

Autoxidation of cholesterol in aqueous dispersions and in monomolecular films

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Abstract The autoxidative formation of 7-ketocholesterol and diols from aqueous cholesterol dispersions and from cholesterol monomolecular films has been studied as a function of time. The rate of oxidation of cholesterol is much faster at the surface than in the bulk. Whereas more than one-half of the cholesterol is 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 period. However, similar rates of oxidation were observed when the dispersions were maintained at 85°C.

Supplementary key words 7-ketocholesterol · 7-hydroxycholesterol

CHOLESTEROL is relatively stable towards 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°C, and the chief products are 7-ketocholesterol and diols, principally 7 α - and 7 β -hydroxycholesterol (3). Oxidation also occurs when cholesterol in thin films is exposed to air and irradiated with ultraviolet 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, Biswas, and Cook (7) have shown a

number of oxidation products of cholesterol to be toxic to organ cultures of rabbit aorta.

It is likely that the autoxidation of cholesterol under the many conditions described in the literature may not be characteristic of that which occurs under *in vivo* conditions, and particularly within the cell membrane. Monomolecular films have been used by numerous workers as a model membrane system and are generally assumed to simulate, though admittedly in a crude way, the conditions existing at the cell membrane (8). Therefore, the autoxidation of cholesterol spread as a monomolecular film should give some insight into the mechanism of this process at the cell membrane.

We (9) recently presented thin-layer chromatographic and surface pressure–surface area (π -A) evidence of autoxidation of cholesterol monomolecular films spread on an aqueous subphase. It is the purpose of this investigation to present more quantitative data on the autoxidation of cholesterol monomolecular films and to compare the rates of these reactions with those occurring in the bulk phase.

EXPERIMENTAL

Bulk studies

2.5 g of cholesterol (Pierce Chemical Co., Rockford, Ill.) was dissolved in 100 ml of absolute alcohol with the aid of heat. 0.5 g of sodium stearate (purified powder) was dissolved in 500 ml of distilled water, which was preheated to 70°C. The cholesterol solution in absolute alcohol was then poured into the sodium stearate solution with constant stirring so as 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 $85 \pm 2^\circ\text{C}$. The air was allowed to

flow for 10 hr, during which time samples of the cholesterol dispersion were removed with the aid of an Agla micrometer syringe (Burroughs Wellcome and Co., London, England) at hourly intervals.

The amount of diols, expressed as 7-hydroxycholesterol, was determined by the method described by Bergström and Wintersteiner (3). 0.03 ml of cholesterol dispersion was mixed with 1 ml of chloroform and 2 ml of Lifschütz reagent. This color reagent was prepared by dissolving 0.1 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 90 ml of glacial acetic acid and then adding 10 ml of concentrated sulfuric acid. After 5 min, a bluish green color develops which remains stable for about 15 min. The absorbance was read at a wavelength of 590 nm with a Beckman spectrophotometer, and the concentration of the diols was determined from the standard curve. The preparation of 7-hydroxycholesterol for use in obtaining the standard curve has been described previously (10).

The amount of 7-ketocholesterol formed was determined by mixing 0.03 ml of cholesterol dispersion and 3 ml of ethyl alcohol. The absorbance was read at a wavelength of 256 nm with a Beckman spectrophotometer. The concentration was then determined from the standard curve of 7-ketocholesterol obtained from Mann Research Laboratories, New York. The purity of this standard was verified by thin-layer chromatography. 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.

Surface studies

The apparatus for this phase of the study consisted of a surface balance and a torsion balance which have been described previously (11). Cholesterol spreading solutions were prepared by dissolving 7.2 mg of cholesterol in 10 ml of *n*-hexane (spectrograde). 0.7 ml of this solution was then spread at room temperature ($26 \pm 1^\circ\text{C}$) with the aid of an Agla micrometer syringe on a distilled water subphase so that the surface pressure was 2 ± 0.5 dynes/cm. Air, flowing at a rate of 100 ml/min, was allowed to flow through glass tubing connected to a short length of perforated Teflon tubing. The latter was affixed to the underside of a Lucite cover in a loop form to maintain the desired gaseous atmosphere over the spread films. The shape of the loop and the positions of the perforations allowed a uniform flow of the gases over the film surface.

The films under investigation were exposed to air for 1, 2, 4, 6, and 8 hr. At the end of each of these time periods, the entire film was removed. Removal of the films was accomplished by applying suction through a glass tube drawn to a fine tip and collecting the lipid

material along with a small volume of the subphase in a 50-ml side arm flask. In order to obtain large enough samples, at least two successive cholesterol films were collected and pooled for each time period.

The samples were then evaporated to dryness and the residues were dissolved in 3 ml of ethyl alcohol. The concentration of 7-ketocholesterol was determined as previously described. The samples were then warmed until the ethyl alcohol evaporated; they were redissolved in 1 ml of chloroform. 2 ml of Lifschütz reagent was added, and the concentration of diols was determined as previously described.

RESULTS AND DISCUSSION

The percentage conversion-time curves for the conversion of cholesterol in bulk dispersion to 7-ketocholesterol and diols are shown in Fig. 1. The amount of 7-ketocholesterol formed increased with time, reaching 44% at the end of 8 hr, while the amount of diols formed reached a level of 19% after 8 hr. These results appeared to be consistent with those of Bergström and Samuelsson (12) at the same temperature (85°C).

Fig. 2 shows the percentage conversion curves of spread films of cholesterol exposed to air. The amount of 7-ketocholesterol formed increased with time and reached a value of 46% at the end of 8 hr, while the

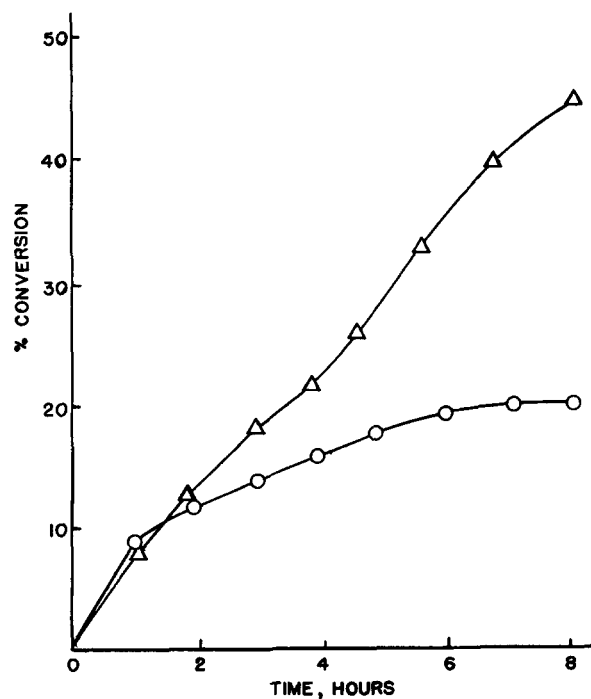


FIG. 1. Percentage conversion of cholesterol dispersions exposed to flowing air at $85 \pm 2^\circ\text{C}$. Key: O, diols; Δ , 7-ketocholesterol.

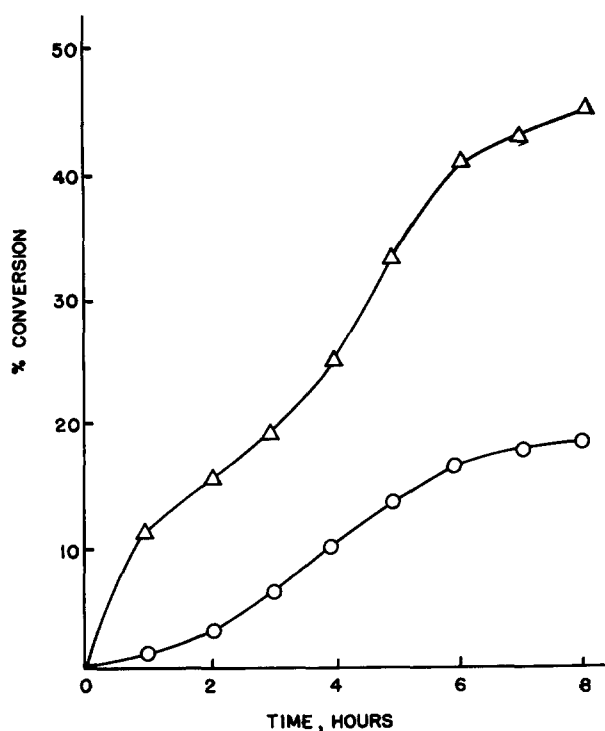


FIG. 2. Percentage conversion of cholesterol monomolecular films exposed to flowing air at $26 \pm 1^\circ\text{C}$. Key: O, diols; Δ , 7-ketocholesterol.

amount of diols formed reached a level of 17.5% at 8 hr.

When cholesterol dispersions were exposed to air for up to 8 hr at room temperature, no conversion was observed within experimental error ($\pm 1\%$). The reason for the large differences in rates in the bulk and surface reactions at room temperature may be due to the fact that at the surface, cholesterol is oriented in a manner so as to expedite attack by oxygen. An alternate explanation is that whereas in the bulk we are dealing with a heterogeneous system, in which only a fraction of the cholesterol molecules are exposed to the reactive gas at any instant, at the surface we are dealing with a concentrated two-dimensional solution where each molecule of cholesterol is in intimate contact with the reactive gas. In any event, whereas cholesterol dispersions are stable at room temperature (3, 13), cholesterol in the

film, which may be in an orientation similar to that found in biological membranes, is highly reactive.

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