erentially adsorbed from the rumen, or are preferentially passed from the rumen, leaving the saturated. These possibilities are most unlikely. The rumen is incapable of absorbing long chain fatty acids, and the resolution of a solution of saturated and unsaturated acids in a churning milieu such as a rumen must be considered impossible.

Another alternative is that the unsaturated acids are oxidized to short chain acids and these are resynthesized to saturated acids. Since the synthesis of saturated fatty acids from acetate is a proven phenomenon, this may take place to some degree, but to the author's knowledge nowhere in all the rather voluminous literature on the short chain acids of the rumen has this been observed to occur. On the contrary, short chain acids are absorbed to a considerable degree directly from the rumen.

There is the possibility that the unsaturated acids are oxidized to short chain acids in the rumen and absorbed there, leaving the saturated acids. However Shorland has shown that the saturated acids in the rumen are mainly stearic. The saturated acids of linseed and cottonseed oil are mainly palmitic.

The interpretation that the dietary linoleic and linolenic acids of the goat are converted in the rumen to oleic and saturated acids explains the high level of stearic acid in the tissues of ruminant animals and the relative lack of influence of dietary fatty acids on the depot fat of these animals. Previous work in this laboratory (7) has shown that the inclusion of 5% of cottonseed oil in an otherwise low fat ration of fattening steers resulted in a fat of higher saturated and lower oleic acid than the controls. Linoleic and linolenic acids were not affected. This paradox was explained by the hypothesis that the exogenous fat, hydrogenated in the rumen, contained more saturated and less oleic than endogenously produced fat. The present data substantiate this interpretation. Endogenous animal fat produced on "fat-free" rations is about 35% saturated and about 65% or more oleic and palmitoleic (12, 13, 14). Rumen fat, after ingestion of cottonseed oil, contained between 50 and 60% saturated acids and only 30 to 50% monoethenoid acid. Thus the addition of cottonseed oil to a low fat cattle ration actually would paradoxically increase the level of the saturated acid and reduce the level of the oleic (monoethenoid) acid. Others have also found that the ingestion or rather large quantities of soybean and other unsaturated fats to steers for 260 days had little influence on the iodine number or firmness of the depot fats (5). No analysis was made of the fatty acid composition.

Summary

Goats were fed alfalfa meal containing 10% cottonseed or linseed oil. After 11 weeks the fatty acids of rumen, stomach, and caecum contents were compared to those of the feed.

It was found that the high levels of linoleic and linolenic acids of the feed were reduced to very low levels in the rumen, with comparable increases in the saturated acids. Monoethenoid acids were increased after linseed oil ingestion and in one animal after cottonseed oil ingestion.

The ratio of monoethenoid to saturated acids in the rumen fat was lower than in the endogenous fat of nonruminant animals. This explains the paradox of the low ratio in the depot fat of ruminants even after the ingestion of highly unsaturated fats.

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Concurrent Oxidation of Accumulated Hydroperoxides in the Autoxidation of Methyl Linoleate[1,2]

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UNDER MILD CONDITIONS OF OXIDATION the yield of hydroperoxides in the initial stages of the autoxidation of methyl linoleate is believed to be virtually quantitative (4, 8, 10, 12, 16), that is, secondary oxidation of hydroperoxides has been considered to be relatively unimportant in the early stages of the reaction. In fact, Bolland (7) suggested that hydroperoxides formed in the autoxidation of ethyl linoleate would be less likely to undergo oxidative attack than ethyl linoleate because less resonance energy would be made available on radical formation.

Notwithstanding, there are a number of observations peculiar to the autoxidation of methyl linoleate that remain unexplained. Notable among these is the amount of diene conjugation formed during the reaction. The average value of about 23,000 for the molecular extinction coefficient for the peroxides formed in the autoxidation of methyl linoleate is much lower than that determined for *cis, trans* diene conjugated methyl octadecadienoate (14, 17). This cannot be explained on the basis of our present knowledge unless one takes the unattractive view that nonconjugated hydroperoxides are produced. Formation of nonconjugated hydroperoxides has not been demonstrated in the autoxidation of methyl linoleate, and from thermodynamic and other considerations it appears unlikely that they are produced in an amount sufficient to account for the low molecular extinction (4, 21, 22).

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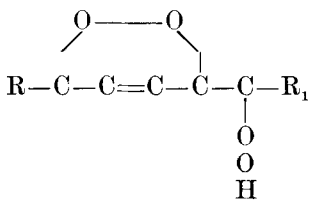
Although *cis, trans* diene conjugated hydroperoxides are the primary reaction products in the early stages of the low temperature autoxidation of methyl linoleate, products containing *trans, trans* diene conjugation are also frequently observed (12, 18, 21). The amount of *trans, trans* diene conjugation progressively increases as the oxidation is continued beyond the initial stages at low temperatures and actually predominates when the autoxidation is carried out at room temperature (12, 18, 21). Also, in spite of the fact that the diene conjugation exists predominantly in the *trans, trans* configuration in autoxidations conducted at elevated temperatures, the absorptivity for diene conjugation is generally no greater than when the product consists primarily of *cis, trans* diene conjugated hydroperoxides (16, 18).

A number of investigators (6, 12, 15), have postulated that diene conjugated monomeric monohydroperoxides may undergo further oxidation by 1, 4 addition of oxygen to the conjugated diene system during the course of the autoxidation of methyl linoleate to form diperoxides of the type



The formation of such compounds has generally been regarded as taking place in the more advanced stages of autoxidation. However a careful study of published data on the products of the autoxidation of methyl linoleate (5, 12, 13, 15, 21) reveals that, although the yield of hydroperoxides is higher under mild oxidative conditions, secondary reactions yielding non-hydroperoxidic products take place even in the initial stages of the reaction.

The present investigation consists, essentially, of a study of the course and mechanism of the oxidation of hydroperoxides isolated from autoxidized methyl linoleate and was undertaken for the purpose of determining the importance of secondary reactions in the early stages of the autoxidation of methyl linoleate.

Some related observations also were made on the autoxidation of reduced hydroperoxides (hydroxy derivatives) and diene conjugated methyl linoleate.

Materials and Methods

Methyl Linoleate. This ester was obtained by urea fractionation and low temperature crystallization of the fatty acids of safflower seed oil. The highly purified acids were esterified with methanol and fractionally distilled through a Podbielniak Hypercal column. The final product had an iodine value of 169.0 (Wijs) and a refractive index of 1.4612 at 23°C. The diene conjugation was essentially nil as no maximum was observed at 230–236 mμ in the ultraviolet spectrum; infrared analysis showed that the final product was free from unnatural geometric isomers.

Methyl Linoleate Hydroperoxide Preparation. The hydroperoxides used in this study were obtained from two sources, autoxidized methyl *cis*-9, *cis*-12-linoleate, and lipoxidase-oxidized sodium *cis*-9, *cis*-12-linoleate.

The method of formation, recovery, and isolation of hydroperoxides obtained from lipoxidase-oxidized sodium *cis*-9, *cis*-12-linoleate is reported in detail elsewhere (19). For the present work the concentrate of hydroperoxides, obtained as described (19), was further purified in the following manner. The concentrate was dissolved in 8.5:1 mixture of Skellysolve F —ethyl ether to give a 10% solution, which was cooled to —70°C. At this temperature the hydroperoxides crystallized in the form of fine white crystals; a dark yellow noncrystalline material also was precipitated. The solution was then allowed to warm slowly to about —50°C. to dissolve the hydroperoxides. The yellow amorphous material remained insoluble at the bottom of the flask at this temperature, and most of the solution containing the hydroperoxides was siphoned off. The hydroperoxides were then crystallized from this solution by lowering the temperature again to —70°C. The supernatant liquid was decanted, fresh solvent added, and the recrystallization repeated. After five such crystallizations the hydroperoxides were recovered and esterified with diazomethane in ethyl ether at 0°C. The resulting esters had a peroxide value of 5900 m.e./kg. and a specific extinction coefficient at 234 mμ of 80.3. The infrared absorption spectrum (Figure 6) showed that the concentrate consisted primarily of *cis, trans* diene conjugated isomers. On the basis of these analyses and previous experience in isolating and purifying hydroperoxides from this source (19), this concentrate was estimated to consist of more than 90% monomeric monohydroperoxide.

The hydroperoxide concentrate obtained from autoxidized methyl linoleate was isolated by countercurrent extraction (20) from a sample autoxidized at 0°C. to a peroxide value of 670 m.e./kg. The final product had a peroxide value of 6250 m.e./kg. and a specific extinction at 234 mμ of 70.0. Analyses on the reduced product showed 0.95 moles OH per mole of ester; 1.87 moles hydrogen uptake per mole of ester; k_{234} mμ, 74.0 This peroxide concentrate was used in only a few experiments to determine if there were any differences between hydroperoxides isolated from autoxidized and lipoxidase-oxidized linoleate. Since the hydroperoxides isolated from both sources appeared to be identical as far as their kinetic behavior was concerned, the compound isolated from the lipoxidase-oxidized linoleate was used in most of the studies because it was more readily available.

The hydroperoxides isolated from either the lipoxidase-oxidized linoleate or autoxidized linoleate by the methods and under the conditions reported here may be presumed to consist primarily of a mixture of two isomers 9-hydroperoxy *trans*-10, *cis*-12-octadecadienoic acid methyl ester, and 13-hydroperoxy *cis*-9, *trans*-11-octadecadienoic acid methyl ester (4, 5, 21). For convenience however this preparation will be referred to in the text as methyl linoleate hydroperoxides.

Reduced Methyl Linoleate Hydroperoxide Preparation. This material (presumed to consist primarily of a mixture of 9-hydroxy, *trans*-10, *cis*-12-octadecadienoic acid methyl ester and 13-hydroxy, *cis*-9, *trans*-11-octadecadienoic acid methyl ester) was obtained by reducing a hydroperoxide concentrate isolated from lipoxidase-oxidized linoleate. The reduction was made with a five-fold excess of stannous chloride dissolved in absolute ethanol (1% solution) at room temperature. The reduced product was extracted with Skellysolve F and recovered by evaporation of the solvent

under reduced pressure. Further purification was made by molecular distillation at 120°C. The final product gave the following analyses: k_{234} 82.0; hydrogen uptake 1.93 moles per mole of ester; hydroxy 0.97 moles per mole of ester. Infrared spectral analysis showed that the product consisted primarily of *cis, trans* diene conjugated isomers. In accordance with the designation of the hydroperoxide concentrate isolated from oxidized linoleate, this preparation will be referred to in the text as *reduced methyl linoleate hydroperoxides.*

Methyl Myristate. This compound was obtained by purifying Eastman methyl myristate by fractional distillation. The final product did not absorb any oxygen under the conditions employed in the oxidation studies, and its refractive index (1.4350/25°C.) was in close agreement with that reported for the pure compound (3).

Methyl cis-9, trans-11-Linoleate. The conjugated fraction of the methyl esters of dehydrated castor oil acids was separated by fractional distillation, and the desired compound was then isolated by low temperature crystallization from acetone, using the procedure described by Jackson *et al.* (14, 17) for the isolation of methyl *trans*-10, *cis*-12-linoleate. Recrystallizations were conducted until no further purification could be obtained, as evidenced by ultraviolet and infrared spectral analysis. Analytical data were as follows: k_{233}, 92.1; n_D^{30}, 1.4704; I.V. (hydrogenation), 174.0. The infrared spectrum (Figure 6) showed the doublet absorption bands at 10.55 and 10.18 μ characteristic of *cis, trans* diene conjugation.

Methyl trans-10, trans-12-Linoleate. Cis-9, *trans*-12-linoleic acid obtained from dehydrated castor oil (14, 19), was alkali-isomerized at 200°C. in 21% KOH diethylene glycol solution. The resulting conjugated fatty acids consisted of a mixture of *cis, trans* and *trans, trans* diene conjugated isomers in which the *trans, trans* isomer was presumed to consist virtually entirely of *trans*-10, *trans*-12-linoleic acid (23). This isomer was isolated by low temperature crystallization from acetone and esterified with diazomethane. The ester was given a final distillation and then analyzed.

The analyses were as follows: n_D^{30} 1.4710; I.V. (hydrogenation) 175.0; K_{231} 107.5. The infrared spectra from 10-11 μ showed a single strong absorption band at 10.12 μ.

Measurement of Oxygen Absorption. Rate of oxidation measurements were conducted under various conditions of temperature, oxygen tension, and peroxide concentration in a Warburg respirometer. The oxidations were conducted on 200–250-mg. samples in 15-ml. conical flasks shaken at 120 oscillations per minute through an amplitude of 2.5 cm. Brodie's solution, specific gravity 1.003, was used in the manometers. The temperature of the bath was controlled within ±0.1°C., and samples were equilibrated in the bath for 5 minutes before the manometers were closed. When spectral and peroxide analyses were performed on samples oxidized to predetermined levels, the analyses were conducted immediately after they were removed from the bath.

Infrared Absorption. The infrared spectra reported in this study were obtained with a Perkin-Elmer Model 21 double beam instrument. Measurements were made on a 10% solution of the compounds in carbon disulphide.

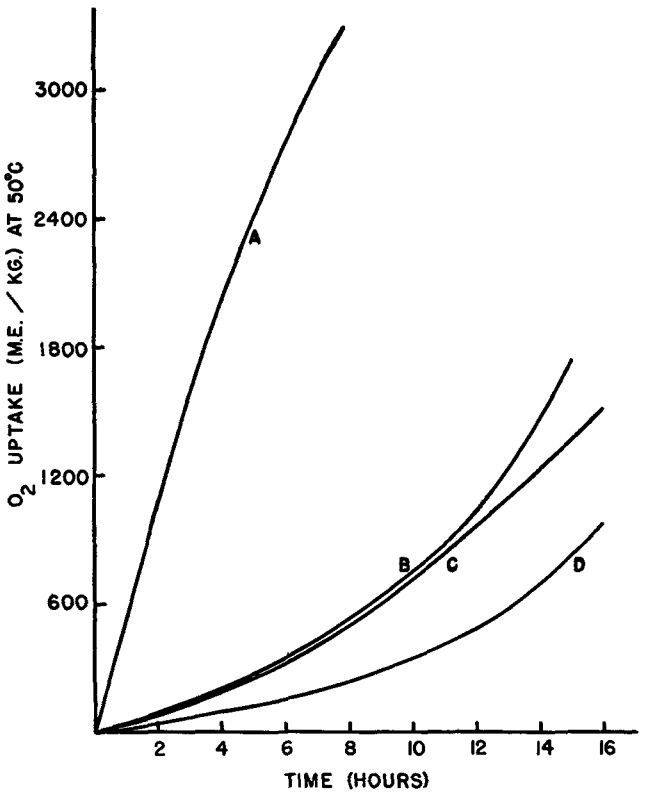


FIG. 1. Rate of oxidation at 50°C. A, methyl linoleate hydroperoxides; B, methyl linoleate; C, methyl *cis*-9, *trans*-11-linoleate; D, reduced methyl linoleate hydroperoxides.

Peroxide Value. A modified iodimetric method was employed in which the solutions were kept under purified nitrogen at all critical stages of the determination (20).

Ultraviolet Absorption. Ultraviolet spectra were obtained with a Beckman model DU spectrophotometer; optically pure 95% ethanol was used as the solvent.

Details of the method for the determination of hydroxyl value have been described in another publication from this laboratory (18). Analytical hydrogenations were conducted at atmospheric pressure in 95% alcohol, using a palladium-on-charcoal catalyst.

Results and Discussion

The general characteristics of the rate of oxidation of methyl linoleate hydroperoxides, methyl linoleate, methyl *cis*-9, *trans*-11-linoleate, and reduced methyl linoleate hydroperoxides are compared in Figure 1, A, B, C, and D, respectively. An important feature of the rate of oxidation of methyl linoleate hydroperoxides, as compared with the other compounds was that in addition to being much faster, it did not follow an autocatalytic pattern. The oxidation of samples of methyl linoleate hydroperoxides proceeded at a steady rate (linear relationship between oxygen uptake and time) for varying periods of time, depending on the temperature. Then the rate gradually fell off as the oxidation was continued, as illustrated by curve A in Figure 1. At 50°C. the steady rate of oxidation continued to an oxygen uptake of about 1400 m.e./kg. of sample (Figure 1), and at 78°C. the rate of oxygen uptake started to fall off after an oxygen uptake of about 850 m.e./kg. of sample. Presumably, at higher temperatures, the rate would start

to fall off still sooner. Thus the steady rate of oxidation that was observed initially at all temperatures represented the initial rate of the primary reaction. All rate measurements were therefore made at this phase of the reaction.

Activation Energy. The activation energy was determined from the slope of the plot of the integrated initial reaction rates as related to initial concentration (Figure 4) *vs.* the reciprocal of the absolute temperature of the reactions (Figure 2). A value of 15.1 k. cal./g. mol was obtained.

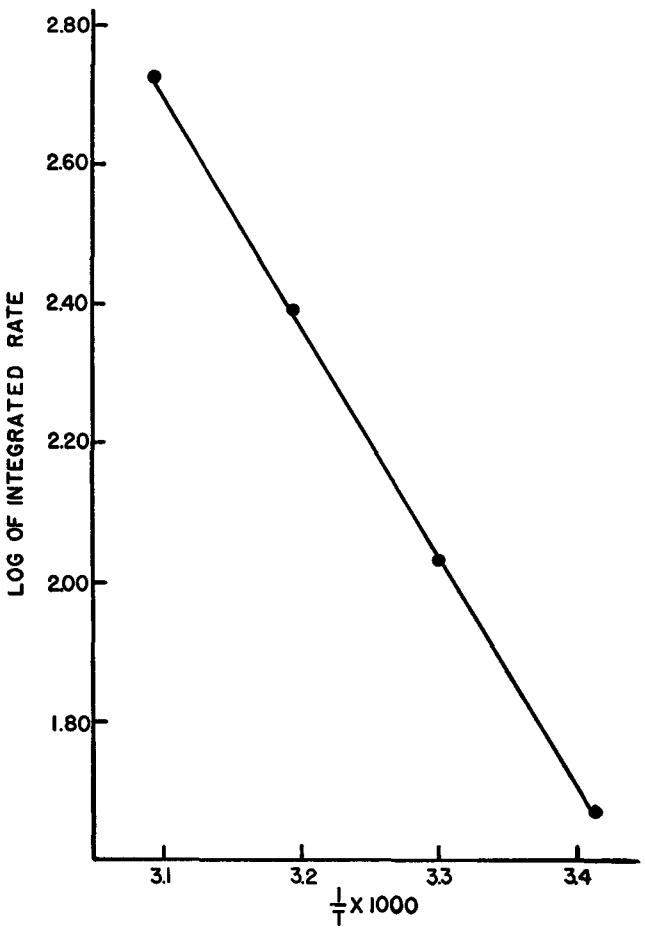


FIG. 2. Effect of temperature on the rate of oxidation of the methyl linoleate hydroperoxides.

For comparison the activation energy for the over-all reaction of the autoxidation of methyl linoleate oxidized to an O_2 uptake of 100 m.e./kg. was determined under similar conditions from the slope of the plot of the log of the integrated rates of oxidation *vs.* the reciprocal of the absolute temperature. A value of 16.2 k. cal./g. mol. was obtained as compared to a value of 17.2 k.cal./mol. by Bolland (7) for the autoxidation of ethyl linoleate at infinite pressure.

The quantitative effect of temperature on the relative rates of autoxidation of methyl linoleate and the oxidation of methyl linoleate hydroperoxides is not so clear from the present work. On the basis of the general observation that secondary reactions take place in greater volume in the autoxidation of methyl linoleate at elevated temperatures, one would expect that the activation energy for the over-all oxidation of accumulated hydroperoxides would be greater than

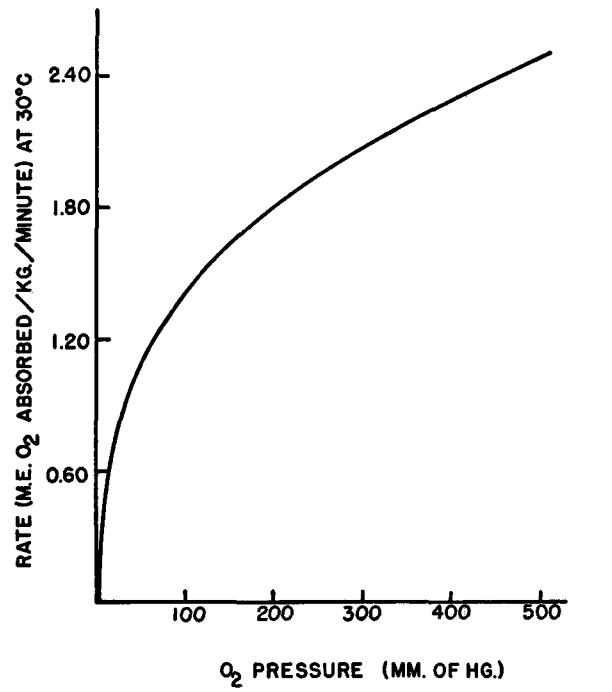


FIG. 3. Effect of oxygen pressure on the rate of oxidation of the methyl linoleate hydroperoxides.

that for the over-all autoxidation of linoleate. The finding of an activation energy for the oxidation of hydroperoxides of the same order as that for the autoxidation of methyl linoleate appears to be anomalous. However since both values represent composite quantities for a number of elementary reactions, the values so obtained may not be strictly comparable. Also this may be a reflection of a situation similar to that existing in the autoxidation of methyl oleate (12A), in which it was found that the highest yields of hydroperoxides were actually obtained in autoxidations at 80°C.

The Effect of Oxygen Pressure on the Oxidation of Methyl Linoleate Hydroperoxides. In contrast to the autoxidation of methyl linoleate in which the reaction was found to be relatively insensitive to oxygen pressures above about 200 mm. (7), the oxidation of methyl linoleate hydroperoxides was sensitive to oxygen pressure up to and beyond about 730 mm. (Figure 3).

The oxidation of methyl linoleate hydroperoxides proceeded so rapidly at 50°C. under atmospheric conditions that unless care was taken to replace the air above the sample frequently, the oxygen was depleted sufficiently in the flasks to change its concentration and cause a premature falling off in the rate due to the sensitivity of the reaction to low oxygen tensions.

Effect of Initial Concentration on the Rate of Oxidation of Methyl Linoleate Hydroperoxide. Various initial hydroperoxide concentrations were obtained by diluting the methyl linoleate hydroperoxides with methyl myristate. A typical sigmoidal relationship was exhibited between the initial peroxide concentration and the initial rate of oxidation (Figure 4).

The oxidation did not proceed in accordance with the kinetics of a simple order, and observations on the effect of antioxidants in giving a well-defined induction period (Figure 5) indicated the strong probability of a chain mechanism.

TABLE I

Relationship Between Peroxide Value, Oxygen Uptake, and Diene Conjugation

Methyl linoleate hydroperoxide preparation oxidized at 30°C.			Reduced methyl linoleate hydroperoxide preparation oxidized at 50°C.			Methyl *trans*-10, *trans*-12-linoleate oxidized at 50°C.			Methyl *cis*-9, *trans*-11-linoleate oxidized at 50°C.		
P.V. m.e./kg.	O_2 uptake m.e./kg.	Absorptivity k_{234}	P.V. m.e./kg.	O_2 uptake m.e./kg.	Absorptivity k_{234}	P.V. m.e./kg.	O_2 uptake m.e./kg.	Absorptivity k_{234}	P.V. m.e./kg.	O_2 uptake m.e./kg.	Absorptivity k_{234}
5900	0	80.3	0	0	82.0	0	0	107.5	0	0	92.1
5830	294	74.0	257	273	78.0	218	408	105.0	247	202	90.6
5820	469	72.5	359	500	76.5	456	841	92.5	460	520	86.4
5950	602	68.0	392	699	72.5	660	1012	90.5	930	1028	80.5
5900	936	62.5	960	1550	66.5	721	1250	86.6			
6400	1985	38.2	1200	2170	54.6	920	1476	83.4			
6000	2930	28.3	1800	3320	43.5						

An insight into the mode of the reaction was indicated from the observation that a sample of methyl linoleate hydroperoxides which had been allowed to absorb 2200 m.e. of oxygen per kilogram then diluted to a P.V. of 2730 (2.23:1 by weight) with methyl myristate oxidized at the same rate as a sample of the original methyl linoleate hydroperoxides diluted to a P.V. of 1745 (3.5:1 by weight) with methyl myristate. This rate corresponded to a peroxide concentration equivalent to the calculated concentration of unreacted hydroperoxide molecules on the basis that one mole of O_2 reacted with one mole of hydroperoxide. Thus the rate of oxidation appeared to depend on the concentration of the unreacted hydroperoxide molecules.

Relation Between Peroxide Value, Oxygen Uptake, and Loss of Diene Conjugation. Another important feature of the oxidation of the methyl linoleate hydroperoxides was the failure of the peroxide value to increase with the absorption of oxygen. There was virtually no increase in the peroxide value as determined by iodimetry even after an oxygen uptake of about 3000 m.e./kg. of sample had taken place in oxidations at 30°C. (Table I). A number of evidences in addition to the peroxide value indicated that the concentration of hydroperoxide groups remained virtually unchanged during the reaction. Although the absorption band at about 2.3 μ in the infrared, usually attributed to OH or OOH, may be due to a number of oxygenated structures, it was significant that it was not altered either in intensity or shape by oxidation of the methyl linoleate hydroperoxides. Further chemical analysis on samples of oxidized hydroperoxides after reduction with stannous chloride also showed virtually no change in derived hydroxyl groups before and after oxidation (Table 2).

Infrared analysis on samples of the reduced methyl linoleate hydroperoxide preparation oxidized to various levels showed that the concentration of hydroxyl groups also remained essentially unchanged throughout the course of the oxidation of this compound. Thus it appeared that little actual destruction of hydroperoxide groups took place during the primary reaction. There is the possibility, however, that the hydroperoxide group is reacted, and the product (a peroxide) is reduced equivalently by both potassium iodide and stannous chloride to yield mostly hydroxyl groups as both of these reagents are general reducing agents for peroxides. Nevertheless, since the rate of the reaction depended on the concentration of unreacted hydroperoxide molecules, after a molecule was reacted, the peroxide group apparently did not exert any further influence on the primary reaction. Thus the apparent role of the hydroperoxide group was to facilitate oxidative attack on the molecule. This may be the mode by which new chains are formed in the reaction and so explain the kinetics of the effect of varying the initial concentration (Figure 4).

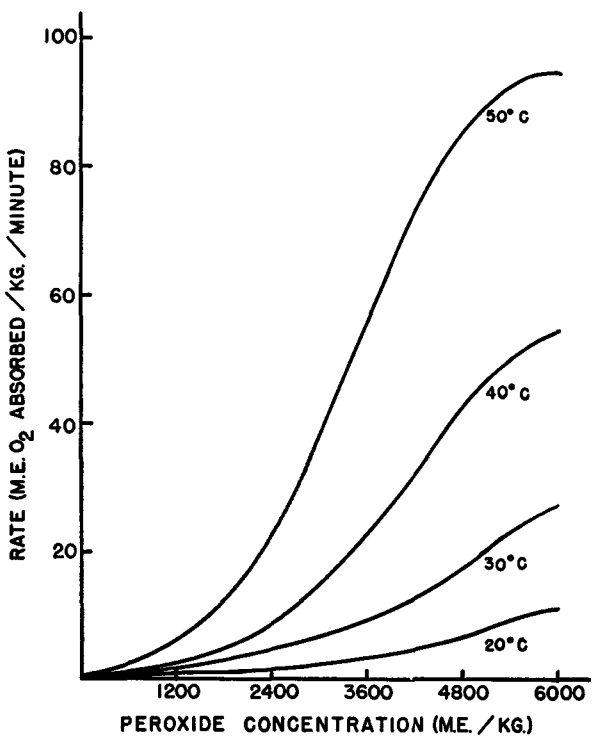


FIG. 4. Effect of initial concentration and temperature on the rate of oxidation of the methyl linoleate hydroperoxides.

Although there was essentially no change in the peroxide value, the diene conjugation progressively decreased during the oxidation. On the basis of the decrease in absorptivity at 234 mμ, on the average, 1.5 moles of diene conjugation were destroyed for each mole of oxygen absorbed. Likewise there was a progressive decrease in the diene conjugation during the oxidation of the reduced methyl linoleate hydroperoxides and the diene conjugated isomers of methyl linoleate (Table I). In the oxidation of these latter compounds it appeared that one mole of diene conjugation was destroyed for each mole of oxygen absorbed. However, except in the case of the all-*trans* compound, the quantitative relationships are clouded by the effect of *cis, trans* isomerism. Nevertheless the primary point of oxidative attack appears to take place at the conjugated diene in the hydroperoxides as well as in the diene conjugated isomers of methyl linoleate.

Peroxides, as determined by iodimetry, accumulated to an appreciable extent during the oxidation

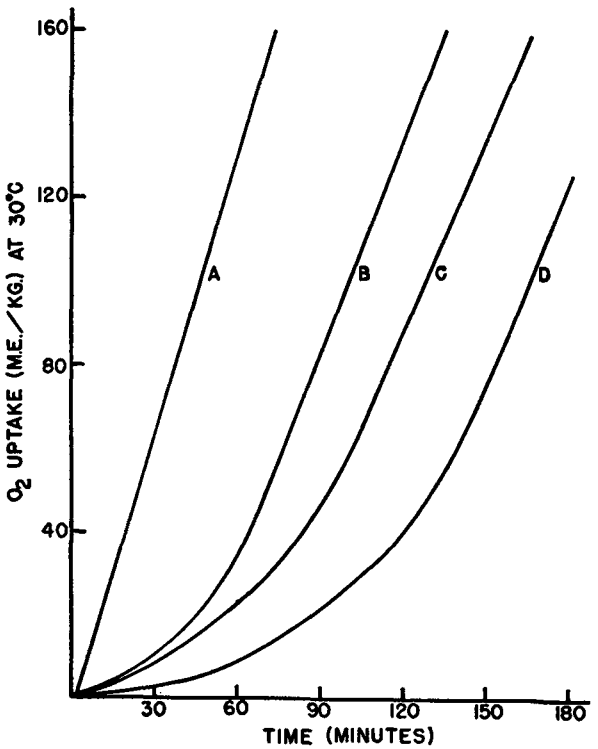


FIG. 5. Effect of antioxidants on the rate of oxidation of methyl linoleate hydroperoxides at 30°C. A, no antioxidant; B, +0.05% hydroquinone; C, +0.05% N.D.G.A.; D, +0.05% propyl gallate.

of the latter compounds (Table I). On the other hand, there was much less accumulation of peroxides in the autoxidation of alkali-isomerized methyl linoleate. When 1006 m.e. of oxygen/kg. had been absorbed in the oxidation of such a compound, which consisted of a mixture of *cis, trans* and *trans, trans* diene conjugated isomers, a peroxide value of only 238 m.e./kg. was obtained. This was similar to the observations of Allen *et al.* (2). At present the nature of the peroxides formed in the autoxidation of diene conjugated methyl linoleate is not known.

Infrared Spectral Analysis. The infrared spectra from about 10 to 11 μ of samples of the methyl linoleate hydroperoxides and methyl *cis*-9, *trans*-11-linoleate before and after oxidation are shown in Figure 6, series A and B, respectively. Examination of these results showed that, as the oxidation of these compounds progressed, *trans, trans* diene conjugated and isolated *trans* double bonds were formed at the expen of the original *cis, trans* diene conjugation. The loss of *cis, trans* diene conjugation was indicated by the diminution of the intensity of the absorption band at 10.55 μ The presence of *trans, trans* conjugation in the oxidized samples was indicated by the increase in the intensity and the shift of the 10.18 μ band relative to the band at 10.55 μ. The band at about 10.15 μ in the oxidized samples is the combined absorption of the 10.18 band of the *cis, trans* doublet and the 10.12 band for *trans, trans* diene conjugation. Thus, as *trans, trans* diene conjugation was formed, the absorption peak shifted toward a wavelength of 10.12 μ and increased in intensity relative to the band at 10.55 μ. The formation of a peak absorption at 10.33 μ was due to the production of isolated *trans* double bonds. Changes similar to these also occurred in the infrared spectra of the reduced methyl linoleate hydroperoxides as they were oxidized.

During the autoxidation of methyl *trans*-10, *trans*-12-linoleate the loss of *trans, trans* diene conjugation was accompanied by the formation of compounds containing isolated *trans* double bonds. This was evidenced by the diminution of the absorption band at 10.12 μ concurrent with the development of a band at 10.33 μ in the infrared spectra.

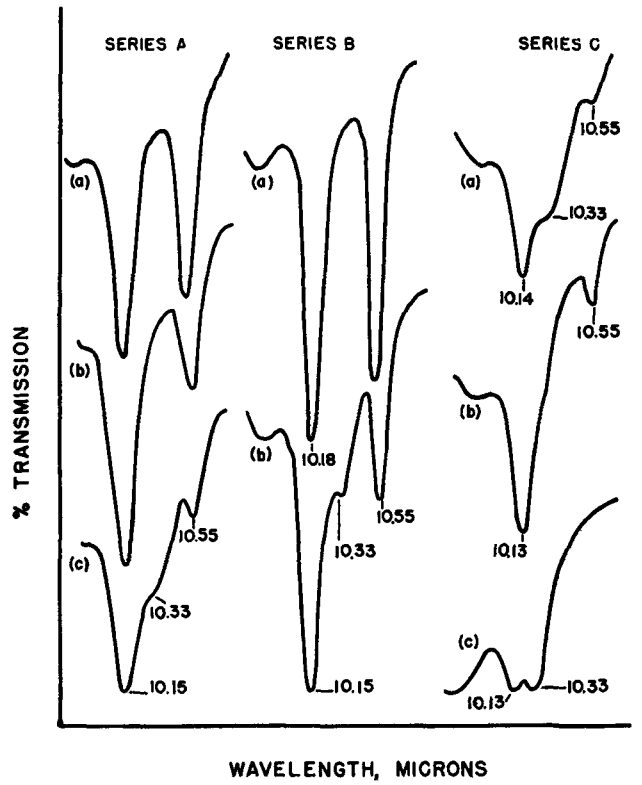


FIG. 6. Infrared spectra. Series A, methyl linoleate hydroperoxides a) before oxidation; b) 7 days exposure to air in a thin film at 0°C.; c) 42 days exposure to air in a thin film at 0°C. Series B, methyl *cis*-9, *trans*-11-linoleate, a) before oxidation; b) O_2 uptake at 50°C. of 1880 m.e./kg. Series C, methyl linoleate hydroperoxides after an O_2 uptake of 2650 m.e./kg. at 30°C., a) before fractionation; b) monomeric fraction; c) polymeric fraction.

Figure 6 also shows the infrared spectra (series C) from 10 to 11 μ of a sample of oxidized methyl linoleate hydroperoxides (O_2 uptake 2650 m.e./kg.) and its component monomeric and polymeric fractions separated by molecular distillation at 120°C. after reduction of the hydroperoxide groups with stannous chloride. Although the fractionation was not as sharp as it might have been, it was evident that the *trans, trans* diene conjugated compounds in the original oxidized hydroperoxide sample were monomeric. The isolated *trans* double bonds, on the other hand, resided in the polymers formed in the reaction.

The chemical and ultraviolet spectral analyses of this sample of methyl linoleate hydroperoxides before and after oxidation are shown in Table II. As observed previously (Table I), there was no increase in the peroxide value in spite of the fact that 2650 m.e/kg. of oxygen had been absorbed. There was also no change in the concentration of hydroxy groups formed on reduction of the peroxides. The increase in the amount of polymer in this sample corresponded roughly with the amount of oxygen absorbed on the basis that the reaction involved one mole of oxygen

per mole of hydroperoxide. Thus the primary oxidation products appeared to be polymers. This is not unexpected since polymers are believed to be the primary reaction products of the oxidation of conjugated fatty acid esters (1, 2, 11, 13). However more experimentation must be undertaken before the quantitative relationship between the amount of polymer formed and the amount of oxygen reacted can be established in the oxidation of fatty hydroperoxides.

TABLE II

Analyses of a Hydroperoxide Preparation Before and After Oxidation at 30°C.

Analysis	Before oxidation	After oxidation
	Unreduced sample	
O_2 uptake (m.e./kg.)	0	2650
Absorptivity (k_{232})	72.6	29.1
P. V. (m.e./kg.)	5540	5230
	Reduced sample	
P. V. (m.e./kg.)	180	145
Hydroxyl (%)	5.18	5.36
I. V. (hydrogenation)	146.8	111.0
% Monomer	79.5	50.6
% Polymer (residue)	20.5	49.4
Absorptivity (k_{232}) of monomer	83.9	41.6
I. V. (hydrogenation) of monomer	154.0	134.5

Generally isolated *trans* double bonds can be detected by infrared spectroscopy only in the more advanced stages of the autoxidation of methyl linoleate. Their absence however does not preclude the presence of polymers because the high general absorption in this region of the spectrum (10–11 μ) in the case of peroxide concentrates and the close proximity of the *trans, trans* and *cis, trans* bands along with the relatively poor sensitivity of the infrared method of analysis makes it virtually impossible to detect the presence of small amounts of isolated *trans* double bonds in these materials. The presence of *trans, trans* diene conjugated absorption may prove to be a more sensitive index of the presence of polymers, even though *trans, trans* conjugation itself is mostly, if not all, monomeric because its formation is associated with the secondary oxidation of the hydroperoxides.

In addition to polymers, other products were formed as evidenced by the analysis of the monomeric fraction separated by molecular distillation. Undoubtedly a certain amount of peroxide decomposition as well as other side reactions, some of which lead to the formation of volatile cleavage products, also occur during the oxidation of the linoleate hydroperoxides.

Oxidation of Hydroperoxides During the Autoxidation of Methyl Linoleate. It is evident from the foregoing studies that the total oxygen absorbed in the autoxidation of methyl linoleate consists of the oxygen consumed in the formation of hydroperoxides by the chain reaction, and the oxygen consumed in the further oxidation of the accumulated hydroperoxides. At constant temperature the rate of oxidation of the accumulated hydroperoxides depends on their concentration. Thus the amount of secondary oxidation of the hydroperoxides during the autoxidation of methyl linoleate may be obtained from a knowledge of the hydroperoxide concentration relative to the extent of the over-all oxidation. A polarographic method has been employed for making direct measurements of the concentration of hydroperoxides in mixtures of products formed in the autoxidation of fats and fatty acid esters (24, 25). Lacking the facilities of such a method an indirect method to arrive at the approximate relationship between the hydroperoxide concentration and the extent of autoxidation of methyl linoleate was used. This consisted of integrating the relationships of a) the rate of autoxidation of methyl linoleate with respect to the extent of the reaction, and b) the rate of oxidation of the methyl linoleate hydroperoxide preparation with respect to its initial concentration. The percentage ratio of the difference between a) and b) of the integrated over-all rate of autoxidation, a), represents the percentage concentration of hydroperoxides at these levels of autoxidation on the assumption that the rates of reaction are directly proportional to the amount of product formed. The criticism of this method is that the integrated rates of oxidation of the hydroperoxides used in the calculation are slightly high because they are calculated on the basis of the extent of autoxidation rather than the true hydroperoxide concentration. However, in the initial stages of the oxidation of hydroperoxides, the rate of oxidation increases relatively slowly with respect to an increase in peroxide concentration (Figure 4). Thus the error from this source is not great. Although there are other possible sources of error as well as the uncertainty that factors not apparent in the isolated oxidation of the hydroperoxide preparation may be important in the original mixture, this method appears to give a valid relationship between the extent of autoxidation of methyl linoleate and the concentration of hydroperoxides. Using this relationship, the extent of the oxidation of accumulated hydroperoxides that took place during the autoxidation of methyl linoleate at 50°C. is shown in Figure 7. Curve B (Figure 7) represents the relationship between the rate of autoxidation of methyl linoleate and the extent of autoxidation; curve D represents the relationship between the rate of oxidation of the accumulated hydroperoxides and the extent of autoxidation, and curve C represents the percentage of the total products of the autoxidation of methyl linoleate arising from the oxidation of accumulated hydroperoxides. It is evident from these relationships that concurrent oxidation of accumulated hydroperoxides cannot be disregarded at any stage of the autoxidation of methyl linoleate.

Curve A, Figure 7, shows the relationship between the diene conjugation of the products of the autoxidation of methyl linoleate and the extent of the reaction. The decrease in the diene conjugation may be attributed to the oxidation of accumulated hydroperoxides. Since the increase in the rate of oxidation of the hydroperoxides is much faster than the increase in the rate of autoxidation, the diene conjugation would be expected to be destroyed faster than it is formed as the oxidation progresses beyond the initial stages. The results confirmed this prediction. The apparent constant relationship between the oxygen uptake and formation of diene conjugation in the early stages of the autoxidation of methyl linoleate (16) may be attributed to the fact that the rate of destruction of the diene over this stage of the reaction parallels its formation. This was indicated by the parallel in the relative rates of the oxidation of the accumulated hydroperoxides and the over-all rate of autoxidation as related to the extent of autoxidation, Figure 7, curves D and B, respectively.

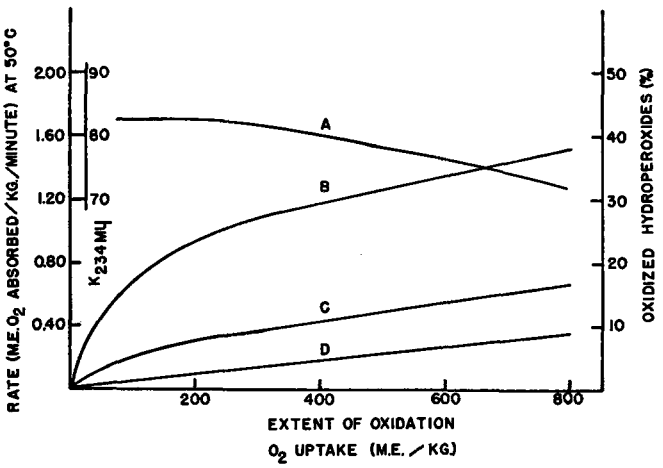


FIG. 7. Relationship between the extent of the autoxidation of methyl linoleate at 50°C. and A, k_{234} of the products of the autoxidation of methyl linoleate; B, rate of oxidation of methyl linoleate; C, percentage oxidation of accumulated hydroperoxides; D, rate of oxidation of accumulated hydroperoxides.

Summary

1. Kinetic studies showed that concurrent oxidation of preformed hydroperoxides may be expected to take place at all stages of the autoxidation of methyl linoleate. The rate of oxidation relative to the rate of autoxidation of unoxidized ester is determined chiefly by the extent of the accumulation of hydroperoxides.

2. Infrared spectral analysis of hydroperoxides oxidized to various degrees indicated that *trans, trans* diene conjugation and isolated *trans* double bonds produced in the autoxidation of methyl linoleate are related to the concurrent oxidation of the accumulated hydroperoxides.

3. The low absorptivity observed for diene conjugation, compared to that which may be expected for the exclusive production of *cis, trans* diene conjugated hydroperoxide isomers during the autoxidation of methyl linoleate is attributed to the concurrent oxidation of accumulated hydroperoxides.

4. The effect of antioxidants in giving a well-defined induction period in the oxidation of hydroperoxides isolated from autoxidized methyl linoleate indicated that the oxidation proceeds by a chain reaction.

5. The primary reaction products of the oxidation of hydroperoxides isolated from autoxidized methyl linoleate were found to be polymers formed in a sequence of reaction involving the diene conjugation.

6. Studies on the autoxidation of methyl *cis*-9, *trans*-11-linoleate showed that *cis, trans* isomerization of the conjugated diene took place with the concurrent production of isolated *trans* double bonds and loss of diene conjugation.

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Viscometric Properties of Higher Fatty Acids and Their Derivatives[1]

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VISCOSITY is the internal friction or resistance to flow displayed by a liquid. One of the most important properties of liquids, it is determined by the size and shape of the molecules of the liquid and by the nature and magnitude of intermolecular forces in the liquid. These factors are, in turn, uniquely determined by the chemical and physical structure of the molecules of the liquid, *i.e.*, chain length, the presence or absence of side chains, the nature of polar groups in the molecule, the existence of hydrogen bonding, and the tendency of the molecules to be linear or coiled, rigid or flexible.

The absolute viscosity of a liquid (expressed as poises) can be calculated from the volume of liquid per unit time that flows under a given pressure differential through a capillary tube of radius r and length l. The equation connecting these variables, known as Poiseuille's Law, is

$$\frac{v}{t} = \frac{\Delta p \pi r^4}{8\eta l} \qquad (1)$$

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