., 2002). Nuclear extract from RCCC tissues also exhibited impairment in binding to TREs. These results indicate that the normal functions of TRs in RCCC tissues were impaired. Together with the aberrant expression patterns, these mutated TRs could contribute to the carcinogenesis of RCCC (Kamiya et al., 2002; Ying et al., 2003; Nygard, 2003).

Expression of mutant hormone nuclear receptors in human hepatocellular carcinoma cell:

To demonstrate the expression and role of thyroid hormone nuclear receptors (TRs) in hepatocarcinogenesis, investigators (Lin et al., 1999) have characterized the TRs in 16 human hepatocellular carcinoma (HCC) specimen, the full-length cDNAs for the two TR subtypes alpha-1 and beta-1, were cloned from several tumours by reverse transcription - polymerase chain reaction. Southern blot analysis indicated that, in addition to the full-length cDNA, truncated TR alpha-1 and TR beta-1 cDNAs were present in nine tumours (53%). In addition, point mutation detected by the mismatch RNAase cleavage assay in TR alph-1 and TR beta-1 were found in 65% and 76% of the tumours, respectively. The mutations were confirmed by DNA sequencing. Most of the TR alpha-1 mutations were in amino acid codons 209-228 and 245-256, two hot-spots in HCC patients. However, no hot-spot was detected in TR beta-1. The expression of TR alpha-1 and TR beta-1 proteins was determined in the tissue extracts by western blotting (Lin et al., 1999). TR beta-1 protein was expressed or elevated in 10 tumours but not in normal livers, whereas the expression of TR
alpha-1 was variable among tumours. The mutant TR proteins were translated in vitro, and their hormone-and DNA-binding activities were evaluated. Abnormal binding to the thyroid hormone elements was observed. The proteins DNA binding activity was either partially impaired or completely lost. The high prevalence of TR mutations found in the tumours of patients with hepatocellular carcinoma suggests that mutant TRs could play an important role in liver carcinogenesis (Lin, et al., 1999).

**Thyroid hormone induced liver oxidative stress in rat:**

Thyroid hormone induced calorigenesis contribute to liver oxidative stress and promotes an increased respiratory burst activity in Kupffer cells, which could conceivably increase the expression of redoxsensitive genes, including those coding for cytokines (Fernandez et al., 2002). To test the hypothesis that L-3, 3', 5-triiodothyronine (T3)-induced liver oxidative stress would markedly increase the production of tumour necrosis factor alpha (TNF - alpha) by Kupffer cells and its release into the circulation, sprague-Dawley rats was fed with a single dose of 0.1 mg T3/kg body weight and determinations of liver O2 consumption, serum TNF - alpha, rectal temperature, and serum T3 levels were carried out at different times after treatment. Hepatic content of total reduced glutathione (GSH) and biliary glutathione disulfide (GSSG) efflux were measured as indices of oxidative stress. Some investigator (Horrium et al., 1985; Fernandez et al., 2002) reported the administration of either (i) the Kupffer cell inactivator gadolinium chloride, (ii) the antioxidants alpha - tocopherol and
N-acetyl-L-cysteine (NAC), or (iii) an antisense oligonucleotide against TNF- alpha (ASO TJU-2755) prior to T3 injection. T3 elicited an 80-fold increase in the serum level of TNF- alpha at 22 hour after treatment which coincide with the onset of thyroid calorogenesis. Pretreatment with Gd Cl3, alpha-tocopherol, NAC and ASO TJU-2755 virtually abolished this effect and markedly reduced T3-induced liver GSH depletion and the increase in biliary GSSG efflux. The hyperthyroid state in the rat increases the circulating levels of TNF- alpha by actions exerted at the Kupffer cell level and these are related to the oxidative stress status established in the liver by thyroid calorogenesis (Fernandez et al., 2002).

**Thyroid and Breast Cancer**:

The coincidence of both breast cancer and thyroid disorders is still a subject of extensive debate.

Some researchers (Sirchia et al., 2000) suggest that there is definite evidence that thyroid abnormalities may influence the progression of breast cancer.

Shomon (2003) reported that treatment for thyroid cancer in younger women increases their risk for breast cancer five to twenty years later. Thyroid hormone has a direct and crucial role in the development of breast cancer. Thyroid hormone is a very powerful co-factor of experimental carcinogenesis.

However, there is no clear evidence of a causal relationship in that one disease causes the other. The preponderence of published work favours an association with hypothyroidism. Geographical
variations in the incidence of breast cancer have been attributed to differences in dietary iodine intake and an effect of iodide on the breast has been postulated. Recent reports have shown a direct association between thyroid enlargement and breast cancer. Although the exact mechanism for the demonstrated association between diseases of the thyroid and breast cancer remains to be elucidated, there is at least the possibility that the presence of thyroid abnormalities may influence breast cancer progression and this alone should stimulate awareness into the concience of the two disorder(Smyth, 1997).

The prevalence of hyperthyroidism and hypothyroidism in patients with breast cancer versus those without breast cancer is very similar. Non-toxic goiter was more than twice as common in the breast cancer patients; 45.5% of breast cancer patients had thyroid enlargement compared with only 10.5% of controls. And, antithyroid peroxidase autoantibodies were twice as common in breast cancer patients than in controls (Shomon, 2003).

**Relevance of autoimmune thyroid disorders in breast malignancy:**

Sirchia *et al.* (2000) reported that the overall prevalence of thyroid disease was 46% in the breast cancer patient studied, versus 14% in the control studied. The prevalence of nontoxic goiter was 27.41 in breast cancer patients, versus 11% in controls. Hashimoto's thyroiditis was found in 13.7% of breast cancer patients, and in 2% of the controls. The prevalence of thyroperoxidase (TPO) antibody was higher in breast cancer patients than in controls (23.5% versus 8%). The study found that the prevalence of thyroid disorders is increased in patients
with breast cancer, and thyroid autoimmune disorders, especially Hashimoto's thyroiditis, account to a large extent for the increased prevalence of thyroid disease in patient with breast cancer (Widschwendter et al., 2000)

**Alterations in genomic profiles during tumour progression in a mouse model of follicular thyroid carcinoma:**

The molecular genetics underlying thyroid carcinogenesis is not well understood. Researchers have recently created a mutant mouse by targeting a mutation (PV) into the thyroid hormone receptor β gene (TR β PV mouse) (Ying et al., 2003). TR βPV/PV mice spontaneously develop follicular thyroid carcinoma through pathological progression of hyperplasia, capsular and vascular invasion, anaplasia and eventually metastasis to distant organs. TR βPV/PV mice provide an unusual opportunity to study the alterations in gene regulation that occur during thyroid carcinogenesis. Among the activated tumour-related gene identified, Cyclin D1, pituitary tumour transforming gene-1, cathepsin D and transforming growth factor α were also found to over-express in human thyroid cancers. Analysis of the gene profiles suggested that the signalling pathways mediated by thyrotropin, peptide growth factor β, tumour necrosis factor-α and nuclear factor -rβ were activated, whereas pathways mediated by peroxisome proliferation activated receptor γ were repressed. These results indicate that complex alterations of multiple signaling pathways contribute to thyroid carcinogenesis. The critical genes associated with thyroid follicular carcinogenesis uncovered in the present study could serve as signature genes for
diagnostic purpose, as well as for possible therapeutic genes (Ying et al., 2003).

Expression of type II iodothyronine deiodinase in brain tumours:

$T_4$, which is major secretory product of the thyroid gland, needs to be converted to $T_3$ by iodothyronine deiodinase to exert its biological activity (Leonard and Koehrle, 1996; Murakami et al., 2000). Two different isozymes have been demonstrated for the iodothyronine deiodinase to catalyze $T_4$ activation (Leonard and Koehrle, 1996). Type II iodothyronine deiodinase (D II) is present in a limited number of tissues, including brain, anterior pituitary, brown fat, and pineal gland in the rat (Leonard and Koehrle, 1996). D II activity increases in the hypothyroid state and plays a critical role in providing local intracellular $T_3$. In human, D II activity has been demonstrated in normal brain tissues and brain tumours (Campos-Barros et al., 1996; Calvo et al., 1998).

Further studies are required to clarify the pathophysiological roles of local $T_3$ production by D II in brain tumours.

It has been demonstrated that thyroid hormones are required for malignant transformation of cultured cells by ionizing irradiation or chemical induction (Guernsey et al., 1980; Borek, 1983; Murakami et al., 1988; Murakami et al., 2000).

Oxidative Stress

Free radicals:

Over the past few decades, free radicals that are highly reactive...
and destructive molecules have come to be appreciated increasingly for their importance in human health and disease. Many common and life threatening human diseases, including cancer, atherosclerosis and ageing have free radical reactions as an underlying mechanism of injury. Over this period of time, the conceptual understanding of the interaction of such reactive oxygen species (ROS) with living organisms has undergone a remarkable evolution (Halliwell and Cross, 1991).

In the modern terminology, a free radical is defined as any atom, group of atoms or molecules having at least one unpaired electron in its outermost orbital which alters the chemical reactivity of the atom or molecule, usually making it more reactive than the non radical (Lunec, 1990).

Free radicals have cationic, anionic or neutral characteristics and are extremely reactive. Gamma radiation, UV light and environmental pollutants are among the many exogenous initiators of free radical reaction, however, the most important source of these radical species in vivo are univalent biochemical redox reactions involving oxygen (Halliwell and Gutteridge, 1984).

Oxygen is required to transform various substrate for the release of energy to oxidize endogenous compounds and to detoxify xenobiotics. During this process, oxygen acts as a terminal 4-electron acceptor and is eventually converted to more stable chemical state-water. Some biological reduction of oxygen occurs by the monovalent pathway and necessarily produces first superoxide radical ($\text{O}_2^-$) and hydrogen peroxide ($\text{H}_2\text{O}_2$) and then, if these are not efficiently scavanged, hydroxyl radical ($\text{OH}^-$) and possible singlet oxygen ($1\Delta g$).
as well. Living cells could not tolerate the high quantity of there highly reactive products. The hydroxyl radical, in particular is incredibly reactive and its production must be minimised. Since it is the third intermediate in the monovalent pathway of oxygen reduction, its production can be avoided by efficiently removing the first two namely \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \). Molecular oxygen or dioxygen is a stable triplet diradical in the ground state, with a kinetic preference for undergoing radical reaction such as initial univalent reduction in enzymatic reaction where \( \text{H}_2\text{O}_2 \) is formed. Free radical damage to cell parts (cell membrane, nuclear membrane, DNA, cell membrane fat and proteins) by:—

- Breaking off membrane proteins and thus destroying the cells identity.
- Fusing together membrane lipid and proteins hardening the cell.
- Puncturing the cell membrane so viruses, bacteria etc. can enter.
- Disrupting the nuclear membrane and thus opening up the nucleus and exposing the genetic material.
- Mutating and destroying genetic material.

Burdening the immune system and threatens its integrity by damaging immune cells. Among the most important of these are the actions of free radicals on the fatty acid side chains of lipids in the various membrane of the cells especially mitochondrial membrane (which are directly exposed to the superoxide anions produced during cellular respiration) (Witting, 1965; Kosler et al., 1986).

Lipid peroxidation (LPO) can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bond (Kale and Sitasawad, 1990).

[60]
Among the different components associated with membrane lipids, the unsaturated fatty acid essential for formation of either phospholipids or sphingolipids is the most susceptible target for oxidative stress induced peroxidation (Witting, 1965).

Lipid peroxidation proceeds by an autocatalytic chain reaction since peroxide products of the reaction also acts as initiator (Porter, 1984). Autooxidation can be triggered by diverse agents such as:

(a) redox metal ion (e.g. iron or copper) in the presence of reductants such as thiols or ascorbates
(b) ionizing or ultraviolet radiation and
(c) photoactivated dyes and pigments.

Non-radical reactions are also known to occur e.g. those involving singlet molecular oxygen as the primary oxidant (Halliwell and Gutteridge, 1984). Lipid peroxidation may also be driven enzymatically, important examples being (i) the xanthine oxidase-induced reaction (Keiiog and Fridovich, 1975, Lynch and Fridovich, 1984). (ii) the NADPH - cytochrome p450 reductase - dependent reaction in microsomes (Aust et al., 1984) and (iii) cyclooxygenase - dependent peroxidation of arachidonate to prostaglandin and thromboxane precursors (Gale and Egan, 1984).

Lipid peroxidation involves three discrete phases of chain reactions that includes initiation, propagation and termination. In the course of LPO initiation, activated oxygen species are produced as a result of consequent one-electron reduction. These species are the oxygen radicals—superoxide anion-radical and the hydroxyl-radical. These oxyradicals are formed with the participation of transient metal.
ions, which often takes place outside the hydrophobic zone of the membrane - on its surface or in aqueous phase. The superoxide anion-radicals have a relatively low reactivity, as a result of which their interaction with the membrane phospholipids is not very important for the initiation of LPO reaction (Witting, 1965; Placby et al., 1974; Halliwell and Gutteridge, 1984; Halliwell and Gutteridge, 1985). Conversely, the interaction of highly reactive OH radicals with membrane lipids (LH) results in the formation of intermediates free radical products of a lipid nature: alkyl (L'), alkoxyl (LO') and peroxyalkyl (LO_2') radicals. This stage of the LPO process already takes place in the hydrophobic zone of the membrane. The maintenance and development of the LPO process as well as the involvement in it of newer lipid substrates (from among membrane phospholipids), are guaranteed by the constant "regeneration" of L', LO' - and LO_2' - radicals and by their interaction with membrane phospholipids. An important source of such lipid radicals are the primary molecular LPO products, hydroperoxides, which are decomposed into alkoxyl or peroxyalkyl radicals in the presence of transition metals (Halliwell and Gutteridge, 1984).

With exception of the initiation stage, in the course of which the primary oxygen and lipid radicals are formed, the most difficult and limiting reaction of the LPO process is the interaction of lipids with peroxide radicals, resulting in the formation of hydroperoxides. The rate constant of this reaction sharply increases when the number of double bonds in the oxidized molecule is increased (Witting, 1965; Emanuel and Lyaskovskaya, 1967). This is the reason for the preferable oxidation of unsaturated lipids in the course of the LPO process in
biomembranes. As a result of these sequence of the reactions, a considerable amount of membrane polyenoic phospholipids can be involved in the LPO process. The formation of LPO products in the biomembranes inevitably leads to their damage as a result of the non-specific involvement of phospholipids in the LPO process.

There are two alternative ways for the oxidation of the polyenoic lipids of biomembranes by means of free radical reaction. Polyenoic fatty acyls can be subjected to free-radical oxidation both after preliminary hydrolysis of phospholipids by type A2 phospholipases as well as in esterified form, directly in the molecules of the membrane phospholipids. In the first case the reaction products catalyzed by cyclooxygenases or lipoxygenases are two groups of physiologically active compounds: (1) prostaglandins, thromboxanes and prostacyclins and (2) leukotrienes, lypoxins and lypoxenes (Vliegenthart and Veldink, 1982). The oxidation of fatty acyls in the phospholipids can also be catalyzed by lipoxygenenes (e.g., lipoxygenase from reticulocytes, microvessels) (Baba et al., 1985) with the formation of stereospecific hydroperoxides. The enzyme or nonenzyme initiation of the peroxidation of the fatty acid residues of phospholipids, taking place at the expense of the interaction with the oxygen radicals, results as a rule, in the formation not of one or several stereoscopic products, but of a wide range of different compounds.

At present, there is evidence that the LPO products are capable of forming single ionic channels in the lipid bilayer (Lebedev et al., 1982; Rostavtseva and Dev, 1986).
Oxidative stress caused by free radicals:

Oxidative stress is defined as the cellular damage caused by oxygen free radicals or reactive oxygen species (Reiter, 1997).

Oxidative damage has long been implicated in both the malignant phenotype and carcinogenesis. Enthusiasm for these correlative studies has been tempered by the perceived lack of specificity of chemical reactions involving oxidative damage (Toyokuni, 1999). Oxidative stress (OS) is a general term used to describe the steady state level of oxidative damage in a cell, tissue or organ, caused by reactive oxygen species. This damage can affect a specific molecule or the entire organism (McCord, 1985; Moslen, 1994).

Because bodies are continuously exposed to free radicals and other ROS, from both external sources and generated endogenously, ROS - mediated oxidative damage is a final common pathway for a number of disease process like —

1. Cancer and other malignancies.
2. Atherosclerosis
3. Degenerative neurological diseases.
4. Ischemia/reperfusion injury
5. Aging
6. AIDS etc.

It follows that almost any disease is likely to be accompanied by increased formation of reactive oxygen species. It is not therefore surprising that the list of diseases in which their formation has been implicated is long and is growing longer (Halliwell and Cross, 1991). For carcinogenesis, atherosclerosis (Esterbauer et al., 1988; Steinberg et
al., 1989; Halliwell and Cross, 1991), rheumatoid arthritis some forms of adult respiratory distress syndrome (ARDS), reoxygeneration injury (Bolli, 1988; McCord, 1985), and traumatic or ischemic damage to the central nervous system, there is reasonable evidence to suggest that oxidative damage by free radical reactions make a significant detrimental contribution to the pathologic process.

**Oxidative stress and cancer:**

Cancer and other malignancies all entail unconstrained cell growth and proliferation based upon changes in the cell’s genetic information. In most cases, for example, one or more genes that normally constrain cell growth and replication are mutated or otherwise inactivated. These genetic deficiencies correspond directly with deletions and sequence changes in the genetic code, resident in the cell’s DNA. A frequently seen final common cause of such DNA damage is oxidative stress (Weitzman and Gordon, 1990). Of the myriad injuries sustained by DNA on a daily basis, most are repaired by normal DNA repair mechanisms within the cell, while some result in cell death. Since such injuries are sporadic and distributed somewhat randomly across the genome, most lethal DNA injuries are clinically inconsequential resulting in the loss of a few cells among millions. However, when a single cell sustains an injury that impairs growth regulation, it can proliferate disproportionally and grow rapidly to dominate the cell population by positive natural selection. The result is a tumour, frequently a malignant one, where the constraint of growth and proliferation is particularly deficient. Therefore, free radical or
oxidative injury to the genetic material is major final common pathway for carcinogenesis (Cerutti, 1985; Weitzman and Gordon, 1990). Products of phagocyte oxygen metabolism, such as hypochlorous acid, chloramines, and oxidized lipids have all been related to DNA damage. Free radical intermediates of xenobiotic chemicals, as well as oxygen radical production by chemical carcinogen have been related to environmental carcinogenesis (Lesko et al., 1978).

These data led investigators (Cerutti, 1985; Cerutti and Trunya, 1991) to suspect that intra or extra cellular generation of free radicals and thereby oxidative stress or an extracellular pro-oxidant state may be important in producing many types of cancer.

**Oxidative injury and DNA strand breaks:**

Critical to the process of initiation in the multi-step model of carcinogenesis is damage to DNA. Birnboim (1983) showed that DNA strand breaks could be induced in murine erythroleukemia cells by phagocytes stimulated by phorbol myristate acetate (PMA), an agent which stimulates phagocytes to initiate the respiratory burst (Birnboim, 1983; Gordon and Weitzman, 1993). There breaks were inhibited by the enzyme catalase, which degrades hydrogen peroxides, but not by the enzyme superoxide dismutase (SOD), which converts the superoxide anion radical to hydrogen peroxide. Data suggests that although hydrogen peroxide is a major oxygen derived DNA - damaging species, it is not the only one. Indeed, there are evidences to suggest that a calastogenic factor made up of oxidized lipids derived from arachidonic acid may also contribute to DNA strand breaks. This
diffusible factor has a longer half life than the short lived hydroxyl radical, and it is likely that this substance acts in the extracellular space or close to the cell surface (Gordon and Weitzman, 1993).

Although it is likely that strand breaks contribute to carcinogenesis, permanent changes in DNA are rare. The repairing enzymes may repair DNA and the cells may survive, but incomplete or inaccurate repair may later contribute to carcinogenesis. An example of this comes from data reported by Cerutti and Trunya (1991), who demonstrated differences in sensitivity to oxidants in JBC clones based on relative amounts of the cellular antioxidant enzymes copper-zinc superoxide dismutase and catalase. One clone had large amounts of these enzymes and had larger numbers of DNA strand breaks, ADP-ribosylation and cytotoxicity when exposed to oxidants; this clone was non-promotable. Another clone was susceptible to tumour promotion by oxidants, but was more resistant to oxidant-induced cytotoxicity and had higher levels of anti-oxidant enzymes (Cerutti and Trunya, 1991).

Cell deficient in major DNA double strand break repair pathway (nonhomologous DNA end joining [NHEJ]) have increased spontaneous chromosome breaks; however, the source of these chromosome breaks has remained undefined. These spontaneous breaks are partially suppressed by reducing the cellular oxygen tension. Conversely, elevating the level of ROS and thereby oxidative damage by overexpressing the antioxidant enzyme superoxide dismutase (SOD), in a transgenic mouse, increases chromosome breakage. The effect of SOD can also be modulated by cellular oxygen tension. The elevated chromosome breakage correlates histologically with a significant increase
in the amount of neuronal cell death in Ku86 (-/-) embryos. Therefore, oxygen metabolism is a major source of the genomic instability observed in NHEJ-deficient cells and, presumably, in all cells (Karanjawala et al., 2002).

**Mutagenesis and chromosome changes:**

Oxidative damage induce mutations (Weitzman and Gordon, 1990) and chromosome aberrations (Cerutti and Trunya, 1991) in mammalian cells. Of some recent interest is the occurrence of base pair mutations at codon 12, 13 and 61 of the H-, K-, and N-ras oncogenes (Bos, 1988). The GC pairs are most frequently mutated, and it is interesting that point mutations in the human p53 and RB tumour suppressor genes also often occur at GC base pairs (Cerutti and Trunya, 1991). Recently, ROS have also been observed to induce amplification of both viral and cellular genes in hamster cells. In addition to experiments using whole phagocytes, investigators have shown that oxidative injury products (including chloramines and lipid peroxidation products) are also mutagenic. Further studies have demonstrated that cultured cells when exposed to human neutrophils stimulated to produce reactive oxygen species, could form both benign and malignant tumours in athymic mice (Zimmerman and Cerutti, 1984) and these cells could be transformed in vitro.

Oxidative damage can cause sequence specific DNA damage and play important role in mutagenesis and thus carcinogenesis. Free hydroxyl radical causes DNA damage with no marked site specificity. Reactive nitrogen species, sulfate radicals, nitrogen centered radicals,
benzoyloxyl radical and alkoxyl radical shows different sequence specificity. Benzoyloxyl radical specifically causes damage to the 5'-G in GG sequence (Kawanishi et al., 2001).

During the past decade, studies on mutagenesis by oxidative stress have been focused on oxidized guanine, 8-hydroxyguanine. Recently, investigators found that oxidized adenine, 2-hydroxyadenine is formed by Fenton-type reactions and that this DNA damage is as mutagenic as 8-hydroxy guanine when present either in DNA or in the nucleotide pool. These results suggest that 2-hydroxy adenine plays an important role in mutagenesis by oxidative stress and thus opening the gateway for formation of preneoplastic cells (Cheng et al., 1992; Kasai, 1997).

**Activation of xenobiotic carcinogens:**

There is evidence that environment carcinogens may undergo metabolic activation in the presence of endogenous free radical intermediates. One group of such potent carcinogens are the polycyclic aromatic hydrocarbons (PAH). These compounds are activated in vivo by cytochrome p450 oxidases to reactive intermediates which interacts with DNA. There are data to show that phagocyte activation can replace the p450 oxidase system in this process (Trush et al., 1985). For example, Dix and Marnett, (1983) has shown that PAH are activated to ultimate carcinogens during lipid peroxidation and Marnett et al. (1987) has recently reviewed data which showed that certain xenobiotics are co-oxidized to mutagens by arachidonic acid co-oxidation, lipid peroxidation, and peroxyl free radicals. Tsurata et al. (1985)
showed that phorbol ester-stimulated neutrophils oxidized the arylamine carcinogenes benzidine, methylaminobenzidine, and amino flourine to reactive intermediates which bind DNA irreversibly by a mechanism requiring oxygen and dependent on myeloperoxidase. Trush et al. (1985) showed that bleomycin A2 and benzo[a]pyrene-7,8-dihydrodiol are oxidized by Phorbol-activated phagocytes to derivatives, which are mutagenic, covalently bind DNA and cause sister chromatid exchanges.

Tumour promotion and oxidative stress:

Many cancers develop in steps — initiation, promotion and the final stage progression (Marnett, 1987; Gordon and Weitzman, 1993). Initiation is defined as an irreversible genetic event, such as a mutation of DNA. Once this has taken place, promotion can occur. In experimental systems, tumour promoters must be applied regularly, or tumours do not develop. Originally, tumour promoters were thought to act by epigenetic mechanisms, but now data has been generated which demonstrates convincingly that cancer develops with the accumulation of a number of DNA lesions such as a activation of oncogenes and inactivation of tumour suppressor genes. Oxidative injury by free radicals have been implicated in the tumour promotion process. Important among these are the stimulation of the arachidonic acid cascade by the calcium-dependent hydrolysis of membrane fatty acids by phospholipase A2, a process which appears to occur concomitantly with phospholipid methylation. Arachidonic acid is in turn oxidized by one or
two pathways, using either cyclooxygenase or lipoxygenase to form thromboxane A2 or leukotrienes respectively. The prostaglandin endoperoxides which occur as intermediates in this cascade can be compounds that serve as a source of free radicals and thus contribute to a persistent oxidative stress environment which finally results in tumour promotion (Birnboim, 1983; Cerutti, 1985).

Tumour progression results in the development of malignant growth from benign lesions. In this stage oxidative stress may play a direct role in the development of cancer characteristics such as uncontrolled growth, genomic instability, chemotherapy resistance, and invasion and metastasis. Tumour cells continually undergo high and persistent oxidative stress in human carcinoma cells than in surrounding normal cells (Toyokuni et al., 1995). This persistent oxidative stress does not appear large enough to induce cell death because tumour cells have a decreased cell sensitivity to oxidative stress (Palozza et al., 1989; Toyokuni, 1995; Klaunig et al., 1998). Cancer cell emerging from the multistep carcinogenic process with inactivated or deleted tumour-suppressor genes or activated oncogenes are much less dependent than normal cells on external growth factors because they can manufacture their own factors. High antioxidants induced by persistent oxidative stress in cancer cells increase the chemotherapy resistance of the cells. Increased protein oxidative damage on certain protease inhibitors facilitates tumour invasion (Toyokuni et al., 1995).

The interaction of inflammatory cells with tumour promoters such as phorbol esters, has been well studied (Gordon and Weitzman, 1993). There is an almost immediate increase in oxygen consumption and
production of superoxide anion and hydrogen peroxide. Evidence suggest (Gordon and Weitzman, 1993) that the phorbols bind to protein kinase C, a phospholipid - dependent kinase. These observations have led to the conclusion that the free radicals so generated are important contributor to the oxidative stress and which in turn contribute to the process of tumour promotion. These conclusions are supported by data which show that certain agents which are able to block tumour promotion in a two-stage mouse skin model are also potent inhibitors of the respiratory burst in human phagocytes. These includes inhibitors of arachidonic acid metabolism, retinoids, protease inhibitors, antioxidants and dihydroxyepiandrosterone.

**Transient change in DNA induced by free radical generated during oxidative damage:**

The role of oxygen - derived free radicals in reactions with various cellular kinase and therefore a role in cellular growth, differentiation and development through signal transduction pathway is suggested by various investigators (Jones *et al.*, 1983; Swann *et al.*, 1990). Oxidative injury can cause an increase in cytosolic free calcium through mobilization of calcium stores in mitochondria and inactivation of plasma membrane associated calcium - ATP ase.

Protein kinase C(PKC) is translocated from cytosol to membrane of mouse epidermal cells by hydrogen peroxide and phosphorylation of the PKC substrate adenosine diphosphoribose (ADPR) is enhanced in cytosolic extracts from mouse epidermal cells exposed to the free radical generating system Xanthine/Xanthine
oxidase (Jones et al., 1983).

Activated oxygen species can induce the early response genes c-fos, c-jun, c-myc and beta actin. The mechanism by which transcription is induced in these genes involves post-translational poly ADP-ribosylation of chromosomal proteins. This is a process which occurs exclusively in the nucleus, unlike the action of some kinases, which function near the cell surface. Cerutti and Trunya (1991) has proposed a model of DNA breakage and poly ADP-ribosylation which involves the action of oxygen-derived free radicals to induce DNA breaks which becomes amplified by activation of endonucleases. This change induces c-fos and other early genes. However, phosphorylation and poly ADP-ribosylation of transcription factors are also involved, and may serve to turn down the process by inactivation of ADPR-transferase and subsequent repair of DNA strand breaks. Oxidant induced strand breaks can be inhibited by the iron chelator desferrioxamine, suggesting that this process is in part dependent on the Fenton reaction, wherein ferrous (Fe²⁺) iron catalyzes the reduction of hydrogen peroxide to produce hydroxyl radicals that initiate or propagate oxidation chain reaction (Cantoni et al.; 1989).

**Effects on nuclear proteins:**

Gordon and Weitzman (1993) showed that following exposure to oxidative stress, certain Nuclear proteins are poly ADP ribosylated in human keratinocytes. This suggests that poly ADP-ribosylation of nuclear proteins may augment the DNA breaks induced by oxidative stress. Free radicals may also directly influence the binding of
transcription factors by altering the redox state of binding proteins. Other examples of this auto-regulatory property of free radicals via alteration of redox state include the oxy-R encoded protein in bacteria, heat shock factor Drosophila, iron-responsive element protein on mammalian cells (Cerutti and Trunya, 1991).

Antioxidant system:

'Antioxidant' are the most effective scavengers of potentially hazardous free radicals produced during normal cell metabolisms as well as during disease condition.

Three groups of antioxidants make up the antioxidant system viz.

1. Primary antioxidant includes superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT), Metal binding proteins e.g. Ferritin and Ceruloplasmin.
2. Secondary antioxidant includes - Vit E, Vit C, β-Carotene, Uric acid and Albumin.
3. Tertiary antioxidants includes - DNA repair enzymes, Methionine sulphoxide reductase.

Antioxidants again can be divided into five classes: (i) enzymes (ii) peptides, (iii) phenolic compounds, (iv) nitrogen compounds, and (v) carotenoids.

Other agents may have antioxidant effects through replenishing mechanisms - Vit. C, for instance, helps to recycle Vit E, and NAC (N-acetyl cysteine) provides an important component of glutathione (Diplock, 1987; Halliwell and Cross, 1991).
Catalase:

Different authors at different point of times have reported isolation and purification experiments on catalase. Waentig and Gierisch (1914) suggested that catalase is a protein. Warburg, as early as 1923, has postulated that catalases are iron enzymes because their activity was inhibited by cyanide. Catalase was first isolated and obtained in crystalline form from beef liver by Sumner and Dounce in 1937 and later from blood and other sources (Aebi, 1983). Catalase can be separated from haemoglobin either by classical precipitation with ethanol formation or better by chromatography on DEAE - Cellulose or by gel filtration (Aebi, 1983).

Catalase in animals, plants and micro-organisms:

Catalytic activity is present in nearly all animal cells and organs. Catalases have been crystallised from three animal sources: liver, erythrocyte and kidney. The catalase activity of tissues varies greatly. It is higher in liver and kidney and low in connective tissue. In tissue it is mainly particle bound such as mitochondria and peroxisomes, whereas it exists in a soluble state in erythrocytes. Human erythrocytes are normally rich in catalase. The catalase activity in blood is practically all due to the erythrocytes. (Aebi, 1983).

Catalytic activity is found in all kinds of plant material. The amounts of catalase always seem to be low, compared to those in liver or erythrocytes. The method for crystallising animal catalases are not applicable to plant catalases.

Catalase is present in all aerobic micro-organism but absent in
the obligate anaerobic ones. In the facultative anaerobic micro-organisms, conditions vary from one species to another. Exceptions are among others, the aerobic Acetobacter peroxydans, where catalase is lacking and anaerobic Actinomyces necrophorus that seem to contain catalase.

**Immunology of catalases:**

The work of Tria (1939) has shown that catalases are good antigens, rabbits developed anticalatalases on injection of beef-, lamb-, horse-, and dog liver catalases. The anticalatalases were not entirely specific for the same antigen; precipitation sometimes occurred with other catalases than the one used as antigen, though always to a lesser extent.

**Physiological role:**

The physiological function of catalase is still a matter of discussion. Probably the catalase located in the cell organelles plays a role of a specific peroxidase. The enzyme pattern of the peroxisomes is noteworthy for the simultaneous presence of H$_2$O$_2$ - producing (e.g. D-amino oxidase, uricase) and consuming enzymes like catalases. In erythrocytes, catalase - like glutathione peroxidase exerts a protective function for haemoglobin and other SH-protein (enzymes, stroma), the importance of which can vary with the species and the experimental conditions. The lower the catalase activity of the erythrocytes the more effective is the action of oxidizing agents (H$_2$O$_2$, ascorbic acid, methyl hydrazine) or X-rays on methaemoglobin formation (Aebi, 1983).
The high catalase content of erythrocytes has been taken as an argument in favour of the protection theory: haemoglobin is known to be easily destroyed by hydrogen peroxide. The protective action of catalase on haemoglobin from peroxide destruction was in doubt and stress was given on the importance of its peroxidatic function (Theorell, 1951).

**Catalase and diseases:**

Greenstein (1947) observed that implantation of a tumour in the rat liver is followed by a rapid and great decrease of the catalase content, which can be promptly reversed by the removal of tumour. The activity of catalase, the peroxisomal enzyme is reported to be altered in various hepatic disorders as hepatitis, cirrhosis and cholestatis (Steraleib and Quintana, 1977; Créamer et al., 1991). Low level of catalase activity is reported in cataract also (Fecondo and Augusteyn, 1983).

Alteration of catalase activity with different cancers is reported by several group of workers (Creamer, 1991; Guner et al., 1996).

**Thyroid hormone and oxidative stress.**

Mitochondria constitute a major source of reactive oxygen species and have been proposed to integrate the cellular responses to stress (Tiwari et al., 2002). One of the major effect of thyroid hormone is to increase mitochondrial respiration (Roodyn et al. 1965, Nishiki et al., 1978) by many complex changes in the number and activity of mitochondrial respiratory chain component. An increase in cellular
respiration mediated by the hormone thyroxine, has been associated with an increase in mitochondrial lipid peroxidation. Chen and Hoch (1977) reported that after 3 days of thyroid hormone treatment, there was mitochondrial membrane lipids. Birds with higher thyroid hormone levels may be more prone to mitochondrial generation of ROS. When thyroid hormone level increase, thyroid receptors in the cell nucleus increase DNA transcription which increases the synthesis of specific mitochondrial proteins which in turn up-regulates mitochondrial energy production (Kadenbach et al., 1995; Nelson et al., 1995) and thus ultimately the consequence is generation of more ROS. It is observed that (Venditti et al., 1997) in tissues of hypothyroid rats the lipid peroxidation was not modified, whereas in hyperthyroid rats lipid peroxidation increased in liver and heart but not in skeletal muscle. Venditti et al. (2004) suggests that liver oxidative damage observed in experimental and functional hyperthyroidism is mediated by thyroid hormone. Thyroid hormone - induced calorigenesis contributes to liver oxidative stress and promotes an increased respiratory burst activity in Kupffer cells. Fernandez et al. (2002) recorded that 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.

3-Methylcholanthrene

Cancer is a multiple step process. One of the characteristics of
chemical or physical carcinogenesis is the usually extended period of
time (latent period) between contact with the carcinogen and the
appearance of a tumour. The latent periods of occupational cancers
may extend from one to several years.

Chemical carcinogens are of synthetic or natural origin, are
extremely diverse in structure without any common feature and are
classified in two categories (i) direct - acting (DNA- reactive, activation
independent, genotoxic) carcinogens that bind covalently to cellular
genomic DNA and are mutagens (ii) Procarcinogens (activation
dependent) that require metabolic conversion to metabolites ("ultimate
carcinogenes") capable of transforming cells and inducing tumours.
Procarcinogens are among the most potent chemical carcinogen.

A variety of carcinogenic chemicals of natural or anthropogenic
origin are known to exist and at least in human beings, based on
industrial exposures, epidemiology and migrant population studies,
chemical agents appear to be of major importance in the induction of
neoplasms (Miller and Miller, 1981). These carcinogens can be referred
to as environmental and divided into two broad classes:
(i) consumables, to which exposure is deliberate and (2) contaminants,
to which exposure is inadvertent.

A direct causative relationship between the agent employed and
the neoplasm produced is not implied. Simply it can be stated that
following the injection or exposure to a procedure, tumours arise in
significantly higher incidence than in untreated animals.

Literally, thousands of compounds have been shown to increase
the occurrence of neoplasms in experimental animals, usually mice or
rats. The compounds usually are given in maximum tolerated dose over the life span of the animals (Doll and Peto 1981). Carcinogenic hydrocarbons act at the point of contact (Berwald and Sachs 1965). They evoke cutaneous carcinomas when painted on the skin and induce sarcomas at the site when injected subcutaneously. Carcinoma of the kidney and stomach, brain tumour and rhabdomyosarcoma have been elicited on injection into appropriate tissues. The feeding of carcinogenic hydrocarbons produce intestinal adenocarcinomas in mice and intravenous injection increase the number of pulmonary tumours.

In human, cancer arises after prolonged and intense exposure to chemical carcinogens. Industrial workers with coal tar, pitch, soot, asphalt, petroleum etc. shows high frequency of skin cancers and compounds of polycyclic hydrocarbon type probably are the active carcinogens in skin cancers.

The scientific study of chemical carcinogenesis started from Percival Pott's description of cancer of skin in chimney sweeps in chirurgical observations in 1775.

Yamagiwa and Ichikawa (1918) produced the first experimental tumours by painting of tar on a rabbit's ear. The hydrocarbon benzpyrene can be isolated from tar and possessed a high degree of carcinogenic activity (Kennaway, 1955). Benzpyrene gave a spectrum with fluorescent light which is very similar to that of synthesized 1:2:5:6 dibenzanthracene. Many other members of this group of polycyclic aromatic hydrocarbons with a benzene or aromatic six membered ring structure proved to be carcinogenic. The carcinogenic hydrocarbons possess their condensed carbon rings to be active and most of them have four. Barry et al. (1935) reported that 1:2 benzananthracene has
practically no carcinogenic activity but the attachment of a new ben-
zene ring in 5:6 position gives it a great carcinogenic power.

Fig. II.1: Carcinogenic hydrocarbons.

Many naturally occurring substances including bile acids and the
sex-hormones have similar condensed polycyclic systems. The
dehydonorcholene, a hydrocarbon prepared by Wieland and
Schlichting from the desoxycholic acid of the bile is a hydro derivative
of 1:2-benzanthracens.

By selenium dehydrogenation of dehydonorcholene Cook and
Haslewood (1933) synthesized the fully aromatic hydrocarbon 3-me-
thylcholanthrene or 20-methylcholanthrene according to the sterol-
bile acid numbering system.
Cook and Haslewood (1933) reported that carcinogenic potency of this compound is of extremely high order. Fieser and Seligman (1935) derived the hydrocarbon from cholic acid which involves an intramolecular perkin condensation between a 12-keto group and the position of the acid side chain. The steps by which the bile acids were converted to methylcholanthrene involved successively oxidation, hydrogenation, cyclization and dehydrogenation.

Methylcholanthrene also has been produced by an unusual pyrolytic degradation of a derivative of cholesterol (Fig. II.3).

A closer resemblance of methylcholanthrene to products of biological origin was drawn from considerations of the adrenocortical steroids (Fieser, 1938). Thus dehydrocorticosterone when condense with an aldehyde, or estrone with pyruvate, followed by aromatization, ring closure, and further dehydrogenation yield the corresponding cholanthrene or its derivatives (Fieser, 1938).

All of them possess the condensed carbon ring skeleton known as the phenanthrene nucleus. The nucleus is also present in the benzpyrene, benzantrhacene and cholanthrene groups of carcinogenic hydrocarbons.

Fieser and Seligman (1935) prepared methylcholanthrene
synthetically which utilize the Elbe reaction. This involves the condensation of p-chlorotoluene (iii) with chloropropionyl chloride. Substitution occurs at both available positions but no separation of isomers (iv). After cyclization it gives a mixture of hydrindenes (v). Clemmensen reduction of both isomers affords a single product.

Fig. II. 3: Precursors of methylcholanthrene.
Fig. II.4: Synthesis of methylcholanthrene.

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which is condensed with -naphthylmagnesium bromide to give the ketone (viii). The ketone undergoes unusually smooth pyrolysis with condensation of the 9-O-methylene group of the five membered ring into the second aromatic nucleus. Pure methylcholanthrene is obtained in overall yield from p-chlorotoluene.

The structural relationship of the chemical carcinogen with the normal constituents of the body suggests that carcinogenic agents are produced within the body as a result of abnormal steroid metabolism.

Cook et al. (1940) however, reported that injection of desoxycholic acid itself may result in the production of connective tissue tumours in mice.

**Carcinogenic activity of 3-methylcholanthrene:**

Methylcholanthrene gave rise to tumours in every one of eight mice in an average time of 9.7 weeks, and acted as rapidly at a dosage of 0.25 mg. as at a 2mg. level (Fieser, 1938).

Lorenz and Stewart (1940) reported a variety of tumour formation with the administration of methylcholanthrene. Severi et al. (1961) also observed tumours in different sites including skin, breast, lungs and lymphoid tissue following the treatment of mice with graded doses of methylcholanthrene. They reported that tumours of the skin appear first then the tumour of the breast; tumours of the lungs and lymphoid tissue were the last to appear.

Engelbreth and Holm (1941) reported that methylcholanthrene accelerate the development of mammary carcinoma of mice and a variety of tumour formation in albino mice following the treatment of 20-methylcholanthrene. These includes the formation of multiple hepatomas, pulmonary adenoma, papilloma, squamous cell carcinoma.
hepatomas, pulmonary adenoma, papilloma, squamous cell carcinoma and skin carcinoma.

Gruenstein et al. (1966) observed the mammary, sebaceous and cutaneous neoplasm and leukemia in male rats following gastric installations of 3-methylcholanthrene. Biliary carcinoma is also reported by Bain et al. (1959) following the treatment with 3-methylcholanthrene.

According to Fieser et al. (1938) the methyl group of 3-methylcholanthrene does not contribute significantly to its carcinogenic activity. But Law and Lewisohn (1941) reported that the elaboration of the cholangrene molecule by substitution and addition beyond the introduction of single methyl group at position 3, decreases the carcinogenic activity. They compared the carcinogenicity of some cholangrene derivatives. It was observed that addition of a methyl group at position 3 and 4 does not significantly alter the incidence of induced sarcoma. However, substitution of methyl group at the 5-position (5-methylcholanthrene) in the cholangrene molecule resulted in a decided decrease in the incidence of sarcomas. These results indicate that a change in the molecule of a carcinogen may affect the neoplastic response of the tissue.

Considerations of the essential nature of the carcinogenic hydrocarbons had indicated that activity was associated with the structure of the molecule. Robinson (1946) pointed out that carcinogenic hydrocarbons usually contained a phenanthrene double bond between 9-10. Pullman (1946) by quantum mechanical study of the electronic structure of the hydrocarbons showed that the carcinogenic activity was dependent on a high electron density of a part of the molecule. Schmidt (1939) had termed this region as the K-region. Pullman (1954) reported that carcinogenic activity in aromatic hydrocarbon is determined by
the existence of an active K-region. However the molecule contains also an L-region which is rather inactive (Fig. II.5). But most of the in vitro reactions generally occur at this region. The K-region takes part only in some more specific reactions. Pullman and Pullman (1955) therefore suggested that the L-region is necessary to entail certain stability. This enable the molecule to resist any fundamental changes till the K-region is able to manifest its activity.

McCabe et al. (1983) reported that neoantigens induced by 3-methylcholanthrene treatment of syrian hamster embryo cells has a relationship to antigens expressed on foetal and 3-methylcholanthrene transformed neoplasic cells. Huggins and Pollice (1958) reported that repeated administration of 3-methylcholanthrene to adolescent rats resulted in (a) a profound, incomplete, and selective depression of certain hypophyseal functions; (b) decreased growth of transplanted mammary tumours; and (c) a retardation of body growth.

The administration of 3-methylcholanthrene resulted in decreased gonadotrophin production by the pituitary and the ovaries were more
drastically affected by the depression of pituitary activity. There was a considerable reduction of the content of alkaline phosphatase in the breast of intact rats treated with 3-methylcholanthrene but atrophy of the mammary epithelium did not occur and hyperplasia of the mammary tree was often observed (Huggins and Pollice, 1958).

The administration of 3-methylcholanthrene considerably slowed the growth of transplanted mammary tumours characterized by high dependence on hormones and the concurrent administration of gonadotrophin restored the growth rate of the tumours.

The administration of 3-methylcholanthrene was only moderately effective in controlling the growth of transplanted mammary tumours characterized by low hormone dependence; the combined administration of these compounds was highly efficacious in retarding the growth of these refractory tumours (Huggins and Pollice, 1958).

3-methylcholanthrene partially retarded the growth of mammary fibroadenomas in hypophysectomized rats.

**Metabolism of 3-methylcholanthrene:**

Chalmers and Peacock, (1936) using the fluorescence method showed that the compound could be detected in the bile of mice, 1-2 hours following intravenous injection. But no original hydrocarbon was detected after 3 hours. Chalmers, (1938) also observed no specific bands of methylcholanthrene in the fluorescent spectrum of urine and faeces after 24 hours of intravenous injection. After painting the skin with methylcholanthrene, Simpson and Cramer (1943) had found no specific spectrum of the hydrocarbon in any layers of the skin. Lorenz and Shimkin (1942) however, observed the hydrocarbon in the lungs after 4 days of intravenous injection.
Dauben and Mabee (1951) by administration of 20-methylcholanthrene (20 MC) labelled in the 11 position, observed the distribution of radioactivity in the mice. The radioactivity was found to be eliminated mainly in the faeces and to a lesser extent in the urine. They observed an appreciable quantity of radioactivity in the tumours induced by methylcholanthrene.

Harper, (1959) detected two metabolites in the faeces of mice following the treatment of the hydrocarbon. A phenol that was either 8- or 10- hydroxy-3-methylcholanthrene and carboxylic acid that has tentatively identified as cholantherene-3-carboxylic acid. Phenolic sulphuric acid and glucuronic acid conjugates were also observed.

Sims (1966) by chromatographic investigation detected the products of metabolism of 3-methylcholanthrene by rat liver homogenates. Mouse liver homogenate converted 3-methylcholanthrene into 1- and 2- hydroxy - 3-methylcholanthrene cis-and trans-1, 2-dihydroxy-3-methylcholanthrene; 11, 12-dihydro-11, 12-dihydroxy-3-methylcholanthrene; 3-methylcholanthrene-1 and -2 one and 1, 2 quinone and the unidentified hydroxy-3-methylcholanthrene. A glutathione conjugate that is S-(11, 12-dihydro-12-hydroxy-3methyl-11-cholanthrenyl) glutathione was also detected. Most of the metabolic activity of 3-methylcholanthrene is centered around the 1- and 2- positions and at the 11, 12-bond (Sims, 1966). The metabolic pathway are shown in the Fig. II.6. Both 1-hydroxy 3-methylcholanthrene and the related ketone have carcinogenic properties when injected subcutaneously into mice (Badgar et al., 1940). Tumours have been obtained in mice injected subcutaneously with the epoxide (Boyland and Sims, 1965). No literature is available on the endocrinial influence on the metabolism of these carcinogen.
Fig II.6: Metabolic product of 3-methylcholanthrene.


Bimboim, H. C., 1983: Importance of DNA strand break damage in

* Original not consulted


Burgos-Trinidad, M., and Koenig, R. J., 1999: Dominant negative activity by thyroid hormone receptor variant alpha-2 and interaction with nuclear corepressor. Mol. Cell.

* Original not consulted

[92]
Endocrinol., 149: 107-114.


* Original not consulted


Foster, Daniel and Jean, W., 1992: William’s Textbook of

* Original not consulted
endocrinology. 8th edition, Philadelphia : W. B. Saunders Co.


* Original not consulted

[96]


*Homolsky, M. W., Freedberg, A. S., Kurland, G. S., and Wolsky, L.,

* Original not consulted

[97]
Horrum, M. A., Tobin, R. B., and Eckland, R. E., 1985: Thyroxine
induced changes in rat liver mitochondria cytochromes.
Molecular and cellular endocrinology., 41:163-169.

Huggins, C., and Pollice, L., 1958. Effect of 3-methylcholanthrene on
the endocrine system and metabolism mammary
tumours hitherto refractory. The journal of


Ingbar, S. H., and Freinkel, N., 1960: Regulation of the peripheral
metabolism of the thyroid hormones. Recent. Prog.


dependent microsomal calcium sequestration during
oxidative stress and its prevention by glutathione.J. Biol.
chem., 261:14628.

41:676.

Kadenbach, B., Barth, J., Akgun, R., Freud, R., Linder, D., and Possekel,
S., 1995: Regulation of mitochondrial energy
generation in health and disease. Biochimica et

Kale, R. K., and Sitasawad, S. L., 1990: Lipid peroxidation and


* Original not consulted


* Original not consulted


McCabe, R. P; Evans, C. H., and Dapaolo, J. A., 1983: Relationship of neoantigens induced by 3-methylcholanthrene treatment of Syrian hamster embryo cells to antigens

* Original not consulted

[101]


* Original not consulted

[102]


Placeby, W. Z., Danyi, J. K., and Kates, M., 1974: Lipid interactions in

* Original not consulted


* Original not consulted

[104]


Shomon, M., 2003: Thyroid disease and breast cancer. The cancer

* Original not consulted
Swann, J., Jones, T.W., and Maki, A., 1990: Sub-lethal oxidative injury cause deregulation of cytosolic free calcium without depression of intracellular soluble thiols or elevation of

* Original not consulted

[106]
Trush, M. A., Seed, J. L., and Kensler, T. W., 1985 : Oxidant-dependent metabolic activation of polycyclic aromatic hydrocarbons by phorbol ester-stimulated human PMNs : Possible link between inflammation and cancer. PNAS

* Original not consulted

[107]


* Original not consulted


* Original not consulted