

Photodynamic action of merocyanine 540 on artificial and natural cell membranes: Involvement of singlet molecular oxygen

(photosensitization/lipid peroxidation/leukemic cells/spin label oximetry)

B. KALYANARAMAN*[†], J. B. FEIX*, F. SIEBER[‡], J. P. THOMAS[§], AND A. W. GIROTTI[§]

*National Biomedical Electron Spin Resonance Center, Department of Radiology, and [§]Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226; and [‡]Midwest Children's Cancer Center, Department of Pediatrics, Medical College of Wisconsin, 1700 West Wisconsin Avenue, Milwaukee, WI 53233

Communicated by H. Beinert, December 29, 1986

ABSTRACT The photochemistry of merocyanine 540 (MC 540), a sensitizing dye that binds preferentially to leukemia and electrically excitable cells, has been investigated. MC 540-mediated photooxidation of histidine, arachidonate, and unsaturated phospholipid vesicles was assessed by spin label oximetry and shown to involve type II (singlet oxygen) chemistry. The dye was also shown to be a potent sensitizer of lipid peroxidation in a natural cell membrane, the erythrocyte ghost. Inhibition by azide, stimulation by ²H₂O, and identification of the cholesterol product 5 α -cholest-6-ene-3 β ,5-diol in this system, all were consistent with singlet oxygen intermediacy. Finally, MC 540 was found to be considerably more phototoxic to K-562 leukemia cells in ²H₂O than in H₂O. We conclude that singlet oxygen plays a major role in the phototherapeutic effects of this dye.

Merocyanine 540 (MC 540) is a fluorescent dye that binds to the outer surface of the plasma membrane. High-affinity binding sites for MC 540 are found on electrically excitable cells, some classes of immature blood cells, and leukemic cells (1–8). Simultaneous exposure to MC 540 and light of a suitable wavelength rapidly kills electrically excitable cells and leukemic cells but spares nonexcitable cells and normal pluripotent hematopoietic stem cells (4–8). In this way leukemic cells have been selectively purged from bone marrow *in vitro*, and attempts to exploit this differential photosensitivity in clinical situations are underway (9).

Although the effectiveness of MC 540 in killing neoplastic cells has been demonstrated (5–8), little is known about the mechanism of cell killing, and those factors on which the selectivity of MC 540 for cancer cells is based are not understood. However, the fact that uptake and toxicity of MC 540 in leukemic cells are augmented in the presence of visible light and oxygen (2) suggests that photodynamic processes are involved. A preliminary report has indicated that singlet oxygen (¹O₂) is one of the reactive intermediates (10). The first detectable event following the production of this species is rapid consumption of molecular oxygen, which can occur as a result of reactions with biological constituents. Thus, we have used the highly sensitive spin label oximetric approach (11, 12) to monitor oxygen consumption during MC 540-sensitive photooxidation. Using this approach, we now report additional evidence for the production of ¹O₂ by the photoactivated dye. The involvement of ¹O₂ in the killing of leukemic cells and in the peroxidation of cell membrane phospholipids and cholesterol is also reported.

MATERIALS AND METHODS

Oximetry Studies Using Model Systems. *Sample preparation.* MC 540 obtained from Eastman was dissolved in a 50:50

mixture of ethanol and water and frozen until further use. Arachidonic acid (Sigma) was dissolved in ethanol. All other chemicals were used as received.

Sample irradiation. Samples for irradiation typically contained 90 μ M MC 540 in deionized water, pH 7.0. 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-yloxy (CTPO; Aldrich) was added to give a final concentration of 1.2×10^{-4} M. Samples, following saturation with a mixture of 50% nitrogen and 50% air from calibrated flow meters (Matheson) were introduced into a Pasteur pipette, which was sealed with capillary tube sealant (Miniseal, American Scientific Products, Stone Mountain, GA) and subsequently positioned inside the electron spin resonance (ESR) cavity.

Samples were irradiated in the ESR cavity with 320- to 600-nm light obtained from an Osram HBO 200 W/2 Hg lamp in combination with a chemical filter (5 cm CuSO₄ solution, 100 g/liter). The fluence rate at the sample surface was measured with a radiometer (Yellow Springs Instruments).

Liposome preparation. Dimyristoylphosphatidylcholine (Myr₂-PtdCho), and 1-palmitoyl-2-oleoylphosphatidylcholine (Pam-OlePtdCho) were purchased from Avanti Polar Lipids. Multilamellar liposomes were prepared as described previously (13). Stock solutions of lipid components in chloroform were mixed in appropriate amounts to give the desired membrane composition. Solvent was removed under a stream of dry nitrogen, and samples were further dried under vacuum. Hydration was accomplished by dispersing the dried samples in 10 mM phosphate/145 mM NaCl, pH 8.0 (PBS). Liposomes were hydrated in buffer containing the desired concentration of MC 540 to ensure that the dye was accessible to all lamellae.

Optical measurements. Absorbance measurements were made in a Perkin-Elmer 320 dual-beam spectrophotometer equipped with temperature control. Binding of MC 540 to liposomes was assessed by pelleting the liposomes (16,000 \times g, Eppendorf Microfuge) and determining the amount of dye remaining in the supernatant. MC 540 concentration in the supernatant was determined from absorbance at 570 nm in 50% (vol/vol) methanol (14). For optical bleaching measurements, 1-cm quartz cuvettes containing MC 540 were irradiated directly in front of the light source and absorption spectra of irradiated samples were measured with time.

ESR measurements. Samples were maintained at 37°C in the cavity of a Varian E-109 spectrometer equipped with a gas

Abbreviations: ESR, electron spin resonance; CTPO, 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-yloxy; MC 540, merocyanine 540; ¹O₂, singlet oxygen; Myr₂-PtdCho, dimyristoylphosphatidylcholine; Pam-OlePtdCho, 1-palmitoyl-2-oleoylphosphatidylcholine; PBS, phosphate-buffered saline (25 mM Na₂HPO₄/NaH₂PO₄, 125 mM NaCl, pH 7.4); Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; 5 α -OH, 5 α -cholest-6-ene-3 β ,5-diol; 7 α -OH, cholest-5-ene-3 β ,7 α -diol; 7 β -OH, cholest-5-ene-3 β ,7 β -diol; 5 α -OOH, 3 β -hydroxy-5 α -cholest-6-ene-5-hydroperoxide.

[†]To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

transfer Dewar and a Varian temperature controller. The superhyperfine structure of the $M_1 = +1$ ESR line of the nitroxide (the resolution of which depends on the oxygen concentration in the medium) was recorded at 1 mW microwave power and 0.04 modulation amplitude.

Studies on Erythrocyte Membranes. *Membrane preparation.* Freshly drawn human blood from anonymous donors was obtained from the Blood Center of Southeastern Wisconsin. Unsealed erythrocyte membranes (ghosts) were prepared by hypotonic lysis, followed by washing and concentration in a Millipore-Pellicon tangential-flow filtration apparatus (15).

Reaction conditions. Stirred membrane suspensions (4–6 ml) were sensitized with MC 540 and irradiated in matched beakers (45-mm diameter) that were thermostatically maintained at 15°C (15). The sources, Duro Test R-40 lamps, were positioned above the suspensions; yellow filters (Corning CS 3-68) were used to restrict incident light to wavelengths maximally absorbed by the dye. Samples from continuously irradiated suspensions were removed periodically for assessment of lipid peroxidation or dye bleaching. Rose bengal-sensitized reactions were carried out similarly.

Measurement of lipid peroxidation. A microiodometric method was used for quantitating total lipid hydroperoxides in photooxidized membranes (16). The thiobarbituric acid assay of peroxidation, often preferred because of its relative simplicity (17), could not be used in this system, because the dye itself made such a large contribution to the absorbance at 532 nm. The conditions for xanthine oxidase-catalyzed lipid peroxidation were described previously (17).

Chromatography of cholesterol products. Cholesterol photoproducts were analyzed by TLC, using adaptations of previously published methods (18, 19). The typical procedure was as follows. A 0.5-ml sample from a membrane reaction mixture (0.11 mg of cholesterol) was mixed with 0.2 mM EDTA and extracted with 0.8 ml of $\text{CHCl}_3/\text{CH}_3\text{OH}$ (2:1). Manipulations were done in subdued light when lipophilic sensitizers were present. The organic phase, 0.4 ml in a polypropylene Microfuge tube, was treated with NaBH_4 (0.5–2 μmol) to reduce lipid hydroperoxides to their relatively stable alcohols. After standing for 10 min at room temperature, the mixture was evaporated to dryness at 50°C under a stream of nitrogen. Typically, two to three identical extracts were combined in this step. Borohydride reduction of 7-ketocholesterol (Sigma) yielded the epimeric 7 α - and 7 β -alcohols, which were used as standards for assessing free radical oxidation. The products were taken up in 10–20 μl of $\text{CHCl}_3/\text{CH}_3\text{OH}$ (2:1), spotted onto a Silica Gel-60 plate (EM Science, Cherry Hill, NJ) and chromatographed, using heptane/ethyl acetate (1:1) as the solvent. In this system it was found that phospholipids remain at the origin, allowing cholesterol derivatives to be clearly identified. The plate was sprayed with 50% H_2SO_4 and warmed briefly at 80°C to enhance color development.

Studies on Leukemic Cells. *Dye-mediated photolysis.* K-562 cells were obtained from B. Lozzio and grown in α -medium (KC Biological, Lenexa, KS) supplemented with 10% fetal bovine serum (KC Biological). The cells were harvested in exponential growth phase and suspended at a density of 1×10^6 cells per ml in Hepes-buffered (10 mM, pH 7.4) α -medium in either distilled water or deuterium oxide supplemented with 15% fetal bovine serum (Reheis, Kankakee, IL), penicillin (100 units per ml) and streptomycin (100 μg per ml). MC 540 was added from a 1 mg/ml stock solution in 50% ethanol to a final concentration of 15 $\mu\text{g}/\text{ml}$. Clear polystyrene tubes (15-mm diameter) containing the cell suspension were mounted on a disk that rotated between two parallel banks of five tubular fluorescent lights (F20T12.CW; General Electric) and illuminated at room temperature for the specified time intervals. The fluence rate at the sample site was $\approx 80 \text{ W}/\text{m}^2$. The

tubes were swirled manually every 10 min to prevent cells from settling. Control cell suspensions were treated with equivalent amounts of 50% ethanol or α -medium instead of MC 540 and exposed to light or were incubated with MC 540 in the dark. Photooxidation was terminated by washing the cells twice with Hepes-buffered α -medium supplemented with 5% fetal bovine serum. This and all subsequent operations were done under subdued lighting.

In vitro clonal assay of K-562 cells. Approximately 400 untreated or up to 80,000 photosensitized K-562 cells were suspended in 1 ml of α -medium supplemented with 0.9% methylcellulose (4000 cPa, Fluka) and 20% fetal bovine serum and plated in 35-mm petri dishes (Greiner, Nürtingen, F.R.G.). The cultures were incubated for 7 days in a humidified atmosphere of 5% CO_2 in air at 36°C. Colonies containing ≥ 20 cells were scored *in situ* with an inverted microscope.

RESULTS

Spin Label Oximetry. Spin label oximetry was chosen to monitor oxygen consumption during MC 540 photosensitization. In the presence of air, dissolved oxygen molecules interact with nitroxide free radicals through Heisenberg spin exchange, leading to a broadening of the ESR lines that is proportional to oxygen concentration. As oxygen is removed from the system, Heisenberg exchange, which broadens the spectral lines, is reduced, and spectral resolution is increased. This behavior is shown for the nitroxide, CTPO, in Fig. 1. Oxygen uptake traces were obtained from calibration curves relating the K parameter (11, 12) to oxygen concentration.

MC 540 Sensitized Oxygen Consumption in the Presence of a Singlet Oxygen Trap. When an aqueous solution containing MC 540, histidine, and CTPO was irradiated with 300–700-nm light ($\approx 100 \text{ W}/\text{m}^2$), a steady increase in O_2 uptake was observed (Fig. 2, $^1\text{H}_2\text{O}$). The rate of O_2 uptake was found to increase linearly with histidine concentration up to at least 9 mM (data not shown). At this low light intensity, no appreciable O_2 was consumed in the absence of either the dye or histidine (Fig. 2). At significantly higher light intensities (e.g., $1750 \text{ W}/\text{m}^2$) O_2 consumption could be measured when solutions of MC 540 alone were irradiated (data not shown). The consumption was enhanced in $^2\text{H}_2\text{O}$, a solvent that increases $^1\text{O}_2$ lifetime (20), suggesting that the dye itself is a substrate for $^1\text{O}_2$. Replacement of H_2O by $^2\text{H}_2\text{O}$ also increased the rate of O_2 uptake by histidine (note 2.5-fold enhancement in Fig. 2). Addition of azide inhibited the rate of O_2 uptake in a dose-dependent manner (data not shown). Thus, MC 540-sensitized photooxidation of histidine appears to be mediated by $^1\text{O}_2$. In the presence of this trap, little or no bleaching of the dye was observed (data not shown).

MC 540-Sensitized Oxygen Consumption in the Presence of an Unsaturated Fatty Acid. Because MC 540 localizes in plasma membranes, we studied its ability to sensitize the photooxidation of an unsaturated fatty acid, *viz.*, arachidonic acid. Illumination of a solution containing MC 540, arachidonic acid, and CTPO with a near-UV visible light (300–700 nm) resulted in a marked dose-dependent increase in the rate of oxygen uptake (Fig. 3A, b–d). No significant uptake was observed when either the dye or fatty acid alone was irradiated under otherwise identical conditions (Fig. 3A, a). Also no reduction of the nitroxide could be observed until after almost all the oxygen had been consumed. This rules out any radical-mediated (type I) process in the photooxidation of arachidonic acid, because if radicals had been produced, we would have expected a decrease in the concentration of the nitroxide due to facile radical-radical recombination reactions. These preliminary results suggest that MC 540-sensi-

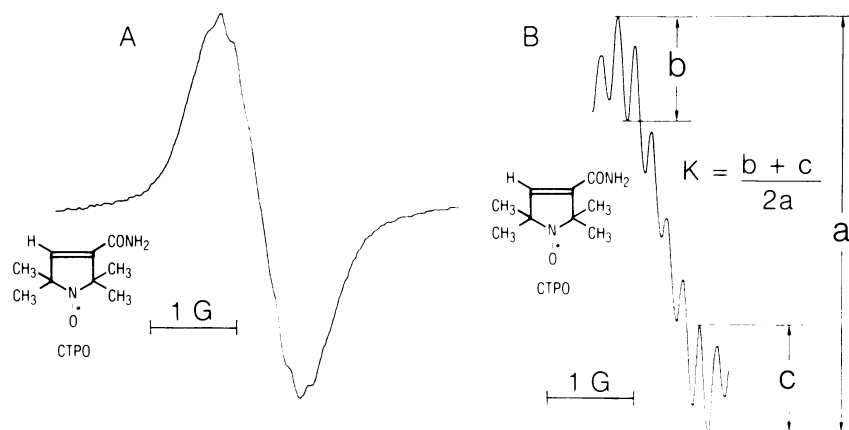


FIG. 1. Low field line of the ESR spectrum of CTPO in air-saturated (A) and partially nitrogen-saturated (B) aqueous solution. [CTPO] = 10^{-4} M; microwave power, 1 mW, modulation amplitude, 0.04 G.

tized photooxidation of arachidonate occurs via the type II ($^1\text{O}_2$) mechanism.

Photooxidation of Liposomes. Irradiation of merocyanine-treated liposomes composed of Pam-OlePtdCho, which contains a cis double bond in the sn-2 alkyl chain, caused a rapid depletion of oxygen from the medium (Fig. 3B). No oxygen was consumed at this relatively low light intensity in the absence of lipid or dye or if dye-treated liposomes were kept in the dark. Similar irradiation of MC 540-treated liposomes composed of Myr₂-PtdCho, a fully saturated phospholipid, resulted in no oxygen consumption (Fig. 3B). In both cases incubation with MC 540 and irradiation were carried out at 37°C, so that both lipids were in the fluid phase. Centrifugation of the liposomes after incubation with MC 540 confirmed that essentially all of the dye was membrane-bound. Thus, the different results with Pam-OlePtdCho- and Myr₂-PtdCho-liposomes were not due to differences in dye binding. This strongly suggests that oxygen depletion in this system is due to lipid peroxidation, i.e., reaction of $^1\text{O}_2$ with the unsaturated fatty acyl group of Pam-OlePtdCho.

Photooxidation of Erythrocyte Ghosts. Irradiation of isolated erythrocyte membranes in the presence of MC 540 resulted in lipid peroxidation, as evidenced by the increase in

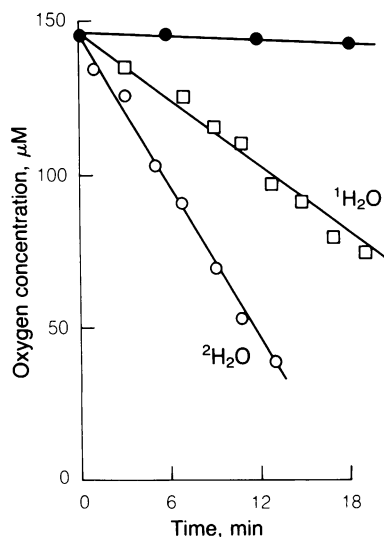


FIG. 2. Photosensitized oxygen consumption during irradiation of a solution containing 90 μM MC 540 in $^1\text{H}_2\text{O}$ (●), 90 μM MC 540, 3 mM histidine in $^1\text{H}_2\text{O}$ (□), or 90 μM MC 540, 3 mM histidine in $^2\text{H}_2\text{O}$ (○). The light intensity was ≈ 100 W/m². Irradiation was started at time zero. Before irradiation the solution was bubbled with a mixture of 50% nitrogen and 50% air.

lipid hydroperoxide content with irradiation time (Fig. 4A). Neither light nor dark controls showed any effect. The increase in lipid hydroperoxide content was linear for at least 1 hr, during which time the dye underwent considerable photobleaching (Fig. 4B). The apparent abatement of peroxidation after 70 min may be at least partially due to dye destruction as a result of direct oxidation and/or reaction with lipid peroxides. Addition of azide to the membrane suspension resulted in a 2.5-fold decrease in the rate of lipid hydroperoxide formation, whereas replacing H_2O in the medium with $^2\text{H}_2\text{O}$ caused a 3-fold increase in this rate (Fig. 4A). Taken together, these results confirm those obtained with model systems (see above) and indicate that $^1\text{O}_2$ plays a significant role in MC 540-sensitized lipid peroxidation. Correspondingly, destruction of the dye itself appeared to involve $^1\text{O}_2$, because this was also slowed by azide and enhanced by $^2\text{H}_2\text{O}$ (Fig. 4B).

Definitive evidence for the participation of $^1\text{O}_2$ in MC 540-dependent lipid peroxidation was obtained by showing that the unique photoproduct of $^1\text{O}_2$ attack on cholesterol is generated, *viz.*, 3 β -hydroxy-5 α -cholest-6-ene-5-hydroperoxide (5 α -OOH). As shown in Fig. 5, the major cholesterol product obtained upon borohydride reduction of MC 540-

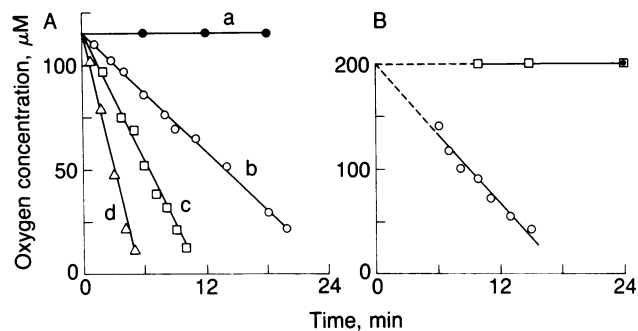


FIG. 3. Oxygen consumption during photooxidation of arachidonic acid (AA) or liposomes composed of (Pam-OlePtdCho). (A) Arachidonic acid: a, AA alone (1 mM); b, MC 540 (90 μM) plus AA (0.25 mM); c, MC 540 (90 μM) plus AA (0.5 mM); d, MC 540 (90 μM) plus AA (1.0 mM). The light intensity was ≈ 100 W/m². Before irradiation the solution was bubbled with a mixture of 50% nitrogen and 50% air. (B) Liposomes: The vesicles were hydrated by the addition of MC 540 (20 $\mu\text{g}/\text{ml}$ in PBS) for 30 min at 37°C in the dark and then mixed with an equal volume of 2.4×10^{-4} M CTPO. The sample was placed in the ESR cavity, and irradiation was begun at time zero with broad spectrum (300- to 700-nm) light at 170 W/m². Samples contained lipid at a final concentration of 100 mg/ml. (○) Pam-OlePtdCho; (□) Myr₂-PtdCho; (●) Pam-OlePtdCho without irradiation.

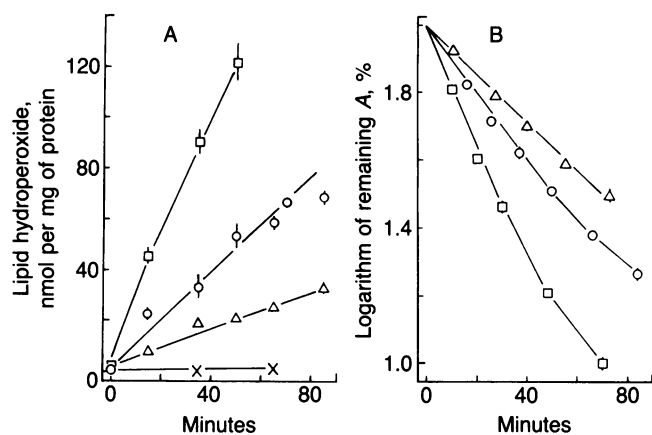


FIG. 4. Irradiation of erythrocyte ghosts in the presence of MC 540: effects of azide and $^2\text{H}_2\text{O}$ on lipid peroxidation and dye bleaching. (A) Lipid hydroperoxide formation, (B) dye bleaching. Membranes (1.0 mg of protein per ml in PBS; ≈ 0.7 mM in phospholipid) were sensitized with $10 \mu\text{M}$ MC 540 and irradiated as such (\circ) or in the presence of 20 mM azide (Δ). The light intensity was ≈ 270 W/m^2 . Aliquots were removed at the indicated times for determining lipid hydroperoxides and residual dye. For the latter determination, samples were diluted 10-fold in 2% NaDodSO₄ and A_{557} readings were recorded (initial value ≈ 0.2). In a companion experiment, the membranes were washed extensively with PBS in $^2\text{H}_2\text{O}$ (pH 7.4) before photooxidizing with $10 \mu\text{M}$ MC 540 (\square). The control (\times) contained dye, but was kept in the dark. Data points are means \pm deviation of values from duplicate experiments.

photooxidized ghosts comigrates on TLC with material produced by rose bengal, a well-known photogenerator of $^1\text{O}_2$. On this basis, the product is identified as 5α -cholest-6-ene- 3β , 5 -diol (5α -OH). A relatively minor component (unidentified) of slight-

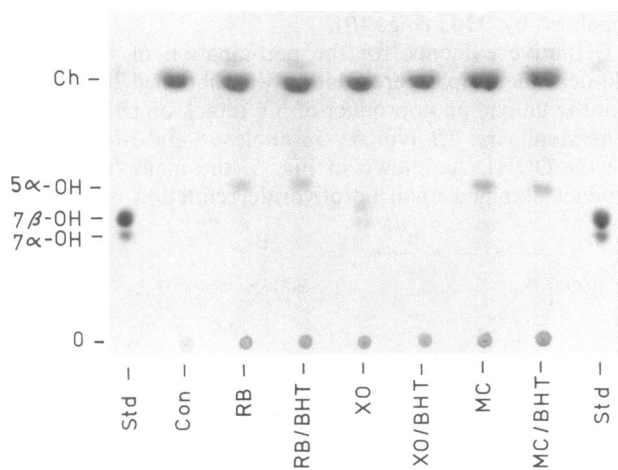


FIG. 5. Cholesterol products obtained by MC 540-sensitized photooxidation of erythrocyte ghosts. Membranes were sensitized with $20 \mu\text{M}$ MC 540 and irradiated for 2 hr in the absence (MC) or presence (MC/BHT) of $25 \mu\text{M}$ butylated hydroxytoluene (light intensity ≈ 270 W/m^2). Extracted lipids were reduced with NaBH₄, subjected to TLC, and sprayed with 50% H₂SO₄. A control (Con) lacking sensitizer was irradiated alongside. Membranes were also sensitized with $5 \mu\text{M}$ rose bengal (RB) and irradiated for 1 hr in the absence (RB) or presence (RB/BHT) of $25 \mu\text{M}$ BHT. In a separate experiment, the membranes were incubated with xanthine oxidase (XO; 0.01 unit/ml), xanthine (1.0 mM), and FeCl₃ (0.1 mM) for 2 hr at 37°C in the absence (XO) or presence (XO/BHT) of $25 \mu\text{M}$ BHT. Concentration of membrane suspensions: 1.0 mg protein/ml in PBS. Sample loads (as cholesterol): 0.24 mg for MC experiments and 0.16 mg for all other experiments. The standard (Std) shows the 7-OH epimers obtained by reducing 7-ketocholesterol (4- μg load). Ch, cholesterol; O, origin.

ly greater mobility than cholesterol was also observed in the photoreactions. Neither the rose bengal- nor the MC 540-sensitized formation of 5α -OOH was affected by butylated hydroxytoluene, a free radical trap, which is consistent with a nonradical reaction mechanism. Similarly, butylated hydroxytoluene had no effect on overall lipid hydroperoxide formation with either photosensitizer (results not shown).

Contrasting results were obtained when ghosts were exposed to a nonphotochemical source of oxygen radicals, *viz.*, xanthine oxidase acting on xanthine in the presence of added Fe(III) ion. In this case, as described previously (21) and anticipated for the free radical oxidation of cholesterol (19), the major products (subsequent to borohydride treatment) were cholest-5-ene- 3β , 7α -diol (7α -OH) and cholest-5-ene- 3β , 7β -diol (7β -OH), which comigrated with the reduction products of the 7-ketocholesterol standard (Fig. 5). There was no evidence for $^1\text{O}_2$ involvement in this reaction by virtue of the absence of 5α -OH. Moreover, butylated hydroxytoluene prevented formation of the free radical-derived products. The trace amounts of 7α -OH and 7β -OH observed in the photochemical reactions are attributed to the slow allylic rearrangement of 5α -OOH (18), although a small contribution of type I (free radical) photochemistry cannot be ruled out.

MC 540-Mediated Photolysis of Leukemia Cells. Simultaneous exposure of K-562 leukemia cells to MC 540 and light caused a rapid decrease of *in vitro* clonogenic cells (Fig. 6). The decrease was even more rapid if irradiation was performed in deuterated medium. This result is compatible with those described above for simpler systems and thus indicates that $^1\text{O}_2$ participated in the cytotoxic events leading to leukemic cell killing.

DISCUSSION

These results are consistent with the production of singlet oxygen during aerobic photosensitization by MC 540. The following scheme (reactions 1-3) is proposed to account for $^1\text{O}_2$ generation.

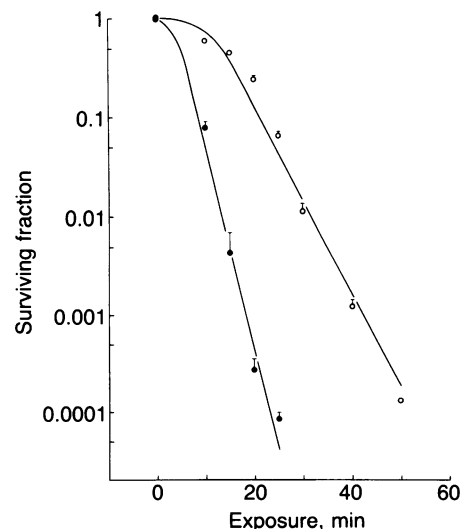
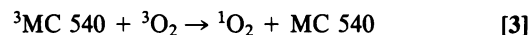
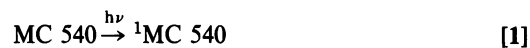


FIG. 6. Representative example of the photosensitization of K-562 leukemia cells in H₂O (\circ) or 85% $^2\text{H}_2\text{O}$ (\bullet). All data points represent mean colony counts of four to eight replicate culture dishes. Vertical bars indicate SE. Colony counts at zero time point were 267 in H₂O and 253 in $^2\text{H}_2\text{O}$, respectively.

MC 540 is photoexcited to the singlet state (reaction 1), which undergoes intersystem crossing to the relatively long-lived triplet state (reaction 2). Subsequent energy transfer to ground state oxygen results in $^1\text{O}_2$ formation (reaction 3).

At high light intensities (Fig. 4 relative to Fig. 2), the dye itself consumed oxygen and was gradually bleached. At least part of this effect is attributed to photooxidation of the dye by $^1\text{O}_2$ (22). It is conceivable, however, that in the presence of membranes (Fig. 4) back attack by lipid peroxides also played a role in photobleaching.

That singlet oxygen has rarely been detected directly in cellular systems is probably due to its rapid reaction with endogenous biological constituents. This report describes oxygen consumption due to photosensitization by a membrane-bound dye. Oxygen was consumed during photoexcitation of MC 540 in unsaturated, but not saturated liposomes, which raises the possibility that peroxidation of unsaturated lipids and consequent membrane disruption play a role in MC 540-sensitized photokilling of leukemia cells. Additional support for this argument is provided by showing that a natural cell membrane, the erythrocyte ghost, undergoes vigorous lipid peroxidation when irradiated in the presence of this dye. Inhibition by azide, stimulation by $^2\text{H}_2\text{O}$, and identification of a unique cholesterol photoproduct, $5\alpha\text{-OOH}$, all indicate the intermediacy of $^1\text{O}_2$ in lipid peroxidation damage. If electron or hydrogen transfer photochemistry were at all involved, it was relatively insignificant, because only trace amounts of cholesterol products ascribed to free radical reactions were observed (Fig. 5). Thus, even though MC 540 interacts strongly with lipid targets in the membrane, energy transfer from dye triplet to oxygen seems to predominate. Photokilling of K-562 cells *in vitro* also appears to involve type II chemistry (Fig. 6), and membrane-damaging lipid peroxidation could mediate cell killing, as suggested above.

We thank G. J. Bachowski, G. J. Krueger, and M. W. Corcoran for excellent technical assistance. This work was supported by National Institutes of Health Grants AM-26950 (B.K.), RR-01008 (B.K.), GM-22923 (J.F.), CA-42734 (F.S.) and National Science

Foundation Grant DCB-8501894 (A.W.G.). F.S. is a Leukemia Society of America Scholar.

1. Easton, T. G., Valinsky, J. E. & Reich, E. (1978) *Cell* **13**, 475-486.
2. Valinsky, J. E., Easton, T. G. & Reich, E. (1978) *Cell* **13**, 487-499.
3. Schlegel, R. A., Phelps, B. M., Waggoner, A., Terada, L. & Williamson, P. (1980) *Cell* **20**, 321-328.
4. Meagher, R. C., Sieber, F. & Spivak, J. S. (1983) *J. Cell. Physiol.* **116**, 118-124.
5. Sieber, F., Spivak, J. L. & Sutcliffe, A. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7584-7587.
6. Sieber, F. & Sieber-Blum, M. (1986) *Cancer Res.* **46**, 2072-2076.
7. Sieber, F., Rao, S., Rowley, S. D. & Sieber-Blum, M. (1986) *Blood* **68**, 32-36.
8. Sieber, F., Stuart, R. K., Rowley, S. D., Sharkis, S. J. & Sensenbrenner, L. L. (1987) *Leukemia Res.* **11**, 43-50.
9. Sieber, F., Craig, A., Krueger, G. J., Smith, R. E. & Ash, R. C. (1986) *Blood* **86**, 292A (abstr.).
10. Kalyanaraman, B. & Sieber, F. (1986) *Photochem. Photobiol.* **43**, 28S (abstr.).
11. Lai, C.-S., Hopwood, L. E., Hyde, J. S. & Lukiewicz, S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1166-1170.
12. Ankel, E., Felix, C. C. & Kalyanaraman, B. (1986) *Photochem. Photobiol.* **44**, 741-746.
13. Feix, J. B., Popp, C. A., Venkataramu, S. D., Park, J. H. & Hyde, J. S. (1984) *Biochemistry* **23**, 2293-2299.
14. Aiuchi, T. & Kobatake, Y. (1979) *J. Membr. Biol.* **45**, 233-244.
15. Girotti, A. W., Thomas, J. P. & Jordan, J. A. (1985) *Photochem. Photobiol.* **41**, 267-276.
16. Girotti, A. W., Thomas, J. P. & Jordan, J. E. (1985) *Arch. Biochem. Biophys.* **236**, 238-251.
17. Girotti, A. W. & Thomas, J. P. (1984) *J. Biol. Chem.* **259**, 1744-1752.
18. Kulig, M. J. & Smith, L. L. (1973) *J. Org. Chem.* **38**, 3639-3642.
19. Smith, L. L., Teng, J. I., Kulig, M. J. & Hill, F. L. (1973) *J. Org. Chem.* **38**, 1763-1765.
20. Nilsson, R., Merkel, P. B. & Kearns, D. R. (1972) *Photochem. Photobiol.* **16**, 117-124.
21. Girotti, A. W. & Jordan, J. E. (1985) *Photochem. Photobiol.* **41**, 24S (abstr.).
22. Byers, G. W., Gross, S. & Henrichs, P. M. (1976) *Photochem. Photobiol.* **23**, 37-43.