findings were in agreement with the theory of rust prevention which postulated orientation of the polar additives at the oil-metal interface and establishment thereby of a barrier to normal mode of entry of the causative agents of rusting, oxygen, and water.

The communications on oil treating of leather were brief treatises on essential factors for water repellency of chrome leather (Pressley—*J. Intern. Soc. Leather Trades' Chem. 30,* 94), fat-liquoring side leather (Meyers—*J. Am. Leather Chem. Assoc. 41,* 427), and uses of fatty materials in the tannery (Meunier—*Bull. assoc, francaise ind. cuir 1943,* No. 17, 321). A patented leather preservative contained fatty oils or fat acids and triaryl phosphates (Benischek—*Ger. 741,056*). A paper on leather polishes gave the analyses of 13 oil types, eight oil-in-water emulsions, three water-in-oil emulsions, two solid without oil, and three solid with oil (Herfeld *et al.—Fette u. Seifen 50,* 576). A general service polishing wax contained wax, oleic acid, morpholine, and a nonsoap detergent (Wassell—*U. S. 2,395,025*).

A miscellaneous group of unrelated fat products was difficult to classify in the preceding paragraphs. A composition for dielectric usage comprised hydrogenated castor oil and an aromatic sulfone (Clark—*U. S. 2,410,715*). Fat acids, recovered from sulfuric acid treated vegetable oil refining foots, served as defoamers in the sugar industry (Larsen—*U. S. 2,412,276*). Cottonseed oil phosphatides were used as spreaders for insecticides (Thurman—*U. S. 2,407,041*). The active ingredient of an insecticide was 2-ethyl-*n*-caproic acid (Jones & Travis—*U. S. 2,396,012*). A flushing liquid for cleansing internal-combustion engines contained light spindle oil, a detergent, fat acids, and sulfurized wool fat (Burk—*U. S. 2,403,169*); another product for the same purpose contained various organic solvents, mineral oil, water, morpholine oleate, and oleic acid (Skinner—*U. S. 2,403,618-9*). A cloth for metal polishing was impregnated with oleic acid, triethanolamine, and powdered abrasive (Morgan & Lowe—*U. S. 2,403,821*).

The Rates of Oxidation of Unsaturated Fatty Acids and Esters *

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ALTHOUGH several studies of the rates of oxidation of fatty acids and their esters have been reported (1), most of these studies concern themselves with small groups of purified substrates or even a single substance. Moreover, data from these studies cannot be directly compared because of the widely different conditions under which they were made. Inasmuch as several fatty acids and esters were available to us and many of these substances have not been studied in this regard, it was thought that a comparative study of the rates of oxidation of a series of pure acids and esters would be of sufficient interest to merit attention. It is to be regretted that some acids and esters were not available, making certain comparisons difficult, and that time did not permit the preparation of missing isomers or homologs before the fatty acid research program was terminated at this laboratory. However, the information gained from the present study throws some light upon the effects of fatty acid composition upon the rate of oxidation.

Experimental

The oleic acid and ethyl oleate used in this study were prepared by F. Greenwood by low-temperature crystallization methods. The linoleic acid, ethyl linoleate, linolenic acid, ethyl linolenate, and methyl arachidonate were prepared through their respective bromides. Trilinolein, trilinolenin, pentaerythritol linoleate, and dipentaerythritol linoleate were prepared by a modification of Wheeler's method (2). The preparation and properties of these latter substances is reported elsewhere (3). Elaidolinolenic acid was prepared by J. P. Kass (4).

The 10,12-linoleic acid was prepared by alkaline isomerization of ethyl linoleate (5). To 300 ml. ethylene glycol containing 50 g. potassium hydroxide at 180° 27 g. of ethyl linoleate was added with stirring. The temperature was maintained at 170-180° for 30 minutes when the mixture was poured into about five volumes of ice and water. The soaps were acidified and the mixture chilled to yield a solid cake of fatty acids which was removed and recrystallized twice from alcohol and twice from petroleum ether at —40°. The resulting product melted at 7.0-7.5° (uncorrected) and had a molar extinction coefficient of 28,600 at 2330 Å in methanol.

The properties of the substances used in this study are presented in Table I.

The autoxidation of the acids and esters was carried out in air at 37° upon small samples in Warburg respirometer vessels of 10-12 sq. cm. bottom area as described previously (6). The rates of enzymatic oxidation of ethyl linoleate, ethyl linolenate, and methyl arachidonate were determined in systems described elsewhere (7). The temperature 13° was chosen be-

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TABLE I.

Substance	M. P. °C.	M. P. of Bromide	I. V.	P. V.	Acetyl No.	% Diene Conj.	% Triene Conj.
Oleic acid	12.5	.....	.....	0	.....	0.14	0.008
Ethyl oleate	.....	.....	80.4	.....	.....	0.49	0.016
10,12 Linoleic acid	7.0-7.5	.....	.....	.....	.....	.....	.....
Ethyl linoleate	.....	.....	160.6	.....	.....	0.4	0.12
Trilinolein	—11.0	81.0	173.0	0	0	0.13	0.06
Pentaerythritol linoleate	—24.5	.....	167.1	.....	0	1.6	0.08
Dipentaerythritol linoleate	.....	.....	161.1	.....	0	.....	.....
Linolenic acid	—10.6	185.5*	268.0	3.16	.....	.....	.....
Ethyl linolenate	.....	.....	243.7	.....	.....	0.54	0.08
Elaidolinolenic acid	27-8	.....	.....	.....	.....	0.17	0.02
Trilinolenin	.....	149.0	255.0	0	0	0.06	0.01
Methyl arachidonate	.....	.....	316.5	.....	.....	10.0	0.10

* These bromides were used for the preparation of the acids used in this study.

cause lipoxidase in the extracts used is rapidly inactivated during the reaction at higher temperatures.

Results and Conclusions

The course of the autoxidation of the family of unsaturated fatty esters ethyl oleate, ethyl linoleate, ethyl linolenate, and methyl arachidonate is shown in Fig. 1. It will be seen that as the unsaturation increases the maximum rate of autoxidation increases markedly. The rate of oxidation of ethyl oleate observed during the duration of the experiment is probably not the maximum rate of oxidation, for it will be noticed that the maximum rate of oxidation of the other substrates is not attained at such low degrees of oxidation. The maximum rate of oxidation of ethyl linolenate is seen to be 2.4 times that of ethyl linoleate, and the rate of oxidation of methyl arachidonate is about twice that of ethyl linolenate. The rate of oxidation of linolenic acid (Table 2) was 2.8 times that of linoleic acid, and the rate of oxidation of trilinolenin was 2.4 times that of trilinolein. In general then, it may be said that the introduction of one additional double bond into a fatty acid at least doubles the rate of oxidation of the fatty acid or its esters. It will be seen then that the rate of oxidation of a fat depends upon the composition of the fat and not merely upon its total unsaturation. This has been strikingly demonstrated in another way by Gunstone and Hilditch (8).

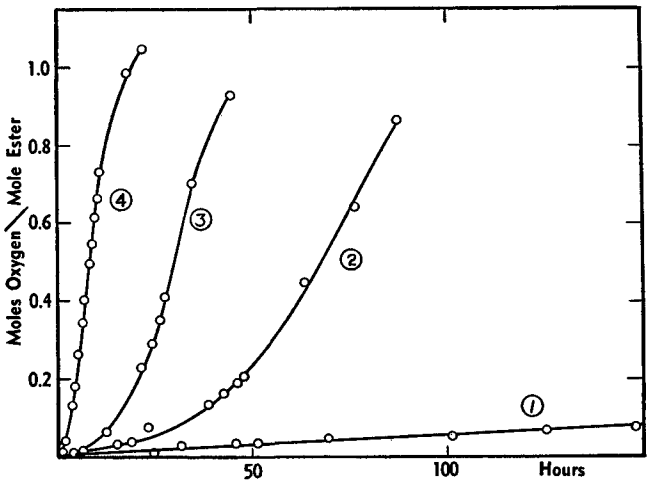

FIG. 1. Autoxidation of fatty esters in air at 37°C.

1.) Ethyl oleate
2.) Ethyl linoleate
3.) Ethyl linolenate
4.) Methyl arachidonate

TABLE II.

The Maximum Rates of Oxidation of Acids and Esters

Substance	Moles O_2 per Acid Equiv. per 100 hr.	Average Sample
		mg.
Oleic acid	0.08	45
Ethyl oleate*	0.04	58
10,12-linoleic acid	2.21	78
Linoleic acid	2.20	91
Ethyl linoleate*	1.63	60
Trilinolein	1.99	69
Pentaerythritol linoleate	2.41	84
Dipentaerythritol linoleate	1.72	77
Elaidolinolenic acid	5.32	43
Linolenic acid	6.18	67
Ethyl linolenate*	3.90	52
Trilinolenin	4.83	84
Methyl arachidonate*	7.78	48

* Shown in Fig. 1.

The average maximum rate of oxidation is listed with the average sample size for each of the substances in Table II. With the exception of dipentaerythritol linoleate all values are averages of two or more samples.

The rate of oxidation of conjugated linoleic acid was not perceptibly different from that of linoleic acid itself, indicating that the presence of the second double bond in a fatty acid has the same activating effect upon the oxidation of the first double bond whether the two are conjugated or in the methylene-interrupted position. This is not the case with the group of triene acids studied by Myers, *et al.* (9), who found that conjugated trienes oxidized at a greater rate than the natural isomer. This seeming lack of agreement will bear further study.

The rates of oxidation of linoleic and linolenic acids were greater than the corresponding esters. This is in agreement with the data of Myers, *et al.*, who showed that both the conjugated and natural isomers of linolenic acid oxidized more rapidly than the esters. The relationship between the rates of oxidation of linoleic acid and its glyceryl, pentaerythrityl, and dipentaerythrityl esters is not so clear, for the glyceryl and dipentaerythrityl esters oxidized more slowly, and the pentaerythrityl esters more rapidly, than did the acid itself. The range of rates of oxidation of the linoleates does not overlap with that of the linolenates.

Elaidolinolenic acid was found to oxidize somewhat more slowly than natural linolenic acid itself, although its rate of oxidation fell within the range of the rates of the other linolenates. Unfortunately only one elaido acid was available for study and little can be concluded from this isolated study concerning the effect of cis-trans isomerism upon the rate of oxidation.

The oxygen uptake during lipoxidase oxidation of ethyl linoleate, ethyl linolenate, and methyl arachidonate is shown in Fig. 2. It will be seen that the

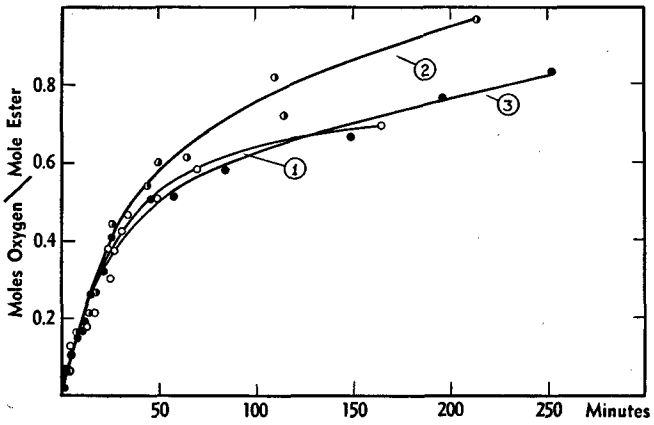

FIG. 2. Lipoxidase oxidation of fatty esters at 13°C.
1.) Ethyl linoleate
2.) Ethyl linolenate
3.) Methyl arachidonate

rates of oxygen uptake by these three lipoxidase substrates during the early stages of oxidation are strikingly similar. This is in marked contrast to the widely different rates of oxidation of these same esters by atmospheric oxygen. It appears that the autoxidation of a double bond of a fatty acid is activated by the proximity of another double bond; whereas the enzymatic oxidation of the methylene-interrupted diene system is not so affected. Unfortunately the data collected on the autoxidation of the acids and esters will not allow an estimation of the number of moles of oxygen a fatty acid can ultimately absorb, but from the data it was clear that many of the substances absorb more than one mole of oxygen per equivalent of acid. However, the enzymatic oxidation of the esters appears to be limited to one mole of oxygen per mole of acid. From previous studies it is clear that the enzymatic oxidation and autoxidation of these fatty esters are very similar in character, leading to the same products (7, 10). It is probable that the enzymatic oxidation is merely the intensively activated oxidation of one of the double bonds in a methylene-interrupted multiple unsaturated system.

Summary

The rates of autoxidation of oleic acid, ethyl oleate, linoleic acid, 10,12-linoleic acid, ethyl linoleate, trilinolein, pentaerythritol linoleate, dipentaerythritol linoleate, elaidolinolenic acid, linolenic acid, ethyl linolenate, trilinolenin, and methyl arachidonate have been studied by oxygen uptake in a Warburg respirometer and the results are compared with the rates of enzymatic oxidation of lipoxidase substrates.

The increase in the number of double bonds in a fatty acid by one increases the rate of oxidation of the fatty acid or its esters by at least a factor or two.

Earlier findings that acids oxidize more rapidly than their esters have been confirmed.

The initial rates of lipoxidase oxidation of ethyl linoleate, ethyl linolenate, and methyl arachidonate were found to be essentially the same.

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