Chapter 2

REVIEW OF LITERATURE

2.1 LIPID PEROXIDATION - AN OVERVIEW

Lipid peroxidation is known since long and has been suggested to be responsible for numerous effects observed in biological systems, especially because after initiation, it concurrently proceeds by a free-radical reaction mechanism. Lipid peroxidation, the degradative oxidation of polyunsaturated fatty acids of intracellular lipids, often chemically induced, leads to the destruction of membrane architecture, the degradation of membrane-bound enzymes and eventually to cell death, and has been associated with ageing, atherosclerosis, liver toxicity, lung tissue injury and many other pathophysiological disturbances.

Rat liver microsomal preparations, as well as whole homogenates and sub-cellular fractions of many other tissues, when incubated in the presence of oxygen, NADPH (or ascorbate), ADP and iron result in lipid peroxidation. NADPH cytochrome P-450 reductase or ascorbic acid catalyze the reduction of iron. The iron in the ferrous state gets conjugated with $O_2$ and the resulting iron-oxygen complex can abstract a hydrogen atom from unsaturated fatty acid (LH) in phospholipids, yielding a lipid radical (L') as an initiator of lipid peroxidation. Molecular oxygen, that
has been often called both benign and malign, on the one hand provides enormous advantages and on the other, it imposes a universal toxicity. This toxicity is largely due to the intermediates of oxygen reduction, i.e. $O_2^-$, $H_2O_2$ and $HO^-$ and any organism that avails itself of the benefits of oxygen does so at the cost of maintaining an elaborate system of defence against these toxic intermediates. After initiation, the process of lipid peroxidation, being a chain reaction, proceeds autocatalytically, until interrupted and numerous products are formed. Among these are hydroperoxides of lipids (LOOH), which breakdown spontaneously on heating or with metal catalysts propagating further chain reactions because lipid alkoxy (LO') or lipid peroxy (LOO') radicals are formed which can undergo cleavage of C-C bonds and give rise to numerous products like alkanes and alkenes. Other products include epoxy fatty or endoperoxide radicals which after rearrangement give rise to malondialdehyde, unsaturated lipid aldehydes and alkenyl or alkyl radicals which can again initiate radical chain reactions. Some other products that have been identified as a result of lipid peroxidation in autocatalytic reactions with fatty acids and phospholipids are polyhydroperoxides, dimers and polymers of fatty acids.

The biological systems have to defend themselves from the deleterious effects of various types of oxygen free radicals. Primary defence systems preventing lipid
peroxidation interact with the initiation step. Normally the biological systems do not readily form oxygen radicals e.g., mitochondrial respiration is strictly coupled under physiological conditions and free iron ions are kept sequestered by ferritin or transferrin so as not to allow their reaction with molecular \( \text{O}_2 \) to initiate lipid peroxidation. If \( \text{O}_2 \) radicals have already formed, a number of defence systems are there to act e.g., superoxide dismutase traps \( \text{O}_2^- \) ions, glutathione peroxidase and catalase remove \( \text{H}_2\text{O}_2 \) which could react with \( \text{Fe}^{2+} \) yielding \( \text{HO}^+ \) radicals and \( \beta \)-carotene or vitamin A trap singlet oxygen (\(^1\text{O}_2\)). These defence systems against lipid peroxidation, in addition, defend the organism against various types of biochemical lesions, such as damage to proteins, nucleic acids and carbohydrates.

Secondary defence systems which are highly specific against lipid peroxidation include vitamin E, which is an efficient chain breaking compound in biological systems because it is specifically incorporated into membranes, which are critical targets for lipid peroxidation. Ascorbic acid and glutathione have been suggested to reduce oxidized form of vitamin E. Another secondary defence system is Se-glutathione peroxidase and nonSe-glutathione peroxidase, which can possibly reduce the hydroperoxy- and epoxy-fatty acids to hydroxy derivatives.

Although the biological systems including animals
and humans are well protected against oxidative stress so that excessive lipid peroxidation is probably rather rare, yet under certain physiological conditions or with a number of xenobiotics, the protective mechanisms can be overwhelmed and deleterious effects of reduced oxygen radicals and products of lipid peroxidation can ensue. As a result of lipid peroxidation, biomembranes lose their ultrastructural integrity, leading to changes in fluidity and permeability. Lysosomal enzymes can be released which can digest cells. A number of enzymes of microsomes and mitochondria are inactivated as a result of lipid peroxidation. It also leads to the depletion of protective chemicals involving antioxidants like glutathione. The reaction products of lipid peroxidation e.g. malondialdehyde and hydroxyalkenals react with DNA in vitro. Similar reactions, if occurring in vivo, could lead to mutagenic, carcinogenic, cytostatic or lethal effects. Cytotoxicity of substances such as paraquat, adriamycin, mitomycin C, 6-hydroxydopamine, alloxan, nitrofurantoin, metronidazole, bleomycin etc. has been attributed to reactive oxygen species generated during redox cycling of these drugs and an enhanced lipid peroxidation has invariably been observed. A large number of compounds including ethanol, carbon tetrachloride, bromobenzene, acrylonitrile, methylethylbenzo-p-dioxin, cocaine, barbiturates, polychlorinated biphenyls, organophosphates and tetrachlorodibenzo-p-dioxin have been shown to stimulate
lipid peroxidation. Toxicity of certain inorganic compounds \( O_3, NO_x, SO_2, \) and of metal ions has also been related to lipid peroxidation. Besides, numerous genetic and acquired diseases have been related to lipid peroxidation e.g. hemolytic anemia, \( \beta \)-thalassemia, sickle cell anemia, ischemia, uremia, inflammation, muscular dystrophy, liver cirrhosis, myocardial infarction, cataract, atherosclerosis and carcinogenesis.

2.2 MECHANISM(S) OF GENERATION OF REACTIVE OXYGEN SPECIES

The reactive oxygen species involved in the initiation and propagation of lipid peroxidation in biological systems are believed to be one or more of partial reduction products of oxygen. Sequence of reactions leading to and dealing with various oxygen partial reduction products is given in Fig.1. Figure 2 represents the redox reactions of biological relevance involving oxygen radicals and Fig.3 shows the electronic distribution in the bonding in diatomic oxygen molecule.

2.2.1 Superoxide Anion Radical \( (O_2^-) \) and Hydroperoxyl Radical \( (HO_2^-) \)

Studies with enzymes and subcellular systems which are able to generate superoxide radicals have supported the view that these radicals play a major role in the process of lipid peroxidation. Gebicki and Bielski. (1981) and Bielski et al. (1983) have suggested that it is only
FIGURE 1. THE PRODUCTS DERIVED FROM THE SUCCESSIVE ONE-ELECTRON REDUCTION OF DIOXYGEN (GREEN et al., 1984)
<table>
<thead>
<tr>
<th>Reaction type</th>
<th>le oxidation (e.g. xanthine oxidase)</th>
<th>le dismutation (e.g. superoxide dismutase)</th>
<th>2e H₂O₂ forming oxidation (e.g. uricase, amino acid oxidase)</th>
<th>2e H₂O forming oxidation (e.g. catalase)</th>
<th>2e dismutation (e.g. catalase)</th>
<th>3e oxidation dismutation (metal catalyzed Haber-Weiss reaction)</th>
<th>4e oxidation (e.g. cytochrome oxidase)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Granulocyte membrane</td>
<td>Cytoplasm, peroxisome</td>
<td>Cytoplasm, granules of granulocytes</td>
<td>Granulocytes</td>
<td>Mitochondrion</td>
<td>FIGURE 2 REDOX REACTIONS OF BIOLOGICAL RELEVANCE INVOLVING OXYGEN RADICALS (Brunori and Rotilio, 1984).</td>
<td></td>
</tr>
</tbody>
</table>
the protonated form of superoxide anion i.e. hydroperoxyl radical (HO$_2^+$), which is present at lower pH (pK = 4.8) and is sufficiently reactive in lipid phases such as membranes, that can become a likely candidate as an initiator of lipid peroxidation. Superoxide anion radicals are produced in many biological sources.

2.2.1.1 Autoxidations : According to Taube (1965), the univalent pathway is the most facile route for the reduction of dioxygen because of the spin restriction. Autoxidation of reduced ferredoxin (Misra & Fridovich, 1971), hemoglobin and catecholamine (Misra & Fridovich, 1972), polyhydric phenols (Marklund & Marklund, 1974), tetrahydropterine (Nishikimi, 1975) and myoglobin (Gotoh & Shikama, 1976) have been shown to produce O$_2^-$. Such autoxidations are often chain reactions, in which O$_2^-$ can serve both as initiator and as chain propagator.

2.2.1.2 Enzymic oxidations : A number of enzymes including xanthine oxidase, aldehyde oxidase, dihydro-orotic dehydrogenase and a variety of flavin dehydrogenases produce O$_2^-$ during their catalytic cycles. The respiratory burst exhibited by granulocytes, when suitably activated, is due to a membrane-bound NADPH oxidase that reduces O$_2$ exclusively to O$_2^-$ (Babior & Peters, 1981).

2.2.1.3 Sub-cellular organelles : Organelles such as chloroplasts (Asada & Kiso, 1973), microsomes (Debey &
Balny, 1973), mitochondria and nuclei (Patton et al, 1980) have been shown to generate $O_2^-$.  

2.2.1.4 Intact cells: $O_2^-$ made within cells cannot be ordinarily detected because of its being scavenged by intracellular superoxide dismutases but human neutrophils have been shown to be producing large amount of superoxide. Chance et al. (1979) have estimated the rate of production of $O_2^-$ to be 24 nmoles min$^{-1}$ g$^{-1}$ in liver cells.

2.2.2 Singlet Oxygen ($^1O_2$)

Singlet oxygen is formed if one of the unpaired electrons of $O_2$ is moved in a way that alleviates the spin restriction. Excitation of $O_2$ to the singlet state can be achieved when several biological pigments, such as chlorophyll, retinal, flavin or porphyrin, are illuminated in the presence of $O_2$ (Foote, 1982). Formation of $^1O_2$ occurs in vivo when chloroplasts (Halliwell, 1981a) and lens and retina (Zigler & Goosey, 1981; Kirschfeld, 1982 and Katz et al. 1982) are illuminated.

Other reactions which generate singlet oxygen include spontaneous dismutation of superoxide anions in contrast to enzyme catalyzed dismutation which generates triplet oxygen ($^3O_2$) (Fridovich, 1976, 1983; Khan, 1970)

\[
\text{Spontaneous } 2O_2^- + 2H^+ \rightarrow H_2O_2 + ^1O_2 \quad (1)
\]

\[
\text{SOD Catalyzed } 2O_2^- + 2H^+ \rightarrow H_2O_2 + ^3O_2 \quad (2)
\]
Singlet oxygen can also be formed according to the reaction (Arneson, 1970)—

\[ \text{O}_2^- + \text{HO}^* + \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O} \]  \hspace{1cm} (3)

According to Sies and Cadenas (1983), the evolution of \( \text{O}_2 \) may be associated with decomposition of lipid peroxides—

\[ \text{LOO}^- + \text{LOO}^- + \text{H}^+ \rightarrow \text{LOH} + \text{LO}^- + \text{O}_2 \]  \hspace{1cm} (4)

Pryor and Castle (1984) have shown that attack by \( \text{O}_2 \) on unsaturated lipids gives rise to lipid hydroperoxides, by a nonradical and nonchain process. Singlet oxygen is next only to \( \text{HO}^- \) in reactivity.

2.2.3 Hydroxyl Radical (\( \text{HO}^- \))

2.2.3.1 Haber-Weiss reaction (1934)

\[ \text{O}_2^- + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{O}_2 + \text{HO}^- + \text{H}_2\text{O} \]  \hspace{1cm} (5)

The \( \text{HO}^- \) radical formed is highly reactive and has been shown to peroxidize lipids. Gutteridge (1982) and Halliwell (1982) found that formation of \( \text{HO}^- \) required traces of transition metal ions to be present in the system and Haber-Weiss reaction has been modified—

\[ \text{O}_2^- + \text{H}_2\text{O}_2 + \text{H}^+ \xrightarrow{\text{catalyst}} \text{O}_2 + \text{HO}^- + \text{H}_2\text{O} \]  \hspace{1cm} (6)

Kellogg and Fridovich (1975) and Lynch and Fridovich (1979) have proposed that singlet oxygen is generated in reaction (6).
In the presence of iron ions the reaction mechanism is presumably the following:

\[ \text{O}_2^- + \text{Fe}^{3+} \rightarrow \text{O}_2 + \text{Fe}^{2+} \]  \hspace{1cm} (7a)

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} + \text{H}^+ \rightarrow \text{HO}^* + \text{Fe}^{3+} + \text{H}_2\text{O} \]  \hspace{1cm} (7b)

The latter is the classical Fenton reaction (1894). Copper ions, are also able to catalyze such a reaction sequence (Gutteridge & Wilkins, 1982 and Rowley and Halliwell, 1983).

The yield of HO' radicals in corresponding systems is relatively low, but in the presence of some metal chelating agents, e.g. EDTA, ADP, ATP, CDP and CTP, the amount of hydroxyl radicals increases considerably and peroxidation of unsaturated fatty acids is observed (Butler & Halliwell, 1982; Cohen and Sinet, 1982; Buettner et al. 1983 and Floyd and Lewis, 1983). The effect of chelators is presumably due to a much faster reduction of the Fe\(^{3+}\) chelate compared to free Fe\(^{3+}\). But some of the chelators have been found to inhibit this reaction in higher concentrations. These in vitro experiments on hydroxyl radical formation do not prove that the above mechanism is really involved in lipid peroxidation in living organisms.

2.2.3.2 Redox-cycling

Youngman and Elstner (1981); Nohl et al. (1982) and Richmond & Halliwell (1982) proposed that HO' can
also be formed if the semiquinone form of ubinqueinone or other semiquinone radicals react with \( \text{H}_2\text{O}_2^- \):

\[
\text{Semiquinone}^- + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{quinone} + \text{HO}^+\text{H}_2\text{O}
\]  
\((8)\)

### 2.3 FREE RADICAL CHAIN REACTIONS AND PRODUCT FORMATION DURING LIPID PEROXIDATION

#### 2.3.1 Free Radical Chain Reactions

It is generally accepted that the lipid peroxidation reaction process starts when some form of reactive oxygen species brings about hydrogen abstraction from an unsaturated fatty acid \((\text{LH} \rightarrow \text{L}^\cdot)\) and the lipid radical \((\text{L}^\cdot)\) thereby formed reacts with \(\text{O}_2\) to produce lipid peroxy radical \((\text{L}^\cdot + \text{O}_2 \rightarrow \text{LOO}^\cdot)\). Beginning with this, radical chain reactions can occur:

\[
\text{LOO}^\cdot + \text{L}^\cdot\text{H} \rightarrow \text{LOOH} + \text{L}^\cdot
\]  
\((9)\)

The reaction products are monohydroperoxides of lipids \((\text{LOOH})\) which breakdown spontaneously on heating or with metal catalysts initiating further radical chain reactions:

\[
\begin{align*}
\text{LOOH} + \text{Fe}^{2+} & \rightarrow \text{LO}^- + \text{Fe}^{3+} + \text{HO}^- \\
\text{LOOH} + \text{Fe}^{3+} & \rightarrow \text{LOO}^\cdot + \text{Fe}^{2+} + \text{H}^+
\end{align*}
\]  
\((10)\), \((11)\)

Lipid alkoxy radicals \((\text{LO}^-)\) and lipid peroxy radicals \((\text{LOO}^\cdot)\) can undergo cleavage of \(\text{C} - \text{C}\) bonds and many products are formed.
Many schemes have been proposed in literature that attempt to explain the initiation, propagation and formation of end products of lipid peroxidation. One of the earliest such schemes was proposed by Hochstein and Ernster in 1963 (Fig. 4) in which the comparison of lipid peroxidation caused by NADPH and ascorbate was done. They proposed the identical nature of ADP-Fe$^{2+}$-O$_2$ complex attacking the lipid (LH) and abstracting a hydrogen atom to generate L' in both types of peroxidation systems. Pederson et al. (1973) established the role of NADPH-cytochrome P-450 reductase in NADPH dependent lipid peroxidation by using an antibody against this enzyme which inhibited lipid peroxidation. Svingen et al. (1979) studied this process in a reconstituted system with purified cytochrome P-450 which enhanced lipid peroxidation just as EDTA-Fe$^{3+}$ does and has proposed a scheme of lipid peroxidation (Fig. 5) according to which the first step, initiation, is catalyzed by the ADP-perferryl ion. The second step, propagation, is dependent upon the lipid hydroperoxide (LOOH) formed during initiation and results in the rapid formation of reactive intermediates and products of lipid peroxidation. Propagation, in their view, accounts for more than 90% of the products formed primarily through radical intermediates. Also perhaps, it is a Fenton type of reaction resulting primarily in the lipid alkoxy radicals. Low levels of hydroperoxides are also produced as mediated by singlet oxygen.
FIGURE 4. SCHEMATIC REPRESENTATION OF LIPID PEROXIDATION LINKED TO ASCORBATE OR TPNH (NADPH) OXIDATION IN MICROSONES AND MITOCHONDRIA (HOCHSTEIN AND ERNSTER, 1963)
FIGURE 5. NADPH-DEPENDENT LIPID PEROXIDATION (SVINGEN et al., 1979)
Ernster et al. (1982) do not agree with the scheme of Svingen et al. (1979) and have proposed the following sequence of reactions that lead to lipid peroxidation, while retaining the theme of their earlier (Fig. 4) scheme:

\[
\text{NADPH} + H^+ + O_2 \rightarrow \text{NADP}^+ + H_2O_2 \tag{12}
\]

\[
\text{LH} + O_2 \rightarrow \text{LOOH} \tag{13}
\]

Reaction (12) may involve the following steps:

\[
\text{NADPH} + 2\text{XPP-Fe}^3+ \rightarrow \text{NADP}^+ + 2\text{XPP-Fe}^{2+} + H^+ \tag{12a}
\]

\[
2\text{XPP-Fe}^{2+} + O_2 \rightarrow 2\text{XPP-Fe}^{2+} - O_2 \tag{12b}
\]

\[
2\text{XPP-Fe}^{2+} - O_2 \rightarrow 2\text{XPP-Fe}^{3+} - O_2^- \tag{12c}
\]

\[
2\text{XPP-Fe}^{3+} - O_2^- + 2H^+ \rightarrow 2\text{XPP-Fe}^{3+} + H_2O_2 + O_2 \tag{12d}
\]

\[
\text{sum: } \text{NADPH} + H^+ + O_2 \rightarrow \text{NADP}^+ + H_2O_2 \tag{12}
\]

where XPP-Fe is a suitable iron chelate (XPP standing for an organic or inorganic pyrophosphate). Reaction (12a) is catalyzed by the flavo-enzyme NADPH-cytochrome P-450 reductase whereas the reactions (12b), (12c) & (12d) are nonenzymic. The dismutation according to reaction (12d) may consist of several steps -
2XPP-Fe$^{3+}$ -O$_2^-$ $\rightarrow$ 2XPP-Fe$^{3+}$ + 2O$_2^-$ (12d$_1$)

2O$_2^-$ + 2H$^+$ $\rightarrow$ H$_2$O$_2$ + O$_2$ (12d$_2$)

sum: 2XPP -Fe$^{3+}$-O$_2^-$ + 2H$^+$ $\rightarrow$ 2XPP -Fe$^{3+}$ + O$_2^-$ + H$_2$O$_2$ (12d)

Step (12d$_2$) is relatively slow at physiological pH in absence of a catalyst and thus reaction (12d) probably is the rate limiting step of reaction 12. This enables XPP -Fe$^{3+}$ -O$_2^-$ to initiate lipid peroxidation and also explains why superoxide dismutase inhibits this process.

The initiation of lipid peroxidation (reaction 13) may involve the following steps:

LH + XPP -Fe$^{3+}$-O$_2^-$ $\rightarrow$ L' + XPP -Fe$^{2+}$-O$_2$H (13a)

L' + O$_2$ $\rightarrow$ LOO' (13b)

LOO' + XPP -Fe$^{2+}$-O$_2$H $\rightarrow$ LOOH + XPP -Fe$^{3+}$-O$_2^-$ (13c)

sum: LH + O$_2$ $\rightarrow$ LOOH (13)

The fact that NADPH and ascorbate induced lipid peroxidations have similar requirements for iron-chelates suggests that it is the reaction (13) rather than reaction (12) that determines this requirement (Ernster et al. 1982).

McCay et al. (1972), Fong et al. (1973) and King et al. (1975) are of the view that NADPH dependent micro-
somal lipid peroxidation occurs via an ADP-Fe$^{2+}$ facilitated Haber-Weiss reaction. They found that NADPH-caused lipid peroxidation was dependent on ADP-Fe$^{3+}$, O$_2^-$, H$_2$O$_2$ and HO'. These authors, therefore, suggested the following scheme for NADPH dependent lipid peroxidation involving O$_2^-$ produced by NADPH-cytochrome P-450 reductase and HO':

$$2 \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (14)$$

$$H_2O_2 + \cdot O_2^- \rightarrow O_2 + HO^- + HO' \quad (15)$$

$$O_2^- + ADP-Fe^{3+} \rightarrow O_2 + ADP-Fe^{2+} \quad (16)$$

$$ADP-Fe^{2+} + H_2O_2 \rightarrow ADP-Fe^{3+} + HO^- + HO' \quad (17)$$

The flow of HO' produced by reactions (14), (16) and (17) was proposed to be much greater than that by reactions (14) & (15) and thus the requirement for catalytic amounts of ADP-Fe$^{3+}$ was necessary.

Tien & Aust (1982a) view that the activated complex capable of free radical formation in PUFA is generated by providing reducing equivalents to a system containing ADP-Fe$^{3+}$ and O$_2$ (Fig.6). In NADPH-dependent lipid peroxidation, reducing equivalents are provided by the NADPH-cytochrome P-450 reductase catalyzed reduction of iron by NADPH. In the O$_2^-$ dependent lipid peroxidation, reducing equivalents are provided by xanthine oxidase catalyzed reduction of O$_2$ by xanthine. Svingen et al. (1979) had
proposed this activated complex to be $\text{ADP-Fe}^{3+} - \text{O}_2^-$ but Tien & Aust (1982a) believe that an oxygen bridged di-iron complex ($\text{ADP-Fe}^{2+}_2 - \text{O}_2 - \text{Fe}^{3+} - \text{ADP}$) intermediate is involved in the mechanism. One of the free radicals formed would be the lipid free radical ($L'$) which reacts with $O_2$ to form lipid peroxyl radical (LOO') which can react with another PUFA to form the lipid free radical and lipid hydroperoxide. The latter can decompose to free radical products by EDTA-$\text{Fe}^{2+}$ or cytochrome P-450. These radicals can participate in the propagation reactions of lipid peroxidation and also breakdown to numerous products as discussed below.

2.3.2 Product Formation During Lipid Peroxidation

Figure 7 shows the simplest scheme for lipid peroxidation reactions and demonstrates that the cleavage of C-C bonds results in the formation of unsaturated fatty acid aldehydes and alkyl radicals, which in turn initiate new radical chain reactions. According to Tappel (1980), Kappus & Sies (1981) and Wendel & Dumelin (1981), the formation of alkanes (ethane, n-pentane etc.) during lipid peroxidation occurs by the mechanism shown in Fig. 7. The $\omega$-3 unsaturated fatty acids yield ethane and the $\omega$-6 acids yield n-pentane. That metal ions are involved in lipid hydroperoxide decomposition has been proved by in vitro experiments of Tappel (1980), Gardner and Crawford (1981),
FIGURE 7. SCHEME REPRESENTING THE FORMATION OF ETHANE DURING LIPID PEROXIDATION INDUCED BY HYDROXYL RADICALS. (KAPPUS, 1985)
Gardner and Jurisinic (1981) and Esterbauer (1982). At least two isolated double bonds are needed to form monohydroperoxides of fatty acids which can form alkanes. Alkenes can also be formed.

The quantity of monohydroperoxides of lipids formed in biological systems is relatively low, as shown by studies measuring ethane and n-pentane. Pryor et al. (1976) and Kappus et al. (1982) have proposed a scheme (Fig. 8) for the formation of reaction products during lipid peroxidation. Endoperoxides arise during lipid peroxidation and if an endoperoxide radical undergoes intramolecular rearrangement followed by reaction with $O_2$, a prostaglandin-like precursor of malondialdehyde is formed (Fig. 8). MDA is the main defined reaction product measurable in biological systems which undergo lipid peroxidation. Wills (1964), Asakawa & Matsushita (1979), Kappus et al. (1982) and Mulianwan & Kappus (1983) have indicated that the decomposition of the cyclic lipid endoperoxyhydroperoxide is catalyzed by metal ions, but this intermediate is probably heat-labile as well. The alkanes are quantitatively minor reaction products of the lipid peroxidation process. It has been estimated that the formation of one molecule of ethane or n-pentane is associated with the formation of 100-500 molecules of malondialdehyde, the latter being about 10% of the total unsaturated fatty acid molecules lost (Kappus, 1985). At least, three isolated double bonds...
**Figure 8. Formation of Malondialdehyde During Lipid Peroxidation (Kappus, 1983)**

**INITIATION**

\[
R-\text{CH=CH-CH}_2-\text{CH}=\text{CH-CH}_2-\text{CH}=\text{CH-R'} \quad \text{LIPID}
\]

\[
\begin{align*}
\text{Fe}^2+ & \text{O}_2^+ & \text{H}^+(\text{HO}^*) \\
\text{Fe}^3+ & \text{H}_2\text{O}_2(\text{H}_2\text{O})
\end{align*}
\]

\[
R-\text{CH-CH-CH}_2-\text{CH}=\text{CH-CH}=\text{CH-R'} \quad \text{LIPID RADICAL}
\]

**Diene conjugation**

\[
\begin{align*}
R-\text{CH-CH-CH}_2-\text{CH}=\text{CH-CH}=\text{CH-CH-R'} & \text{LIPID} \\
\text{O}_2 & \\
R-\text{CH-CH-CH}_2-\text{CH}=\text{CH-CH}=\text{CH-R'} & \text{PEROXY RADICAL}
\end{align*}
\]

**INTRAMOLECULAR REARRANGEMENT**

\[
R - \text{CH} - \text{CH} - \text{CH} - \text{CH} = \text{CH} - \text{CH} - \text{CH}-R'
\]

**RADICAL CHAIN REACTION**

\[
\begin{align*}
R - \text{CH} - \text{CH} - \text{CH} - \text{CH} = \text{CH} - \text{CH} - \text{CH}-R' & \text{LIPID} \\
\text{HO}^+ & \text{Fe}^2+ \\
\text{Fe}^3+ & \text{O}^-
\end{align*}
\]

\[
'R-\text{CH}=\text{CH}_2 + \text{O=CH-CH=CH-R'} + \text{O=CH-CH}_2-\text{CH}=\text{O} \quad \text{ALDEHYDE + MDA}
\]

**ALKYL RADICAL+LIPID**

**ALKOXY RADICAL**

**ENDOPEROXY RADICAL**
in unsaturated fatty acids are necessary to form malondialdehyde. Besides MDA, the breakdown of the cyclic endoperoxidehydroperoxides yields unsaturated lipid aldehydes and alkenyl or alkyl radicals, which again initiate radical chain reactions. The cyclic endoperoxidehydroperoxide would lead to a monohydroperoxide (Pryor et al. 1976).

Many other products have been detected from auto-oxidation experiments with unsaturated fatty acids or phospholipids. Coxon et al. (1981), Frankel (1982), Gutteridge & Wilkins (1982), Miyashita et al. (1982), Neff et al. (1981, 1982) and Toyoda et al. (1982) have detected additional compounds, such as hydroxy- and epoxy-derivatives, ketones, polyhydroperoxides, dimers and polymers of fatty acids which are formed during radical chain reactions. But, in biological systems, only a few of these lipid peroxidation products have been identified. For example, malondialdehyde, 4-hydroxynonenal and the alkanes have been identified after lipid peroxidation reactions in biological systems (Tappel, 1980; Halliwell, 1981; Esterbauer, 1982). Hughes et al. (1983) have found similar lipid auto-oxidation products after treatment of animals with CCl₄, a well known hepatotoxic agent which induces lipid peroxidation in vitro and in vivo.

Pryor (1982), Sies et al. (1982), Sutherland & Gebicki (1982) and Thomas et al. (1982) have suggested that lipid hydroperoxides react with superoxide radical...
initiating new radical chain reactions:

\[ \text{LOOH} + \text{O}_2^- + \text{H}^+ \rightarrow \text{LO}^- + \text{O}_2 + \text{HO}^- \]  

Metal ions are probably not required for this step, which may occur distant from that cell compartment where lipid peroxides originate.

Kappus & Sies (1981) found that lipid peroxidation involves a consumption of tremendous amount of \( \text{O}_2^- \). This may lead to critical conditions in cells where the oxygen supply is limited. Many of the lipid peroxides and other reaction products formed during lipid peroxidation are still chemically reactive and can change other cell components or can lead to products which are resistant to catabolism and can, therefore, accumulate. Since, such reactive free radicals are produced \textit{in vivo} in amounts sufficient to overcome the normally efficient protective mechanisms, it can be expected that metabolic and cellular disturbances occur in various ways (Slater et al. 1986) which are summarized below (Fig. 9).

\[ \text{Lipid peroxidation} \rightarrow \text{toxic products} \]  

(Fig. 9)
2.4 ENZYMES INVOLVED IN LIPID PEROXIDATION

2.4.1 Enzymes of Microsomes

The occurrence of enzymically induced peroxidation of lipids in rat liver microsomes was first reported in 1963 by Hochstein and Ernster. An year later, Hochstein et al. (1964) reported that the reaction required, besides molecular oxygen, the presence of NADPH, and was greatly enhanced by nucleoside di- or triphosphates as well as inorganic pyrophosphate, in combination with ferrous or ferric ions.

It is established that the most susceptible cellular location for lipid peroxidation is the endoplasmic reticulum. In particular, microsomes originating from the endoplasmic reticulum of liver, kidney, brain and other organs have been examined (Plaa & Witschi, 1976; Buege & Aust, 1978; Bus & Gibson, 1979; Halliwell, 1981 and Hornsby & Crivello, 1983). Microsomes have been used to measure enzymatic and non-enzymatic lipid peroxidation. In the latter case, the microsomes serve exclusively as a good peroxidizable phase according to Searle & Wilson (1983). However, it is the enzyme catalyzed lipid peroxidation that is more important physiologically.

Evidence has been presented by Orrenius et al. (1964), Nielson & Lehninger (1964) and Ernster et al. (1982) that the enzymatically induced lipid peroxidation involved
the microsomal flavoprotein known as NADPH-cytochrome C reductase, subsequently identified as NADPH-cytochrome P-450 reductase and it is likely to be involved in the reduction of Fe\(^{3+}\) to Fe\(^{2+}\), either free or chelated.

Tien et al. (1981), Ernster et al. (1982) and Morehouse et al. (1983) have found that during lipid peroxidation, NADPH cytochrome P-450 reductase is able to release superoxide radical. This radical in the presence of H\(_2\)O\(_2\) and transition metal ions can yield HO\(^-\) radicals. But lipid peroxidation induced by microsomes or by isolated NADPH-cytochrome P-450 reductase is not inhibited by catalase, a prerequisite for the involvement of the iron-catalyzed Haber-Weiss reaction. Therefore, formation of and involvement of hydroxyl radicals according to these authors is ruled out.

Hildebrandt et al. (1982) and Orrenius et al. (1982) have suggested that the cytochrome P-450 and other components of microsomal electron transport chain could also be involved in microsomal lipid peroxidation by the so-called uncoupling phenomenon which is provoked by a number of drugs, leading to superoxide anion and hydrogen peroxide instead of hydroxylated drugs. However, according to Bast et al. (1983), it is unlikely that this phenomenon contributes to the microsomal lipid peroxidation.

indicate that ferrous-oxygen complexes react with lipids non-enzymatically. According to these authors Fig.10 NADPH-cytochrome P-450 reductase is involved in the reduction of ferric to ferrous ions, which then can initiate lipid peroxidation. Direct reduction by the enzyme or indirect reduction by superoxide released from the enzyme are both possible. That superoxide could be involved in this step has been shown by the inhibition of lipid peroxidation by superoxide dismutase in a system of liposomes, cytochrome P-450 reductase, NADPH and complexed iron ions. However, superoxide dismutase has been found to be unable to inhibit iron ion induced lipid peroxidation in microsomes. Probably superoxide dismutase cannot penetrate microsomes to react with $O_2^-$ formed inside the membrane. But, low molecular weight copper complexes have been used to efficiently inhibit microsomal lipid peroxidation. NADPH-cyt. P-450 reductase is probably involved in the breakdown of lipid hydroperoxides by reducing ferric to ferrous ions.

Plaa & Witschi (1976), Bus & Gibson (1979) and Recknagel et al. (1982) have reported that conjugated dienes of lipids are detectable in microsomes after treatment of animals with several drugs. Lokesh et al. (1981) and Wills et al. (1985) have indicated that the susceptibility of the endoplasmic reticulum to lipid peroxidation can be modulated by changes in the degree of unsaturation.
FIGURE 10. SCHEME REPRESENTING LIPID PEROXIDATION INDUCED BY MICROSOMAL NADPH-CYTOCHROME P-450 REDUCTASE IN THE PRESENCE OF IRON IONS AND OXYGEN (KAPPUS, 1985)
of fatty acids of the microsomal membranes. Plaa & Witschi (1976), and Tien & Aust (1982b) have found considerable differences in susceptibility to lipid peroxidation of microsomes obtained from different species. Also varying microsomal lipid peroxidation of different organs could be either due to differences in their fatty acid composition or due to different microsomal enzymic activities.

2.4.2 Enzymes of other Cell Compartments

Zimmermann et al (1973), Tayler (1975), Chance et al. (1979), Halliwell (1981), Fujimoto et al. (1982) and Meszaros et al. (1982) have studied lipid peroxidation in mitochondria. In most experiments lipid peroxidation was initiated by a non-enzymatic mechanism or by adding an enzyme which catalyzes lipid peroxidation. It is clear that the enzyme NADH-oxidase of the electron transport chain is involved in the enzymatic mitochondrial lipid peroxidation, which strictly depends on the presence of iron ions. Mechanism of lipid peroxidation in mitochondria is not established but it is likely that some enzymes are involved in the reduction of ferric ions. According to Iida et al. (1982) and Klimek et al. (1983) in adrenal mitochondria, iron induced lipid peroxidation seems to be quite similar to that in liver microsomes. This may be due to the presence of NADPH-cyt. P-450 reductase which is involved in the cytochrome P-450-dependent hydroxylation of steroids.
A number of cytosolic enzymes could be involved in cellular lipid peroxidation. The example is xanthine oxidase which produces superoxide anion radicals in the presence of xanthine, acetaldehyde or other substrates. Tien et al. (1981) have indicated that even with this enzyme, the hydroxyl radical is not responsible for the initiation of lipid peroxidation. The enzyme might be functioning by reducing ferric to ferrous ions, which initiate lipid peroxidation. Many other systems which also produce superoxide radicals, e.g. hemoglobin and various peroxidases could be acting in the same way.

Baehner et al. (1982), Fantone & Ward (1982) and Kanner & Kinsela (1983) have suggested that myeloperoxidase of neutrophils is able to initiate or catalyze lipid peroxidation in the presence of H₂O₂. This has been deduced from experiments with lactoperoxidase, an enzyme with a reaction mechanism similar to that of myeloperoxidase.

2.5 ANTIOXIDANT DEFENCE MECHANISMS AGAINST REACTIVE OXYGEN SPECIES AND LIPID PEROXIDATION

Detoxication of reduced oxygen species is one of the pre-requisites of aerobic life, and the multiple lines of defence, which exist in biological systems are listed below:
<table>
<thead>
<tr>
<th>System</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-enzymatic</strong></td>
<td></td>
</tr>
<tr>
<td>Tocopherols (Vitamin E)</td>
<td>Membrane bound</td>
</tr>
<tr>
<td>Ascorbate (Vitamin C)</td>
<td>Water soluble</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Plant antioxidants (rutin, quercetin, etc.)</td>
</tr>
<tr>
<td>β-Carotene and Vitamin A</td>
<td>Singlet oxygen quenchers</td>
</tr>
<tr>
<td>Urate</td>
<td>Singlet oxygen quencher and radical scavenger</td>
</tr>
<tr>
<td>Plasma proteins</td>
<td>Ceruloplasmin, ferritin, transferrin and albumin</td>
</tr>
<tr>
<td>Glucose</td>
<td>HO' radical scavenger</td>
</tr>
<tr>
<td>Chemicals</td>
<td>Food additives</td>
</tr>
<tr>
<td><strong>Enzymatic</strong></td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutases</td>
<td>Cu-Zn enzyme, Mn enzyme</td>
</tr>
<tr>
<td>GSH peroxidase</td>
<td>Selenoenzyme, nonSe-enzyme (some GSH-S-transferases)</td>
</tr>
<tr>
<td>Catalase</td>
<td>Heme enzyme (peroxisomal matrix)</td>
</tr>
<tr>
<td><strong>Ancillary enzymes</strong></td>
<td></td>
</tr>
<tr>
<td>NADPH-quinone oxidoreductase</td>
<td>Two electron reduction, dicoumarol-sensitive</td>
</tr>
<tr>
<td>Epoxide hydrolase</td>
<td>Hydrolyses fatty acid epoxides</td>
</tr>
<tr>
<td>Conjugation enzymes</td>
<td>UDP-glucuronyl-transferase, sulfotransferase, GSH-S-transferase</td>
</tr>
<tr>
<td>GSSG-reductase</td>
<td>Regenerates GSH</td>
</tr>
<tr>
<td>NADPH supply</td>
<td>Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, malic enzyme, energy linked transhydrogenase</td>
</tr>
</tbody>
</table>
The repertoire to counteract the potentially hazardous reactions initiated by oxygen metabolites includes all levels of protection, prevention, interception and repair. It comprises nonenzymatic scavengers and quenchers denoted by the term 'antioxidants' and also enzymic systems. These enzymes, of course, are the superoxide dismutases and various hydroperoxidases such as glutathione peroxidase, catalase and other hemoprotein peroxidases. They are characterized, in general, by high cellular activity, by specific organ and sub-cellular localization which often overlap in a complementary way, and by a specific form of metal involvement in the catalysis including copper, zinc, iron, manganese and selenium.

2.5.1 Prevention

Primary defence systems preventing lipid peroxidation interact with the initiation step. Many biological systems do not readily form oxygen radicals, e.g. during mitochondrial respiration because it is strictly coupled under physiological conditions and the free radicals do not escape from the site of generation. Further, transferrin and ferritin sequester iron ions so that these are normally not available to catalyze the Haber-Weiss reaction to initiate lipid peroxidation by reacting with molecular oxygen to bring about the decomposition of lipid hydroperoxides. Similarly albumin can bind copper tightly and iron weakly,
whereby copper can catalyze the formation of HO' which are just 'frozen' on the surface of albumin and are scavenged by it and thus serves as a sacrificial antioxidant and chain reactions do not ensue. Ceruloplasmin is probably an extracellular defence system, because it keeps iron ions in the oxidized state (Fe$^{3+}$) which are not involved in the initiation of lipid peroxidation (Yamashoji & Kajimoto, 1983).

2.5.2 Interception

If oxygen radicals have already been formed, a number of defence systems exist that can intercept them and minimize the possibility of lipid peroxidation or other forms of oxidative damage. Among these are:

1. Superoxide dismutases trap superoxide anions, which can reduce iron (Fridovich, 1983)

2. Glutathione peroxidase (Se enzyme) and catalase remove H$_2$O$_2$, which could react with Fe$^{2+}$, yielding HO' radicals (Kappus & Sies, 1981; Sies et al.1982 and Sies & Cadenas, 1983)

3. In some organisms, especially plants, B-carotene and Vitamin A, which can trap $^{1}$O$_2$, are available in relatively high amounts (Halliwell, 1981) and urate in animals can do the same job (Ames et al.1981)

4. Ascorbate and glutathione have also been suggested to be intercepting the free radicals.

All these primary defence systems are active not only against lipid peroxidation but also defend against
numerous biochemical lesions, such as damage to proteins, nucleic acids, sugars, metabolic intermediates etc.

Another compound which is highly specific against lipid peroxidation reaction is Vitamin E which is comprised of 4 different derivatives, α-tocopherol being the most important. Tocopherol (TH₂) reacts with lipid radicals, thereby breaking the free radical chain (Witting, 1980; Tappel, 1980; Fukuzawa et al. 1981; Halliwell, 1981; Hicks & Gebicki, 1981; Burton & Ingold, 1983 and Hornsby and Crivello, 1983).

\[
L' + TH_2 \rightarrow LH + TH' \quad (19)
\]

\[
LOO' + TH_2 \rightarrow LOOH + TH' \quad (20)
\]

The tocopherol semiquinone (TH') formed is relatively stable and can be reduced by cellular components. Packer et al. (1979), Leung et al. (1981) and Bascetta et al. (1983) presented evidence to show that ascorbic acid is involved in the regeneration of tocopherol, whereas the role of glutathione in this connection was suggested by Reddy et al. (1982).

Other naturally occurring quinonoid chemicals such as ubiquinone, catechin and synthetic radical scavengers such as BHT, propylgallate, promethazine, DPPD etc. are not as efficient as Vitamin E in vivo because they distribute equally in the lipids of the organism and are rapidly metabolized.
2.5.3 Repair

Under certain circumstances, due to increased oxidative stress, the lipid peroxidation can occur and the polyunsaturated fatty acids in membranes can become oxidized to epoxy- or hydroperoxide derivatives. Even then, there are certain enzyme systems which can repair this damage. Among these are epoxide hydrolases, glutathione peroxidases, glutathione transferases and phospholipase A₂. Lipid peroxidation increases the activity of membrane-bound phospholipase A₂ which hydrolyzes the damaged or altered fatty acids in β-position of phospholipids. Glutathione peroxidase (both or any of Se and nonSe), then acts on the oxidized fatty acids and reduces them to hydroxy fatty acids which may become a substrate for β-oxidation.

2.5.4 Ancillary Enzyme Systems

There are a number of additional or ancillary enzymes which are of crucial importance in defence against lipid peroxidation e.g. many of the radical or non-radical reactions in cells may lead to thiol oxidation to the disulfides i.e. the oxidation of glutathione to form GSSG. Thus, the regenerative reaction of re-reduction of GSSG to GSH as catalyzed by glutathione reductase can become pivotal in antioxidant defence. Likewise, the provision of reducing equivalents in the form of NADPH to this enzyme is essential. Therefore, the NADPH regenerating systems
like HMP shunt are very important links in defence against oxidative damage.

Similarly, diminution of the steady state levels of reactive compounds capable of generating reactive oxygen species also results in a decreased expression of oxidative stress. In this context, the two electron reduction of quinones by NADPH-quinone oxidoreductase or DT diaphorase and subsequent conjugation reactions undergone by the hydroquinone are part of the antioxidant defence. Also the export of the reactive species in free or conjugated form serves as a detoxication function, so that transport of conjugates and GSSG from the cells falls in the defensive line.

2.5.5 Synergism in Antioxidant Defensive Enzymes

The dismutation of $\text{O}_2^-$ whether spontaneous or enzyme catalyzed produces $\text{H}_2\text{O}_2$, which is also a threat to the chemical integrity of cells. There are, however, catalases and peroxidases dedicated to the task of elimination of $\text{H}_2\text{O}_2$. The defensive team is thus comprised of superoxide dismutases, to lower the steady state level of $\text{O}_2^-$; and catalases and peroxidases to do the same for $\text{H}_2\text{O}_2$. These enzymes are also mutually protective and are, therefore, synergistic, when both $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ are being made. Superoxide anion inactivates catalase by converting the resting ferric enzyme to the poorly active
ferro-oxy form (Compound-III) and also by converting the perferryl intermediate (Compound I) to the inactive ferryl state (Compound III) (Kono & Fridovich, 1982). Heme containing peroxidases are also inhibited by conversion to Compound III. Superoxide dismutase protects catalase and peroxidase against inactivation. At the same time, \( \text{H}_2\text{O}_2 \) inactivates two of the three known types of superoxide dismutases (Beauchamp & Fridovich, 1973; Bray et al., 1974 and Asada et al., 1975) but catalase or peroxidase prevent this. SOD plus catalase or GSH peroxidase thus constitute a mutually supportive defensive team.

2.6 BIOLOGICAL RELEVANCE

2.6.1 Lipid Peroxidation and Damage to Biomembranes

It has become evident that lipid peroxidation reactions destroy the biological membranes in which they occur. This results in an increased or decreased influx or efflux of various cellular and extracellular components (Bridges et al., 1983; Kunimoto et al., 1981 and Weiss et al. 1983). Another deleterious effect that has been observed is the release of hydrolytic enzymes from lysosomes which can digest cells. Lysis of cells has been found to occur in cases where plasma membrane itself was seriously damaged during lipid peroxidation (Yagi, 1982; Hornsby and Crivello, 1983 and Mak et al., 1983). Some
workers including Nielsen (1981) and Narabayashi et al. (1982), Marshansky et al. (1983) and Wolff et al. (1986) have observed that lipid peroxidation interferes with the functions of membranal enzymes and receptors. Best known are the losses of cytochrome P-450 and glucose-6-phosphatase in microsomes and the inactivation of the respiratory chain in mitochondria. The alteration of membranal enzymes and receptors can be caused simply by the loss of membrane structure (resulting from an alteration in unsaturated fatty acids in the phospholipids) as the enzymes or the receptors might undergo some changes resulting in their activation or inactivation. Direct attack of oxygen radicals, lipid peroxides and lipid degradation products on membrane enzymes and receptors is also likely to occur but little is known about the mechanism of these alterations during lipid peroxidation.

2.6.2 Lipid Peroxidation, Oxidative Damage to DNA and Carcinogenesis

Any type of damage of DNA, if not repaired, can result in cancer and there are numerous chemicals that can cause oxidative damage to DNA. Halogenated carcinogens such as PCB's, dieldrin, DDE, DDT, CCl₄ and CHCl₃, which are not mutagenic (Ames et al. 1982) all generate free radicals and enhance lipid peroxidation. Many other chlorine or bromine containing organics have been shown to cause lipid
peroxidation. It appears highly likely that the carcino-
genicity of these halogen containing chemicals is due
to their ability to form free radicals which cause lipid
peroxidation which being a chain reaction causes the pro-
duction of a considerable quantity of active forms of
oxygen such as HO\textsuperscript{•} and ROOH which can damage DNA. Other
carcinogens not detected as mutagens in Ames Test such
as lead, cadmium and substituted hydrazines may be active
by causing lipid peroxidation and then damage to DNA (Ames,
1982).

Halliwell (1981), Summerfield and Tappel (1981),
Yonei & Furul (1981) and Schauenstein (1982) have emphasized
the relevance of reactions of DNA with products of lipid
peroxidation like MDA and hydroxalkenals but DNA adducts
have not been isolated from whole cells. The changes
in DNA could lead to mutations and carcinogenesis.

Slater (1972), T'so et al. (1977) & Demopoulos
et al. (1980) have shown that many chemical carcinogens
can be metabolically activated to free radical inter-
mediates. According to Nagata et al. (1982), polycyclic
hydrocarbons can be metabolized to the free radical inter-
mediates and if these are formed close to DNA (by activa-
tion in the nuclear membrane), then consequences of the
activated carcinogens may ensue (Baired et al., 1980 and
Bachur et al., 1982). An important aspect of carcinogen
activation is that this may occur during and as a result
of lipid peroxidation. Dix & Marnett (1983) have shown that polycyclic hydrocarbons are metabolized to epoxy derivatives when included in a peroxidizing microsomal system. Marnett et al. (1977) had previously shown that prostaglandin synthetases can also catalyze benzopyrene metabolism during a co-oxidation of arachidonate and the endoperoxide PGG$_2$ can promote the formation of benzopyrene quionone (Marnett & Reed, 1979).

2.6.3 Oxygen Free Radicals, Lipid Peroxidation and Diseases

Oxygen radicals and lipid peroxidation have been implicated as important causative agents of ageing and of several human diseases, including cancer, multiple sclerosis, Parkinson's disease, autoimmune disease & senile dementia (Halliwell and Gutteridge, 1986). Some other genetic and acquired disorders which have also been related to lipid peroxidation include hemolytic anemia, uremia, $\beta$-thalasemia, ischemia, inflammation, muscular dystrophy, myocardial infarction, liver cirrhosis, cataract and atherosclerosis (Halliwell, 1981; Pryor, 1982 and Yagi, 1982).
2.7 DIETARY LIPID AND DRUG METABOLISING ENZYMES

Dietary PUFA appear to induce greater activity of drug metabolizing enzymes. Castor et al. (1968), Wade et al. (1969) and Norred & Wade (1972) observed greater hepatic mixed function oxidase activity in rats fed 3-10% corn oil for three weeks. Marshall and McLean (1971) reported increased aniline hydroxylase in liver from rats fed 15% herring oil supplemented diet for 10 days but Century (1973) reported decreased drug metabolism in rats fed corn oil up to 10%. Synthetic diet containing groundnut oil (6%) for 6 weeks has been found to increase ethylmorphine and aniline hydroxylase in rat liver (Patel and Pawer, 1972). Agradi et al. (1975) also reported a decreased level of benzopyrene hydroxylation by feeding 10% rapeseed oil, olive oil and safflower oil but aniline hydroxylase was increased by feeding 10% safflower oil. Wade et al. (1978) obtained evidence that indicated that increase in microsomal enzyme activity induced by dietary lipid intake was due to increase in enzymatic protein synthesis.

Shakman (1974) concluded that the toxicity of the chlorinated hydrocarbon, dieldrin, is increased in states of PUFA deficiency. PUFA, if not deficient, would have protected against the toxicity of dieldrin, by undergoing peroxidation and thus saving other vital macromolecules and toxicity would have been decreased.
2.7.1 Cytochrome P-450 Level

Cytochrome P-450 level was significantly decreased after feeding a synthetic fat free diet to male and female rats but feeding corn oil (10%) to these rats resulted in substantial increase in Cyt P-450 level (Norred and Wade, 1972).

Even hydrogenated coconut oil supplemented diet increased the microsomal cytochrome P-450 content by 70% as compared to rats fed standard laboratory feed (Kaschnitz, 1970). Wade et al (1972) found no significant alteration in cytochrome P-450 levels in livers of rats given arachidonic acid (0.3-100 μL/day) or menhaden oil (0.001-100 μL/day) for 3 weeks.

2.7.2 NADPH-Cytochrome P-450 Reductase and G-6-PD Levels

A 3 week feeding of diets containing 3% or 10% corn oil to female rats resulted in a significant increase in the activity of NADPH-Cytochrome P-450 reductase (Wade et al. 1978), but earlier in 1972, Wade et al. had not found a significant alteration in its activity by feeding corn oil, arachidonate or menhaden oil to male rats.

Glucose-6-phosphate dehydrogenase activity in hepatic cytosol was markedly reduced in rats fed high level of linseed oil, menhaden oil or arachidonate (Century, 1972 & 1973 and Wade et al, 1972).
2.7.3 Dietary Fat and Cancer

Armstrong and Doll (1975) and Knox (1977) have observed a close association of total dietary fat with colon cancer and to lesser extent rectal cancer. Phillips (1975), Dales et al. (1978) and Jain et al. (1980) have observed a direct association between consumption of high fat foods, total fat intake and colon cancer whereas Berg and Howell (1974) and Howell (1975) have seen a link between meat consumption and colon cancer but Graham et al. (1978) and Bjelke (1978) have found contradictory results. Still others like Armstrong and Doll (1975) and Kolonel et al. (1981) have suggested an association of dietary fat and cancers of testis, corpus uteri, ovary and pancreas.

2.7.4 Dietary Fat and Chemical Carcinogenesis

Many workers have demonstrated a relationship between dietary fat and cancer incidence in colon (Wynder et al. 1969); Burkitt, 1971) skin (Tannebaum, 1944) and mammary gland (Cammal et al. 1967; Carroll & Khor, 1970 and Hopkins et al. 1976).

Tannebaum & Silverstone (1957) observed that tumor incidence was greater in obese mice than in normal mice and that caloric restriction inhibited mammary tumorigenesis in normal mice. However, Lavik and Baumann (1943)
had observed that calories appear to have a greater effect than fat on 3-methyl cholangthrene induced skin tumors. Carroll & Khor (1971) and King et al. (1979) noticed that high fat diets enhance breast carcinogenesis by 7, 12 dimethyl benzo(a)anthracene. Carroll (1980) further observed that breast tumorigenesis appears to be enhanced when high fat diets were fed after, but not before, tumor initiation. High fat might be acting as a promotor.

Carroll & Khor (1971) found that diets containing 20% polyunsaturated fats enhance tumorigenesis more effectively than do diets containing 20% saturated fats. But Reddy et al. (1974) observed no clear difference between the effect of polyunsaturated fat and saturated fat at 20% level.

2.7.5 Dietary Fat and Lipid Peroxidation in Tissues

The membranes of subcellular organelles like mitochondria, endoplasmic reticulum, lysosomes, nuclei etc. contain a substantial amount of unsaturated phospholipids which are very important for the structure and function of the cell. The peroxidation of phospholipids of membranes, if induced, can result in its damage and impairment of function and finally cell death. Studies of Bunyan et al. (1967), Glavind (1972), Iritani (1980), and Saito (1981) have raised doubts about the presence of higher levels of lipid peroxides in blood and tissues
of animals consuming diets rich in lipids containing unsaturated fatty acids.

2.7.6 Feeding of Oxidized Oils and Biological Effects

Crampton et al. (1953) and Kauntiz et al. (1955) conducted studies by feeding fats that had undergone severe damage by heat or oxidation and observed diarrhoea, edema of the gut and enlarged livers and kidneys in animals consuming such diets, and growth was diminished. Nolen et al. (1967) conducted a long term feeding study for 2 years at 15% level of oxidized oil (partially hydrogenated soyabean oil, cottonseed oil and lard). Although they observed decreased growth but otherwise no adverse clinical effects were noted. The results of the experiment indicated that although prolonged heating of fats could produce certain toxic products, yet may be that their level is so low as not to have any significance biologically.

Kaunitz and Johnson (1973) conducted a feeding study in which olive oil, corn oil, soyabean oil, coconut oil, chicken fat, butter fat and other types of fats and oils were used after 40 hours of aeration at 60°C. They observed significantly decreased body weight and life span was shortened.

2.7.7 Fried Oils and Mutagenesis

The formation of mutagens and toxic compounds
in cooked foods has been reported by many workers. Lizinsky and Shubik (1964) reported the occurrence of benzo(a) pyrene and other hydrocarbons in smoked meats. Sugimura and Nagao (1979) also detected potent mutagens in protein pyrolysates obtained by heating proteins at temperatures above 300°C. Commoner et al. (1978) and Pariaza et al. (1979) have detected mutagens in beef fried at normal frying temperatures. But according to Taylor et al. (1982), no mutagenic activity was there in commercially available foods containing deep fried fats. Taylor et al. (1983) again did not find any mutagens in used deep frying fats obtained from commercial establishments.

Sugimura and Nagao (1979) conducted a survey and found that various pyrolysis products obtained from tryptophan are produced at temperatures well above 200°C. Cigarette smoke could generate such compounds but rarely these could be there in cooked foods. But Weisburger et al., (1981) have demonstrated that different mutagens are formed during normal frying, broiling and boiling of meat.

It has been quite in vogue to use unsaturated oil instead of saturated fat so as to decrease the risk of atherosclerosis and other heart ailments but with this the risk to mutagenesis and carcinogenesis can always increase (Ames, 1983; Matsushita, 1986).