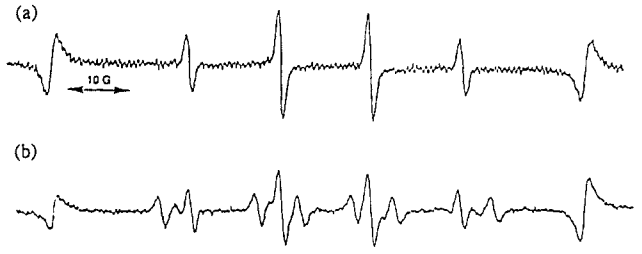

Figure 1. ESR spectrum of the hydroxyl radical spin adduct of DMPO produced during photoirradiation of hydroperoxide **7**. (a) The sample solution containing 1 μL of hydroperoxide **7** (10 mM) in acetonitrile, 5 μL of DMPO (29.2 mM), 10 μL of sodium cacodylate (20 mM), and 84 μL of water was irradiated with transilluminator (302 nm) at ambient temperature for 10 min. An aliquot (5 μL) of the solution was taken up in a capillary, and the ESR was measured at room temperature. The magnetic fields were calculated by the splitting of Mn^{2+} in MnO (ΔH_{3-4} = 86.9 G) (signals at both ends). The 1:2:2:1 pattern of four lines can be interpreted in terms of equivalent hyperfine splitting constants (a_N = a_H = 14.8 G, g = 2.0058) due to both the nitroxide nitrogen and the β-hydrogen.[14] (b) Addition of ethanol (2.5 mM), a hydroxyl radical scavenger, to the reaction system described above collapsed the signals of the DMPO–OH adduct, resulting in the production of signals due to $CH_3\dot{C}HOH$. Similar ESR spectra were obtained in the photoirradiation of **2** and **9**.

position is consistent with the free-radical oxidation initiated by hydroxyl radical.[11] The photooxidation of cyclododecane with **2** was almost completely inhibited by the addition of typical hydroxyl radical scavengers such as 2-propanol or dimethyl sulfoxide.[12] A similar photooxidation was also observed with **5**, **7**, and **9**. Irradiation of **7** (2 mM) and adamantane (10 mM) in acetonitrile for 7 h under similar conditions provided 1-adamantanol (28%), 2-adamantanol (9%), and adamantanone (5%) together with formation of **11** (85%).[13] In contrast, irradiation of **3** or **6** in the presence of adamantane produced less than 10% of the total oxidation products obtained in the photooxidation using **2** under identical conditions. The generation of hydroxyl radical from **2** and **7** was further confirmed by an ESR spin trapping method using 5,5-dimethylpyrroline *N*-oxide (DMPO). As shown in Figure 1, the only detectable ESR signals were those assignable to the hydroxyl radical–DMPO spin adduct. These results clearly indicate that phthalimides possessing a secondary hydroperoxy group at the γ-position are excellent photochemical hydroxyl radical generators.

We then examined the DNA cleaving activity of these hydroperoxides by using supercoiled circular φX 174 RFI DNA (form I). When a solution of form I DNA and hydroperoxide **2** or **5** was irradiated with transilluminator (302 nm) at 0 °C, single-strand and, to a lesser extent, double-strand breaks were observed, as evidenced by the production of form II (relaxed circular) and form III (linear) DNA, respectively (Table I). Hydroperoxides **7** and **9** also cleaved DNA efficiently. Particularly, **9** cleaved DNA at only 1 mM concentration upon irradiation with 366-nm light at 0 °C, whereas **3** and **6** induced DNA cleavage at more than 200 mM concentrations by 302-nm irradiation.

The present work has demonstrated a unimolecular generation of hydroxyl radical from readily available phthalimide hydroperoxides with long-wavelength light (>300 nm) and their use as a photochemical DNA cleaver. Design of a new class of bifunctional molecules comprising a DNA binding component linked to phthalimide hydroperoxide, as well as an examination of the mechanistic aspects of the photochemistry of these hydroperoxides, is underway in our laboratory.

Table I. Cleavage of Supercoiled Circular φX 174 RFI DNA (Form I) into Nicked Circular DNA (Form II) and Linear DNA (Form III) by Photoirradiation of Hydroperoxides **2** and **5**[a]

hydroperoxide	concn, μM	% form I[a]	% form II[b]	% form III[b]
2	200	44.9	55.1	
5	200	5.3	82.9	11.8
2	20	75.3	24.7	
5	20	48.2	46.8	5.0
DNA alone[c]		87.0	13.0	

[a] The reaction mixtures containing 25 μM DNA (form I) and hydroperoxide **2** or **5** at varying concentrations in 50 mM sodium cacodylate buffer (pH 7.0) were irradiated at a distance of 10 cm from transilluminator (302 nm) at 0 °C for 1 h and analyzed by agarose gel electrophoresis. [b] Forms were obtained from densitometry reading after ethidium bromide staining and photography. [c] The DNA used contained a small amount of form II DNA.

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Constraint of the Spontaneous Intermembrane Movement of Sitosterol by Its 24α-Ethyl Group

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The predominant plant sterol β-sitosterol (referred to as sitosterol below) differs from cholesterol only by the presence of a 24α-ethyl group. This structural difference results in a marked decrease in the amount of dietary sitosterol absorbed compared with dietary cholesterol and a preferential excretion of sitosterol in bile. As a result of these processes, the level of sitosterol and other plant sterols normally found in mammalian tissues and plasma is very low (<1% of total plasma sterols).[1] A loss of sterol recognition capacity occurs in the inherited lipid-storage disease called sitosterolemia with xanthomatosis. This disorder is characterized by the abnormal hyperabsorption of ingested dietary sitosterol[2] and shellfish sterols,[3] with a concomitant increase in the concentrations of plant and shellfish sterols[2,3] and 5α-saturated stanols[4] in the plasma.[5]

Cholesterol is thought to be transported into the lymphatic circulation by a passive diffusion process that depends on bile salts for absorption.[6] A number of processes have been considered

(11) The C2/C3 ratio for the selectivity at secondary vs tertiary positions is 0.56. For related examples, see: (a) Fossey, J.; Lefort, D.; Massoudi, M.; Nedelec, J.-Y.; Sorba, J. *Can. J. Chem.* **1985**, *63*, 678. (b) Lau, T.-C.; Che, C.-M.; Lee, W.-O.; Poon, C.-K. *J. Chem. Soc., Chem. Commun.* **1988**, 1406. (c) Broun, R. B.; Hill, C. L. *J. Org. Chem.* **1988**, *53*, 5762. (d) Saito, I.; Takayama, M.; Matsuura, T. *Tetrahedron Lett.* **1989**, *30*, 2297.

(12) Irradiation of **2** (4.6 mM) and cyclododecane (23 mM) in acetonitrile for 2.5 h gave cyclododecanol (4%) and cyclododecanone (11%), whereas in the presence of 2.3 M of 2-propanol or dimethyl sulfoxide, formation of only small amounts of cyclododecanone (less than 3%) was observed under the conditions.

(13) For analogous photochemical δ-hydrogen abstraction of phthalimide,[6] see also: Kanaoka, Y.; Nagasawa, C.; Nakai, H.; Sato, Y.; Ogiwara, H.; Mizoguchi, T. *Heterocycles* **1975**, *3*, 55.

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(5) There is also a defect in the ability of the liver to concentrate these sterols preferentially into bile; since sterol esters are not secreted into bile, esterification of these sterols with fatty acids (which is retarded in normal cells) may be responsible for this defect.[3] Loss of the normal sterol substrate specificity of enzymes may result in other modifications of plant and shellfish sterols, contributing to their increased absorption.

(6) (a) Treadwell, C. R.; Vahouny, G. V. In *Handbook of Physiology*; Code, C. F., Heidel, W., Eds.; American Physiological Society: Washington, DC, 1968; Vol. 3, pp 1407–1438. (b) Westergaard, H.; Dietschy, J. M. *J. Clin. Invest.* **1976**, *58*, 97–108.

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to be involved in the ability of normal intestinal cells to absorb cholesterol preferentially to sitosterol during lipid digestion, including differences in (a) solubility of the sterols in the aqueous intestinal contents as a mixture of mixed micelles and unilamellar vesicles,[7] (b) partitioning of sterols between micelles and membrane lipids and interactions with individual phospholipids,[8] (c) recognition by mucosal plasma membrane proteins[7c,9] or other proteins that mediate sterol uptake into the intestinal brush-border membranes,[10,11] (d) intracellular reactions with membrane-bound enzymes, such as differential rates of esterification by acyl-CoA cholesterol acyltransferase[12] or hydroxylation by cholesterol 7α-hydroxylase,[13] and (e) partitioning between subcellular membranes[14] and uptake into chylomicrons or other lipoproteins.[15] Uptake of sterol from solutions containing bile salts into cells is complicated by many factors, including dependence of micelle structure on lipid structure and concentration,[6b,16] perturbation of membrane structure by physiological concentrations of bile salts, interactions with many membrane proteins and lipids, and subsequent metabolic events.

We sought to determine whether the greater extent of uptake of cholesterol by intestinal cells observed in normal individuals is correlated with a difference in the spontaneous exchange rate between phospholipid membranes. The rate-limiting step in sterol exchange from donor vesicles to an excess of acceptor vesicles is desorption of the sterol from the donor species.[17] The cholesterol desorption rate is sensitive to interactions between cholesterol and its nearest-neighbor molecules in the membrane.[18] Although enzymes and other proteins that have specific binding sites for the isooctyl side chain of cholesterol may contribute to the ability of intestinal cells to take up exogenous cholesterol selectively from the micellar phase in vivo, the results communicated here suggest that competition between micelles and plasma membranes for sterol is an important factor in absorptive recognition. The ethyl group at C_{24} of the sitosterol side chain decreases the rate of desorption of the sitosterol molecule from the donor lipid system (dipalmitoylphosphatidylcholine (dipalmitoyl-PC) and egg PC) by 4–7-fold at a sterol concentration of 1 mol %, at which sterol-induced increases in acyl-chain conformational order are minimal. A similar rate difference was found for exchange of these sterols between unilamellar vesicles composed of a binary mixture of egg PC and egg sphingomyelin with 6 mol % sterol.

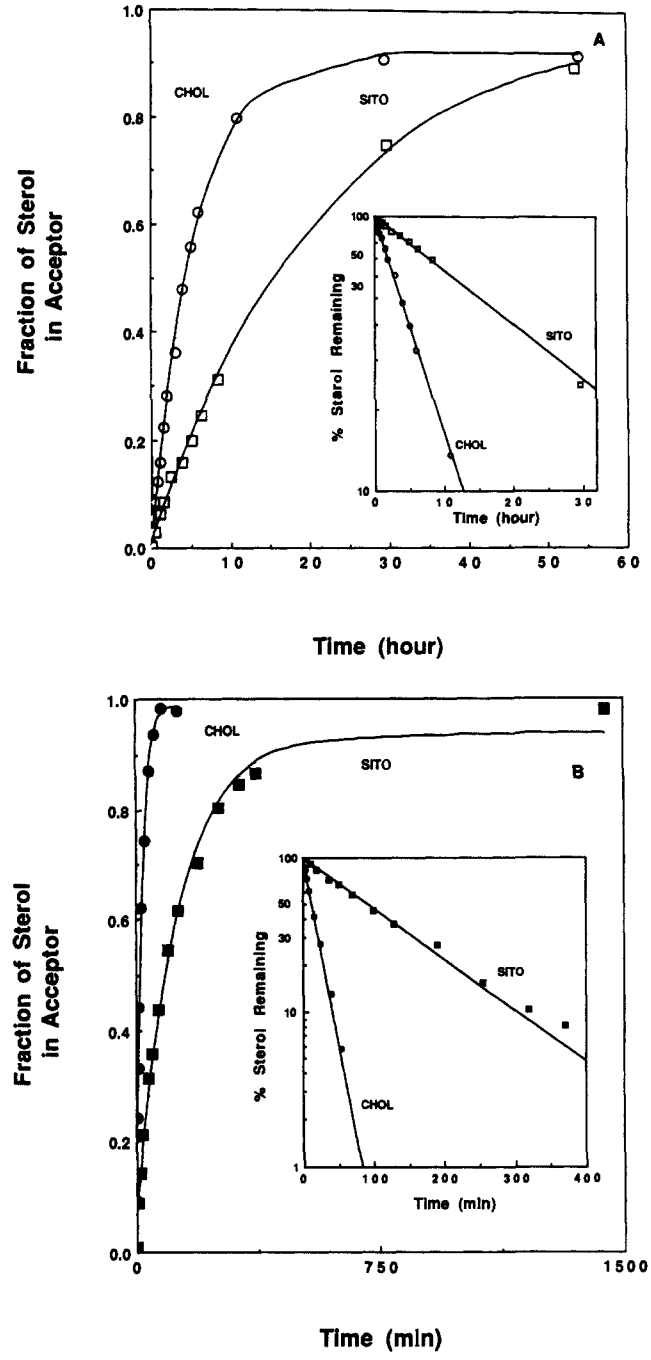

Figure 1. Kinetics of exchange of [4-^{14}C]cholesterol (○, ●) and [4-^{14}C]sitosterol (□, ■) between unilamellar vesicles containing 1 mol % sterol at 50 °C. Donor and acceptor vesicles were prepared from (A) dipalmitoyl-PC and (B) egg PC. Inset: Semilog plots of the exchange data. The preparation of sonicated vesicles and kinetic analysis were conducted by modification of methods reported previously.[17,19] Donor vesicles contained radiolabeled sterol (0.08 μCi) as the exchangeable lipid and 15 mol % of dicetyl phosphoric acid to confer negative charge, and acceptor vesicles contained a trace (0.046 μCi) of [9,10-^{3}H(N)]glycerol trioleate as a nonexchangeable marker to monitor their recovery. Albumin (2% w/v) was present in the incubation medium. Neutral acceptor vesicles were eluted from aliquots of the incubation mixture applied to short columns of diethylaminoethyl-Sepharose CL-6B.[19] Recovery of the neutral acceptors in the column eluate was 85–90%; <0.5% of the negatively charged donors was eluted. Oxidation of sterol during incubation was precluded (as judged by GC/MS and TLC analysis of lipid extracts) by including 1 mM EDTA in the buffer (20 mM sodium phosphate, pH 6.0) used to disperse the lipids.

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(17) For criteria indicating that desorption of cholesterol molecules from the donor bilayer into the aqueous phase is rate limiting for exchange in vesicle systems, see: (a) McLean, L. R.; Phillips, M. C. *Biochemistry* **1981**, *20*, 2893–2900. (b) Backer, J. M.; Dawidowicz, E. A. *Biochemistry* **1981**, *20*, 3805–3810. (c) Reviews.[18] We postulate that sitosterol and cholesterol have a common rate-determining step for exchange from donor vesicles to an excess of acceptor vesicles. Both sterols exhibit first-order kinetics in which the rate constant of exchange increases with an increase in fatty acyl chain unsaturation of the phospholipids in the host matrix (see Table I). Moreover, both sterols show a preference for partitioning into dipalmitoyl-PC over egg PC vesicles when both phospholipids are in the liquid-crystalline state: Rujanavech, C.; Silbert, D. F. *J. Biol. Chem.* **1986**, *261*, 7215–7219. These observations are consistent with a rate-determining desorption step.

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Table I. Comparison of Half-Times for [^{14}C]Cholesterol and [^{14}C]Sitosterol Exchange between Vesicles Prepared with Different Phospholipid Composition[a]

phospholipid composition	$t_{1/2}$, min cholesterol	$t_{1/2}$, min sitosterol
dipalmitoyl-PC	222.3 ± 43.2	990.8 ± 76.0
egg PC	14.9 ± 1.3	109.1 ± 12.7
egg PC/egg sphingomyelin	71.6	419.2

[a] The exchange experiments were carried out at 50 °C for dipalmitoyl-PC and egg PC vesicles and at 45 °C for egg PC (44 mol %)/egg sphingomyelin (35 mol %) vesicles. The sterol content was 1 mol % for dipalmitoyl-PC and egg PC vesicles and 6 mol % for egg PC/egg sphingomyelin vesicles. At least two different vesicle preparations were used in the experiments with dipalmitoyl-PC and egg PC. The sources and purities of the lipids have been cited elsewhere.[19,26] Photon correlation spectroscopy was used to estimate donor vesicle sizes at 50 °C. Measurements were made at a 90° scattering angle by using a 15-mV He-Ne laser of wavelength 632.8 nm together with a Brookhaven Instruments Corp. (Holtsville, NY) Model Bi-2030AT 128-channel digital correlator. A temperature-controlled scattering cell holder was used to maintain the samples at 50.0 ± 0.1 °C. The diameters of our sitosterol- and cholesterol-containing preparations were very similar. For dipalmitoyl-PC vesicles with 1 mol % sterol: sitosterol, 177-196 nm (index of polydispersity, 0.17-0.23); cholesterol, 245-264 nm (index of polydispersity, 0.23-0.29). For egg PC/egg sphingomyelin vesicles with 6 mol % sterol: sitosterol, 208 nm (index of polydispersity, 0.20); cholesterol, 206-240 nm (index of polydispersity, 0.19-0.25). Differential scanning calorimetry (Hart Scientific, Provo, UT) was used at a scanning rate of 15 °C/h to determine the phase-transition temperature of aqueous dispersions of dipalmitoyl-PC with 1 mol % sterol; no difference was found in the sitosterol- and cholesterol-containing preparations.

The kinetics of sterol exchange between dipalmitoyl-PC vesicles was examined over a prolonged period of time, appearing to reach equilibrium after about 60 h (Figure 1A). The exchange of radiolabeled sterol from negatively charged donor vesicles to a 10-fold excess of neutral acceptor vesicles was monitored as described previously.[19] The half-times for sterol exchange are more than 4-fold higher for sitosterol than for cholesterol in dipalmitoyl-PC bilayers (Table I). In egg PC bilayers (Figure 1B), the rates of sterol exchange are much faster than in dipalmitoyl-PC bilayers; this has been observed for cholesterol by several investigators[17,18a,19,20] and is considered to reflect the increased lateral packing density at the lipid-water interface in saturated relative to unsaturated PC bilayers. Table I shows that the half-time for sitosterol exchange is about 7 times higher than that for cholesterol exchange in egg PC bilayers. Incorporation of egg sphingomyelin (35 mol %) into egg PC bilayers results in a decrease in the rate of sterol exchange, with sitosterol undergoing exchange 6-fold more slowly than cholesterol.[21] The ability of sphingomyelin to lower the cholesterol exchange rate in mixed PC-sphingomyelin and sphingomyelin bilayers has been reported previously[20,22] and is explained by the greater lateral packing density in the lipid-water interface, which decreases the rate of cholesterol desorption. The magnitude of the difference in exchange rates between sitosterol and cholesterol was unexpected, since only a small difference in the promotion of acyl-chain order was noted in previous comparisons of their effects on phospholipid bilayers and monolayers,[23] furthermore, a membrane-*disordering* effect of sitosterol has been proposed in yeast.[24]

We suggest that the constraint in the intermembrane movement of sitosterol compared with cholesterol observed in our studies of exchange rates could account for *some* of the 5-10-fold-lower rate of absorption of sitosterol by intestinal mucosal cells. The results reported here support the model of the transition state postulated for cholesterol transfer between membranes, in which the cholesterol molecule is proposed to be attached by the tip of its hydrophobic side chain.[18a] The additional van der Waals interactions[25] between the sitosterol side chain and acyl chains of phospholipids appear to contribute significantly to the basis of discrimination in absorption of cholesterol and sitosterol. Future work will seek to evaluate whether the sterol to phospholipid molar ratio is an important factor in determining the relative absorptive discrimination of sterols. Comparative studies with ^{14}C-labeled sitosterol and cholesterol in biological membranes as the donor species would be worthwhile for evaluating the role of proteins in the transfer of these sterols between membrane surfaces.

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Registry No. Cholesterol, 57-88-5; β-sitosterol, 83-46-5; dipalmitoyl-PC, 2644-64-6.

Supplementary Material Available: A figure showing the kinetics of exchange of [^{14}C]cholesterol and [^{14}C]sitosterol from egg PC (44 mol %)/egg sphingomyelin (35 mol %)/dicetyl phosphate (15 mol %)/sterol (6 mol %) donor vesicles to a 10-fold excess of egg PC (94 mol %)/sitosterol (6 mol %) acceptor vesicles (1 page). Ordering information is given on any current masthead page.

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2D and 3D NMR Spectroscopy Employing ^{13}C-^{13}C Magnetization Transfer by Isotropic Mixing. Spin System Identification in Large Proteins

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For large proteins (>10 kDa), proton NMR signals are difficult to assign due to the overlap of a large number of broad resonances.[1] The large line widths not only decrease the sensitivity and resolution but limit the number of correlations that can be obtained from 2D *J*-correlated experiments, since a rapid decay of proton magnetization occurs during the relatively long time required for coherence transfers involving small proton-proton *J* couplings.

In this communication, we describe a group of novel 2D and 3D NMR experiments that rely on magnetization transfer between

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