Component Acids of Suinsh Blubber and Liver Fats

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(Received 29 August 1955)

Pathak & Agarwal (1952) have studied the component acids of the fats from different parts of a freshwater fish *Catla buehanani*. Fats from the body and the viscera of two other Indian freshwater fishes, *Labeo rohita* and *Cirrhina mrigala*, have been investigated by Pathak, Pande & Mathur (1954). The present investigation deals with the blubber and liver fats from an Indian freshwater mammal, a Ganges dolphin (*Platanista gangetica*; local name, suinsh) caught in the River Ganges near Banaras in May 1952. The mammal was a female about 4 ft. in length and weighing about 100 lb.

EXPERIMENTAL

Portions of the blubber and of the liver of the mammal were cut up into small pieces and separately extracted several times with hot acetone. The acetone was removed and the residue was treated with light petroleum (b.p. 40–60°) at room temperature, and the lipids were extracted. The lipids were recovered from the solution and dried under vacuum, and kept in 10 vol. of acetone at 0° for several weeks to separate the phosphatides, but none separated.

The mixed fatty acids obtained in the usual manner were resolved into groups of simpler mixtures of acids differing in unsaturation by the lithium salt–acetone method (Tsujimoto, 1920; Tsujimoto & Kimura, 1923) followed by the lead salt–ethanol method (Hilditch, 1949). Each group of acids was separately converted into methyl esters in the usual manner (Bjarnason & Meara, 1944) and fractionated through an electrically heated and packed column (Pathak *et al.* 1954). Saponification equivalents and iodine values of each of the subfractions were determined and the composition was calculated from them (Hilditch, 1949). The mean unsaturation, expressed by the fractional number of the hydrogen atoms short of saturation (e.g. –2.0, mono-ethenoid), was determined as usual by interpolation or extrapolation from the respective ester fractions in each group. The compositions of the original fats were obtained from those of the separated groups of fatty acids.

RESULTS

Blubber fat. The mixed fatty acids (247.0 g., iodine value 98.0) were resolved into three groups. The results are recorded in Table 1. The component acids of each group and of the whole fat are recorded in Table 2.

Table 1. *Fractions of suinsh-blubber fatty acids*

Oil (270.0 g.: saponification equivalent 280.8; iodine value 98.6) gave on saponification 253.0 g. of mixed fatty acids. The Reichert–Meissl and Reichert–Polensky values were 0.2 and 0.5 respectively.

Fraction	Description	Wt. (g.)	Wt. (%)	Iodine value
A	Lead salt, ethanol-insoluble	46.7	18.9	12.2
B	Lithium salt, acetone-insoluble, but lead salt, ethanol-soluble	164.0	66.4	101.5
C	Lithium salt, acetone soluble	36.3	14.7	158.6

Table 2. *Component acids of suinsh-blubber fat*

Acid	Component acids in groups as percentage of total			Component acids in whole fat (excluding unsaponifiable fraction)		
	A (18.9 %)	B (66.4 %)	C (14.7 %)	% (w/w)	% (mol.)	Mean unsaturation (H atoms/mol.)
Lauric	—	0.3	—	0.3	0.4	—
Myristic	1.4	2.6	—	4.0	4.7	—
Palmitic	11.2	2.8	—	14.1	14.7	—
Stearic	4.3	3.1	—	7.3	7.0	—
Unsaturated fractions						
C ₁₄	Trace	3.1	1.1	4.2	5.0	–2.0
C ₁₆	0.5	18.7	2.9	22.1	23.4	–2.1
C ₁₈	1.5	34.0	3.9	39.5	37.6	–2.7
C ₂₀	—	1.8	3.6	5.4	4.7	–5.7
C ₂₂	—	—	3.1	3.1	2.5	–8.5(?)
Unsaponifiable fraction	Trace	Trace	0.1	—	—	—

Liver fat. The quantity of the liver fat available (30.0 g.) was too small for resolution into simpler mixtures of acids by the lithium-salt or the lead-salt method, hence the esters of the mixed acids were directly fractionated in the same way as pahuna-liver fat (Pathak & Agarwal, 1952). The calculated composition of the fat is recorded in Table 3.

Table 3. *Component acids of suinsh-liver fat*

A sample (30.0 g.) of the liver fat (iodine value 95.2; saponification equivalent 284.5) gave 27.3 g. of mixed fatty acids.

Acid	% (w/w)	% (mol.)
Myristic	7.7	9.1
Palmitic	7.4	7.8
Stearic	4.5	4.2
Unsaturated fractions		
C ₁₄	5.8	6.9
C ₁₆	13.3	14.0
C ₁₈	30.3	30.5
C ₂₀	26.1	24.1
C ₂₂	4.0	3.4
Unsaponifiable fraction	0.9	—

DISCUSSION

The mammal *Platanista gangetica* belongs to the zoological suborder *Dontoceti* (Delphinidae), to which also belong sperm whales, dolphins and porpoises. Few totally aquatic mammals inhabit fresh waters exclusively, and this is one of the rare species that has left the sea and inhabited the rivers. A few other species belonging to this family are found in the rivers of South America.

Blubber fat. Negligible values for Reichert-Meissl (0.2) and -Polenske (0.5) estimations indicate the absence of lower acids in suinsh-blubber fat.

The species is rare, so that comparative data are not available. The composition of the fat lies between that of typical marine-mammal fat and that of the typical freshwater fish. For comparison the fatty acid compositions of fats of some marine mammals and some freshwater fishes are given in Table 4.

The fat largely resembles the sperm-whale fat; the important difference is its lower content of unsaturated C₂₀ acids, which is made up by a higher content of palmitic and stearic acids. The general unsaturation is higher than that of the sperm-whale fat. There is a close resemblance between the two in their content of other acids.

The saturated acids of suinsh fat differ from those of porpoise fat chiefly in the absence of *iso*-valeric acid, a lower content of lauric and myristic acids, and a higher content of palmitic and stearic acids. In the unsaturated portion of suinsh fat the chief feature is the much higher proportion of unsaturated C₁₈ acids than that in porpoise fat. Incidentally, a higher content of unsaturated C₁₈ acids is characteristic of fats of freshwater fishes. The contents of unsaturated C₁₆, C₂₀, and C₂₂ acids are slightly higher in porpoise fats.

Suinsh fat shows a somewhat closer resemblance to dolphin fat than to porpoise fat. The saturated portion differs from both porpoise and dolphin fat in a similar manner. In the unsaturated portion the similarity to dolphin fat is closer, both in the percentages of acids of different carbon content and in the degrees of their unsaturation, except for the much higher content of unsaturated C₂₀ acids in dolphin fat (18.6%) than in suinsh fat (5.4%). The absence of *iso*valeric acid and reduced proportions of lower saturated acids in the blubber fat from an animal of the same family as porpoise and dolphin is notable.

Table 4. *Component acids of blubbers of some marine mammals and body fats of freshwater fishes*

Amounts of the acids are given in % (w/w). Figures in parentheses show the mean unsaturation.

Acids	Marine mammals				Freshwater fish body fat		
	Suinsh	Sperm whale*	Porpoise†	Dolphin†	Nain‡	Rohu‡	Bhakur§
<i>iso</i> Valeric	—	—	13.6	3.2	—	—	—
Lauric	0.3	1.0	3.5	1.0	—	—	—
Myristic	4.0	5.0	12.1	7.2	1.9	3.4	2.9
Palmitic	14.1	6.5	4.7	8.6	21.4	21.2	29.0
Stearic	7.3	—	—	0.8	3.1	11.5	6.5
Arachidic	—	—	—	—	—	0.5	—
Unsaturated fractions							
C ₁₄	4.2 (-2.0)	4.0 (-2.0)	4.7 (-2.0)	4.7 (-2.0)	3.7 (-2.0)	3.7 (-2.0)	0.2 (-2.0)
C ₁₆	22.1 (-2.1)	26.5 (-2.0)	27.2 (-2.0)	25.9 (-2.0)	32.6 (-2.2)	8.1 (-2.2)	25.3 (-2.7)
C ₁₈	39.5 (-2.7)	37.0 (-2.0)	16.7 (-2.8)	24.1 (-3.3)	29.5 (-3.6)	32.2 (-3.3)	17.9 (-2.5)
C ₂₀	5.4 (-5.7)	19.0 (-2.5)	10.5 (-4.8)	18.6 (-6.5)	5.0 (-4.9)	12.4 (-5.2)	7.5 (-5.7)
C ₂₂	3.1 (-8.5)	1.0 (-4.0)	7.0 (-4.9)	5.9 (-7.6)	2.8 (-6.0)	6.7 (-6.9)	10.1 (-9.0)
C ₂₄	—	—	—	—	—	0.3 (-8.0)	0.6 (-11.0?)

* Hilditch & Lovern (1928, 1929).

† Pathak, Pande & Mathur (1954).

‡ Lovern (1934).

§ Pathak & Agarwal (1952).

As suinsh inhabits fresh water, it is natural that its fat has some similarities to fat of freshwater origin. This becomes evident by comparing its composition with that of nain-body fat and of bhakur-body fat (Table 4). It is observed that the palmitic acid content of suinsh fat is lower than that of the fats of freshwater fishes, but higher than that of the fats of the marine mammals. The situation is similar with the C_{16} unsaturated acids, except in rohu fat, where the C_{16} unsaturated acid content is rather low.

richer in lower-molecular-weight saturated acids, as well as in unsaturated acids, than the seal-liver fat. Suinsh- and porpoise-liver fats resemble each other closely in their content of palmitic, stearic and unsaturated C_{20} acids, but differ widely in their content of myristic and unsaturated C_{14} , C_{16} , C_{18} and C_{22} acids.

The content of unsaturated C_{18} acids in pahuna-liver fat is similar to that in suinsh-liver fat. With all the other acids there is the similarity generally found with lower-molecular-weight acids predomi-

Table 5. *Component acids of the liver fats of some aquatic animal fats*

Amounts of the acids are given in % (w/w). Figures in parentheses show the mean unsaturation.

Acid	Marine mammals			Freshwater fishes	
	Suinsh	Common seal*	Porpoise†	Pahuna‡	Bhakur‡
Myristic	7.8	0.2	—	1.5	0.6
Palmitic	7.5	11.4	7.6	14.2	19.0
Stearic	4.5	7.8	5.5	4.0	6.4
Arachidic	—	0.1	—	—	3.1
Unsaturated fractions					
C_{14}	5.8 (-2.0)	—	—	0.4 (-2.0)	Trace
C_{16}	13.4 (-2.0)	8.6 (-2.0)	6.1 (-2.0)	8.4 (-2.0)	7.0 (-2.0)
C_{18}	30.6 (-2.6)	27.9 (-2.4)	42.5 (-2.8)	32.6 (-2.4)	25.4 (-2.7)
C_{20}	26.3 (-7.8)	23.7 (-6.2)	27.3 (-5.4)	19.8 (-5.3)	26.5 (-4.8)
C_{22}	4.1 (-10.0?)	20.3 (-11.0?)	11.0 (-6.5)	19.1 (-8.0)	9.4 (-6.5)
C_{24}	—	—	—	—	2.6 (-8.0)

* Hilditch & Pathak (1949).

† Lovern (1934).

‡ Pathak & Agarwal (1952).

The comparatively greater proportion of unsaturated C_{18} acids (nearly 40%) in fat from suinsh may probably be explained by its adoption of a freshwater habitat, since increased proportions of unsaturated C_{16} and C_{18} acids and smaller proportions of unsaturated C_{20} and C_{22} acids are characteristic of freshwater animal fats; this is evident from the compositions of nain- and bhakur-fish fats given in Table 4. Suinsh fat resembles crocodile fat (Pathak & Pande, 1955) closely, except for its higher content of unsaturated C_{16} acids, although the total content of C_{16} acids remains practically the same.

When suinsh-body fat and suinsh-liver fat are compared it is noticed that the relationship between them is very much the same as that described by Pathak & Agarwal (1952) for bhakur fats. The body fat has a higher content and the liver has a lower content of saturated acids, especially of palmitic acid.

A general survey of these two fats confirms the view expressed by Lovern (1942) that the chemical composition of the fat is greatly influenced by the biological origin and the environment.

Suinsh-liver fat. Suinsh-liver fat resembles common seal-liver fat (Hilditch & Pathak, 1949) in the total saturated-acid content (Table 5). In the unsaturated-acid portion the contents of C_{18} and C_{20} acids are similar in both animals. Suinsh fat is

nating in suinsh fat. The content of unsaturated C_{20} acids is more in suinsh fat. It resembles bhakur-liver fat in the content of C_{20} acids, but in other acids suinsh fat is generally richer in lower-molecular-weight acids and poorer in higher-molecular-weight acids.

SUMMARY

1. The compositions of blubber and liver fats of suinsh (*Platanista gangetica*) have been studied. Preliminary separations of the fatty acids into groups differing in their content of unsaturated acids were made with lithium and lead salts in acetone and ethanol respectively, and their compositions were studied by the ester-fractionation procedure.

2. Suinsh is a mammal which has left its original habitat, the sea, and has inhabited fresh water. Its blubber fat resembles dolphin and porpoise fats in composition to some extent, but also resembles a typical fat from freshwater fishes. This supports the present views on the effect of the biological origin and of the environment on the composition of the fat. The suinsh-blubber fat does not contain isovaleric acid, which is characteristic of dolphin and porpoise fat, and in this respect it is unique.

3. The liver fat shows the usual significant differences in composition from the body fat.

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Comparative Studies of 'Bile Salts'

9. THE ISOLATION AND CHEMISTRY OF HYOCHOLIC ACID*

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(Received 14 June 1955)

The bile of the domestic pig is known to contain 3 α :6 α -dihydroxycholanolic acid (hyodeoxycholic acid; Windaus & Bohne, 1923), 3 α :7 α -dihydroxycholanolic acid (chenodeoxycholic acid; Ido & Sakurai, 1939), 3 β :6 α -dihydroxycholanolic acid (Kimura, 1937) and 3 α -hydroxy-6-oxoallocholanolic acid (or 3 α -hydroxy-6-oxocholanolic acid; Fernholz, 1935), all probably in conjugated form. Shimizu (1953) isolated 3 α -hydroxycholanolic (lithocholic) acid from the bile, and Trickey (1950) suggested that an acid of formula $C_{27}H_{44}O_6$ might be present. Pig bile acids are largely conjugated with glycine, but taurine derivatives also occur (Haslewood & Sjövall, 1954). The glycine conjugates were examined chromatographically by Haslewood & Sjövall (1954), who detected a substance which on paper ran rather more slowly than glycocholic acid. The parent trihydroxy acid, $C_{24}H_{40}O_5$, probably corresponding to this compound, was later isolated (Haslewood, 1954a) and named hyocholic acid. The present report describes the isolation and properties of this substance, and gives an account of experiments which have almost entirely elucidated its chemical constitution. In this work unexpected difficulties were encountered.

RESULTS

Pig bile salts were hydrolysed by recognized methods in an autoclave or, by the use of ethylene glycol, at atmospheric pressure. The ethylated crude bile acids were separated on alumina columns, and fractions were examined by paper chromatography as previously described (Haslewood, 1954b).

* Part 8: Haslewood & Sjövall (1954).

Esters of hyodeoxycholic and chenodeoxycholic acids were easily recognized. There were also spots corresponding to at least two unidentified substances. One of these remains unidentified, but is not ethyl 3 β :6 α -dihydroxycholanate or ethyl 3 α -hydroxy-6-oxoallocholanate; the other was shown to be ethyl hyocholate. A simple chromatographic process then permitted the isolation in substance of hyocholic acid, which could also be obtained, without chromatography, from crude 'hyodeoxycholic acid', as usually prepared. Girard separation of the ethylated bile acids removed ethyl 3 α -hydroxy-6-oxoallocholanate almost quantitatively in a single operation; it did not remove ethyl hyocholate. Hyocholic acid was converted into a crystalline ethyl ester, diacetyl ethyl ester, triacetyl methyl ester, triacetyl ethyl ester and other derivatives, discussed below. Triacetyl methyl hyocholate and triacetyl ethyl hyocholate readily formed characteristic crystals, resembling those of cholesterol. The triacetyl ethyl ester was useful in the isolation of hyocholic acid from mixtures.

The most obvious property of hyocholic acid itself was its similarity to hyodeoxycholic acid, from which it could not easily be separated by ordinary crystallization. Purified hyocholic acid had m.p. 188–189° and $[\alpha]_D + 5.5^\circ$ in ethanol; the mixed m.p. with hyodeoxycholic acid (m.p. 197–198°, $[\alpha]_D + 7^\circ$) was 184–196°. Other mixtures of hyodeoxycholic and hyocholic acids melted sharply; for example, a laboratory specimen of 'hyodeoxycholic acid' of m.p. 191–192° was shown to contain at least 20% of hyocholic acid. Much work may have been done in the past on 'hyodeoxycholic acid' contaminated in this way.