easily be identified at −10C. 18:4 on these columns falls before 20:1, 16:3ω4 and 17:1 overlap and also 16:2ω4 and 17:0.

Myristic acid, 14:0, although completely saturated, is still quite soluble at −10C due to its short chain length, and conversely, 22:5 only shows slight enrichment in spite of five double bonds in the molecule. Isomers of 22:4 and 20:3 are usually present in marine oils in small amounts, but are not enriched by this method. The concentration of isomers of 20:2, when present, decreases slightly.

The method described has been used routinely in this laboratory for some time, although only methyl esters from marine lipids have been segregated. The method should, however, also be useful in the analysis of some animal and vegetable lipids with complex fatty acid compositions.

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Autoxidation of Cholesteryl Linoleate in Aqueous Colloidal Suspension[1]

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Abstract

The autoxidation of the cholesteryl moiety of cholesteryl linoleate stabilized in aqueous colloidal suspension with sodium dodecyl sulfate has been studied at 85C. The overall rate of this oxidation is more rapid than that for unesterified cholesterol and oxidation also occurs to a greater extent for the linoleate ester. These results are in contrast to those for more saturated fatty acyl esters of cholesterol which show diminished susceptibility to attack by oxygen in such a system. Autoxidation of cholesteryl linoleate by an intramolecular free-radical mechanism is considered.

INFORMATION CONCERNING the autoxidation of cholesteryl esters is relatively meager whereas studies of autoxidation of unesterified cholesterol have been reported somewhat extensively. Bergstrom and Wintersteiner (1) reported that esterification of cholesterol by acetate, palmitate, or oleate greatly diminished the susceptibility of the cholesterol in aqueous colloidal suspension to attack by oxygen. Cook (2) compared the oxidation of cholesteryl acetate with unesterified cholesterol in xylene containing phthalocyanine. The literature of the autoxidation of cholesterol has been reviewed (3).

Since cholesterol occurs in the animal body in both the unesterified and esterified forms it was felt that the chemical reactivity of the esters, particularly those of the more unsaturated fatty acids, of cholesterol in aqueous suspension should be elucidated further. Cholesteryl linoleate was chosen for study. It was hypothesized that the high susceptibility of the linoleate 9,12 diene system to autoxidative attack might render the cholesteryl moiety of cholesteryl linoleate rather more susceptible to autoxidative attack than is the case for cholesteryl acetate, palmitate, and oleate (1). Experimental study revealed that cholesteryl linoleate in aqueous colloidal suspension has a markedly different susceptibility to autoxidative attack than the more saturated fatty acyl esters. It is the purpose of this communication to report this finding.

Experimental

The procedure of autoxidation was similar to that of Bergstrom and Wintersteiner (1). Modifications involved the amounts of cholesterol [cholesterol or cholesteryl esters (99% pure, Applied Science Laboratories, Inc., State College, Pa.) were used in equimolar amounts] and sodium dodecyl sulfate. pH was adjusted to 7.0 ± 0.2 with M/15 phosphate buffer. Autoxidation was carried out at 85C. Aliquots of the reaction mixture were acidified, extracted with diethyl ether, the ether extract water-washed, the solvent evaporated, and the extract analyzed for unoxidized cholesterol by the Sperry-Webb (4) procedure. Use of this analytical method for following the time course of autoxidation of cholesterol has been described (5). As a further check of the analytical procedure we have tried the method on standard solutions of 7-ketocholesterol, 7-β-hydroxycholesterol, and 3β, 5α, 6β-cholestanetriol. The first two compounds behaved

TABLE I

The Autoxidation of Cholesterol and Its Esters by Aeration in Aqueous Colloidal Suspension, pH near neutrality, 85C, 5 hr.

Compound	Percent of Cholesterol Autoxidized	
	Bergstrom-Wintersteiner (1,6)	Norcia-Janusz
Cholesterol	60	60
Cholesteryl acetate	16[a]	....
Cholesteryl palmitate	10	....
Cholesteryl oleate	<5	0
Cholesteryl linoleate	....	78

[a] Autoxidation for 4 hr.

[1] Presented at the AOCS Meeting in Houston, Texas, April, 1965.
[2] Work conducted during the tenure of a Summer Research Student Fellowship.

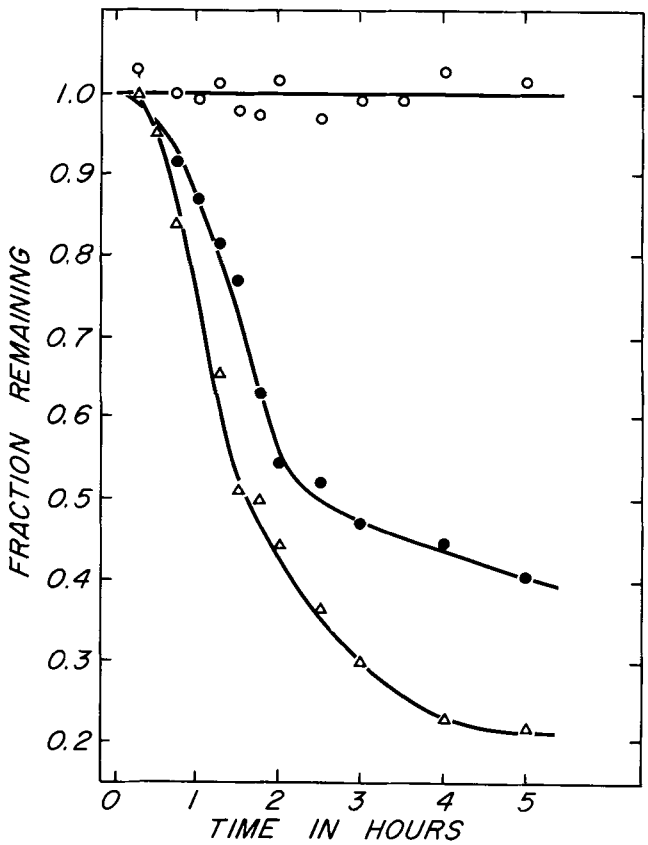

FIG. 1. Fraction of cholsterol remaining during aeration of cholesterol and cholesteryl esters in aqueous collodial suspension at 85C: ●—cholesterol, ○—cholesteryl oleate, △—cholesteryl linoleate.

according to prediction (5). The triol available to us did produce some yellow color with the Liebermann-Burchard reagent having about one-third the absorbance at 625 mμ of similarly treated cholesterol. However, the triol did not precipitate with digitonin under the assay conditions.

Results

Data from autoxidation experiments on cholesterol, cholesteryl oleate, and cholesteryl linoleate are presented in Figure 1. The time course and extent of autoxidation of cholesterol and cholesteryl oleate are shown to be very closely similar to the results reported by Bergstrom and Wintersteiner for these compounds (1,6). No rate inhibition is shown for the linoleate ester, it being autoxidized at a faster overall rate and to a greater extent than unesterified cholesterol. Data from experiments on cholesterol and its esters that have been studied under these conditions are presented in the summary table.

Discussion

The results obtained with cholesteryl linoleate suggest the possibility of oxidative attack of the cholesteryl moiety by an intramolecular free-radical mechanism. Such a scheme may take the form shown in Fig. 2. The reaction scheme is not intended to exclude participation by the resonance hybrids of linoleate free-radicals or the several positional isomers of linoleate hydroperoxide. Such an intramolecular free radical mechanism might be expected to show some stereospecificity with respect to products formed. We have not studied the products of the autoxidation of cholesteryl linoleate.

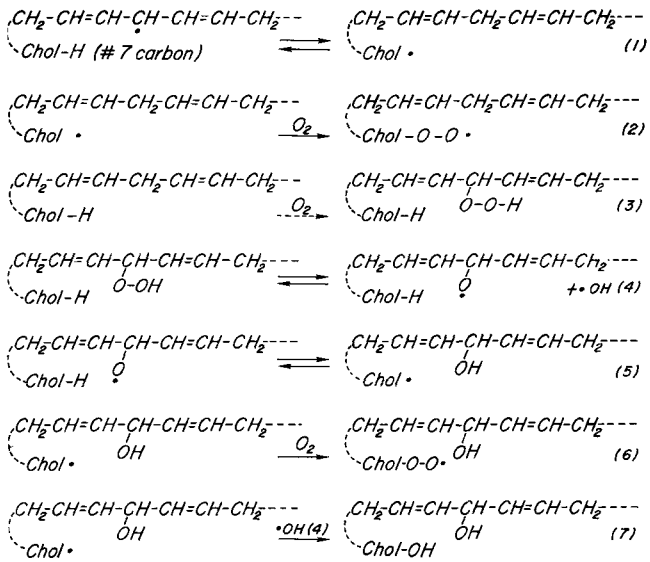

FIG. 2. Reaction schema.

The possibility of intermolecular propagative reactions appears less likely considering the organization and orientation of molecules in a colloidal system. The ratio of cholesterol or cholesteryl ester molecules to dodecyl sulfate ions in the reaction systems studied was 1/7 to 1/9.

An alternative possibility is that cholesterol linoleate autoxidation is facilitated by a surface phenomenon. Introduction of oxygen functions by autoxidation on the linoleate 9, 12 diene system would alter the polarity of the ester molecule. This altered polarity might cause a reorientation of the molecule on the surface of the micelle thus rendering the cholesteryl moiety susceptible to oxidative attack. Autoxidation studies of cholesteryl esters with oxygen functions introduced into the fatty acyl chain might yield information relative to this point.

Diminished susceptibility to autoxidative attack by the esters more saturated than the linoleate ester might be due to steric hindrance. In the colloidal system the fatty acyl moiety may fold back over the cholesteryl moiety, and hinder the sensitive α-methylenic group at position 7 of the cholesteryl moiety.

The study reported here suggests a generalization for autoxidation of cholesteryl esters in aqueous colloidal suspension. Saturated and monoethenoic fatty acyl esters of cholesterol have diminished susceptibility to autoxidation of the cholesteryl moiety compared with unesterified cholesterol, while diethenoic and other polyunsaturated fatty acyl esters of cholesterol in all probability do not have this diminished susceptibility.

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