

CHAPTER XIV

OXIDATION BY ATMOSPHERIC OXYGEN (AUTOXIDATION)

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1. Introduction.....	1387
(a) Background Material.....	1387
(b) Modern Trends.....	1390
2. Development of the Concepts of Autoxidation.....	1390
(a) Cyclic Peroxide Theory.....	1391
(b) Ethylene Oxide Theory.....	1393
(c) Hydroperoxide Theory.....	1393
(d) Kinetics of Autoxidation.....	1411
(e) Secondary Products of Autoxidation.....	1414
(f) Miscellaneous.....	1418
3. Quantitative Determination of the Products of Autoxidation.....	1422
(a) Peroxides.....	1422
(b) Other Oxygen-Containing Products of Autoxidation.....	1424
4. Miscellaneous Investigations.....	1425
5. Biological Effects of Autoxidized Fats.....	1430
6. Detection of Oxidation Products.....	1432
7. Oxidation Reaction vs. Temperature.....	1434
References.....	1436

1. Introduction

(a) *Background Material*

Atmospheric oxygen is the most universally prevalent, as well as economically important, oxidizing agent for fats and fatty acids. Its action on fats and fatty products may be beneficial or deleterious depending on the conditions and circumstances under which it occurs. It is advantageously employed in the production of blown oils and in various oxidation and poly-

merization products in the drying oil industry. The process of film formation in applied protective coatings is essentially an oxidative process. The ultimate failure of these same protective coatings, however, is a result of prolonged and excessive oxidation. Rancidification and other forms of deterioration of many fats and fat-containing materials are likewise attributable to atmospheric oxidation. These reactions are highly undesirable and result in serious economic losses.

It is generally assumed that the favorable as well as the unfavorable results of atmospheric oxidation follow from the same type of reactions between oxygen and the unsaturated constituents of fats or fatty acids. Consequently a very great amount of effort has been expended in attempting to unravel the mystery which has enshrouded the fundamental reactions involved in these processes. A vast literature has appeared on the subject since about 1830 but it is only since the 1940's that a reasonably clear understanding of the reaction mechanisms has been obtained.

Because of the spontaneous nature of the reaction between atmospheric oxygen and unsaturated fats and fatty acids, the process is frequently referred to as autoxidation. Without attempting to justify this terminology, it is used here because of its convenience and because it affords a means of avoiding the repetitious use of the cumbersome phrase "oxidation with atmospheric oxygen."

Light, heat, concentration of oxygen, moisture, and the presence of catalysts or inhibitors affect the reaction between oxygen and unsaturated fatty acids, often with seemingly very different results. However, it is probable that the same or very similar mechanisms are involved or would ultimately be involved if the reaction process were permitted to run its course. It is sometimes difficult to evaluate the effect of a specific environmental factor in the over-all oxidation process because in most cases several of these factors are simultaneously active. Of the several factors which may be operating, one may predominate under one set of conditions and quite a different one under another. For example, in a relatively thick layer of fat or fatty acid maintained in a completely filled glass container at room temperature and exposed to sunlight or ultraviolet light, the absorbed radiant energy may be the predominant factor influencing the oxidation reaction, and the temperature and concentration of oxygen may be quite secondary. On the other hand, in the process of blowing oils the temperature and concentration of oxygen are the predominant factors and the effect of light is secondary.

Catalysts or inhibitors of various types may be added to or may be present naturally in fats and oils, thus markedly influencing the reaction velocity.

For example, traces of copper accidentally introduced in a fat or fatty acid increase the rate of oxidative rancidification. The addition of metal oxides or metallic salts in the manufacture of boiled oils accelerates the oxidation and subsequent polymerization reactions in this process, and similar use of these substances in protective coatings enhances their rate of drying and film formation. On the other hand, the presence of antioxidants acts to inhibit these same reactions.

In planning and executing an investigation in the autoxidation of fats, the careful worker attempts to minimize the number of variables which may affect the course of the reaction, or at least control them within relatively narrow limits. Thus, the effect of light may be avoided by excluding it entirely from the reaction, or the effect of heat may be minimized by maintaining a constant low temperature in the reacting system. Unfortunately, the literature contains reports of many investigations in which little or no control was exercised over the various environmental factors, or if they were controlled the author failed to mention it. In such cases it is often difficult to evaluate the data reported or determine the validity of the conclusions drawn by the investigator.

Underlying all investigations of the autoxidation of fats is a desire not only to learn the nature of the products formed but also to understand the mechanisms involved in their production, since only by control of these mechanisms can the desired products be produced or undesirable products be avoided. Natural fats are generally too complex to permit drawing far-reaching generalizations concerning the mechanisms involved in autoxidative processes. Hence, much work involving these reactions, especially since the late 1930's, has been carried out with simple substances, such as oleic, linoleic, or similar acids and their monoesters, because they can be obtained in pure form. Generalizations made on the basis of the results with these simple substances have then been applied to natural fats. In some cases such generalizations may be valid, but as in all cases of reasoning by analogy they may not be entirely justified. It should therefore be borne in mind that applying the results and conclusions derived from one substrate, such as methyl oleate at 100°C., to a quite different substrate, such as cottonseed or soybean oil at room temperature, may not be entirely justified. It is known, for example, that peroxides are relatively unstable at or above 100°C., whereas at room temperature they are relatively stable; therefore quite different end products may be produced under the two reaction conditions. Of course, if the time were sufficiently extended at the lower temperature the same end result might ultimately be achieved although it cannot be assumed that this will be the case. If these limitations

are borne in mind, however, it is entirely possible to proceed from the simple to the more complex with reasonable prospects of resolving ultimately the whole of the complicated phenomena involved in the autoxidation of fats.

(b) *Modern Trends*

Prior to about 1940 most investigators studied the autoxidation of the fats themselves. This approach not only introduced many additional and complicating reactions but it made the interpretation of analytical results difficult, if not impossible. In addition, it soon became evident that some analytical methods were not reliable when applied to autoxidation mixtures, thus rendering some of the early conclusions of dubious validity. Furthermore, before 1940 modern instruments, such as ultraviolet and infrared spectrophotometers and the polarograph, and efficient methods of separation, such as urea-complexing techniques, countercurrent distribution, gas-liquid and column chromatography, molecular and fractional distillation, and low temperature crystallization, were either not available or were incompletely developed.

Modern investigators availed themselves of new instruments and techniques of separation and they also studied a large number of highly purified model compounds. Many of these were not derived from fats but contained structure known to be present in fats and in autoxidizing systems. The kinetics of the autoxidation process was studied in detail and led to a better understanding of the phenomena of catalysis and inhibition.

The increased interest in the phenomenon of autoxidation resulted in a flood of publications. For the reader who wishes to pursue this subject in more detail than can be given here, it is recommended that he refer to some of the excellent reviews that have been devoted to the various aspects of the autoxidation process (1-13).

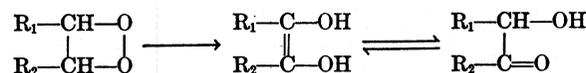
2. Development of the Concepts of Autoxidation

Any explanation of the process of autoxidation must begin, as in all related oxidation reactions, with an understanding of the nature of the first reaction of oxygen with the double bond system. Until this initial step is known the subsequent steps of the process must remain more or less speculative. It is for this reason that every theory which has been evolved with regard to the autoxidation of fats has been founded on some concept concerning this initial reaction and upon the chemical nature of the product thus formed.

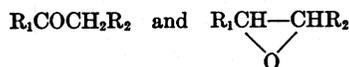
(a) *Cyclic Peroxide Theory*

Although the autoxidation of fatty materials was studied more than a hundred years ago by de Saussure (14) and shortly thereafter by others (15,16), the first observation of autoxidation of a carbon-carbon double bond has been attributed to Schönbein (17), who also investigated the reactions of a variety of oxidizing agents with unsaturated substances, such as almond oil and turpentine. The modern theories of autoxidation, however, are generally assumed to date from about 1900 with the work of Bach (18) and of Engler and co-workers (19-21), who investigated the role of organic peroxides in slow oxidation processes and introduced the term "activated oxygen." Prior to the work of these investigators, molecular oxygen was presumed to be broken down, at least to a small extent, into atomic oxygen in a manner analogous to the liberation of oxygen from hydrogen peroxide, and this "active oxygen" was presumed to be responsible for the slow oxidation observed in various unsaturated organic substances. Bach and Engler, however, believed that autoxidation by atmospheric oxygen was molecular and not atomic in nature, that is, a molecule of oxygen added at the double bond to form a peroxidized compound corresponding to the formula $R_1-O-O-R_2$ and that this compound, like hydrogen peroxide, could in turn oxidize another oxidizable substance. The "activated oxygen" was not considered to be a free oxygen atom but was, rather, chemically bound in such a manner that it could readily be liberated to enter into autoxidative reactions.

In an attempt to explain the action of driers in the formation of linseed oil films, Fahrion (22) and later Ellis (23) assumed that an autoxidation occurred in the fatty acid portion of the molecule to form a cyclic peroxide which underwent rearrangement to a dihydroxyethyleneic or a hydroxyketo configuration as follows:

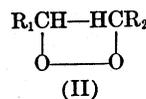
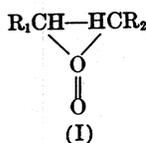


It was also presumed that the cyclic peroxide might give rise to more stable products corresponding to the formulas:



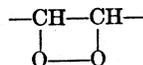
Staudinger (24) proposed a theory of autoxidation based on the assumption that the oxidation reaction originally proposed by Bach and by Engler was probably not the first but the second step in the autoxidation of

ethylenic compounds. He assumed that a molecule of oxygen added at the ethylene bond to form a moloxide, probably corresponding to formula (I), which subsequently underwent a rearrangement to form a cyclic peroxide, (II):



This theory was predicated on an investigation of the autoxidation of *asym*-diphenylethylene, $(\text{C}_6\text{H}_5)_2\text{C}:\text{CH}_2$. Diphenylethylene peroxide was isolated and found to be relatively stable, whereas the product of first addition of oxygen, which could not be isolated, was unstable, and exploded when heated in a steel bomb to 40–50°C. Subsequently, it was learned that the isolable peroxide was a polymer and not a cyclic peroxide.

Although the majority of workers in the field accepted for the next several decades the theory of the formation of a heterocyclic peroxide of the formula

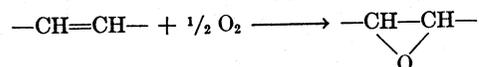


as the primary step in the autoxidation process, all the evidence for the existence of such peroxides was indirect, and no product containing this grouping was isolated or identified from an oxidized fat or fatty acid. The existence of cyclic fatty peroxides was assumed on the basis of certain analytical data which were interpreted as substantiating the existence of such structures in autoxidized unsaturated acids. These analytical determinations included iodine, thiocyanogen, and diene values, total absorbed oxygen, peroxide value, molecular weight, saponification, hydroxyl, and carbonyl values. If the autoxidized acid or ester contained a single oxidation product only, and the methods gave quantitative and reliable results, the structure of the primary autoxidation product might have been deduced on the basis of these data, at least for the simpler unsaturated acids. As a general rule, however, all of these methods were applied to unfractionated, autoxidized acids or esters (and even to fats) in which oxidation had proceeded to the point where there existed in the reaction mixture a number of oxidation, degradation, and polymerization products. Consequently, the interpretation of the analytical data was extremely complicated. Furthermore, the methods were not entirely quantitative or specific in their applica-

tion, especially in the presence of the considerable number of oxidation and degradation products that existed in the reacting system.

(b) *Ethylene Oxide Theory*

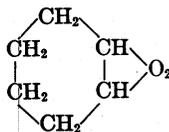
In 1909, Fokin (25) proposed a theory in which the first step in the autoxidation of an ethylenic bond was the formation of an ethylene oxide ring:



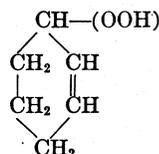
Fatty acids or esters containing the ethylene oxide (oxirane) ring have also been called epoxides, monoxides, and oxido compounds. This structure is now known to be formed during epoxidation of unsaturated fatty materials with perbenzoic, performic, peracetic, and other peracids, and many of these pure epoxy derivatives have been isolated and characterized (26,27). Although epoxy compounds have been isolated from autoxidation mixtures (23,28), it is doubtful that they are primary products. Experimental work in support of the ethylene oxide theory is the least extensive of any of the proposed mechanisms, and it is no longer seriously considered by investigators in the field of autoxidation.

(c) *Hydroperoxide Theory*

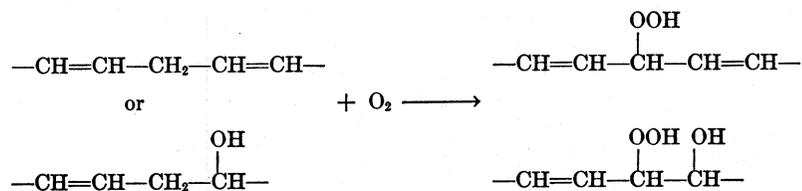
Much information regarding the mechanism of autoxidation of compounds derived from fats has been obtained by studying the oxidation of simple, monounsaturated, nonfatty compounds, such as cyclohexene, which can be prepared readily in a high degree of purity. In 1928, Stephens (29) reported the isolation of a peroxide of cyclohexene, $\text{C}_6\text{H}_{10}\text{O}_2$, which he obtained by treating cyclohexene with oxygen in daylight. He assumed, on the basis of the theories of oxidation accepted at that time, that the product was saturated:



Further research, however, by Criegee and co-workers (30,31) Hock (32-35), and notably by Farmer and Sundralingam (36), established the fact that Stephens' product was a hydroperoxide and that a double bond was present:



It was also determined by Farmer's group that 1-methyl-1-cyclohexene and 1,2-dimethyl-1-cyclohexene behaved similarly when autoxidized. The isolation of purified hydroperoxides from these and many other autoxidized olefins was a tremendous step forward, and cast considerable doubt on the validity of the older concepts of olefin oxidation. Rieche (37,38) postulated that unsaturated fats and oils probably behaved similarly. He suggested that the autoxidation of mono- or polyunsaturated substances may occur through the formation of oxygen-activated methylene groups according to the following scheme:



To Farmer and his co-workers (39-43), however, is due the major credit for developing the hydroperoxide hypothesis of autoxidation, especially in its application to fatty acids, and for substantiating it with convincing experimental evidence. According to Farmer, the autoxidation of practically all nonconjugated olefinic compounds proceeds by a chain reaction involving addition of a molecule of oxygen to the carbon atom adjacent to the double bond to form a hydroperoxide having an intact double bond:



However, as will become evident later, this reaction is probably not the primary step, but is the main chain-propagating reaction.

The concepts of hydroperoxide formation are discussed in detail as they apply to (1) autoxidation of monounsaturated fatty compounds, (2) autoxidation of nonconjugated polyunsaturated fatty compounds, (3) autoxidation of conjugated polyunsaturated fatty compounds, and (4) autoxidation of saturated fatty compounds.

Autoxidation of Monounsaturated Fatty Compounds. The autoxidation of pure monounsaturated fatty acids and esters, although autocatalytic, is slow at temperatures below 60°C. Ultraviolet radiation, metal catalysts,

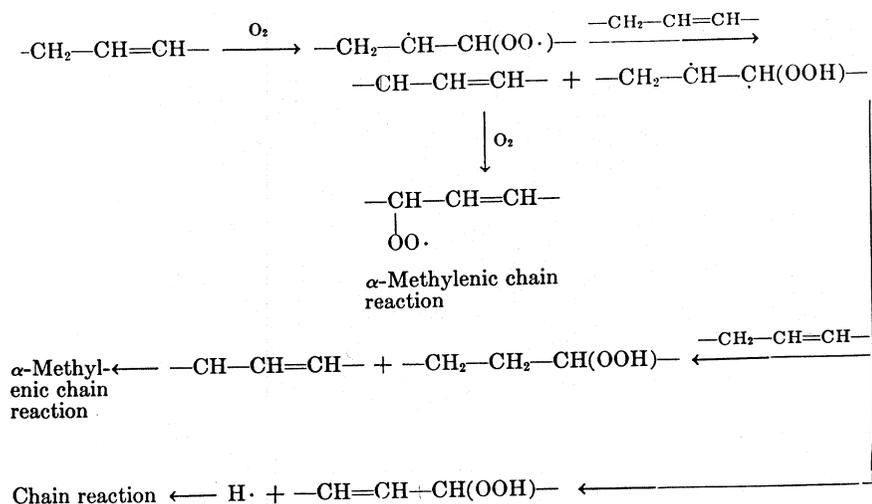
and higher temperatures have been used to speed up the reactions. The rates of oxygen absorption of pure methyl oleate, methyl linoleate, and methyl linolenate have been shown to be about 1:10-12:16-25, at comparable temperatures (44,45). Quantitative data of this type serve to confirm the theory of hydroperoxide formation because if oxygen added to the double bonds exclusively, as proposed by many earlier workers, the relationship just described should be closer to 1:2:3.

The early studies of reaction mechanism were oriented toward the isolation of reaction products, usually after extensive autoxidation. These investigations served to confirm the complexity of autoxidation but did not reveal the nature of the initial reactions.

In a logical extension of the earlier work on pure monoolefins of relatively low molecular weight, Farmer and Sutton (41) isolated nearly pure methyl octadecenoate hydroperoxides from mixtures of products from methyl oleate autoxidized in the presence of ultraviolet to low peroxide levels. Molecular distillation and chromatographic adsorption were used to separate the products of autoxidation. The concentrated or isolated hydroperoxides were shown to contain approximately the theoretical content of peroxide oxygen. Catalytic hydrogenation of the hydroperoxidized methyl oleate yielded a mixture of monohydroxystearates and partial reduction with aluminum amalgam yielded a mixture of hydroxyoctadecenoates, thus demonstrating the presence of a double bond in the peroxide. Oxidation of methyl elaidate under similar conditions also yielded hydroperoxides (46).

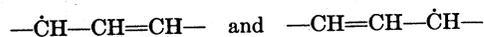
Later, Swift *et al.* (47) employed low temperature solvent crystallization to obtain concentrates containing 90% of hydroperoxides from autoxidized methyl oleate. In 1951, Fugger *et al.* (48), using countercurrent distribution, and in 1953, Privett *et al.* (49), using a modified extraction procedure, fractionated autoxidized methyl oleate between aqueous ethanol and hydrocarbon solvents and obtained concentrates containing 80-90% peroxide. Zilch and Dutton (50) examined numerous model compounds known or suspected to be present in autoxidation mixtures. For large-scale laboratory preparations, Coleman *et al.* (51) employed the urea complex technique to precipitate unoxidized methyl oleate, thereby concentrating the peroxides in the filtrate. Concentrates containing 90% hydroperoxides were consistently obtained when autoxidation did not exceed 15-20%.

According to Farmer, methyl oleate, yields a mixture of mono- and dihydroperoxides (the former predominating) in which the hydroperoxide group is attached to the 8th or 11th carbon atom. As Ross *et al.* (52), and others (53,54) have shown, however, the hydroperoxide group may be at the 8th, 9th, 10th, or 11th carbon atom:

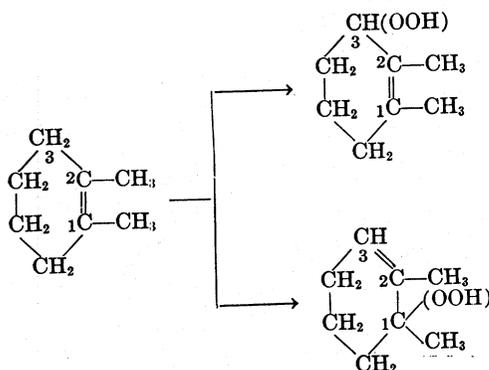


This reaction scheme is completely satisfactory kinetically (65) and thermochemically (65).

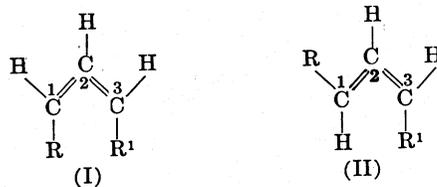
In the free radical mechanism, resonance between the three-carbon systems will occur (43); when a molecule of oxygen and an atom of hydro-



gen are incorporated into the oxidizing molecule, there should be an approximately equal tendency for the hydroperoxide group to appear at positions 1 and 3, thus fixing some of the double bonds in the original position and the remainder at the adjacent pair of carbon atoms. This shift of the double bond, which had been demonstrated spectrophotometrically by Farmer *et al.* (43) in the case of polyunsaturated compounds (discussed later), was suggested by them for monoolefins, although at that time no experimental evidence was offered. Evidence for this conclusion was subsequently obtained in the air oxidation of purified methyl oleate (52-54). Additional chemical evidence that a shift of double bonds occurs during the oxidation of monoolefins and, therefore, that a free radical mechanism is probably applicable in these and comparable oxidations, was also advanced by Farmer and Sutton (53). They studied the oxidation of 1,2-dimethyl-1-cyclohexene and concluded that both 1,2-dimethyl-1-cyclohexene-3-hydroperoxide and 1,2-dimethyl-2-cyclohexene-1-hydroperoxide must have formed:



In 1951 Knight *et al.* (54) demonstrated by infrared measurements that in the early stages of autoxidation of methyl oleate under ultraviolet light, the formation of peroxides is accompanied by the appearance of *trans*-double bonds in an amount approximating 90% of the peroxide formed. Among the possible mechanisms for this isomerization, the following was given. In the free radicals that are formed, the atoms probably lie in a plane which provides maximum resonance energy. The radical could then have two isomeric forms:



Addition of oxygen to carbon 1 of either (I) or (II) or to carbon 3 of (I) would yield a *cis*-hydroperoxide. Infrared measurements suggest that most of the radicals assume configuration (II) and add oxygen to carbon 3, yielding a mixture of *trans*-octadecenoates. This preferential attack may be favored by steric factors.

The foregoing description of autoxidation refers to mild conditions and the initial stages of the reaction. These reactions may be and probably are followed, at least in part, by others at later stages of the autoxidation, at higher temperatures, and in the presence of catalysts. These more vigorous conditions are conducive to accelerated decomposition of peroxides, and the various and perhaps numerous radicals formed probably alter the course of the oxidation to one in which the predominant attack may be at the double bond.

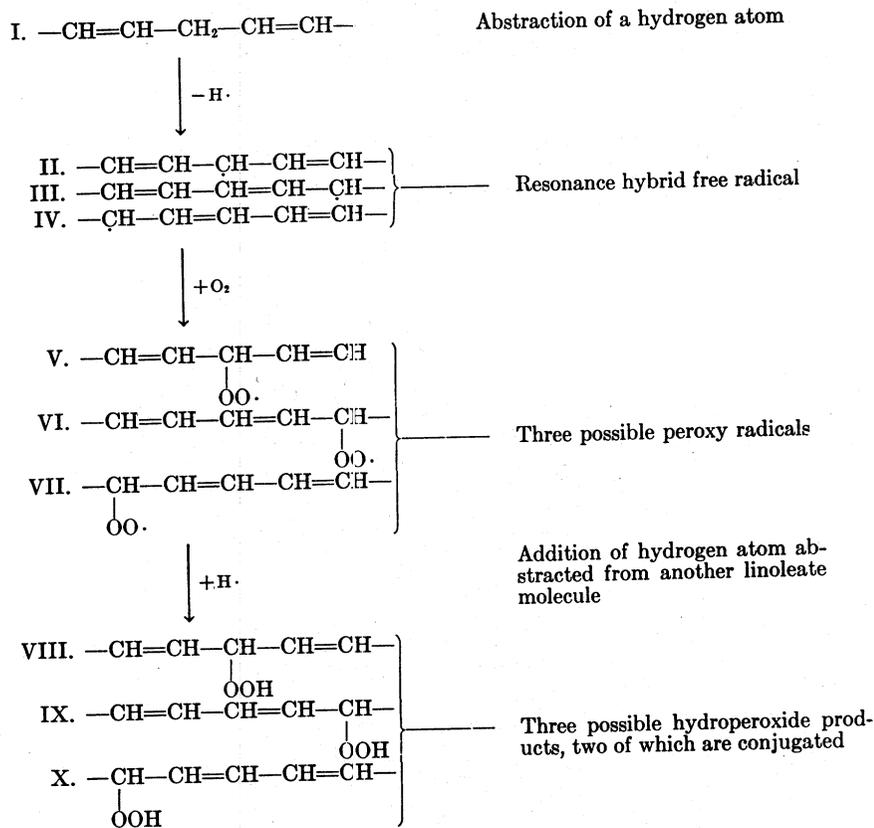
Autoxidation of Nonconjugated Polyunsaturated Fatty Compounds.

The rate of oxidation of methylene-interrupted polyunsaturated systems is more rapid than that of monoethenoic systems because of the activation of a methylene group by the presence of two adjacent double bonds in compounds of this type. This double activation results in oxidation rates twenty to forty times greater than those occurring in compounds containing a single double bond. The polyethenoic acids are, therefore the main source of oxidative rancidity. The oxidation of the polyunsaturated hydrocarbons—dihydromyrcene, dihydrofarnesene, and squalene—was studied by Farmer and Sutton (67), who showed that during the early stages of oxidation substantially all the absorbed oxygen was in the form of hydroperoxides and that the original unsaturation of the compounds was unaffected.

The modern theories of autoxidation of nonconjugated, polyunsaturated fatty compounds began to develop when it was observed that conjugation of double bonds occurred in autoxidizing fish-oil acids (41). When oils containing linoleate or more highly unsaturated systems are autoxidized, the diene conjugation, measured by the absorption of ultraviolet light at 2340 Å, increases proportionately to the uptake of oxygen and the formation of peroxides in the early stages of oxidation. Holman *et al.* (68) demonstrated that the ultraviolet absorption was not associated with the structure of the peroxides because thermal decomposition of the peroxides did not diminish the absorption.

The spectral changes occurring in autoxidizing fatty materials have been observed independently by several investigators and have been studied in considerable detail. The spectral changes accompanying oxidation are qualitatively similar for fatty acids containing two or more double bonds interrupted by methylene groups. Oxidized linoleate has a principal absorption at 2300–2360 Å, due to diene conjugation, and a secondary smooth absorption maximum at 2600–2800 Å, probably due to small amounts of unsaturated ketones. The principal bond is the same for linoleate, linolenate, and arachidonate, but the greater the degree of unsaturation, the lower the diene conjugation absorption per mole of absorbed oxygen. Conversely, the more unsaturated the fatty ester, the greater the light absorption caused by secondary reaction products.

In the oxidation of ethyl linoleate, the monohydroperoxide that forms was shown by ultraviolet absorption measurements to contain approximately 70% of conjugated diene isomers (68a). This calculation was based on spectral data available at that time (1945). The mechanism of reaction originally proposed by Bolland and Koch (68a) is as follows:



The calculated value of 70% of conjugated diene isomers, in the monohydroperoxide fraction was taken as evidence supporting the concept of random attack of oxygen on the free radical from linoleate, thereby giving rise to three products, two-thirds of which were conjugated diene hydroperoxide. In 1945 Bergström (69), however, reported the separation by chromatography of the hydrogenated products of autoxidized linoleate. He isolated and identified 9- and 13-hydroxystearates but was unable to detect any of the 11-isomer which would have been produced if oxygen attacked the resonance hybrid at random. These results do not prove conclusively that the 11-hydroperoxide had not formed because, under the conditions of hydrogenation, rearrangement of the nonconjugated hydroperoxide to conjugated isomers could have taken place.

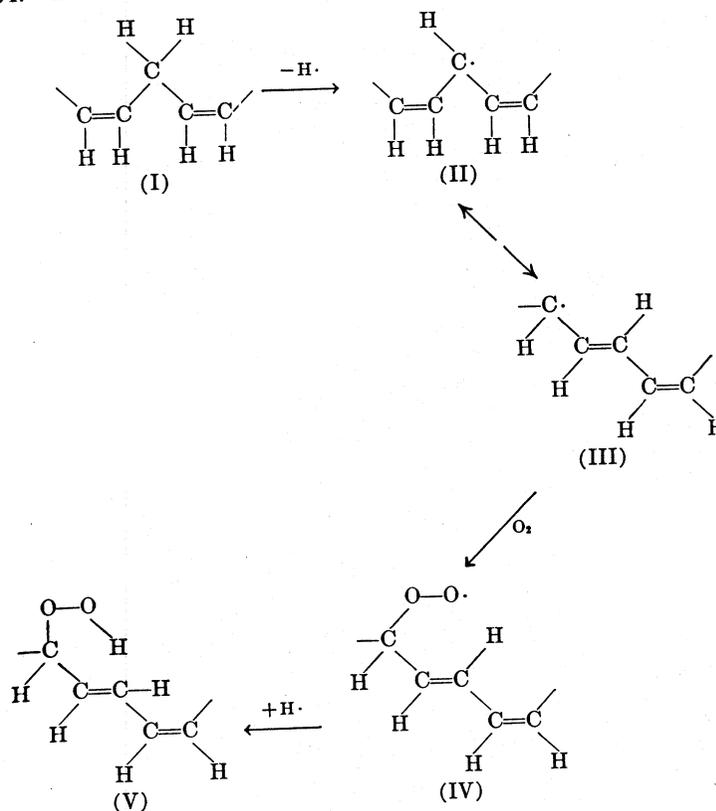
Only thermodynamic evidence (70,71) supported Bergström's observations for the formation of conjugated hydroperoxide in excess of random

amounts until 1954, when Khan *et al.* (72) reported certain evidence obtained by chromatography. Methyl linoleate was autoxidized at -10°C . in the dark, under visible or under ultraviolet light, or in the presence of copper catalyst. The resultant peroxides were separated by countercurrent extraction and then reduced by stannous chloride. These hydroxylinoleates were then separated by displacement chromatography. The products prepared under the above-mentioned conditions consisted almost entirely of conjugated compounds, but in the case where the oxidation was activated by the presence of chlorophyll and irradiation, a nonconjugated product was isolated. These observations suggest strongly that the observed conjugation was not induced by the reduction, and that autoxidation results largely in conjugated products. Sephton and Sutton (72a) reported similar results.

Much of the uncertainty regarding the exact extent of conjugation produced during autoxidation arises from lack of adequate standards of comparison. The data reported in the literature are based upon comparison with *trans,trans*-10,12-octadecadienoic acid which has a molar extinction coefficient of about 32,000. However, the effect of the peroxide group in the molecule, and the effect of *cis,trans*-isomerism were not considered in making these comparisons.

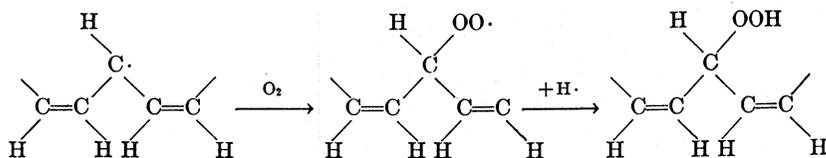
Later investigations of the infrared spectra of the *cis,trans*-isomers of linoleic acid (73) indicated that the conjugated linoleate hydroperoxide is not a *trans,trans* compound and that estimates of the extent of the conjugation present in the product must be revised upward. Subsequent studies of conjugated linoleate isomers obtained by alkali isomerization of linoleate indicated that these isomers have lower extinction coefficients at 2320 Å. than the better known *trans,trans* isomers (74,75). Privett and co-workers (76) and Dutton and co-workers (77) have investigated the infrared spectra of preparations of linoleate hydroperoxides of high purity and found that the products are conjugated to the extent of at least 90% and consist largely of *cis,trans*-isomers. The degree of conjugation calculated from ultraviolet absorption of the peroxide and of known conjugated *cis,trans*-linoleate also indicated at least 90% conjugation. These higher proportions of conjugated *cis,trans*-hydroperoxide were found only in preparations oxidized near 0°C . Oxidation at 24°C . yielded concentrates of peroxides in which appreciable proportions of conjugated *trans,trans*-forms existed. The investigators suggested that conjugated *cis,trans*-isomers were initially formed and that the thermodynamically more stable conjugated *trans,trans*-isomers arose as a result of some catalytic reaction probably initiated by peroxides.

In the light of the afore-described observations, a simplified mechanism for the main course of autoxidation of linoleates was proposed by Holman (4) in 1954. It is indicated schematically as follows:

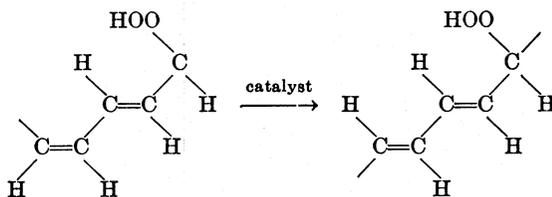


In this scheme the linoleate (I) loses a hydrogen atom to some radical and becomes a free radical (II). This free radical is a resonance hybrid, the two extreme forms of which are shown as (III). Oxygen adds to the resonating radical, predominantly at the ends of the resonating system, to yield two types of hydroperoxy radicals (IV). These radicals accept hydrogen atoms from other linoleate molecules to become isomeric conjugated *cis,trans*-hydroperoxides, and in so doing perpetuate the cycle. Other reactions which occur to a limited extent under ideal conditions are as follows:

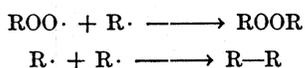
(1) Oxygen may add to the intermediate form of the free radical (II) to yield nonconjugated peroxides:



(2) The conjugated *cis,trans*-linoleate peroxide may be isomerized to a *trans,trans*-form:

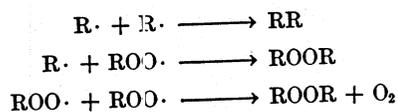


(3) Polymers may be formed by addition of radicals (II), (III), (IV) with each other:



The mechanism involved in the formation of *trans*-double bonds during autoxidation may be similar to that operating during alkali isomerization (77a).

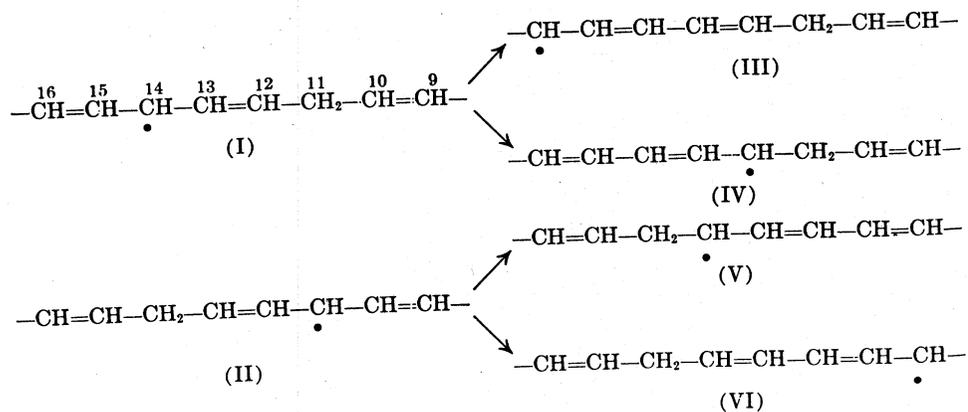
The cyclic or chain nature of the autoxidation reaction is well established. The entire mechanism, however, involves three types of reactions, namely, chain initiation, chain propagation, and chain termination. The reaction chain can be initiated by the attack of any free radical upon linoleate. The most probable radicals to initiate chains are those formed by the decomposition of a peroxide. It was formerly believed that peroxide was not required for chain initiation and Bolland (70) showed that ethyl linoleate had a low but measurable rate of oxidation at 0% oxidation. Lundberg and Chipault (78), however, demonstrated that highly purified linoleate had a long induction period, that is, it had no measurable oxidation rate for several hours after exposure to oxygen. Therefore it would appear that the first chain must be initiated by some nonperoxidic free radical or by stray radiation. Parallel oxidation chains are initiated by radicals formed by decompositions of hydroperoxide. The higher the concentration of peroxide the more rapid is its rate of decomposition. Thus, as the oxidation proceeds it generates its own catalyst, i.e., the reaction is autocatalytic. The reaction chains can be stopped by collision of two radicals, for example:



If the radicals attack molecules of other substances present in the reaction mass, products may be formed which do not decompose to form radicals and are thus incapable of propagating the reaction chain.

Other concepts of the mechanism of the autoxidation of linoleates have been published by Hilditch (79) and especially by Gibson (80), who presents the mechanism of oxidation in a rather unique manner. The reader may wish to consult these publications.

In the autoxidation of ethyl linolenate (43) there should be two initial radical forms (I and II) which could rearrange to III, IV, V, and VI, as indicated in the following scheme. The hydroperoxides derived from III through VI would then show diene conjugation. If both the active methylene groups were attacked consecutively by oxygen, which would happen frequently only in advanced stages of oxidation, then numerous diperoxide forms would become possible, some of which would show diene conjugation, some triene conjugation, and one the original state of unconjugation. The autoxidation of linolenates is thus even more complicated than that of linoleates. The autoxidation shows the characteristics of a chain reaction, forming hydroperoxides and migration of double bonds, but the fine details have not yet been developed (81). At 0°C., 60% of the products are monomeric *cis,trans*-conjugated diene monohydroperoxides.



Methyl docosahexaenoate (41,43), which is an extremely complex substance, also displays an increased absorption in the ultraviolet, indicating

that conjugated compounds are formed, but no quantitative data have been reported for the autoxidation of this compound.

In the case of squalene and rubber (43) no appreciable increase in ultraviolet absorption was observed which could be ascribed to the formation of conjugated units. In these materials the reactive α -methylene groups are flanked on only one side by a double bond; hence the olefin possesses a much lower reactivity and requires two peroxidations to occur in any diene unit of the chain, $-\text{C}=\text{C}-\text{C}-\text{C}-\text{C}=\text{C}-$, before conjugation can appear. There is little doubt, however, that double-bond migration occurs and it is unfortunate that the ultraviolet absorption technique is inapplicable to such systems.

Autoxidation of Conjugated Polyunsaturated Fatty Compounds. The autoxidation of conjugated compounds has received much less study than that of the nonconjugated polyunsaturated fatty compounds, even though the former are important constituents of tung, oiticica, and dehydrated castor oils which are used in the manufacture of protective coatings. The mechanism of oxidation of conjugated polyunsaturated fatty compounds differs from that of the nonconjugated compounds, and the reaction products are not the same.

Studies with model conjugated hydrocarbons by Kern and co-workers (82-86) have shown that oxidation occurs by addition of oxygen to the diene systems, a conclusion confirmed by Privett (8a), to yield mainly polymeric noncyclic peroxides, although monomeric cyclic peroxides can also form in some instances. Both 1,2 and 1,4- addition occur, depending upon the diene hydrocarbon. Hock and Siebert (87), however, have reported that monomeric and polymeric cyclic peroxides are formed by 1,4-addition simultaneously with the formation of α -hydroperoxides.

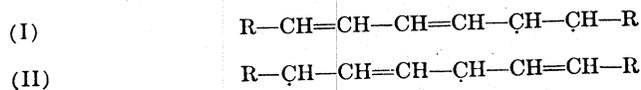
Pioneering work in the autoxidation of conjugated polyunsaturated fatty compounds was done by Morrell and co-workers (88,89), and by Miller and Claxton (90). The former group, unfortunately, investigated an extremely complex system, namely, the adducts of methyl eleostearate with maleic anhydride. Morrell and co-workers as well as Miller and Claxton carried the oxidation to advanced stages and followed the reaction by analytical means which are now known to be fallacious. They concluded that oxygen-containing polymers were formed and that ketol and enol groups were present in the products.

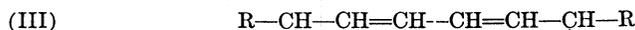
Brauer and Steadman (91) followed the oxidation of β -eleostearic acid by means of ultraviolet spectrophotometry and observed that the absorption in the region of 2600-2800 A., which is attributed to conjugated triene, decreased as the oxidation proceeded and that absorption due to conjugated

diene increased. This was subsequently shown to occur also with pseudo-eleostearic, α -eleostearic, and β -licanic acids (92). Allen *et al.* (93) compared the oxidation of methyl 9,12- and 10,12-linoleate and found that in the early stages of oxidation of 9,12-linoleate all the oxygen absorbed was found as peroxide, whereas in the oxidation of the conjugated isomer no peroxide accumulated in the first stages of the process. The disappearance of conjugated double bonds was proportional to the oxygen absorbed, suggesting that carbon-oxygen rather than carbon-carbon polymerization occurred. The work of Jackson and Kummerow (94) on the oxidation of the two isomeric linoleates in the presence of metallic naphthenate driers indicated that the driers had less effect upon the oxidation of conjugated linoleate than upon nonconjugated linoleate, which would suggest that peroxide decomposition is not a major factor in the mechanism of oxidation of conjugated substances.

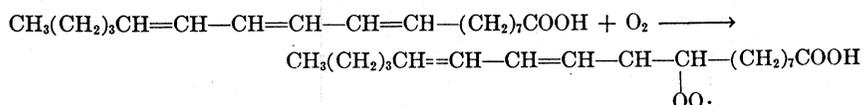
The oxidation of conjugated unsaturated substances is accompanied by less decomposition than the oxidation of the related nonconjugated compounds. The conjugated triene fatty acids and esters oxidize more rapidly than the corresponding nonconjugated isomers, according to Myers *et al.* (95), who compared the oxidation of small amounts of trienoic acids and esters on filter paper. Comparison of the rates of oxidation of normal linoleic and 10,12-linoleic acids, however, showed no essential difference (96). On the other hand, Allen *et al.* (93) reported that oxidation of the conjugated methyl linoleate proceeded more slowly than oxidation of methyl linoleate.

The most thorough investigation on the oxidation of the eleostearate is that reported by Allen and Kummerow (97). They found that the amount of triene conjugation lost, and the amount of diene conjugation formed, were proportional to the amount of oxygen absorbed. The primary product of oxidation was isolated by low temperature crystallization and found to possess strong absorption in the ultraviolet region corresponding to conjugated diene. The effect of alkali upon this absorption was minimal. Hydrogenation of this oxidation product yielded mostly methyl dihydroxystearates. Oxidation of these hydroxylated esters with alkaline permanganate yielded only valeric and azelaic acids, indicating that the original oxidative attack had been confined within the triene system. The attack of oxygen upon the conjugated system was postulated to be at the 1,2-, 1,4-, or 1,6-positions, probably yielding the following partial structures:

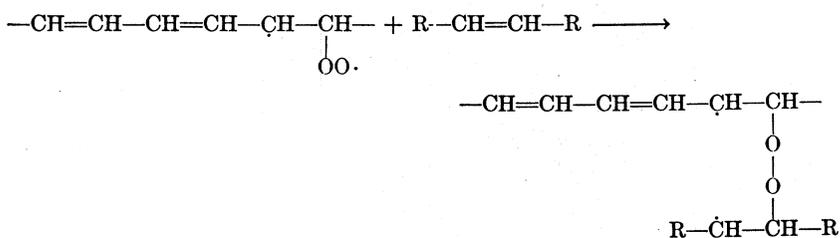




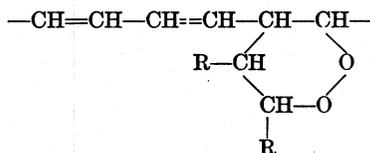
Two of these three isomers possess residual diene conjugation. The isolated primary product of oxidation had a specific extinction coefficient of 62, compared with a calculated value of 71. Upon hydrogenation at least three isomeric dihydroxystearates were obtained, only one of which contained an α,β -dihydroxy group. In accord with these data and a study of the kinetics of the reaction, the following mechanism was proposed:



The foregoing diradical is stabilized by resonance along the unsaturated system, allowing addition at any of the carbons of the triene system. Reaction with another unsaturated molecule would yield a dimer still possessing free radical centers:



This dimeric diradical could stabilize itself internally to give a cyclic peroxide:



However, the dimeric radical could again add oxygen and olefin thus building a polymeric chain in which the repeating unit would be $-\dot{C}HR-CHR-OO-$. Oxidation of pure material would favor the latter course, whereas formation of the first-mentioned dimer would be favored in diluted media. On a basis of the kinetic studies, the rate of reaction of eleostearate oxidation may be summarized by the equation:

$$dO_2/dt = K(\text{product})^{1/2} (\text{ester})$$

The rates of oxygen uptake for various conjugated unsaturated systems

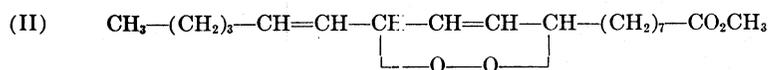
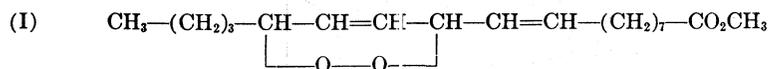
diminish markedly when the total oxygen uptake approaches two moles of oxygen per mole of ester or acid. This is true even for α - and β -parinaric acids which possess conjugated tetraene systems (98). Apparently polymer formation increases steric hindrance, thereby increasing the difficulty of further oxidative attack. Increasing viscosity and diminished diffusion of oxygen is apparently a lesser factor, which would appear to be confirmed by the observation of Chipault *et al.* (99) that trieleostearin, pentaerythritol eleostearate, and an eleostearic alkyd became hard at a very early stage of oxidation, long before the maximum oxygen uptake had occurred. On the other hand, in the oxidation of similar linoleate and linolenate compounds the films set and hardened at later stages.

The above-described reaction mechanism does not account for all the recorded observations of the oxidation of conjugated polyenes. The oxidation of the eleostearates has been shown to be autocatalytic by Brauer and Steadman (91) in solutions, and by Myers, Kass, and Burr (95) in thin films. According to the diradical reaction mechanism of Kummerow and his associates, propagation of the chain reaction occurs only by polymerization. The formation of low molecular weight polymers such as the dimers found by Brauer and Steadman results from early termination of the reaction chains. Chain reaction leading to monomeric or dimeric products would appear to occur only by a monoradical mechanism which would involve the abstraction of a hydrogen from an eleostearate molecule.

The postulated mechanism makes no provision for autocatalysis. The chain polymerization reaction is not autocatalytic. Moreover, it is implied that polymerization occurs primarily through carbon-oxygen bonds, whereas it is known that eleostearate sets to a hard film very early in the process of oxidation, before sufficient oxygen has been absorbed to account for cross-linking. Polymerization in this case probably occurs through carbon-carbon linkages. A free radical reaction mechanism for conjugated polyene oxidation is not necessarily obligatory. The simple 1,2- or 1,4-addition of oxygen may account for many of the observations concerning the autoxidation of conjugated polyene fat products, except for autocatalysis. It would explain the partly conjugated primary product of oxidation isolated by Allen and Kummerow, and early polymerization could be stimulated by peroxide catalysis. The mechanism of this reaction can by no means be considered as completely settled.

O'Neill (7) investigated the autoxidation of methyl eleostearate exposed to ultraviolet radiation. He found that after 1.2 atoms of oxygen had been absorbed per mole of substrate, the reaction mass consisted of unchanged ester (38%), crystalline peroxide (7%), monomeric peroxidic, hydroxylic,

and ketonic products (35%), polymers (15%), and cleavage products (5%). Analysis of the crystalline peroxide suggested that it consisted of about 75% of an easily reducible 1,4-cyclic peroxide (I or II) and about 25% of a more difficulty reducible and probably isomeric peroxide.



The remaining monomeric fraction consisted of other peroxidic material including about 15% of 1,2-cyclic peroxide, probably some 1,6-peroxide and unidentified hydroxylated and ketonic material.

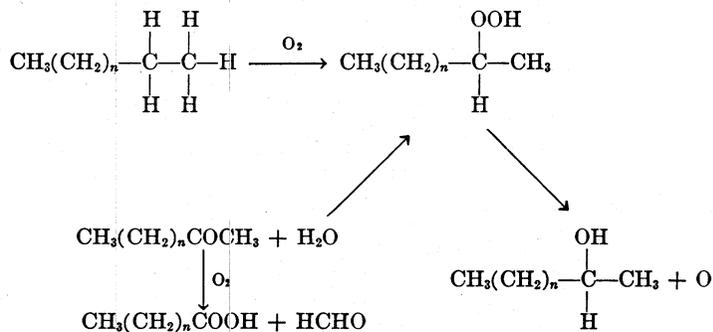
The polymeric material, the yield of which was unexpectedly low considering that a tung oil film will dry at an oxygen uptake of about one atom per fatty acid radical, was of higher molecular weight (number average about 1000) than the polymeric material obtained from experiments with the nonconjugated esters, and light-scattering measurements suggested the presence of some higher polymeric material. It contained peroxide groups which could not be readily hydrogenated and did not react with potassium iodide, but reacted with hydrogen iodide. The reaction with hydrogen iodide, however, yielded only small amounts of monomeric material, indicating that the chains were not linked by peroxide groups but were carbon-carbon bonded.

On oxidation of nonconjugated fatty esters, conjugated double bond systems are developed and the later stages of oxidation of these esters would be expected to follow the same course as the oxidation of the conjugated esters. Evidence of the presence of difficultly reducible peroxides in the autoxidation of nonconjugated fatty esters has been obtained by first reducing hydroperoxides by hydrogenation with platinum at atmospheric pressure. The reaction product then contains peroxides which will react with hydrogen iodide but not with potassium iodide, and which can be hydrogenated to hydroxy compounds with Raney nickel at 7 atmospheres.

Autoxidation of Saturated Fatty Compounds. Under the mild conditions usually prevailing in autoxidation, saturated compounds have often been assumed to be practically unreactive. It is true that the main problems of autoxidation are associated with unsaturated fatty acids, but it is now known that saturated fatty acids undergo a slow autoxidation particularly at or above 100°C. Stirlon *et al.* (44) have shown that at 100°C.

the ratio of rates of oxygen absorption of methyl stearate:methyl oleate:methyl linoleate:methyl linolenate is 1:11:114:179.

Much work has been published on the autoxidation of pure saturated hydrocarbons, because such studies are important in problems of petroleum lubrication. The results of these investigations are also applicable to saturated fatty acids (100-103a). At 110°C. saturated normal hydrocarbons autoxidize at rates which are increasingly proportional to the chain length. *n*-Decane, $\text{CH}_3(\text{CH}_2)_8\text{CH}_3$, and *n*-hexadecane, $\text{CH}_3(\text{CH}_2)_{14}\text{CH}_3$, absorb oxygen slowly during the early stages (first 20 hours) of autoxidation, and then an autocatalytic acceleration to a constant rate occurs (101). Autoxidative attack is probably random (103a,104), and the primary product is a hydroperoxide (100,102-103a,104a). Decomposition of the hydroperoxide yields a ketone, an alcohol, or products of chain cleavage, indicated schematically as follows:



Attack can also occur at other carbon atoms, giving rise to other species of compounds. The aldehydes, ketones, and acids among the reaction products are subject to further oxidation and polymerization. Hexadecane (cetane) $\text{CH}_3(\text{CH}_2)_{14}\text{CH}_3$, may be cited as an example of these oxidation. It absorbs oxygen at the rate of approximately 0.002 mole per hour at 110°C. There is a slight increase in rate of oxidation with increased chain length. Normal and branched chain paraffins have similar autoxidation curves and typical induction periods. The reaction is autocatalytic and is accelerated by metallic ions and inhibited by metal-binding compounds or antioxidants. The reaction is believed to be of the chain type.

The oxidation of saturated fatty acids or derivatives has been investigated by Paquot and de Goursac (105). Ethyl palmitate and ethyl caprate were autoxidized by blowing with air at 120°C. in the presence of 1% of nickel phthalocyanine as catalyst. About 40-50% of the original esters were recovered unchanged, 0.5% was lost as carbon dioxide, 5% was lost

by entrainment, and the remainder was autoxidized. Sodium and potassium soaps were also autoxidized by these investigators. The principal products were shorter chain acids and oxalic acid, thus suggesting that attack at the β -carbon atom predominated. Minor amounts of lactones were also isolated, indicating that some attack occurred at the γ - and δ -carbon atoms. Methyl ketones were also isolated in even lesser amounts.

It is probable that during the early stages of the "drying" process the saturated components of an oil play little or no part in the reactions that are occurring, but that as oxygenated components accumulate, particularly peroxides, the rate of reaction of long-chain saturated compounds may become significant. It is also quite possible that the oxidation of the saturated components of an oil may play an important role in the ultimate deterioration of paint films and similar coating materials.

d. Kinetics of Autoxidation

Accurate comparisons of oxidation rates for different unsaturated fatty acids and esters cannot be made on the basis of data found in the literature because experimental results are highly dependable upon the reaction temperature and the manner of relating time with peroxide data. Various investigators have shown, however, that the linolenates oxidize approximately twice as fast as the linoleates (44,45). This increased rate may be correlated with the fact that linolenate has twice as many active methylene groups as linoleate. Oleates which contain no methylene groups between unsaturated carbons, are reported to autoxidize at rates ranging from one-twelfth to one-fiftieth of that for linoleate. When the double bonds in fatty acids are separated by more than one methylene group, the reaction rate for each double bond approaches that of monounsaturated acids.

Allen (93) found that when the active methylene group is missing, as in conjugated 10,12-linoleic acid, the oxidation rate is one-third that of the nonconjugated 9,12-linoleic acid. Furthermore, the 10,12-isomer absorbed one mole of oxygen per mole of ester before any appreciable amount of peroxides were formed. In compounds with more than two conjugated double bonds, conjugation increased the rate of oxidation. Myers *et al.* (95) found that conjugated triene esters oxidized more rapidly than nonconjugated triene esters.

The effect of the presence of small proportions of linoleate upon the rate of oxidation of oleate was investigated by Gunstone and Hilditch (45). From the results of these studies it is apparent that a high purity of any unsaturated substrate is important for use in investigations of autoxidation.

Highly purified oleate esters are extremely resistant to autoxidation (106).

Bolland (107) investigated the kinetics of the autoxidation of linoleate, in the course of which he observed the effect of the concentration of ethyl linoleate on the reaction of ethyl oleate. The relative rate of oxidation was a linear function of the molar concentration of linoleate, indicating that the oleate acted merely as a diluent.

The more common unsaturated acids oxidize at maximum rates which at times are twice as great as that of their esters (96). This effect probably results from participation of the carboxyl groups in the decomposition of peroxides, which is indicated by the work of Privat *et al.* (49), who observed that the addition of free linoleic acid to methyl linoleate peroxide accelerated its decomposition. Moreover, it has been observed that the total oxygen uptake by free oleic acid is less than that of its esters (108,109). One of the earliest quantitative investigations of the kinetics of autoxidation of fatty acids was reported in 1942 by Henderson and Young (110), who derived the following expression for the rate of autoxidation of oleic acid:

$$-dO_2/dt = k_1 + k_2 (O_2)^{1/2}$$

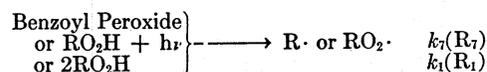
For oxygen pressures between 0.5 and 1.0 atmosphere the average value for k_1 was observed to be 2.2 and for k_2 , 226.

The detailed investigations of the kinetics of autoxidation reported by Bateman (1), Bolland (2), and their co-workers are among the foremost contributions to our knowledge of the mechanism of autoxidation of unsaturated fatty materials. These investigators demonstrated unequivocally that the low-temperature, liquid-phase oxidation of olefins occurs by a chain mechanism. They demonstrated that if inhibitors or initiators are added to these reactions marked decreases or increases in the respective reaction rates are observed; and also that if the reaction is accelerated photochemically, the quantum yield may exceed unity. Comparison of the experimentally determined rate equations for the interaction of oxygen with olefins in the presence of benzoyl peroxide, ultraviolet radiation, or in the dark in the absence of added initiators shows a parallelism in the way the rate of oxidation depends on $[O_2]$ and $[RH]$. Kinetic analysis shows that photo- and nonphoto-catalyzed oxidations proceed by the same basic mechanisms. The rate equations reduce to the common form (1,2):

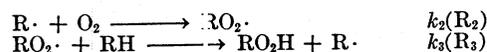
(R_1 is the rate of formation of chain carriers)

The kinetic characteristics embodied in this generalized relation can be accounted for in terms of a single chain-reaction mechanism as follows:

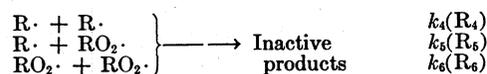
Initiation (Production of radicals $R\cdot$ or $RO_2\cdot$) R_1



Propagation



Termination



The rate of oxidation is related to the velocity coefficient of the various elementary reactions (k_1 - k_7) by

$$-d[O_2]/dt = R_1^{1/2} (k_3/\sqrt{k_6}) [RH] \frac{k_2 \sqrt{k_6} [O_2]}{k_3 \sqrt{k_4} [RH] + k_2 \sqrt{k_6} [O_2] + \sqrt{k_4 k_6 R_1}} \quad (B)$$

The correspondence between the experimental (A) and the theoretical (B) is complete since the term $\sqrt{k_4 k_6 R_1}$ becomes negligible at chain lengths as great as those encountered in these autoxidations.

The efficiencies with which the initiation process (R_1) and the termination reaction (R_4) occur are sensibly the same for a variety of nonconjugated unsaturated hydrocarbons. The resultant influence of the propagation and termination reactions involving the R-type chain carrier is negligible. The remaining chain-propagation step (R_3) is sufficiently sensitive to the nature of RH to introduce considerable variations in oxidation chain length; R_3 must thus be regarded as the key reaction in controlling rates of autoxidation. Further details and discussion of these equations are given in the review by Bolland (2).

Chlorophyll also accelerates the autoxidation of oleic acid in the light and dark, but the mechanism of the reaction is not known (107a).

Khan *et al.* (108), Max and Deatherage (111), and Khan (112) also studied and compared the rates of autoxidation of methyl oleate, methyl 9,10-dideuterooleate, and 8,8,11,11-tetradeutero-*cis*-octadecene. These investigators showed that deuterium compounds oxidize at a slower rate than do the corresponding hydrogen compounds. They concluded that

initial autoxidative attack is at the double bond, and the main sustaining reaction is attack at the α -position.

(e) *Secondary Products of Autoxidation*

Various investigations have been made of the secondary products of autoxidation of fatty materials. In the early studies the isolation of reaction products, usually after extensive autoxidation had occurred, was a major objective of many workers who presumed that the reaction mechanism could be developed on the basis of the results of a careful separation and quantitative estimation of the resulting products of oxidation. The large number of products formed and the dependence of yields on reaction conditions made this approach relatively fruitless and merely reaffirmed the complexity of the autoxidation reaction. Unfortunate, also, was the fact that many of the early studies were carried out with impure starting materials, thereby further complicating the problem.

One of the earliest systematic investigations of this type was that of Skellon (113), who air-oxidized pure oleic acid at 100°–120°C. for long periods. From the oxidized mass he isolated two 9,10-dihydroxystearic acids, a monohydroxystearic acid, and a number of compounds arising from chain cleavage. More than twenty years later, Feuill and Skellon (113a) reinvestigated the same reaction and showed that, in addition to the products previously reported, the reacted product contained oxiranes, unsaturated carbonyls, and dimers. Ellis (23,28) oxidized oleic and elaidic acids in the presence of cobaltous elaidate as catalyst until from one to three moles of oxygen were absorbed at 55–80°C. Epoxide accounted for at least 20% of the products formed; this was interpreted as evidence that oxygen attacked the double bond, a conclusion now known to be untenable. Cleavage products included nonanoic, octanoic, suberic, azelaic, and oxalic acids, as well as carbon dioxide and water. Peroxides constituted a minor proportion of the oxidation products. Deatherage and Mattill (109) confirmed most of these observations and in addition showed that epoxy derivatives were among the chief products of autoxidation. Swern *et al.* (114) fractionated by molecular distillation the products of a cobalt salt-catalyzed extensively autoxidized, methyl oleate and showed that they contained oxygen-linked polymers. Knight *et al.* (115) autoxidized oleic acid and methyl oleate in acetic acid in the presence of a cobalt salt catalyst. Autoxidation of oleic acid at 65°C. gave 64–68% of mixed cleavage products (mono- and dibasic acids) and 12–17% of high-melting 9,10-dihydroxystearic acid, thus accounting for about 80%

of the original oleic acid, but the cleavage products consisted of C₆-C₁₂ mono- and dibasic acids, no one of which predominated.

Later, Coleman *et al.* (116) showed that in the uncatalyzed autoxidation of methyl oleate substantially all of the ester undergoes single attack by oxygen or peroxides before any significant proportion of the chain is subjected to multiple attack. After the peak in peroxide formation had been passed in the autoxidation of methyl oleate, the composition of the oxidation product corresponded to 30-35% peroxide, 25-30% hydroxy compounds, 20-25% oxirane compounds, 15-20% α,β -unsaturated carbonyl compounds, and some residual methyl oleate, cleavage products, polymers, and multiattacked methyl oleate. Hydrogenation of such mixtures with Raney nickel and palladium catalysts produced high yields of monohydroxystearic acids (117).

α,β -Unsaturated carbonyl compounds were isolated by Ellis (118,119) from autoxidized oleic and elaidic acids. These products appear to result from the corresponding α -methylene hydroperoxides by the loss of a molecule of water. 12-Ketoelaidic and 12-ketooleic acids, isomers of the α,β -unsaturated carbonyls produced during autoxidation, have been synthesized by controlled oxidation of ricinelaidic and ricinoleic acids (119). 12-Ketoelaidic acid is relatively stable toward autoxidation, whereas 12-ketooleic acid absorbs oxygen even at 0°C. These relative autoxidizabilities are particularly significant because it has been shown that most, if not all, of the hydroperoxides formed from methyl oleate have *trans*-configurations (54). The α,β -unsaturated carbonyl compounds derivable from these hydroperoxides should also have *trans*-configurations because dehydration does not involve the double bond. Although the α,β -unsaturated carbonyls formed during autoxidation are not identical with 12-ketoelaidic acid, they are closely related to it and it might be assumed that they too would be resistant to further autoxidative attack in the absence of catalysts. The fact that single attack of the chain predominates during autoxidation of methyl oleate supports this conclusion; otherwise a significant amount of multiattack would occur before all the ester had been autoxidized.

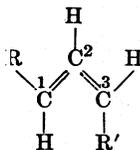
It is known that oxirane compounds are also resistant to further autoxidative attack at moderate temperatures, and it would appear that the unsaturated hydroxy compounds must be too. Although this view has not been verified experimentally, it is probable that the hydroxy compounds, as well as some of the oxiranes, are converted to esters, thereby enhancing their stability.

King (63) reported the results of a detailed analytical study of the prod-

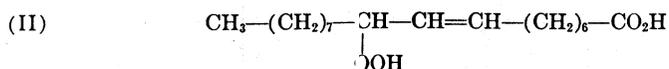
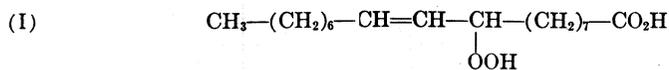
ucts of elaidic acid autoxidized at 47° and 78°C. with and without a cobalt salt catalyst. The formation of ketol derivatives was confirmed, and he identified formic acid among the volatile products of autoxidation. Methods were also described for estimating the proportion of ketol and other carbonyl compounds in the autoxidized product.

Skellon and Thruston (119a) investigated the catalytic oxidation of elaidic acid, methyl elaidate, and *n*-propyl elaidate. About 33% peroxides were formed with methyl elaidate at 98°C., but only 10–15% with elaidic acid, which parallels the experience with methyl oleate and oleic acid (96,108,109). Hydroperoxides decomposed with the formation of (a) monomeric oily complexes containing carbonyl groups, (b) other secondary products such as oxiranes and keto esters, and (c) cleavage products:

Ellis (28) showed that autoxidation of oleic and elaidic acids produces *trans*-9,10 epoxystearic acid in about 20% yield. That both a *cis*- and a *trans*-compound should yield the same, rather than different geometric forms by the identical method of oxidation was indeed surprising and remained unexplained for many years. With the development of infrared spectrophotometric methods for identifying *trans*-configurations in autoxidized and other materials, it was deduced (54,115) that the majority of radicals of autoxidized oleic (and elaidic) acid have the configuration:



and add oxygen at carbon atom 3. The resulting hydroperoxides, 9-hydroperoxido-10-*trans*-octadecenoic acid and 10-hydroperoxido-8-*trans*-octadecenoic acid (I and II), on homolytic cleavage yield the radicals HO· and RO· (III and IV). Both III and IV, by shift of one π -electron of the double bond to couple with the odd electron on oxygen, would form the oxirane ring in the 9,10-position. Reacquisition of a hydrogen atom then yields *trans*-9,10-epoxystearic acid.



tion has been devoted to those derived from monounsaturated fatty materials. Swift *et al.* (124), however, have identified saturated and unsaturated aldehydes as oxidation products from decomposed hydroperoxides of cottonseed oil. Linoleic acid is the principal fatty acid constituent as well as the most readily oxidized component of this oil. Johnson *et al.* (124a) isolated methyl ethyl ketone, acetaldehyde, α -pentenal, crotonaldehyde, and an unknown 5 carbon carbonyl compound in the volatile decomposition products of the oxidative polymers of ethyl linolenate. Similar cleavage products have been isolated from autoxidized soybean (124b, 124c) corn and avocado (124d) oils, and methyl linoleate (124b).

f. Miscellaneous

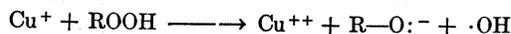
Peroxide Catalysis. In the autoxidation of unsaturated substances, the induction period is that initial time during which no appreciable oxidation takes place, and during which peroxide does not accumulate rapidly or in measurable quantity. When peroxide (or partially oxidized material) is added to a fat it has a pro-oxidant action because, in effect, the stage of oxidation is advanced beyond the induction period. Peroxides are unstable substances and have appreciable thermal decomposition rates; hence they provide free radicals to initiate new chains. Benzoyl peroxide is well known as an initiator of polymerization reactions and has been used as a source of chain-starting radicals in investigating autoxidation, as has linoleate hydroperoxides (65,107), peracids (124e), free radicals (124f), hydroperoxides, and peroxides (124g).

Metal Catalysis. In the drying oil industry, pro-oxidants called "dryers" are often used to promote rapid oxidation. These substances are usually salts of heavy metals of organic acids. The effect of dryers upon the drying time of linseed oil was investigated by Lund (125) and the action of copper upon the oxidation of linoleic acid was studied by Smith and Stotz (126). Skellon (127) investigated the action of various metallic salts upon the oxidation of oleates and concluded that lead, aluminum, and barium are good catalysts in the primary stages of oxidation, but that zinc is a good catalyst for formation of ketone from peroxide. Linoleates were more effective catalysts than oleates, elaidates, or ricinoleates.

Metallic catalysts appear to act primarily through the decomposition of peroxides to form free radicals. No experimental work in the field of fats appears to have been directed toward this phenomenon, but it is generally believed that the decomposition of peroxides occurs via the metallic salts to yield free radicals and thus promotes polymerization (128) and oxidation (129). It is clear, however, from the work reported

by Jackson and Kummerow (94) that in the presence of dryer, the peroxide value of oxidizing linoleic acid is held to a lower level than in the absence of dryer.

Many organic peroxides are able to abstract electrons from and yield electrons to metallic cations, depending upon oxidation-reduction potential:



The metallic catalysts function by maintaining a uniform concentration of active hydroxy radicals.

High polyvalency of metallic catalysts is not absolutely requisite for good activity, although valence changes are characteristic of the best catalysts (129a). According to Skellon (129b), catalysts appear to function through a simple cycle of valency changes which may be described as follows:

(1) A loose complex forms between metal catalyst in a lower state of valency and oxygen, in the course of which the metal is raised to a higher valency condition.

(2) This higher condition of valency is more stable but is still reactive toward oxygen; in this state the catalyst can react further with oxygen and oxidizable substance, donating oxygen continuously to the latter without permanently reverting to its lower valence state, that is, the catalyst-oxygen complex acts as an oxygen donor.

(3) Ultimate interaction between the catalyst in its higher valency and a quantum of oxidized material causes reversion of the metal to its lower valency accompanied by changes in the character of the oxidized material.

Film formation in the presence of dryers is marked by a shortened induction period, an increase in rate of oxidation, formation of a film at a lower oxygen content of absorbed oxygen and a lower content of total oxygen during subsequent aging (129a).

Andersson (130) has given a slightly different view of the metal-catalyzed oxidation of methyl linoleate. He observed that after the induction period the rate increased to a constant value r and could be related to the concentration of dryer c by the following relation:

$$r^2 + \alpha r = \beta c$$

in which α and β are constants that are dependent upon the concentration of linoleate. The induction period increased with the concentration of

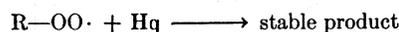
dryer, suggesting that it is related to the oxidation of the cations to a higher valence. Ninety per cent of the absorbed oxygen in the autoxidized linoleate was found as peroxide. His concepts of the mechanisms of catalyzed and uncatalyzed autoxidations are the same except that the chain carrier is an addition product of the catalyst rather than the peroxide radical ROO·.

Reviews of the action of dryers and methods of manufacture of these products have been published by Klebsattel (130a) and by Kastens and Hansen (130b).

Surface Catalysis. The influence of the nature of the material of the vessel upon the induction period and rate of oxidation has been known for some time. The more commonly observed surface catalysis is frequently due to contact of fat products with atoms of the transition metallic elements in the vessel, and is therefore representative of ordinary metal catalysis. George (131) made a systematic study of surface catalysis by addition to the test samples of inert powders containing micro amounts of transition elements as impurities. In common with metal-catalyzed and benzoyl peroxide-catalyzed oxidation, the surface-catalyzed oxidation of tetralin yielded hydroperoxide as the primary product. Surface catalysis is often responsible for initiation and termination of reaction chains.

Antioxidants. Antioxidants are substances that in small quantities prevent or delay the oxidation of an oil. Such substances are present in many crude fats and oils, and contribute to their stabilities or resistance to oxidation, a property which is often lost or reduced by purification. The literature pertaining to the theory of antioxidant action and practices relating thereto has been reviewed by Lundberg (132).

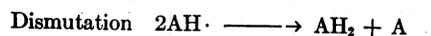
The mechanism of inhibition or negative catalysis was recognized by Alyea and Bäckström (133) to be related to the breaking of reaction chains and oxidation of the inhibitor. Bolland and ten Have (134) investigated the kinetics of the oxidation of ethyl linoleate in the presence of hydroquinone (Hq) and concluded that the inhibitor terminates chains by interaction with peroxide radicals:



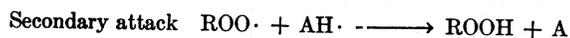
From kinetic evidence they also concluded that the hydroquinone underwent chemical change. The intense yellow color of the oxidized mixture suggested that the product of the oxidation of hydroquinone was benzoquinone. Golumbic (135) found that tocopherol was rapidly oxidized during the induction period of fat oxidation; when it had disappeared, the induction period ended. Lundberg *et al.* (136) studied the kinetics

of the oxidation of hydroquinone, catechol, nordihydroguaiaretic acid, and gallic acid in oxidizing lard. They found in each case that the concentration of antioxidant diminished during the initial stages of oxidation and that peroxide did not attain high values until most of the antioxidant had disappeared.

Taylor (137) and Michaelis (138) have discussed the mechanism of antioxidant action. Michaelis explained the phenomenon of antioxidant action on the basis of compulsory univalent oxidation-reduction. Aside from synthetic sulfur compounds, two types of natural antioxidants are known, one of which can be reversibly oxidized to quinone, and another (tocopherol) which cannot. The tocopherols have the common property of being reversibly oxidized to semiquinone radicals. Semiquinones are well known, and Michaelis demonstrated the existence of the semiquinone of tocopherol. Solutions of tocopherol in ethanol, diethyl ether, and pentane were cooled to glassy noncrystalline masses. When these rigid solutions were irradiated with ultraviolet light, intense red colors were produced, which disappeared when the glassy solutions melted, thus allowing dismutation of the free radicals to occur. On the basis of these observations it would appear that the most plausible mechanism of antioxidant activity occurs as follows:



or



Synergists. Synergists are substances that reinforce the effect of antioxidants. The synergists may or may not possess antioxidant activities. Synergists are usually dibasic or polybasic organic or inorganic acids. The synergists that have received most attention to date are ascorbic, phosphoric, and citric acids. Golumbic (139) stated that a synergist functions to regenerate the antioxidant at the expense of the synergist, and that the latter acts as a source of hydrogen.

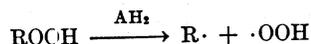
Privett and Quackenbush (140) have pointed out that three factors are incompatible with such a mechanism, namely, (1) some very effective combinations of antioxidants and synergists do not form oxidation-reduction systems; (2) in the system tocopherol-ascorbic acid, no evidence has been presented defining the rate of destruction of ascorbic acid; and (3) it has been shown that phosphoric acid reacts with quinone in the absence of hydrogen donors to give an apparent reaction for the presence

of tocopherol in the iron-bipyridyl color test. Privett and Quackenbush found that citric and ascorbic acids suppress the initial accumulation of peroxides which takes place at "pro-oxidant" levels of nordihydroguaiuretic acid and tocopherol in autoxidizing lard. It was also found that ascorbic acid and tocopherol exerted sparing actions on each other in oxidizing lard.

The work of Privett and Quackenbush indicates that synergists suppress "pro-oxidant" action of phenolic antioxidants. This "pro-oxidant" action is a catalytic decomposition of peroxide stimulated by the antioxidant, particularly when it is present in the reacting system in high concentration. In an autoxidizing fat containing both antioxidant and synergist, the antioxidant has two actions, one of which is to terminate oxidation chains by reacting with peroxide radicals:



The antioxidant also catalyzes the decomposition of the peroxides, the extent of which is dependent upon the concentration of antioxidant.



The function of the synergist is probably to suppress the catalysis of peroxide decomposition by the antioxidant. By suppressing the catalysis, additional chain formation is prevented, and thus the molecules of antioxidant are spared their function of stopping such chains.

The reader who wishes more details on the mechanism of action of antioxidants and their practical evaluation should consult the review of this subject by Riemenschneider (140a).

3. Quantitative Determination of the Products of Autoxidation

(a) Peroxides

Quantitative determination of peroxides is the most common analytical method for following the course of autoxidation reactions. Many methods for determining peroxides have been described; they have been discussed critically by Barnard and Hargrave (141). Several of these have also been examined statistically by Ricciuti *et al.* (142).

Iodimetry. Peroxides are most commonly determined by iodimetry. Iodimetric methods are all based on the assumption that potassium iodide (or other salt) and hydriodic acid when brought in contact with fatty peroxides liberate iodine quantitatively in some simple stoichiometric manner, e.g., two atoms of iodine per one equivalent of active oxygen.

The peroxide methods of Lea (143) and of Wheeler (144) are widely used, and it has been shown that they can be made more precise by exclusion of oxygen from the reagents and reaction flask and also by excluding light (145,145a).

Modified Wheeler Method (144,145a,146). Twenty milliliters of acetic acid-chloroform solution (3:2) is introduced into a glass-stoppered 250-ml. iodine flask. A weighed sample is transferred to the flask, which is flushed with nitrogen. Two milliliters of freshly prepared 50% solution of potassium iodide in water is added, and the flask is again flushed quickly with nitrogen. After 15 minutes, 50 ml. of water is added, and the liberated iodine is immediately titrated with 0.1 *N* sodium thiosulfate. The entire procedure is so arranged that the contents of the reaction flask is not in contact with any appreciable amount of dissolved or atmospheric oxygen at any time until the water is added.

Under these conditions, the liberated iodine reaches a maximum concentration in less than 15 minutes, and thereafter remains constant. Peroxide values obtained with reaction times from 15 minutes to 2 hours are identical.

% Peroxide oxygen = ml. of thiosulfate

× normality × 0.008 × 100/wt. of sample

Polarography. Polarography has been used in the analysis of fat oxidation products. Lewis *et al.* (57,58) observed a linear relationship between wave height and the peroxide value in the early stages of the oxidation of fats. A more detailed examination of the polarographic behavior of the products of autoxidation was carried out by Willits *et al.* (55) and later by Kuta *et al.* (58a), who found that peroxides, hydroperoxides, aldehydes, and ketones conjugated with a double bond, and α -diketones can be measured polarographically, and especially that hydroperoxides can be determined quantitatively in the presence of other types of peroxides (142).

Miscellaneous Methods for Determining Peroxide. *Ferric Thiocyanate Method.* Chapman and co-workers (147) and Erdmann and Seelich (147a) described a method for the determination of peroxide based on the development of a colored reaction product with ferric thiocyanate. Peroxide values determined with this method are appreciably greater than those found by iodometric methods. Lea (148) observed that excluding oxygen from the reaction diminished the color to about one-fourth that obtained in the presence of oxygen, and that the corresponding calculated peroxide values were lower than theoretical. The fer-

ric thiocyanate method is useful in comparative work where rapid determinations are essential, but for good reproducibility it requires rigid exclusion of air. Values obtained in the presence of air are proportional to, but higher than those obtained by iodometry. The method is more sensitive than the iodometric method and hence is useful in determining relatively small quantities of peroxide.

Dichlorophenol-Indophenol Method. The dichlorophenol-indophenol method was introduced by Hartmann and Glavind (149). Like the ferric thiocyanate method it yields high values in the presence of air (150), but peroxide values obtained with its use are reproducible. It is useful principally in comparative determinations. Perhaps its most significant value is in the histochemical detection of peroxides (151).

Stannous Chloride Method. Barnard and Hargrave (141) proposed a titrimetric modification of the stannous chloride method of determining peroxide which requires only about 1 milliequivalent of peroxide for determination. A weighed sample containing 0.75 to 1.0 milliequivalent of peroxide is dissolved in acetic acid (10 ml.) in a 250-ml. Erlenmeyer flask, which is then evacuated to 20 mm. of mercury and filled with nitrogen. Fifteen milliliters of 0.1 *N* stannous chloride solution is added from a pipette, and the flask is immediately re-evacuated and filled with nitrogen. The latter procedure is repeated twice. After standing for 1 hour at room temperature, a boiling solution consisting of 5 ml. of stock ferric solution, 1 g. of ammonium chloride, and 45 ml. water is added. The mixture is kept at 75°C. for 30 seconds and then rapidly cooled, and 10 ml. of phosphoric acid solution is added. The ferrous ion is titrated with 0.05 *N* potassium dichromate solution and 6 drops of indicator solution (0.25% solution of diphenylamine sulfonic acid in water). The end point is a sharp transition from green to violet. Blank determinations are carried out in a similar manner.

% Peroxide oxygen = (blank - titer)

× dichromate normality × 0.008 × 100/wt. of sample

(b) *Other Oxygen-Containing Products of Autoxidation*

The determination of autoxidation products other than peroxides is not simple. Early investigators did not realize that conventional methods of fat analysis were not necessarily applicable to autoxidized products; consequently much of the early work in this field, particularly on the composition of mixtures of autoxidation products, is of questionable validity.

A report of a detailed re-examination of analytical methods applicable to autoxidation systems was published by Knight and Swern (152). Knight *et al.* (152a) applied these modified procedures to autoxidizing methyl oleate. It was shown that in the absence of peroxide and oxirane groups the analytical procedures are reliable. When peroxides are present, which is usually the case, high and variable values for carbonyl oxygen are obtained, and the iodine and saponification values are generally unreliable. Determination of hydroxyl oxygen is interfered with by large proportions of oxirane compounds but apparently not by peroxides. Determinations of acid values and peroxide and oxirane oxygen are reliable in the presence of all other functional groups investigated. Procedures were described for accurately determining functional groups when peroxide and oxirane groups are present. Feuell and Skellon (152b) described methods for estimating ketonic groups in complex oxidation products.

Ahlers and McTaggart (123) developed infrared spectroscopic methods of estimating quantitatively various products of autoxidized fatty materials which require only 20 milligrams of sample. The accuracy with which each oxidation product can be estimated compares favorably with that obtainable with the chemical method for determining the same product. This procedure offers much promise for the rapid and accurate analysis of small quantities of autoxidation products (see Chapter V for more detailed discussion of application of infrared spectrophotometry).

4. Miscellaneous Investigations

Acetylenic Compounds. Acetylenic fatty acids occur in many natural oils, although most of them are rarely found in commerce. Like olefinic oils, acetylenic oils undergo autoxidation, but these reactions have not been extensively investigated. Khan *et al.* (108) investigated the autoxidations of stearolic acid and its methyl ester and compared them with the autoxidation of oleates. In contrast to oleates, stearolic acid and methyl stearolate exhibited no induction period and oxidation began at its maximum rate. The acetylenic compounds absorbed oxygen at a greater rate (4 times) than did the oleates. Oxidation of stearolate was accompanied by polymerization and the residues contained a considerable amount of carbonyl oxygen. No diketostearic acid was found. Acid and ester groups were present, but only small amounts of peroxide and hydroxyl groups. The volatile products of oxidation of stearolate consisted of water, carbon dioxide, and other organic products. With methyl stearolate, the evolved water equaled more than 0.8 mole per mole substrate

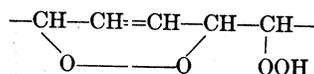
and carbon dioxide more than 0.1 mole per mole substrate. The non-aqueous volatile products of oxidized stearolate had a rancid odor but gave a negative Kreis test, whereas the corresponding product from oleate gave a positive test. These differences in reactions velocities and products of oxidation imply marked differences in the mechanisms of oxidation of oleate and stearolate. Khan *et al.* (108) have suggested that α -methylene attack of acetylenic compounds is the predominant type of oxidation rather than addition of oxygen to the double bond.

Oxidation of so-called "matricaria" methyl ester (methyl 2,8-decadien-4,6-dienoate), $\text{CH}_3\text{CH}:\text{CHC}:\text{C}:\text{C}:\text{CCH}:\text{CHCOOCH}_3$, by Holman and Sorensen (153) indicated that in this conjugated system the addition of oxygen followed a biphasic curve. Likewise, the absorption in the ultraviolet at 3550 Å. in alkaline solution increased rapidly initially, whereas the increase in absorption at 6000 Å. corresponded to the second phase of oxygen addition. The oxidation was accompanied by rapid polymerization and intense deepening of color of the oxidized mass. The mechanism of oxidation of conjugated unsaturated systems involving triple bonds, e.g., matricaria ester and isano oil, probably differs from that occurring with isolated triple bonds. The mechanism of oxidation of conjugated systems composed of mixed double and triple bonds may be similar to that occurring with conjugated polyenes, because oxidation is accompanied by rapid polymerization in both types of unsaturated systems. It might be expected that oxygen would add to both types of resonating systems in similar manners.

Physical State of Substrates. The degree of dispersion of unsaturated substrates has a marked effect upon their oxidation. The spreading of oil films on porous material can, and often does, lead to spontaneous combustion of the oil. Honn *et al.* (154) studied the oxidation of drying oils adsorbed on the surface of finely divided silica gels. They observed that a critical oil/solid ratio existed which corresponds to a maximum rate of oxygen uptake. Their results were interpreted as indicating the existence of a closely packed monomolecular layer of oil on the adsorbent at the critical ratio, and that this arrangement is most favorable for promoting oxidation. At concentrations of oil below the critical oil/solid ratio, the molecules of oil are separated by distances which depend upon the ratio, and hence the rate of oxidation depends upon the average distance between the molecules of oil. Above the critical ratio, the molecules of oil form multimolecular films, and the rate of oxidation is decreased, because diffusion of oxygen becomes a limiting factor. This interpretation was verified by the observation that at the critical ratio the calculated area

occupied by that amount of oil as a monolayer nearly equaled the area available on the silica gel surface.

In aqueous colloidal solution, the oxidation of sodium linoleate appears to occur in a slightly different manner than the oxidation of a mass of linoleate esters. Bergström *et al* (155) found that in the former system the rate of oxidation was dependent upon copper ions; that maximum uptake of oxygen is two moles of oxygen per mole of linoleate; and that the spectral changes which occurred were similar to those observed in oxidizing linoleate esters. Isolation of the reaction products in the form of an oil of slight viscosity indicated that polymerization had been inhibited, and the sharp termination of the reaction after absorption of two moles of oxygen per mole of linoleate suggested that the product of oxidation might be reproducible. The authors hypothesized that monomeric diperoxides corresponding to the structure,



may have been formed by the oxidation of the primary peroxide. This method might be used to prepare an oxidation product of linoleate which would contain two moles of oxygen per molecule.

Emulsified oils are susceptible to oxidation, but because of the instability and nonreproducibility of emulsions, little work has been done on the oxidation of fat systems of this type, with the exception of enzymic studies. However, emulsions are of biological and medical interest. Oil in the dispersed phase is subject to the same type of oxidation as oil in mass, but the presence of water-soluble catalysts in the former influences the oxidation. Metallic salts and hemoproteins are particularly important catalysts in oxidation of biological systems. Much of the oxidative catalysis in animal tissue fats, which has been considered to be enzymic in character, is due to oxidation catalyzed by hemoglobin, myoglobin, and catalase (156).

Most of the research on the oxidation of fats has been carried out in liquid systems. Oxidation of solid fats is inhibited by the presence of considerable amounts of saturated fatty acids which act as diluents. Moreover, oxidation of fatty substances is limited in the solid state because penetration and diffusion of oxygen are much reduced.

Restriction of unsaturated fatty acids and esters within the crystal structure of urea complexes is effective in inhibiting oxidation of these readily oxidizable substances. The inhibition of oxidation in this case may result from the failure of oxygen to penetrate the system, or from

prevention of chain reaction because of the rigid lattice to which the substrate is restricted (157).

Oxidation in the gaseous phase is extremely rapid, but little is known of the mechanism of this reaction. Contact of hot vapors of fatty acids or esters with air can lead to explosive oxidation.

Irradiation. The oxidation of unsaturated acids and esters is stimulated and accelerated by various types of irradiation. The absorbed radiant energy activates the molecules of the substrate to the energy level necessary for chemical reaction to take place. Infrared and visible radiation are somewhat effective, but ultraviolet light, because of its higher energy, is much more effective. X-rays have been found to be effective in inducing the oxidation of fats.

Irradiation with ultraviolet light was used by Farmer and Sutton (41) to promote the oxidation of methyl oleate to the hydroperoxide. Sutton (46) also applied a similar form of radiation in oxidizing methyl elaidate to the hydroperoxide. Swift *et al.* (47) oxidized methyl oleate rapidly with the aid of ultraviolet radiation in the preparation of hydroperoxide in 90% purity.

Bateman and Gee (158) investigated the kinetics of the photooxidation process, using cyclohexene, 1-methylcyclohexene, 2,6-dimethyl-2,6-octadiene, and ethyl linoleate as substrates. They concluded that photooxidation proceeds by a chain mechanism in which the generation of free radicals as a consequence of light absorption is the chain-initiation mechanism. The predominant initiation process is the photolysis of the hydroperoxides. When the intensity of the light is fixed and wavelength selected to provide weak absorption, the photooxidation is autocatalytic, because the reaction products are more strongly light-absorbing than the original substrate, and their photolysis leads to additional chain formation.

Photooxidation is modified by the presence of chlorophyll in the oxidation system (71,159). The photooxidation of methyl oleate and methyl linoleate in the presence of chlorophyll was investigated by Khan *et al.* (159). The energy of the visible light absorbed by the chlorophyll is transferred by an unknown mechanism to the substrate, thereby activating it to an energy level sufficiently high for oxidative attack to take place. The products of autoxidized methyl linoleate exhibited a lower spectral absorption at $234\text{ m}\mu$, i.e., in the ultraviolet region, which is characteristic of the absorption of conjugated dienes, whereas the products isolated from thermal and nonchlorophyll-catalyzed photooxidation are very similar in light absorption. The photooxidized product exhibited considerable absorption in the infrared at $10.36\ \mu$, indicative of the presence of isolated *trans*-

material in the product, in contrast to the products of nonchlorophyll-catalyzed photooxidation. Methyl linoleate photooxidized in the presence of chlorophyll yielded a peroxide concentrate of high peroxide value, which after reduction to the alcohol and chromatographic separation yielded a significant fraction of product which was nonconjugated. These results suggest that the 11-hydroperoxidooctadecadienoate can exist and attain a significant proportion of the total autoxidation product by the action of chlorophyll, despite the greater thermodynamic stability of the conjugated isomers (see Chapter V for further discussion of this subject).

Mead (160) reported that x-irradiation of linoleate resulted in autoxidative chain reaction. He measured the quantum yield and found that with increasing linoleate concentration, the ionic yield increases. With increasing concentration of cysteine, the ionic yield decreases, indicating that sulfhydryl compounds are effective in protecting linoleate against oxidation. Presumably, the mechanism of x-ray-stimulated oxidation of linoleate is the same or similar to that occurring in autoxidation. It is also possible that radiation-initiated chain oxidation of unsaturated fat accompanies radiation injury of animals.

Enzymes. Lipoxidase is an enzyme widely distributed throughout the plant kingdom and primarily active in the oxidation of polyethenoid acids and their esters. The enzyme and its activity have been subjects of many investigations which have been reviewed by Holman and Bergström (156,161), Franke (162), and others.

Lipoxidase attacks essential fatty acids, yielding hydroperoxide as a primary product. The enzyme-catalyzed oxidation of linoleate is accompanied by the complete conjugation of the double bonds and the formation of optically active hydroperoxides (162a). The double bonds are *cis,trans* and *trans,trans* (162a,163a). The enzyme is active from the freezing point of the solution to somewhat above room temperature, but becomes inactivated at higher temperatures.

The most plausible mechanism for lipoxidase-catalyzed oxidation of linoleate probably involves contact between the enzyme and each molecule of the substrate. Tappel *et al.* (163) found that the enzyme was capable of oxidizing antioxidants in the presence of linoleate without the concomitant oxidation of linoleate itself.

The lipoxidase-catalyzed oxidation of polyphenolic antioxidants in the presence of linoleate suggests that the latter may play a role analogous to a coenzyme or prosthetic group in the oxidation of polyphenols and other substances. The reported lipoxidase-catalyzed oxidation of amino acids and polyphenols may occur by such a mechanism, in which case the

enzyme may be as important in the oxidation of these secondary substrates as in the direct oxidation of polyunsaturated fatty acids.

Strain (163b) has pointed out that the direct catalytic formation of peroxides in the presence of oxidase occurs only with those compounds containing $-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CO}-$ with a *cis*-configuration.

5. Biological Effects of Autoxidized Fats

The adverse effect of the ingestion of rancid fat is well known. The subject was reviewed by Burr and Barnes (164) in 1943 and by Quackenbush (165) in 1945. These authors concluded from the evidence available at that time that one of the chief deleterious effects of rancid fat in the diet is the destruction of vitamins and perhaps other dietary essentials. On the other hand, many symptoms associated with the ingestion of rancid fats are not explained solely in terms of the destruction of vitamins.

Rancid (oxidized) fat in the diet results in biotin deficiency through oxidation of biotin which is synthesized in the intestine (166). This is in agreement with observations that biotin is inactivated by rancid fats *in vitro* (167). Ascorbic acid may be partially destroyed as a consequence of its synergistic antioxidant action. Pyridoxine and pantothenic acid have been found to relieve acrodynia in rats fed rancid fat (168). Tocopherol and carotene are easily oxidized; vitamin D is subject to oxidation under conditions which prevail in dietary preparations (169). Deficiencies in vitamin K (170), vitamin A (171), and riboflavin (172), as well as in the effect of cortisone (173), are intensified by ingestion of autoxidized fat. Fresh fat, however, exerts a protective effect on the toxicity of heated and aerated cottonseed oil (174).

Destruction of Essential Fatty Acids. It is obvious that if rancidity is caused largely by the oxidation of polyunsaturated fatty acids, these essential fatty acids may be partly or completely destroyed by this process. This aspect of rancid fat toxicity has had little attention until recently. In the study of the ability of rancid fat and oxidized esters to relieve the symptoms of acrodynia, a deficiency disease caused by removal of pyridoxine and essential fatty acids from the diet, it was observed that oxidized methyl linoleate relieved the symptoms but was not as effective as fresh ester. The addition of tocopherol did not improve the response to the rancid preparations and highly oxidized preparations were completely ineffective (168).

It has been suggested that one role of tocopherol *in vivo* may be to provide an antioxidant status for the protection of essential fatty acids (poly-

unsaturated fatty acids). To test this hypothesis Witten and Holman (175) attempted to simulate pro-oxidant status by feeding benzoyl peroxide with a supplement of essential fatty ester, and to simulate antioxidant status by feeding added tocopherol with the supplements. The conversions of linoleate and linolenate to more highly unsaturated fatty acids by fat-deficient rats were used as indices of essential fatty acid utilization. It had been previously found that in rats the ingestion of linoleate induces synthesis of arachidonate, and linolenate induces synthesis of hexaenoate, with other cross-conversions also taking place (176). When benzoyl peroxide plus linoleate was fed as a supplement to fat-deficient rats, growth response was greatest, and least when the benzoyl peroxide was fed alone. Fat synthesis was found to be stimulated by ingestion of benzoyl peroxide plus unsaturated ester. Benzoyl peroxide plus linoleate led to the formation of hexaenoic acid, but other conversions appeared to be unaffected by either tocopherol or benzoyl peroxide. Thus, contrary to expectation, the pro-oxidant, which is toxic when fed alone, proved to be beneficial according to criteria of growth and fat synthesis.

From the above results it would appear that oxidized essential fatty acids are involved in certain *in vivo* conversions. Holman (4) administered to fat-deficient rats the following supplements: ethyl linoleate, partially oxidized ethyl linoleate, ethyl linoleate with dietary benzoyl peroxide, ethyl linoleate hydroperoxide, thermally decomposed ethyl linoleate hydroperoxide, and conjugated ethyl linoleate. Fresh linoleate, oxidized linoleate, and linoleate plus benzoyl peroxide relieved the dermal symptoms of fat deficiency, reduced the water consumption of the rats, and stimulated the synthesis of arachidonate. Linoleate peroxide, decomposed peroxide, and conjugated linoleate were ineffective, judged by these criteria. The concentrated products of oxidized linoleate and conjugated linoleate could not be utilized as essential fatty acid, but fresh linoleate, slightly oxidized linoleate, and linoleate plus dietary benzoyl peroxide were curative. The beneficial effect of feeding the catalyst for autoxidation, benzoyl peroxide, plus linoleate together in supplement suggested that a more oxidizable medium may be better utilized by the animal. The influence of pro-oxidant and antioxidant conditions upon the metabolism of essential fatty acid is still not clear, and much experimental work will be needed before the subject is completely understood.

Effects of Autoxidatively Produced Polymers. There is increasing evidence that the abnormal nutritional properties of highly autoxidized fats are related in part to the formation of polymers during autoxidation. Kaunitz *et al.* (177,178) isolated polymers from highly autoxidized cotton-

seed oil and lard and fed them to rats. Diets containing 20% of autoxidatively produced polymeric residue led to diarrhea and rapid death, but when only 10% of the product was fed most of the animals developed a tolerance to it. When fed at levels of 4 or 7%, it was well tolerated but growth was reduced. There were no distinctive histological lesions and withdrawal of the polymer permitted immediate realimentation without evidence of subsequent injuries.

The polymeric residue from autoxidized cottonseed oil exerted a greater growth-depressant effect than that from lard. Addition of fresh fat to the polymeric residue decreased the growth-depressant effect. On diets marginal in protein, autoxidatively produced polymers intensified the deficiency effect when fed at levels which had little or no effect in normal diets (178).

Effect of Oxidized Fat on Enzyme Systems. Bernheim *et al.* (178a) demonstrated an inhibition of enzyme action in vitro by the products of oxidized fatty acids. Washed tissue suspensions or mitochondria, when incubated with ascorbic acid, lose enzymic activity. The decrease in the activities of succinoxidase, cytochrome oxidase, and choline oxidase has been found to parallel the amount of oxidized unsaturated fatty acid as measured by the thiobarbituric acid test. The enzyme inactivation can be prevented by quercetin, which inhibits oxidation. Oxidized methyl linolenate also inactivates the enzymes. These observations are of interest particularly in the case of cytochrome oxidase, which is known to be associated with lipid containing some highly unsaturated acids. It may be that the inhibition is due to the oxidative destruction of essential fatty acids that are a part of the active enzyme system.

6. Detection of Oxidation Products

The Kreis Test. The Kreis test has been used widely for assaying oxidized fats and oils, but the method is highly empirical. The test as ordinarily carried out involves two-phase systems or other means of separation of the active components. Modification of the test to make it suitable for colorimetry has led to some improvement in the method (179). Phloroglucinol has been shown to yield colored reaction products with epihydrinaldehyde, malonic dialdehyde, or acrolein treated with hydrogen peroxide (180), but the presence of these compounds has not been demonstrated in an oxidized fat. It is possible, however, that they may be formed as secondary products of oxidation. The test, although valuable for the qualitative detection of oxidation, has been of little value in fundamental investigations of fat oxidation. Apparently the Kreis reaction detects

substances formed by the decomposition of peroxides, and measures terminal oxidation products; hence it may prove valuable in investigating secondary oxidations and decomposition of peroxide.

Thiobarbituric Acid Test. The thiobarbituric acid test for rancidity of fats appears to be related to the Kreis test. It was originally applied by Kohn and Liversedge (181), who observed that animal tissues, after aerobic incubation, gave a color reaction with thiobarbituric acid. Bernheim and co-workers (182,183) related the color development to reaction with products of oxidation of unsaturated fatty acids, principally linolenic acid. Patton and Kurtz (184) investigated the reaction and applied the test to detection of oxidation in milk fat. They found that malonic dialdehyde gave a strong reaction and that the thiobarbituric acid test is much more sensitive than the Kreis test, which does not yield measurable amounts of color until after the appearance of a decided oxidized flavor, whereas the thiobarbituric acid test gave measurable responses during the development of perceptible rancidity. Methyl oleate hydroperoxide also gives the color reaction (184a) with thiobarbituric acid. The test appears, therefore, to be somewhat more useful than the Kreis test, especially because it can be applied to detect oxidation in its early stages.

Stamm Test. The Stamm test depends upon reaction of oxidized fat with *sym*-diphenylcarbazide to yield a color (185). The test has been correlated (186) with the appearance of organoleptic rancidity in fat products.

Dicarbonyl Compounds. Dicarbonyl compounds have been reported to be present in the products of oxidation of fats. O'Daniel and Parsons (187) have postulated that the color developed by oxidized fats with alkali is attributable to the formation of quinones from such compounds by double aldol condensation. They suggested that the color developed by fats in contact with alkali, the so-called "alkali color," is a test for α -dicarbonyls. Prill also developed a color test involving the dioximes (188). The "alkali-color" test has been shown to be affected by the concentration of fat and by time (186). Nevertheless, the α -dicarbonyl test was considered the best method for estimating rancidity because the dicarbonyl value of vegetable oils is not affected by heat treatments during processing.

Alkali Color. When oxidized fats are dissolved in an alcoholic alkaline medium, intense orange or red colors are produced. These colors result from the production of chromophores whose principal absorption is in the near ultraviolet. The color is merely absorption in the visible region by ultraviolet chromophores. The alkali color has been examined spectro-

photometrically by Holman *et al.* (189), who concluded that the color was probably not due to quinones formed from dicarbonyl in the oxidized fat. Jaspersen *et al.* (190) also concluded that dicarbonyl color tests obtained with oxidized fats are fundamentally different from those obtained with model compounds. Present opinion is that the development of so-called "alkali color" arises from the condensation of unsaturated carbonyl compounds. Lundberg and Chipault (191) reported that the alkali color results from two reactions, one of which is instantaneous, and another which continues over long periods.

Hendrickson *et al.* (192) applied ultraviolet spectrophotometry in studying the relation between alkali color and the oxidative changes taking place in film formation. Chipault *et al.* (99,191,193) likewise used the formation of this color to evaluate the chemical changes occurring during the formation of films from pure triglycerides, pentaerythritol esters, and alkyds prepared from pure fatty acids.

Ultraviolet Absorption. Oxidation of polyunsaturated fatty acids is accompanied by increased ultraviolet absorption. The magnitude of change is not readily related to degree of oxidation because the effects upon the various unsaturated acids vary in quality and magnitude (194). However, the changes in the ultraviolet spectrum of a given substance can be used as a relative measure of oxidation, and probably is best applied in detecting oxidation rather than measuring it. The examination of ultraviolet spectrum is a rapid and assured method for evaluating the purity and freshness of unsaturated fatty materials. The greater the ultraviolet absorption of a fat, the greater has been the extent of oxidative degradation.

Infrared Analysis. Henick (195) has applied infrared analysis to the detection of oxidation products in milk fat. Spectral changes were detected before off flavors developed, and both loss of flavor and development of off flavors were correlated with definite absorption bands. Dugan *et al.* (195a) examined autoxidized methyl linoleate in the infrared absorption regions of hydroxyl and carbonyl. They assigned the region of 3430–3445 cm.^{-1} to the absorption --OOH group. Honn *et al.* (195b) and Crecelius *et al.* (195c) employed infrared spectrophotometry to follow the autoxidation of linseed oil. For more detailed information on the application of all forms of spectrophotometry to oxidized fats see Chapter V.

7. Oxidation Reaction vs. Temperature

Classification of Oxidation Reactions on a Temperature Basis. The effect of temperature on the oxidation mechanism and the products of

oxidation of unsaturated fats or fatty acids is such that for practical purposes three temperature ranges are readily distinguished; many investigations have been confined to one or another of these ranges. These temperature ranges are generally delimited as follows: (1) atmospheric temperature or 0°–40°C. (32°–104°F.); (2) 60°–120°C. (140°–248°F.); (3) 200°–300°C. (392°–572°F). Many of the oxidation reactions involved in the rancidification of fats occur at the lowest temperature mentioned. Blown oils, used in the drying oil industry, are generally prepared at temperatures between 60° and 120°C., and it is in this temperature range that most of the investigations on accelerated fat deterioration are conducted. The highest temperatures are employed in the manufacture of boiled oils, stand oils, and related polymerized fat products.

Atherton and Hilditch (196) measured changes in iodine value in methyl oleate and concluded that at 20°C. oxygen reacted with the methylenic group to form hydroperoxides, and also with the olefinic linkage. At 120°C. the reaction appeared to occur exclusively at the olefinic linkage and to be followed by secondary reactions at other points. Paquot (197) concluded that at 20°C. hydroperoxide formation predominated while at 120°C. a moloxide was formed.

From a study of the oxidation of elaidic acid and its esters, Skellon and Thruston (198) concluded that at 55° and 85°C. the oxidation reaction is marked by an induction period and that chemical changes at these temperatures are slight. At 120°C. the induction period is said to be absent and carbonyl compounds, carbon dioxide, and polymers are formed.

The oxidation products of triglycerides have also been classified in this manner. The products of the oxidation of fatty oils were reported by Gillam (199) in 1949 to fall into four groups that pass into each other with considerable overlapping. This author classified the products on the basis of the temperature of the oxidation and concluded that the products of the oxidation at any temperature are a mixture of all types.

A difference in chemical nature of the oxidation products of lard formed at different temperatures has been demonstrated by Lewis and Quackenbush (57) by polarographic analysis.

The effects that differences in reaction temperature exert on the induction period and oxypolymerization of raw linseed oil have been investigated by Hess and O'Hare (200). They found three distinct temperature regions, each characterized by different types of oxidative changes. The regions are: above 130°, between 84° and 130°, and below 84°C. Temperature was found to have an effect on the induction period and on the peroxide values of the oil. Ultraviolet absorption analyses indicated

that the formation of conjugated diene systems reached a maximum and was never appreciably greater than 5% at temperatures between 84° and 200°C. Higher values were obtained at lower temperatures. In addition, these investigators proposed a chelate-type of intermediate in autoxidatively produced polymerization.

Ricinoleic Acid and Methyl Ricinoleate. Ellis (201) obtained unsaturated ketones from the autoxidation products of ricinoleic acid and showed that there is considerable polymerization at high temperatures. Autoxidation of methyl ricinoleate (10) with uranyl ricinoleate as catalyst gave typical oxygen absorption curves at 55°, 85° and 120°C. At 85°C. rapid peroxidation occurred with little change in iodine value, suggesting addition of oxygen to the molecule without affecting the unsaturation. At later stages, the peroxide and iodine values decreased together.

Erucic and Brassidic Acids. Skellon and Taylor (202) have described the autoxidation of erucic acid and of methyl and propyl erucates at 55°, 85°, and 120°C. Changes in the observed content of oxygen followed the usual course, but the percentage of active oxygen was highest in the autoxidized propyl ester. The influence of alkyl groups was marked. The oxidation products consisted of small percentages of epoxides and cleavage products, ketohydroxy, dihydroxy, and aldehyde derivatives, together with oily complex products. Unsaturated keto derivatives were not conclusively identified.

The autoxidation of brassidic acid at 73° and 120°C. has also been studied (203). In contrast to erucic acid, monoperoxy acids were not isolated from the autoxidation products. After saponification, dihydroxybehenic acid (8%) and an oily monomer (72%) were recovered. The latter may be either acyloin or an unsaturated ketonic derivative.

These results are qualitatively similar to those reported earlier by Dorée and Pepper (204), who employed cobalt erucate as catalyst in most of their work. Yields of epoxides were as high as 15%.

10-Hendecenoic Acid. The autoxidation of 10-hendecenoic acid or its methyl ester at 80°C. yields some sebacic acid, 10,11-dihydroxyhendecanoic acid, and polymer (205). The polymer may arise from aldol condensation of oxidation products containing carbonyl groups. For additional information on fatty acid condensation and polymer reactions reference should be made to Chapter X.

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