

## Effect of Ionizing Radiation on Cholesterol in Aqueous Dispersion

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### ABSTRACT

Aqueous sodium stearate dispersions of cholesterol were irradiated at 0–2°C with absorbed doses ranging from 2.5 to 50 kGy. The resulting mixture of cholesterol derivatives was isolated and examined for 7-ketocholesterol and cholesterol 5 $\alpha$ ,6 $\alpha$ -epoxide and 5 $\beta$ ,6 $\beta$ -epoxide content. Concentrations of all three compounds increased with dose, while the ratio of 7-ketocholesterol to total epoxides decreased with increasing dose. The ratio of 7-ketocholesterol to the epoxides was approximately 1 or below at all dose levels while the same ratio in autoxidations of cholesterol in dispersions was normally 6 or greater. The change in the keto/epoxide ratio may be a means for determining whether meat or other foods containing cholesterol have been subjected to ionizing radiation.

### INTRODUCTION

THE EFFECT of ionizing radiation on fatty acids, lipids and fat-containing foods has been under investigation for over thirty years. Most of the work through 1977 has been summarized in two reviews (Nawar, 1977, 1983). The types of chemical changes initiated in lipids by ionizing irradiation are similar to those occurring in the autoxidation process. Both processes are initiated by free-radical generating species, and some of the products generated are common to both processes. However, because of the higher energy of the initiating species, radiation results in the formation of additional products not observed in autoxidation (Kucera et al., 1984).

Cholesterol is known to be sensitive to oxidation, and over 60 products resulting from autoxidation, photo-oxidation and enzymatic action have been described (Smith, 1981). Current knowledge of cholesterol autoxidation has been summarized in two recent reviews (Maerker, 1987; Smith, 1987).

Autoxidation of cholesterol has been studied in a variety of media including organic solvents (Muto et al., 1982), aqueous dispersions (Kimura et al., 1979) and absorbed monolayers (Weenen and Porter, 1982). A significant number of autoxidation studies have been carried out in aqueous sodium stearate dispersions of cholesterol, since this system may simulate the dispersed state of the sterol in aqueous fluid of animal tissue (Smith, 1981). Although quantitation was not always performed, previous researchers (Bergström, and Wintersteiner, 1942; Kimura et al., 1976) determined that under these conditions the predominant autoxidation product was 7-ketocholesterol (3 $\beta$ -hydroxycholest-5-en-7-one) and that the isomeric 5,6-epoxides (cholesterol-5 $\alpha$ , 6 $\alpha$ -epoxide and cholesterol 5 $\beta$ ,6 $\beta$ -epoxide) were produced in relatively lower quantities. Typically, the 7-keto/5,6-epoxide ratio in cholesterol autoxidation reactions in aqueous media ranged from six to ten.

Chromatographic resolution and direct quantitation of the isomeric cholesterol 5,6-epoxides are relatively recent developments. In earlier work the relative amounts of these isomers were determined by indirect means, e.g. by reduction to hydroxy derivatives. Despite these difficulties, several earlier researchers and some more recent ones (Gumulka et al., 1982;

Maerker and Bunick, 1986) determined that the  $\beta$ -epoxide isomer is produced in large excess over the alpha-isomer in autoxidation in aqueous dispersions or in solution. On the other hand, Ansari and Smith (1979) reported that hydroxyl radicals produced by radiolysis of aqueous cholesterol dispersions, generated more  $\alpha$ -epoxide than the  $\beta$ -isomer. Kucera et al. (1984) irradiated cholesterol emulsified in water/ethyl acetate at dose rates from 13 kGy to 120 kGy and determined the G values (number of molecules affected by 100 e.v. absorbed) of 7-ketocholesterol, the  $\alpha$ -epoxide and the  $\beta$ -epoxide to be 1.48, 0.26 and 0.24, respectively.

Except for two aforementioned studies, there do not appear to be any other relevant investigations on the effects of ionizing radiation on cholesterol in aqueous dispersions. This work was undertaken to determine whether the effects of ionizing radiation on cholesterol can be distinguished from those caused by autoxidation.

### MATERIALS & METHODS

#### Reagents

Cholesterol (99+%), 6-ketocholestanol, 7-ketocholesterol (5-cholesten-3- $\beta$ -ol-7-one) and cholesterol 5 $\alpha$ ,6 $\alpha$ -epoxide were purchased from Sigma Chemical Co. (St. Louis, MO). Cholesterol 5 $\beta$ ,6 $\beta$ -epoxide was prepared from cholesterol via 5 $\alpha$ -cholestane-3 $\beta$ ,5,6 $\beta$ -triol (Fieser and Rajagopalan, 1963) and the corresponding triacetate (Davis and Petrow, 1949) by the method of Chicoye et al. (1968). Labelled cholesterol (4-<sup>14</sup>C), 55mCi/mM was obtained from Amersham Corp. (Arlington Heights, IL). Stearic acid, technical grade, was purified by double recrystallization from acetone. Thin layer chromatography plates, silica gel GHL (250  $\mu$ m) were purchased from Analtech, Inc. (Newark, DE). Silicic acid, 100 mesh, was obtained from Mallinckrodt, Inc. (St. Louis, MO). All solvents used were "distilled in glass grade" and chemicals were of reagent grade quality. Water was double-deionized, glass-distilled.

#### Cholesterol dispersions

Stearic acid (500 mg), dissolved in 9 mL ethanol, was added to 300 mL of water at 80°C. Sodium hydroxide (0.1 N) was then added until the solution was clear. The solution was allowed to cool to room temperature and adjusted to pH 8.0. The solution was reheated to 80°C, covered with an atmosphere of nitrogen, and 300 mg of cholesterol dissolved in 6 mL ethanol was added slowly with stirring. When the dispersion had cooled to room temperature, 25 mL aliquots were transferred to 50 mL glass vials, covered with an atmosphere of nitrogen, capped with a Teflon lined screw cap and placed overnight in a refrigerator at 4°C.

#### Gamma radiation

Cholesterol dispersions were irradiated using a <sup>137</sup>Cs source (0.12 kGy/min) at 0°C to 2°C to the following dosage levels: 2.5, 5.0, 10, 20, 50 kGy. The samples were stored with a nonirradiated control in a refrigerator for analysis the following day.

#### Extraction and quantitation

To 25 mL aliquots of each of the cholesterol dispersions was added an internal standard (15  $\mu$ g 6-ketocholestanol dissolved in ethyl acetate). The samples were extracted three times in a separatory funnel with 25 mL of ethyl acetate. Combined extracts were dried over an-

hydrous sodium sulfate and filtered through washed glass wool. Solvent was removed under vacuum on a rotary evaporator. The samples were then reconstituted with a minimum volume of 10% ethyl acetate in cyclohexane (v/v) and added to a column (2.5 cm i.d.) containing 3 g silicic acid slurred in the same solvent mixture. The silicic acid, sea sand and glass wool were purified by extraction with ethyl acetate for 6 hr in a Soxhlet extractor, followed by activation of the silicic acid at 100°C for 10 hr. The silicic acid columns were eluted with 50 mL of 10% ethyl acetate in cyclohexane; this fraction contained the bulk of the cholesterol and was discarded. The cholesterol oxides were eluted from the column with 40 mL ethyl acetate. The eluate was evaporated under vacuum and reconstituted to 250  $\mu$ L with ethyl acetate. A portion of the solution of oxides (~150  $\mu$ L) was streaked onto a 20  $\times$  20 cm scored TLC plate [previously washed in chloroform/methanol 2/1, (v/v) and activated] and developed in a benzene/ethyl acetate/acetic acid (60/40/1, v/v/v) solvent system. The left and right scored sections, which had been spotted with standard solutions containing 6-ketocholestanol, 7-ketocholesterol and  $\alpha$ -epoxide, were snapped off, sprayed with 50% sulfuric acid and heated to visualize the region containing these cholesterol oxides. An area 1 cm above and below, adjacent to these spots, was scraped off the unsprayed center portion of the TLC plates and extracted with ethyl acetate and centrifuged. The supernatant was filtered through a 0.5  $\mu$ m FH Millipore filter to remove fines and any insoluble material. The samples were evaporated under nitrogen and reconstituted with 200  $\mu$ L ethyl acetate prior to gas chromatography.

#### Gas chromatography

Underivatized samples were analyzed by GC as described previously (Maerker and Unruh, 1986; Maerker and Bunick, 1986).

#### GC-mass spectroscopy

Gas chromatographic analysis was performed on a model 3400 Varian GC interfaced with a Finnigan 8230 mass spectrometer (magnetic sector). Separation was achieved using a J&W 30m DB 5 bonded phase column, 0.25  $\mu$  film with a 0.25 mm i.d. The same temperature program was used as described above. The data were processed using a Finnigan SS 300 data integrator. The mass spectral library used was that of the National Bureau of Standards. Mass spectral identification was carried out by Thomas G. Hartman at the Center for Advanced Food Technology, Cook College, Rutgers Univ., New Brunswick, NJ.

## RESULTS & DISCUSSION

SINCE it was intended to irradiate aqueous dispersions of cholesterol at 0–4°C, the stability of these dispersions near the freezing point of water was of concern. Previous workers studied aqueous stearate dispersions of cholesterol in the 50–85°C range (Maerker and Bunick, 1986; Kimura et al., 1976; Kimura et al., 1979; Korahani et al., 1982) but not at lower temperatures. Ansari and Smith (1979) prepared aqueous dispersions of cholesterol without dispersing agent. These required filtration after standing overnight at room temperature and precipitated particulate cholesterol after  $^{60}\text{Co}$  irradiation for 16–24 hr at an unspecified temperature.

In the current study, attempts were made to assess the stability of aqueous sodium stearate dispersions of cholesterol adjusted to pH 8, at room temperature and below. These dispersions were prepared by the procedure described in the Experimental section, and all contained 1 mg/mL stearic acid. The cholesterol concentration of these dispersions ranged from 0.5 mg/mL to 1.2 mg/mL and all contained  $^{14}\text{C}$  labelled cholesterol. Centrifugation of a 0.5 mg/mL dispersion for 20 min at 1000 rpm at room temperature gave no apparent precipitate and showed no loss of cholesterol from the supernatant. There was also no cholesterol loss when a 1.2 mg/mL dispersion was stored at room temperature for 24 hr and then centrifuged at 2500 rpm for 20 min. On the other hand, when the more concentrated dispersion was stored at 4°C for 12 hr and then centrifuged at 4°C for 10 min at 1000 rpm, a 14% loss of activity (cholesterol) in the supernatant was observed. This indicated that near the freezing point of water some cholesterol

separated from the dispersion. A 14% loss from the supernatant also occurred when the dispersion that had been kept at 4°C was cooled further to –20°C and was then brought back to 4°C and centrifuged. Cooling the 4°C solution to –80°C for 2 hr and returning it to 4°C resulted in a 19% loss after centrifugation. These cooling experiments provided an idea of how a temporary or local loss of temperature control in the liquid nitrogen cooled irradiator might affect the stability of the dispersion. It was concluded that actual freezing of the dispersion was not more injurious to its stability than just cooling to 4°C. Most subsequent experiments of this study were carried out at a dispersion cholesterol concentration of 1 mg/mL.

Previous authors have studied a number of different surface active agents as dispersants in cholesterol oxidation studies at elevated temperatures (Kimura et al., 1976; Maerker and Bunick, 1986). A number of nonionic surfactants were evaluated here to test their effectiveness at low temperatures. The most effective of those tested was Tween 60 at a 1% (10 mg/mL) level. However, use of this surfactant was not pursued, because a minor component of this surfactant interfered with the determination of the cholesterol 5,6-epoxides. Sodium stearate was considered superior to the nonionic surfactants tested and was used in all subsequent experiments.

While autoxidation of cholesterol in aqueous dispersions typically proceeds until 60% or more of the cholesterol present has been converted to products (Kimura et al., 1979), much lower yields of products can be expected when such dispersions are subjected to low-dose irradiation. For instance, Ansari and Smith (1979) obtained an estimated 1% conversion by irradiation of a dispersion sparged with  $\text{N}_2\text{O}$ . In this study, low conversions to products required removal of the bulk of the stearic acid (1.67 mg/mL) and the unreacted cholesterol (essentially 1 mg/mL) present in the irradiated dispersion, before the small amounts of cholesterol derivatives (perhaps 1–10  $\mu\text{g/mL}$  dispersion) could be resolved adequately by TLC. Adsorption chromatography was the method of choice and several adsorbents, e.g., Florisil, various types of alumina, were tested but were found inferior to silicic acid. The latter adsorbent permitted a sharp separation between stearic acid and cholesterol eluted with cyclohexane: ethyl acetate (9:1, v/v) and the cholesterol oxides eluted with ethyl acetate. Careful pre-extraction with ethyl acetate of the adsorbent and all the column materials (sand, glass wool) in a Soxhlet extractor was necessary to prevent introduction of artifacts into the sample. Studies indicated that with the use of this procedure 95% of the cholesterol 5,6-epoxides and 92% of 6-ketocholestanol (internal standard) were recovered.

Several of the cholesterol oxides generated by irradiation in aqueous dispersion are identical with those formed by autoxidation (Ansari and Smith, 1979). It was therefore necessary to evaluate the extent to which autoxidation might occur while cholesterol was being irradiated. We subjected a cholesterol dispersion, formed as described in the Experimental section, to a flow of air (Maerker and Bunick, 1986) at 0–4°C. The dispersion was sampled periodically over a period of 2 weeks during which it showed no signs of physical break-down. Work-up of the samples gave no detectable amounts of cholesterol oxidation products after 2 weeks. It was concluded that autoxidation at 0–4°C was so slow that it was not likely to compete with irradiation which required about 7 hr to complete at the highest dosage used.

It has been reported (Gumulka et al., 1982) that irradiation of sodium stearate dispersions of cholesterol gives rise to a distinctly different ratio of  $\alpha$ -epoxide to  $\beta$ -epoxide (3.5) than that obtained by autoxidation (0.1). If confirmed, such a large difference in  $\alpha/\beta$ -epoxide ratio might be useful in distinguishing between products formed as a result of ionizing radiation and those resulting from autoxidation. Sevanian and McLeod (1987), who irradiated unilamellar vesicles containing cholesterol, reported data that were in conflict with the earlier find-

ings. Their  $\alpha/\beta$ -epoxide ratio was 0.5-1.0, slightly higher than the 0.3-0.4 reported to have been obtained by autoxidation in dispersions (Maerker and Bunick, 1986), but only marginally so. We have determined the concentrations of  $\alpha$ -epoxide and  $\beta$ -epoxide in irradiated aqueous sodium stearate dispersions of cholesterol and have calculated  $\alpha/\beta$ -epoxide ratios (see Table 1). Our ratios were generally below 1.0 and mostly in the 0.3-0.4 range. We, therefore, consider epoxide ratios of irradiated dispersions insufficiently different from those of oxidized dispersions to permit a clear distinction between the two processes.

The ratio of 7-ketocholesterol (3 $\beta$ -hydroxycholest-5-en-7-one) to total cholesterol 5,6 epoxide ( $\alpha + \beta$ ) is quite another matter, however. In the earliest studies of the autoxidation of aqueous cholesterol (Bergström and Wintersteiner, 1942), it was recognized that 7-ketocholesterol was the major component among the oxidation products and that its concentration was approximately twice that of the next most prominent component, the mixture of isomeric 7-hydroxycholesterols (cholest-5-ene-3- $\beta$ -7 $\beta$ -diols). Later workers confirmed these relative amounts of 7-ketocholesterol and 7-hydroxycholesterol (Smith, 1981; Kimura et al., 1979; Zulak and Maerker, 1989). They also determined that the isomeric 5,6-epoxides were generated in much lesser amounts than either of the major components, so that the ratio of 7-ketocholesterol to the sum of the two 5,6-epoxides ( $\alpha + \beta$ ) was about 6-10 (Kimura et al., 1979; Terao et al., 1985; Zulak and Maerker, 1989). By contrast, both Ansari and Smith (1979) and Sevanian and McLeod (1987) irradiated cholesterol in aqueous environments and obtained slightly more 5,6-epoxides than 7-ketocholesterol, i.e., a ratio of 1. Ratios of 7-ketocholesterol to total 5,6-epoxides in the present study are shown in Table 2. It is apparent from these data that the ratio is close to 1 at low doses and decreases as the dose increases.

The recognition that the ratio of concentrations of 7-ketocholesterol/total epoxycholestanols is substantially different for irradiated cholesterol (ratio = < 1) than for autoxidized cholesterol (ratio = > 6) has not been pointed out previously. This is a significant finding, because it implies the possibility of distinguishing experimentally between cholesterol that has been subjected to autoxidation (and perhaps photooxidation) from cholesterol that has been irradiated. It is also conceivable that the difference in ratios might be employed in the food field to distinguish cholesterol containing foods that have been irradiated from those that have been allowed to autoxidize, if the results obtained in dispersions can be translated to the more highly organized biological systems. This may be of some importance to regulatory agencies.

While it is apparent on inspection that the 7-keto/total epoxide ratio is different for cholesterol irradiation than it is for autoxidation, the relationship between that ratio and the absorbed dose is less clear. Statistical analysis (ANOVA) of the data obtained from the analysis of the 6 sets of cholesterol samples (1 mg/mL), irradiated at 2.5, 5.0, 10, 20, 50 kGy and at pH 8, indicated that there was a highly significant ( $p < 0.01$ ) correlation between dose and the increase in the concentration of both the  $\alpha$ -epoxide and the  $\beta$ -epoxide. A significant ( $p < 0.05$ ) increase in the levels of the 7-ketocholesterol formed as a result of irradiation was also determined. Linear regression of the concentration versus dose is presented in Table 3. Analy-

Table 1—Ratios of cholesterol 5 $\alpha$ ,6 $\alpha$ -epoxide / cholesterol 5 $\beta$ ,6 $\beta$ -epoxide resulting from irradiation

Study #	Absorbed dosage (kGy)				
	2.5	5.0	10	20	50
1	0.3	0.4	0.3	0.4	0.2
2	0.5	0.4	0.4	0.3	0.3
3	0.7	1.0	0.5	0.7	0.2
4	0.4	0.9	1.1	N.D.	0.6
5	N.D.*	0.3	0.3	0.3	0.4
6	0.5	N.D.*	0.4	0.3	0.2

\* N.D. = Not determined.

Table 2—Ratios of concentrations of 3 $\beta$ -hydroxycholest-5-en-7-one / cholesterol 5 $\alpha$ ,6 $\alpha$ -epoxide + cholesterol 5 $\beta$ ,6 $\beta$ -epoxide

Study #	Absorbed dosage (kGy)				
	2.5	5.0	10	20	50
1	0.5	0.4	0.4	0.4	0.2
2	0.8	0.7	0.4	0.4	0.2
3	1.2	1.1	0.4	1.0	0.4
4	1.5	1.2	0.4	N.D.*	0.2
5	N.D.*	1.0	0.7	0.8	0.6
6	1.1	N.D.*	1.0	0.7	0.5

\* N.D. = Not Determined.

Table 3—Linear regression analysis of the concentration of cholesterol derivatives versus the absorbed dose yields

5,6-alpha-Epoxide
Concentration = 4339.5 $\times$ dose + 38301
$r^2 = 0.623$ , $n = 24$ , $p < 0.01$
5,6-beta-Epoxide
Concentration = 18504 $\times$ dose + 45627
$r^2 = 0.710$ , $n = 24$ , $p < 0.01$
7-Ketocholesterol
Concentration = 6485.6 $\times$ dose + 120335
$r^2 = 0.443$ , $n = 24$ , $p < 0.01$

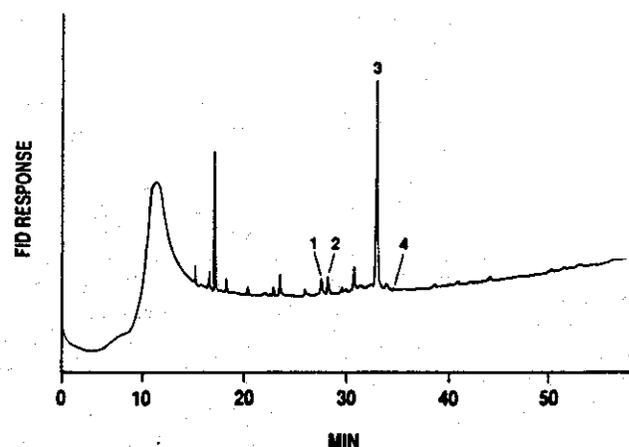


Fig. 1—Cholesterol oxides in nonirradiated cholesterol dispersion. GC trace of highly concentrated extract from TLC band. Peak identities are as follows: (1) cholesterol 5 $\beta$ ,6 $\beta$ -epoxide; (2) cholesterol 5 $\alpha$ ,6 $\alpha$ -epoxide; (3) 6-ketocholestanol [internal Standard]; (4) 7-ketocholesterol.

sis of the data of Table 2, i.e., ratio vs absorbed dose, was performed as well. ANOVA and regression analysis indicated highly significant negative correlation of ratios with dose.

Difficulties in reproducibility between studies were encountered, especially in the 2.5 to 10 kGy range as is evident from the data in Table 2. This is partly due to low conversion to products and partly due to the need for further refinement of the analytical technique. While rigorous quantitation was not attempted, it was estimated that at the highest dose employed, 50 kGy, cholesterol conversion to products was probably less than 1%. At 2.5 kGy conversions were, of course, much lower. At such low conversions it was difficult to concentrate products sufficiently, by the techniques employed, to obtain an accurate measure of the desired compounds. At 50 kGy, on the other hand, it became apparent that along with 7-ketocholesterol and the isomeric 5,6-epoxides, many additional compounds were generated by the irradiation of cholesterol. This can be seen from a comparison of Fig. 1 and Fig. 2. Figure 1 is the GC trace of a highly concentrated sample from a TLC scraping of the 7-keto/epoxide band from a non-irradiated cholesterol dispersion. Figure 2 is the equivalent sample from a cholesterol dispersion irradiated at 50 kGy. It is clear that irradiation generated many more compounds than those which were deter-

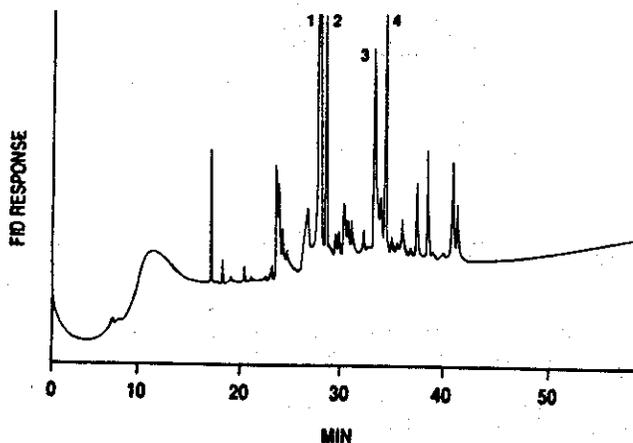


Fig. 2—Cholesterol oxides in cholesterol dispersion irradiated at 50 kGy - GC trace of extract from TLC band. Peak identities are as follows: (1) cholesterol 5 $\beta$ ,6 $\beta$ -epoxide; (2) cholesterol 5 $\alpha$ ,6 $\alpha$ -epoxide; (3) 6-ketocholestanol [Internal Standard]; (4) 7-ketocholesterol.

mined. The compounds seen in Fig. 2 only represent those that have  $R_f$  values similar to that of 7-ketocholesterol and are contained in the narrow TLC band scraped.

To examine the possibility that some or many of the extraneous compounds seen were derived from stearic acid, a dispersion of the latter, free of cholesterol, was irradiated at 50 kGy and then isolated and quantitated in the same manner as dispersions containing cholesterol. GC analysis demonstrated the absence of stearic acid derivatives with retention times of 7-ketocholesterol or the epoxides. In addition the identities of the  $\alpha$ - and  $\beta$ -epoxides and the 7-ketocholesterol formed as a result of the irradiation of cholesterol were confirmed by co-chromatography of the irradiated samples with authentic standards on capillary GC. GC-MS also confirmed the identities of the epoxides and of 7-ketocholesterol.

Quantitation of the isomeric epoxides and of 7-ketocholesterol was accomplished by use of 6-ketocholestanol as internal standard (Maerker and Unruh, 1986). Detector response vs concentration for the four compounds was linear and response factors compared to 6-ketocholestanol for 7-ketocholesterol,  $\alpha$ -epoxide and  $\beta$ -epoxide were 1.09, 1.10 and 0.94, respectively.

In the autoxidation of cholesterol in aqueous dispersion the first oxidation product formed is the 7-hydroperoxide from which both 7-ketocholesterol and the isomeric epoxides are formed, although by different pathways (Maerker, 1987). In the irradiation of cholesterol in aqueous dispersion, the pathway leading to 7-ketocholesterol and the 5,6-epoxides is less well known, although the hydroxyl radical generated in the aqueous phase is thought to be involved (Ansari and Smith, 1979). The same authors pointed out the absence of 7-hydroperoxycholesterol among the cholesterol irradiation products. Irradiated cholesterol samples were specifically examined for the presence of hydroperoxides, especially 7-hydroperoxides, with the use of TLC. No hydroperoxides were detected by spraying with Wurster dyes (Smith and Hill, 1972) or by sulfuric acid spraying and charring. It is unlikely that 7-hydroperoxycholesterol is an intermediate in the formation of 7-ketocholesterol and cholesterol 5,6-epoxides.

The observation (Table 2) that the ratio of concentrations of 7-ketocholesterol to 5,6-epoxides decreased with increasing dose suggested the possibility that 7-ketocholesterol was less stable to irradiation than the 5,6-epoxides. Sodium stearate dispersions containing 7-ketocholesterol,  $\alpha$ -epoxide or  $\beta$ -epoxide individually (each at 0.5 mg/mL) and free of cholesterol were subjected to 50 kGy (absorbed dose) radiation. The irradiated dispersions after extraction with ethyl acetate were streaked on

TLC plates, the plates were developed as usual, the areas from cholesterol to the origin were scraped, and the scrapings were extracted and analyzed by GC. Although the data were not suitable for quantitation, it was apparent from inspection of the GC traces that all three compounds had given rise to additional derivatives on exposure to radiation. The order of degradation by radiation seemed to be 7-ketocholesterol >  $\beta$ -epoxide >  $\alpha$ -epoxide judging from the number and size of the new peaks generated. It is not known, and at present there is insufficient information to judge, whether degradation of initial products on further irradiation is responsible for the decreasing ratio of 7-ketocholesterol to total 5,6-epoxides as a function of dosage.

The information developed in this study suggests that cholesterol might be useful in the determination of whether an animal-derived food had a past history of exposure to ionizing radiation. This problem will be investigated further in this laboratory in the future.

## References

- Ansari, G. A. S. and Smith, L. L. 1979. The oxidation of cholesterol by hydroxyl radical. *Photochem. Photobiol.* 30: 147.
- Bergström, S. and Wintersteiner, O. 1942. Autoxidation of sterols in colloidal aqueous solution. III. Quantitative studies on cholesterol. *J. Biol. Chem.* 145: 309.
- Chicoye, E., Powrie, W. D., and Fennema, O. 1968. Isolation and characterization of cholesterol 5 $\beta$ ,6 $\beta$ -oxide from an aerated aqueous dispersion of cholesterol. *Lipids* 3: 335.
- Davis, M. and Petrow, V. V. 1949. Steroids and related compounds. Part VI. The stereochemical configuration and dehydration of the isomeric androstane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol-ones. *J. Chem. Soc.* p. 2536.
- Fieser, L. F. and Rajagopalan, S. 1963. Selective oxidation with *n*-bromosuccinimide. II. Cholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol. *J. Am. Chem. Soc.* 71: 3938.
- Gumulka, J., St. Pyrek, J., and Smith, L. L. 1982. Interception of discrete oxygen species in aqueous media by cholesterol: Formation of cholesterol epoxides and secosterols. *Lipids* 17: 197.
- Kimura, M., Jin, Y., and Sawaya, T. 1979. Autoxidation of cholesterol and behavior of its hydroperoxide in aqueous medium. *Chem. Pharm. Bull.* 27: 710.
- Kimura, M., Kawata, M., and Sawaya, T. 1976. Autoxidation of cholesterol in aqueous colloidal dispersions with different detergents. *Chem. Pharm. Bull.* 24: 2258.
- Korahani, V., Bascoul, J., and Crastes de Paulet, A. 1982. Autoxidation of cholesterol fatty acid esters in solid state and aqueous dispersion. *Lipids* 17: 703.
- Kučera, J., Schwarz, V., and Sykora, M. 1984. Radiation-induced reactions of cholesterol in an aqueous medium. *J. Radioanal. Nucl. Chem.* 87: 219.
- Maerker, G. 1987. Cholesterol autoxidation. *Current status.* *J. Am. Oil Chem. Soc.* 64: 388.
- Maerker, G. and Bunick, F. J. 1986. Cholesterol oxides II. Measurement of the 5,6-epoxides during cholesterol oxidation in aqueous dispersions. *J. Am. Oil Chem. Soc.* 63: 771.
- Maerker, G. and Unruh, J. Jr. 1986. Cholesterol oxides I. Isolation and determination of some cholesterol oxidation products. *J. Am. Oil Chem. Soc.* 63: 767.
- Muto, T., Tanaka, J., Miura, T., and Kimura, M. 1982. Iron-catalyzed autoxidation of cholesterol in the presence of unsaturated long-chain fatty acid. *Chem. Pharm. Bull.* 30: 3172.
- Nawar, W. W. 1977. Radiation chemistry of lipids. In "Radiochemistry of Major Food Components," P. S. Elias and A. J. Cohen (Ed.), Ch. 3, p. 21. Elsevier Scientific Publishing Co., New York, NY.
- Nawar, W. W. 1983. Radiolysis of nonaqueous components of foods. In "Preservation of Food by Ionizing Radiation," E. S. Josephson and M. S. Peterson (Ed.), Vol. II, Ch. 2, CRC Press, Inc., Boca Raton, FL.
- Sevanian, A. and McLeod, L. L. 1987. Cholesterol autoxidation in phospholipid membrane bilayers. *Lipids* 22: 627.
- Smith, L. L. and Hill, F. L. 1972. Detection of sterol hydroperoxides on thin layer chromatography by means of Wurster dyes. *J. Chromatogr.* 66: 101.
- Smith, L. L. 1981. "Cholesterol Autoxidation." Plenum Press, New York.
- Smith, L. L. 1987. Cholesterol autoxidation 1981-1986. *Chem. Phys. Lipids* 44: 87.
- Terao, J., Sugino, K., and Matsushita, S. 1985. Fe<sup>2+</sup> and ascorbic acid induced oxidation of cholesterol in phosphatidylcholine liposomes and its inhibition by  $\alpha$ -tocopherol. *J. Nutr. Sci. Vitaminol.* 31: 499.
- Weenen, H. and Porter, N. A. 1982. Autoxidation of model membrane systems: Cooxidation of polyunsaturated lecithins with sterols, fatty acids, and  $\alpha$ -tocopherol. *J. Am. Chem. Soc.* 104: 5216.
- Zulak, I. M. and Maerker, G. 1989. Cholesterol Oxides III. Autoxidation of cholesterol in sodium stearate and sodium linoleate dispersions. *J. Am. Oil Chem. Soc.* Submitted.

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