

# A-Ring Oxidation Products from $\gamma$ -Irradiation of Cholesterol<sup>d</sup> in Liposomes

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$\gamma$ -Irradiation of cholesterol in multilamellar vesicles (MLV) at 0–4°C causes oxidation of the A-ring. Two A-ring oxides formed in considerable amounts are cholest-4-en-3-one (10) and cholest-4-ene-3,6-dione (12) in addition to the usual B-ring oxides. Lesser amounts of cholesta-4,6-dien-3-one (11) are also generated. Compounds 10 and 12 were detected and measured in cholesterol irradiated at less than 0.5 kGy in liposomes containing saturated or unsaturated phospholipids. Lesser amounts of 10 and 12, as well as lesser amounts of other cholesterol oxides, were formed when a major constituent of the MLV was dilinoleoyl-phosphatidylcholine. Autoxidation of cholesterol in MLV also gave rise to small amounts of 10, 11 and 12.

**KEY WORDS:** A-ring oxides, cholesterol, cholesterol oxides, irradiation, liposomes.

Further research has been carried out to explore the potential identification of irradiated meat and poultry by determining the effect that  $\gamma$ -radiation has on cholesterol. In continuation of previous work (1,2), these studies were carried out on cholesterol incorporated into multilamellar vesicles (MLV). In the earlier investigations, the emphasis was on cholesterol oxides that are more polar than the parent compound, *i.e.*, derivatives in which the B-ring of cholesterol has been oxidized and in which the A-ring retains its original  $\beta$ -hydroxy function. By contrast, the current work focuses on compounds in which cholesterol has undergone oxidation in the A-ring. These cholesterol oxidation products are less polar than cholesterol, *i.e.*, they migrate ahead of the parent compound in normal thin-layer chromatog-

raphy (TLC). As before, the effort in the current work was to distinguish the effect of  $\gamma$ -radiation from that of a potentially competing free-radical reaction: autoxidation.

In the compounds of principal interest in this study, the  $3\beta$ -hydroxy function of cholesterol has been oxidized to a 3-keto function, and the 5,6-double bond has moved to the 4,5-position, in conjugation with the carbonyl group. (For chemical and trivial names of cholesterol derivatives see Table 1. For structures of selected compounds see Fig. 1).

The simplest of these three compounds is cholest-4-en-3-one (10). It was first reported as an oxidation product of crystalline cholesterol in 1961 (3). Its separation from other cholesterol oxidation products by high-performance liquid chromatography (HPLC) (4) and by gas chromatography (GC) (5) has since been reported, and its C-13 nuclear magnetic resonance spectrum has been described (6). Enzymatic oxidation of cholesterol has long been known to yield cholest-5-en-3-one, which isomerizes readily to 10 (6,8). The enzymatic oxidation of cholesterol is efficient and has been used in attempts to lower the cholesterol content of foods, particularly milk (9,10).

A second A-ring oxidation product, cholesta-4,6-dien-3-one (11), also has been detected among the autoxidation products of crystalline cholesterol (3). Three different pathways for its formation in autoxidation have been suggested (3), all of which require the intermediacy of a hydroperoxide of cholesterol. This diene has been isolated from human aortic tissue, human plasma and pig spleen (3), and it has been characterized by capillary GC-mass spectrometry (MS) (11).

The third in this group of compounds is cholest-4-ene-3,6-dione (12). It is one of forty products that have been detected in air-oxidized solid cholesterol (3), and it has been partly

TABLE 1

Cholesterol Oxides Discussed<sup>a</sup>

Compound number	Chemical name	Trivial name	Purity (%)	TLC R <sub>r</sub>	GC response factor		GC RRT <sup>c</sup>
					<i>vs.</i> 5 $\alpha$ -Cholestane	<i>vs.</i> Desmostrol <sup>b</sup>	
1	5 $\alpha$ -Cholestane-3 $\beta$ ,5,6 $\beta$ -triol	Triol	98.0	0.03	1.60	1.32	2.30
2	Cholest-5-ene-3 $\beta$ ,7 $\alpha$ -diol	7 $\alpha$ -Hydroxycholesterol	97.5	0.10	1.33	1.17	1.70
3	Cholest-5-ene-3 $\beta$ ,7 $\beta$ -diol	7 $\beta$ -Hydroxycholesterol	98.4	0.14	1.48	1.14	1.73
4	3 $\beta$ -Hydroxy-5 $\alpha$ -cholestan-7-one	7-Ketocholestanol	99.5	0.22	N.D. <sup>d</sup>	1.08	1.80
5	5,6 $\alpha$ -Epoxy-5 $\alpha$ -cholestan-3 $\beta$ -ol	$\alpha$ -Epoxide	98.7	0.23	1.24	1.05	1.60
6	3 $\beta$ -Hydroxycholesta-5-en-7-one	7-Ketocholesterol	99.3	0.24	1.26	1.11	1.94
7	5,6 $\beta$ -Epoxy-5 $\alpha$ -cholestan-3 $\beta$ -ol	$\beta$ -Epoxide	97.9	0.25	1.34	1.09	1.56
8	3 $\beta$ -Hydroxy-5 $\alpha$ -cholestan-6-one	6-Ketocholestanol	97.2	0.26	1.29	1.10	1.88
9	Cholest-5-en-3 $\beta$ -ol	Cholesterol	98.5	0.48	1.18	0.97	1.33
10	Cholest-4-en-3-one	4-En-3-one	90.0	0.63	1.06	N.D.	1.55
11	Cholesta-4,6-dien-3-one	4,6-Dien-3-one	97.5	0.62	1.05	N.D.	1.61
12	Cholest-4-ene-3,6-dione	4-Ene-3,6-dione	93.9	0.68	1.45	N.D.	1.89

<sup>a</sup>TLC, thin-layer chromatography; GC, gas chromatography.

<sup>b</sup>Cholesta-5,24-dien-3 $\beta$ -ol.

<sup>c</sup>Relative retention time with respect to 5 $\alpha$ -cholestane.

<sup>d</sup>Not determined.

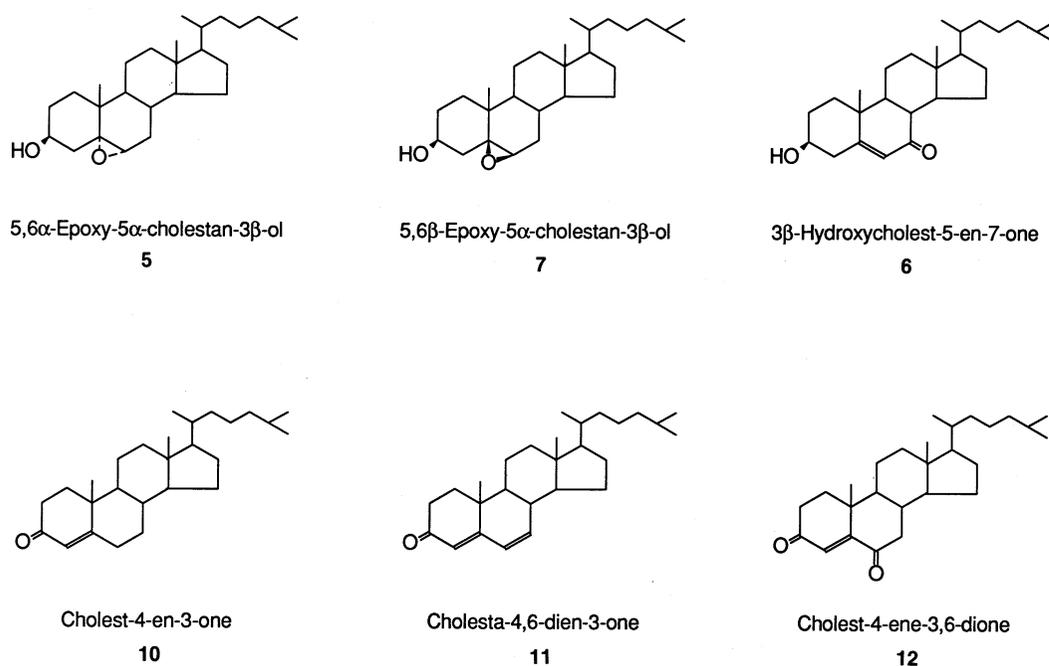


FIG. 1. Chemical structures of selected cholesterol oxides. For additional structures see Reference 13.

resolved from other cholesterol oxidation products by HPLC (4). In autoxidation it may be formed from 10 *via* the 6-hydroperoxide.

It is apparent that the above A-ring oxidation products are formed when solid cholesterol is subjected to air oxidation or to  $\gamma$ -irradiation in air (7). It is less clear whether such products are also formed by autoxidation of cholesterol in solutions or dispersions. Among the many recent studies concerned with autoxidation of cholesterol, the attention of the authors was focused on the common B-ring oxidation products including the isomeric 3,7-diols (2 and 3), the isomeric 5,6-epoxides (5 and 7) and 7-ketocholesterol (6) (12, 13). Exceptions have been the detection of 10 in heated butterfat (14) and in whole milk sterols stored for three months (15).

This work was undertaken to investigate the extent to which A-ring oxides are formed when cholesterol in MLV is exposed to low-dose ionizing radiation, and to compare the results with those obtained when cholesterol in MLV is subjected to autoxidation.

## EXPERIMENTAL PROCEDURES

**Materials and reagents.** Cholesterol (cholest-5-en-3 $\beta$ -ol, 9), 6-ketocholestanol (3 $\beta$ -hydroxy-5 $\alpha$ -cholestan-6-one, 8),  $\alpha$ -epoxide (5,6 $\alpha$ -epoxy-5 $\alpha$ -cholestan-3 $\beta$ -ol, 5), 5 $\alpha$ -cholestane and Sephadex G50-80 were purchased from Sigma Chemical Co. (St. Louis, MO),  $\beta$ -epoxide (5,6 $\beta$ -epoxy-5 $\alpha$ -cholestan-3 $\beta$ -ol, 7), 7 $\alpha$ -hydroxycholesterol (cholest-5-ene-3 $\beta$ ,7 $\alpha$ -diol, 2), 4,6-dien-3-one (cholesta-4,6-dien-3-one, 11), 7-ketocholestanol (3 $\beta$ -hydroxy-5 $\alpha$ -cholestan-7-one, 4) and 4-en-3,6-dione (cholest-4-ene-3,6-dione, 12) were from Research Plus, Inc. (Bayonne, NJ); 7 $\beta$ -hydroxycholesterol (cholest-5-ene-3 $\beta$ ,7 $\beta$ -diol, 3), triol (5 $\alpha$ -cholestane-3 $\beta$ ,5,6 $\beta$ -triol, 1) and 4-en-3-one (cholest-4-en-3-one, 10) were obtained from Ste-roids, Inc. (Wilton, NH). Dipalmitoylphosphatidylcholine

(PPPC), 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), dioleoylphosphatidylcholine (OOPC), dinoleoylphosphatidylcholine (LLPC) and dipalmitoylphosphatidylglycerol (PPPG) were purchased from Avanti Polar Lipids, Inc. (Pelham, AL). Celite 545 was obtained from Fisher Scientific (Malvern, PA) and TLC plates, silica gel GHL (250  $\mu$ m) from Analtech, Inc. (Newark, DE). All solvents used were "distilled-in-glass grade," and chemicals were reagent-grade quality. Water was double-deionized distilled in glass.

**Liposome preparation.** MLV were prepared from 96.0  $\mu$ moles of the appropriate phosphatidylcholine, 23.1 mg (32.0  $\mu$ moles) of PPPG and 24.8 mg (64.0  $\mu$ moles) of cholesterol, (9). The procedure used for the liposome preparation was described previously (1).

**Irradiation of cholesterol in liposomes.** Triplicate 1-mL aliquots of MLV were irradiated in a  $^{137}\text{Cs}$  source (0.114 kGy/min) at 0–4°C at dose levels of 0.48, 0.97 or 4.83 kGy. The dose rate was established with National Physical Laboratory (Middlesex, United Kingdom) dosimeters. Variations in dose were minimized by the use of small samples placed in a uniform portion of the radiation field. Samples were maintained at the desired temperature during irradiation by the injection of liquid nitrogen into the irradiation chamber. Controls consisted of unirradiated, refrigerated (0–4°C) aliquots. Controls and irradiated samples were extracted immediately after irradiation.

**Autoxidation of cholesterol in liposomes.** A liposome suspension (12 mL) was placed in a 50-mL screw-top test tube equipped with a magnetic stirring bar. The tube was sealed with air in the headspace. The tube was suspended in a waterbath kept at 45°C and stirred for 48 h. Unoxidized aliquots were kept at 0–4°C. Triplicate 1-mL portions of oxidized samples and unoxidized controls were analyzed.

*Dry column extraction of cholesterol oxides.* The procedure described previously (1) was followed.

*Isolation of cholesterol oxidation products.* Each dry-column eluate was evaporated to dryness with nitrogen. The residue was reconstituted in 250  $\mu\text{L}$  chloroform, and the solution was streaked on the middle 10-cm section, 2 cm above the lower edge, of a scored 20  $\times$  20 cm TLC plate. The plate had been prewashed with chloroform/methanol (2:1, vol/vol) and again with ethyl acetate and activated at 110°C. The residue container was rinsed with 150  $\mu\text{L}$  chloroform, and the rinse was streaked over the previously applied sample. The end sections of the scored plate were spotted with 2  $\mu\text{L}$  of an ethyl acetate solution containing 1  $\mu\text{g}/\mu\text{L}$  of each of the following cholesterol oxide standards: 4-en-3-one, 7-ketocholesterol, cholesterol, 7 $\alpha$ -hydroxycholesterol, 7 $\beta$ -hydroxycholesterol and 6-ketocholestanol. The plate was developed with benzene/ethyl acetate (60:40, vol/vol), and development continued for 10 min after the solvent front had reached the top of the plate. The end sections, intended for visualization, were snapped off and sprayed with 50% aqueous sulfuric acid and heated. These end sections were used to locate the A-ring oxidation products and the band containing 7-ketocholesterol and the two 5,6-epoxides in the middle section of the plate. For the A-ring oxidation products, a 4-cm wide band of the middle section, beginning 0.5 cm above cholesterol, was scraped. To isolate 7-ketocholesterol and the two epoxides, a 2-cm wide band was scraped centered on the doublet formed by the 7-ketocholesterol and 6-ketocholestanol standards. The two scraped bands were extracted separately with 2  $\times$  5 mL acetone. After each extraction, the mixtures were centrifuged to pellet the silica. The extracts from each band were pooled, filtered through a 2- $\mu\text{m}$  Nylon 66 filter and evaporated to dryness under nitrogen. To each residue was added 5 $\alpha$ -cholestane (8  $\mu\text{L}$ , 1  $\mu\text{g}/\mu\text{L}$  in ethyl acetate) as internal standard, and the solvent was removed under nitrogen. The samples were reconstituted in 200  $\mu\text{L}$  of ethyl acetate for gas-chromatographic analysis.

*Determination of cholesterol content of liposomes.* The method was described previously (1).

*GC.* The parameters have been described (1).

*GC-MS.* The GC-MS work was carried out under the conditions reported earlier (2), except that the initial injector temperature was 50°C. Direct-probe MS analyses were conducted with the same instrument and scan conditions as the GC work. Several micrograms of sample were placed into a glass capillary tube, which was inserted directly into the MS ion source and heated to vaporize the compounds. The heating ramp was 50 to 450°C at a rate of 10°C per second. GC-MS and MS were performed by the Center for Advanced Food Technology, Cook College, Rutgers University (New Brunswick, NJ).

*GC response factors.* Response factors of cholesterol and of ten cholesterol oxidation products relative to the internal standard 5 $\alpha$ -cholestane were determined by the method of Lee *et al.* (16). Response factors relative to desmosterol (cholesta-5,24-dien-3 $\beta$ -ol) were determined earlier (2).

## RESULTS AND DISCUSSION

Three A-ring oxidation products of cholesterol are considered in this manuscript. These are cholest-4-en-3-one

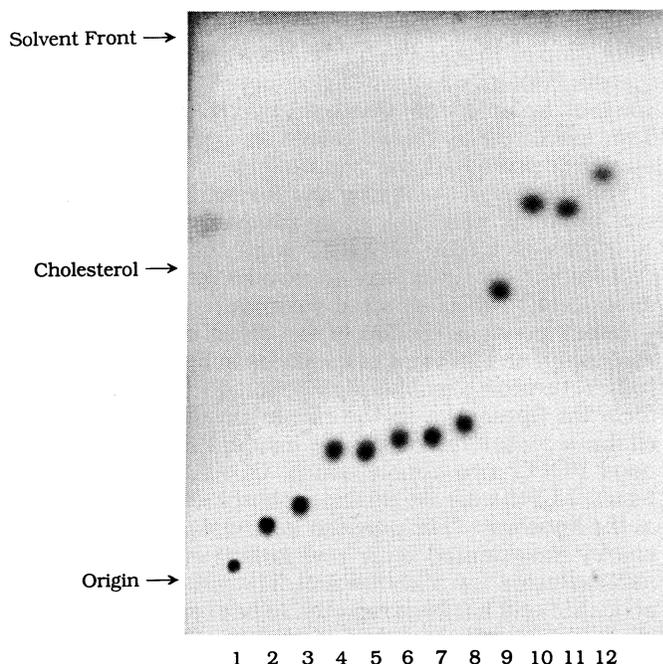


FIG. 2. Thin-layer chromatography profile of cholesterol oxide standards. Compound numbers on the abscissa are those of Table 1.

(10), cholesta-4,6-dien-3-one (11) and cholest-4-ene-3,6-dione (12). They are less polar than cholesterol (9) and its better-known B-ring oxidation products (1-8) and are readily separable from the latter by TLC (see Fig. 2). Although the A-ring oxides have long been known to be formed in the oxidation of crystalline cholesterol (4,5,7), scant attention has been given to their generation by cholesterol autoxidation in organic solvents or in aqueous suspensions (13). On the other hand, enzymatic oxidation of cholesterol to 10 and the physiological effects of this compound have been examined repeatedly (8,17-19).

To examine the formation of the A-ring oxides in the current study, we used isolation and quantitation procedures similar to those described earlier (1) for B-ring oxides. After an initial dry-column extraction of the irradiated, autoxidized or control liposomes, the solvent was removed from the eluate, and the residue was applied to a TLC plate. TLC was a crucial part of the procedure, because it permitted a clean separation of the A-ring from the B-ring oxides and from the bulk of the unchanged cholesterol as well as from residual phospholipids (and their potential radiolysis products) in one step. Because 85-95% of the cholesterol present in the irradiated liposomes remained unchanged, its removal before GC analysis was desirable to permit greater sensitivity in the measurement of the oxidation products. Removal of the relatively nonvolatile phospholipids before direct on-column injection of the samples was also necessary to prevent contamination of the capillary column.

With the GC conditions used, it was necessary to inject and measure the A-ring and the B-ring oxides separately. It can be seen from the relative retention times (GC/RRT—Table 1) that the  $\beta$ -epoxide (7) and cholest-4-en-3-one (10) are not resolved, nor are the  $\alpha$ -epoxide (5) and

cholesta-4,6-dien-3-one (11) or 6-ketocholestanol (8) and 4-en-3,6-dione (12). No attempt was made to improve the resolution of these pairs by modification of the GC parameters. Triol (1), a hydration product of the two epoxides, was not isolated and measured by GC, because its low TLC mobility (see Table 1) made its separation from the immobile phospholipids impractical.

The identity of the A-ring and B-ring cholesterol oxidation products was inferred by comparison of their TLC  $R_f$  values and their GC RRT with those of authentic standards. Identities were confirmed by GC-MS and by direct-probe MS of selected samples.

In our previous studies of the effect of  $\gamma$ -radiation on cholesterol and on some of its oxides in liposomes (1,2,20), fully saturated phosphatidylcholine, PPPC, was used to form the liposomes. In the current work, phosphatidylcholines containing no double bonds, PPPC, one double bond POPC, two double bonds, OOPC, or four double bonds, LLPC, were the principal phospholipid components of the liposomes. The purpose was to study the effect of nearby unsaturated fatty acyl groups on the radiation-induced oxidation of cholesterol. The rate of autoxidation of linoleic acid has been reported to be about 62 times that of oleic acid (21), and that of cholesterol has been estimated to lie somewhere between these two rates (22). It seems reasonable, but is not known, that this relationship might hold approximately for radiation-induced oxidation. If this were so, it suggests that the linoleoyl group, but perhaps not the oleoyl, is preferentially oxidized over cholesterol, *i.e.*, it might act as a protective agent that competes with cholesterol oxidation.

Formation of the liposomes was accompanied by increasing viscosity and opacity with increasing unsaturation of the suspensions after curing unsaturation. The more unsaturated MLV also passed through the molecular sieve column more slowly than the saturated, and the

finished preparations were more dilute with respect to cholesterol. Cholesterol concentrations of the various MLV are given later in Table 4.

Irradiation of cholesterol in saturated MLV gave rise to significant amounts of A-ring oxidation products (Table 2), in addition to the B-ring products reported previously (1). 4,6-Dien-3-one (11) is omitted from this tabulation because this compound was formed inconsistently in low concentrations at 4.83 kGy and only in traces at lower doses. At the highest dose, 4-en-3-one (10) is formed in larger amounts than 7-ketocholesterol (6), the oxide that predominates in the autoxidation reaction (see below), and 4-en-3,6-dione (12) approaches the level of the 7-ketone. Even at the lowest radiation dose of almost 0.5 kGy, the sum of 10 and 12 exceeds the amount of 6 generated.

Unsaturation in the lecithin acyl functions causes no great changes in the relative amounts of the products as is revealed by an inspection of the data presented in Table 3. However, the absolute amounts of the products formed decrease with increasing unsaturation. This is due, in part, to the lower cholesterol content of the unsaturated liposome preparations. For those MLV containing POPC or OOPC, the lower cholesterol content appears to be the principal cause, as can be shown by normalizing the product amounts to the cholesterol content of the liposomes (Table 4). In the irradiated liposomes containing LLPC, the amounts of cholesterol oxides formed are well below those expected from the cholesterol content. A reason may be that the linoleoyl groups, being more readily oxidized than cholesterol, in a sense protect cholesterol from radiation-initiated oxidation.

Lipid peroxidation in liposomes has been studied in detail and reviewed (23). It is generally assumed that the hydrophobic hydrocarbon phase of liposomes is segregated from the aqueous phase. However, the autoxidation of LLPC liposomes can be inhibited by water-soluble anti-

TABLE 2

Effect of  $\gamma$ -Radiation on Cholesterol in Liposomes Containing PPPC<sup>a</sup>

Dose kGy	4-En-3-one 10	4-En-3,6-dione 12	$\beta$ -Epoxide 7	$\alpha$ -Epoxide 5	7-Ketocholesterol 6
4.83	3.7 $\pm$ 0.9	2.1 $\pm$ 0.8	7.3 $\pm$ 1.5	1.7 $\pm$ 0.3	2.4 $\pm$ 1.2
0.97	0.9 $\pm$ 0.5	0.8 $\pm$ 0.3	5.5 $\pm$ 1.6	1.2 $\pm$ 0.3	1.7 $\pm$ 0.5
0.48	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	3.0 $\pm$ 1.0	0.7 $\pm$ 0.2	0.8 $\pm$ 0.1
none	—	—	trace	trace	trace

<sup>a</sup>n = 12; Amounts are  $\mu$ g/mL liposome suspension; PPPC, dipalmitoylphosphatidylcholine.

TABLE 3

Effect of  $\gamma$ -Radiation (4.83 kGy) on Cholesterol in Saturated and Unsaturated Liposomes<sup>a</sup>

Phospholipid <sup>b</sup>	4-En-3-one 10	4-Ene-3,6-dione 12	$\beta$ -Epoxide 7	$\alpha$ -Epoxide 5	7-Ketocholesterol 6
PPPC <sup>c</sup>	3.7 $\pm$ 0.9	2.1 $\pm$ 0.8	7.3 $\pm$ 1.5	1.7 $\pm$ 0.3	2.4 $\pm$ 1.2
POPC <sup>d</sup>	2.6 $\pm$ 1.0	1.3 $\pm$ 0.0	7.5 $\pm$ 2.1	2.3 $\pm$ 0.9	1.8 $\pm$ 0.4
OOPC <sup>d</sup>	2.1 $\pm$ 0.2	0.9 $\pm$ 0.0	6.4 $\pm$ 0.8	2.3 $\pm$ 0.5	0.9 $\pm$ 0.1
LLPC <sup>d</sup>	1.2 $\pm$ 0.5	0.4 $\pm$ 0.1	1.5 $\pm$ 0.2	0.6 $\pm$ 0.1	0.8 $\pm$ 0.1

<sup>a</sup>Amounts are in  $\mu$ g/mL liposome suspension.

<sup>b</sup>PPPC, dipalmitoylphosphatidylcholine; POPC, palmitoyloleoylphosphatidylcholine; OOPC, dioleoylphosphatidylcholine; LLPC, dilinoleoylphosphatidylcholine.

<sup>c</sup>n = 12.

<sup>d</sup>n = 6.

TABLE 4

Amounts of Cholesterol Oxides Produced by Irradiation as a Function of Liposome Cholesterol Content

Phospholipid <sup>a</sup>	Cholesterol content <sup>b</sup> mg/mL	4-En-3-one (%) 10	4-En-3,6-dione (%) 12	$\beta$ -Epoxide (%) 7	$\alpha$ -Epoxide (%) 5	7-Ketocholesterol (%) 6
PPPC	1.30	0.29	0.16	0.56	0.13	0.19
POPC	1.13	0.23	0.11	0.67	0.19	0.15
OOPC	0.878	0.24	0.10	0.73	0.26	0.10
LLPC	0.717	0.16	0.06	0.21	0.08	0.11

<sup>a</sup>See Table 3 for full names.<sup>b</sup>Of unirradiated liposomes.

oxidants (24). The implication is that in radiation-induced lipid oxidation, OH radical, a radiolysis product of water (25), could be involved in the initiation of the oxidation of linoleate as well as that of cholesterol. The involvement of OH radical in radiation-induced lipid hydroperoxide generation in liposomes has been demonstrated (26), and its involvement in the formation of cholesterol radiolysis products is suggested by the difference in cholesterol oxide composition patterns resulting from irradiation and autoxidation (see below). In the present study, the fatty acid composition of LLPC before and after irradiation was not analyzed.

Liposomes, containing PPPC and prepared with water in the same manner as those used for the radiation studies, were heated at 45°C for 48 h in the presence of air. The purpose was to discover whether cholesterol A-ring oxidation products are formed in liposomes under autoxidation conditions, and, if so, to compare their amounts relative to that of 7-ketocholesterol. After 48 h the amounts of products were (in  $\mu\text{g/mL}$ ): 4-en-3-one (10), 1.2; 4,6-dien-3-one (11), 1.1; 4-ene-3,6-dione (12), 2.2; 7-ketocholesterol (6), 30. On further heating in air, the amounts of all products increased, but 6 increased much faster than the A-ring products. When liposomes containing PPPC were prepared with phosphate buffer (pH 7.5), there was no evidence of autoxidation after 48 h, but after three days, autoxidation was proceeding, and the relative amounts of 6 and of the A-ring oxides were essentially as above. In liposomes prepared with LLPC, both buffered and unbuffered, autoxidation at 45°C proceeded normally, and while small amounts of A-ring oxides formed, the amounts of 7-ketocholesterol (6) generated far exceeded them.

Comparison of the irradiation and the autoxidation data indicates that A-ring oxides are formed by both processes, although in the irradiated samples, 4,6-dien-3-one (11) can be measured only at higher doses. The two processes differ significantly, however, in the amounts of A-ring derivatives relative to 7-ketocholesterol (6). The latter, of course, is well known to be the most prominent product of cholesterol autoxidation in liposomes (27,28) as well as in homogenous solution (29) and in aqueous dispersion (30). At any given point of autoxidation, the amount of 6 far exceeds that of 10 or 12, and as the process continues, the ratio 6/10 or 6/12 may exceed 100. In irradiated cholesterol, however, one often finds more 10 than 6, and the amount of 12 may approach or surpass that of 6.

## REFERENCES

- Maerker, G., and K.C. Jones, *Lipids* 26:139 (1991).
- Maerker, G., and K.C. Jones, *J. Am. Oil Chem. Soc.* 69:451 (1992).
- Smith, L.L., in *Cholesterol Autoxidation*, Plenum Press, New York, 1981.
- Smith, L.L., and G.A.S. Ansari, *J. Chromatogr.* 175:307 (1979).
- Korahani, V., J. Bascoul and A. Crastes de Paulet, *Ibid.* 211:392 (1981).
- Parish, E.J., H. Honda, S. Chitrakorn and P. Livant, *Lipids* 26:675 (1991).
- Ansari, G.A.S., and L.L. Smith, *Chem. Phys. Lipids* 22:55 (1978).
- Owen, R.W., A.N. Mason and R.F. Bilton, *J. Lipid Res.* 24:1500 (1983).
- Xiansheng, W., T.V. Hung, P.G. Drew and K. Versteeg, *Austral. J. Dairy Techn.* 45:50 (1990).
- Smith, M., C. Sullivan and N. Goodman, *J. Agric. Food Chem.* 39:2158 (1991).
- Brooks, C.J., W.J. Cole, T.D. Lawrie, J. Maclachlan, J.H. Borthwick and G.M. Barrett, *J. Steroid Biochem.* 19:189 (1983).
- Smith, L.L., *Chem. Phys. Lipids* 44:87 (1987).
- Maerker, G., *J. Am. Oil Chem. Soc.* 64:388 (1987).
- Amer, M.A., Abstract, *Ibid.* 63:452 (1986).
- Cleveland, M.Z., and N.D. Harris, *J. Food Protection* 50:867 (1987).
- Lee, M.L., F.Y. Yang and K.D. Bartle, in *Open Tubular Column Chromatography. Theory and Practice*, John Wiley & Sons, New York, 1984, p. 222.
- Starka, L., J. Tallova and K. Motlik, *Endocrinol. Exp.* 15:191 (1981).
- Kutryk, M.J.B., T.G. Maddaford, B. Ramjiawan and G.N. Pierce, *Circ. Res.* 68:18 (1991).
- Liu, K., B. Ramjiawan, M.J.B. Kutryk and G.N. Pierce, *Mol. Cell Biochem.* 108:49 (1991).
- Moriarty, N.J., and G. Maerker, *J. Am. Oil Chem. Soc.* 67:923 (1990).
- Pryor, W.A., in *Free Radicals in Biology*, Vol. I, edited by William A. Pryor, Academic Press, New York, 1976.
- Korahani, V., J. Bascoul and A. Crastes de Paulet, *Lipids* 17:703 (1982).
- Chatterjee, S.N., and S. Agarwal, *Free Radical Biol. Med.* 4:51 (1988).
- Barclay, L.R.C., S.J. Locke, J.M. MacNeil, J. van Kessel, G.W. Burton and K.U. Ingold, *J. Am. Chem. Soc.* 106:2479 (1984).
- von Sonntag, C., and H.-P. Schuchmann, *Angew. Chem., Int. Ed. Engl.* 30:1229 (1991).
- O'Connell, J.J., and A. Garner, *Int. J. Radiat. Biol.* 44:615 (1983).
- Terao, J., K. Sugino and S. Matsushita, *J. Nutr. Sci. Vitaminol.* 31:499 (1985).
- Lijana, R.C., M.S. McCracken and C.J. Rudolph, *Biochem. Biophys. Acta* 879:247 (1986).
- Muto, T.J. Tanaka, T. Miura and M. Kimura, *Chem. Pharm. Bull. (Tokyo)* 27:499 (1985).
- Kimura, M., Y. Jin and T. Sawaya, *Ibid.* 27:710 (1979).

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