TABLE I.

MINIMUM, MAXIMUM AND AVERAGE CAROTENE CONTENT OF BUTTERFAT SAMPLES WHICH MATCHED IN COLOR VARIOUS CONCENTRATIONS OF POTASSIUM DICHROMATE SOLUTION.

Stock dichromate solution (0.2 per cent) in dilute standard	Strength of dilute dichromate standard	Number of samples	Carotene content micrograms per gram of fat			
			Minimum*	Maximum*	Average	Calculated average**
ml.	per cent					
2	0.016	13	0.10	0.40	0.23	0.23
3	.024	25	0.40	1.15	0.60	0.67
4	.032	16	1.01	1.70	1.26	1.11
5	.040	23	1.18	2.00	1.55	1.54
6	.048	5	1.05	2.24	1.64	1.97
7	.056	8	1.81	3.58	2.42	2.41
8	.064	7	2.21	3.74	3.03	2.85
9	.072	6	2.20	3.98	3.07	3.28
10	.080	8	2.20	5.15	4.16	3.72
11	.088	2	4.17	4.41	4.29	4.15
12	.096	4	3.98	4.74	4.18	4.58
13	......	....	......	......	......	5.02
14	......	....	......	......	......	5.46
15	.120	5	4.90	7.15	5.86	5.89
16	......	....	......	......	......	6.33
17	.136	10	5.56	8.84	6.62	6.76
18	......	....	......	......	......	7.20
19	.152	1	......	......	7.50	7.63
20	.160	5	6.93	10.31	8.25	8.07

* Spectrophotometric determinations.

** The figures in this column were obtained with the formula $y = ax + b$ (see text).

The carotene content of the fat in micrograms per gram can be obtained from the figures given in Table 1 of the text or calculated with the formula

$$x = \frac{y - b}{a}$$

in which x is the micrograms of carotene per gram of fat, y is the concentration of the matching dichromate solution in per cent, and b and a are factors, 0.012 and 0.018 respectively.

REFERENCES

1. Barnett, H. M. The determination of carotene in butterfat. J. Biol. Chem., *105*, 259-267. 1934.
2. Baumann, C. A., and Steenbock, H. Fat-soluble vitamin A. xxxvi. The carotene and vitamin A content of butter. J. Biol. Chem., *101*, 547-560. 1933.
3. Gillam, A. E. A modified spectrophotometric method for the assay of carotene and vitamin A in butter. Biochem. J., *28*, 79-83. 1934.
4. Goldblatt, H., and Barnett, H. M. Carotene and vitamin A. Proc. Soc. Exp. Biol. Med., *30*, 201. 1932.
5. Hand, D. B., and Sharp, P. F. Rapid photoelectric determination of carotenoid in milk fat. J. Dairy Sc., *22*, 729-735. 1939.
6. Leuschen, M. E., Kunerth, B. L., Kramer, M. M., and Riddell, W. H. Vitamin A activity of butters determined by various methods. J. Nutri., *14*, 247-259. 1937.
7. Palmer, L. S. Carotenoids and related pigments. Amer. Chem. Soc. Monograph, New York. 1922.
8. Shrewsbury, C. L., and Kraybill, H. R. The carotene content, vitamin A potency and antioxidants of butterfat. J. Biol. Chem., *101*, 701-709. 1933.
9. Treichler, R., Grimes, M. A., and Fraps, G. S. The relation of the color and carotene content of butterfat to its vitamin A potency. Texas Agr. Exp. Sta. Bull., 513. 1935.

The Mechanism of the Autoxidation of Fats*

By H. A. MATTILL

DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA, IOWA CITY, IOWA

THE autoxidation of fats and oils exhibits some of the typical characteristics of an autocatalytic reaction: a latent or induction period of variable length during which the amount of oxygen absorbed is small, followed by a period of rapidly accelerating oxygen absorption. Not all fats show a sharply defined termination of the induction period; the reasons for this varying behavior are inherent in the source and nature of the fat.

The changes occurring during the induction period are very different from those that follow it. They are less obvious, but from a practical point of view they are more important because once the induction period is past the damage is done. Arbitrarily the end of the induction period usually coincides with or immediately precedes the first appearance of the products of organoleptic rancidity. For their detection and estimation various tests have been devised and standardized and these tests have been employed in making almost countless observations on the deterioration of fats (1).

The first event is probably the addition of oxygen at the vulnerable double bonds of unsaturated fat acids, with the formation of fat acid oxides. For such oxides several formulas have been proposed, ethylene oxide, peroxide, moloxide, dative or oxonium peroxide, ozonide,—all of which have been assumed to occur among the primary products of oxidation. Attempts have recently been made by variations in the titration methods, to distinguish between different kinds of fat peroxides (2). Some of the fat peroxides are pro-oxygenic and their removal by a properly chosen adsorbent may make for greater stability of the fat; others may be quite inactive; some are highly unstable, others very stable. Some are volatile, others non-volatile.

The fact that some of these early oxidation products are chemically more active than others may be explained by differences in the configuration of the oxygen linkage to the fat but differences between the original fat molecules may also be recognized.

In their survey of autoxidation Moureu and Dufraisse (3) recall the conception of Arrhenius that in a fluid made up of a definite chemical species, all of the molecules are not in the same state; a small proportion of them may be described as activated. At a given moment only a small portion of the entire number of molecules can enter into reaction. The velocity of a reaction is regulated precisely by the proportion of active molecules, and by the speed with which they are formed. It is possible to calculate the additional energy which must be acquired by molecules in the mean state to become activated for a given reaction. This minimum additional energy requirement Arrhenius called the critical increment of energy.

Many factors may facilitate the acquisition of a sufficient critical increment of energy by fat molecules in the average state. The agencies may be mechanical, such as collision of the molecules with the walls of the containing vessel; physical, such as light, especially ultraviolet, and heat, or they may be chemical, such as metals like copper and iron and their salts, and the fat peroxides themselves. The actual state of an activated molecule may be visualized in various ways, such as the possession of a higher potential, or of molecular valence electrons (4). Not only does it possess a sufficient amount of energy to take part in a reaction, but when it does so, a highly reactive product molecule is formed, whose excess energy is transferred to another average reagent molecule, enabling

---

*Presented in a symposium on Oxidation, Rancidity, and Flavor Reversion of Fats and Oils at the Fall Convention of the American Oil Chemists Society, Chicago, October, 1940.

it, in turn, to react. This transfer of excess energy is the fundamental idea underlying the conception of chain reactions. If the transfer is accomplished without loss, the rate of the reaction remains constant; if not every activated product molecule succeeds in transferring its extra energy to a normal reagent molecule, or if the energy is dissipated by some means, then the reaction does not continue at the same rate. If each active reaction product molecule could perchance activate two average reagent molecules the rate of the reaction would increase and might approach explosive proportions.

In its early stages the oxidation of fat probably partakes of the nature of a chain reaction. More than one chain mechanism may be operating. During the induction period a gradual activation of molecules is proceeding, and active fat peroxide molecules are being formed; accumulation of these and a greatly accelerated rate of oxygen absorption coincide with the first appearance of decomposition products arising from the rupture of the fat acids at the double bonds, and the rapid oxidation of the fragments to aldehydes, peracids and acids. Unsaturation may not be a limiting factor since new double bonds may be created. Individual ethylenic linkages of a polyethenoid unsaturated acid autoxidize at different rates and the resultant peroxides have different stabilities (5). Peroxides modify the progress of many organic chemical reactions (6). In their presence substances that are ordinarily somewhat stable are easily subject to induced or coupled oxidation. The vagueness of our ideas regarding the meaning of the induction period is due largely to lack of information about the chemistry of fat acid oxides and the kinetics of their formation and decomposition.

Whether a perfectly pure fat or fat acid substrate has an induction period is a debatable question. If the length of this period is a function of the balance of pro- and anti-oxygenic substances present, a fat substrate completely free of both might perhaps still have an induction period depending on the ratio of active to average molecules. Stephens (7) has adduced evidence to show that the induction period is indicative of inhibiting effects, chemical and physical, quite apart from antioxidants, and he argues that autocatalysis plays a very subordinate role in these so-called autoxidations.

The possible role of hydrogen peroxide has had considerable speculative consideration. In some quarters (8) it has been given the same pivotal position in the scheme of fat oxidation as it holds in aqueous media. A search of the literature has revealed no trustworthy evidence for this point of view; the tests for peroxide are not specific (9). Hydrogen has indeed been found in the gas mixture above autoxidizing fat, even during the induction period, but the darkening of a photographic plate by emanations from rancidifying fat is not evidence of the presence of hydrogen peroxide (10); some of the organic peroxides appearing in rancid fats are highly volatile and the vapors are bactericidal.

Following the transition from the period of induction to that of active oxidation, by whatever empirical method this transition is determined, peroxide oxygen does not ordinarily continue to increase. If it did, visible combustion might occur; sometimes this does occur, especially in connection with textiles. But in food fats and oils under usual conditions the further progress of the oxidation is accompanied after a time by a decline in the concentration of active oxygen, at a rate sometimes approaching that at which it accumulated, continuing at a lower level or gradually falling to the initial level, depending on conditions and on the character of the substrate. The energy and oxygen of peroxides are passed on to other intermediate and final products of oxidation as they accumulate. The definite character and actual amounts of these varied products can be determined only with difficulty because of lack of discriminating methods of analysis, and because of the great variability of products from different substrates or from the same substrate under different conditions.

In unraveling such a tangled skein of events no progress can be made until reliable quantitative methods are applied to the rigorously controlled oxidation of highly purified fatty substrates. Such experiments were undertaken in our laboratory by Olcott, French, Hamilton and Deatherage (11) (12), and their careful and painstaking work has afforded a little glimpse of what may be taking place. The quantitative methods included accurate and well-established procedures for determining oxygen absorbed, double bond destroyed, peroxide oxygen, and water formed. In addition hydroxyl groups, free and combined carboxyl groups, and aldehydes were also determined. For purposes of comparison the highly purified substrates were all closely related: oleic acid, methyl oleate, oleyl alcohol and cis-9-10-octadecene; the constant conditions were secured by bubbling oxygen through the substrate at constant pressure and temperature (75° C.) in a specially designed apparatus. The partially oxidized volatile products were condensed in traps immersed in a mixture of solid carbon dioxide and ether; carbon dioxide and the last traces of water were absorbed by dehydrite and ascarite in weighing tubes. The runs were 45 to 150 hours long, and duplicate runs showed fair agreement throughout.

All of the substrates had comparable short induction periods and all of the curves of oxygen absorbed were of the sigmoid type. Since inhibitors were probably not present in the highly purified substrates, these sigmoid curves suggest that the induction period may represent something more than the effect of antioxidants.

The amounts of oxygen absorbed and the analytical data obtained from the oxidation residues and trap contents indicated that the fundamental processes were similar, some of the reactions being more prominent in one substrate than in another. Thus oleyl alcohol absorbed most oxygen, oleic acid the least, methyl oleate and octadecene being intermediate.

Each of the residues was fluid and, with the exception of that from octadecene, somewhat insoluble in light petroleum ether. All substrates showed a general increase in —OH except oleyl alcohol, whose terminal hydroxyl may have been oxidized. No significant differences appeared in the amount of double bond remaining or in the peroxide figures; following the initial increase, the longer the run the more peroxide disappeared. Total carboxyl increased in all cases; free carboxyl increased in all substrates except oleic acid where the increase in combined carboxyl was greatest. The aldehyde content of the autoxidizing mixtures was never great; once formed, aldehydes apparently either react with other autoxidation products or are themselves autoxidized. A small amount of freshly redistilled pelargonic aldehyde added to inert paraffin oxidized exceedingly fast, partly to per-pelargonic acid, most of it to pelargonic acid, probably in the usually accepted manner. The residue and trap contents were negative to the Kreis test, and no water was detected.

Of the volatile oxidation products the larger fraction was caught in the freezing traps, the remainder in the first dehydrite trap where it formed a dark brown resin. In the freezing traps water and water-soluble substances were present in very small amounts. Most of the material was a clear colorless oil, moderately volatile at room temperature and with the typical stinging odor of rancidity; it contained very active peroxide oxygen which attacked rubber almost as vigorously as does ozone. These observations do not entirely agree with those of Roschen and Newton (13) who steam-distilled rancid lard. This somewhat volatile oil gave a more intense Kreis test than the residue in the reaction vessel; this is contrary to the idea of Powick (14) that the substance responsible for the Kreis test is non-volatile.

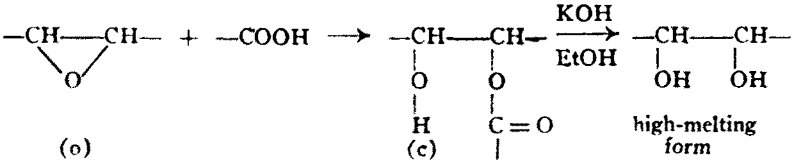
In the first trap the molar content of peroxide oxygen was larger than that of acid. On standing at room temperature, the acid increased while the peroxide decreased; the peroxides oxidized aldehydes to acids or themselves spontaneously decomposed to acids. Contrary to many reports, Deatherage (12) found carbon dioxide in insignificant amounts. Calcium chloride is often used instead of dehydrite to stop water, and since the volatile oil passes calcium chloride and is absorbed by ascarite, some of this oil has probably heretofore been weighed as carbon dioxide.

Although the oxidation products, both volatile and non-volatile, from the various substrates, were somewhat similar in kind and amount, certain differences, when examined more carefully, proved to be enlightening. The residue from oleic acid was very viscous and 50 to 70% of it was insoluble in light petroleum ether. Since it was practically odorless, most of the lower molecular weight compounds must have been removed; since oleic acid is miscible with petroleum ether, most of the unoxidized acid had also disappeared. Compared with the total residue, the petroleum ether insoluble portion contained less free carboxyl groups, much more combined carboxyl and hydroxyl groups, and only slightly less double bonds and peroxides; the double bonds were not those of free oleic acid. The nature of this material was revealed by the following considerations.

In the hands of several investigators the saponification of autoxidized oleic acid has yielded the higher-melting dihydroxystearic acid (132° C.) This was also isolated by Deatherage (12) from the products of oleic acid oxidation but from the other substrates, only traces or none of the corresponding hydroxy compounds could be obtained. Furthermore, oleic acid was the only compound whose free carboxyl groups decreased during the course of autoxidation. If the carboxyl group reacted with some other group formed during oxidation, this could not well be a free hydroxyl because more water should have been formed from oleic acid than from the other compounds and this was not the case. A functional group which reacts readily with a carboxyl group to form an ester, but which will not form water in the process, is the ethylene oxide group. In confirmation of the work of Ellis (15) oxidoöleic acid was not found. Perhaps when oxidoöleic acid is formed, it quickly reacts with a carboxyl group to form a half ester of dihydroxystearic acid and thus prevents appreciable accumulation of oxidoöleic acid. On this assumption, and if all the other substrates were autoxidized in the same fundamental manner, their oxido derivatives should be found in the respective residues.

The presence of these compounds in considerable quantity was demonstrated by heating their residues with glacial acetic acid for several hours and subsequently saponifying the resulting esters to form the corresponding dihydroxy derivatives.

With appropriate modifications of the methods, high melting octadecanediol was obtained from autoxidized octadecene, high melting dihydroxystearic from methyl oleate and high melting octadecanetriol from oleyl alcohol. On the basis of this evidence ethylene oxide derivatives play an important role in the autoxidation of unsaturated fat acids and related substances as illustrated by Equation 1:



(o) (c) (1)

Compound (o) was found in autoxidized octadecene, in esters of oleic acid, and in oleyl alcohol, but not in oleic acid: (c), an ester, was found in appreciable amounts only in autoxidized oleic acid, and appears to have had its origin in oxidoöleic acid and a molecule of oleic acid.

The analogous reaction for the other substrates was not prominent because no original —COOH was available. For this reason also the oxido derivatives of the other materials appeared in relatively large amounts; a similar reaction occurs when their autoxidation residues are esterified with glacial acetic acid.

There is excellent confirmatory evidence that ethylene oxides are prominent oxidation products. When dihydroxy stearic acid is produced through the agency of peroxides, such dihydroxy acid is always of the low-melting variety, 95° C. In other words, the oxido compounds encountered in this work were not formed through the reaction of peracids with the ethylene linkage, a reaction which forms the basis of one of the many proposed mechanisms of autoxidation (16).

Half esters of dihydroxystearic acid could be formed by the reaction of oxidoöleic acid with any free carboxyl group as in oleic acid, oxidoöleic acid, or short-chain acids. The ethylenic bond that might occur in such a compound (c) could also undergo characteristic autoxidation reactions.

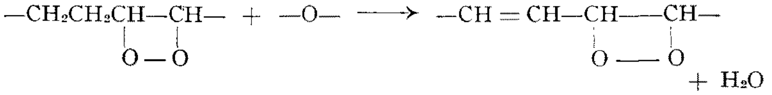
The formation of these half esters of dihydroxy stearic acid from oleic acid converts each molecule of such altered oleic acid to the status of a saturated aliphatic compound that is no longer autoxidizable, and the absorption of oxygen by oleic acid should fall off more rapidly than with the other substrates. This was the case.

The largest and most rapid uptake of oxygen occurred with oleyl alcohol, perhaps by oxidation of the terminal hydroxyl to aldehydes in the presence of peroxides. The additional water formed, the increased total carboxyl, and the decreased hydroxyl all substantiate this interpretation. With oleyl alcohol, 1.58

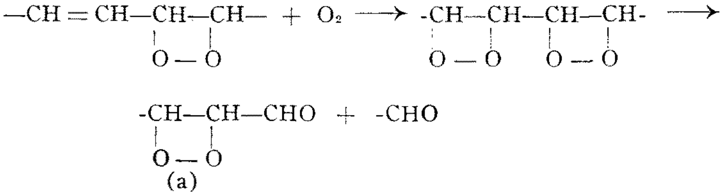
moles of water were formed per mole of double bond destroyed; all of the other substrates yielded 1 mole or less.

Water was an important autoxidation product of all of the compounds studied. Octadecene produced a somewhat smaller amount than the other substrates; it consumed somewhat less oxygen and more volatile products were formed. These volatile materials, largely peroxides and peracids, are probably responsible for the oxidation of the carbon chain with the formation of water and the creation of new double bonds; they in turn undergo autoxidation reactions. The escape of volatile peroxides from the reaction mixture thus delays oxidation of the chain, and less water is formed. The volatility of the autoxidation products of octadecene thus influences not only the total oxygen consumption but also the amount of water formed.

The following series of hypothetical equations may account for the formation of water, the oxidation of the products formed, the high content of peroxide found, and the intense Kreis test given by the volatile oil caught in the traps:



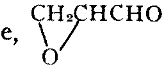
$$\mathrm{-CH_2CH_2}\underset{\mathrm{O-O}}{\mathrm{CH-CH}}\mathrm{-} + \mathrm{-O-} \longrightarrow \mathrm{-CH=CH-}\underset{\mathrm{O-O}}{\mathrm{CH-CH}}\mathrm{-} + \mathrm{H_2O} \qquad (2)$$

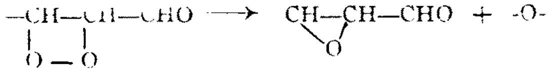


$$\mathrm{-CH=CH-}\underset{\mathrm{O-O}}{\mathrm{CH-CH}}\mathrm{-} + \mathrm{O_2} \longrightarrow \mathrm{-}\underset{\mathrm{O-O}}{\mathrm{CH-CH}}\mathrm{-}\underset{\mathrm{O-O}}{\mathrm{CH-CH}}\mathrm{-} \longrightarrow \underset{\substack{\mathrm{O-O}\\ \mathrm{(a)}}}{\mathrm{-CH-CH}}\mathrm{-CHO} + \mathrm{-CHO} \qquad (3)$$

The peroxide aldehyde (a), is typical of a number of volatile autoxidizable aldehydes that could be formed and that may polymerize in a way similar to the observed polymerization of volatile products as a brownish resin in the first dehydrite trap.

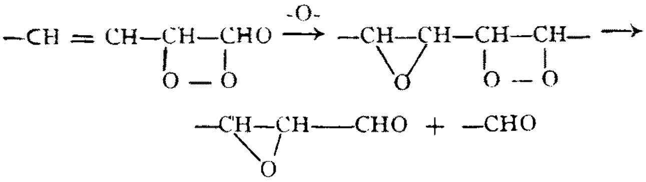
Such an unstable peroxide is probably formed because the water-insoluble material in the first freezing trap contained a greater molar concentration of peroxide oxygen than of carboxyl group. Much of the active oxygen was therefore contained in a volatile peroxide which was not an acid; also, the contents of this trap gave a strong test for aldehydes.

Powick (13) demonstrated that the Kreis test is due to epihydrinaldehyde,  $\underset{\mathrm{O}}{\mathrm{CH_2CHCHO}}$ either present as such, or produced by the reaction of hydrochloric acid with a fat acid chain that had been oxidized at several places. The large amount of volatile material giving the Kreis test and the small amount of oxygen consumed are not compatible with such a process. Hence it must be concluded that a positive Kreis test may be produced by substances other than epihydrinaldehyde, possibly by its higher homologs. Such compounds could be formed by the reduction of products of equation 3



$$\underset{\mathrm{O-O}}{\mathrm{-CH-CH}}\mathrm{-CHO} \longrightarrow \underset{\mathrm{O}}{\mathrm{CH-CH}}\mathrm{-CHO} + \mathrm{-O-}$$

or by the partial oxidation and cleavage of the products of equation 2:



$$\mathrm{-CH=CH-}\underset{\mathrm{O-O}}{\mathrm{CH-CH}}\mathrm{-CHO} \xrightarrow{\mathrm{-O-}} \mathrm{-}\underset{\mathrm{O}}{\mathrm{CH-CH}}\mathrm{-}\underset{\mathrm{O-O}}{\mathrm{CH-CH}}\mathrm{-} \longrightarrow \mathrm{-}\underset{\mathrm{O}}{\mathrm{CH-CH}}\mathrm{-CHO} + \mathrm{-CHO}$$

No all-inclusive theory of autoxidation can yet be formulated. Once the obscure induction period is past, events seem to follow thick and fast in greatest confusion, but like the devastating speed and apparent disorder of a blitzkrieg, everything nevertheless goes according to plan. The plan is flexible and is in part dictated by the events themselves. Further careful study is needed to indicate what the events are and why they take place.

LITERATURE CITED

1. Lea, C. H., Rancidity in Edible Fats, New York, Chem. Publ. Co., 1939; Special Report No. 46, Food Investigation, London, H. M. Stationery Office, 1938.
2. Morrell, R. S. and Phillips, E. O., J. Soc. Chem. Ind., *58*, 159 (1939).
3. Moureu, C. and Dufraisse, C., Chem. Rev., *3*, 113 (1926).
4. Milas, N. A., Chem. Rev., *10*, 295 (1932).
5. Franke, W. and Jerchel, D., Ann., *533*, 46 (1937).
6. Kharash, M. S. and McNab, M. C., J. Am. Chem. Soc., *56*, 1425 (1934) and many later papers.
7. Stephens, H. N., J. Am. Chem. Soc., *58*, 219 (1938).
8. Coe, M. R., Oil and Soap, *15*, 230 (1938).
9. Schales, O., Ber., *71 B*, 447 (1938).
10. Keenan, G. L., Chem. Rev., *3*, 95 (1926).
11. French, R. B., Olcott, H. S., and Mattill, H. A., Ind. Eng. Chem., *27*, 724 (1935); Hamilton, L. A. and Olcott, H. S., Ibid., *29*, 217 (1937).
12. Deatherage, F. E. and Mattill, H. A., Ibid., *31*, 1425 (1939).
13. Roschen and Newton, Oil and Soap, *11*, 226 (1934).
14. Powick, J. Agr. Research, *26*, 323 (1923).
15. Ellis, Biochem. J., *26*, 791 (1932).
16. Hyman, J. and Wagner, C. R., J. Am. Chem. Soc., *52*, 4345 (1930).