THE OXIDATION OF SOLUBILISED AND EMULSIFIED OILS

PART III. THE OXIDATION OF METHYL LINOLEATE IN POTASSIUM LAURATE AND CETOMACROGOL DISPERSIONS

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Oxidation rates of aqueous dispersions of methyl linoleate emulsified and solubilised by potassium laurate and cetomacrogol, have been measured manometrically at 40°. Emulsions of methyl linoleate oxidised at a greater rate than solutions. The relation between concentration of surface-active agent and oxidation rate of emulsions is complex, and appears to depend on the relative amounts of emulsified and solubilised oil present. Poor agreement was obtained between oxidation rates calculated from peroxide values, chemical and polarographic, and those calculated from oxygen uptake. The results are discussed. An increase in light absorption at 234 mμ in the early stages of oxidation was observed. An uptake of 2·5 mol. oxygen per mol. of methyl linoleate was estimated to occur during the primary oxidation reaction in the presence of cetomacrogol, and 1·5 mol. oxygen per mol. of methyl linoleate in potassium laurate dispersions. The possible mechanism of the reaction and the nature of the oxidation by-products are discussed.

THE use of aqueous solutions of surface-active agents for the solubilisation of essential oils, steroids and oil soluble vitamins, is becoming of increasing importance. The stability of solubilised vitamin A has been reported by Kern and Antoshkiw[1] and Coles and Thomas[2] to be greater than the corresponding oily solution. In our previous paper[3] we reported the stability of benzaldehyde solubilised with potassium laurate and cetomacrogol. Solubilised benzaldehyde was found to be more resistant to atmospheric oxidation than emulsified benzaldehyde.

The autoxidation of methyl linoleate in the absence of water has been extensively studied and detailed mechanisms of oxidation have been suggested[4]. The primary products of autoxidation are conjugated hydroperoxides. The formation of these conjugated products can be detected by spectrophotometry since they show characteristic absorptions in the 230–236 mμ region. An accurate estimation of the amount of conjugated diene peroxide is impossible unless the relative proportions of the geometric isomers are known, since the *cis-trans* and *trans-trans* conjugated hydroperoxides have widely different extinction coefficients.

The oxidation of fat emulsified by gelatin and soya bean phosphatides for use by intravenous alimentation has been reported by Gayer and others[5]. They found that emulsification lowered the rate of oxidation of the fat, except when gelatin was used. They suggested that possible mechanisms for the lower oxidation rate were retardation of oxygen diffusion, an outer network of groupings able to scavenge metal ions and so prevent catalysis or the prevention of an optimal spacial arrangement for oxidation of the oil. They suggested that only materials which are

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soluble in both the water and oil phases could act as a protective film. No evidence was given for the mechanisms suggested.

The rate of oxidation of irradiated methyl linoleate emulsions has been estimated[6] from chemical peroxide values and the conjugated diene content. The experimental details for this latter method were not specified and an attempt to correlate the degree of oxidation obtained from the two values showed wide discrepancies. The presence of up to 50 per cent water has been shown to retard the onset of autoxidation, but not the final rate attained[7] and a peroxide differing from the normal conjugated hydroperoxide has been reported by Bergstrøm and others[8] for the oxidation of sodium linoleate in water.

In this paper it is shown that emulsions of methyl linoleate oxidise at a greater rate than solutions. The ratio of emulsified methyl linoleate to solubilised methyl linoleate appears to play a predominant role in determining the rate of oxidation.

The solubility of methyl linoleate in potassium laurate and cetomacrogol solutions is reported elsewhere[9].

EXPERIMENTAL

Materials

Methyl linoleate was obtained from the Hormel Institute. The stated characters of this material were as follows. Wij's iodine number was 261·4. The theoretical value is 260·4. Infra-red analysis showed the compound to be completely *cis-cis* in character. Conjugated constituents, as methyl esters of C_{18} fatty acids per cent were; dienoic, less than 0·05, trienoic, a trace; tetraenoic, a trace. Peroxide was absent.

Surface-active agents. The potassium laurate and cetomacrogol are those used and described in previous studies[3].

Methods

Preparation of dispersions. Potassium laurate dispersions were prepared by dissolving the methyl linoleate in concentrated solutions of the soap and then diluting with water and catalyst solution to produce the required concentration. Dispersions in cetomacrogol solutions were prepared by adding the methyl linoleate to the molten cetomacrogol and shaking to disperse, before adding the water and catalyst solutions.

Measurement of oxygen uptake. The oxygen uptake was measured at 40° in the Warburg apparatus using the technique previously described[3].

Measurement of iodometric peroxide values. This was based on the method of Boehm and Williams[10] modified as follows. 1 ml. of the oxidising dispersion was added to 10 ml. of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform in a glass stoppered flask, the atmosphere of which was replaced by nitrogen. 1 ml. of a saturated solution of potassium iodide was added and the flask placed in the dark for 10 minutes. The solution was then titrated with 0·005 N sodium thiosulphate solution using 1 ml. of a 1 per cent starch solution as indicator. Blank determinations on the reagents were also made. The

results were calculated as milli-equivalents of oxygen per kg. of methyl linoleate.

Measurement of polarographic peroxide values. 1 ml. of the oxidising dispersion was diluted with 4 ml. of lithium chloride:lithium acetate buffer solution[11] and made up to 10 ml. with 96 per cent ethanol. 3 ml. of this solution was placed in the polarographic cell and the remainder

Fig. 1. The oxidation of dispersions of methyl linoleate in potassium laurate solutions at 40°. Methyl linoleate, 3·2 mg./ml. Catalyst 1×10^{-5} M $CuSO_4$. Potassium laurate: A = 0; B = 0·01; C = 0·04; D = 0·07; E = 0·1; F = 0·2; G = 0·6; H = 0·8 molar.

.... Suspension. —— Emulsions. ––– Solutions.

in the second of two wash bottles, the first one of which contained 96 per cent ethanol. The cell and wash bottles were connected, placed in a water bath at 20° ± 0·1° and nitrogen passed through the cell for 10 minutes to deoxygenate. The current voltage curve between 0 and −2 volts was measured with a manual polarograph (Electrochemical Laboratories) and the polarographic peroxide value was calculated from the formula given by Willits and others[11].

Measurement of ultra-violet absorption spectra. A Unicam SP500 spectrophotometer modified by Morton[13] was used for all ultra-violet determinations. 1 ml. samples were diluted with water, or in the potassium laurate dispersions, absolute ethanol to give a suitable solution. The results were plotted as E (1 per cent, 1 cm.).

<div align="center">RESULTS</div>

Oxygen Uptake

The uptake of oxygen by dispersions of methyl linoleate is shown in Figures 1 and 2. Figure 1 represents the results of two series of experiments. During the overnight periods the taps of the Warburg apparatus were left open so that the total uptake was unknown, and only the rate of oxidation could be measured after the first day.
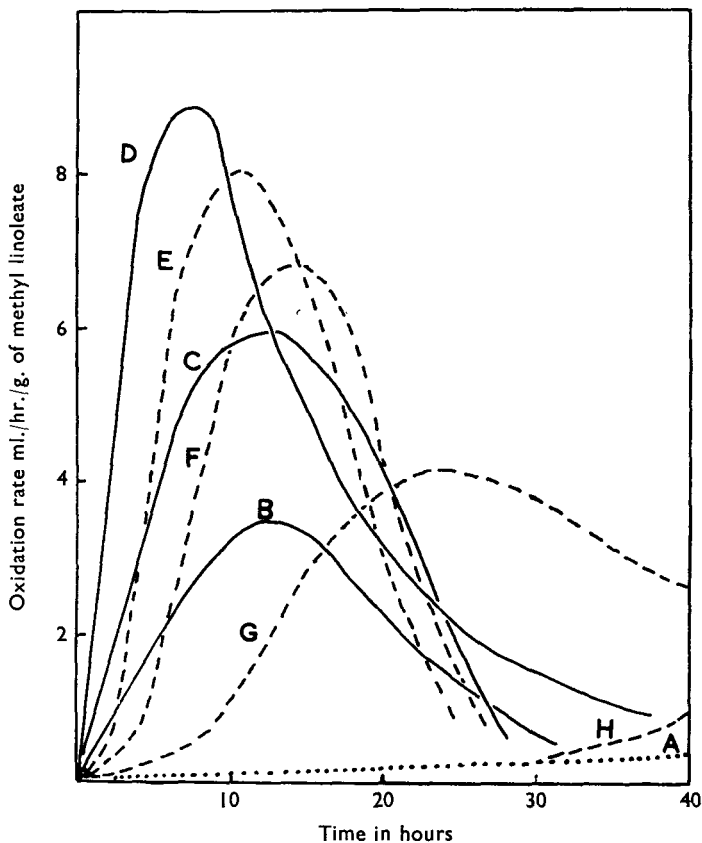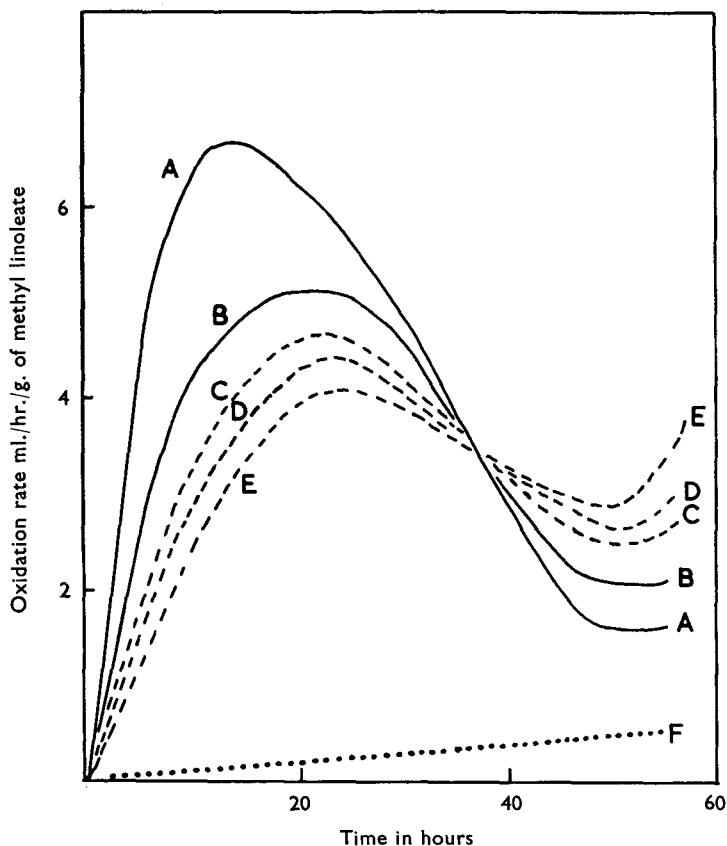

FIG. 2. The oxidation of dispersions of methyl linoleate in ceto-macrogol solutions at 40°. Methyl linoleate, 2·43 mg./ml. Catalyst 1×10^{-5} M $CuSO_4$. Cetomacrogol: A = 0·01; B = 0·03; C = 0·047; D = 0·065; E = 0·09; F = 0 molar.

.... Suspension. —— Emulsions. — — — Solutions.

Suspensions of methyl linoleate in water in the absence of surface-active agents oxidised at a very low rate. The emulsions formed by the addition of potassium laurate oxidised at a higher rate, which increased with soap concentration. When sufficient potassium laurate was added to solubilise the oil, the rate of oxidation fell. Reference to Figure 1 shows the pattern of oxidation rates as the oil is emulsified or solubilised with different amounts of potassium laurate. It can be seen that a reduction in the concentration of soap in the solutions resulted in a decreased induction period and a higher maximum rate of oxidation. Emulsions showed no induction period.

In cetomacrogol dispersions the maximum rate of oxidation fell with increasing soap concentration irrespective of the state of the dispersion (Fig. 2). Solutions showed no marked induction period as did the potassium laurate dispersions.

It also appears from Figure 2 that a second reaction takes place in cetomacrogol dispersions, although a noticeable velocity is only reached as the rate of the primary reaction decreases to a low value. The secondary reaction was more rapid in the solubilised systems.

The concentration of potassium laurate was maintained at 0·32M and that of cetomacrogol at 0·065M whilst studying the effect of methyl linoleate concentration on the oxidation rate. The rate of oxidation of all the dispersions passed through a maximum. The oxidation rates after four hours showed a sigmoid curve, the higher rates occurring with emulsions of methyl linoleate. In cetomacrogol systems it was found that concentrations of methyl linoleate greater than 9 mg./ml. resulted in a lower oxidation rate per gram of oil probably because of diffusion of oxygen becoming a limiting factor. When the concentration of oil was reduced to 0·19 mg./ml the oxidation rate was found to be 48 ml./hour/g. of methyl linoleate, but a tenfold increase in linoleate concentration caused a fall of oxidation rate to only 5 ml./hour/g. The rapid oxidation of dilute solutions of olefinic materials has been previously reported[14] and it was suggested that it proceeded by a mechanism differing from the normal chain reaction.

Methyl linoleate added directly to dilute cetomacrogol solutions formed an emulsion and no solubilisation could be detected even after several months of storage. It was found that these dispersions oxidised at a rate of 1·95 ml./hour/g. of methyl linoleate irrespective of the concentration of cetomacrogol used, over the range 0·02 to 0·08M. Thus in the

TABLE I
THE TOTAL OXYGEN UPTAKE DURING THE PRIMARY OXIDATION
REACTION OF METHYL LINOLEATE IN CETOMACROGOL

Concentration of cetomacrogol M	Concentration of methyl linoleate M	State of dispersion	Mol. of oxygen/ mol. methyl linoleate
0·01	2·40	Emulsion	2·80
0·03	2·44	Emulsion	2·57
0·047	2·44	Solution	2·50
0·065	2·43	Solution	2·43
0·09	2·44	Solution	2·48

absence of solubilised oil the amount of soap does not affect the oxidation rate of these emulsions.

The estimation of total uptake of oxygen by methyl linoleate was obtained graphically from Figures 1 and 2, by measuring the areas under the curves, after extrapolation of the curves to the time axis. This method would tend to overestimate the amount of oxygen used in the primary reaction occurring in cetomacrogol dispersions, due to the overlap of the secondary reaction; a total uptake of approximately 2·5 mol. of

TABLE II

THE INFLUENCE OF CATALYST CONCENTRATION ON THE RATE OF OXIDATION
OF EMULSIONS AND SOLUTIONS OF METHYL LINOLEATE

Soap	Concentration of soap M	Concentration of methyl linoleate M	Concentration of copper sulphate M	State of dispersion	Oxidation rate* ml./hr./g.
Cetomacrogol	0·045	0·0085	0 1 × 10⁻⁶ 1 × 10⁻⁵ 5 × 10⁻⁵ 1 × 10⁻⁴	Solution	0·5 0·7 0·8 0·9 1·0
Cetomacrogol	0·046	0·0165	0 1 × 10⁻⁶ 1 × 10⁻⁵ 5 × 10⁻⁵ 1 × 10⁻⁴	Emulsion	4·2 6·1 6·6 7·3 7·5
Potassium laurate	0·32	0·0211	0 1 × 10⁻⁶ 1 × 10⁻⁵ 5 × 10⁻⁵ 1 × 10⁻⁴	Solution	4·2 4·7 4·8 4·9 4·2
Potassium laurate	0·32	0·0394	0 1 × 10⁻⁶ 1 × 10⁻⁵ 5 × 10⁻⁵ 1 × 10⁻⁴	Emulsion	9·2 9·7 10·1 10·8 11·8

* Measured after four hours.

oxygen per mol. of methyl linoleate was found (see Table I). In potassium laurate dispersions where no secondary reaction interferes, approximately 1·5 mol. of oxygen per mol. of methyl linoleate was found. Bergstrøm and others[8] reported finding a total uptake of 2 mol. of oxygen per mol. of linoleate with aqueous solutions of sodium linoleate.

Table II shows the effect of copper catalyst concentration on the oxidation of emulsions; the rate being measured after four hours. Increases in catalyst concentration produced only a relatively small increase in the rate of oxidation.

Peroxide Values

The iodometric and polarographic peroxide values of the emulsions and solutions of methyl linoleate appear in Table III, together with the corresponding values of the oxygen uptakes of the systems. There was no apparent correlation of either of these two peroxide values and the oxygen uptake. In a further series of experiments, Table IV, of longer duration, the iodometric peroxide value reached a maximum and then began to fall, although the oxidation rate continued to increase; a similar

TABLE III

CHANGES IN THE PEROXIDE VALUE DURING THE OXIDATION OF EMULSIONS AND SOLUTIONS OF METHYL LINOLEATE

Soap	Concentration of soap M	Concentration of methyl linoleate M	State of dispersion	Time hours	Oxygen uptake mol. O_2/ml. linoleate	Chemical peroxide value m-equiv. O_2/kg. linoleate	Calculated oxygen uptake from chemical peroxide value mol. O_2/mol. linoleate	Polarographic peroxide value m-equiv. O_2/kg. linoleate	Calculated oxygen uptake from polarograph peroxide value mol. O_2/mol. linoleate
Potassium laurate	0·08	0·0096	Emulsion	0	0	30	0·002	10	0·000
				10	0·72	270	0·019	280	0·020
				25	1·69	410	0·030	510	0·037
				36	2·48	480	0·035	870	0·064
Potassium laurate	0·20	0·0105	Solution	0	0	30	0·002	10	0·000
				10	0·46	280	0·021	150	0·011
				25	1·12	350	0·026	440	0·033
				36	1·41	390	0·028	480	0·035
Cetomacrogol	0·02	0·0093	Emulsion	0	0	20	0·001	0	0·000
				10	0·36	1,200	0·088	270	0·019
				25	0·99	5,200	0·383	680	0·050
				36	1·45	5,960	0·439	590	0·044
Cetomacrogol	0·06	0·0091	Solution	0	0	30	0·002	10	0·000
				10	0·23	1,140	0·084	240	0·017
				25	0·58	3,210	0·237	470	0·035
				36	0·83	4,870	0·358	890	0·066

TABLE IV

CHANGES IN THE IODOMETRIC PEROXIDE VALUE OF METHYL LINOLEATE OXIDISING
IN POTASSIUM LAURATE AND CETOMACROGOL DISPERSIONS

Soap	Concentration of soap M	Concentration of methyl linoleate mg./ml.	State of dispersions	Time hours	Oxidation rate ml./hr./g.	Peroxide value m-equiv./kg.
Potassium laurate	0·2	3·10	Solution	0 3 9 27	0 0·3 1·6 4·6	20 220 410 360
	0·04	3·16	Emulsion	0 2 12 27	0 1·6 5·8 4·2	10 280 410 380
Cetomacrogol	0·06	2·54	Solution	0 35 82 99	0 4·1 5·4 6·5	0 3860 6750 6270
	0·02	2·57	Emulsion	0 27 53 70	0 3·7 4·9 7·2	0 5130 6270 4930

FIG. 3. Polarogram at 20° of oxidising methyl linoleate.

—— Cetomacrogol dispersions.

Cell concentration of methyl linoleate 0·32 mg./ml.
Cell concentration of cetomacrogol 0·006 M.
Oxygen uptake ml./g. of methyl linoleate:A = 0;
B = 4; C = 17; D = 38; E = 61.

- - - - Potassium laurate dispersions.

Cell concentration of methyl linoleate 0·46 mg./ml.
Cell concentration of potassium laurate 0·01 M.
Oxygen uptake ml./g. of methyl linoleate: A = 0;
F = 48; G = 86.

sequence of events occurs during the oxidation of anhydrous methyl linoleate[4].

Comparing the chemical with the polarographic peroxide values the following points are evident. In the potassium laurate systems, the polarographic values are higher than the iodometric, whilst in the ceto-macrogol systems the iodometric values were from 5 to 8 times greater than the polarographic values.
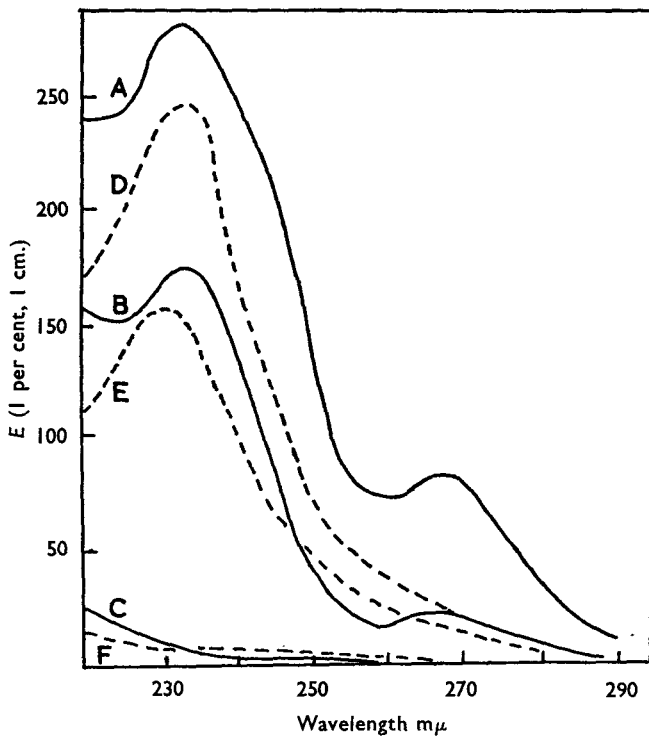
FIG. 4. The ultra-violet absorption spectrum of oxidising methyl linoleate. Oxygen uptake ml./g.: A = 138; B = 58; C = 0; D = 111; E = 33; F = 0.

—— Oxidised potassium laurate dispersions.
- - - - Oxidised cetomacrogol dispersions.

The polarographic waves of the oxidised methyl linoleate in potassium laurate and cetomacrogol differed from one another. In cetomacrogol dispersions two steps in the wave were observed, half-wave potential ($E_{\frac{1}{2}}$) of the first step was —0·04 volts and of the second step, which began at —0·35 volts, the $E_{\frac{1}{2}}$ was —0·55. This second step would appear to be due to the hydroperoxide although it is lower than previously reported values[12,15]. It does however come within the range of $E_{\frac{1}{2}}$ for hydroperoxides found by Willits and others[15]. The step, $E_{\frac{1}{2}}$ —0·04 volts corresponds with the small wave found by Willits and others[12], who suggested that it was due to a small amount of some other unidentified

peroxide. Kalbag and others[17] have also reported finding a similar preliminary wave. Typical polarographic curves appear in Figure 3.

In potassium laurate dispersions the polarographic wave had an $E_{\frac{1}{2}}$ of -0.82, which agreed closely with the published $E_{\frac{1}{2}}$ of methyl linoleate[12,15].

Ultra-violet Absorption Spectra

The formation of conjugated material during the oxidation of the methyl linoleate dispersions was detected by the appearance of an absorption peak at 233–234 mμ (Fig. 4). In potassium laurate dispersions, at higher oxygen uptakes, an absorption peak in the region of 265 mμ was also found. This indicates the possible formation of a diketone such as is normally found as a secondary reaction product[16]. This peak did not appear in the cetomacrogol dispersions. Diketones give intense yellow coloration in alkaline solution and it was noted that potassium laurate emulsions became yellow on oxidation, which also suggests that ketone formation was occurring.

DISCUSSION

The oxidation of methyl linoleate proceeds by a chain reaction in which the free radicals produced by oxygen attack on the α-methylene group propagates the reaction[18]. The mechanism for anhydrous methyl linoleate oxidation may be summarised as follows.

$$
\begin{array}{lll}
RH \; + \; O_2 & \longrightarrow & RO_2\cdot + H \quad \text{Initiation} \\
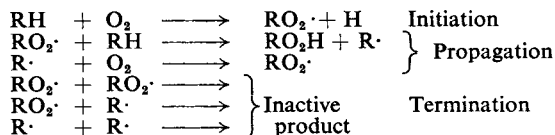RO_2\cdot \; + \; RH & \longrightarrow & RO_2H + R\cdot \; \left.\right\} \; \text{Propagation} \\
R\cdot \; + \; O_2 & \longrightarrow & RO_2\cdot \\
RO_2\cdot \; + \; RO_2\cdot & \longrightarrow & \left.\right\} \\
RO_2\cdot \; + \; R\cdot & \longrightarrow & \left.\right\} \text{Inactive} \quad \text{Termination} \\
R\cdot \; + \; R\cdot & \longrightarrow & \left.\right\} \text{product}
\end{array}
$$

The addition of copper catalyses the reaction by breaking down the RO_2H into further free radicals $RO\cdot$ and $RO_2\cdot$[18]. A similar chain reaction for benzaldehyde has been reported in our previous paper[3].

In all examples studied the rate of oxidation of methyl linoleate dispersed in soap solutions was higher than for suspensions in water. The relation between oxidation rate and soap concentration is a complex one as it appears that the proportion of solubilised to emulsified methyl linoleate greatly influences the rate. In suspensions there is no dissolved oil in the aqueous phase but when soap is included to form an emulsion some of the methyl linoleate will be solubilised. The solubilised methyl linoleate appears to increase the rate of oxidation. When sufficient soap is present to solubilise all the methyl linoleate then the rate of oxidation is below that of the emulsion. This shows that oxidation takes place most rapidly in the emulsion droplets although the need for solubilised oil to be present can be inferred from the above results.

It is suggested that the oxidation initiation stage commences in the micelle and the free radicals formed are transferred to the emulsion droplet where propagation is fast due to the large number of oil molecules available for oxidation. The slower rate of oxidation of micellar material could be due to the small number of methyl linoleate molecules resulting

in a greater chance of radical-radical termination reactions. In the drop-
let more methyl linoleate is available for oxidation once the reaction has
been initiated and the chance of radical-radical termination would be
reduced because of the relatively large number of oxidisable molecules
in the droplet.

Because of the very small quantities of methyl linoleate present it was
found impossible to isolate any of the reaction products. In both surface-
active agents the formation of conjugated material having a peak density
of 234 mμ indicates that the initial radical formation occurs by reactions
similar to the anhydrous oxidation[4]. The height of this peak does not
parallel the oxygen uptake after the initial stages of oxidation, and in
cetomacrogol dispersions decreases in intensity with time, showing that
the conjugated system is being broken down. In potassium laurate dis-
persions, the peak at 265 mμ indicates the possible formation of a di-
ketone which is normally found as a secondary reaction product[16]. Its
presence is unlikely to be detected by the polarographic method since
diketones have polarographic waves with a similar $E_{\frac{1}{2}}$ to hydro-
peroxides[15]. The iodometric peroxide values of methyl linoleate in ceto-
macrogol are the same as those formed during the oxidation of the
anhydrous oil[12]. The large difference between the high iodometric value
and the polarographic value could be due to the presence of a peroxide
form which is not polarographically reducible or which possesses a
polarographic wave with markedly different characteristics from that
of methyl linoleate hydroperoxide. Willits and other[15] have shown
that ether linked peroxides have a half-wave potential of lower than
-0.2 volts which enables one to differentiate between this type of
peroxide and hydroperoxides which have a half-wave potential of not
lower than -0.5 volts. The preliminary reduction wave in the polaro-
grams of oxidising methyl linoleate in cetomacrogol dispersions, is in the
region expected of the ether type of peroxides and it is possible that
a diperoxide of the type suggested by Bergstrøm and others[8] (I) is
formed. He found that sodium linoleate in solution ceased to oxidise at
an oxygen uptake of 2 molecules of oxygen per molecule of linoleate.

$$
\begin{array}{c}
CH = CH \\
\diagup \qquad \diagdown \\
-CH \qquad\qquad CH - CH- \qquad (I)\\
\diagdown \qquad \diagup \qquad | \\
O - O \qquad OOH
\end{array}
$$

This diperoxide contains both an ether-linked and a hydroperoxide
grouping. The formation of such a compound would account for the
fall in ultra-violet peak density as it possesses no conjugation. Berg-
strøm found that his oxidising dispersions exhibited a maximum ultra-
violet density at an oxygen uptake of 1.0 mol. of oxygen per mol. of
linoleate and a similar observation was made in the present study. The
reaction $RH \longrightarrow RO_2H$ would require an overall oxygen uptake of
1.0 mol. of oxygen per mol. of methyl linoleate. From the data in
Figure 2 the oxygen uptake at the end of the primary reaction was approxi-
mately 2.5 mol. of oxygen per mol. of linoleate compared with the figure

of 2 found by Bergstrøm and others. It therefore appears probable that a diperoxide of the type suggested by Bergstrøm and others is formed during the oxidation of methyl linoleate dispersed in cetamacrogol. In potassium laurate dispersions an uptake of 1·5 mol. of oxygen per mol. of methyl linoleate was found. The increased oxygen uptake over and above the theoretical hydroperoxide formation is possibly due to the formation of secondary decomposition products as indicated previously.

Whilst the final products of the oxidation of emulsions and solutions of methyl linoleate appear to vary considerably from those normally found in the oxidation of the anhydrous oil, the general mechanism of the oxidation appears to be unchanged. Thus a conjugated hydroperoxide appears to be the initial oxidation product as in the oxidation of the anhydrous oil, but in emulsions and solutions it is rapidly broken down to secondary products.

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