Influence of Lecithins on Autoxidation of Cholesterol from Aqueous Dispersions at 85°

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Abstract □ The autoxidative formation of 7-ketocholesterol and 7-hydroxycholesterol from aqueous cholesterol dispersions was studied in the absence and in the presence of various lecithins at 85 ± 2°. Whereas more than one-half of the cholesterol is oxidized within 8 hr. in the absence of phospholipids, less than 10% oxidation occurs in the presence of the lecithins. The *in vivo* significance of these data is discussed.

Keyphrases □ Lecithins—influence on autoxidation of aqueous cholesterol dispersions □ Cholesterol dispersions, aqueous—influence of various lecithins on autoxidation at 85°, *in vivo* significance □ Autoxidation—aqueous cholesterol dispersions, influence of various lecithins, *in vivo* significance □ Phospholipids—influence of lecithins on autoxidation of aqueous cholesterol dispersions

Cholesterol is relatively stable toward air oxidation in the macrocrystalline state, but when finely divided and exposed to excess oxygen, oxidation takes place. These conditions are met, in particular, when cholesterol, in colloidal dispersion stabilized with sodium stearate, is aerated (1–3). The reaction is rapid at 85°, and the chief products are 7-ketocholesterol and diols, principally 7α- and 7β-hydroxycholesterol (3). Oxidation also occurs when cholesterol in thin films is exposed to air and irradiated with UV light (4).

It was found that the oxidation of cholesterol under physiological conditions of pH and temperature takes place, although at a slower rate. Bergström and Wintersteiner (2, 3) suggested that a conversion of cholesterol to the 7-oxygenated sterols might occur *in vivo* and that an attack on the sensitive 7-position of the cholesterol molecule might be involved in the biological degradation of this sterol. It has also been suggested that the oxidation of cholesterol *in vivo* may be related to the development of a variety of pathological conditions such as carcinoma (5), cholelithiasis, and atherosclerosis (6). In addition, MacDougall *et al.* (7) showed a number of oxidation products of cholesterol to be toxic to organ cultures of rabbit aorta.

In a recent series of papers (8–10), the autoxidation of cholesterol in aqueous dispersions and in monomolecular films was reported. The rate of oxidation of cholesterol was much faster at the surface than in the bulk. Although more than one-half of the cholesterol was oxidized at the surface within 8 hr. at room temperature, no noticeable reaction was observed for the oxidation of cholesterol from aqueous dispersions at room temperature during this time. However, similar rates of oxidation were observed when the dispersions were maintained at 85°.

Although it is conceivable that some cholesterol oxidation does occur *in vivo* (2, 3), these reaction rates must be significantly slower than those reported for *in vitro* systems. Surface pressure–surface area data for mixed cholesterol–phospholipid films (8) did indicate that the phospholipids present in the film protected the cholesterol from attack by oxygen. The mechanism of this protection appears to be physical rather than chemical in nature. Apparently, it involves intermolecular interactions between the phospholipids and cholesterol which serve to protect the reactive groups on the cholesterol molecule from attack by oxygen. Since membrane associated cholesterol is always associated with phospholipids, it appears likely that this association could be a significant factor in the *in vivo* protection of cholesterol oxidation.

It was, therefore, of interest to quantitate the effects of phospholipids on the air oxidation of cholesterol in aqueous dispersions.

EXPERIMENTAL

Two and one-half grams of cholesterol[1] was dissolved in 100 ml. of absolute alcohol with the aid of heat. The purity of cholesterol was verified by TLC (a 250-mcg. sample yielded a single spot). Sodium stearate, 0.5 g. (purified powder), was dissolved in 500 ml.

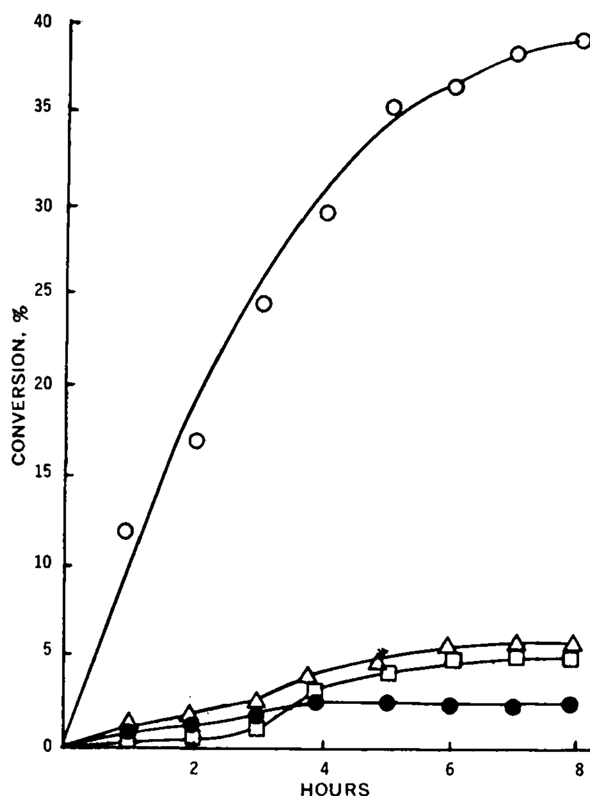

Figure 1—*Percentage conversion of cholesterol to 7-ketocholesterol for dispersions exposed to flowing air at 85 ± 2°. Key:* ○, *no lecithin;* ●, *vegetable lecithin;* □, *egg lecithin; and* △, *dipalmitoyl lecithin.*

[1] Pierce Chemical Co., Rockford, Ill.

of distilled water, which was preheated to 70°. The cholesterol solution in absolute alcohol was then poured into the sodium stearate solution with constant stirring to obtain a colloidal dispersion. Dry air was allowed to pass through a flowmeter at a rate of 100 ml./min. and was directed into the cholesterol dispersion, which was maintained at a temperature of 85 ± 2°. The air was allowed to flow for 8 hr., during which time samples of the cholesterol dispersion were removed with the aid of a micrometer syringe[2] at hourly intervals.

The amount of diols, expressed as 7-hydroxycholesterol, was determined by the method described by Bergström and Wintersteiner (3). Cholesterol dispersion, 0.03 ml., was mixed with 1 ml. of chloroform and 2 ml. of Lifschütz reagent. This color reagent was prepared by dissolving 0.1 g. of ferric chloride hexahydrate in 90 ml. of glacial acetic acid and then adding 10 ml. of concentrated sulfuric acid. After 5 min., a bluish-green color developed which remained stable for about 15 min. The absorbance was read at a wavelength of 590 nm. with a spectrophotometer[3], and the concentration of the diols was determined from the standard curve. The preparation of 7-hydroxycholesterol for use in obtaining the standard curve was described previously (9).

The amount of 7-ketocholesterol formed was determined by mixing 0.03 ml. of cholesterol dispersion and 3 ml. of ethanol. The absorbance was read at a wavelength of 256 nm. with the spectrophotometer[3]. The concentration was then determined from the standard curve of 7-ketocholesterol[4]. The purity of this standard was verified by TLC. The use of standard mixtures demonstrated that 7-hydroxycholesterol did not interfere with the analysis of 7-ketocholesterol and that the presence of 7-ketocholesterol did not interfere with the analysis of 7-hydroxycholesterol.

Similar experiments were performed for systems in which vegetable lecithin[5], egg lecithin[6], or dipalmitoyl lecithin[7] was added. In each case, the lecithin was dissolved with the cholesterol in 100 ml. of absolute alcohol to yield 1:1 cholesterol–lecithin molar ratios.

To correct for the presence of lecithins in the assays of 7-ketocholesterol and 7-hydroxycholesterol, standard curves for mixtures of the steroids and lecithins were used.

RESULTS AND DISCUSSION

Figures 1 and 2 show the percentage conversion–time curves for the conversion of cholesterol to 7-ketocholesterol and 7-hydroxycholesterol, respectively, in the absence and in the presence of the lecithins.

As can be seen, all lecithins studied are very effective inhibitors of cholesterol oxidation. Although the mechanism of inhibition cannot be stated with certainty, it appears to be physical rather than chemical in nature since: (*a*) the intermolecular interactions between cholesterol and lecithins are strong and involve the sensitive 3-hydroxy group of cholesterol, and (*b*) dipalmitoyl lecithin, a fully saturated phospholipid, is very effective in inhibiting oxidation.

Although the systems studied here certainly cannot be easily extrapolated to *in vivo* systems, the data indicate that a possible mechanism for the protection of membrane-bound cholesterol from oxidation may be the interactions with phospholipids.

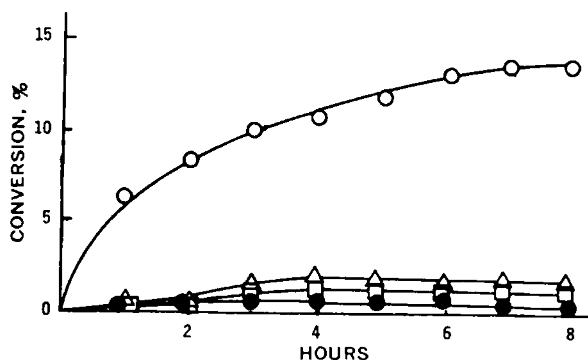

Figure 2—*Percentage conversion of cholesterol to 7-hydroxycholesterol for dispersions exposed to flowing air at 85 ± 2°. Key:* ○, *no lecithin;* ●, *vegetable lecithin;* □, *egg lecithin; and* △, *dipalmitoyl lecithin.*

[2] Agla, Burroughs Wellcome and Co., London, England.
[3] Beckman.
[4] Mann Research Laboratories, New York, N. Y.
[5] Ruger Chemical Co., Long Island City, N. Y.
[6] Becton Dickinson & Co., Orangeburg, N. Y.
[7] Mann Laboratories, Inc., New York, N. Y.

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