itative and quantitative parameters of the teratogenicity of isoproterenol were compared to those of trypan blue.

The author wishes to express his sincere thanks to Miss Karin Landeen for her technical assistance.

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Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.

The Metabolism of Glyceryl Thioethers* (33746)

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Alkyl O-ethers of glycerol are readily cleaved by a pteridine-requiring enzyme found in mammalian livers (1, 2). The activity of the cleavage enzyme is lower in other tissues and varies among species (2); neoplasms that contain high levels of ether-linked lipids (3) appear to lack the cleavage enzyme (4). Although the general reaction sequence for the cleavage has been postulated (1), experimental evidence on the actual mechanism is lacking.

We believed that the glyceryl thioethers (S-ethers) would be valuable model compounds to shed light on the reaction steps involved in the biocleavage of the *O*-alkylglycerols, since we could isotopically label the thioethers at the three critical positions of the glyceryl ether molecule (the ether linkage with ^{35}S, the alkyl chain with ^{3}H or ^{14}C, and the glycerol moiety with ^{3}H or ^{14}C). Glyceryl thioethers have not yet been isolated in nature nor is anything known about their behavior in biological systems although their chemical synthesis (5) and their physical properties and quantitative analysis (6) have been investigated. Therefore, our initial feeding experiments with rats were directed toward comparing the metabolism of alkyl O- and S-ethers of glycerol having identical 1-^{14}C-alkyl moieties.

Methods. The synthesis and radiopurity of the 1-^{14}C-hexadecyl-O-glycerols (6.35 μCi/mg) used in this study were previously described in detail (7). The 1-^{14}C-hexadecyl-S-glycerol (2.01 μCi/mg) and 1-^{14}C-octadecyl-S-glycerol (1.95 μCi/mg) were synthesized (5) from 1-thioglycerol and 1-^{14}C-hexadecyl bromide or 1-^{14}C-octadecyl bromide, respectively, under conditions similar to those described by Lawson *et al.* (8). The 1-isomers were racemic mixtures. Radiopurities of all compounds used in our study were >98% as determined by zonal profile scans (9, 10) prepared after thin-layer chromatography on Silica Gel G in a solvent system of hexane:diethyl ether:methanol:acetic acid (80:20:10:1, v/v). The chemical purities of the O-ethers

* This investigation was supported in part by the U. S. Atomic Energy Commission and by Public Health Research Grant GM12562-04.

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(7) and S-ethers (6) used in this investigation have been reported.

Twenty μCi of the glyceryl (O and S) ethers were administered in 1 ml of corn oil by stomach intubation to 15 female Carworth Farm Nelson strain rats (175–200 g) maintained on Gambril and Dietrich laboratory chow; 0.5 ml of dextrose (150 mg) was administered immediately after the corn oil. The rats were kept in metabolism cages to permit the collection of urine and feces. In some experiments $^{14}CO_2$ was collected in hydroxide of Hyamine (1 *M* in methanol; Packard Instrument Company, Inc., Downers Grove, Ill.) using a special apparatus (11) for this purpose; collections were made at 15-min intervals at 0–3 hr and 23–26 hr after administering the labeled compounds.

The extraction (12) and measurement of total lipids from tissues and the chromatography (13) and radioassay (9, 10) measurements were carried out as previously described. Aliquots of total urine samples from these experiments were extracted with multiple portions of diethyl ether or butanol.

Other procedures used in attempts to liberate the glyceryl thioethers or their metabolic products from any complexed form in the urine were saponification (13) and $LiAlH_4$ reduction (14) followed by subsequent extraction into diethyl ether. We also attempted to prepare isopropylidene (15) and trimethylsilyl ether (16) derivatives to determine whether any ^{14}C-labeled glyceryl *S*-ethers or derivatives could be extracted from the urine. Chromatography of aliquots of urine and other samples were carried out on 250 μ layers of Silica Gel G in a solvent system of ethanol:NH_4OH:H_2O (80:4:16, v/v) (17) and on anion-exchange resins (18).

Results and Discussion. Our studies showed that the glyceryl S-ethers and the glyceryl O-ethers are not metabolized in the same way. A very high percentage of radioactivity from the ^{14}C-labeled *S*-ethers (45–87%) was found in the urine the first day after administration, but only a small amount of the O-ethers given (<5%) (Table I) showed up in the first-day urines. In general, most lipids or their products are not found in normal urine; we had previously established that only 0.8% ^{14}C was found in a 24-hr urine collection after feeding 1-^{14}C-labeled palmitic acid (17).

TABLE I. Urinary Excretion of ^{14}C after Glyceryl O- and S-Ethers Were Fed to Rats.[a]

Glyceryl ether	^{14}C excreted (% of injected ^{14}C dose)
18:0-1 (S)	87
16:0-1 (S)	45[b]
16:0-1 (O)	5
16:0-2 (O)	3

[a] Total radioactivity in urine collected during first day after oral administration of ^{14}C glyceryl ethers.

[b] A 6.9% portion of the ^{14}C was collected during the first 6 hr.

None of the radioactivity found in the urine after feeding O- and S-ethers was extractable with diethyl ether or butanol. The labeled components did not appear to be conjugated complexes, since they were not released by acid or alkaline hydrolysis. The attempted preparation of several derivatives that reduce the polarity of alcohol and amino groups also failed to allow extraction of the metabolic products.

We did not attempt to chromatograph the small quantities of ^{14}C found in the urine after feeding the O-ethers, but earlier experiments (17) produced at least four ^{14}C urinary components. A single peak of urinary activity was detectable by thin-layer chromatography after feeding the 1-^{14}C hexadecyl glyceryl thioether to rats (Fig. 1). This peak was somewhat more polar than that of the original ^{14}C-labeled glyceryl thioether administered [Fig. 1; the upper ^{14}C-profile scan demonstrates the high degree of radiopurity (>98%) of the S-ethers used in this investigation].

We thought that the oxidation of the glyceryl thioether to a sulfone or some other product might be possible, and therefore tried to oxidize a standard glyceryl thioether in the presence of urine with H_2SO_4 but did not detect any alteration in the TLC pattern of the glyceryl thioethers. Zonal ^{14}C-profile scans of thin-layer chromatograms of glyceryl

thioethers that had been added to urine and exposed to air and room temperature for 6 days were similar to scans from the original thioethers. The thioethers could be extracted from this urine with diethyl ether.

Although the radioactivity appearing in the urine after we fed the glyceryl thioether was contained in a single TLC peak, it was resolved into two major and three minor components by anion-exchange chromatography (Fig. 2). We hope that this separation of the ^{14}C-components by ion-exchange chromatography will permit us to isolate large enough quantities of these metabolic products to ascertain their chemical structures.

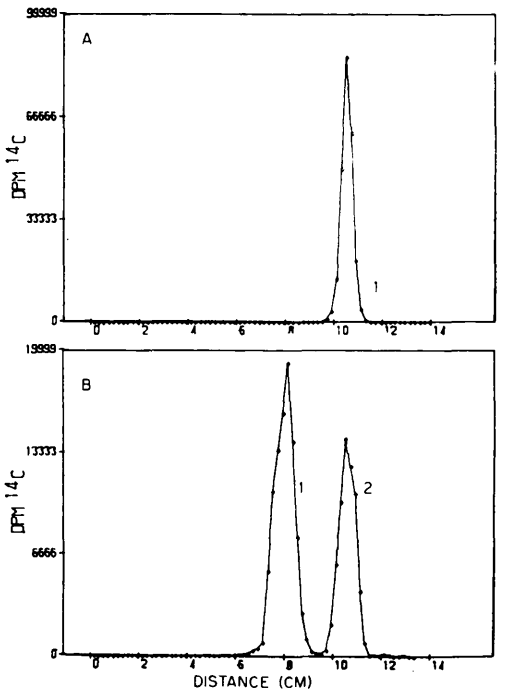

FIG. 1. Zonal ^{14}C-profile scans; (A) 1-^{14}C-hexadecyl glyceryl thioether used in metabolic experiments and as a "spike," and (B) urine sample containing radioactive metabolite (peak 1) after feeding the labeled glyceryl thioether plus the "spike" (peak 2).

The distribution of ^{14}C in lipids of various rat tissues 27 hr after feeding the glyceryl thioethers and 1- and 2-isomers of the glyceryl O-ethers is shown in Table II. The highest specific activities were obtained after administration of the O-ethers, especially in the blood, liver, spleen, and kidney. The muscle, intestinal tract, and intestinal contents also had high specific activity-lipids in animals fed the 2-isomer. Distribution of radioactivity from 1- and 2-isomers in the lipid classes of rat liver was similar to that which we observed after giving these compounds intravenously (13). In contrast, only the intestinal contents and the feces of rats fed ^{14}C-labeled glyceryl thioethers contained high specific activities of lipid (Table III). This unabsorbed radioactivity in feces and intestinal contents occurred primarily as the unesterified glyceryl thioether. The specific activities of lipid in other tissues of the rats fed glyceryl thioethers were very low and the sample size was too small for reliable chromatographic radioassay.

Collection of $^{14}CO_2$ during the first 3 hr and during the 23–26-hr period after we fed

TABLE II. ^{14}C Distribution in Lipids of Various Rat Tissues 27 hr after ^{14}C-Labeled Glyceryl O- and S-Ethers Were Fed.

Tissue	Glyceryl ether fed		
	16:0-1 (S)	16:0-1 (O)	16:0-2 (O)
	(spec act.: cpm per mg of lipid)		
Plasma	1500	2700	4100
RBC	1400	1400	2000
Liver	900	3100	6000
Spleen	1600	3700	3900
Kidney	—	2000	3500
Muscle	200	1100	2100
Perirenal fat	0	100	1500
GI tract	500	1000	4100
GI contents	3000	1000	2000
Feces	7000	1000	1200

TABLE III. Metabolic Fate of Glyceryl Thioethers (16:0-1) Fed to Rats.[a]

TLC area	Sample ^{14}C (%)	
	Feces	GI contents
Lysophosphatidyl choline	1.1	0.8
Sphingomyelin	1.6	3.3
Phosphatidyl choline	1.8	7.9
Phosphatidyl serine	4.3	6.2
Phosphatidyl ethanolamine	7.4	6.7
Glyceryl thioether (unesterified)	83	74

[a] 27 hr after oral intubation.

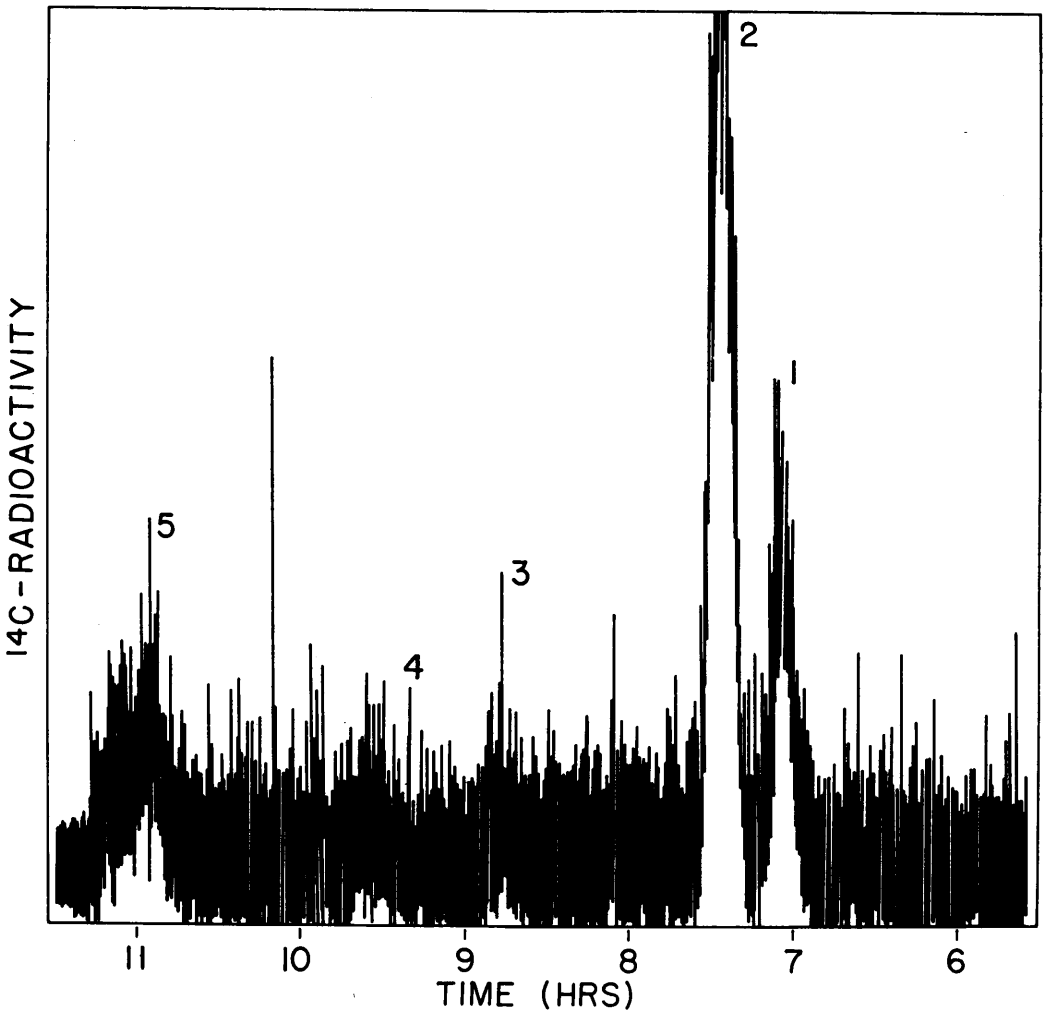

FIG. 2. A portion of a tracing of ^{14}C-constituents isolated from urine of rats fed 1-^{14}C-glyceryl thioether; five ^{14}C-components were resolved by anion-exchange chromatography (18) of a total urine aliquot. No other radioactive peaks were observed during the entire chromatographic run.

the O- and S-ethers containing 16:0 1-^{14}C alkyl moieties revealed that the O-ethers were oxidized at a much higher rate than the S-ethers (Fig. 3). The 2-isomer of the O-ethers was oxidized faster than the corresponding 1-isomer, but the difference was quite small. The higher rate of metabolism exhibited by the 2-isomer, the isomeric form which does not occur in nature, has previously been noted (2, 12, 19).

Our data indicate that the S-ethers of glycerol are not suitable as model compounds for examining the biochemical mechanism of ether cleavage. On the other hand, the marked difference in the biological behavior of the O- and S-ether bond has raised some intriguing questions on the biochemistry of ether linkages.

Summary. The metabolism of 1-^{14}C-labeled glyceryl thioethers ($C_{16:0}$ and $C_{18:0}$) was investigated in feeding experiments with rats. These studies revealed that the glyceryl S-ethers are not metabolized in the same way as are the glyceryl O-ethers. A very high percentage (45–87%) of the ^{14}C from the S-ethers administered was found in the urine during the first day after administration, and essentially none of the label appeared as

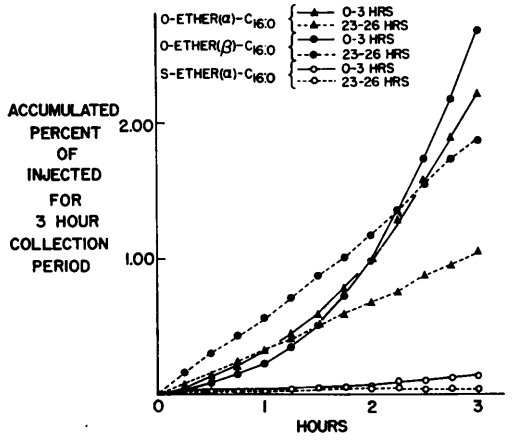

FIG. 3. Metabolism of the O- and S-ethers of glycerol: the production of $^{14}CO_2$ during the first 3 hr and at 23–26 hr after feeding 1-^{14}C-hexadecyl (O-ethers) and S-ethers of glycerol; the 1-isomers are designated by (α) and the 2 isomers by (β); the data are plotted as accumulative percentages at 15-min intervals.

$^{14}CO_2$. The urinary ^{14}C was isolated as a single peak by thin-layer chromatography and as five peaks by anion-exchange chromatography; none of the urinary-^{14}C was extractable with diethyl ether or butanol. Only small quantities of ^{14}C from the absorbed glyceryl S-ethers were found in tissues. Most of the unabsorbed S-ether in the feces and intestinal contents was unchanged. These results obtained *in vivo* indicate that the S-ethers of glycerol cannot serve as model compounds for examining the biochemical mechanism of ether cleavage.

The authors thank Edgar A. Cress for technical assistance in these experiments. We express our appreciation to Drs. C. D. Scott, C. Burtis, and K. S. Warren for doing the analysis of the urinary ^{14}C-constituents by anion-exchange chromatography.

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Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.