in compound I and also the bridge methine protons[14] in II and III at room temperature.

Temperature-dependent ^1H nmr spectral changes for I and III dissolved in 1,1,2,2-tetrachloroethane (bp 146°) are shown in Figure 2. As the temperature is raised, the peaks broaden, coalesce, and gradually sharpen. The changes have all been shown to be completely reversible with temperature. Compound II gives spectral changes similar to those of III. These results illustrate that the compounds I–III display a fluxional behavior.

The above fluxional phenomenon is best explained by the intramolecular rearrangement of the metal–carbonyl group among the four ring nitrogens of porphyrin and also movement of the N–H; it can also be regarded as an intramolecular substitution at rhenium or technetium. A solution containing II and excess free mesoporphyrin IX dimethyl ester in relative amounts 1:2.5 showed no broadening of the free ligand bridge methine proton peak in the fast exchange region for compound II. Free ligand is thus not involved in the exchange process in this system. The colescence temperatures, moreover, were not shifted by changes in the concentrations of the complexes within the standard deviations of the experiment (*ca.* ±5°). These results lend support to the fact that the thermal rearrangement process is intramolecular rather than intermolecular. Dissociation and recombination of the metal–carbonyl moieties and the porphyrin ligand or interchanges of two such ligands between two molecules at high temperature are ruled out due to the fact that attempts at the conversion of $Mp[Re(CO)_3]_2$ to compound II by reflux in decalin (bp 195°) with excess porphyrin were unsuccessful.[8]

The free energy of activation, $\Delta G^{\pm} = 19.3 \pm 2$ kcal/mol, was estimated[15] for III from the coalescence of the bridge methine proton signals.

Further studies are now being undertaken to more fully elucidate the nature and scope of these fluxional characters for out of plane organometalloporphyrin compounds.

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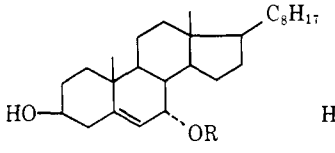
Sterol Metabolism. XXIX. On the Mechanism of Microsomal Lipid Peroxidation in Rat Liver[1]

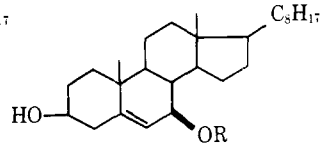
Sir:

Speculation on the participation of electronically excited (singlet) molecular oxygen in enzymic oxidations is current, such participation having been suggested for the actions of soybean lipoxygenase[2] and horseradish peroxidase[3] and for mammalian liver NADPH-dependent microsomal[4] and mitochondrial[5] lipid peroxidations. However, experimental evidence does not infer such participation for lipoxygenase[6,7] or peroxidase,[6] and the claim associated with lipoxygenase[2a] has been retracted.[8] Moreover, singlet molecular oxygen is not implicated in the oxidation of xenobiotic substances by mammalian liver microsomes.[9]

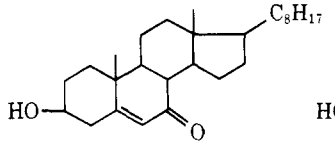
We have now examined the NADPH-dependent hepatic microsomal lipid peroxidation system using cholesterol as a probe to test the participation of singlet molecular oxygen.[10] Cholesterol oxidation in this system previously yielded cholest-5-ene-3β,7α-diol (Ia),



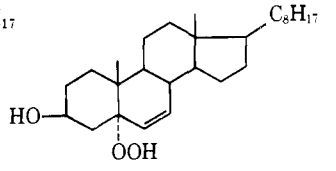
Ia, R = H
b, R = OH



IIa, R = H
b, R = OH



III



IV

cholest-5-ene-3β,7β-diol (IIa), 3β-hydroxycholest-5-en-7-one (III), and 5α-cholestane-3β,5,6β-triol.[11] Hydroperoxides, though suspected, have not heretofore been demonstrated.[11a,c] However, we have established cholesterol 7α-hydroperoxide (Ib) and 7β-hydroperoxide (IIb) but not 3β-hydroxy-5α-cholest-6-ene 5-hydroperoxide (IV) as initial products of cholesterol oxidation by air[12] and by lipoxygenase and peroxidase.[6] Subse-

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Table I. Oxidation of [1,2-^{3}H]Cholesterol by Rat Liver Subcellular Fractions

	Product yield, %[a]								
		Microsomal fraction[c]				Microsomal + soluble fractions			
Sterol product	Mitochondrial fraction[b]		With linoleate[d]	With linoleate hydroperoxides[e]	Soluble fraction[f]		Without NADPH[g]	With EDTA[h]	With gallate[i]
Ia	0.19	0.43	1.06	1.06	0.40	0.33	0.12	0.25	0.24
Ib	0.01	0.09	0.16	0.83	0.00	0.05	0.00	0.01	0.00
IIa	0.36	1.19	2.89	1.98	1.12	1.00	1.26	0.26	0.29
IIb	0.03	0.12	0.93	3.97	0.01	0.07	0.03	0.03	0.00
III	0.83	2.58	6.92	8.94	2.35	3.40	0.98	0.33	0.98
Total	1.42	4.41	11.96	16.78	3.88	4.85	2.39	0.88	1.51

[a] Radioactivity incorporated over control without enzyme. [b] 10,000*g* pellet. [c] 105,000*g* pellet. [d] With 0.03 mmol ethyl linoleate. [e] With 0.01 mmol mixed ethyl linoleate hydroperoxides (soybean lipoxygenase derived). [f] 105,000*g* supernate. [g] NADPH and NADPH-generating system omitted. [h] With 4 mg EDTA. [i] With 0.01 mmol propyl gallate.

quent thermal decomposition of Ib and IIb yielded Ia, IIa, and III as secondary products.[13] In that neither the 5α-hydroperoxide IV nor its unique pyrolysis product cholesta-4,6-dien-3-one[13] was found, the participation of singlet molecular oxygen was excluded from those oxidations.

Subcellular fractions of liver from adult (500 g) male Sprague-Dawley rats incubated with [1,2-^{3}H]cholesterol and a NADPH-generating system (0.015 *M* Tris–HCl, pH 6.8) at 30° for 2 hr under oxygen yielded the 7-hydroperoxides Ib and IIb, the corresponding 3β,7-diols Ia and IIa, and the 7-ketone III, identified chromatographically.[13b,14] Neither the 5α-hydroperoxide IV nor cholesta-4,6-dien-3-one was detected despite a careful search.

Data of Table I show cholesterol oxidation was greatest in the microsomal and combined microsomal-soluble fractions. Sterol peroxidation was diminished in the absence of NADPH, by boiling the enzyme or by preincubation with trypsin, and was completely inhibited by 1 m*M* EDTA, by 1 m*M* propyl gallate, or by dithionite. Carbon monoxide was without effect, as was pretreatment of rats with phenobarbital. Additions of ethyl linoleate or ethyl linoleate hydroperoxides markedly stimulated peroxidation.

The 7-ketone III predominated in most experiments and accounted for approximately half of measured products. The 7α- to 7β-oxygenated product ratio ranged from 1 : 1 to 1 : 5 for the 7-hydroperoxides Ib and IIb and from 1 : 1 to 1 : 10 for the 7-alcohols Ia and IIa, thus emphasizing preference for the quasiequatorial[15] epimers as previously demonstrated in other studies.[6,12,15] The 7-hydroperoxides were not specifically detected in every experiment where the secondary products Ia, IIa, and III were found. However, it is clear that Ia, IIa, and III derived from Ib and IIb and that complex transformations of sterol hydroperoxides occur in liver[11a,c,16] which may or may not be enzymic. The same products Ia, IIa, and III result from pyrolysis[13] and from bimolecular disproportionation[17] of Ib and IIb.

These sterol oxidations by liver microsomes are thus similar to free radical oxidations of cholesterol by air[12] and by lipoxygenase and peroxidase.[6] The obvious difference is the diminished stability of the initial product hydroperoxides Ib and IIb in the microsomal preparations. Our present data suggest that the lipid peroxidation system involves free radical processes and not singlet molecular oxygen. Neither a putative direct activation of ground-state molecular oxygen nor disproportionation to peroxide anion and singlet molecular oxygen of superoxide radical anion implicated in lipid peroxidations[4c,d,5a,18] is supported by our results.[19] Speculations implicating singlet molecular oxygen in lipid peroxidation in rat liver, indeed in enzyme reactions, should now be viewed with reservation.

Formation of the 7-hydroperoxides Ib and IIb from cholesterol, while formally representing the action of a microsomal dioxygenase, may not be enzymic or involve a sterol–dioxygenase complex. Rather, the cholesterol 7-radical[12,17,20] necessary to formation of Ib and IIb may derive in conjunction with generation of other lipid radicals,[11d] superoxide radical anion,[4c,d,5a,18] hydroxyl radicals resulting from interaction of superoxide and peroxide anions,[21] or yet other radicals.

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Kinetic Analysis of an Intramolecular Addition of a Grignard Reagent to an Alkene

Sir:

Many cyclizations of unsaturated Grignard reagents and (the reverse reactions) ring openings of strained