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Distribution of Alpha Glyceryl Ethers in Animal Tissues.* (27876)

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Ethers of glycerol with long chain fatty alcohols were first discovered in 1922 by Tsujimoto and Toyama(1) in the non-saponifiable fraction of the liver oil of certain elasmobranchs. Progress on the chemistry and biological properties of these substances has been reviewed(2,3). Little is known of the distribution of the ethers in animal tissue. Karnovsky and co-workers(4) developed a quantitative method of analysis for the ethers based on measurement of formaldehyde liberated by periodate. This was applied to the non-saponifiable fraction from a variety of fish oils and tissues(5,6,7). Among land animal tissues the ethers have been found in intestine and liver of rats(5), in bovine yellow bone marrow(8), erythrocytes(9), brain(10) and butter fat(11), pig spleen(12), and human erythrocytes(13) and atherosclerotic arteries(14). Carter, Smith and Jones(15) isolated a glyceryl ether ethanolamine phosphatide from egg yolk which was resistant to alkaline hydrolysis. Inasmuch as several dog tissues contain appreciable amounts of alkali resistant ethanolamine lipids other than plasmalogen(16) it was of interest to determine if this fraction might be identified with the glyceryl ether phosphatide.

Method. Preparation of lipid hydrolysates. Tissue lipid extracts were prepared, purified and analyzed for phosphorus using methods described previously(16). The glyceryl ethers were liberated by the following hydrolysis procedure. A dried aliquot equivalent to about 0.3 mmole of lipid phosphorus was refluxed on a sand bath for 4 hours in 40 ml 1 N ethanolic KOH. 13.3 ml concentrated HCl were added and the mixture refluxed for another 12 hours. After cooling the alcohol was evaporated *in vacuo* and the hydrolysate extracted 3 times with a total of 50 ml chloroform. The chloroform extract was dried *in vacuo* and taken up in 20 ml petroleum ether.

Isolation of glyceryl ethers. The petroleum ether solution was then fractionated on a silicic acid column by the method of Hirsch and Ahrens(17). In this procedure neutral lipids are first eluted with petroleum ether and mixtures of petroleum ether and ethyl ether (Fractions 1 to 6 inclusive). The glyceryl ethers are then eluted with ethyl ether (Fraction 7) and finally the phosphatides are eluted with methanol (Fraction 8). Hydrolysis recovery experiments with standard samples of batyl alcohol added to synthetic mixtures and to whole lipid extracts are shown in Table I. The batyl alcohol was well recovered from the synthetic mixtures, less so from the natural lipid extracts. It was found only in Fraction 7, as observed by Hirsch and Ahrens. If very large amounts of batyl alcohol were used, small amounts were carried over into Fraction 8.

Hydrolysis recovery experiments with triacetylsphingosine alone or added to synthetic mixtures are shown in Table II. Sphingosine was completely recovered in Fraction 8 on the basis of the nitrogen(16) content of the fractions.

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TABLE I. Recovery of Batyl Alcohol from Hydrolysis and Chromatographic Fractionation Procedures.

Exp. No.	Composition of recovery mixture, μ moles	μ moles HCHO found, Fraction 6	7	8	μ moles batyl alcohol recovered, Fraction 6	7	8	% batyl alcohol recovered, Fraction 6	7	8
1.	Batyl alcohol 33.9, palmitic acid 1024, NaH_2PO_4 167		35.0			35.0			103.	
2.	Batyl alcohol 41.4, NaH_2PO_4 720., palmitic acid 1500., glycerol 700., choline 196., ethanolamine 103., serine 24.1, sphingosine 47.8, inositol 32.8, stearaldehyde 25., cholesterol 82.1		40.8			40.8			98.6	
3.	Intestine lipids containing 301 P.	—	3.06	3.95	—	—	—	—	—	—
4.	Batyl alcohol 42.6, intestine lipids containing 301. P.	.0	34.6	10.3	.0	31.5	6.35	.0	74.0	14.9
5.	Intestine lipids containing 274 P.	—	2.78	3.60	—	—	—	—	—	—
6.	Batyl alcohol 27.6, intestine lipids containing 274. P.	.0	26.3	2.34	.0	23.5	.0	.0	85.2	.0
7.	Heart lipids containing 348. P.	—	2.40	1.31	—	—	—	—	—	—
8.	Batyl alcohol 27.6, heart lipids containing 348. P.	.0	20.0	2.57	.0	17.6	1.26	.0	63.7	4.6

TABLE II. Recovery of Sphingosine from the Hydrolysis and Chromatographic Fractionation Procedures.

Exp No.	Composition of recovery mixture, μmoles	μmoles N found, Fraction 7	8	μmoles HCHO formed, Fraction 7	8	% N recovered in Fraction 8
1.	Triacetylsphingosine 47.0	4.0	43.9			93.4
2.	Triacetylsphingosine 96.4, palmitic acid 1500., cholesterol 92.3, NaH_2PO_4 371.	5.9	91.3	.0	13.2	94.8
3.	Triacetylsphingosine 49.4, palmitic acid 410.	2.5	48.6			98.4

Determination of the ethers. Fractions 6 and 7 were oxidized with periodic acid by the method of Karnovsky and Rapson(4) and the formaldehyde formed was determined colorimetrically with chromotropic acid using the method of Frisell and Mackenzie(18). Fraction 6 contained insignificant amounts of formaldehyde-forming substances. Table III summarizes the alpha glyceryl ether content of the several tissues on the basis of formaldehyde liberated from Fraction 7.

Discussion. The specificity of this method for alpha glyceryl ethers depends on the separation of these from most other lipid hydrolysis components and on the formation of formaldehyde by periodic acid oxidation. Recovery experiments with batyl alcohol and

TABLE III. Alpha Glyceryl Ether Content of Dog Tissues.

Tissue and animal No.	Molar ratio to lipid phosphorus	μmoles per g of dry lipid-free tissue
Lung 1	.0027	.41
" 2	.0043	.70
Kidney 1	.0015	.29
" 2	.0086	.80
Liver 1	.0026	.31
" 2	.0077	1.16
" 2	.0071	1.07
Spleen 1	.0048	.67
Intestine 1	.0102	1.35
Heart 1	.0069	.59
Aorta 1	.0039	.20
Skeletal muscle 1	.0157	1.02
" " 1	.0159	1.03
Bone marrow 1 (Rabbit)	.157	8.73
Bone marrow 2 (Rabbit)	.360	14.1

sphingosine demonstrate the general validity of the method although recovery of batyl alcohol was not as good from natural lipid extracts as from synthetic mixtures.

Two possible sources of error might be considered. Fraction 7 might contain unknown substances capable of liberating formaldehyde on periodate oxidation. Incomplete hydrolysis of the glyceryl ether compounds would result in failure to recover them in Fraction 7, giving low results. The drastic conditions of hydrolysis would argue for complete liberation of the ethers from the parent lipids.

The total amount of alpha glyceryl ether found in most of the tissues is very low and would represent from 0.2 to 1.4% of total phospholipid if all of it were present as ethanolamine phosphatide(15). This is much smaller than the amounts of the alkali resistant ethanolamine lipid in these tissues (16). However, bone marrow lipid was found to be exceptionally high in alpha glyceryl ethers by this method as anticipated from the isolation from this source by Holmes *et al.*(8).

Summary. A method is described for determination of lipid alpha glyceryl ethers in animal tissues. Purified tissue lipid extracts are hydrolyzed and the lipid soluble fraction of the hydrolysate is fractionated on a silicic acid column. The glyceryl ether fraction is oxidized with periodic acid and the formaldehyde formed is determined colorimetrically. The alpha glyceryl ethers are a minor component of all of the tissues examined except bone marrow. The tissues other than bone marrow range in content from 0.20 to 1.35 micromoles per gram of dry lipid-free tissue and from 0.002 to 0.010 molar ratio to lipid phosphorus.

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