Colloidal ion lengths of the anionic (20–25 Å) and nonionic (49–56Å) surfactants may help to explain the ability of the latter to accommodate the length of triolein (26 Å), in addition to the nonionic aggregate size.

The heat of solubilization, $\triangle H_s$, for triolein in 0.25% solution of tridecanol-10 EO adduct, calculated by the Clausius-Clapeyron equation, was 15 kcal/mole. This was approximately 12 times greater than the adduct's heat of micellization, $\triangle H_m$, estimated at 1.3 kcal/mole from cmc data.

Solubilization of triolein by 0.25% tridecanol-10 EO increased linearly with triolein level up to the saturation point (0.044%) for equilibrium data at 60°C.

No attempt was made at correlation between solubilization, soil removal, and critical micelle concentration, reserving this for another presentation.

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Analysis of Fat Acid Oxidation Products by Countercurrent Distribution Methods. V. Low-Temperature Decomposition of Methyl Linoleate Hydroperoxide

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Methyl *cis,trans* diene conjugated linoleate hydroperoxide isolated by countercurrent distribution from 4°C. autoxidation of methyl linoleate was stored in atmospheres of oxygen and of nitrogen at 4°C. in darkness. Besides manometric changes, infrared and ultraviolet characteristics, peroxide value, diene conjugation, and molecular weights were followed on samples removed at various periods of storage up to 53 days. These same analyses were obtained on fractions obtained by countercurrent distribution.

Evidence for the reaction that occurs on storage in oxygen may be summarized thus: 1 mole oxygen absorbed by linoleate hydroperoxides destroys 1 mole *cis,trans* diene conjugation, ½ mole peroxide group, and 1 mole linoleate hydroperoxide; dimers of varying polarities, scission acids, and isolated *trans* bonds are formed.

Since no volume changes were observed in the nitrogen storage of methyl linoleate hydroperoxide, changes in chemical and physical characteristics can only be related to time of storage. Storage in nitrogen at 4°C. destroys diene conjugation, peroxides, and linoleate hydroperoxide and produces dimers of varying polarities, scission acids, and isolated *trans* bonds. Destruction of diene conjugation was one-fourth as rapid in a nitrogen atmosphere as in oxygen. While differences in reactions and products were observed between oxygen and nitrogen storage, particularly in rates and in countercurrent distribution patterns, the similarity of products from oxygen and nitrogen storage is remarkable. Once methyl linoleate hydroperoxide is formed regardless of storage atmosphere, dimerization and attendant destruction of double bonds and peroxides proceed.

SEVERAL REVIEWS and original papers have been published on the autoxidation of methyl linoleate within the past 10 years (2,4–6,8,9). General agreement exists that methyl linoleate oxidizes to yield methyl *cis,trans* diene-conjugated linoleate hydroperoxide (MLHP) as the primary oxidation product.

Previous work at this laboratory concerned the autoxidation of methyl linoleate at low temperature (0°C.) to give mixtures of methyl linoleate, MLHP, and their reaction products. To interpret these results fully, it was necessary to study the decomposition of MLHP by itself and under comparable and controlled conditions. This paper therefore reports low-temperature decomposition of MLHP in atmospheres of oxygen and nitrogen.

Experimental Procedures

MLHP formed by the 4°C. light-catalyzed autoxidation of 233 g. of methyl linoleate was separated from the parent methyl linoleate by large-scale countercurrent extraction (12). The isolated material (82 g.) was further purified by a 100-tube countercurrent distribution in the system 80% aqueous ethanol and pentane-hexane; the recovered MLHP had the chemical and physical constants given in Table I.

The reaction of MLHP with oxygen was studied at 4°C. Two simultaneous runs designated as A and B were made in manometric equipment, which consisted of a flask, approximately 25 ml. in size, sealed to a 50-ml. mercury-filled gas burette and equipped with magnetic stirrers. The side arm of flask A was closed with a rubber serum cap in order that samples could be periodically withdrawn by use of syr-

TABLE I

Analysis of Methyl Linoleate Hydroperoxide

Absorptivity at 233 mμ	75.1
Absorptivity molar at 233 mμ	24,500
Molecular weight (cryoscopic–theory 326.47)	321.6
Partition coefficient (80% EtOH–pet. ether)	0.57
Elementary analysis (theory calculated for $C_{19}H_{34}O_4$: C, 69.90; H, 10.50)	C–69.09 H–10.30
Peroxide value (theory 6125 meq./kg.)	5,253
Iodine value [after reduction of OOH group (Benham and Klee) theory 166.2]	148.9

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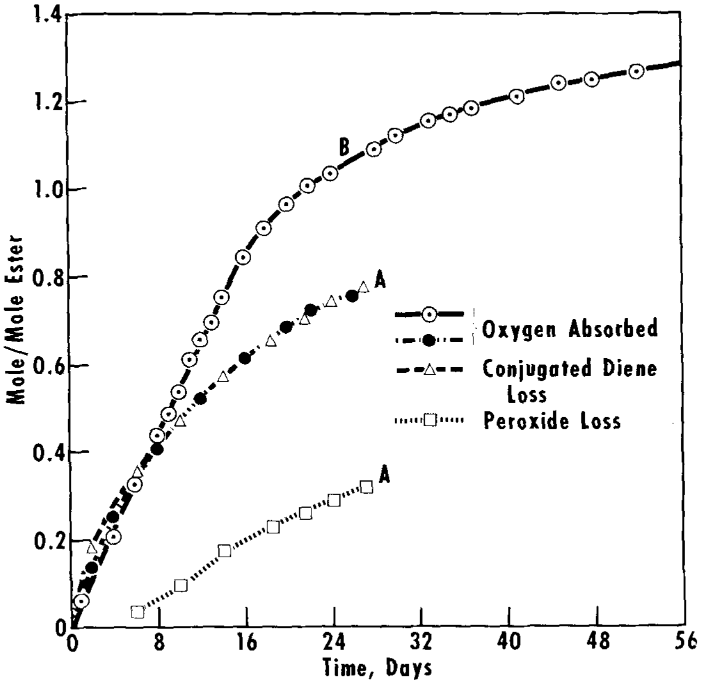

FIG. 1. The change in oxygen absorption, conjugated diene loss and peroxide loss with time.
(A) Sampled manometer
(B) Sealed manometer

inge and needle. To assure freedom from leaks, the second flask B was completely sealed in glass except for the mercury manometer. This manometer was not opened until the end of the experiment. Except for the sampling outlet of A, flasks A and B were exactly alike. After the burettes were filled with oxygen and placed in darkness at 4°, samples were introduced, the flasks closed, and the stirrers started. Oxygen uptake was followed periodically, and the systems were maintained at near atmospheric pressure (Figure 1).

Samples were withdrawn from flask A at intervals and analyzed for peroxide values (3), ultraviolet, and infrared spectral absorbances.

Countercurrent distributions were then performed at certain storage intervals in a 24-tube apparatus, using the 80% ethanol and pentane-hexane system. At the final sampling date the distribution was performed in 200-tube automatic countercurrent distribution equipment, and 400 transfer stages were applied.

Molecular weights were determined on combined fractions from the distribution, as well as on stored samples, by a cryoscopic method, using cyclohexanol as the solvent (4,10). The sample solution was introduced into a capillary tube *via* syringe and needle, and the tube was sealed off. This tube was placed in a water bath and was fastened to a solenoid-operated agitator that lifted the tube once every 4 seconds. As the temperature of the water bath was permitted to rise toward room temperature, melting points were observed. The molecular weights were calculated from melting-point depressions.

Conjugated dienes were determined on weighed samples in methanol, using either a Cary Model 14 spectrophotometer or a Beckman Model DU spectrophotometer at λ 233 with an extinction coefficient for methyl linoleate hydroperoxide of 81.3 (1).

Nitrogen storage of the MLHP was similar except that the volume of nitrogen over the sampled flask A was not measured.

Results and Discussion

Oxygen Storage. The results of the measurements on the sealed manometer B indicate that oxidation at 4°C. (Figure 1) is linear with time during the initial period up to approximately 14 days; then the rate drops off. Oxygen uptake data show this reaction to be nonautocatalytic as observed by Privett and Nickell (7).

Figure 2 shows the results obtained on samples withdrawn from flask A. The decrease in conjugated double bonds is about twice the decrease in peroxide values. Thus it is evident that the measurement of diene conjugation provides a more sensitive insight into the decomposition of MLHP than does peroxide determination. This evidence seems to differ from that of Privett and Nickell (7), who found at 30°C. no reduction in peroxide value during the oxidation of MLHP although the diene conjugation was reduced to almost one-third of the starting value.

As determined by the isolation of residual MLHP with countercurrent distribution, Figure 2 also reveals that 1 mole of *cis,trans* diene conjugation is lost for each mole of oxygen absorbed by the MLHP and that ½ mole of the peroxide is destroyed. The two data points shown as loss in weight of MLHP in Figure 2 come from countercurrent distribution of samples plotted in Figures 4 and 5 and correspond to storage of 14 and 27 days. The rate of decrease in weight of MLHP leads to the conclusion that 1 mole of recoverable MLHP is lost for every mole of oxygen absorbed. After 3 days' storage the first definite decrease in peroxide values is evident. The 24-tube countercurrent distribution is presented in Figure 3. The three curves are the total weight per tube, the weight of peroxide material, and the weight of conjugated diene material. Tubes to the right contain

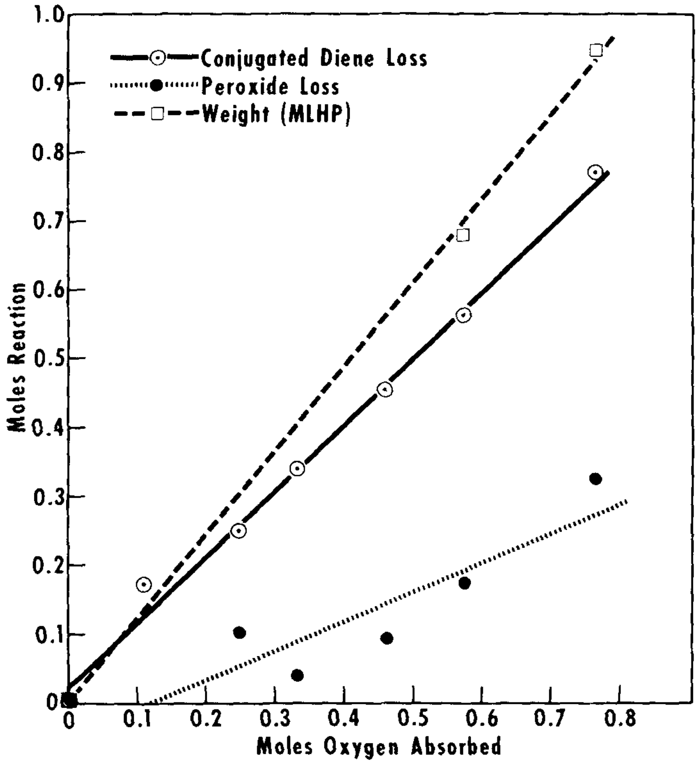

FIG. 2. Relation of moles of oxygen absorbed to the loss in conjugated diene and peroxide groups and to the disappearance of recoverable methyl linoleate hydroperoxide (MLHP).

the more petroleum ether-soluble materials, those to the left the more 80% aqueous ethanol-soluble. The countercurrent distribution curve for the starting methyl linoleate differs from these curves in that it has its maximum in tube 10 and all three curves coincide. This distribution, and subsequent 24-transfer stage distributions, can be characterized by delineating three portions. First, tubes 10 through 16, where the three curves coincide, represent reisolation of the starting material, MLHP. Second, tubes 0 through 6 contain decomposition compounds which are more polar than the original MLHP and with reduced peroxide and conjugated diene contents. Third, tubes 7 through 9 represent an unresolved mixture of the MLHP and its secondary decomposition products.

After 14 days' storage MLHP shows a significantly different countercurrent distribution pattern (Figure 4). The bulk of the material has shifted to the more alcohol-soluble tubes. Curves for peroxide and diene conjugation tend to approach each other in tubes 10 through 16, as is characteristic of unaltered, reisolated MLHP. Throughout the course of storage and oxidation of the MLHP it is apparent that the position of the original MLPH peak remains constant whereas the percentage of the total weight of this material decreases. Also in these same tubes a relative increase in the amount of material other than MLHP is apparent as well as a decrease in MLHP.

Figure 5 is the graph for a 400-transfer stage countercurrent distribution of the 27-day storage sample. The 200 withdrawn tubes were combined but do not appear on the graph.

The MLHP, as identified by the coincidence of the weight, peroxide, and conjugated diene curves for tubes 145 through 165, is partially separated from the other oxidation products. Tubes 0 through 120 contain material that has little diene conjugation. The material in these tubes is approximately one-half the peroxide value of pure MLHP.

Also given in Figure 5 are molecular weights of the combined fractions. Tubes containing similar constituents were combined, as indicated in the figure, to get more working material. Data indicate that the monomeric MLHP forms a dimer during storage and during this reaction loses one hydroperoxide group. This fact suggests conversion of a peroxide group to oxygen bond in line with the suggestions of Witting, Chang, and Kummerow (11) on their work with linolenate.

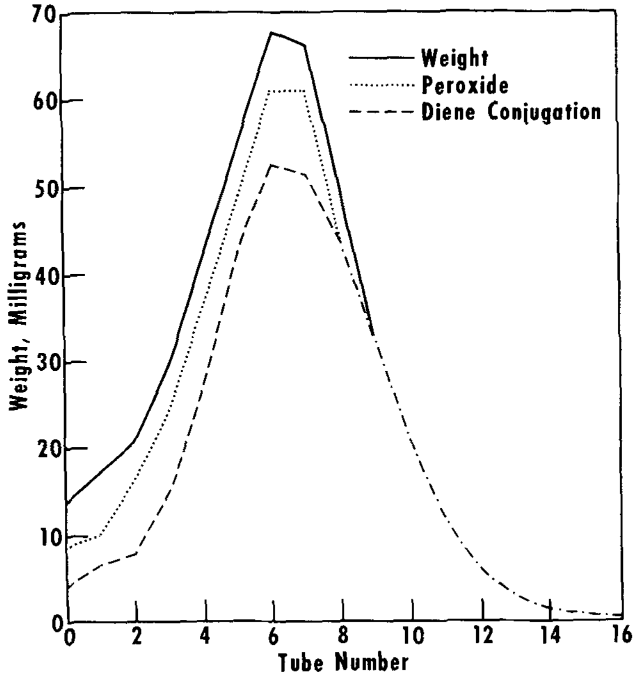

FIG. 3. Countercurrent distribution (24 tube) after 3 days' storage of methyl linoleate hydroperoxide in oxygen.

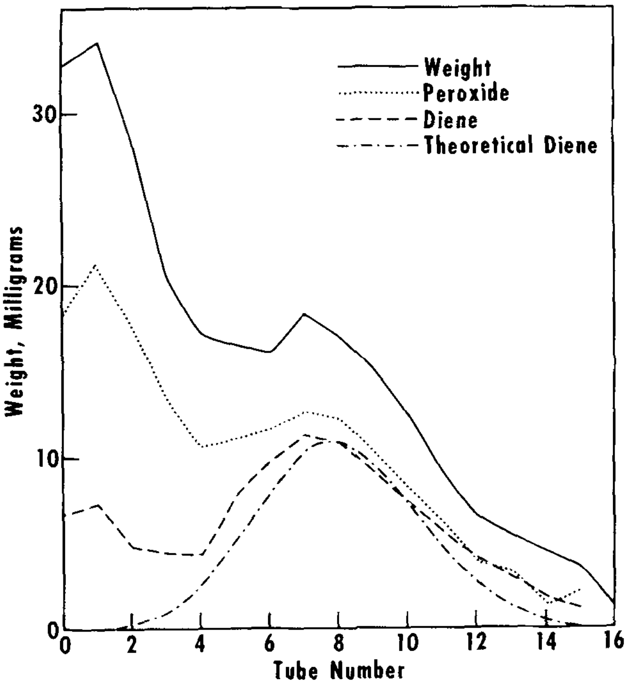

FIG. 4. Countercurrent distribution (24 tube) after 14 days' of methyl linoleate hydroperoxide in oxygen.

Tubes 120 through 145 (average molecular weight of 548) are composed of dimers unresolved from monomeric MLHP. Conversely the presence of dimers in the identified MLHP, tubes 145 through 165, explains its higher-than-theoretical molecular weight.

A maximum in average molecular weight, approaching that of a dimer, is reached somewhere between tube 85 and tube 120. As the material becomes more polar toward 0 tube, the molecular weight drops; this decrease is explained by the presence of acidic materials presumably of shorter chain-length and originated by oxidation scission. Since volatile scission products were lost in the evaporation step, the acids titrated are probably the nonvolatile residue of dimers.

Infrared analyses of samples withdrawn at 2, 4, 6, 10, 14, and 27 days' storage lead to additional information concerning a) the structural configuration and rearrangement of conjugated double bonds and b) the amount and degree of association of hydroperoxide groups.

The samples were prepared for infrared analysis by dissolving as a 2% solution (weight-volume) in carbon disulfide. Infrared transmissions were obtained with a Perkin-Elmer Model 21 infrared spectrophotometer and with a Baird atomic KM-1 instrument.

Figure 6 shows curves typical of those obtained during storage of MLHP in oxygen, starting with the pure material on the top curve and progressing down to the bottom curve, comparable to 27 days' storage.

The most significant regions of these curves are: first, the 10–11 micron region, which reflects the geometrical configuration for *cis,trans* conjugation (maxima at 10.18 μ–10.56 μ), *trans,trans* conjugation (maximum at 10.13 μ), and isolated *trans* double bonds (maximum at 10.36 μ); second, the 2–3 micron region, which contains absorption bands for the hydroxyl and hydroperoxide groups.

In considering the 10–11 micron region first, the original MLHP has conjugated *cis,trans* double bonds, as indicated by the pair of bands at 10.18 and 10.56. At subsequent periods of storage this pair decreases, and a new band at 10.36 microns characteristic of an isolated *trans* bond becomes more apparent. The observation confirms the loss in diene conjugation measured in the ultraviolet.

In the hydroxyl region from 2–3 microns there are two bands—one at 2.87 microns, corresponding to nonassociated hydroxyl groups and the other at 2.95 microns, corresponding to associated hydroxyl groups. The pure hydroperoxide itself has a greater percentage of nonassociated than associated hydroperoxide groups in a 2% solution in CS_2. As the time of storage increases, more and more associated hydroxyls are formed. No inference can be drawn at this time concerning the structural changes responsible.

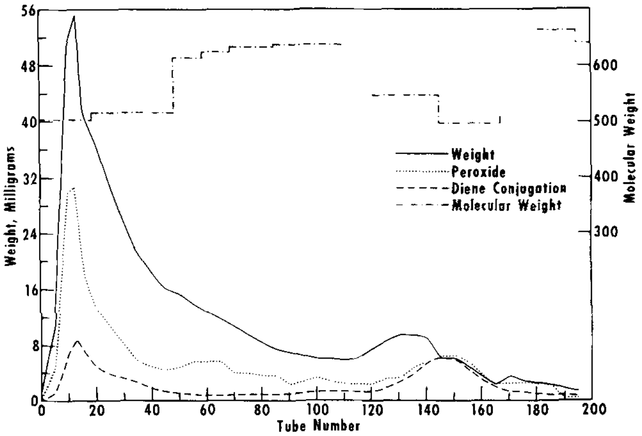

FIG. 5. Countercurrent distribution (400 transfer stages) after 27 days' storage of methyl linoleate hydroperoxide in oxygen.

As seen in Figure 5, tubes 0 through 100 have materials with absorption spectra comparable to the bottom curve in Figure 6 and exhibit mainly isolated *trans* with traces of *cis,trans* conjugated material. Materials in these tubes also contain hydroxyl or hydroperoxide groups which are largely associated, as seen in the 2.95 μ region. Tubes 110 through 120 correspond in appearance with the curve second from the bottom in Figure 6. The *cis,trans* conjugated bonds are present in small amounts. Tubes 146 through 167 are typical of the original MLHP with *cis,trans* conjugation exclusively and with hydroxyl groups largely nonassociated.

Nitrogen Storage. MLHP was stored in a nitrogen atmosphere, and pressure was followed manometrically. As anticipated, no change in the volume of gas in the sealed container was observed over the experimental period. Consequently it appears that no oxygen exchange is involved during storage. Figure 7 shows the results obtained by analyses on samples withdrawn. In a manner similar to that observed in oxygen storage, data show the decrease in conjugated diene to be about twice as rapid as the decrease in peroxide values, and again diene conjugation would seem to give a more sensitive index for following the decomposition of the hydroperoxides than does the peroxide value. It should be noted that decomposition in oxygen is about four times as fast as in nitrogen, based on loss of diene conjugation.

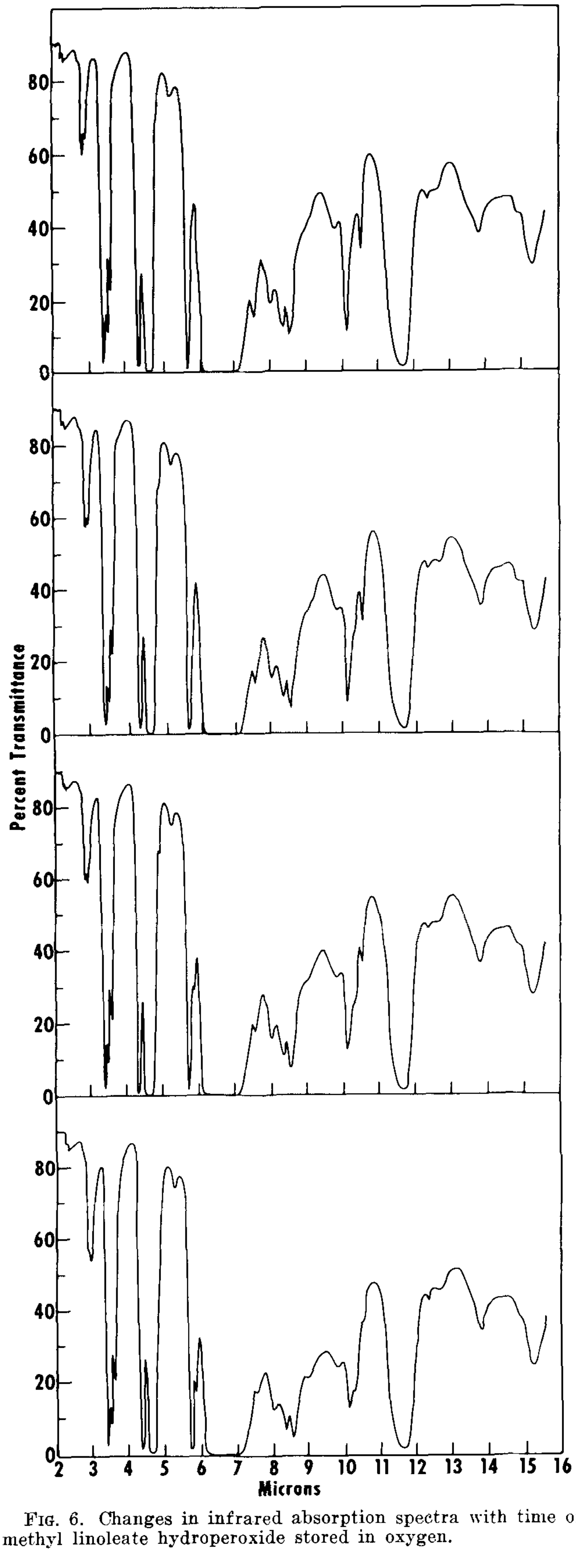

FIG. 6. Changes in infrared absorption spectra with time of methyl linoleate hydroperoxide stored in oxygen.

After 18, 40, and 53 days, samples were withdrawn and submitted to countercurrent distribution in the system 80% aqueous ethanol and pentane-hexane. In Figure 8 is given the results of a 400-transfer stage countercurrent distribution of the 53-day nitrogen storage. Some differences are found in compounds formed under nitrogen as compared to oxygen. A peak occurs at tube 133 on the oxygen storage curve (Figure 5) but not on the nitrogen. Tube 60 shows a peak on the nitrogen curve but not on the oxygen. The peak at tube 60 is a little more than half the peroxide value of pure MLHP but contains proportionately little diene conjugation. It is the lack of diene conjugation that clearly differentiates this peak from the more polar peak at tube 10 in Figure 8.

Also shown on Figure 8 are some molecular weights on the combined fractions. This pattern is also similar to the molecular weight data for oxygen storage (Figure 5). Again lower molecular weight toward the more polar area of the curve is explained by shorter chain-length scission acids and is confirmed by increased titratable acidity. Less acidity per unit of weight was observed in nitrogen storage than in oxygen and can be explained by the effect of limited oxygen supply on the production of oxidative scission products.

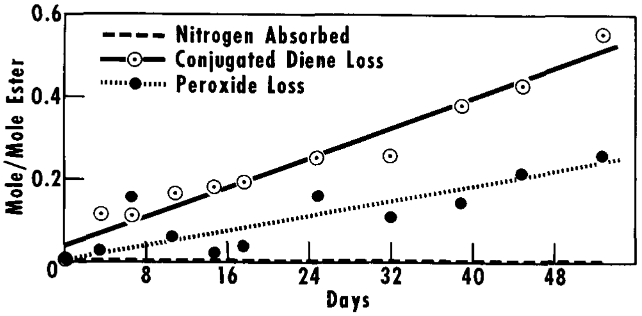

FIG. 7. Storage of methyl linoleate hydroperoxide in nitrogen.

The average molecular weights obtained for material on this curve are somewhat lower than for oxygen storage, and this difference is accounted for, in part, by the 2 moles of oxygen absorbed per mole of dimer formed during oxygen storage; thus in those dimers where no scission takes place, the added oxygen would increase the molecular weight. There is also the possibility that small amounts of trimer or higher molecular weight polymer are formed.

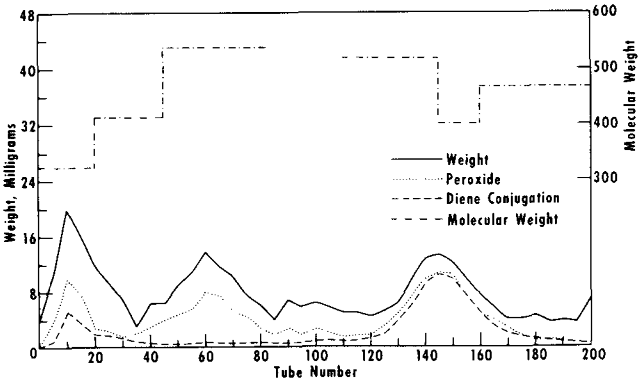

FIG. 8. Countercurrent distribution (400 transfer stages) after 57 days' storage of methyl linoleate hydroperoxide in nitrogen.

Infrared curves run on the nitrogen samples also showed the same pattern of a shift from *cis,trans* to isolated *trans* as the reaction progresses.

Acknowledgment

The assistance of E.H. Melvin and C.A. Glass for infrared spectral data is greatly appreciated, Mrs. Boneta Heaton for elemental analyses, and of J.C. Cowan through his interest and encouragement.

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Carbonyls in Oxidizing Fat. V. The Composition of Neutral Volatile Monocarbonyl Compounds from Autoxidized Oleate, Linoleate, Linolenate Esters, and Fats

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The steam volatile monocarbonyl compounds in mildly autoxidized esters of oleic, linoleic, linolenic acids, and animal and vegetable fats were quantitatively estimated. The major aldehydes in oleate and linoleate were those that might be expected from the scission of reported monomeric hydroperoxide isomers. The predominance of hept-2,4-dienal and propanal in linolenate suggested that the major monomeric hydroperoxides were 12- and 16-hydroperoxy conjugated dienoic isomers. The number of minor aldehydes increased with degree of unsaturation of the fatty acid. The amounts of monocarbonyl compounds in the fats examined generally agreed with their average fatty acid composition. Appreciable amounts of heptanal in lamb and beef fat and heptanal and decanal in butterfat, under the conditions of oxidation, could not have come from the three unsaturated acids. Heating at 165°C. in all samples increased the proportions of the most unsaturated major aldehydes.