The radical intermediates and the nonradical products of peroxidized lipids are both effective in polymerizing RNase. The radicals formed by the fission of LAHPO may initiate a chain reaction resulting in the formation of protein radicals. These protein radicals react together to form polymeric products. This conclusion is in agreement with the observation of Roubal (1966), who suggested that the radical intermediates initiate the production of protein radicals which in turn polymerize as $P-(P)_n-P\cdot$. This mechanism is further strengthened by the effects of AsA and BHT on the polymer formation of RNase by LAHPO. The pronounced effect of AsA on the polymer formation of RNase by LAHPO suggests that AsA has increased the rate of the production of alkoxy or peroxy radicals. These radicals react with the proteins, affecting the formation of the protein radicals, which are also being degraded to SP. The antioxidative behavior of BHT further supports the radical mechanism of LAHPO.

The reaction of SP is time dependent and SP did not produce polymers within a short incubation period but showed a relatively high fluorescence in contrast to LAHPO. LAHPO did not produce the fluorescence at 425 nm, which is due to a carbonylamine condensation reaction resulting in the formation of a conjugated chromophoric Schiff's base system (Chio and Tappel, 1969).

From the differences observed in the incorporation, inactivation, and relative damage to the amino acid residues with respect to LAHPO and SP, it was deduced that different amino acid residues are able to be attached by LAHPO or SP. Among the four enzymes under consideration, the structural conformations are different. This results in differences of the surface exposed groups susceptible to attachment by LAHPO or SP, and such exposed groups may be influenced by pH. For instance, at pH 8.0 amino groups of protein would be more unprotonated and better nucleophiles than at pH 5.0. The occurrence of a conformational change of protein structure can also be expected from a change in pH. Also, pH may affect the formation of reactive groups, for instance, the differences of radical formation by the decomposition of LAHPO at pH 5.0 and 8.0.

The specific interactions are very complicated, and it can only be said that specific interactions of oxidized products of linoleic acid and proteins are caused by several factors, pH, specific structure of proteins, etc.

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Involvement of Cytochrome P-450 in the Intracellular Formation of Lipid Peroxides

Peter J. O'Brien* and Anver Rahimtula

Organic hydroperoxides greatly increase the effectiveness of hemoproteins in catalyzing lipid peroxidation. Using this technique it has been shown that the heat-labile catalyst responsible for liver microsomal lipid peroxidation was cytochrome P-450. Evidence for this included inhibitor studies and reconstitution studies. Cytochrome P-450 had similar kinetics and turnover number to plant lipoxygenases and was more effective than other hemoproteins and hematin. A mechanism is proposed for lipid peroxidation in which the catalyst acts as a peroxidase with lipid as a hydrogen donor. A similar mechanism involving other hemoproteins is also presumably involved in the oxidative deterioration of lipid containing foods.

Polyunsaturated fatty acids, a major component of many vegetable oils, undergo autoxidation at ambient temperatures to yield hydroperoxides and a wide range of secondary degradation products associated with subsequent rancidity. Thus, high peroxide values occur after prolonged storage, exposure to sunlight, increased temperatures, and contact with iron or copper as in cooking vessels (Frazer, 1962). Dietary fatty acid hydroperoxides can be toxic to the gastrointestinal tract and can be carcinogenic (Cutler and Hayward, 1974; Cutler and Schneider, 1973). The identification of the catalysts in animal tissues which are responsible for the oxidation of unsaturated fats

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is therefore important, not only because of its pertinence to food preservation, but also for health reasons. Peroxidation of the lipids of animal tissue *in vivo* may also occur and be enhanced by a vitamin E deficient diet, in the chemically induced liver injury by ethanol, carbon tetrachloride, or orotic acid, in oxidant induced lung damage as during air pollution, in oxygen toxicity, and in some phases of atherosclerosis (reviewed by Tappel, 1973; DiLuzio, 1973). The administration *in vivo* of antioxidants greatly reduces the associated biochemical and pathological changes of these conditions.

The lipid peroxidations of subcellular fractions reported by various investigators were mediated by ferrous iron maintained in the reduced form enzymically by NADPH (Hochstein and Ernster, 1963) or nonenzymically by ascorbate (Barber, 1963). However, hemoproteins in the ferric form were also shown to catalyze the oxidation of linoleate with concurrent destruction of the hemoprotein (Tappel, 1953, 1955) and were suggested to participate in the lipid peroxidation of homogenates (Wills, 1966).

Lipid peroxides are readily reduced intracellularly by NADPH and NADH. The NADPH oxidation mechanism was shown to involve glutathione, glutathione peroxidase and glutathione reductase of the cytosol fraction (Little and O'Brien, 1968) and has now been confirmed in a perfused liver system (Sies *et al.*, 1973). Two NADH oxidation mechanisms have been discovered. One mechanism located in the microsomal fraction involves NADH:cytochrome P-450 reductase and cytochrome P-450 (Hrycay and O'Brien, 1974), while the other located in the mitochondrial and microsomal fractions involves ascorbate and semidehydroascorbate reductase (Green and O'Brien, 1973). The lipid peroxide is reduced by the cytosol pathway to a monohydroxy product whereas the microsomal and mitochondrial fractions produce a complex range of products presumably by free-radical interactions and similar to those formed with heme compounds and iron or copper compounds (O'Brien, 1969; O'Brien and Little, 1969). It was later shown that cytochrome P-450 was responsible for most of the microsomal peroxidase activity for organic hydroperoxides and thus could be an intracellular source of free radicals (Hrycay and O'Brien, 1971).

EXPERIMENTAL SECTION

Reagents. Most chemicals were of Analar grade and were supplied by British Drug Houses or Canlab Ltd. Cumene hydroperoxide was purchased from Matheson Coleman and Bell, Norwood (Cincinnati, Ohio). *p*-Menthane hydroperoxide and *tert*-butyl hydroperoxide were obtained from K&K Laboratories, Inc., Plainview, N. Y. *n*-Butyl hydroperoxide was a gift from Dr. G. R. Schonbaum. Linoleic acid hydroperoxide was prepared by the method of O'Brien (1969). Egg yolk lecithin, linoleic acid, and arachidonic acid were of the highest purity supplied by Supelco Co., Bellefonte, Pa. Hemoproteins were the purest available from Sigma Ltd. The cytochrome P-450 preparation was a gift from Dr. M. J. Coon of the Department of Biological Chemistry, the University of Michigan, Ann Arbor, and was prepared by the method of Van der Hoeven and Coon (1974), and had a specific activity of 8 nmol/mg of protein.

Procedure. Oxygen uptake was measured in a Clark oxygen electrode. The reaction mixture contained 3 mg of microsomes in 0.1 *M* Tris-HCl (pH 7.4) in a final volume of 2.0 ml at 25° and was started by the addition of 1.5 m*M* cumene hydroperoxide. The reconstituted reaction mixture contained a cytochrome P-450 fraction (4.4 μM) and egg yolk lecithin (10 m*M*) instead of microsomes. Malonaldehyde was measured by the colorimetric method of Ottolenghi (1959).

Difference Spectra. Difference spectra in the ultraviolet region were carried out on microsomal suspensions at a protein concentration of 0.1 mg of protein/ml using an Aminco DW2 spectrophotometer.

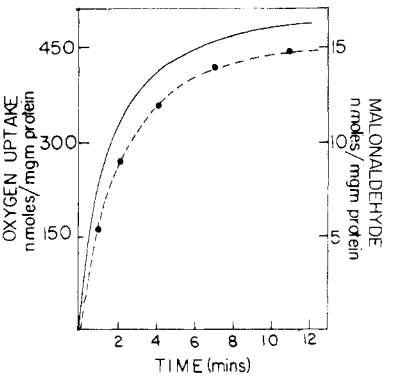

Figure 1. Time course of oxygen uptake and malonaldehyde formation during microsomal lipid peroxidation. The reaction medium at 25° contained, in a final volume of 2.0 ml, 0.1 *M* Tris-HCl buffer (pH 7.4), 2.0 mg of microsomal protein, and 1.5 m*M* cumene hydroperoxide. Malonaldehyde (●---●) determinations were carried out at specific time intervals during the oxygen uptake measurements (—).

Hydroperoxide Decomposition. Cumene hydroperoxide decomposition was measured as reported by Thurman *et al.* (1972). Microsomes (2.0 mg/ml) were incubated with 0.1 m*M* cumene hydroperoxide in a final volume of 15.0 ml. The reaction was terminated by the addition of 0.4 ml of 15% Cl_3CCOOH to 1.6 ml of microsomal suspension. After removal of the precipitate by centrifugation, 0.2 ml of 10 m*M* ferrous ammonium sulfate and subsequently 0.1 ml of 2.5 *M* potassium thiocyanate were added to a 1.0-ml aliquot of the supernatant. The absorbance of the red ferrithiocyanate complex formed was measured at 480 nm and compared to H_2O_2 standards.

RESULTS

Microsomal Lipid Peroxidation Catalyzed by Hydroperoxides. A rapid oxygen uptake occurred when hydroperoxides were added to microsomes (Figure 1). A characteristic rancid odor could be detected and a concurrent production of malonaldehyde accompanying the oxygen uptake was observed. No induction phase was observed and the time curves were hyperbolic in shape. Malonaldehyde production followed the oxygen uptake closely at a rate 3.4% of the oxygen uptake on a molar basis. The rate of oxygen uptake with 1.5 m*M* cumene hydroperoxide was 150 nmol of O_2/min per mg of protein.

Effect of Microsome and Oxygen Concentration on the Lipid Peroxidation. The initial rates of malonaldehyde formation and oxygen uptake were proportional to the concentration of microsomes present. After a time both malonaldehyde production and oxygen uptake reached a maximum value. These maximum values were proportional to the quantity of microsomes and were independent of the hydroperoxide concentration below 0.4 m*M* cumene hydroperoxide. Further addition of hydroperoxide after oxygen uptake had ceased had no effect indicating that either all available lipid had been oxidized and/or the catalyst had been destroyed. However, addition of egg yolk lecithin, linoleate, or arachidonate at a concentration of 3 m*M* resulted in a further increase in oxygen uptake. A similar total increase in oxygen uptake was observed if the lipid was added to the microsomes before the cumene hydroperoxide. It can be concluded that oxygen uptake ceases when the available lipid has been oxidized. A total oxygen consumption of 0.52 mol of O_2/mg of protein was found.

The rate of oxygen uptake was independent of the oxygen concentration at the lowest oxygen concentration tried

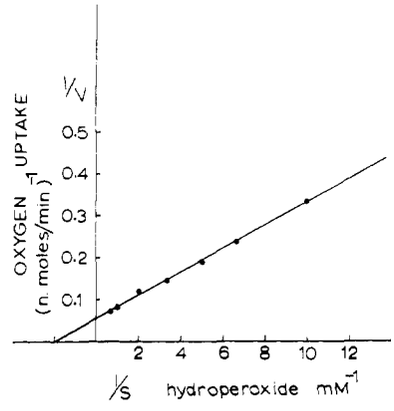

Figure 2. Lineweaver–Burk plot of the cumene hydroperoxide dependence of the microsomal lipoxygenase activity. The reaction medium was similar to that in Figure 1 except that 0.4 mg of microsomal protein was used.

(30 μM). This would indicate that the apparent K_m for oxygen is less than 15 μM.

Effect of Hydroperoxide Concentration on the Lipid Peroxidation. The rate of oxygen uptake and malonaldehyde formation was proportional to the hydroperoxide concentration. The double reciprocal Lineweaver–Burk plot was linear and the apparent K_m from the plot was 0.5 mM (Figure 2).

Various hydroperoxides were tried and found to be effective. In order of effectiveness they were linoleic acid hydroperoxides, cumene hydroperoxide, *p*-menthyl hydroperoxide, and *tert*-butyl hydroperoxide. The primary hydroperoxide, *n*-butyl hydroperoxide, was ineffective.

Changes in Ultraviolet Spectra during Lipid Peroxidation. Since autoxidations of polyunsaturated fatty acids are known to be accompanied by conjugation of the double bond system with resulting absorption at 232 nm, it was of interest to know if similar changes occurred during the microsomal lipid peroxidation. Addition of cumene hydroperoxide (10–30 μM) to a dilute suspension of rat liver microsomes (0.1 mg of protein/ml) in 0.1 M Tris-HCl buffer (pH 7.4) at 26° resulted in rapid spectral changes with a generalized increase in absorbance throughout the ultraviolet range from 350 to 233 nm. The rate of increase at 270 nm was initially 45% of the rate of increase at 233 nm. However, after 30 min the rate of increase at 233 nm had slowed considerably unlike the increase at 270 nm. After 1 hr the increase at 270 nm was 80% of the 233-nm increase. These spectral changes did not occur in the presence of 0.1 mM Mn^{2+} or 0.1 mM glutathione or under anaerobic conditions.

Enzymic Nature of Lipid Peroxidation. When the microsomes were heated at 80° for 1 min a loss of 80% of the activity occurred. Furthermore, addition of egg yolk lecithin, linoleate, or arachidonate at a concentration of 3 mM did not restore the activity indicating that the catalyst had been destroyed.

Effect of Temperature of Incubation on Lipid Peroxidation. The rates at 8 and 37° were, respectively, 25 and 160% of the rate at 25°. A similar total amount of malonaldehyde formation and oxygen uptake occurred at all temperatures. The oxygen uptake rates were corrected for the effect of temperature on the oxygen tension of the medium.

Effect of Inhibitors on Lipid Peroxidation. The effects of various kinds of inhibitors on oxygen uptake and malonaldehyde formation are shown in Table I. Inorganic cations such as Mn^{2+} and Co^{2+} are known to inhibit lipid peroxidation reactions (May and McCay, 1968). Both of these cations prevented lipid peroxidation at concentrations as low as 50 μM and were instantaneously effective if added 1 min after the initiation of the lipid peroxidation.

Table I. Peroxide-Catalyzed Lipid Peroxidation (Per Cent Inhibition)[a]

Modifier	Mechanism	Microsomes	Reconstituted system
Cyanide (1 mM)	Type II	45	25
Metyrapone (0.1 mM)	Type II	50	40
Imidazole (2 mM)	Type II	51	61
Progesterone (0.5 mM)	Type I	57	35
SKF 525A (1 mM)	Type I	55	66
p-CMB (1 mM)	P-450 conversion	20	50
Urea (6 M)	P-420 conversion		70
TMPD (10 μM)	Antioxidant	52	71
α-Naphthol (50 μM)	Antioxidant	60	65
Benzphetamine (1 mM)	Antioxidant or I	64	89
Aniline (1 mM)	Antioxidant or II		88
Mn^{2+} (0.1 mM)	Antioxidant 100	100	65

[a] The reaction medium at 25° contained, in a final volume of 2.0 ml, 0.1 M Tris-HCl buffer (pH 7.4), 2.0 mg of microsomal protein, and 1.5 mM cumene hydroperoxide. The initial rate of oxygen uptake was measured.

The antioxidants α-naphthol (50 μM) and tetramethylphenylenediamine (10 μM) were particularly effective. Since ethylenediaminetetraacetic acid did not affect the reaction rate, inorganic metal ions are unlikely to be involved.

Hydroperoxide Decomposition during Lipid Peroxidation. During the microsomal lipid peroxidation reaction catalyzed by hydroperoxide, hydroperoxide decomposition as measured by the ferrithiocyanate method occurred simultaneously. The initial rate of hydroperoxide decomposition was 60 nmol of hydroperoxide/min per mg of protein. Under the same conditions the rate of oxygen uptake was 58 nmoles of O_2/min per mg of protein. Furthermore, the hydroperoxide decomposition rate was affected by type I and II inhibitors to the same extent as lipid peroxidation. Lipid peroxidation also ceased instantaneously when glutathione (0.1 mM) was added to reduce the hydroperoxide and thus the lipid peroxidation is dependent on hydroperoxide decomposition.

Effect of Inhibitors of Drug Metabolism on Microsomal Lipid Peroxidation. Cyanide (1 mM) and imidazole (2 mM), which can form ligands with heme iron, form type II spectra with cytochrome P-450, and inhibit P-450 dependent reactions (Jefcoate *et al.*, 1969; Orrenius *et al.*, 1972), also inhibited lipid peroxidation 45 and 51%, respectively. Metyrapone (0.1 mM) which also forms type II spectra with cytochrome P-450 (Hildenbrandt, 1972) inhibited 50%. Aniline (1 mM) which can form type II spectra with cytochrome P-450 was also an effective inhibitor but its antioxidant properties could be an explanation. Carbon monoxide inhibited only 20%.

Various drug and steroid substrates for cytochrome P-450 form spectrally distinct complexes known as type I complexes. Progesterone (0.5 mM) and SKF 525A (1 mM) inhibited the lipid peroxidation 57 and 55%, respectively. Benzphetamine (1 mM) was also an effective inhibitor but may also act as an antioxidant.

The conversion of microsomal cytochrome P-450 into its inactive P-420 form is brought about by a wide variety of

Table II. Lipoxygenase Activity of Cytochrome P-450[a]

System	Oxygen uptake, nmol/min per nmol of P-450
Microsomes + HPO	250
P-450 + lecithin + HPO	495
P-450 + lecithin	5
P-450	75
Lecithin + HPO	5

[a] The complete reaction mixture contained cytochrome P-450 (0.9 μM), egg yolk lecithin (10 mM), and cumene hydroperoxide HPO (1.5 mM) in 2 ml of 0.1 M Tris buffer (pH 7.4) at 25°. Initial oxygen uptake rates were measured.

reagents such as proteases, sulfhydryl reagents, lipophilic substances, surface active agents, strong oxidizing agents, and acidic or alkaline pH (Ichikawa *et al.*, 1969). The effects of *p*-CMB (1 mM) and urea (6 M) resulted in a 20 and 40% inhibition of the lipid peroxidation, respectively.

Reconstitution of Microsomal Lipoxygenase. Since the previous results have implicated cytochrome P-450 as responsible for the microsomal lipid peroxidation system, a study was undertaken to determine whether the cytochrome P-450 solubilized from the membrane bound enzyme system and separated from other membrane components could carry out peroxidation of added lipids as effectively and in a similar manner to that of microsomes. A comparison was also made with cytochrome b_5, another major hemoprotein of the microsomal fraction.

As shown in Table II, cytochrome P-450 was found to be a very effective lipid peroxidation catalyst in the presence of hydroperoxide with egg yolk lecithin. Both cytochrome b_5 prepared by detergent extraction (Spatz and Strittmatter, 1973) and that solubilized with trypsin (Omura and Takesue, 1970) were ineffective. Various unsaturated lipids and polyunsaturated fatty acids also acted as good substrates for the lipid peroxidation with cytochrome P-450. Table III shows that hemin and other hemoproteins were less effective than cytochrome P-450. One interesting observation was that if the lipids or unsaturated fatty acids were slightly oxidized, addition of cytochrome P-450 resulted in rapid lipid peroxidation in the absence of hydroperoxide. Presumably, the lipid peroxides present in the lipid catalyze the lipid peroxidation reaction in the same way that cumene hydroperoxide can.

Lipoxygenase Activity of Cytochrome P-450. The initial rate of oxygen uptake was proportional to the concentration of cytochrome P-450. The total oxygen uptake was also dependent on the concentration of P-450 and the concentration of lipid. Further addition of hydroperoxide after oxygen uptake had ceased had no effect. Furthermore, unlike microsomal lipid peroxidation addition of lipid had no effect. However, addition of cytochrome P-450 resulted in a further oxygen uptake indicating that the catalyst had been destroyed.

The dependency of the rate of oxygen uptake on the concentration of cumene hydroperoxide was similar to that observed with microsomes. The double reciprocal Lineweaver–Burk plot was linear and the apparent K_m from this plot was 0.55 mM.

The dependency of the rate of oxygen uptake on the concentration of lipid is shown in Figure 3. As shown in Table III, under maximal conditions the rate of oxygen uptake by cytochrome P-450 with 20 mM egg yolk lecithin and 1 mM cumene hydroperoxide was 2970 nmol of O_2/min per mg of protein and corresponds to a turnover number for cytochrome P-450 of 495.

Effects of Inhibitors on the Reconstituted Model Systems. As the lipoxygenase activity of cytochrome P-450 in the reconstituted model system was active enough to account for the microsomal lipoxygenase activity, the effects of various inhibitors of the microsomal activity were studied on the cytochrome P-450 system. As can be seen from Table I, the model system was also inhibited by type I and II compounds and by conversion of the P-450 to P-420. The antioxidants Mn^{2+}, α-naphthol, and tetramethylphenylenediamine were also as effective at inhibiting the reconstituted system as the microsomal system.

Table III. Comparative Efficiencies of Heme Catalysts for Lipid Peroxidation by Cumene Hydroperoxide[a]

Catalyst	Oxygen uptake, nmol/min per nmol of heme
P-450	495
Cytochrome b_5	35
Methemoglobin	110
Oxyhemoglobin	60
Myoglobin	90
Cytochrome c	50
Catalase	20
Hematin	300

[a] The complete reaction mixture contained catalyst (1 μM), egg yolk lecithin (10 mM), and cumene hydroperoxide (HPO) (1.5 mM) in 2 ml of 0.1 M Tris-HCl buffer (pH 7.4) at 25°. Initial oxygen uptake rates were measured.

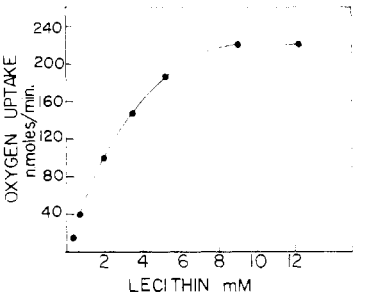

Figure 3. Lipid dependence of the lipoxygenase activity of cytochrome P-450. The reaction medium at 25° contained, in a final volume of 2.0 ml, 0.1 M Tris-HCl (pH 7.4), 4.4 μM cytochrome P-450, 1.5 mM cumene hydroperoxide, and varying amounts of egg yolk lecithin emulsion. The 100 mM lecithin emulsion was prepared using a Vortex mixer.

DISCUSSION

Lipid peroxidation in model systems in the absence of a metal catalyst is autocatalytic and the oxidation rate is sigmoidal with time showing a continuous increase in the reaction rate. The latter increase is caused by chain reactions involving unstable radicals acting as chain initiators and formed from the slowly formed lipid hydroperoxides. However, the lack of an induction period when hydroperoxides were added to microsomes and the decrease in the oxidation rate with time indicates that this lipid peroxidation reaction was not catalytic. Further evidence against a free-radical chain reaction was the requirement for continuous hydroperoxide decomposition to sustain the lipid peroxidation. Finally, the initial rate of hydroperoxide decomposition was also similar on a molar basis to the rate of oxygen uptake.

The rate of autocatalytic lipid peroxidation is proportional to the lipid concentration (Bolland, 1948) and oxygen concentration (Marcuse and Fredriksson, 1968). However, an investigation of the kinetics of the microsomal lipid peroxidation showed that the rate of lipid peroxidation was zero order with respect to lipid concentration

above 10 mM and zero order with respect to oxygen concentration. The lowest concentration of O_2 investigated was 30 μM. The dependency of lipid peroxidation on the cumene hydroperoxide concentration showed an apparent K_m for the hydroperoxide of 0.5 mM. These kinetics again indicate an autocatalytic lipid peroxidation mechanism is not involved and suggest a mechanism more like that of lipoxygenases.

The heat-labile catalyst responsible for microsomal lipid peroxidation was identified as cytochrome P-450 as compounds which form type I and II spectra with cytochrome P-450 inhibited the lipid peroxidation. The reconstitution of the microsomal lipid peroxidation system from cytochrome P-450 and polyunsaturated lipids and the finding that cytochrome b_5 has very low activity are convincing proof that cytochrome P-450 was responsible for microsomal lipid peroxidation. Further confirmation of this was the finding that the reconstituted system was inhibited by type I and II compounds and by conversion of the P-450 to P-420 to the same extent as microsomal lipid peroxidation. The rate of lipid peroxidation catalyzed by cytochrome P-450 showed the same apparent K_m for cumene hydroperoxide of 0.55 mM and was therefore similar to that observed with microsomes. Organic hydroperoxides also greatly increased the effectiveness of hemoproteins, cytochromes, and hematin in catalyzing lipid peroxidation. Cytochrome P-450 was, however, more effective than the other catalysts. The order of effectiveness of the catalysts in lipid peroxidation was similar to their order in decomposing hydroperoxide (O'Brien, 1969). The rate of lipid peroxidation catalyzed by cytochrome P-450 was also first order with respect to cytochrome P-450 concentration at saturating concentrations of lipid. It was also previously shown that the rate of decomposition of hydroperoxide by heme compounds was first order with respect to the heme catalyst (O'Brien, 1969).

During the decomposition of linoleic and hydroperoxide by hemoproteins it was previously shown that there was a decrease in the conjugated diene peak accompanied by an increase in the absorption at 280 nm (O'Brien, 1969). The latter compounds were presumably formed by scission of the carbon chain at the double bond with the simultaneous production of such decomposition products as carbonyl compounds. The difference spectral changes found during microsomal lipid peroxidation in which the ratio of the 270-nm peak to that of the 233-nm peak increases as the oxidation proceeds could be explained if cytochrome P-450 catalyzes not only lipid peroxidation but also the decomposition of the lipid peroxides formed (Hrycay and O'Brien, 1971).

The function of cytochrome P-450 in liver microsomes is believed to be the hydroxylation of fatty acids, steroids, drugs, alkanes, and other xenobiotics. Cytochrome P-450 functions as a mixed function oxidase in this system. The discovery that cytochrome P-450 can catalyze lipid peroxidation probably indicates that in its role as a lipoxygenase, cytochrome P-450 is acting as a dioxygenase. The previous discovery that cytochrome P-450 can also act as a powerful peroxidase (Hrycay and O'Brien, 1971) suggests a mechanism for lipid peroxidation in which lipid can also act as a peroxidase hydrogen donor. Lipid peroxides could be formed from the reaction of oxygen with the resulting lipid radicals *via* a series of intermediates and without involving a free-radical chain reaction (Smith and Lands, 1972).

Cytochrome P-450 was similar in thermolability, kinetics, and turnover number to the plant lipoxygenases and could be the animal equivalent of this enzyme. Both catalysts were also similar in their requirement for peroxide and inhibition by various antioxidants. However, the plant lipoxygenase has a much higher specificity for peroxide (Tappel, 1963) and also forms an optically active hydroperoxide which is usually one particular isomer, *e.g.* the 9 or 13 hydroperoxide of linoleic acid. On the other hand hematin compounds are nonspecific and catalyze the oxidation of many olefin compounds (Tappel, 1963).

Cytochrome P-450 could be a principal mechanism for intracellular lipid peroxidation in the liver. A similar mechanism involving the other hemoproteins is also presumably involved in the oxidative deterioration of lipid containing foods.

It has also been found that maximal induction of cytochrome P-450 by drugs requires the presence of dietary peroxidized lipids (Marshall and McLean, 1971a,b). It seems likely that this induction effect is either related to detoxification of these reactive molecules, which are present in most natural diets, or that intracellular lipid peroxidation is involved in the induction. Thermally oxidized corn oil may also induce cytochrome P-450 (Andia and Street, 1974).

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