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The Component Acids of the Milk Fat of a Grey Atlantic Seal

By M. L. MEARA

Department of Industrial Chemistry, University of Liverpool

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The milk fats of many land animals are characterized by the presence of fatty acids of lower molecular weight than those obtaining in their respective depot fats. This phenomenon is most marked in the case of the milk fats of the ruminants which contain appreciable amounts, especially when expressed as a molar percentage, of the lower even-membered saturated acids from butyric to myristic, which are either absent or occur only in small amounts in their respective depot fats. It is not so pronounced, however, in the case of the milk fats of other herbivora, e.g. horse and ass, while sow milk fat has been shown (De la Mare & Shorland, 1944) to resemble closely that of a normal pig depot fat. The Reichert and Polenske values recorded for milk fats of a number of other animals (e.g. cat, dog and mouse) also indicate the absence therefrom of short-chain acids.

Little, however, is known of the composition of the milk fats of marine animals. Klem (1935) has recorded data for various species of whales, and indicated that the fat content of the milk ranged from 19 to 46 %, thus far exceeding that found in the milk of land animals (usually 3–6 %). He also noted that the mean unsaturation of the milk fat of a blue whale was considerably higher (iodine value 171.8) than that of the fat from any other site in the same animal (iodine values 94.0–102.8), though these latter values are considerably lower than the values obtained for fat obtained from the same sites in healthy males and non-lactating females of the same

species (iodine values 120–140). He further showed that the blue whale milk fat contained the following acids: myristic 8.4, palmitic 16.8, stearic 1.8, unsaturated acids C_{14} 1.2 (–2.0H), C_{16} 6.2 (–2.0H), C_{18} 26.8 (–3.3H), C_{20} 25.9 (–8.5H), C_{22} 12.9 (–11.0H) % (w/w). Similarly, Schmidt-Nielsen & Frog (1933) showed that there are no acids of lower molecular weight than lauric acid present in the milk fat of the finner whale.

An opportunity for further study of the milk fat of a marine animal was made possible by the kindness of Prof. E. C. Amoroso, Dr S. J. Folley, F.R.S., and Dr S. J. Rowland, who placed a small quantity of the milk of a grey (Atlantic) seal (*Halichoerus grypus*), caught off the Pembrokeshire coast in 1950, at the disposal of this laboratory.

METHODS AND RESULTS

The milk was pale cream in colour, of a thick viscous consistency and had a pronounced fishy odour. On extraction of 235 g. milk with light petroleum (b.p. 40–60°) (emulsions readily broken by addition of ethanol) 118 g. (50.0 %, w/w) of a pale oil, saponification equivalent 295.9, iodine value 146.2, unsaponifiable matter 1.5 %, was obtained. The fat (101 g.) gave 95.2 g. mixed fatty acids, after saponification and acidification of the soaps. The steam distillate from the mixed fatty acids required 1.8 ml. 0.1 N-KOH to neutralize the steam volatile acids, this being equivalent to 0.03 g. decanoic acid (or a proportionally smaller amount of the lower steam-volatile acids) indicating the virtual absence of this group of fatty acids.

Component fatty acids of the seal milk fat

The mixed acids (86.3 g.) were crystallized first from acetone at -70° , the deposited acids being subjected to a further recrystallization from acetone at this temperature. The deposited acids were then crystallized from ether at -40° . In this manner the acids were resolved into three fractions consisting of mainly saturated, mainly mono-ethenoid and mainly polyethenoid acids respectively, their proportions and unsaturation being recorded in Table 1.

Table 1. *Low temperature crystallization of seal milk fatty acids*

	Weight		Iodine value
	(g.)	% of total	
A Insoluble in ether at -40°	16.85	19.5	3.4
B Soluble in ether at -40°	22.30	25.8	87.5
C Soluble in acetone at -70°	47.15	54.7	234.2

After methylation, according to the procedure recommended by Bjarnason & Meara (1944), each group of esters was fractionally distilled, the analytical data obtained for each ester fraction being recorded in Table 2, together with the spectroscopic analysis of the acids recovered from fractions B4 and C3. From these results the composition of each ester fraction is calculated according to the methods adequately described by Hilditch (1947, pp. 498-510) and Gupta & Hilditch (1951), and therefrom the composition of each group of acids, leading subsequently to the component acids of the whole fat (Table 3).

DISCUSSION

It is now established that whilst the component fatty acids of whale oils vary but little from season to season (Armstrong & Allan, 1924; Tveraen, 1935; Hilditch & Maddison, 1948), seal blubber oils show an abnormally large variation in the proportions of their component fatty acids (Hilditch & Pathak, 1949; Winter & Nunn, 1950*a, b, c*). These latter authors have shown (1950*b*) that significant differences occur, not only in the blubber oils of the elephant seals from different localities (Heard Island and Macquarie Island), but also in the blubber oils of elephant seals caught in the same locality (Heard Island) and concluded that the influence of any of the three factors, diet, sex and species, was not sufficient to account for the observed differences.

Comparison of the component acids recorded in Table 4 shows that no fundamental difference exists between the blubber oils of the seals so far reported and the seal milk fat, such as occurs between the depot and milk fats respectively of the ruminants and some other land animal species.

There are, however, notable differences in the proportions of the component acids of the seal milk fat and the blubber fat of an animal of the same species, and indeed differences from the blubber fats of all the species of seals so far recorded. Thus,

although the content of both myristic and stearic acids in the milk fat is of the same order as that which obtains in the blubber oils of both British coastal and Antarctic seals, the milk fat differs markedly, in that the palmitic acid content rises to about 16% as compared with the more usual 10-12% in the blubber oils. This increase in the palmitic, and therefore in the total saturated acid content of the milk fat does not appear to be at the expense of any particular unsaturated acid group, if the recorded analysis of grey seal blubber fat can be considered to be typical for that species.

Table 2. *Fractionation data for seal milk ester fractions*

Fraction	Weight (g.)	Saponification equivalent	Iodine value
Methyl esters of acids A			
A1	1.40	245.8	1.1
A2	1.87	266.8	0.8
A3	2.55	269.1	1.0
A4	2.60	269.3	0.8
A5	3.00	270.5	0.5
A6	3.40	278.0	5.6
A7	2.22	333.1*	16.4
Total	17.04	—	—
Methyl esters of acids B			
B1	1.77	262.0	52.8
B2	2.05	286.0	78.2
B3	2.06	294.8	82.3
B4	2.70	295.3	83.7
B5	2.69	295.3	83.9
B6	2.80	297.1	85.5
B7	3.19	297.3	85.8
B8	2.77	298.7	86.1
B9	2.72	323.2*	96.9
Total	22.65	—	—
Methyl esters of acids C			
C1	1.67	241.6	64.2
C2	2.31	263.0	89.4
C3	2.80	267.5	98.0
C4	3.61	271.0	98.2
C5	4.42	281.0	106.7
C6	6.51	292.3	111.6
C7	7.01	302.2	143.8
C8	6.83	325.8	337.6
C9	3.57	334.9	389.6
C10	3.38	336.9	386.9
C11	2.30	338.2	376.4
C12	3.47	363.8*	233.3
Total	47.88	—	—

* Equivalents of esters (freed from unsaponifiable matter): A7, 300.4; B9, 306.7; C12, 330.4.

Spectroscopic analysis of acids recovered from fractions B4 and C3

	B4	C3
$E_{1\text{ cm.}}^{1\%}$, 232 m μ . (unisomerized)	—	5
$E_{1\text{ cm.}}^{1\%}$, 270 m μ . (unisomerized)	—	1*
$E_{1\text{ cm.}}^{1\%}$, 234 m μ . (180°/60 min.)	3*	42
$E_{1\text{ cm.}}^{1\%}$, 268 m μ . (170°/15 min.)	1*	17

* Neglected.

Table 3. *Component acids in groups A, B, C, and in the whole seal milk fat*

Acid	A (19.5%)* %†	B (25.8%)* %†	C (54.7%)* %†	Total fatty acids excluding unsaponifiable	
				(%, w/w)	(% by mol.)
Myristic	8.7	0.9	1.5	2.8	3.4
Palmitic	71.0	4.5	2.2	16.4	17.8
Stearic	14.4	—	—	2.8	2.8
Unsatd. C ₁₄	0.1 (-2.0)	0.8 (-2.0)	2.6 (-2.0)	1.7 (-2.0)	2.0
Unsatd. C ₁₆	0.5 (-2.0)	5.3 (-2.0)	20.3 (-2.2)	12.7 (-2.2)	13.9
Unsatd. C ₁₈	2.5 (-2.0)	80.3 (-2.0)	27.8 (-2.7)	36.6 (-2.3)	36.3
Unsatd. C ₂₀	1.4 (-2.0)	7.6 (-3.7)	20.5 (-7.2)	13.6 (-6.6)	12.4
Unsatd. C ₂₂	—	—	24.4 (-11.2)	13.4 (-11.2)	11.4
Unsaponifiable	1.4	0.6	0.7	—	—

* Group as % (w/w) of total fat.

† Component acids as % (w/w) of group.

Figures in brackets denote mean unsaturated of each group of acids (Hilditch, 1947, p. 23).

Table 4. *Component fatty acids of seal blubber oils and grey seal milk fat (% w/w)*

	Elephant seal			Leopard seal, Heard Island	Common seal, Norfolk coast		Grey Atlantic seal, Pembroke coast	
	Macquarie Island		Heard Island					
	Mature male	Mature female	mature male	mature male	Suckling female	Yearling male	Blubber fat	Milk fat
Iodine value of oil	136.4	153.5	114.8	130.7	140.4	145.4	162.2	146.2
Acid:								
Lauric	0.3	0.1	—	—	—	—	—	—
Myristic	5.2	4.4	3.4	4.0	2.4	2.2	3.7	2.8
Palmitic	11.4	11.5	9.6	7.4	10.3	10.6	10.5	16.4
Stearic	2.4	2.4	2.5	1.6	2.5	4.4	2.0	2.8
Arachidic	—	—	0.3	0.2	0.3	0.3	—	—
Unsatd. C ₁₄	1.7	1.3	1.2	2.1	2.9	2.2	1.6	1.7
Unsatd. C ₁₆	14.5	13.0	10.5	12.7	25.7	20.8	15.5	12.7
Unsatd. C ₁₈	35.1	33.0	39.8	42.3	32.6	33.7	30.8	36.6
Unsatd. C ₂₀	18.8	20.6	18.0	17.3	12.1	13.6	16.5	13.6
Unsatd. C ₂₂	10.6	13.7	14.7	12.4	11.2	12.2	18.1	13.4
Unsatd. C ₂₄	—	—	—	—	—	—	1.3	—
Mean unsaturation of:								
Unsatd. C ₁₄	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0	-2.0
Unsatd. C ₁₆	-2.1	-2.1	-2.1	-2.1	-2.1	-2.2	-2.2	-2.2
Unsatd. C ₁₈	-2.7	-2.4	-2.2	-2.2	-2.7	-2.4	-2.7	-2.3
Unsatd. C ₂₀	-5.4	-5.9	-4.0	-4.6	-5.9	-7.2	-5.7	-6.6
Unsatd. C ₂₂	-10.0	-10.8	-7.4	-9.4	-11.0	-11.0	-10.6	-11.2
Unsatd. C ₂₄	—	—	—	—	—	—	-11.0	—

Although there is considerable variation in the amounts of the unsaturated acids of the various blubber oils, the grey seal milk fat does not conform to any type so far examined. Thus the C₁₆ unsaturated acids more nearly resemble those of the blubber fats of the Antarctic seals rather than those from British coastal waters, whereas curiously, with the exception of leopard seal blubber, the C₁₈ unsaturated acid content of the milk fat shows the least resemblance to that of the grey seal blubber fat. Again, although there is no correlation between the C₂₀ unsaturated acids with those of the Antarctic species, the milk fat much more nearly resembles those values recorded for the blubber of the common seal than that of the grey seal, this latter observation also holding true for the C₂₀ unsaturated acids.

It appears, therefore, that although they are broadly similar there may be no simple correlation between the milk fat of the grey seal and that of its blubber fat, neither does any correlation exist with the blubber fat of other species so far examined.

A further point of interest lies in the fact that the grey seal milk fat is considerably less unsaturated (iodine value 146.2) than that recorded for grey seal blubber oil (iodine value 162.2). In this respect seals might seem to differ entirely from whales, since the milk fat of a blue whale was found to be more unsaturated than that of the fat from any other site in the body of the same animal.

Clearly much more information is necessary before the apparent abnormalities of seal oils are understood, and to that end it is hoped that in the

near future an opportunity may be afforded of studying the blubber and milk fats of a seal at various stages during lactation, and also the blubber fat of the calf.

SUMMARY

1. Grey (Atlantic) seal (*Halichoerus grypus*) milk resembles whale milk in that its fat content is of the order of ten times that found in the milks of land animals.

2. The composition of seal milk fat has been investigated: it contains only those acids which occur in its blubber fat.

3. Qualitatively grey seal milk fat resembles that of the blubber oil of the same and of a number of other species, notable differences, however, occurring in the proportions of palmitic and unsaturated acids.

4. Grey seal milk fat is relatively more saturated than that of the blubber oil in contradistinction to that of the blue whale, for which the reverse holds true.

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The Liberation of Chloride Ions from Organic Chloro Compounds by Tissue Extracts

By H. G. BRAY, W. V. THORPE AND D. K. VALLANCE
Department of Physiology, Medical School, University of Birmingham

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There have been several reports of the liberation of chloride ions from organic chloro compounds. Liebreich (1869), who introduced chloral hydrate as a hypnotic, stated that this compound was in part excreted as inorganic chloride. This was disputed by von Mering & Musculus (1875), von Mering (1882) and Külz (1882) who found that chloral hydrate was eliminated by man and dogs as urochloralic acid (trichloroethyl- β -glucuronide). Kast (1887) failed to find an increased chloride excretion after administration of chloral hydrate to dogs, although he observed that chloride excretion was considerably increased after chloroform anaesthesia. The increase in man was slight. Willstätter, Straub & Hauptmann (1922) detected only small amounts of urochloralic acid in urine when therapeutic doses of chloral hydrate were given to man or rabbits. They maintained that urochloralic acid was formed when large doses were given, but that chloral was broken down

mainly to inorganic constituents when therapeutic doses were given. The only metabolic product detected in urine after giving trichlorourethane to man, rabbits or dogs was inorganic chloride. Akamatsu & Wasmuth (1923), using rabbits, showed that even after large doses of chloral hydrate no more than half could be accounted for as urochloralic acid. More recently Heppel & Porterfield (1948) claimed the discovery of an enzyme in rat liver, kidney and spleen which liberated halide ions from halogenated aliphatic hydrocarbons.

Since metabolic studies on a number of chloro-compounds were being undertaken in this laboratory it was desirable to confirm the existence of an enzyme such as that claimed by Heppel & Porterfield, especially as these workers did not appear to have considered the possibility of chloride ions being liberated as a result of interaction between chloro compounds and SH compounds. Several instances