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On the Occurrence of 1-*O*-Alkylglycerols and 1-*O*-(2-Methoxyalkyl)glycerols* in Human Colostrum, Human Milk, Cow's Milk, Sheep's Milk, Human Red Bone Marrow, Red Cells, Blood Plasma and a Uterine Carcinoma

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1-*O*-Alkylglycerols and 1-*O*-(2-methoxyalkyl)-glycerols were isolated from the neutral lipids and phospholipids of human colostrum, human milk, cow's milk, sheep's milk, human red bone marrow, red cells, blood plasma, and a uterine carcinoma. Human colostrum has a higher content of unsubstituted glycerol ethers in the neutral lipids than human milk. Human milk contains nearly 10 times more unsubstituted glycerol ethers than cow's milk and twice as much as sheep's milk. The highest percentage of unsubstituted glycerol ethers in neutral lipids was found in the human red bone marrow and the uterine carcinoma. The methoxy-substituted glycerol ethers were found both in the neutral lipids and in the phospholipids of all the tissues studied but only in trace quantities. Glycerol ethers with 16 and 18 carbon atoms in the long hydrocarbon chains are the principal components of both the unsubstituted and the 2-methoxy-substitued glycerol ethers. A poly-unsaturated methoxy-substituted glycerol ether, 1-*O*-(2-methoxydocosahexaenyl)glycerol, was found in the neutral lipids and phospholipids of red blood cells.

The physiological effect of the glycerol ethers that is best documented is the stimulation of the bone marrow. Sandler [1] reported that batyl alcohol (1-octadecylglycerol) had a stimulatory effect on the erythrocyte count of both normal rats and those poisoned with benzene. It was confirmed by several investigators that both optically active and racemic batyl alcohol stimulated erythropoesis, thrombopoesis and granulopoesis.[2–10] Chimyl alcohol also has a stimulatory effect on haemopoesis [9] but selachyl alcohol (*cis*-9-octadecenylglycerol) has no haemopoetic activity.[7,10]

The glycerol ethers occur in the tissues in the form of diesters [11,12] and alkyl acyl phosphatides.[13–17] A high level of glycerol ether lipids was found in a variety of transplantable tumours in animals [18–19] and in human tumours.[20]

2-Methoxy-substituted glycerol ethers have been isolated from Greenland shark liver oil.[21] These substituted glycerol ethers have antibiotic activity and also inhibited the dissemination and growth of several experimental tumours in mice.[22,23] The aim of the present investigation was to study methoxy-substituted as well as ordinary, unsubstituted glycerol ethers in human milk, red bone marrow, red blood corpuscles, and blood plasma. A human uterine tumour has also been studied. Milk from two ruminant species, cow and sheep, is included for comparison with human milk. The composition of the two groups of glycerol ethers has also been determined.

MATERIAL AND METHODS

Material. The human milk was pooled milk from a milk bank at Sahlgren's hospital, Gothenburg. Colostrum was collected during the first two days after delivery, "transition milk" during days 3 – 7 and the third sample of milk was from lactating women 8 days to 3 months

* "Alkyl" also includes unsaturated carbon chains except those with a double bond adjacent to the glycerol ether oxygen.

after delivery. The cow's milk was fresh pasteurized milk and the sheep's milk was fresh but unpasteurized.

Red blood cells and plasma were obtained by centrifugation of blood from blood donor bottles. The cells were washed twice with one volume of 0.9 % NaCl. The red bone marrow was collected from the long bones of several autopsy cases free from any blood diseases.

The uterine carcinoma was obtained from the Department of Gynecology, Sahlgren's hospital. The tumour could not be dissected out from the part of the uterus obtained. The large tumour analyzed thus included some normal uterine tissue.

Extraction of lipids. The blood plasma and the milk were freeze-dried before the extraction of the lipids. The red bone marrow, the red cells and the uterine carcinoma were extracted directly. One part by weight of the freeze-dried or the fresh material was extracted with 12 volumes of a chloroform – methanol mixture, 2:1 (v/v) or a mixture of chloroform – ethanol, 2:1 (v/v). The mixture was boiled under reflux for 90 min. After cooling at room temperature, it was filtered. The residue was boiled for another 90 min in chloroform – methanol or chloroform – ethanol. The extract was evaporated to half its volume and then partitioned against one-fifth volume of 0.1 % NaCl. After separation of the chloroform phase, the NaCl solution was extracted 3 times with chloroform. The solvents were evaporated from the lipid extract and the residue was dried under a stream of nitrogen.

Separation of neutral lipids, phospholipids, glycerol ethers, and isopropylidene derivatives of glycerol ethers. 10 g of lipid material was dissolved in chloroform and applied to a column of 1350 g of silicic acid (silicic acid for lipid chromatography, Bio-Rad Lab., Richmond, Calif.). The neutral lipid fraction was eluted with chloroform and chloroform – methanol 9:1 (or chloroform – ethanol 9:1). The main phospholipid fraction was eluted with chloroform – methanol 1:1 (or chloroform – ethanol 1:1) but due to tailing the last parts had to be eluted with methanol (or ethanol).

The neutral lipid fraction was saponified by boiling in 1 M KOH in ethanol for 1 h. The phospholipids were treated according to Thompson and Lee.[24] The material was refluxed with acetic acid – acetic anhydride 3:2 for 8 h. The mixture was made alkaline with 6 M KOH in ethanol and refluxed for a further 2 h. The non-saponifiable material was extracted into ether. Fatty acid salts remaining in the material were removed by treatment with a small amount of lithium aluminium hydride in ether solution. In a few cases the lipid fraction was directly treated with lithium aluminium hydride to cleave the ester bonds. After acidification of the reduction mixture with hydrochloric acid, the organic material was extracted into ether. The material was chromatographed on silicic acid columns. Hydrocarbons, sterols and alcohols were eluted with a mixture of light petroleum (b.p. 60 – 80 °C) and ethyl ether (19:1 v/v). The unsubstituted and methoxy-substituted glycerol ethers were then eluted with ethyl ether. The column chromatography was checked by thin-layer chromatography (Silica gel G, Merck. Developing solvent: trimethylpentane/ethyl acetate/methanol, 50:40:5). To transform the glycerol ethers into their isopropylidene derivatives the material from the fractions containing glycerol ethers was treated with acetone in the presence of perchloric acid.[25] The crude mixture of isopropylidene derivatives was purified by chromatography on silicic acid columns (and checked by TLC). The isopropylidene derivatives of the unsubstituted glycerol ethers were eluted with a mixture of 1 % ether in light petroleum (b.p. 60 – 80 °C) and those of the methoxy-substituted glycerol ethers with a mixture of 5 % ether in light petroleum. The amounts of unsubstituted and methoxy-substituted glycerol ethers were determined by weighing the fractions after evaporation of the solvents under a stream of nitrogen. In some cases with very small amounts of material available, preparative thin-layer chromatography was used (Silica gel G, layer 0.5 – 1 mm). When trimethylpentane/ethyl acetate, 70:20, was used as developing solvent, the isopropylidene derivatives of the unsubstituted and the methoxy-substituted glycerol ethers were obtained from the bands with $R_F \approx 0.6$ and $R_F \approx 0.3$, respectively. The isopropylidene derivatives were used for determination of the compositions by means of gas chromatography and mass spectrometry.

Gas-liquid chromatography. The gas chromatography was performed with a Perkin-Elmer F 11 instrument equipped with a flame ionization detector. A 180 cm × 2 mm i.d. stainless steel column, packed with Gas Chrom Q 80 – 100 mesh containing 1 % Apiezon L was used. The column temperature was in most cases 220 °C. The flow was 30 ml helium/min.

Gas-liquid chromatography-mass spectrometry. The GLC – MS was carried out with the LKB 9000 combination instrument. The operating conditions of the mass spectrometer were: electron energy 70 eV, ion source temperature 270 °C, trap current 60 μA and accelerating voltage 3.5 kV.

The gas chromatography on the combined instrument was carried out at 220 °C using a 300 cm × 2 mm i.d. glass column packed with Gas Chrom Q 80 – 100 mesh, containing 1 % Apiezon L.

RESULTS AND DISCUSSION

The quantitative data on the amounts of glycerol ethers in the lipids are given in Table 1. The content of lipids in human colostrum as

Table 1. The content of unsubstituted and methoxy-substituted glycerol ethers in neutral lipids and in phospholipids isolated from human colostrum, human milk, cow's milk, sheep's milk, human red bone marrow, human red cells, human blood plasma, and a human uterine carcinoma.

Material	Lipids % (w/w)	Neutral lipids (N) and phospholipids (P) in total lipids % (w/w)		Unsubstituted glycerol ethers in N and P [a] % (w/w)		Methoxy-substituted glycerol ethers in N and P % (w/w)	
		N	P	N	P	N	P
Human colostrum, 1–2 days	2.9	96.4	3.6	0.19	0.16	trace	trace
Human "transition milk", 3–7 days	2.8	98.2	1.8	0.14	0.20	trace	trace
Human milk, 8 days–3 months	2.8	99.0	1.0	0.10	0.18	trace	trace
Cow's milk	2.9	99.0	1.0	0.01	0.16	trace	trace
Sheep's milk	7.2	99.6	0.4	0.02	0.14	trace	trace
Human red bone marrow		98.2	1.8	0.33	2.0	trace	trace
Human red blood cells	0.3	32.5	67.5	0.08	0.75	trace	trace
Human blood plasma	0.5	64.6	35.4	0.05	0.63	trace	trace
Uterine carcinoma	1.0	48.3	51.7	0.42	0.50	trace	trace

[a] The figures should be multiplied by a factor of about 2.0–2.5 to get the content of the original glycerol ether lipids in the lipid fractions.

well as in transition milk and milk collected from 8 days to 3 months after delivery is about the same. The percentage of phospholipids in the lipids decreases from 3.6 % in the colostrum to 1.0 % in the milk from the second week of lactation and onwards. The content of glycerol ethers in the phospholipids is about the same, 0.2 %, in the tree types of human milk, whereas the glycerol ether content in the neutral lipids is highest in colostrum, 0.19 %, and decreases to 0.14 % during the first week and then to 0.10 %. The neutral lipids of cow's milk and sheep's milk contain 0.01 and 0.02 % glycerol ethers, respectively. A comparison of human milk with cow's and sheep's milk shows that human milk per volume contains nearly 10 times more glycerol ethers than cow's milk and twice the amount found in sheep's milk. Trace quantitites of (2-methoxyalkyl)glycerols were found in the neutral lipids as well as in the phospholipids in all the samples of milk studied.

The phospholipids of human red bone marrow, red blood cells and blood plasma are fairly rich in alkylglycerols. The highest figure, 2 %, was found for the red bone marrow. The neutral lipids of the bone marrow are also rich in glycerol ethers in contrast to the neutral lipids of the red blood cells and the blood plasma. In both the neutral lipids and the phospholipids of the uterine carcinoma high levels of glycerol ethers were found. Minute amounts of 1-*O*-(2-methoxyalkyl)glycerols were found in both the neutral lipids and the phospholipids of all tissues investigated.

Composition of the glycerol ethers. The composition of the unsubstituted glycerol ethers of the neutral lipids and of the phospholipids is given in Table 2. The compounds with even-numbered, long chains are predominant; the compounds with odd-numbered chains usually constitute less than 5 % of the whole mixture. The unsubstituted glycerol ethers in the neutral lipids of cow's milk and sheep's milk contain somewhat more odd-numbered, long chains than human milk and also branched alkyl chains. The glycerol ethers with 16 and 18 carbon atoms in the long chains (16:0 chimyl, 18:0 batyl, and 18:1 selachyl alcohol) are the principal components. The compounds with 16 carbon atoms in the long chains are mainly

Table 2. Percentage composition of unsubstituted glycerol ethers from neutral lipids (N) and phospholipids (P).

Long chain component	Human colostrum, 1–2 days		Human "transition milk", 3–7 days		Human milk 8 days–3 months		Cow's milk		Sheep's milk		Human red bone marrow		Human red blood cells		Human blood plasma		Uterine carcinoma	
	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P	N	P
< 14:0			0.2	0.4			0.8*[a]		0.1[e]					0.2	0.5	0.4		
14:0	0.5	0.7	0.5	0.7	0.9	0.8	7.6[b]		4.5[f]	tr.	0.5	0.7	3.2	0.6	3.1	1.4	0.9	0.4
15:0	0.5	0.4	0.5	0.4	0.5	0.6	7.7[c]		2.6[g]	tr.	0.4	0.5	0.7	0.4	0.9	1.0	0.4	0.1
16:0	33.8	32.5	26.0	27.7	24.8	25.3	32.0[d]	46.9	29.2[h]	27.6[k]	33.2	27.9	23.8	30.3	19.5	35.1	29.4	25.6
16:1	1.5	1.7	1.1	1.6	3.2	4.6	1.4	tr.	0.3	1.3	tr.	4.3	16.8	tr.	9.2	tr.	1.0	6.3
17:0	0.7	1.1	1.2	3.8	1.4	1.8	0.9		3.6[i]		1.1	0.7	0.8	1.1	0.7	2.0	0.7	0.9
17:1	0.6	1.0	tr.	1.1	1.6	1.5	1.8		tr.				1.6	1.0	1.7	1.9	1.3	1.2
18:0	21.2	19.1	21.5	17.8	21.8	19.0	29.5	38.0	41.9	52.8	27.0	19.3	11.1	34.8	11.1	19.1	16.3	23.7
18:1	29.7	29.2	38.3	34.1	37.5	34.7	17.7	15.1	17.8	18.3	20.3	28.6	31.8	18.3	41.0	21.7	43.8	37.9
19:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1		tr.		0.2	0.2	0.6	0.1	0.2	0.1	0.1	0.1
19:1	0.3	0.3	0.1	0.2	0.4	0.5	tr				0.2	0.4	0.8	0.2	0.4	0.4	0.3	0.2
20.0	1.6	2.1	1.4	1.8	0.9	1.6	0.2		tr.		3.0	3.1	1.4	2.4	1.4	3.5	0.8	1.1
20:1	1.2	2.4	1.4	2.1	1.7	2.4	0.3				4.2	3.9	2.3	2.4	3.2	4.1	1.8	1.1
21:0 and 1	0.1	0.1	0.1	0.3	0.3	0.5					0.2	0.3	tr.			0.2		
22:0	1.3	1.4	1.0	1.2	0.7	0.9					1.4	1.6	tr.	1.3	1.2	2.2	2.8	0.4
22:1	3.0	3.7	3.1	2.9	2.7	2.8					5.5	4.6	2.1	3.7	5.2	6.9	0.4	1.0
23:0 and 1	0.3	tr.	0.2	0.2	0.1	0.6												
24:0	0.5	0.4	0.3	0.6	tr.	0.4					0.2	0.4	tr.	0.6	0.1			
24:1	3.1	3.8	3.0	3.0	1.4	1.9					2.6	3.5	3.0	2.6	0.6			

* Glycerol ethers with branched hydrocarbon chains: [a] 0.3 % [b] 1.8 % [c] 4.5 % [d] 4.2 % [e] tr. [f] 0.7 % [g] 1.0 % [h] 1.3 % [i] 1.4 % [k] 3.1 %.

Table 3. Percentage of saturated components in the glycerol ethers of neutral lipids and phospholipids from various human material.

	Sat. % Neutral lipids	Sat. % Phospholipids
Colostrum	61	58
"Transition milk", 3–7 days	53	55
Milk, 8 days – 3 months	51	51
Red bone marrow	67	55
Red blood cells	42	72
Blood plasma	39	65
Uterine carcinoma	51	52

saturated. The degree of unsaturation increases with the chain length and the compounds with 24 carbon atoms are mainly unsaturated. The proportion of saturated glycerol ethers in the neutral lipids is higher in the colostrum than in other human milk samples. The red bone marrow has a higher proportion of saturated lipids than other human tissues studied (see Table 3). Only the saturated glycerol ethers, chimyl and batyl alcohol, have been found to stimulate the formation of blood cells.[1–10] The glycerol ethers of the neutral lipids in the bone marrow might be precursors to the glycerol ether phospholipids in the blood cells. It is interesting to note that the phospholipids of the red cells are relatively saturated (Table 3).

The composition of the methoxy-substituted glycerol ethers occurring in neutral lipids and phospholipids is demonstrated in Table 4. As the methoxy-substituted glycerol ethers are found only in minute quantities it was difficult to isolate them in a pure form. The principal components are 2-methoxy-substituted hexadecyl-, hexadecenyl-, and octadecenyl glycerol ethers. A characteristic feature of the methoxy-substituted glycerol ethers is the high content of ethers with 16 carbon atoms in the long alkyl chain. A poly-unsaturated methoxy-substituted glycerol ether with 6 double bonds, 1-*O*-(2-methoxydocosahexaenyl)glycerol was found in both the neutral lipids and the phospholipids of red blood cells. This poly-unsaturated glycerol ether was first found in Greenland shark liver oil.[26]

In the present study we have thus demonstrated that 2-methoxy-substituted glycerol ethers are of common occurrence but in minute

Table 4. Percentage composition of methoxy-substituted glycerol ethers from neutral lipids (N) and phospholipids (P).

Long chain component	Human "transition milk", 3–7 days		Human milk, 8 days–3 months		Cow's milk		Sheep's milk	Human red bone marrow		Human red blood cells		Uterine carcinoma
	N	P	N	P	N	P	P	N	P	N	P	P
14:0	1.5	0.7	tr.	tr.						2.2	tr.	2.1
15:0	0.9	1.1	tr.	tr.						2.0	tr.	1.0
16:0	71.6	84.9	75.8	77.4	92.0	91.3	predominant	26.8	15.2	54.7	28.6	77.1
16:1					5.3	8.7	tr.	21.4	49.1		27.7	
17:0	0.8	0.5	0.6	0.3				2.5	0.6	2.2	2.1	0.2
17:1	3.5	1.0	3.3	2.7				6.4	3.5	2.2	8.2	2.7
18:0	1.8	2.6	1.8	1.2	2.0	tr.	tr.	7.8	2.1	1.7	3.7	0.9
18:1	19.9	8.0	18.5	16.7	0.7	tr.	tr.	20.0	16.4	20.7	19.3	16.0
19:0		0.5		0.7				5.1	2.7	0.4	0.6	
19:1		0.7		0.1				3.2	4.5	1.1	2.3	
20:0				0.2						tr.	tr.	
20:1				0.2				4.5	1.8	3.2	tr.	
21:0				0.1								
21:1				0.2				2.3	2.6			
22:0				tr.							tr.	
22:1				0.2				tr.	1.5			
22:6										9.6	7.5	

quantities. In some experiments the isolation of the glycerol ethers has been performed using ethanol instead of methanol to allay any suspicion that the methoxy-substituted glycerol ethers could be artefacts from methanol treatment.

The physiological functions of the two types of glycerol ethers, the unsubstituted and the 2-methoxy-substituted ones, have not been clarified. The chimyl and batyl alcohols stimulate blood cell formation [1-10] and the 2-methoxy-substituted glycerol ethers have inhibitory effects on tumour growth and dissemination of metastases.[22,23] Further studies to elucidate the metabolism and physiological functions of the methoxy-substituted as well as of the unsubstituted, ordinary glycerol ethers are therefore indicated.

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