The glyceryl ethers in man and cow*

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SUMMARY

The glyceryl ethers were studied in lipids from human bone marrow, spleen, and milk; from yellow bone marrow and milk of cow; and from the yolk of hens' eggs. The highest concentrations were found in human red bone marrow and in human milk. No glyceryl ethers could be demonstrated in red cells or egg yolk. Besides the main components (chimyl, batyl, and selachyl alcohols), several new glyceryl ethers were found. The glyceryl ethers in man and cow were generally much more saturated than those in the liver oils of elasmobranch fish, although a fairly high degree of unsaturation was found in the glyceryl ethers of human milk.

A method has been described for the isolation and gas-liquid chromatographic (GLC) analysis of glyceryl ethers from elasmobranch fish oils with a high content of these compounds (1, 2). The same technique was used in the present study of glyceryl ethers in different human tissues and tissues from land animals. In these sources, the content of glyceryl ethers is often very low. In the human body, the highest percentage was found in the bone marrow. It is possible that the glyceryl ethers are important for cell growth. We therefore analyzed human milk, cow milk, and egg yolk for their content of glyceryl ethers. Since these compounds are reported to stimulate blood cell formation, we also looked for them in human red cells and human spleen.

MATERIALS AND METHODS

Human Material. The red bone marrow was collected from the long bones of several autopsy cases free from any blood diseases. The spleens were taken from three cases in which death was accidental. The milk was pooled human milk obtained from a milk bank.

Red blood cells were obtained by centrifugation of blood from blood donor bottles. The cells were washed twice with one volume of 0.9% NaCl.

Animal Material. The yellow bone marrow was taken from the long bones of young cows. Fresh, unpasteurized cow milk was used. The fresh egg yolk came from the eggs of domestic hens.

Extraction of Lipids. The red cells and the cream of the milk separated by centrifugation were lyophilized before the extraction of the lipids. Bone marrow, spleen, and egg yolk were extracted directly. One part by weight of the lyophilized or the fresh material was extracted with 12 volumes of a chloroform-methanol mixture, 2:1 (v/v). The mixture was warmed in a water bath at 50° for 15 minutes and allowed to stand for at least 16 hours at room temperature. It was then filtered and the solution partitioned against one-fifth volume of 0.1% NaCl (3). After 24 hours, the mixture had separated into two phases. The lower phase, mainly chloroform, which contained the lipids, was concentrated by evaporating most of the chloroform under reduced pressure. An aliquot was taken for gravimetric determination of the lipids.

Hydrolysis and Extraction of the Nonsaponifiable Matter and Free Fatty Acids. The alkaline hydrolysis of the lipids and the extraction of the nonsaponifiable matter were performed as previously described (2), but, in addition, the ether extracts were washed with water containing sulfuric acid (pH lower than 2) to convert the contaminating soaps into free fatty acids. The sulfuric acid remaining in the ether phase was removed by repeated washings with small amounts of distilled water. Finally, the ether solution was dried over MgSO$_4$, filtered, and evaporated under reduced pressure, the temperature not exceeding 30°. The mixture of nonsaponifiable matter and free fatty acids was treated with diazomethane to esterify the fatty acids.

Chromatography on Activated Alumina. The mixture of nonsaponifiable matter and methyl esters of fatty acids was dissolved in methylene chloride and applied to a column of activated alumina pretreated as de-

scribed by Asselineau (2, 4). As there are only small amounts of glyceryl ethers in animal tissues, we had to start with fairly large quantities of material. In the case of human milk, for example, 2.7 g of the mixture of nonsaponifiable matter and methyl esters of free fatty acids was applied to a column of 100 g of alumina, 25 mm in diameter and 15 cm in height. The methyl esters, carotenoids, and other substances of low polarity were eluted with methylene chloride. After 10 column volumes, the cholesterol appeared, often together with some orange substances. There was some tailing of the cholesterol, but after 10 more column volumes, the polarity of the solvent was increased by adding 10% of methanol to the methylene chloride. The main part of the "crude glyceryl ethers" now left the column rapidly; but as there was a little tailing, the elution was continued until 15 to 20 column volumes of the last eluant had passed the column. The eluants were collected in fractions of 1 column volume. Each fraction was evaporated, and the residue was weighed and taken for further examinations. The glyceryl ethers from this chromatography were purified by rechromatography on alumina. One gram of adsorbent was used for every 25 mg of glyceryl ethers.

The glyceryl ethers were then converted into their dimethoxy derivatives (5), which were purified as previously described (2). The composition of the glyceryl ethers was determined by GLC as described in detail earlier (2).

Quantitative Determination of the Glyceryl Ethers. The glyceryl ethers were determined gravimetrically after rechromatography on alumina. The determinations were checked by comparison with the yield of dimethoxy derivatives; this yield varied between 50% and 75%. In the case of the spleen lipids, there was a large contamination of the glyceryl ether fraction with other substances. The content of glyceryl ethers in the spleen was therefore calculated from the amount of dimethoxy derivatives obtained.

RESULTS

Isolation of Glyceryl Ethers. The quantitative data on the amounts of glyceryl ethers in the lipids analyzed are given in Table 1. The largest amount, 0.2%, was found in human red bone marrow. A more detailed description of the results in the different tissues follows.

The cholesterol fraction obtained from alumina accounted for 7% of the original lipids of spleen. After rechromatography, the fraction of "crude glyceryl ethers" was 0.33% of the total lipids. However, on treatment with boron trifluoride (BF₃) and diazomethane, the yield of the dimethoxy derivatives of the glyc-

TABLE 1. PERCENTAGE OF GLYCERYL ETHERS IN LIPIDS FROM SOME HUMAN AND ANIMAL SOURCES*

Source	Glyceryl Ethers in Lipids, %
Human bone marrow (red)	0.2
Human spleen	0.05
Human red blood cells	< 0.01
Human milk	0.1
Cow bone marrow (yellow)	0.01
Cow milk	0.01
Egg yolk	†
Liver oil of elasmobranch fish	10–30

* The glyceryl ethers were determined gravimetrically after chromatography on activated alumina.
† Not found.

eryl ethers corresponded only to 0.05% of the spleen lipids. A further treatment of the nonreacting residue with BF₃ and diazomethane gave no more dimethoxy derivatives. This residue was analyzed by paper chromatography using a solvent system of tetrahydrofuran-diisobutyl ketone-water, 45:5:6 (v/v) according to Beiss and Armbruster (6). A series of yellow spots was obtained with dipicrylamine. The R_f of the main spot was 0.29. In none of the spots could phosphorus be demonstrated by the reagents of Hanes and Isherwood (7).

The content of lipids in the red cells is very low. Starting with 5.5 g of red cell lipids, we were unable to demonstrate any glyceryl ethers by GLC. Glyceryl ethers could not be more than 0.01% of the lipids of red cells.

Some preliminary studies showed that the red cells contained glyceryl ether-phosphatides. When these compounds were treated with acetic anhydride and acetic acid (8), they were converted into the diacetates of glyceryl ethers, which gave chimyl, batyl, and selachyl alcohols after saponification. Brohult and Holmberg (9) have reported the finding of glyceryl ethers in human red cells. In their study the nonsaponifiable material from the red cells was oxidized by periodic acid.[1]

Cholesterol amounted to 25% of the lipids of the red cell. Because of this high cholesterol concentration, the separation of the other components in the nonsaponifiable material from red cells is a difficult task.

Human milk had a surprisingly large content of glyceryl ethers, 0.1% of the lipids. In cow milk, by contrast, only 0.01% of the lipids were glyceryl ethers. A low percentage of glyceryl ethers, 0.01% of the lipids, was also found in the yellow bone marrow of cow. This figure, however, is not directly comparable to the figure for human marrow as the latter refers to a sample of red

[1] Holmberg, J., personal communication.

marrow. No glyceryl ethers could be demonstrated in egg yolk by GLC. For comparison, the high figures for the levels of glyceryl ethers in the liver oils of elasmobranch fish have been included in Table 1.

Composition of the Glyceryl Ethers. The identification of the glyceryl ethers in the present study is based on the same technical procedures as used in our previous paper (2). Gas-liquid chromatography before and after hydrogenation and mass spectrometry were used. As only minute amounts of substances are needed for these analyses, it has been possible to study the glyceryl ethers in animal tissues even when they are present only in trace amounts.

The composition of the glyceryl ethers from human bone marrow, spleen, and milk is given in Table 2. This table clearly demonstrates that there are several components other than chimyl, batyl, and selachyl alcohols, in conformity with our findings in the elasmobranch liver oils (2). In human bone marrow, the chimyl, batyl, and selachyl alcohols together constituted about 70% of the glyceryl ethers. A remarkably high proportion of glyceryl ethers with odd numbers (17 and 19) of carbon atoms in the long-chain moiety of the molecule was found in the bone marrow. The GLC analysis on Reoplex 400 after hydrogenation indicated that 45% of the C_{17} and 30% of the C_{19} alkyl chains were branched. On Reoplex 400, the separation of normal and branched isomers is fairly good and the dimethoxy derivatives of the odd-numbered glyceryl ethers separated into two peaks. The glyceryl ethers in the human bone marrow were much more saturated (71% saturated components) than those in liver oils of elasmobranch fish (28.5%

saturated in the case of *Squalus acanthias*). The presence of an octadecadienyl glyceryl ether could not be demonstrated by GLC or by mass spectrometry, even after the concentration of unsaturated C_{18} glyceryl ethers was increased in the following manner. The dimethoxy derivatives of the glyceryl ethers were chromatographed on alumina. The more unsaturated ethers were concentrated in the tail of the peak. This material was then fractionated by GLC on a silicone grease column. The first part of the C_{18} peak, containing the unsaturated components, was collected and rechromatographed on Reoplex 400. However, no octadecadienyl ether was found. A fairly large amount of glyceryl ethers with C_{20} and C_{22} alkyl chains was found, about 6% of each.

In human spleen, the chimyl, batyl, and selachyl alcohols constituted about 85% of the glyceryl ethers. As in human bone marrow, the saturated ethers predominated (65% of the total). Only small amounts of components with odd-numbered chains were present. Of the glyceryl ethers of the spleen, 27.6% was selachyl alcohol as compared with 16.7% in the bone marrow. The C_{20} and C_{22} ethers were found in about the same percentage as in the bone marrow.

The GLC analysis of the mixture of glyceryl ethers from milk before hydrogenation is given in Figure 1, and the pattern of the same mixture after hydrogenation in Figure 2. The glyceryl ethers of human milk were more unsaturated than those of human marrow fat. The saturated ethers in milk constituted 55% of the total. The rest were primarily monounsaturated but there were small amounts of an octadecadienyl glyceryl ether. The finding of the glyceryl ether with

TABLE 2. PERCENTAGE COMPOSITION (WEIGHT) OF GLYCERYL ETHERS FROM HUMAN BONE MARROW, HUMAN SPLEEN, AND HUMAN MILK

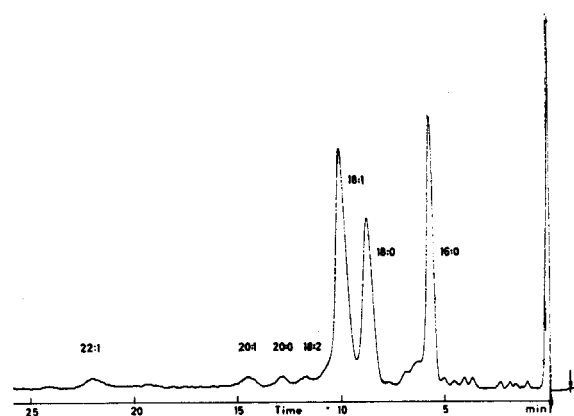| Long-chain Component in Gyceryl Ether	Human Bone Marrow	Human Spleen	Human Milk
Unidentified components	3.8		2.0
16:0	29.4	33.0	23.9
16:1			trace
17*	7.6	1.0	3.6
18:0	24.6	25.8	22.8
18:1	16.7	27.6	33.8
18:2			1.4
19*	6.1	?	2.4
			1.6
20:0	2.9 }	7.3	
20:1	3.2 }		
			2.3
			0.7
22:0	0.7 }	5.2	
22:1	5.1 }		
			3.4
24			2.1

* Normal & branched.

FIG. 1. Gas-liquid chromatogram of the dimethoxy derivatives of the glyceryl ethers in human milk before hydrogenation. Stationary phase: succinate diethylene glycol polyester. Temperature: 243°. Sample size: 1.4 μl of a concentrated solution. The figures beside the peaks refer to the long-chain part of the molecule, the figures before the colons denote the number of carbon atoms, and the figures after the colon the number of double bonds.
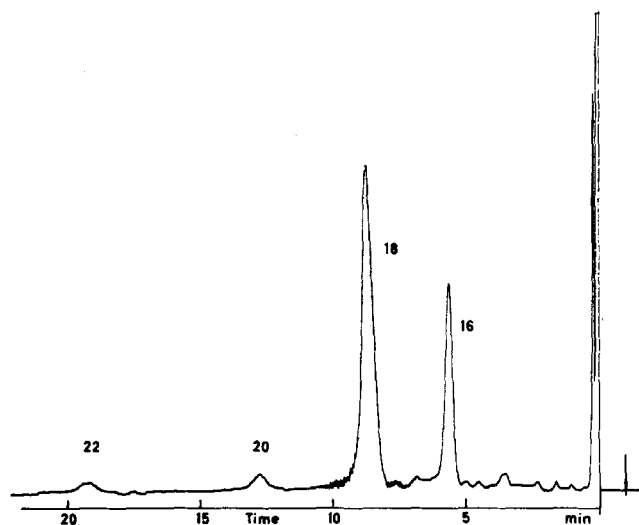
FIG. 2.　Gas-liquid chromatogram of the dimethoxy derivatives of the glyceryl ethers in human milk after hydrogenation. Column and temperature the same as in Figure 1. Sample size: 1.1 μl of a concentrated solution. The figures beside the peaks denote the number of carbon atoms in the long-chain part of the molecule.

a hexadecenyl chain further emphasizes the high degree of unsaturation.　About 4% each of the C_{20} and C_{22} ethers were found.　Furthermore, about 2% of a C_{24} ether could be demonstrated on a silicone column loaded with 5 mg of the hydrogenated mixture (Fig. 3).

Because of the small amounts of glyceryl ethers present, we are only able to give the ratio between the main components, namely chimyl, batyl, and selachyl alcohols in the cow marrow fat and in the cow milk. These figures together with those for the human bone marrow, spleen, and milk have been collected in Table
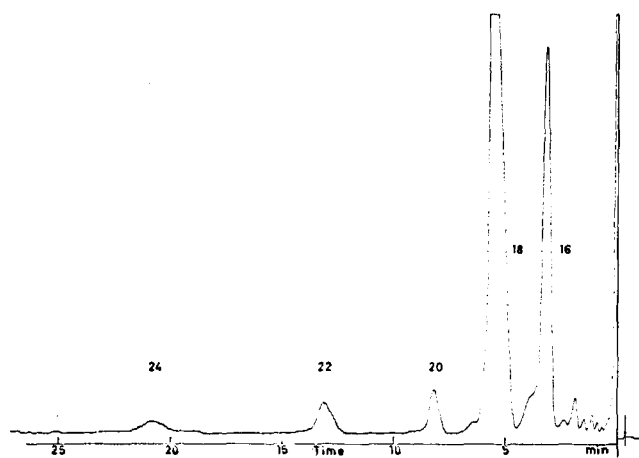

FIG. 3.　Gas-liquid chromatogram of a large sample of the dimethoxy derivatives of the glyceryl ethers in human milk after hydrogenation. Stationary phase: silicone grease. Temperature: 265°.　Sample size: 7 μl of a concentrated solution containing about 5 mg of substance. The figures beside the peaks denote the number of carbon atoms in the long-chain part of the molecule.

TABLE 3.　RELATIVE AMOUNTS OF THE MAIN COMPONENTS OF GLYCERYL ETHERS IN SOME FATS FROM MAN AND COW *

Glyceryl Ether/ Batyl	Human			Cow	
	Milk	Spleen	Bone marrow	Milk	Bone marrow
Chimyl/batyl	1.05	1.28	1.20	1.15	0.60
Selachyl/batyl	1.48	1.07	0.68	0.73	0.30

* The figures represent the ratios between the amounts of the glyceryl ethers shown and the amounts of batyl alcohol in the fats studied.

3.　The glyceryl ethers from cow are much more saturated than those from man.　The ratio between the unsaturated (selachyl) and the saturated (batyl + chimyl) alcohols could be calculated and was 0.72 for human milk and only 0.34 for cow milk.　This quotient was 0.31 in human red bone marrow and 0.20 in the yellow bone marrow of cow.

DISCUSSION

The presence of glyceryl ethers in land animals was first demonstrated by Holmes et al. (10), who isolated batyl alcohol from the yellow bone marrow of cattle. By oxidation with periodic acid, Karnovsky et al. (11) estimated the content of glyceryl ethers of fats from several animal and vegetable materials.　In the marrow fat of the common domestic ox, they found the glyceryl ether content, calculated as selachyl dioleate, to be 0.2% of the lipids.　The free glyceryl ethers thus constituted 0.08% of the lipids.　This figure is of the same order of magnitude as that for the amount of batyl alcohol isolated from yellow bone marrow of cattle by Holmes et al. (10).

No studies on the occurrence of glyceryl ethers in human bone marrow have been published.[2]　Prelog, Ruzicka, and Stein (12) have isolated batyl alcohol in trace amounts from pig spleen; only 85 mg was obtained from 1,500 kg of spleens.　They extracted the nonsaponifiable matter from the acetone-soluble fat (15.7 kg) with warm methanol.　The diester of batyl alcohol might partly have remained in the glycerides (12 kg), which would explain the very low yield.　Hardegger, Ruzicka, and Tagmann (13) separated 285 mg of batyl alcohol (0.008% of the oil) from 3,450 g of fat obtained from 370 atherosclerotic human aortas.　Bodman and Maisin (14) isolated glyceryl ethers from perinephric fat of neonatal calves.　The concentration fell very rapidly after birth.　These authors also identified glyceryl ethers in the meconium.

[2] Brohult and Holmberg (personal communication) have demonstrated the occurrence of glyceryl ethers in the nonsaponifiable material from human bone marrow by oxidation with periodic acid and estimation of the formaldehyde generated.

No investigations on the content of glyceryl ethers in milk have hitherto been made. The finding of glyceryl ethers in milk is interesting as these compounds have been found in fetal tissues or rapidly reproducing ones such as bone marrow. The glyceryl ethers in milk might stimulate the growth of the newborn individual. Brohult (15) has shown that selachyl alcohol has a growth stimulating effect on *Lactobacillus lactis*.

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