U.D.C. 547.915.5

Biochemical Importance of Lipid Peroxides†

V.Karnojitzky

The review deals with the peroxides of unsaturated lipids; the process involving their formation is described and the catalytic action of lipoxidase and haematin compounds is illustrated. The modifying effects of various metals and their chelate complexes are discussed. The mechanism of their decomposition with formation of carbonyl, hydroxy-, epoxy-, and polymeric compounds is described.
The effect of these peroxides on haematin compounds protein thiols, enzymes, and vitamins of various types has been investigated. Various pathological changes induced by the introduction of polyunsaturated lipids and the toxicity of various oils and fats in food after heating in air have been examined. A study has been made of the damage caused by the formation of unsaturated lipid peroxides in various parts of the living cell, such as mitochondria, microsomes, lysosomes, and nuclei and of the toxicity of various types of lipid peroxides injected into living organisms, The appearance of lipid peroxides during photosynthesis and their involvement in the formation of cutin are described.
The bibliography includes 359 references.

CONTENTS

I.	Introduction	630
II.	Lipoxidase	631
III.	Haematin compounds	632
IV.	Various metals and their chelate complexes	633
V.	Nature of the compounds formed on decomposition of lipid peroxides	634
VI.	Effect of lipid peroxides on various substrates	637
VII.	The role of lipid peroxides in the deterioration of food products	639
VIII.	Changes in living organisms induced by lipid peroxides and their decomposition products	639
IX.	Pathological changes induced *in vivo* by a diet containing various lipids heated in the presence of air	640
X.	Changes in tissue cells occurring on formation of peroxides	643

I. INTRODUCTION

An earlier paper by the present author and Viel dealt with the biochemical role of organic peroxides and their synthesis [1] and a paper by Latarjet [2] was concerned with their radiobiology. This review is devoted exclusively to lipid peroxides and covers publications up to 1970, the majority of which appeared in print after 1965. Thus, apart from the results of quite recent studies, the review considers certain questions relating to the effect of lipid peroxides on various types of enzymes and vitamins, their role in the breakdown of certain parts of the cell, and the inhibiting effect of vitamin E in the formation of lipid peroxides *in vivo*, i.e. problems which were only touched upon or not discussed at all in earlier publications.

It is noteworthy that the biochemistry of lipid peroxides constitutes a fairly well ordered and homogeneous field. The mechanisms of their formation and decomposition are known and many of the decomposition products have been identified. There is fairly accurate information concerning the effect of peroxides on haematin compounds, protein thiols, and various enzymes and vitamins, about the inhibiting effect of vitamin E and the changes occurring as a result of the formation of lipid peroxides *in vivo*, and also about the toxicity of various oxidised oils and fats in food. In view of all the above considerations, the author thought it necessary to write the present review dealing with lipid peroxides.

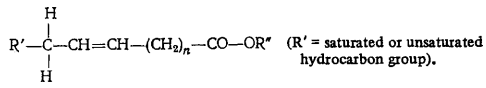
Lipid peroxides are formed as a result of the autoxidation of mono- and poly-unsaturated aliphatic acids which are present in the form of triglycerides in plant oils and animal fats. These acids can also exist in the form of phospholipids and sterol esters in various animal tissues. They are mainly oleic, linoleic, linolenic, and arachidonic acids containing respectively one, two, three, and four double bonds. Oleic acid is very common in both plants and animals and linoleic acid is particularly prevalent in plants. Linolenic acid is encountered much more rarely than the other acids but nevertheless its content is exceptionally high in linseed oil (50%) and in horse fat (16%). Arachidonic acid is present almost exclusively in animals, in which it is synthesised from linoleic acid introduced with food products. Apart from the acids enumerated above, tetra-, penta-, and hexa-enoic acids are present in animal tissues. Considerable amounts of a C_{20} acid with five double bonds and a C_{22} acid with six double bonds are present in cod liver oil‡.

At an elevated temperature and for higher degrees of unsaturation, the induction period in the autoxidation reaction becomes shorter and the rate of reaction increases. The mechanism of the autoxidation of unsaturated fatty acid derivatives has been described by Bolland [3] and Farmer [4,5] and has been discussed in detail in a number of other publications [6-9].

† The review was written originally in French specially for the journal "Uspekhi Khimii" and was translated into Russian by T. Va. Chernysheva. This is an English translation of the Russian version.

‡ Henceforth unsaturated acids will be designated by symbols such as 20 : 5 for the C_{20} acid with five double bonds, 22 : 6 for the C_{22} acids with six double bonds, etc. (Editor of Russian translation).

The first step is the abstraction of a hydrogen atom from the methylene group in the α-position relative to the double bond of the unsaturated compound:



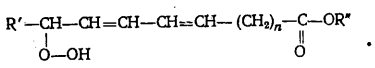
(R' = saturated or unsaturated hydrocarbon group).

The $R'-\dot{C}H-CH=CH-(CH_2)_n-CO-OR''$ radical formed in this way reacts with molecular oxygen, giving rise to the peroxy-radical $RO-O^{\cdot}$ [$R=R'-\dot{C}H-CH=CH-(CH_2)_n-CO-CR''$] which abstracts a hydrogen atom linked to the methylene carbon atom in the α-position of another lipid chain with formation of an α-methylene hydroperoxide and a radical $R^{\cdot}$:

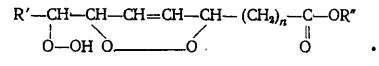
$$RO-O^{\cdot} + HR \rightarrow ROOH + R^{\cdot} .$$

The hydroperoxide in its turn decomposes into the radicals $RO^{\cdot}$ and $HO^{\cdot}$ which initiate new reaction chains.

The formation of the hydroperoxide is accompanied by the migration of the double bonds which are conjugated in polyunsaturated systems:



In the initial stages it may be accompanied by the formation of more complex derivatives of the hydroperoxide–peroxide type:



It is interesting to note that such structures were previously proposed for polyisoprene [10-18] and squalene [17] peroxides. Thus the term "lipid peroxides" refers to a mixture of several hydroperoxides (with a large number of conjugated double bonds), glycerides, phospholipids, and sterol esters of several unsaturated fatty acids incorporating in all probability peroxy-compounds of the hydroperoxide–peroxide type.

There are two main types of effective catalysts of the autoxidation of unsaturated lipids: lipidoxidase, which is highly specific for the determination of configurations, and haematin compounds—non-specific catalysts active in relation to many compounds undergoing autoxidation which are not unsaturated lipids. The speed of their action may be greatly altered in the presence of certain metals and their chelate complexes.

II. LIPOXIDASE

Lipoxidase is absent from animal tissues [19,20] but does occur in plants, particularly in Leguminosae (soya, peas, beans, wheat, groundnuts, clover, vetch, alfalfa, kidney beans, etc.). It has also been detected in radishes and in potatoes. According to quite recent studies, the wheat and pea lipoxidases [21] as well as the soya lipoxidase [22] contain several coenzymes. The presence of lipoxidase in microorganisms is doubtful. In fact *Pseudomonas fluorescens* and *Mycobacterium phlei* are the only bacteria which rapidly oxidase unsaturated fatty acids, but this oxidation is not catalysed by lipoxidase; it has been detected in the *Aspergillus* and *Rhizopus* moulds [23] but the actual oxidation catalyst was not determined exactly.

The preparation of pure lipoxidase from soya flour has been described in detail [24,25] and a new improved method [26] based on the extraction with water of defatted soya flour at pH 4.5 and room temperature was used recently. The enzyme is isolated after successive salting-out with aqueous ammonium sulphate solutions and subsequent electrophoresis.

Pure lipoxidase consists of colourless lamellar microcrystals with molecular weight ranging from 90 000 to 100 000. [25] Since it cannot be inhibited by fluorides, cyanides, azides, pyrophosphates, and diethyl dithiocarbamate prosthetic groups must be absent [26]. Similarly the absence of inhibition by mercury chlorobenzoate demonstrates the absence of SH groups, which is confirmed by chemical determinations [27].

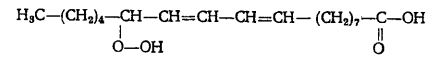
The catalytic activity of pure lipoxidase was studied in an aqueous 1.5×10^{-2} M solution of sodium linoleate in the temperature range from 0° to 37°C at different pH. [27] The results of this study showed that the maximum rate of oxidation occurs at pH 9.4, decreasing slightly at alkaline pH and diminishing appreciably at acid pH. Thus at pH 6.5 its activity is only one fifth of the activity at pH 9.4. The initial rate of oxidation at 37°C is higher than at 0°C but in the former case it decreases rapidly with time. Thus after 10 min the content of O_2 absorbed is identical for both temperatures, amounting to 0.15 mole mole^{-1}, but after 40 min it is only 0.4 mole mole^{-1} at 37°C and 0.6 mole mole^{-1} at 0°C.

The use of certain crude lipoxidases for the oxidation of derivatives of polyunsaturated acids at temperatures between 0° and 37°C has been described [20,28-36].

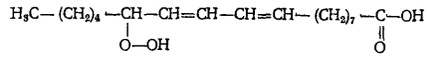
A comparative study [37] of the catalytic activities (at 20°C and pH 7) of soya, pea, groundnut, wheat, *Phaseolus mungo*, and *Phaseolus aurens* lipoxidases in the oxidation of linoleic and arachidonic acids by ammonium salts showed that none of the lipoxidases have prosthetic groups, while the *Phaseolus mungo* and *Phaseolus aurens* lipoxidases probably contain SH groups.

It follows from the above findings that lipoxidase is a highly specific catalyst in the oxidation of unsaturated lipids. Indeed it catalyses the oxidation of only penta-1,4-diene compounds $-CH=CH-CH_2-CH=CH-$ with the *cis*-configuration of the double bonds (as in natural fatty acids). It has been found that lipoxidase does not affect the rate of oxidation of mono- and di-unsaturated conjugated compounds or pentadiene compounds with the *cis-trans*- or *trans-trans*-configuration; it should be noted that the latter behave as inhibitors in oxidation reactions catalysed by *cis-cis*-pentadiene compounds. Moreover, the rate of oxidation of highly unsaturated compounds catalysed by lipoxidase is independent of the degree of their unsaturation. Thus the ammonium salts of linoleic, linolenic, and arachidonic acids are oxidised at the same rate [37]; the same can also be said concerning the esters of these acids [33,38]. Hence it follows that the catalytic action of lipoxidase diminishes with increasing degree of unsaturation of the substrate. The study [38] of the autoxidation and the oxidation catalysed by lipoxidase at 13°C of the ethyl esters of linoleic and linolenic acid showed that O_2 is absorbed (mole mole^{-1}) over a period ranging from 0.2–0.9 to 50 h in the autoxidation, while in the oxidation catalysed by lipoxidase it is absorbed over a period between 0.5 and 50 min for both esters.

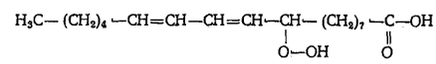
A recent study [39] of the oxidation of soya, linoleic acid, and its methyl ester catalysed by lipoxidase demonstrated that O_2 attacks only the 13-C carbon atom with formation of a single conjugated hydroperoxide,



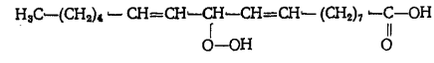
with the *cis-trans*-configuration (and not *cis-cis*), while in autoxidation and in oxidation catalysed by haematin compounds the attack is directed simultaneously to 13-C and 9-C, which leads to a mixture of two conjugated hydroperoxides:



and



formed in equal amounts. Moreover there is a certain amount (of the order of 10%) of the non-conjugated hydroperoxide

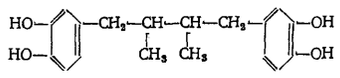


It is noteworthy that the hydroperoxides obtained on oxidation catalysed by lipoxidase are optically active in contrast to the hydroperoxides formed on autoxidation. These results demonstrate the stereospecificity of the catalytic action of lipoxidase.

The mechanism of the oxidation catalysed by lipoxidase has been discussed in detail [37]. Probably initially a transition enzyme–substrate complex is formed and in the second stage a free radical should be generated at the α-methylene carbon atom as a result of the capture of one electron by the lipoxidase protein. In the presence of molecular oxygens, this free radical should lead to the classical autoxidation reaction (the mechanism of which is quoted above).

The inhibiting effect of various phenolic compounds on the oxidation of a 7×10^{-3} M aqueous solution of sodium linoleate at pH 9 catalysed by soya bean lipoxidase has been studied [31] at temperatures ranging from $-6°$ to 30°C. The degree of inhibition (measured as a percentage decrease of the rate of absorption of O_2) increases with temperature. For nordihydroguiaretic acid at a concentration of 10^{-4} M, it amounts to 100% at 30°C and only 32% at 0°C. At 30°C and the same concentration, the relative degrees of inhibition for α-tocopherol, hydroquinone, propyl gallate, and nordihydroguiaretic acid are respectively 27, 38, 100, and 100% . Nordihydroguiaretic acid (NDGA) also proved to be very active in oxidation reactions catalysed by various crude lipoxidases, namely the oxidation of the ammonium salts of linoleic and arachidonic acids (at 20°C and pH 7). The use of propyl gallate, hydroquinone, and α-tocopherol led to different results depending on the nature of the lipoxidase [37].

NDGA, which will be frequently mentioned from now on, is 2,3-dimethyl-1,4-bis-(3,4-dihydroxyphenylbutane):



NDGA is widely used in the USA as an inhibitor of the oxidation of many food products at a concentration of the order of 10^{-4} M.

Apart from the studies described above, the catalytic action of lipoxidase is dealt with in four extensive publications which also contain important historical data [8,40–42].

III. HAEMATIN COMPOUNDS

Haemoglobin, myoglobin, haemin, and cytochrome c are the most important biological catalysts of the autoxidation of unsaturated animal lipids. They are involved in many physiological *in vivo* reactions and also in the oxidation of food products during storage (rancidity).

In contrast to lipoxidase (which catalyses the oxidation of pentadiene systems with the *cis-cis*-configuration only), haematin compounds catalyse the oxidation not only of all the unsaturated lipids, but also of many aliphatic-aromatic and alicyclic hydrocarbons [43]. However, their catalytic activity is shown only in a colloidal medium or in an emulsion but not in a homogeneous system (lipoxidase is active in both heterogeneous and homogeneous media). Thus in the oxidation of linoleic acid catalysed by haemin, the absorption of oxygen ceases on passing from an aqueous emulsion to a homogeneous solution (as a result of the addition of acetic acid, dioxan, or pyridine) and restarts when the heterogeneous medium is restored by the addition of water [44].

The rate of oxidation increases with the degree of unsaturation of the substrate. Thus in the oxidation of the methyl esters of oleic, linoleic, and linolenic acids (at a concentration of 2×10^{-2} M) in an emulsion at 0°C catalysed by haemin (at a concentration of 2×10^{-5} M), the rates of absorption of oxygen are respectively 1.3 and 6. [45]

The rate of oxidation is proportional to the square root of the concentration of the haematin compound. This was confirmed for the oxidation of an aqueous emulsion of 0.1 M ammonium linoleate catalysed by haemoglobin [45], cytochrome c [45], and myoglobin [46] with the catalyst concentration varying from 1×10^{-6} to 7×10^{-6} M.

It is very significant that at high concentrations (ranging from values of the order of 10^{-3} M to 10^{-2} M for 1 M substrate) haematin compounds inhibit the oxidation of unsaturated lipids. A study [46] of the oxidation of an aqueous emulsion of 17×10^{-3} M linoleic acid showed that haemoglobin, which is an active catalyst at a concentration of 4×10^{-6} M, becomes an inhibitor at a concentration of 4×10^{-5} M: haemin and cytochrome c also become inhibitors at concentrations in excess of 10^{-4} M. Likewise it has been found [47] that cytochrome c has an inhibiting effect at high concentrations in the oxidation of linoleic acid.

In the oxidation process catalysed by haematin compounds, the latter decompose with the liberation of iron. This has been observed in the oxidation of aqueous 0.2 M emulsions (pH 6.8) of linoleic and linolenic acid catalysed by haemoglobin and haematin [48].

The finding that the catalytic action of haematin compounds is not inhibited by carbon monoxide and changes only slightly on formation of haemochromes with nitrogenous bases despite an appreciable change in the oxidation-reduction potential suggests that the valence of iron (in the haematin compound) is not altered during the oxidation.

The usual autoxidation inhibitors such as phenols and amines proved to be active in the oxidation of various unsaturated lipids catalysed by haematin derivatives.

α-Naphthol, catechol, p-aminophenol, and dimethylalanine are effective inhibitors in the oxidation of aqueous emulsions of oleic acid and linseed oil at 38°C catalysed by haemin [49]. The action of various enolic inhibitors, α-tocopherol, NDGA, and 2,6-di-t-butyl-4-methylanisole was examined in a study [50] of the oxidation of colloidal sodium linoleate (0.2 M) at 37°C catalysed by haemoglobin and cytochrome c. The concentration of the haematin compound was 2×10^{-5} M. It was found that α-tocopherol

is a weaker inhibitor than NDGA. Indeed in the oxidation catalysed by haemoglobin a 30 m induction period is attained at an NDGA concentration as low as 2×10^{-5} M, whereas the concentration of α-tocopherol required to produce the same effect must be higher by a factor of 10. Similar results were obtained also with cytochrome c. The action of various enolic inhibitors and synergistic agents (such as aminoacids and ascorbic acid) on the oxidation of methyl oleate, methyl linoleate, pig fat, cod liver oil, and linseed oil at 37°C, catalysed by haemin, haemoglobin, and cytochrome c, has been examined in detail [50,51].

As in the previous case, the inhibiting effect of α-tocopherol is weaker than that of NDGA, 2,6-di-t-butyl-4-methylphenol, and 2,6-di-t-butyl-4-methylanisole.

In the oxidation of pig fat at 37°C, catalysed by haemoglobin, ascorbic acid proved to be an excellent synergistic agent over a narrow range of concentrations: 10^{-4} to 10^{-3} mole kg^{-1}. The optimum concentrations of the inhibitor and the synergistic agent are as follows in moles per kilogramme:

NDGA	10^{-3}	NDGA	10^{-4}
Ascorbic acid	10^{-3}	2,6-Di-t-butyl-4-methylphenol	10^{-4}
		Ascorbic acid	10^{-4} .

These compositions increase the induction period by factors of 85 and 300 respectively. The oxidation catalysed by haematin derivatives may be inhibited also by other compounds and not only the usual inhibitors of chain autoxidation. Thus in the autoxidation of a 0.1 M emulsion of oleic acid at 37°C, leuco-Methylene Blue (at a concentration of 10^{-3} M) increases the rate of absorption of oxygen by a factor of 20, while in the oxidation catalysed by haemin (at a concentration of 10^{-5} M) the rate decreases by 20%. Probably there is a coordination between the N and O atoms with formation of a complex more resistant to oxidation.

Haematin compounds catalyse the decomposition of hydroperoxides in an inert atmosphere. The decomposition [43] of a 0.1 M emulsion of the peroxide of sodium linoleate at 40°C, catalysed by haemin (at a concentration of 2×10^{-4} M), showed that the peroxide content decreases faster in the presence of haemin than in its absence, particularly at the beginning of the reaction. Thus after 10 h the corresponding degrees of decomposition are 70 and 40% and after 60 h 96 and 84%. The initial content of hydroperoxide was 0.08 mole per mole of linoleate.

The catalytic action of various haematin compounds (catalase, haemoglobin, haematin, and cytochrome c) in the decomposition of sodium linoleate hydroperoxide has been discussed [52]. The formation of RO· radicals in the decomposition process is explained by the high yield of the corresponding carbanions in the presence of compounds (hydrogen donors) such as polyphenols [53].

Thus the corresponding alcohol is formed in 94% yield from a mixture of methyl linoleate hydroperoxide (1 M), hydroquinone (2 M), and haemoglobin (5×10^{-3} M) when it is allowed to stand in alcoholic solution at room temperature [53]. Similar results were obtained for cumenyl hydroperoxide [53] but with a lower yield (80%) of cumenyl alcohol, owing to some decomposition of the $C_6H_5(CH_3)_2CO$· radicals.

The fact that the rate of the autoxidation reaction (catalysed by haematin compounds) is proportional to the content of hydroperoxide (formed or added) and that in an inert atmosphere haematin compounds decompose the hydroperoxide ROOH with formation of RO· radicals suggests that the hydroperoxide should be formed initially without the involvement of haematin compounds. Then, evidently, the hydroperoxide may decompose with formation of RO· radicals which probably initiate the oxidation reaction, the chain mechanism of which may be inferred from the action of the usual effective autoxidation inhibitors. Nevertheless the mechanism of the decomposition of the hydroperoxide formed differs somewhat from that of the reaction catalysed by variable-valence metals:

$$ROOH + M_n \rightarrow RO\cdot + {}^{-}OH + M_{n+1} .$$

The constant valence of iron in the oxidation process suggests that an electron is abstracted from the haematin protein and not from the metal, as in the preceding case.

Apart from the classical chain termination reaction $ROO\cdot + {}^{\cdot}OOR \rightarrow ROOR + O_2$, the peroxy-radicals probably also interact with haematin compounds [45], which leads to shorter chains and a higher content of carbonyl compounds than in simple autoxidation.

The mechanism of the oxidation catalysed by haematin compounds has been discussed in detail in several earlier publications [8,43,44,52-55].

IV. VARIOUS METALS AND THEIR CHELATE COMPLEXES

The oxidation of 0.017 M aqueous emulsions of linoleic and linolenic acids at 37°C in the pH range 4.5–7.5 catalysed by various metals and their chelate complexes has been the object of serious study [56]. Among the many metals tested, very few are active. Thus Co^{2+} and Mn^{2+} are very active, while Cu^{2+}, Fe^{2+}, and Fe^{3+} are much less active; at a concentration of 10^{-3} M (one mole for ten moles of linoleate) Fe^{2+}, Fe^{3+}, Cu^{2+}, V^{2+}, Mn^{2+}, and Co^{2+} increase the absorption of O_2 by factors of 2, 3, 9, 14, 136, and 170 respectively. The catalytic activity of Fe^{2+} ions has been investigated in the oxidation of colloidal linoleic acid (0.02 M) at 30°C and pH 7 as a function of their concentration [57].

We may note that the catalytic activity of Fe^{3+} increases markedly in the presence of a reducing agent such as ascorbic acid or cysteine and depends greatly on the pH, in contrast to haematin catalysis which is very little sensitive to changes in the pH. Thus in the presence of ascorbic acid the maximum rate of oxidation (which exceeds that of autoxidation by a factor of 54) occurs at pH 5 and Fe^{3+} and ascorbic acid concentrations of 10^{-2} and 10^{-3} M respectively.

In contrast to the behaviour in the presence of Fe^{3+}, the catalytic activity of haematin compounds is weakened in the initial stage as a result of the addition of ascorbic acid, the reaction becoming autocatalytic subsequently owing to the formation of free iron by the decomposition of haematin compounds.

Thus in the oxidation of linoleic acid catalysed by haemoglobin (5×10^{-6} M) or cytochrome c (10^{-5} M) the addition of ascorbic acid (10^{-3} M) gives rise to a 30 min induction period, while in its absence absorption of O_2 begins immediately. Moreover, ascorbic acid does not affect the catalytic action of Cu^{2+} but greatly inhibits that of Co^{2+}.

At a concentration in excess of 0.1 M, phosphates inhibit the catalytic action of Co^{2+} and the Fe^{3+}–ascorbic acid system but their optimum concentrations have not yet been determined.

The rate of oxidation of linoleic acid does not change in the presence of 8-hydroxyquinoline and $\alpha\alpha'$-dipyridyl, while o-phenanthroline (2×10^{-3} M) increases it slightly;

however, the rate does not change at all in the simultaneous presence of the metals and complex-forming agents mentioned above. All the metals were used at a concentration of 10^{-3} M (except for 5×10^{-4} M Co^{2+}) with the ratio

$$K = \frac{\text{moles of complex-forming agent}}{\text{gram-atoms of metal}}$$

varying from zero to 5.

When *o*-phenanthroline is employed, with K varying from zero to 2, the rate of absorption of O_2 increases by a factor of 60 for Fe^{2+} and decreases by a factor of 170 for Co^{2+}, the increase for Cu^{2+} being only by a factor of 2.

8-Hydroxyquinoline has a less pronounced modifying effect than *o*-phenanthroline. Indeed, when K is varied from 0 to 2, the rate of absorption of O_2 increases only by a factor of 16 for Fe^{2+} and decreases by factors of 2 and 4 for Cu^{2+} and Co^{2+} respectively.

Blood serum albumin and egg albumin (at a weight concentration of 1%) increase respectively by factors of 1.9 and 3.5 the rate of O_2 uptake in the oxidation of linoleic acid catalysed by the Fe^{3+} (10^{-3} M)–ascorbic acid (10^{-3} M) system.

The catalytic activity of Cu^{2+} also increases in the presence of certain proteins. In one of the studies[58] on the oxidation of 0.1 M colloidal solution of ammonium linoleate (0°C and pH 7) catalysed by Cu^{2+} ions (10^{-3} M) it was shown that egg albumin (10^{-4} M) increases the rate of absorption of O_2 by a factor of 5. This must mean that haemocyanin catalyses the oxidation of unsaturated fatty acids.

On the other hand, horse serum, even at a dilution by a factor of 50, greatly reduces the activity of the Fe^{3+}–ascorbic acid system and completely inhibits it at a dilution of 20. It shows a similar but less marked effect on the catalytic activity of Co^{2+}, while the catalytic activity of haematin compounds changes little in its presence. Apart from weakening the action of certain catalytic systems in the oxidation of linoleic acid, it has a very pronounced inhibiting effect on the formation of lipid peroxides (pH 7) in rat brain homogenate[59]. The inhibiting effect of this serum must be a consequence of the formation of a Fe^{3+} complex of serum siderophilin.

Among numerous aminoacids, only histidine and its derivative histidylhistidine proved very effective inhibitors of the oxidation of linoleic acid catalysed by Co^{3+} (2×10^{-4} M). The inhibiting action depends greatly on the pH, becoming completely insignificant in an acidic medium. For example at pH 7 histidine (4 moles per gramme atom of Co^{2+}) decreases by a factor of 50 the rate of absorption of O_2, while at pH 5 the rate of absorption of oxygen decreases only by 1%.

At pH 7 the catalytic action of Co^{2+} is also weakened by phenylalanine but to a much less extent than by histidine.

Cysteine acts in the presence of Fe^{3+} like ascorbic acid, the maximum acceleration occurring at pH 4 and becoming zero at pH 8. At pH 5 the Fe^{3+} (10^{-3} M)–cysteine (3.3×10^{-3} M) system increases the rate of oxidation of linoleic acid by a factor of 10.

V. NATURE OF THE COMPOUNDS FORMED ON DECOMPOSITION OF LIPID PEROXIDES

The unsaturated lipid with the general formula $R-CH_2-CH=CH-R'$ (R' = saturated or unsaturated groups) initially gives rise to the hydroperoxide $R-\underset{\underset{O-OH}{|}}{CH}-CH=CH-R'$, which undergoes homolytic decomposition into the radicals $R\dot{C}H-\underset{\underset{O\cdot}{|}}{CH}=CH-R'$ and $\cdot OH$.

The $\cdot OH$ radicals can interact with the initial lipid, attacking double bonds with formation of the dihydroxy-compound $R-CH_2-CHOH-CHOH-R'$, which may lead to the corresponding hydroxyketone and diketone as a result of dehydration.

The $\cdot OH$ radicals can also attack the α-methylene carbon atom with formation of H_2O and the $R-\dot{C}H-CH=CH-R'$ radical (which is involved in the chain oxidation reaction).

The $R-\underset{\underset{O\cdot}{|}}{CH}-CH=CH-R'$ radicals can participate in the chain reaction, attacking the initial lipid with formation of the monohydroxy-compound $R-CHOH-CH=CH-R'$ and regeneration of the $R-\dot{C}H-CH=CH-R'$ radical. They can also decompose in two ways: (1) with dissociation of the double bond in the α-position and formation of the aldehyde RCHO and the radicals $\cdot CH=CH-R'$, which combine with the $\cdot OH$ radicals to give the unstable enol $HO-CH=CH-R'$, isomerising into the aldehyde $OCH-CH_2-R'$; such dissociation leads to the formation of two unsaturated aldehydes (with a saturated group R'); (2) with dissociation of the double bond in the β-position and formation of the radicals $R^{\cdot}$ and the aldehyde $O=CH-CH=CH-R'$.

The radicals $R^{\cdot}$ combine with $\cdot OH$ radicals to give the alcohol ROH. The greater content of carbonyl compounds compared with alcohols, particularly at low temperatures is probably due to the greater ease of dissociation in the α-position compared with the β-position.

The aldehydes thus formed undergo in turn degradative oxidation via two parallel reactions. Thus the C_n aldehyde with the general formula RCH_2CHO may be oxidised to the peroxide $R-CH_2-\underset{\underset{O}{\|}}{C}-O-OH$, which then decomposes into the C_{n-1} alcohol RCH_2OH and carbon dioxide[60-62] (or reacts with the initial aldehyde to form the acid RCH_2COOH).

The aldehyde can be also oxidised to an aldehyde hydroperoxide:

$$R-\underset{\underset{O-OH}{|}}{CH}-CHO .$$

The peroxide decomposes homolytically with subsequent elimination of hydrogen atoms from the α- or β-position relative to the carbonyl group[63]. Dissociation in the α-position leads to a C_{n-1} aldehyde and formic acid:

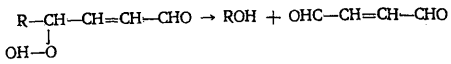
$$R-\overset{\overset{H}{|}}{\underset{\underset{O-OH}{|}}{C}}-CHO \rightarrow RCHO + HCOOH .$$

Dissociation in the β-position yields a C_{n-2} alcohol ROH and glyoxal.

With a C_n α-unsaturated aldehyde $R-CH_2CH=CH-CHO$, a hydroperoxide should be formed in the α-position relative to the double bond (i.e. in the γ-position relative to the carbonyl group), which can probably decompose owing to the dissociation in the α-position into a C_{n-3} aldehyde $R-CHO$ and malonic dialdehyde:

$$R-\underset{\underset{O-OH}{|}}{CH}-CH=CH-CHO \rightarrow R-CHO + HO-CH=CH-CHO \rightarrow OCH-CH_2-CHO$$

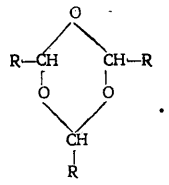
or into a C_{n-4} alcohol ROH and fumaric dialdehyde as a result of dissociation in the β-position:



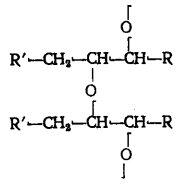
The aldehyde α-hydroperoxide R—CH(O—OH)—CHO can also decompose into H_2O and an α-ketoaldehyde RCOCHO.

The foregoing considerations account for the formation of a large number of carbonyl and hydroxy-compounds with short chains as well as formic acid in the oxidation of many unsaturated lipids. Malonic dialdehyde merits particular mention; its reaction with thiobarburic acid results in a red colour. This reaction has been frequently used for a quantitative determination of peroxides in biological media and has been discussed[64]. Mention should also be made of the formation of formates[65] which are probably produced as a result of the esterification of alcohols by formic acid.

In the presence of formic acid, aldehydes can lead to the formation of simple or mixed trioxans:

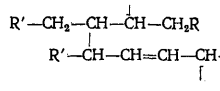


Polymers of two types may be produced. In the first case two carbon atoms are linked via an oxygen atom; the polymers correspond to the general formula



and are formed as a result of the dehydration of dihydroxy-compounds. They are produced in the presence of oxygen at a moderate temperature.

In the second case a bond is formed between carbon atoms:



In this case polymers are produced as a result of the action of dihydroxy-compounds on the olefinic compound with elimination of water. They are formed in the thermal decomposition of the hydroperoxide in an inert atmosphere. It should be noted that the polymers may contain in their chain functional groups (HO, CO) produced as a result of attack on the α-methylene carbon atom or the double bond.

At a high temperature (>250°C) and even in the absence of O_2, polymers with cyclohexene rings may be formed owing to the Diels—Alder addition of a conjugated polyolefinic compound to the initial non-conjugated polyolefinic compound. The mechanism of the formation of various types of polymers and also their determination have been the subject of a publication with 60 literature references[66].

The autoxidation of unsaturated lipids is discussed in a long paper with 192 references[67]. Another publication deals with various peroxides of unsaturated fatty acid esters[68].

Among monounsaturated lipids, the oleic acid derivatives have been most thoroughly investigated. The hydroperoxide of methyl oleate has been isolated with a purity of 85% and its properties have been described[68-70]. It is a colourless and odourless liquid readily soluble in organic solvents, decomposing slowly at room temperature, rapidly at 120°C, and in 15 min at 150°C. The autoxidation of methyl oleate has been studied[71-81] and it has been shown[78] that the *cis*-form (oleate) is converted into the *trans*-form (elaidate) during the autoxidation process.

It is clear from the above studies that the principal compounds formed in the autoxidation of methyl oleate are water, mono- and di-hydroxymethyl sterates, saturated C_7—C_9 aldehydes, C_{10} and C_{11} unsaturated aldehydes, and the hemialdehydes of suberic and azelaic acids. We may mention that α-undecenal is the main compound formed by the direct decomposition of the hydroperoxide of methyl oleate at 150°C. The formation of the above carbonyl compound can be accounted for on the basis of the considerations described in the preceding section, since the OOH group can occupy positions ranging from the 7th to the 12th as a result of double bond migration. Apart from the products mentioned above, numerous hydroxy- and carbonyl compounds are produced in consequence of the subsequent autoxidation of the aldehydes produced in the initial stage of the hydroperoxide decomposition.

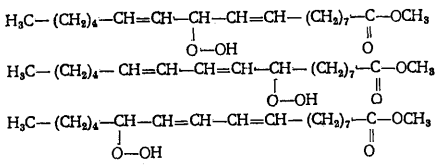
The polymer chains formed in the autoxidation of methyl oleate at 65°C [in the presence of cobalt(II) oleate] contain ester linkages[79]. The polymers obtained in the decomposition of hydrogen peroxide at 210°C[82] consist of carbon chains.

The autoxidation of oleic acid esters which are higher homologues of methyl, ethyl, and propyl oleates has also been investigated[83], but the corresponding hydroperoxides were not isolated. It is noteworthy that the maximum concentration of hydroperoxide at a given temperature decreases with increasing molecular weight of the ester. The autoxidation of oleic acid has been examined in a number of investigations[73-81,84-86]. In the latest study[86] carried out in thin layers at 60—80°C special attention was paid to the isolation and characterisation of the volatile products formed in the autoxidation.

The autoxidation of higher homologues of oleic acid and their methyl esters has also been the subject of several investigations. One fo them concerns the autoxidation of erucic acid and its methyl and propyl esters[87] and another deals with the autoxidation of brassidic acid[88]. The investigations have shown that, as with oleic and elaidic acids, the *cis*-isomer (erucic acid) undergoes autoxidation faster than the *trans*-isomer (brassidic acid). Dihydroxybehenic acid was detected among the autoxidation products. A further investigation dealt with the autoxidation at 40°C of the methyl esters of oleic, elaidic, erucic, and α-undecenoic acids[89]; it showed that the rate of autoxidation decreases in the sequence indicated above. In a recent study[90] on the autoxidation of methyl undecenoate at 50°C, a hydroperoxide (in two stereoisomeric forms) and the corresponding epoxy-compounds were characterised.

The autoxidation of methyl linoleate has been the subject of several investigations[72,91-95]. The last study was concerned with the action of various inhibitors on the autoxidation of the *cis-cis*- and *trans-trans*-isomers. In the dark at 40—60°C there is an induction period of 15 min, while at 80°C the autoxidation begins almost at once.

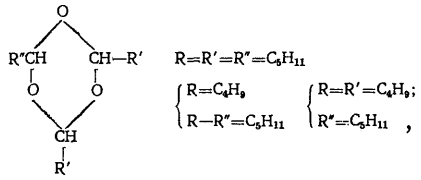
The corresponding hydroperoxide has been isolated [91-93] with purities of 84, 92, and 92% respectively. It consists of a mixture of three isomers with the following formulae:



The isomers with a conjugated diene group having the *cis-trans*-configuration constitute 90% of the total amount of hydroperoxide. Nevertheless, according to a recent investigation [94] of the autoxidation of methyl and ethyl linoleates at 60°C in chloroform solution, conjugated diene groups appear only when the hydroperoxide content is of the order of 100–200 millimoles per kilogramme of ester.

The primary decomposition products of the peroxides of methyl linoleate consist of saturated aldehydes (hexanal, pentanal), octenal, deca-2,4-dienal, and the hemialdehydes of suberic and azelaic acids.

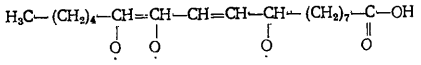
The above aldehydes were isolated also in the autoxidation at 37°C of an aqueous 2×10^{-2} M solution of ammonium linoleate [96]. The aldehydes obtained in the first stage of the decomposition of the above hydroperoxides may condense into trioxans, may be oxidised to the corresponding acids, or can undergo further oxidative degradation with formation of various carbonyl and hydroxy-compounds with lower molecular weights. The formation of 2,4,6-trialkyl-1,3,5-trixoans, simple or mixed, corresponding to the general formulae



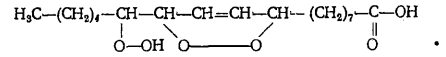
was observed in the oxidation of methyl linoleate in a thin layer at room temperature. The trioxans were obtained as a result of the polymerisation and copolymerisation of pentanal and hexanal. Among the decomposition products of the initial aldehydes, special mention should be made of the dicarbonyl compounds consisting of glyoxals, a C_6 ketoaldehyde, fumaric dialdehyde, and hex-3-ene-1,6-dial, which were isolated in the oxidation of methyl linoleate at 45°C. [97-99].

The polymers formed as a result of the autoxidation of methyl linoleate at 4°C [82] consist of a mixture of dimers and trimers with linkages via oxygen atoms. Each chain contains C=O and O–H groups and a certain number of double bonds. The polymers obtained [100] as a result of the autoxidation of methyl linoleate at 33°C and subsequent decomposition at 100°C in an inert atmosphere also consist of a mixture of dimers and trimers but here the linkages are between carbon atoms.

The autoxidation of linoleic acid was studied recently by several investigators [101-103]. The first two studies were concerned with the identification and isolation of numerous volatile compounds and the third with the kinetic relations for autoxidation at low O_2 pressures in the presence of various inhibitors. In autoxidation in thin layers at 20° and 40°C, [101,102] saturated aldehydes ranging from C_2 to C_7, alcohols ranging from C_1 ot C_5, and esters [65] (mainly formate esters with pentyl formate predominating) were isolated. Apart from the above compounds obtained in the decomposition of the monohydroperoxide, trihydroxy-, triketo-, and hydroxyketo-compounds may also be formed as a result of the disproportionation of the radicals:



The latter are products of the homolytic cleavage of the hydroperoxide–peroxide



The autoxidation of ethyl linoleate has also been the subject of several investigations [3,104-106], in which a hydroperoxide was isolated [3]. The structure of the polymers formed in the autoxidation is similar to that of the polymers produced from methyl linoleate with linkages via oxygen atoms [106].

Two studies [107,108] have been made on the autoxidation of methyl linolenate at 37°C with isolation of the corresponding hydroperoxide having a purity of the order of 80%.

The hydroperoxide is monomeric and has three double bonds, two of which are incorporated in the conjugated group; the *cis-trans*-transfiguration predominates (the amount of the *trans-trans*-configuration is insignificant). Since the OOH group can occupy the 16-, 13-, 12-, and 9-positions, the primary decomposition products of the hydroperoxide consist of propanal, pentanal, and hepta- and octa-2,4-dienals.

The dicarbonyl compounds produced as the result of a subsequent autoxidation of the above aldehydes consist of malonic dialdehyde [109] and hex-3-ene-1,6-dial [110]. The formation of carbonyl compounds in the autoxidation of methyl linolenate (and also oleate and linoleate) is discussed in two large publications [111,112]. The structure of the polymers formed during autoxidation at 30°C is similar to that of the polymers from the linoleate hydroperoxide (linkages via oxygen atoms). Saturated C_2–C_6 aldehydes, 2,4-diethyl-6-propyltrioxan, 2,4-diethyl-6-pentyltrioxan, pentanal, acids ranging from C_1 to C_6, and their various esters were isolated quite recently in a study of the autoxidation of linolenic acid at 20° and 40°C. [113] Hydroperoxides derived from methyl linoleate, methyl linolenate, ethyl arachidonate, methyl eicosapentenoate, and ethyl esters of menhaden§ fat were obtained in 95% purity in a recent investigation [114].

The autoxidation of esters was initially carried out at 4°C over a period of 80 days and then at room temperature for 148 h until the content of hydroperoxides reached 28 moles for 100 moles of ester. The peroxides were isolated from the autoxidation product thus obtained with the aid of thin-layer chromatography.

In two important investigations [115,116] of saturated lipids, a study was made of the autoxidation at 60°, 150°, and 200°C of very pure methyl palmitate. It was found that at 60°C hydroperoxide was not formed over a period of 56 days, while at 150°C its content reached 300 millimoles per kilogramme after 3 h, and at 200°C only hydroxycarbonyl compounds and carboxylic acids formed as a result of the decomposition of the peroxide could be detected. It follows from these observations that the attack of the saturated esters by molecular oxygen at 150°C leads primarily to the formation of a double bond as a result of dehydrogenation. Although probably all the carbon atoms are attacked, the hydroperoxide is formed preferentially in the centre of the molecule. The above

§ Menhaden is a fish of the herring family (Clupeidae) (Editor of Russian translation).

two papers criticise all previous investigations and quote numerous references to studies on the autoxidation of saturated lipids.

Natural fats and oils consist of triglycerides with various contents of saturated and unsaturated acids depending on their nature. Thus carbonyl compounds, formed in oxidative degradation, are of the type which would probably be formed simultaneously from oleic and linoleic acids and in some cases also from linolenic and arachidonic acids.

A lipid containing a relatively small amount of linoleic acid and in particular linolenic acid can give rise to considerable amounts of carbonyl compounds, derived from these polyunsaturated acids, in consequence of a much less extensive autoxidation of oleic acid.

There are publications [111,112] with detailed data on saturated aldehydes, unsaturated aldehydes, and dienals formed from mutton, beef, and pork fats, cocoa oil, and also from ground nut, soya bean, and linseed oils (as well as from methyl oleate, trioleate, and linolenate and ethyl linoleate). These lipids were subjected to autoxidation at room temperature and under ultraviolet irradiation until the formation of different amounts of hydroperoxide; they were then decomposed at room temperature or at 165°C.

Hexanal, oct-2-enal, and deca-2,4-dienal were obtained by the decomposition at 100°C of autoxidised cotton seed oil (with a peroxide content of 125 millimoles per kilogramme) [117]. Numerous saturated aldehydes, unsaturated aldehydes, and dienals, as well as oct-1-en-3-ol and oct-1-en-3-one were obtained from sunflower seed oil autoxidised at 37°C. [118] Saturated aldehydes ranging from C_2 to C_9, unsaturated aldehydes from C_5 to C_9, 2,4-dienals (from C_6 to C_{10} except C_9) were isolated from the autoxidation products of soya bean oil [119]; the configuration of hexa- and octa-2,4-dienals has been discussed [120]. Hexanal, oct-2-enal, and deca-2,4-dienal have been isolated [121]; the last probably consists of a mixture of two stereoisomers—2-*trans*-4-*cis*-(28%) and 2-*trans*-4-*trans*-(72%). Numerous aldehydes, ketones, and hydroxy-compounds have been identified [122,123] as well as *cis*-hex-3-en-1-al. [124] The formation of 2,4-dienals from autoxidised pork fat has been studied [125]. The isolation of octadecadienoic acids (the 11,15-isomer and a small amount of the 10,15-isomer) from beef and mutton fat is noteworthy [126]. The autoxidation was carried out at 37°C until the attainment of a peroxide content of 2 millimoles kg^{-1} with subsequent decomposition at 140°C in an inert atmosphere.

The first hydroperoxide is probably a precursor of nona-2,6-dienal:

$$H_3C{-}CH_2{-}CH{=}CH{-}CH_2{-}CH_2CH{=}CHCH{-}(CH_2)_4{-}C{-}OH \rightarrow$$
$$\rightarrow H_3C{-}CH_2{-}CH{=}CH{-}CH_2{-}CH_2{-}CH{=}CH{-}CHO,$$

and the second is a precursor of hept-4-enal:

$$H_3C{-}CH_2CH{=}CH{-}CH_2{-}CH_2{-}CH{-}CH{=}CH(CH_2)_7{-}C{-}OH \rightarrow$$
$$\rightarrow H_3C{-}CH_2{-}CH{=}CH{-}CH_2{-}CH_2CHO.$$

Probably the above unsaturated aldehydes are partly responsible for the odour of green cucumbers which appears in beef fat during its storage.

An attempt has been made to relate certain chemical characteristics to the organoleptic parameters of alimentary fats [127]. It was found that a very small peroxide content may be very important for the estimation of the freshness of these fats. Thus the appearance of an unpleasant taste was noted for a peroxide content of 0.10 millimole kg^{-1}.

VI. EFFECT OF LIPID PEROXIDES ON VARIOUS SUBSTRATES

The formation of epoxy-compounds and α-epoxy alcohols as a result of the decomposition of α-olefinic hydroperoxides was observed with cyclohexene [128] and many olefins [122,129]. The autoxidation of unsaturated fatty acids at the double bonds has been the subject of an intensive investigation [130–132] which established the following facts.

In the presence of haemin (weight concentration 2×10^{-5}) in a stoichiometric mixture of oleic acid and the peroxide of ethyl oleate (at room temperature and at a reduced pressure), the degree of unsaturation of the acid gradually diminishes, with an accompanying decrease of the overall content of active oxygen.

In addition the ratio

$$r=\frac{\text{unsaturated acid content}}{\text{active oxygen content}}$$

becomes close to unity.

In a mixture of unsaturated fatty acids (with an iodine number of 126) and the peroxide of oleyl alcohol, the peroxide oxygen is transferred to the double bonds of the acid. Quantitatively this phenomenon depends greatly on the nature of the haematin catalyst (weight concentration 3×10^{-3}). Thus at room temperature after 1 h the ratio $r = 0.31$ in the absence of a haematin catalyst or in the presence of oxidised cytochrome; r becomes 0.96 in the presence of reduced cytochrome [131].

The role of reduced cytochrome in the transfer of peroxide oxygen to the double bonds of unsaturated fatty acids has been discussed in detail by Dubouloz and coworkers [133].

Epoxides may be formed immediately in the autoxidation process in consequence of the addition of ROO· radicals to the double bond:

$$R'CH{=}CH{-}R'' + ROO\cdot \rightarrow R'\underset{\underset{O{-}OR}{|}}{CH}{-}\dot{C}H{-}R'' \rightarrow R'{-}\underset{\diagdown\,\diagup}{CH{-}CH}\,O + \cdot OR.$$

The reaction mechanism has been discussed in detail [134].

Epoxyalcohols are probably formed in the decomposition of the hydroperoxide with subsequent addition of ·OH radicals to the double bond [53]:

$$R{-}\underset{\underset{O{-}OH}{|}}{CH}{-}CH{=}CH{-}R' \rightarrow R\underset{\underset{O\cdot}{|}}{CH}{-}CH{=}CH{-}R + \cdot OH \rightarrow$$
$$\rightarrow R{-}\underset{\diagdown\,\diagup}{CH{-}CH}{-}\dot{C}H{-}R' \rightarrow R{-}\underset{\diagdown\,\diagup}{CH{-}CH}{-}\underset{\underset{OH}{|}}{CH}{-}R'.$$
$$O \qquad\qquad O$$

The epoxidation of unsaturated compounds in the presence of haematin compounds by hydroperoxides probably takes place via the addition of ROO· radicals to the double bond, since the latter are formed as a result of a series of reactions [135]:

$$ROOH \xrightarrow{+e} RO\cdot + :OH,$$
$$RO\cdot + HOOR \rightarrow ROH + \cdot OOR.$$

The study [136] of the action of the peroxide of ethyl oleate (peroxide content 0.36 mole per mole of ester) on cysteine and degraded egg white albumin showed that the thiol groups of the protein enter fully into the reactions in the presence of an excess of the peroxide. The reaction products consist of disulphides and their derivatives containing oxygen. The biological importance of this reaction has been discussed in detail and the methods employed have been criticised [136].

Another study [137] dealt with the autoxidation in emulsion of linoleic acid (2×10^{-3} M) at 37°C and pH 7.4, catalysed by haemin (10^{-5} M) or haemoglobin (2×10^{-6} M) in the presence of 10^{-3} M cysteine or glutathione. The rate of

degradation of the SH groups of the last two compounds is proportional to the content of peroxide in the emulsion. The cysteine oxidation product consists of cysteine, cysteic acid, and cysteine disulphoxide.

The action of an aqueous 2×10^{-3} M emulsion of linoleic acid peroxide (37°C and pH 7.4) on various enzymes has been the subject of a major investigation[138]. It was shown that the activity of all enzymes with SH groups as well as that of cholinesterase, choline dehydrogenase, glyoxalase, β-amylase, succinate dehydrogenase, and urease is strongly inhibited. Fairly strong inhibition occurs also with other enzymes not containing SH groups, for example lipase, invertase, pepsin, trypsin, papain, and cytochrome oxidase, while xanthine oxidase, tyrosinase, catalase, and α-aminoacid oxidase are not affected. It is noteworthy that enzymes such as cholinesterase, lipase, invertase, and pepsin are also inhibited by an aqueous emulsion of palmitic acid. Here the inhibition is probably caused by the nautralisation of the positive charges of the proteins by the negative charges of the acid. Attempts have been made several times to relate the degree of inhibition of the enzymes to the content of peroxide in the emulsion. Thus an 80% degree of inhibition obtains for papain and urease at peroxide concentrations of 0.22×10^{-3} and 0.12×10^{-3} M respectively. In another study an emulsion of fatty acids from linseed oil was employed instead of the emulsion of linoleic acid. We may note that the inhibition under irradiation is more pronounced in the presence of linoleic acid than in its absence.

Thus after 2 h succinate dehydrogenase was inhibited only to the extent of 14%, while in the presence of an emulsion of linoleic acid (4×10^{-7} M) the degree of inhibition reached 63%. The degree of inhibition of succinate dehydrogenase (at a linoleic acid concentration of 4×10^{-7} M) was investigated as a function of time and it was shown that the degree of inhibition reaches 20% after 15 min, 60% after 45 min, and then tends asymptotically to 80%.

It is interesting that these enzymes can be protected from the action of peroxides by more labile enzymes containing SH groups. The protection of urease by cysteine against the action of peroxides formed in the autoxidation of linseed oil has been discussed[138]. A study has also been made[139] of the inactivation of glyceraldehyde-phosphate dehydrogenase by linoleic acid hydroperoxide and of the inactivation of ribonuclease by the same hydroperoxide[140].

A special investigation[141] of the inactivation of enzymes with SH groups by peroxy-acids has been made.

The degradation of carotene in the autoxidation of unsaturated lipids catalysed by soya bean lipoxidase has been the subject of numerous investigations[142-144]. The degradation occurs in alfalfa on storage in the field[145] as a result of the action of peroxides formed in the autoxidation of unsaturated lipids catalysed by the lipoxidase of this plant.

The study of the autoxidation[146] of colloidal methyl linoleate (3.4×10^{-2} M) initiated by X-rays in the presence of vitamin A showed that the degree of degradation of vitamin A increases with the peroxide content. Thus at a peroxide concentration of 5×10^{-5} M, only 33% of the initial vitamin A remains. The degradation of vitamin A does not occur solely under the influence of X-rays in the absence of linoleate.

The study of the autoxidation of ethyl linoleate[147] (25° and 37°C) in petroleum ether solution in the presence of carotene (weight concentration 2.5×10^{-4} in relation to linoleate) showed that the decomposition of carotene is complete over a period of 48 h. Certain autoxidation inhibitors proved effective in the stabilisation of carotene. Thus pyrogallol, hydroquinone, and α-tocopherol at a weight concentration of 3×10^{-5} relative to linoleate (at 37°C over a period of 48 h) prevent oxidation to the extent of 100, 74, and 76% respectively). A gradual breakdown of vitamin A occurs in margarine during its autoxidation at room temperature. Thus the degree of decomposition of vitamin A, reaching 6.5% for a peroxide content of 0.7×10^{-3} mole kg^{-1}, becomes 43% when the peroxide content is 10^{-2} mole kg^{-1}.[148]

The degradation of vitamin A in halibut-liver oil during its oxidation at 25°, 80°, and 98°C and also its protection by NDGA alone or NDGA in the presence of a synergistic agent have been investigated in detail[149].

The study of the degradation of vitamin A during the autoxidation of olive oil at 55°C in thin layers showed[150] that vitamin A decomposes gradually. Thus after 2 h the degree of its degradation is 69%, while in the presence of 0.048% of α-tocopherol it amounts to only 5%, reaching 36% after 10 h. Another study[151] dealt with the degradation of carotene in the autoxidation of ground nut oil at different temperatures (from 4° to 98°C) and also its protection against oxidation with the aid of α-, β-, and γ-tocopherols and gossypol.

A large publication[152] has been devoted to the degradation of carotene at 35°C under the influence of the peroxides of ethyl oleate, methyl linoleate, methyl linolenate and cotton seed, ground nut, and soya bean oils as well as to protection against oxidation with the aid of α-, β-, and γ-tocopherols and gossypol. The protective action of α-, β-, and γ-tocopherols against the degradation of carotene and bixin by the peroxide of methyl linoleate is also examined in two other papers[32,153].

In a study of the degradation of vitamin A and carotene[154] during the autoxidation at 37°C of a colloidal solution of ammonium linoleate (0.2 M, pH 7) catalysed by 2×10^{-5} M haemoglobin or cytochrome c showed that, following the absorption of 500 cm^3 of oxygen, the degree of degradation of vitamin A and carotene is respectively 88 and 53%, while in the presence of 5×10^{-3} M α-tocopherol, the degree of degradation is only 63 and 5% respectively.

Another study[50] dealt also with the question of the protection of vitamin A and carotene with the aid of α-tocopherol in the course of the autoxidation of an aqueous emulsion of oleic acid (37°C, 0.1 M, pH 7) catalysed by haemin (2×10^{-5} M). After 3 h, 1400 cm^3 of oxygen is absorbed and the degree of degradation of vitamin A is 88%, while in the presence of 6×10^{-3} M α-tocopherol the degree of degradation is only 63% and 300 cm^3 of oxygen is absorbed. The behaviour of carotene is similar to that of vitamin A. When 1400 cm^3 of oxygen has been absorbed, carotene is degraded to the extent of 63%, while in the presence of 5×10^{-3} M α-tocopherol the degree of degradation is only 5%.

Usually diets rich in unsaturated lipids but deficient in vitamin E and A cause a diminution of the vitamin A reservoir in animal livers. The addition of α-tocopherol and other antioxidants to the diet has a protective effect in relation to vitamin A, as shown by the following findings.

After seven months the vitamin A reservoir in the livers of male albino rats maintained on a diet containing 5% of pork fat and deficient in vitamins A and E is almost completely exhausted. The addition to the diet of each rat of 1 mg of α-tocopheryl acetate over a period of one week leads to a degree of degradation of vitamin A of only 71% during the same time.

The addition of α-tocopherol, leuco-Methylene Blue, phenothiazine, and tetramethylthiuram disulphide to a diet including 10% of cod liver oil and deficient in vitamin E increases the vitamin A content in chicken livers compared with the diet free from additives [155].

The addition of Methylene Blue or phenothiazine to a diet including 10–20% of cod liver oil and deficient in vitamin E increases the content of vitamin A in rat livers over a period of 14 weeks compared with the diet free from additives. The effect of the additives diminishes markedly when cod liver oil in the diet is replaced by pork fat [156,157]. The protective action of vitamin E, promoting the retention of vitamin A in rat livers, has been described in two papers [158,159].

Ethyl linoleate peroxide (250 millimole kg^{-1}) in contact with crystalline biotin leads to 96% degradation of the latter at 37°C after 12 h. As for vitamin A, α-tocopherol has a protective effect: the degree of inhibition is 44% in 48 h at a 4% concentration of this compound. Another study [160] also demonstrated the degradation of biotin at 25° and 37°C in the presence of the peroxide of corn oil (100 millimole kg^{-1}) and in the case of diets containing 5% of lipids of which 3% is cod liver oil and 2% cod liver fat; the overall peroxide content in the diet is 5 millimole kg^{-1}.

A study of the action of pyridoxine and riboflavin [161] showed that riboflavin decomposes lipid peroxides and thereby protects pyridoxine from their action.

VII. THE ROLE OF LIPID PEROXIDES IN THE DETERIORATION OF FOOD PRODUCTS

It has been already seen that carbonyl and mono- or poly-functional hydroxy-compound may be formed from lipid peroxides. The monocarbonyl compounds consist of a mixture of saturated and unsaturated aldehydoacids and in all probability are responsible for oxidative rancidity and reversion. These frequently encountered phenomena have been discussed in detail in a number of publications [11–15,162].

Cow's milk. The overall composition of the acids constituting milk lipids is as follows [163]:

Unsaturated acids (60%): 12% of acids ranging from C_4 to C_{12}, 11% of myristic acid, 28% of palmitic acid, and 9% of stearic acid.

Unsaturated acids (40%): 34% of oleic acid, 5% of linoleic acids, and 1% of others.

Acids entering into the composition of phospholipids [164]: 41% of saturated acids, 40% of mono-olefinic acids, 2.3% of conjugated and 8.8% of conjugated diolefinic acids, 3.6% of triolefinic acids, 2% of tetraolefinic acids, and 2.2% of pentaolefinic acids.

Evidently milk phospholipids have a higher content of polyunsaturated acids than glycerides.

It has been shown [165] that the content of peroxide in powdered milk allowed to stand in air increases with time. In the same study [166] it is recommended that various antioxidants (particularly of the phenolic type) be employed at a weight concentration of 10^{-4}. The formation of peroxides has been investigated in various specimens of powdered milk during storage at 2° and 38°C over periods ranging from 4 months to 1 year [13,113]. When the peroxide content is of the order of 6–10 millimoles per kilogramme of lipids, a rancid odour appears. The maximum content of peroxide formed over a period of 4 and 9 months is 5 and 14 millimoles per kilogramme of lipids.

C_2–C_{18} saturated aldehydes, C_5–C_{11} unsaturated aldehydes, C_8 and C_9 2,4-dienoic aldehydes, oct-1-en-3-one, and oct-1-en-3-ol were identified in the lipids of sour milk [166–168]. Alkyl methyl ketones $RCOCH_3$ ranging from C_1 to C_{13} are also formed [169]. Probably they are derived mainly from myristic and palmitic acids.

The nature and mechanism of the formation of various compounds in the autoxidation of milk lipids have been discussed in detail [166–174]. One of the papers [172] criticises preceding investigations.

Data on the content of peroxides and carbonyl compounds in various animal fats and edible vegetable oils as a function of storage and irradiation conditions have been published [13,175,178]. The study of the storage of raw vegetables, in particular peas [179–182], showed that the content of glucides and nitrogen remains more or less constant.

No changes occur also in blanched vegetables, i.e. those subjected to the action of boiling water which decomposes lipidoxase. On the other hand, the peroxide content and acidity of the lipid component of raw vegetables may greatly increase [181]. There is also a possibility of a decrease of the iodine number—for example, in raw peas it decreases from 98 to 89 in 5 years. We may also note that chlorophyll is degraded in peas to the extent of approximately 30% in 5 years.

Lipids, which constitute only about 0.3% of dry potato [183], contain 75% of polyunsaturated fatty acids, 53% of 18 : 2 acid, and 22.5% of 18 : 3 acid. The saturated component consists almost entirely of a C_{16} acid (17.4%). After exposure to oxygen at room temperature for 5 months, C_2–C_6 saturated aldehydes and C_1–C_5 saturated hydrocarbons were isolated. The latter are probably formed from aldehydes:

$$R{-}CHO \rightarrow RH + CO.$$

Potato chips fried at 185°C in a mixture consisting of 50% of cotton seed oil and 50% of corn oil and stored at room temperature in air were very carefully investigated [179]. 18 different carbonyl compounds were isolated from freshly fried potato chips and 19 from less fresh specimens. During ageing, the content of saturated aldehydes (particularly hexanal), α-unsaturated aldehydes (more of C_7 than C_8), and alkyl methyl ketones (more of 2-pentanone than 2-hexanone) increases. On the other hand, there is a rapid decrease of the content of deca-2,4-dienal.

On storage pork frequency becomes rancid [184]. The rancidity is accompanied by a change in colour, which becomes greyish-brown instead of pink. The rancid meat has an unpleasant odour and taste and is somewhat toxic. The rancidity is a consequence of the oxidation of oleic acid triglycerides and small amounts of polyunsaturated acids (particularly linoleic acid) and subsequent degradation of haemoglobin and myoglobin accompanied by the liberation of inorganic iron compounds. This reaction is catalysed by haematin compounds of the meat itself. The very rapid deterioration of horse meat in the presence of air is probably a consequence of an analogous phenomenon, which, however, is much more rapid owing to the higher content of linoleic acid (16%) in horse fat.

VIII. CHANGES IN LIVING ORGANISMS INDUCED BY LIPID PEROXIDES AND THEIR DECOMPOSITION PRODUCTS

The presence of peroxides at a concentration of several tens of millimoles per kilogramme of dry material was observed in the lipid component of green leaves of certain plants: sunflower, wheat, peas, beans, waterweed (Elodea) [185], and white lucerne [186]. Peroxides could not be

detected in the stalks, roots, and seeds of these plants [185]. The localisation of peroxides in the leaves and their decomposition under conditions where photosynthesis but not tissue respiration ceases suggests that photosynthesis is closely related to the formation of peroxides. This hypothesis is based on the finding that a flavin-lipoprotein compound containing iron and manganese and capable of reducing carbon dioxide was isolated from lucerne leaves [186]. The reduction was accompanied by the formation of a peroxide of the lipid component of the leaf. The role of peroxides of glycolic and glyoxylic acids in the decomposition of chlorophyll has been examined [187,188]. It is interesting that the leaves, seedlings, and seeds of certain oil-bearing plants contain substances capable of causing the decomposition of peroxides [189-191].

The polymers obtained as a result of the decomposition of lipid peroxides the formation of which is catalysed by lipoxidase, are probably components of cutin [160], an impermeable film coating part of the plants, which had been acted upon by air.

Even a balanced diet containing between 5 and 20% of highly unsaturated lipids and with a low content of vitamin E may cause the pathological changes enumerated below, which may vary depending on the type of animal [162,192,235]:

1. Yellow colour of fatty tissues.
2. The formation of the ceroid pigment in rat livers.
3. Brown colour of the rat uterus.
4. Atherosclerosis and peripheral damage of the viens in man.
5. Exudative diathesis in chickens.
6. Encephalomalacia in chickens.
7. Decrease of the capacity for egg bearing and embryo generation in hens.
8. Depigmentation of incisor teeth in rats.
9. Muscular dystrophy in ruminants.
10. Liver necrosis and pulmonary haemorrhage.

These ten pathological changes have been discussed in two large reviews [233,234]. Certain pathological changes, such as, for example, the yellow colour of the fatty tissue in certain animals, the brown colour of the rat uterus, the formation of the ceroid pigment in rats, and the appearance of exudative diathesis and encephalomalacia in chickens may be directly linked to the action of the oxidation products of unsaturated lipids on tissue proteins which results in their damage. The differences between the behaviour of chickens and rats in relation to diets with a high polyunsaturated lipid content are probably a consequence of the different structures of their capillary vessels. In other diseases, for example in muscular dystrophy or liver necrosis, which occur also in the absence of lipids from the diet, the role of polyunsaturated lipids is less clear. However, in liver necrosis the decomposition of sulphoaminoacids under the influence of lipid peroxides probably plays a considerable role. It is quite likely that muscular dystrophy is a consequence, at least partly, of the decomposition of vitamin E under the influence of these peroxides.

It is noteworthy that different antioxidants such as NDGA, Methylene Blue, diphenyl-*p*-phenylenediamine, phenothiazine, and tetraethylthiuram may be replaced in the diet by vitamin E and play a protective role against one or several of these diseases. For example, selenium compounds (at a concentration of $10^{-7}-10^{-6}$ M) can fulfil a protective role against liver necrosis and exudative diathesis but not against encephalomalacia. The action of these different antioxidants has been reviewed [233,234] and has been described in a special paper on the action of SeO_2. [235]

IX. PATHOLOGICAL CHANGES INDUCED *IN VIVO* BY A DIET CONTAINING VARIOUS LIPIDS HEATED IN THE PRESENCE OF AIR

Edible lipids consist of a mixture of triglycerides of stearic, palmitic, oleic, and linoleic acids. Animal fats contain much more of unsaturated acids (40–60%) than vegetable oils (10–30%) and much less of linoleic acid. Beef and mutton fats contain between 1 and 2% of this acid and pork fat contains 6–10%, while in many vegetable oils its content reaches 60%. Horse fat, which contains, apart from a small amount (4.5%) of stearic acid, 5.3% of linoleic acid and a considerable amount of linolenic acid (16%), is an exception.

Various fried products sold commercially are heated at 180–200°C. Under these conditions, there is usually an increase of the content of hydroxy-compounds, free acids, esters, conjugated dienes, and particularly polymers (the initial content of which is low, namely 1–2%). The content of carbonyl compounds initially increases to a maximum and then falls and that of peroxides is more or less constant. This can decrease or increase slightly but remains insignificant, its concentration rarely exceeding 10 millimole kg^{-1} (provided that the food product had not been subjected to preliminary ionising irradiation).

The degree of overall unsaturation (iodine number), and particularly the content of linoleic acid, decreases appreciably. These chemical changes are reflected in an increase of the refractive index, the dielectric constant, and particularly the viscosity [236-239].

The physicochemical changes occurring during the frying of various food products containing lipids have been discussed in a number of publications [240-244], the last of which is devoted to different types of meat.

Apart from temperature and the duration of frying, the content of the peroxides and lipids and their decomposition products is influenced by the area of contact between the lipid and air, which is determined by the shape, dimensions, and material of the frying-pan and also the duration of the preliminary heating and cooling. Indeed, peroxides are formed during the preliminary heating and then decomposed during frying and are reformed during cooling. Thus, evidently, the content of different peroxide derivatives will be higher if the heating is interrupted than when it is carried out continuously and will increase with the number of preliminary heating and cooling cycles. Nevertheless, we may note that carbonyl compounds may be partly eliminated by evaporation or can undergo further oxidative degradation: this in fact accounts for their maximum content when the heating process is of limited duration. Thus, when corn oil [236] was heated for 24 h at a constant air flow rate of 150 $cm^3\ kg^{-1}\ min^{-1}$, the content of peroxides at 120°, 160°, and 200°C was respectively 40, 3, and 1 millimole kg^{-1} (the initial content was 0.5 millimole kg^{-1}). After 24 h at 200°C (at an air flow rate varied from 0 to 2400 $cm^3\ min^{-1}$), the content of carbonyl compound changed from 86 to 116 millimole kg^{-1} (with a maximum of 122 millimole kg^{-1} for an air flow rate of 1200 $cm^3\ min^{-1}$).

When cotton seed oil was heated continuously at 175°C for 62 h [238] and with interruptions (heating for 7–8 h followed by 12–16 h of storage at room temperature), the results presented in Table 1 were obtained.

Lipid peroxides introduced into the alimentary canal by a probe gradually disappear during digestion. Thus the content of the peroxide of ethyl oleate after 5 h was only 12% of the initial value, which was 10^{-4} mole [245].

Moreover, lipids originating from various parts of the organism, particularly from the lymph (specimens of which were removed during digestion), contain no peroxides at all [245-247].

Table 1.

Substance	Content, millimole kg^{-1} heating	
	continuous	interrupted
Peroxides	2.3	3.6
Carbonyl compounds	158	155
Hydroxy-compounds	130	241
Free acids	280	560
Polymers (%)	14.8	13.8
Iodine number	103	98

Probably the peroxide of the lipid ester or triglyceride is hydrolysed by lipase with retention of the OOH functional group, which is then partly reduced by the pancreatic secretion. The above facts and also the absence of peroxides from excrement suggest that lipid peroxides are mainly decomposed on the mucous membrane of the intestine [245].

Experiments [248] performed on rats fed on a diet containing 10% of *Vernonia* oil containing 70% of 12,13-epoxyoleic acid showed the presence of this acid in adipose tissues, the liver, kidneys, and the heart and also in excrement. On the other hand, epoxides were absent from these tissues when *Vernonia* oil was replaced by corn oil. These findings suggest that epoxides are not decomposed in the digestive organs (which is surprising in view of the presence of 0.1 *N* HCl in the stomach and the sensitivity of epoxides to acid hydrolysis) and are normally stored in various tissues together with other lipids.

Hydroxy- and carbonyl compounds are readily absorbed [249]; thus the content of carbonyl compounds in the lymph may increase by a factor of 10.

A diet containing 10–20% of lipids heated in air usually causes a retardation of growth, a decrease of the degree of assimilation of food, and its efficiency, and also an increase of the amount of water required.

On such a diet, certain animal organs, particularly the liver, greatly increase in size. However, it should be noted that the restoration of a normal diet leads to the recovery of the sick animals. Vitamins (including vitamin E) ameliorate but do not eradicate these diseases. Fresh lipids as well as proteins also have a favourable effect.

The toxicity of heated lipids increases with the content of linoleic acid (with the exception of horse fat, the content of the 18 : 3 acid in normal lipids is very low), as shown by the results obtained in two studies on rats [250-252] fed on a diet containing 20% of lipids.

The toxicity increases with the number and duration of preliminary heating and cooling cycles (consequently one should avoid using the same oil in several successive frying operations) and decreases as a function of the weight of the lipid. For example, the harmfulness of various heated lipids employed in commercial frying involving large amounts of product is much less than that of lipids heated in the laboratory in much smaller amounts, as shown by studies [253-259] on rats fed on a diet containing 23% of lipids.

Since the mucous membrane of the intestine decomposes lipid peroxides, their intrinsic action must be restricted to the level of digestive enzymes. Apart from the enzymes mentioned above [138], the inhibition of xanthine oxidase by the peroxide of soya bean oil has been demonstrated [247]. Such decomposition of enzymes should be reflected in a reduced assimilability of food, which entails a retardation of growth. Thus the content of peroxides in lipids treated at 200°C is low, and consequently their role in the overall pattern of the induced diseases is very small.

However, on heating at lower temperature (60–100°C), when the content of peroxides is fairly high, their presence may be responsible for the overall toxicity of the heated lipids. Thus the inhibition of the growth of rats fed on a diet containing 20% of soya bean oil subjected to autoxidation at 60°C increases with the peroxide content [247,259]. A study on cotton seed oil autoxidised at 60°C showed that the incidence of illness increases when the maximum peroxide content is reached [240].

The relation between toxicity and the content of peroxides was observed not only with cotton seed and soya bean oils autoxidised at 60°C but also with the ethyl esters of the highly unsaturated fatty acids in the fat of the cuttle fish. The autoxidation products obtained at 30° and 100°C contained respectively 970 and 75 millimole kg^{-1} of peroxides and proved to be very toxic to rats [260].

In other studies lipid peroxides introduced via the alimentary canal proved to be non-toxic. For example the hydroperoxide of methyl oleate introduced in an amount of 5% into the diet of rats is altogether non-toxic [261,262].

Thus there is no definite relation between the degree of toxicity of the heated lipid and its peroxide content, particularly for the same lipid at low heat-treatment temperatures in the initial autoxidation stage.

It should be noted that in pathological cases, where the regeneration of the intestinal epithelium (decomposed by lipid peroxide) is slow, lipid peroxides can apparently overcome the protective effect of the intestine [263]. Studies during which methyl linoleate labelled with ^{14}C at 1-C was introduced via the digestive tract, showed that this peroxide is extensively decomposed in the intestine and the decomposition products are incorporated into various tissue lipids.

The epoxides which are deposited together with other lipids in various cell tissues are not toxic at first sight. Indeed, the introduction of 5% of methyl *cis*-epoxystearate [261,264] or 7% of epoxyoleic acid (in the form of 10% *Vernonia* oil) [248] into the diet of rats is not harmful. However, according to other investigations [265], epoxides appear to be carcinogenic.

Hydroxy-compounds, at least those with long chains, are not toxic. For example, methyl mono- and dihydroxystearates are not harmful to rats when their content is 5%. [261]

Carbonyl compounds are probably to some extent responsible for the overall toxicity of heated lipids, which decreases after their treatment with semicarbazide. They can act on digestive enzymes, since they inhibit the activity of lipase. One should examine the effect of each type of aldehydes as a function of the degree of their unsaturation; probably aldehydes with conjugated double bonds should be more toxic.

Polymers are usually toxic. Their activity evidently depends on the degree of diene conjugation or, in other words, on the content of linoleic acid in the initial lipid. This has been demonstrated for pork fat and cotton seed oil after their heat treatment in air at 95°C for 300 h and after distilling off the autoxidation products, obtained in

this way, at 300°C at a very low pressure [264]. The residue from cotton seed oil (40% of the total quantity of the oil), introduced in an amount of 20% into rat diet, caused in one week the death of all the animals, while the residue from pork fat (17% of the total quantity) does not cause death but inhibits growth [264]. We may also note that the distillation residue may contain thermal polymerisation products with cyclohexene fragments which are not formed in frying on a commercial scale [266] but the present author believes that they can be formed in the decomposition of polymers at 300°C.

On the other hand, the degree of assimilation of toxic polymers decreases with increase in molecular weight. Therefore the heaviest fractions are not always the most toxic.

Table 2. Composition of certain animal fats (%).

Fat	Acids				
	18:0	16:0	18:1	18:2	18:3
Mutton fat	34	24	44	2	traces
Beef fat	21	32	45	2	traces
Pork fat	16	30	45	7	traces
Chicken fat	7	26	38	20	traces
Horse fat	5	26	34	5	16
	(+5% 14:0)		(+7% 16:1)		

Table 3. Composition of cod liver fat (%).

Acid		% in fat	Acid		% in fat
20% of unsaturated triglycerides	18:0	3	2% of triolefinic acids	18:3	
	16:0	12		20:3	
	14:0	3		22:3	
	15:0	1	tetraolefinic acid	18:4	2
	17:0	1			
45% of mono-olefinic triglycerides	18:1	25	10% of pentaolefinic acids	20:5	9
	20:1	13		22:5	1
	22:1	6	hexaolefinic acid	22:6	9
	14:1	traces			
	16:1				

Table 4. Composition of certain vegetable oils (%).

Acid	Linseed oil	Soya bean oil	Cotton seed oil	Safflower oil	Corn oil	Groundnut oil	Olive oil
18:3	47—60	4—8	traces	traces	1.5	traces	traces
18:2	15—24	49—56	40—50	77	58	20—3	4—12
18:1	15—19	24—34	20—30	13	27	50—60	70—85
Saturated	10—15	12—17	20—30	10	12.5	10—20	7—17

In addition, one must bear in mind the existence of trioxans, particularly derivatives of 2,4-dienoic aldehydes, such as deca-2,4-dienal, in which corn oil heated at 200°C becomes enriched [237]. The importance of 1,3-conjugation has been emphasised by Holman and Greenberg [267], who discuss all compounds with two conjugated double bonds, for example the hydroperoxide of ethyl linoleate, and its reduction and decomposition products, as well as ethyl linoleate derivatives which are not involved in the normal metabolism of linoleic acid [267]. The biochemical activity of various fractions of linseed oil [266,268], herring oil [269,271], and pork fat [271] has been investigated. Several excellent papers have been devoted to the toxicity of heated lipids [249-252,256,272,273]. The compositions of various lipids are given by Hilditch and Williams [274]. The compositions of the main animal fats and vegetable oils according to the literature data [111,274] are quoted in Tables 2, 3, and 4.

X. CHANGES IN TISSUE CELLS OCCURRING ON FORMATION OF PEROXIDES

Earlier it was stated that lipid peroxides administered to rats with food are largely decomposed by the mucous membrane of the intestine and only their traces can be detected in the lymph.

Nevertheless the conditions for the formation of lipid peroxides are favourable even in the mammalian organism in consequence of the simultaneous presence in vascular endothelium also of light unsaturated lipids (in the form of an emulsion), haematin compounds, and oxygen [245]. The capacity of the tissue for the formation of lipid peroxides increases with the content of unsaturated acids and the degree of their unsaturation as well as in the presence of catalytic systems such as haematin iron or non-haematin iron together with ascorbic acid, and decreases in the presence of inhibitors, for example vitamin E.

It is noteworthy that the composition, particularly as regards polyunsaturated lipids, not only of the triglycerides of the fat reserves but also of the phospholipids in various tissues is greatly influenced by the type of lipids in the diet. Thus an increase in the content of linoleic acid in the diet increases simultaneously the content of linoleic acid in the storage lipids and the content of linoleic and arachidonic acids in various tissues. Polyunsaturated acids (other than the main acids), such as those of the 20:5 and 22:6 type, in cod liver oil are deposited in various animal tissues without change.

The correlation between the degree of unsaturation of lipids entering into the composition of tissues and the degree of unsaturation of lipids in the diet accounts for the pathological changes observed *in vivo* under the influence of a diet enriched in polyunsaturated lipids.

However, we may note that the formation of polyunsaturated 20:3 acids may occur also in tissues if the diet is deficient in linoleic acid and includes saturated lipids such as those of coconut oil. The composition of lipids in various tissues as a function of the type of lipids in the diet has been discussed in detail [275-279].

The presence of peroxides in connection with various pathological changes in tissues occurring due to a diet with a high content of polyunsaturated lipids and deficient in antioxidants has already been mentioned. Lipid peroxides were also detected after the exposure of a live animal to ionising radiation in the presence of atmospheric oxygen [280-286].

The formation of lipid peroxides under the influence of ionising radiation is in all probability initiated by $^{\cdot}OH$ and $^{\cdot}OOH$ radicals formed in the aqueous phase [13]. The analogy between the diseases caused by ionising radiation and lipid peroxides, in particular the decomposition of various enzymes, has been discussed in detail [2,287]. The detection of peroxides in the tissues of live animals has

been questioned in two publications [288,289]. Indeed, when lipids were extracted in an inert atmosphere, even traces of peroxides could not be detected in any of the organs of rats (the chromatographic method was used [290]) subjected to preliminary irradiation with X-rays in air or fed on a diet containing 20% of fresh soya bean oil or its peroxide [288]. Similar results were obtained in the study of various organs from chickens fed on a diet containing 10% of cotton seed oil. Blood lipids, which induce the formation of small amounts of a very unstable peroxide, are an exception. A study [289] based on 33 different tissues confirmed the validity of earlier results [288].

Hence it probably follows that live tissues do not contain peroxides (or at most traces in adipose tissues) and that the latter are formed *in vitro* during extraction in contact with an atmosphere from which oxygen has not been completely exhausted. A deficiency of vitamin E in the tissue probably appreciably increases the rate of formation of the peroxide *in vitro*.

Whereas the presence of lipid peroxides in live tissues is doubtful, the formation of peroxides *in vivo* is indicated by the products obtained on decomposition of lipid peroxides and the fact that such formation is prevented by vitamin E and other antioxidants. In this case lipid peroxides probably undergo gradual transformations from the instant of their formation, which lead to various physiological (decomposition of protein thiols and various enzymes) and structural changes (rupture of membranes in mitochondria and lysosomes).

When aqueous emulsions of lipid extracts from various rat organs were exposed to ionising radiation (5–100 krad), the formation of peroxides with a lipid content of several tens of millimoles per kilogramme was observed.

Peroxides are also formed during aerobic incubation of various live tissues at 37°C. However, in the majority of investigations the peroxides were determined only indirectly with the aid of a colour test for malonic dialdehyde (one of the decomposition products of lipid peroxides) with thiobarbituric acid. This very sensitive test yields interesting results in comparative studies, but does not reflect the true content of peroxides (see the discussion in Wills's paper [292].

The considerations concerning the formation of peroxides *in vivo* and the catalysis of the oxidation of aqueous emulsions of linoleic acid by metal chelate complexes are applicable, in broad outline, to the determination of the capacity for the *in vitro* formation of endogenic peroxides in various live tissues in the presence of atmospheric oxygen, particularly when one considers the catalytic activity of various forms of iron (haematin and non-haematin) and ascorbic acid and the effect of chelating agents and of phosphate concentration. The rate of formation of peroxides in the tissue increases with its overall iron content as well as with the content and degree of unsaturation of its component lipids. In the presence of non-haematin iron, the content of ascorbic acid in the tissue is very important. Thus the onset of the formation of the peroxide on addition of ascorbic acid to homogenates of various tissues is explained by the presence in them of non-haematin iron. It has been shown [293] that in various tissues of scorbutic guinea pigs, less peroxides are formed than in the tissues of healthy guinea pigs and that the addition of ascorbic acid to tissues affected by scurvy restores their normal capacity for the formation of peroxides. It is interesting to note that tissues in a state of constant cell division, such as bone marrow, the mucous membrane of the intestine, and cancer cells, do not form peroxides.

Discussions concerning the formation of peroxides as a result of the *in vitro* aerobic incubation of various tissue homogenates have been published [292–299]. The formation of endogenic peroxides in the homogenates of various rat organs and also the catalytic effect of these homogenates in the autoxidation of linoleic acid in emulsion was discussed in detail in one of these papers.

Mitochondria usually contain considerable amounts of polyunsaturated lipids (particularly in the form of phospholipids) derived from acids with 3,4,5, and 6 double bonds, such as 18:3, 20:3, 20:4, 22:4, 22:5, and 22:6 acids. The distribution of lipid molecules in the vicinity of various cytochromes makes them particularly susceptible to autoxidation.

The composition of the liver and heart mitochrondria in various sea fish and birds and also several marine mammals has been described [301,302]. Data have also been published [302] on the composition of the mitochondria in the chicken liver and the ox heart. The composition of rat liver mitochrondria has been studied [303,304].

Suspensions of mitochrondria at 37°C in a sucrose or sodium chloride solution (with a phosphate buffer) absorb oxygen with subsequent formation of malonic dialdehyde. Ascorbic acid usually catalyses these reactions and vitamin E inhibits them.

The phenomena described below are accompanied by the degradation of enzymes such as succinate dehydrogenase, TPNH cytochrome reductase, choline oxidase, and amino-acid oxidase (on the other hand, the liver alkaline phosphatase and brain cholinesterase resist the formation of this peroxide). The degradation of the enzymes is revealed by a weakening of the oxidative phosphorylation and oxidation of α-oxoglutaric acid.

Apart from the degradation of enzymes, the mitochondrial membranes swell and are lysed with liberation of free fatty acids and soluble proteins.

Vitamin E, introduced *in vitro* into suspensions of mitochondria, decreases the absorption of oxygen and the formation of malonic dialdehyde, protecting thereby the above enzymes against oxidative degradation and the membrane against swelling and lysis. The introduction of 0.47 and 4.7% of vitamin E into mitochrondria from rabbit heat tissue decreases the rate of absorption of oxygen by factors of 7 and 28 respectively [305]. It is noteworthy that the capacity of mitochrondria for the formation of peroxides depends also on the composition of polyunsaturated lipids and antioxidants (in particular vitamin E) in the diet. Thus when a diet deficient in vitamin E and containing 3% of pork fat and 2.5% of fish fat is administered, the rate of absorption of oxygen and the amount of malonic dialdehyde formed decrease respectively by factors of 2.5 and 10 when 10 mg of α-tocopherol is added to the diet [306].

Degradation of mitochondrial enzymes has been discussed in Refs. 307 and 308 and the swelling and lysis phenomena in Refs. 309 and 310. The last two papers deal with the role which ascorbic acid plays in the occurrence of these processes.

The formation of peroxides in the mitochondria of rat liver and rabbit heart cells has been thoroughly investigated [305,306]. The inhibiting effect of vitamin E (in relation to the formation of peroxides in the mitochondria of rabbit heart cells and rat and rabbit liver cells) has been studied [311–313].

Microsomes contain 30–40% of lipids, particularly phospholipids, and twice as much of polyunsaturated lipids (in relation to nitrogenous bases) as in mitochondria. Certain molecules of these lipids are located near cyto-

chrome molecules, which gives them a particularly high capacity for the formation of peroxides [314].

Applications to rat liver cell microsomes of the same methods as for mitochondria [305] led to the discovery that the absorption of oxygen accompanied by the formation of malonic dialdehyde takes place.

The formation of peroxides in microsomes leads to a modification of the synthesis of nucleic acids and proteins and to a decrease of the synthesis of ascorbic acid and also to an inhibition of the activity of gulonolactone oxidase [315]. The microsomes themselves degenerate, their altered shape being clearly visible through an electron microscope.

As for complete homogenates and mitochondria, vitamin E plays the role of an inhibitor. Thus addition of α-tocopherol (0.25 g/gN) leads to degrees of inhibition of 72 and 69% respectively in the absorption of oxygen and the formation of malonic dialdehyde.

The formation of peroxides in the microsomes of rat liver and rabbit heart cells has been described in broad outline [314]. The inhibition of gulonolactone oxidase has been investigated [315,316], the second study [316] being concerned in addition with the dependence of the degree of inhibition of the synthesis of ascorbic acid on the content of malonic dialdehyde. The effect of TPNH and ATP on the formation of peroxides in microsomes has been studied [317].

Although lysosomes contain less lipids than mitochondria and microsomes, they nevertheless have only a simple membrane. They form peroxides with difficulty but the membrane is easily ruptured on formation of peroxide even in insignificant amounts. Thus the rupture of the membrane in rat liver cell lysosomes under the influence of ultraviolet light and γ-radiation and the product of the autoxidation of methyl linoleate is accompanied by the liberation of hydrolytic enzymes: cathepsin, glucuronidase, acid phosphatase, and aryl sulphatase. The addition of ascorbic acid increases and that of vitamin E decreases the amount of such liberated enzymes.

The formation of peroxides in lysosomes associated with the liberation of enzymes has been discussed [318-320].

The haemolysis of red blood corpuscles is associated with the rupture of membranes as a result of the formation of lipid peroxides. The haemolysis is promoted by a diet deficient in vitamin E, while the introduction of vitamin E or other antioxidants, for example reduced Methylene Blue, into the diet interferes with haemolysis. The haemolysis of red blood corpuscles has been discussed in a number of publications [321-325].

The inhibition of cell division under the influence of ultraviolet light is probably also induced, at least partly, by the action of lipid peroxides [326].

Indeed, in a study of the fertilised eggs of the worm *Chaetopterus pergamentaceus* and the sea urchin (*Lytechornus variegatus*), it was established that ultraviolet light and irradiated methyl linoleate inhibit cell division. Probably such inhibition is a consequence of the repression of the activity of the enzymes and of the depolymerisation of nucleoproteins.

Vitamin E is the usual component of the majority of living organisms. It is encountered in the tissues of all animals and is widely distributed in plants. It has also been found in unicellular organisms such as yeasts and moulds. Its antioxidant activity, which is usually shown in an increase of the duration of the induction period and in a decrease of the rate of absorption of oxygen and the rate of formation of hydroperoxides and their decomposition products, has been frequently noted in this review.

A similar situation obtains in the oxidation of linoleate catalysed by lipoxidase and haematin compounds and in the oxidation of mitochondria and microsomes.

Although α-tocopherol behaves as a weaker antioxidant *in vitro* than other tocopherols, it accumulates in the animal organism (particularly in phospholipids) and therefore under *in vivo* conditions exhibits a pronounced antioxidant activity. Its distribution in various animal fats and vegetable oils as well as in various rat organs has been discussed in two publications [16,327].

Various tocopherols do not exhibit synergism but form strongly inhibiting systems with ascorbic, citric, and phosphoric acids, which probably also play a very important role in the stabilisation of tissue lipids [326,328,329].

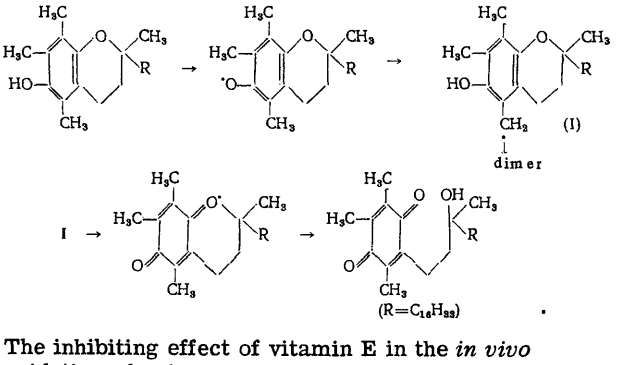
The degree of degradation of α-tocopherol in tissues usually increases with the content and degree of polyunsaturation of lipids in the diet. However, the loss of tocopherol from a tissue on a diet from which it is absent greatly depends on the nature of the tissue. We may note its very tenacious retention by the brain [330]. The decomposition products of α-tocopherol *in vivo* consist of α-tocopherylquinone (formed as a result of the opening of the pyran ring) and dimeric α-tocopherol (with an unaffected heterocyclic structure).

Indeed the presence of tocopherylquinone was established in the liver and in the muscles of mice and pigs [331] and its content in adipose tissues increases with the degree of unsaturation of lipids in the diet. (It is higher by a factor of 3 for methyl linoleate than for coconut oil [332].)

The simultaneous presence of the dimer and the quinone was also observed in the livers of mice and rats after intra-abdominal injection of α-tocopherol into animals which had been fed for a year on a diet deficient in vitamin E. [331,333] The dimer and the quinone are also formed on simultaneous oxidation (60° and 100°C) of α-tocopherol and methyl linoleate by atmospheric oxygen [334].

The effect of ROO· radicals formed as a result of the action of iron(III) salts on the hydroperoxide of methyl linoleate and the peroxides of methyl linolenate, ethyl arachidonate, and methyl eicosapentanoate on α-tocopherol has been investigated [335] and kinetic data have been published.

α-Tocopherol is converted into α-tocopherylquinone as a result of the abstraction of a hydrogen atom (from the OH group) followed by the rupture of the pyran ring, rearrangement, and fixation of the OH group [335]:



The inhibiting effect of vitamin E in the *in vivo* autoxidation of polyunsaturated lipids has been examined [16,233,234,336-338].

Ubiquinone, which is usually found in mitochondria in larger amounts than α-tocopherol, can be reduced to ubiquinol [339,340]. On the other hand, the latter can be

reoxidised by peroxides to ubiquinone. Ubiquinol proved to be an inhibitor approximately as effective as α-tocopherol in the oxidation of an emulsion of arachidonic acid catalysed by 2×10^{-6} M haemoglobin (37°C, pH 7.4) and also in the photochemical oxidation of the mitochondria in ox liver cells. These findings suggest that ubiquinol and ubiquinone can play a very important role as antioxidants for mitochondria.

Squalene [341], which is present in considerable amounts in fish lipids, can behave as an antioxidant, at least at the beginning of the reaction. In fact, a comparative study [341], of the autoxidation of methyl linoleate and methyl oleate at 63°C in the presence of 0.02% of tocopherol or squalene showed that in the first five days the content of the hydroperoxide is lower in the presence of squalene than in the presence of added α-tocopherol; after this period the formation of the peroxide in the presence of squalene is faster and after 8 days it is as rapid as in the absence of inhibitors.

We have already seen that inorganic compounds [337,338,342-344] introduced with food can prevent certain pathological changes induced by a diet with a high content of polyunsaturated lipids and deficient in vitamin E. A study [337] has shown that Na_2SeO_3 introduced into the diet gradually reduces the formation of peroxides in the liver, heart, and muscles of chickens. Selenocystine and selenomethionine were detected in the hydrolysate of liver proteins.

In addition various aminoacids containing selenium at concentrations of 10^{-4} and 2×10^{-4} play the role of inhibitors, which may be compared with α-tocopherol in the oxidation of an emulsion of methyl linoleate catalysed by haemoglobin.

These findings suggest that selenium introduced with food in the form of inorganic compounds is deposited in various tissues of the chicken as selenoproteins which *in vivo* probably behave as antioxidants. The mechanism of inhibition by selenium compounds consists mainly in the decomposition by these compounds of the hydroperoxide formed, but not via a radical mechanism. In addition, selenium compounds, which decompose peroxides, protect coenzyme A in the absence of cystine [343].

The role of selenium compounds in metabolism has been discussed in detail [338,343] and the mechanism of their interaction with hydroperoxides has been considered in another study [344].

Apart from α-tocopherol, which is widely distributed in plants [16,327], many polyhydroxy-compounds, such as simple aromatic polyphenols or heterocyclic derivatives (hydroxychromans, hydroxycoumarones [345]) can play a fairly important role in inhibition. In certain special cases their inhibiting activity may be the main function, as in the case of NDGA, the antioxidant activity of which *in vitro* has been considered in this review.

A thorough study has been made [346] of the toxic effect of various types of lipid peroxides administered intravenously and intra-abdominally to rats and chickens. It was shown that the toxicity depends to a large extent on the nature of the compound in which the lipids are bound and on the method of injection. Thus, peroxides of the triglycerides derived from olive and nut (groundnut?) oils are much less toxic than free fatty acids. Their ester and phospholipid peroxides, which are of the same type, are much more toxic when administered intravenously than intra-abdominally.

The retention of lipid peroxides by various tissues depends also on the type of peroxy-compound and the nature of the emulsifying agent. Probably it is greater the lower the toxicity of the peroxide. The peroxides of triglycerides are retained to a considerable degree by blood, lungs, the liver, the spinal column, kidneys, and the intestine. A moderate amount is retained in the heart and the muscles and a very insignificant amount in the brain, the pancreas, and in adipose tissues. The degree of retention in the blood is very considerable over a period of 1 h, is insignificant after 2 h, and becomes zero after 500 h.

Possibly the mechanism of the elimination of triglyceride peroxides differs from that for peroxides of other types. It is interesting to note that intravenous administration yields identical results with chickens and rats but after intra-abdominal administration the retention of triglyceride peroxides by various rat organs is less than by chicken organs.

We may note that in the presence of 0.5 millimole per kilogramme of the hydroperoxides of ethyl linoleate and $(CH_3)_3COOH$ [t-butyl hydroperoxide], symptoms of methaemoglobinaemia appear.

Numerous quantitative data concerning the retention of various types of peroxides by rat and chicken organs as a function of the dose administered and time and also as a function of the content of methaemoglobin in the case of ethyl linoleate and $(CH_3)_3COOH$ have been published [346].

The toxicity of certain autoxidation products of lipids and of certain organic peroxides administered to rats intra-abdominally only has been examined [2,347]. According to the data of the second study, the hydroperoxide of methyl linoleate and particularly of $(CH_3)_3COOH$ should be less toxic than was suggested in another investigation [346]. Nevertheless, it follows from the above studies [346,347] that lipid peroxides (and probably other peroxides) are much more toxic when introduced with food, which is reasonable, bearing in mind their decomposition by the mucous membrane of the intestine, which occurs in such a way that only some of their decomposition products pass into the lymph. Their toxicity when introduced by injection is undoubtedly a consequence of the combined effects resulting from attack on double bonds (which entails the degradation of the principal fatty acids, namely the 18:2 and 20:4 acids) and the degradation of haematin nuclei, protein thiols, various vitamins (particularly vitamins A and C), various enzymes, and membranes (which may lead to the haemolysis of red blood corpuscles).

Oxidation catalysed by variable-valence elements has been examined in two papers [348,349] and the general theoretical questions concerning the inhibition of autoxidation have been considered by Knorre et al. [350] Moreover, several papers and publications on synthesis after 1960 were devoted to organic peroxides in general [351-359], some of which [351-353] analyse various methods of their determination.

REFERENCES

1. V. Karnojitzky and C. Viel, Produits et Problèmes Pharmaceutiques, 21, 245 (1966).
2. R. Latarjet, "Peroxydes Organiques en Radiobiologie", Masson, 1958.
3. J. Bolland and H. Koch, J. Chem. Soc., 445 (1945).
4. E. Farmer and D. Sutton, J. Chem. Soc., 122 (1943).
5. E. Farmer, Trans. Faraday Soc., 12, 229 (1946).
6. J. Skellon and D. Wharry, Chem. Ind. (London), 929 (1963).
7. D. Swern, in "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1961, Vol. 1, pp. 2–54.

8. A. L. Tappel, "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1961, Vol. 1, pp. 325–366.
9. J. F. Mead, "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1961, Vol. 1, pp. 299–323.
10. E. M. Bevilacqua, in "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1962, Vol. 2, pp. 857–918.
11. W. O. Lundberg, "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1962, Vol. 2, pp. 451–456.
12. J. R. Chipault, "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1962, Vol. 2, pp. 477–542.
13. J. H. Mitchell and A. S. Henick, "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1962, Vol. 2, p. 543–592.
14. J. C. Cowan and C. D. Evans, "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1962, Vol. 2, pp. 593–628.
15. O. S. Privett, "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1962, Vol. 2, pp. 985–1044.
16. E. Aaes-Jorgensen, "Autoxidation and Antioxidants", J. Wiley and Sons, New York–London, 1962, Vol. 2, pp. 1045–1094.
17. J. Bolland and H. Hugues, J. Chem. Soc., 492 (1949).
18. A. Tobolsky and A. Mercurio, J. Amer. Chem. Soc., 81, 5538 (1959).
19. A. Tappel, Food Res., 18, 104 (1953).
20. D. Boyd and C. Adams, Canad. J. Biochem. Physiol., 33, 191 (1955).
21. A. Güss, J. Cereal. Chem., 44, 607 (1967).
22. S. Hale, T. Richardson, J. Van-Elbe, and D. Hagedun, Lipids, 4, 209 (1969).
23. H. Fuinba, J. Agr. Chem. Soc. Japan, 26, 167 (1952).
24. H. Theorell and R. Holman, Arch. Biochem. Biophys., 14, 250 (1947).
25. H. Theorell and R. Holman, Acta Chem. Scand., 1, 571 (1947).
26. N. Catsimpaolas, Arch. Biochem. Biophys., 131, 185 (1969).
27. R. Holman, Arch. Biochem. Biophys., 15, 403 (1947).
28. A. Tappel and W. Lundberg, J. Biol. Chem., 199, 267 (1952).
29. S. Bergström, Nature, 161, 55 (1948).
30. O. Privett, C. Nickell, and W. Lundberg, J. Amer. Oil Chem. Soc., 32, 505 (1955).
31. A. Tappel, W. Lundberg, and P. Boyer, Arch. Biochem. Biophys., 42, 293 (1953).
32. H. Kunkel, Arch. Biochem. Biophys., 30, 306 (1951).
33. R. Holman, Arch. Biochem. Biophys., 10, 519 (1946).
34. A. Siddiqi and A. Tappel, Arch. Biochem. Biophys., 60, 91 (1956).
35. A. Siddiqi and A. Tappel, Plant. Physiol., 31, 321 (1956).
36. W. Frank and H. Frehse, Z. Physiol. Chem., 235, 333 (1953).
37. A. Siddiqi and A. Tappel, J. Amer. Oil Chem. Soc., 34, 529 (1957).
38. H. Holman and O. Elmer, J. Amer. Oil Chem. Soc., 24, 127 (1947).
39. A. Dolev, W. Rohwedder, and H. Dutton, Lipids, 2, 28 (1967).
40. S. Bergström and R. Holman, Adv. Enzymol., 8, 425 (1948).
41. W. Frank, Ergebn. Enzymforsch., 12, 90 (1951).
42. H. Frehse and W. Frank, Fette u. Seifen, 58, 403 (1956).
43. A. Tappel, J. Biol. Chem., 217, 721 (1955).
44. F. Haurovitz and P. Schwerin, Enzymologia, 9, 197 (1941).
45. A. Tappel, Arch. Biochem. Biophys., 44, 378 (1953).
46. S. Lewis and E. Wills, Biochim. Biophys. Acta, 70, 338 (1963).
47. A. Banks, Chem. Ind. (London), 40, 41 (1961).
48. F. Haurovitz, P. Schwerin, and M. Yensen, J. Biol. Chem., 140, 353 (1941).
49. G. Barron and C. Lyman, J. Biol. Chem., 123, 229 (1938).
50. A. Tappel, Arch. Biochem. Biophys., 56, 473 (1954).
51. Y. Lew and A. Tappel, Food Technol., 10, 235 (1956).
52. V. Maier and A. Tappel, J. Amer. Oil Chem. Soc., 36, 8 (1959).
53. V. Maier and A. Tappel, J. Amer. Oil Chem. Soc., 36, 12 (1959).
54. B. Tarlagdis, J. Amer. Oil Chem. Soc., 38, 479 (1961).
55. W. Brown, L. Harris, and H. Olcott, Arch. Biochem. Biophys., 101, 14 (1963).
56. E. Wills, Biochim. Biophys. Acta, 98, 238 (1965).
57. G. Smith and L. Dunkley, Biochim. Biophys. Acta, 98, 46 (1962).
58. A. Tappel, J. Amer. Oil Chem. Soc., 32, 252 (1955).
59. A. Barber, Arch. Biochem. Biophys., 92, 38 (1961).
60. M. Loury, Compt. rend., 255, 2456 (1962).
61. D. Lefort, C. Paquot, and J. Sorba, Bull. Soc. chim. France, 1385 (1959).
62. D. Lefort, J. Sorba, and D. Rouillard, Bull. Soc. chim. France, 2219 (1961).
63. M. Loury, Compt. rend., 256, 2870 (1963).
64. K. Wilbur, F. Bernheim, and O. Shapiro, Arch. Biochem. Biophys., 24, 305 (1949).
65. M. Loury and A. Prevot, Compt. rend. (C), 266, 560 (1968).
66. D. Firestone, J. Amer. Oil Chem. Soc., 40, 247 (1963).
67. H. Wexler, Chem. Rev., 64, 591 (1964).
68. V. Karnojitzky, Peintures, Pigments et Vernis, 39, 144 (1963).
69. C. Swift, F. Dollear, and R. O. Connor, Oil and Soap, 23, 355 (1946).
70. E. Farmer and D. Sutton, J. Chem. Soc., 119 (1943).
71. D. Atherton and T. Hilditch, J. Chem. Soc., 105 (1944).
72. F. Gunstone and T. Hilditch, J. Chem. Soc., 836 (1945).
73. F. Gunstone and T. Hilditch, J. Chem. Soc., 1022 (1946).
74. C. Swift, F. Dollear, and L. Brown, J. Amer. Oil Chem. Soc., 25, 39 (1948).
75. J. Ross, A. Greenhart, and I. Gerlcht, J. Amer. Chem. Soc., 71, 283 (1949).
76. J. Skellon, J. Chem. Soc., 343 (1948).
77. I. Gold, J. Chem. Soc., 934 (1958).
78. N. Knight, Q. Reddy, and D. Swern, J. Amer. Oil Chem. Soc., 28, 113 (1951).
79. D. Swern, N. Knight, and W. Ault, J. Amer. Chem. Soc., 67, 1132 (1945).
80. C. Paquot, Ann. Nutrition Aliment., 134, 327 (1958).
81. O. Privett, Fette u. Seifen, 61, 842 (1959).
82. E. Frankel, C. Evans, and J. Cowan, J. Amer. Oil Chem. Soc., 37, 418 (1960).

83. A. Feuell and J. Skellon, J. Chem. Soc., 59 (1952).
84. W. Ellis, Biochem. J., 46, 129 (1950).
85. G. King, J. Chem. Soc., 587 (1956).
86. M. Loury and C. Lechartier, Rev. Fr. des Corps Gras, 9, 133 (1962).
87. J. Skellon and C. Taylor, J. Chem. Soc., 1813 (1952).
88. J. Skellon and C. Taylor, J. Chem. Soc., 1433 (1953).
89. C. Paquot and J. Mercier, Rev. Fr. des Corps Gras, 9, 275 (1962).
90. J. Mercier, Compt. rend. (C), 271, 944 (1970).
91. O. Privett, W. Lundberg, and N. Khan, J. Amer. Oil Chem. Soc., 30, 61 (1953).
92. J. Cannon and K. Zilch, J. Amer. Oil Chem. Soc., 29, 447 (1952).
93. A. Johnston, K. Zilch, and H. Dutton, J. Amer. Oil Chem. Soc., 38, 367 (1961).
94. J. Pokorny, Nahrung, 12, 747 (1968).
95. K. Fukusimi and N. Ikeda, J. Amer. Oil Chem. Soc., 46, 64 (1969).
96. H. Badings, J. Amer. Oil Chem. Soc., 36, 648 (1959).
97. R. Harvat, W. McFadden, H. Lane, and A. Shilpherd, J. Amer. Oil Chem. Soc., 43, 351 (1966).
98. W. Colb and E. Day, J. Amer. Oil Chem. Soc., 42, 420 (1965).
99. D. Lillard and E. Day, J. Amer. Oil Chem. Soc., 41, 549 (1964).
100. L. Williamson, J. Appl. Chem., 3, 301 (1953).
101. M. Loury and M. Forney, Rev. Fr. des Corps Gras, 15, 367 (1968).
102. M. Loury and M. Forney, Rev. Fr. des Corps Gras, 15, 663 (1968).
103. M. Marcuse and P. Fredericksen, J. Amer. Oil Chem. Soc., 46, 269 (1969).
104. J. Bolland and P. Ten-Have, Trans. Faraday Soc., 43, 201 (1947).
105. J. Bolland and P. Ten-Have, Discuss. Faraday Soc., 2, 252 (1947).
106. S. Chang and F. Kummerow, J. Amer. Oil Chem. Soc., 30, 403 (1953).
107. O. Privett, C. Nickell, W. Tolberg, R. Paschke, D. Wheeler, and W. Lundberg, J. Amer. Oil Chem. Soc., 31, 23 (1954).
108. E. Frankel, C. Evans, D. McCannel, and H. Dutton, J. Org. Chem., 26, 4663 (1961).
109. T. Kevon and D. Olcott, Nature, 210, 214 (1966).
110. F. Kawahara, H. Dutton, and J. Cowan, J. Amer. Oil. Chem. Soc., 29, 633 (1952).
111. A. Gaddis, R. Ellis, and C. Currie, J. Amer. Oil Chem. Soc., 38, 371 (1961).
112. R. Ellis, A. Gaddis, C. Currie, and S. Pawel, J. Amer. Oil Chem. Soc., 45, 553 (1968).
113. M. Loury and M. Forney, Rev. Fr. des Corps Gras, 16, 167 (1969).
114. E. Cruger and A. Tappel, J. Chromatog., 40, 277 (1969).
115. M. Brodnitz, W. Nawar, and I. Fagerson, Lipids, 3, 59 (1968).
116. M. Brodnitz, W. Nawar, and I. Fagerson, Lipids, 3, 65 (1968).
117. C. Swift and F. Dollear, J. Amer. Oil Chem. Soc., 26, 297 (1949).
118. P. Swoboda and C. Lea, J. Sci. Food Agric., 16, 680 (1965).
119. H. Von-Pezold, Fette u. Seifen, 61, 1019 (1959).
120. C. Hoffmann, J. Amer. Oil Chem. Soc., 98, 31 (1961).
121. C. Hoffmann and J. Keppler, Nature, 185, 311 (1960).
122. T. Samouse and S. Chang, J. Amer. Oil Chem. Soc., 44, 508 (1967).
123. B. Mookheijee and S. Chang, J. Amer. Oil Chem. Soc., 40, 232 (1963).
124. C. Hoffman, J. Amer. Oil Chem. Soc., 38, 1 (1961).
125. N. Kimoto and A. Gaddis, J. Amer. Oil Chem. Soc., 46, 403 (1969).
126. C. Hoffman and P. Mejloom, J. Amer. Oil Chem. Soc., 46, 620 (1969).
127. M. Loury and G. Lechartier, Rev. Fr. des Corps Gras, 13, 345 (1966).
128. E. Farmer and H. Sundralingham, J. Chem. Soc., 121 (1942).
129. E. Hawkins and D. Quin, J. Appl. Chem., 6, 1 (1956).
130. P. Dubouloz and J. Gandonnière, Bull. Soc. chim. France, 1339 (1956).
131. P. Dubouloz, J. Gandonnière, and R. Merville, Compt. rend. Soc. biol., 152, 356 (1957).
132. P. Dubouloz, J. Gandonnière, and R. Merville, Compt. rend. Soc. biol., 151, 154 (1957).
133. P. Dubouloz and J. Fondarai, Bull. Soc. Chim. biol., 40, 1521 (1958).
134. W. Brill, J. Amer. Chem. Soc., 85, 141 (1963).
135. D. Denney and J. Rosen, Tetrahedron, 20, 271 (1964).
136. P. Dubouloz and J. Fondarai, Bull. Soc. Chim. biol., 35, 819 (1953).
137. S. Lewis and E. Wills, Biochem. Pharmacol., 11, 901 (1962).
138. E. Wills, Biochem. Pharmacol., 7, 7 (1961).
139. C. Little and P. O. Brien, Biochem. J., 103, 13p (1966).
140. D. Menzel, Lipids, 2, 83 (1967).
141. E. Wills, Biochem. Pharmacol., 2, 276 (1959).
142. H. Tauber, J. Amer. Chem. Soc., 62, 2251 (1940).
143. R. Sumner, J. Biol. Chem., 134, 535 (1940).
144. R. Sumner, J. Biol. Chem., 146, 215 (1942).
145. A. Kaineth and S. Hauge, J. Agric. Food Chem., 1, 1001 (1953).
146. B. Polister and J. Mead, J. Agric. Food Chem., 2, 199 (1954).
147. F. Quackenbusch, R. Cox, and H. Stenbock, J. Biol. Chem., 145, 169 (1942).
148. T. Hilditch, Chem. Ind. (London), 67 (1944).
149. J. Dassow and M. Stansby, J. Amer. Oil Chem. Soc., 26, 475 (1949).
150. C. Robson and J. Bexter, J. Amer. Chem. Soc., 65, 940 (1943).
151. E. Hove and L. Hove, J. Biol. Chem., 156, 623 (1944).
152. H. Hove and L. Hove, J. Biol. Chem., 156, 611 (1944).
153. H. Kunkel and W. Nelson, J. Biol. Chem., 183, 149 (1950).
154. A. Tappel, Arch. Biochem. Biophys., 47, 223 (1953).
155. H. Dam, I. Prange, and E. Sondergaard, Acta Pharmacol. Toxicol., 8, 1 (1952).
156. H. Dam, I. Prange, and E. Sondergaard, Acta Pharmacol. Toxicol., 8, 23 (1952).
157. H. Dam and H. Granados, Acta Pharmacol. Toxicol., 8, 47 (1953).
158. H. Dam, P. Harris, and M. Woodside, Nature, 150, 91 (1952).
159. T. Moore, Biochem. J., 34, 1321 (1940).
160. P. Pavelc and G. Shull, J. Biol. Chem., 146, 351 (1942).
161. J. Wizzing, T. Nishida, O. Johnston, and E. Kummerow, J. Amer. Oil Chem. Soc., 34, 421 (1957).

162. C. Lea, Chem. Ind. (London), 1303 (1953).
163. J. Pien, "Mises au Point de Chim. Anal. Organ. Pharmac. et Bromatol.", 3rd Series, Masson, Paris, 1955, p. 85.
164. L. Smith and E. Jack, J. Dairy Sci., 42, 767 (1959).
165. J. Adda, Le Lait, 42, 378 (1962).
166. W. Stark and D. Forss, J. Dairy Res., 29, 173 (1962).
167. W. Stark and D. Forss, J. Dairy Res., 31, 256 (1964).
168. E. Hammond and F. Hill, J. Amer. Oil Chem. Soc., 41, 180 (1964).
169. R. Lawrence and J. Hawke, Nature, 197, 1276 (1963).
170. D. Forss, G. Pont, and W. Stark, J. Dairy Res., 22, 31 (1955).
171. D. Forss, E. Dunstone, and W. Stark, J. Dairy Res., 27, 211 (1960).
172. E. Day, J. Dairy Res., 43, 1360 (1960).
173. D. Forss, E. Dunstone, and W. Stark, J. Dairy Res., 27, 381 (1960).
174. D. Forss, E. Dunstone, and W. Stark, J. Dairy Res., 27, 373 (1960).
175. J. Mercier, Ann. Nutrition Aliment., 16, No. 3, 59 (1962).
176. R. Hannon, Nature, 169, 152 (1952).
177. R. Hannon and S. Sheppard, Nature, 170, 1021 (1952).
178. J. Chipault, Ind. Eng. Chem., 49, 1713 (1957).
179. E. Lee and A. Wagenknecht, Food Res., 16, 239 (1951).
180. E. Lee and A. Wagenknecht, Food Res., 17, 343 (1952).
181. E. Lee, Food Res., 19, 215 (1956).
182. E. Lee, Food Res., 21, 605 (1956).
183. R. Buttery, C. Hendel, and M. Biggs, J. Agric. Food Chem., 9, 241 (1961).
184. B. Watts and D. Peng, J. Biol. Chem., 170, 441 (1947).
185. A. Kozin and P. Shkol'nikov, Dokl. Akad. Nauk SSSR, 65, 789 (1949).
186. E. A. Boichenko and G. N. Saenko, Dokl. Akad. Nauk SSSR, 138, 1453 (1961).
187. P. Kolesnikov, Biokhimiya, 13, 370 (1948).
188. P. Kolesnikov, Biokhimiya, 14, 124 (1949).
189. P. Dubouloz and R. Merville, Compt. rend. Soc. biol., 146, 1756 (1952).
190. P. Dubouloz, J. Laurent, and J. Fondarai, Bull. Soc. chim. France, 669 (1953).
191. P. Dubouloz and J. Laurent, Compt. rend. Soc. biol., 150, 216 (1956).
192. A. Davies and T. Moore, Nature, 147, 794 (1941).
193. H. Dam and H. Granados, Science, 102, 327 (1945).
194. K. Robinson and E. Coly, Nature, 168, 997 (1951).
195. B. Beadle, O. Widler, and H. Kraybill, J. Biol. Chem., 175, 221 (1941).
196. J. Oldfield, R. Sinhuber, and A. Rasheed, J. Amer. Oil Chem. Soc., 40, 357 (1963).
197. A. Tappel, Arch. Biochem. Biophys., 54, 266 (1963).
198. A. Venolia and A. Tappel, J. Amer. Oil Chem. Soc., 35, 135 (1958).
199. T. Nishida and F. Kummerow, J. Lipid Res., 1, 450 (1959).
200. K. Narayan and E. Kummerow, J. Amer. Oil Chem. Soc., 40, 339 (1963).
201. K. Narayan, M. Pugal, and F. Kummerow, J. Amer. Oil Chem. Soc., 41, 204 (1964).
202. I. Andrews, J. Bjorksten, and E. Trink, J. Amer. Oil Chem. Soc., 42, 778 (1965).
203. K. Narayan and F. Kummerow, J. Amer. Oil Chem. Soc., 35, 51 (1958).
204. I. Desai and A. Tappel, J. Lipid Res., 4, 204 (1963).
205. W. Roubal and A. Tappel, Arch. Biochem. Biophys., 113, 5 (1966).
206. W. Roubal and A. Tappel, Arch. Biochem. Biophys., 113, 150 (1966).
207. S. Hartroft, Science, 113, 678 (1951).
208. B. Casselman, Biochim. Biophys. Acta, 11, 445 (1953).
209. B. Casselman, Biochim. Biophys. Acta, 11, 446 (1953).
210. B. Lalor, W. Leoschke, and C. Elvehjem, J. Nutrit., 45, 183 (1951).
211. J. Glavind and S. Hartman, Acta Path. Microbiol. Scand., 30, 1 (1952).
212. J. Glavind and S. Hartman, Acta Path. Microbiol. Scand., 29, 73 (1971).
213. H. Dam, J. Nutrit., 27, 193 (1944).
214. H. Dam, K. Nielsen, I. Prange, and E. Sondergaard, Nature, 182, 809 (1958).
215. H. Dam, J. Nutrit., 28, 297 (1944).
216. E. Greech, M. Bahman, B. Reid, and J. Couch, J. Nutrit., 64, 55 (1958).
217. B. Century and M. Horvitt, Arch. Biochem. Biophys., 104, 416 (1964).
218. J. Machlin, J. Amer. Oil Chem. Soc., 40, 368 (1963).
219. J. Machlin, S. Gordon, J. Morr, and W. Pope, J. Nutrit., 76, 284 (1962).
220. J. Machlin, Fed. Proc., 21, 474 (1962).
221. H. Granados and H. Dam, Science, 101, 250 (1945).
222. E. Aaes-Jorgensen, H. Dam, and H. Granados, Acta Pharmacol. Toxicol., 7, 170 (1951).
223. J. Glavind, H. Granados, and S. Hartman, Experientia, 5, 84 (1949).
224. T. Moore, Biochem. J., 37, 112 (1943).
225. J. Irving, Nature, 184, 645 (1959).
226. C. McKenzie and M. McCollum, Science, 94, 216 (1941).
227. H. Draper, Nature, 180, 1419 (1957).
228. H. Dam and H. Granados, Acta Pharmacol. Toxicol., 7, 181 (1951).
229. W. Witt and K. Schwarz, Experientia, 14, 28 (1958).
230. J. McLean and J. Beveridge, J. Nutrit., 52, 499 (1954).
231. J. McLean and J. Beveridge, J. Nutrit., 41, 55 (1952).
232. O. Lindau and E. Work, Biochem. J., 48, 301 (1951).
233. H. Dam, Experientia Suppl., 1, 195 (1953).
234. H. Dam, Pharmacol. Rev., 9, 1 (1957).
235. F. Christiansen, H. Dam, I. Prange, and E. Sondergaard, Acta Pharmacol. Toxicol., 15, 181 (1958).
236. O. Johnson and F. Kummerow, J. Amer. Oil Chem. Soc., 34, 407 (1957).
237. L. Wischner and M. Keenly, J. Amer. Oil Chem. Soc., 42, 776 (1965).
238. E. Perkins, J. Amer. Oil Chem. Soc., 42, 782 (1965).
239. D. Firestone, W. Horivitz, and G. Shue, J. Amer. Oil Chem. Soc., 38, 253 (1961).
240. C. Poling, W. Warner, P. Mone, and F. Rice, J. Amer. Oil Chem. Soc., 39, 315 (1962).
241. T. Dornseifer, S. Keith, and J. Powers, J. Amer. Oil Chem. Soc., 42, 1073 (1965).
242. R. Kisnamourthy, T. Kawada, and S. Chang, J. Amer. Oil Chem. Soc., 42, 878 (1965).
243. D. Melnick, F. Luckmann, and C. Gooding, J. Amer. Oil Chem. Soc., 35, 271 (1958).

244. S. Chang and B. Watts, J. Amer. Oil Chem. Soc., 29, 334 (1952).
245. P. Dubouloz, J. Fondarai, and C. Lagarde, Biochim. Biophys. Acta, 3, 371 (1949).
246. J. Glavind and N. Tryding, Acta Physiol. Scand., 49, 97 (1960).
247. J. Andrews, H. Griffith, J. Mead, and R. Stein, J. Nutrit., 70, 199 (1960).
248. A. Chaharidjan, L. Morris, and R. Holman, J. Nutrit., 76, 52 (1962).
249. M. Hanson, Chem. Ind. (London), 1541 (1964).
250. C. Lea, Chem. Ind. (London), 244 (1965).
251. O. Johnson, T. Sekuragi, and F. Kummerow, J. Amer. Oil Chem. Soc., 33, 433 (1956).
252. J. Raulin, "Journée d'Étude sur l'Alteration Oxydative des Corps Gras", Marseille, 23 mars 1967, pp. 57–70.
253. F. Rice, C. Poling, P. Mone, and W. Warner, J. Amer. Oil Chem. Soc., 37, 606 (1960).
254. O. Johnson, E. Perkins, M. Sugai, and F. Kummerow, J. Amer. Oil Chem. Soc., 34, 594 (1957).
255. D. Melnick, J. Amer. Oil Chem. Soc., 34, 351 (1957).
256. A. Dungoumau, F. Berluerau, and H. Debruyne, Rev. Fr. des Corps Gras, 4, 541 (1957).
257. A. Dungoumau, D. Boussagnol, and H. Debruyne, Rev. Fr. des Corps Gras, 5, 613 (1958).
258. N. Raju, N. Narayana, and R. Rajagoplan, J. Amer. Oil Chem. Soc., 42, 774 (1965).
259. J. Andrews, J. Mead, and W. Griffith, Fed. Proc., 15, 918 (1956).
260. W. Matsuo, J. Biochem. (Japan), 41, 647 (1954).
261. H. Kaunitz and C. Stanetz, Fed. Proc., 14, 408 (1955).
262. R. Holman and S. Greenberg, J. Amer. Oil Chem. Soc., 35, 707 (1958).
263. P. O. Brien and A. Frazer, Proc. Nutrit. Soc., 25, 9 (1966).
264. H. Kaunitz, C. Stanetz, and H. Johnson, J. Amer. Oil Chem. Soc., 93, 630 (1956).
265. P. Kotin and H. Falk, Rad. Res. Suppl., 3, 3 (1963).
266. J. Raulin and J. Petit, Arch. Sci. Physiol., 14, 143 (1960).
267. R. Holman and S. Greenberg, Arch. Biochem. Biophys., 49, 49 (1954).
268. B. Potteau, J. Leclere, J. Causeret, and R. Cluzan, Rev. Fr. des Corps Gras, 13, 379 (1966).
269. J. Raulin and J. Petit, Arch. Sci. Physiol., 16, 77 (1962).
270. J. Raulin and T. Terroine, Arch. Sci. Physiol., 16, 89 (1962).
271. R. Barnes, M. Clausen, I. Rusoff, and H. Hanson, Arch. Sci. Physiol., 2, 313 (1948).
272. P. Dubouloz, Rev. Fr. des Corps Gras, 4, 24 (1957).
273. E. Perkins, Food Technol., 14, 508 (1960).
274. T. Hilditch and F. Williams, "Chemical Constitution of Natural Fats", Chapter and Co., London, 1964.
275. M. Horvitt, C. Harvey, and B. Century, Science, 130, 917 (1959).
276. L. Witting, C. Harvey, B. Century, and M. Horvitt, J. Lipid Res., 2, 412 (1961).
277. L. Machlin, C. Mareo, and R. Gordon, J. Amer. Oil Chem. Soc., 39, 229 (1962).
278. K. Bernhard, Oleagineux, 13, 19 (1958).
279. E. LeBreton, Oleagineux, 13, 25 (1958).
280. P. Dubouloz and J. Dumas, Compt. rend. Soc. biol., 144, 1080 (1950).
281. P. Dubouloz and J. Dumas, Compt. rend. Soc. biol., 146, 1350 (1952).
282. P. Dubouloz and J. Dumas, Compt. rend., 234, 2575 (1952).
283. P. Dubouloz and J. Dumas, Bull. Soc. Chim. biol., 36, 983 (1954).
284. J. Dumas, These doctorat Sci. Nat., Marseille, 1954.
285. P. Dubouloz, J. Fondarai, J. Laurent, and R. Marville, Analyt. Chim. Acta, 15, 84 (1956).
286. P. McGray, Nature, 198, 98 (1963).
287. R. Gerschman, D. Gilbert, and S. Nye, Science, 119, 623 (1954).
288. N. Milas, R. Harris, and A. Golbolic, Rad. Res. Suppl., 3, 71 (1963).
289. S. El-Kabib, U. Chenau, M. Carpenter, and R. Caputto, Nature, 201, 188 (1964).
290. N. Milas and N. Belic, J. Amer. Chem. Soc., 81, 3358 (1959).
291. E. Wills and J. Rotblatt, Int. J. Rad. Biol., 8, 551 (1964).
292. E. Wills, Biochem. J., 99, 667 (1966).
293. H. Abramson, J. Biol. Chem., 178, 179 (1949).
294. F. Bernheim, Rad. Res. Suppl., 3, 17 (1963).
295. A. Barber, Rad. Res. Suppl., 3, 33 (1963).
296. E. Pritchard and H. Singh, Biochem. Biophys. Res. Comm., 2, 184 (1960).
297. J. Bieri and A. Anderson, Arch. Biochem. Biophys., 90, 105 (1960).
298. A. Barber, Lipids, 1, 146 (1966).
299. N. Wolfson and K. Wilbur, Exp. Cell. Res., 10, 556 (1956).
300. E. Hill, J. Amer. Oil Chem. Soc., 40, 360 (1963).
301. T. Richardson, A. Tappel, L. Smith, and C. Houle, J. Lipid Res., 3, 344 (1969).
302. T. Richardson, A. Tappel, and C. Cruger, Arch. Biochem. Biophys., 94, 1 (1961).
303. A. Marjorie, A. Swanson, and C. Arton, J. Biol. Chem., 187, 281 (1950).
304. M. Spiro and J. McKiblin, J. Biol. Chem., 217, 643 (1956).
305. A. Tappel and H. Zalkin, Arch. Biochem. Biophys., 80, 326 (1959).
306. A. Tappel and H. Zalkin, Arch. Biochem. Biophys., 80, 333 (1959).
307. E. Bernheim, K. Wilbur, and C. Kenaston, Arch. Biochem. Biophys., 38, 177 (1952).
308. A. Ottolenghi, E. Bernheim, and K. Wilbur, Arch. Biochem. Biophys., 56, 156 (1955).
209. A. Ottolenghi, Arch. Biochem. Biophys., 79, 355 (1959).
310. S. Fortney and W. Lynn, Arch. Biochem. Biophys., 104, 241 (1964).
311. H. Zalkin and A. Tappel, Arch. Biochem. Biophys., 88, 113 (1960).
312. L. Corwin, Arch. Biochem. Biophys., 97, 51 (1962).
313. H. Kinura and F. Kummerow, Arch. Biochem. Biophys., 102 (1963).
314. A. Tappel and H. Zalkin, Nature, 185, 35 (1960).
315. A. Kitabchi, P. McCay, M. Carpenter, R. Trucco, and R. Caputto, J. Biol. Chem., 235, 1591 (1960).
316. R. Caputto, R. Trucco, and A. Kitabchi, Ann. Acad. Sci. N. Y., 92, 79 (1961).
317. P. Hochstein and L. Ernster, Biochem. Biophys. Res. Comm., 12, 388 (1963).
318. D. Desai, P. Sawant, and A. Tappel, Biochim. Biophys. Acta, 86, 277 (1964).
319. E. Wills and A. Wilkinson, Biochem. J., 99, 657 (1966).
320. R. Heath and L. Packer, Biochem. Biophys. Res. Comm., 19, 716 (1965).

321. C. Tsen and H. Collier, Canad. J. Biochem. Physiol., 38, 981 (1960).
322. J. Bunyan, J. Green, E. Edwin, and A. Diplock, Biochem. J., 77, 47 (1960).
323. F. Christiansen and H. Dam, Acta Pharmacol. Toxicol., 7, 167 (1951).
324. F. Christiansen, H. Dam, R. Gortner, and E. Sondergaard, Acta Physiol. Scand., 35, 215 (1956).
325. M. Horvitt, C. Harvey, and B. Meyer, Fed. Proc., 17, 245 (1958).
326. O. Privett and F. Quackenbusch, J. Amer. Oil. Chem. Soc., 31, 321 (1954).
327. E. Have and P. Harris, J. Amer. Oil Chem. Soc., 28, 405 (1951).
328. A. Tappel, W. Brown, H. Zalkin, and V. Maier, J. Amer. Oil Chem. Soc., 38, 5 (1961).
329. C. Golumbic and H. Matill, J. Amer. Chem. Soc., 63, 1279 (1941).
330. O. Gloor, F. Weber, J. Wush, and O. Wiss, Helv. Chim. Acta, 46, 2457 (1963).
331. S. Csallany and H. Draper, Arch. Biochem. Biophys., 96, 142 (1962).
332. F. Weber and O. Wiss, Helv. Physiol. Pharmacol. Acta, 21, 131 (1962).
333. S. Csallany and H. Draper, Arch. Biochem. Biophys., 100, 335 (1963).
334. S. Csallany, M. Chiu, and H. Draper, Lipids, 5, 63 (1970).
335. F. Gruger and A. Tappel, Lipids, 5, 326 (1970).
336. F. Kummerow, Fed. Proc., 23, 1053 (1964).
337. H. Zalkin, A. Tappel, and P. Jordan, Arch. Biochem. Biophys., 91, 117 (1960).
338. L. Witting, Fed. Proc., 24, 912 (1965).
339. A. Mellas and A. Tappel, J. Biol. Chem., 241, 4353 (1966).
340. J. Green, A. Diplock, J. Bunyan, E. Eldwin, and D. McHale, Nature, 190, 318 (1961).
341. G. Rao and K. Achaya, J. Amer. Oil Chem. Soc., 45, 297 (1968).
342. J. Bieri, Nature, 184, 1148 (1959).
343. J. Bieri and E. Andrews, J. Amer. Oil Chem. Soc., 40, 365 (1963).
344. A. Tappel, Fed. Proc., 24, 73 (1965).
345. C. Columbic, J. Amer. Oil Chem. Soc., 63, 1142 (1941).
346. J. Glavind, E. Sundergaard, and H. Dam, Acta Pharmacol. Toxicol., 18, 267 (1961).
347. V. Horgan, L. Philpot, W. Portner, and D. Roodyn, Biochem. J., 67, 551 (1957).
348. N. Uri, Chem. Rev., 50, 875 (1952).
349. E. T. Denisov and N. M. Emanuel', Uspekhi Khim., 29, 1409 (1960) [Russ. Chem. Rev., No. 12 (1960)].
350. D. G. Knorre, Z. K. Maizus, L. K. Obukhova, and N. M. Emanuel', Uspekhi Khim., 26, 417 (1957).
351. A. Martin, "Organic Analysis", New York, 1960, Vol. 4, pp. 1–58.
352. V. Karnojitzky, "Mises au Point de Chim. anal. organ. pharmac. et bromatol.", 12th Series, 1964, pp. 43–106.
353. R. Johnson and I. Siddiqi, "The Determination of Organic Peroxides (Monographs on Organic Functional Group Analysis)", Pergamon Press, London, 1970.
354. E. Hawkins, "Organic Peroxides", Spon Ltd., London, 1961.
355. A. Davies, "Organic Peroxides", Butterworth, London, 1961.
356. J. Edwards, "Peroxide Reaction Mechanisms", J. Wiley, New York, 1962.
357. H. Winter, "Peroxyde organische", Ullmanns Encylopädie des Technischer Chem., 1962, Vol. 13, pp. 249–258.
358. O. Mageli, C. Shappard, I. Edwards, and R. Curie, "Encyclopaedia of Chemical Technology", Second Edition, Kirk-Othmer, 1967, Vol. 14, pp. 766–834.
359. D. Swern, "Organic Peroxides", Wiley Interscience, New York, 1970.

O. N. E. R. A., Chatillon,
France