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The Component Acids and Glycerides of the Depot Fat of an Indian Panther (*Panthera pardus fusca*)

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Little is known about the fatty acid composition and glyceride structure of depot fats of land animals, particularly of those of the Carnivora, and quantitative data for the panther (*Panthera pardus fusca*) fat are not available. A study was therefore undertaken of the fatty acid composition and the nature of the glycerides of the depot fat of a panther of Indian origin.

EXPERIMENTAL

A light-cream-coloured fat was obtained from the abdominal depots of a freshly killed male panther from the forest of Indore (Central India). The saponification equivalent (s.e.) of the fat was 285.6 and iodine value (i.v.) was 62.4 with 3.2% of free fatty acid (as oleic). It was purified by washing an ethereal solution with dilute aqueous alkali, when the s.e. and i.v. of the neutral fat were 284.8 and 62.8 respectively.

Determination of component acids. A sample (140 g.) of the fat was saponified and the mixed fatty acids (130 g.; i.v. 65.5) were recovered. The latter were then resolved into two groups (1 and 2) of simpler mixtures of fatty acids by the lead salt-ethanol method (Hilditch, 1956). Each group of fatty acids was separately converted into the methyl esters. The esters were then subjected to fractional distillation under high vacuum (0.1 mm.) through an efficient electrically heated and packed column (Longenecker, 1937). The composition of each subfraction was calculated from their respective saponification equivalents and iodine values according to the method recommended by Hilditch

(1956). The final composition of the fat was then computed from these figures.

Determination of component glycerides. A portion (195 g.) of the neutral fat (i.v. 62.7) was dissolved in acetone (1.95 l.) and the solution kept at 0°, when 20.6 g. (i.v. 40.8) of glycerides crystallized out. The soluble portion, 174.4 g. (i.v. 63.5), was again crystallized from acetone (1.74 l.) at -12°. The soluble portion (93 g.; i.v. 65.7) and the insoluble portion (81.4 g.; i.v. 59.4) were further crystallized separately from acetone (20 g./100 ml.) at -12°; the former gave an insoluble portion (11 g.) with i.v. 60.3 and a soluble portion (82 g.) with i.v. 66.4. The latter gave an insoluble portion (35.5 g.) with i.v. 51.4 and a soluble portion (45.9 g.) with i.v. 62.6. Attempts were made to crystallize all the above fractions from ether at various temperatures, but were unsuccessful.

Different fractions thus obtained were combined according to similarity of iodine values. The procedure gave three fractions of 56.1 g. (i.v. 47.5), 56.9 g. (i.v. 62.1) and 82.0 g. (i.v. 66.4) respectively which were named A, B and C. The mixed acids obtained by the usual method from these fractions were further subjected to the lead salt-ethanol method of separation. These acid fractions were called AA, AB, BA, BB, CA and CB respectively. Each fraction was converted into the methyl ester and fractionated by distillation. Lastly, the possible component glycerides were calculated from the composition of the individual group in the manner described by Hilditch & Meara (1942).

A separate determination of the fully saturated glycerides by the method of Hilditch & Lea (1927) was done to check the computed values.

RESULTS

Table 1 gives data relating to the fractional crystallization of the mixed fatty acids of panther fat by the lead salt-ethanol method.

The component acids of the panther fat are recorded in Table 2.

Table 3 summarizes the results obtained by crystallization of neutral fat from acetone at various temperatures.

Table 4 presents the separation of the mixed acids of glyceride fractions *A*, *B* and *C* by the lead salt-ethanol method.

The component acids of glyceride fractions *A*, *B* and *C* are given [% (mol.)] in Table 5.

The possible component glycerides of the panther fat are given in Table 6, along with the negative result of fully saturated glyceride content in the fat.

DISCUSSION

Fatty acid composition. The panther feeds generally on wild animals such as deer and sheep and occasionally on calves, cows and buffaloes. Only a few of the carnivorous animal fats have been analysed so far; some are given in Table 7 for comparison with the present work, which shows that panther fat falls into line with other carnivorous animal fats.

The compositions of the fats in Table 7 are broadly similar. Palmitic and stearic acids are major components among the saturated acids. The present panther fat resembles lion fat in having a lower content of stearic acid than tiger fat, but the arachidic acid is a minor component in all the cases cited above.

In general these fats appear to have been synthesized from the fats in the animals' diet. Lion fat (Hilditch, Sime & Maddison, 1942), tiger fat (Pathak & Agarwal, 1952) and panther fat are very similar in composition and appear to have been synthesized mainly from the depot fat of herbivorous ox (Hilditch & Longenecker, 1937) or sheep (Hilditch & Srivastava, 1949) (Table 7). This is an example of the fact that the composition of depot fat depends on the diet of the animal. Among the

Table 1. *Separation of the acids of panther fat by the lead salt-ethanol method*

Group	Solubility of lead salt in ethanol at 15°	Weight		Iodine value
		(g.)	(%)	
1	Insoluble	40.0	33.9	6.6
2	Soluble	78.0	66.1	89.2

Table 2. *Component acids in groups 1 and 2 from the whole fat of the panther*

Figures in parentheses indicate unsaturation, e.g. -2.0H means monoethenoid. The figures for component acids are derived from the quantities of fractions actually obtained on fractionation; loss was little and ignored. Hence the figures total 100.

Total 100.					
	Component acids of groups (%)			Fatty acids in the whole fat, excluding non-saponifiables	
Acids	1	2	Total (%)	% (w/w)	% (mol.)
Saturated					
Myristic	1.4	0.9	2.3	2.3	2.8
Palmitic	18.1	1.9	20.0	20.1	21.4
Stearic	10.8	2.9	13.7	13.7	13.2
Arachidic	1.1	0.6	1.7	1.7	1.5
Unsaturated					
C ₁₄	—	1.8 (−2.0H)	1.8 (−2.0H)	1.8	2.2
C ₁₆	0.5 (−2.0H)	10.3 (−2.0H)	10.8 (−2.0H)	10.8	11.6
C ₁₈	1.6 (−2.0H)	39.7 (−2.1H)	41.3 (−2.1H)	41.3	40.0
C ₂₀	0.4 (−2.0H)	7.9 (−3.1H)	8.3 (−3.1H)	8.3	7.3
Unsaponifiable	0.04	0.06	0.1	—	—

Table 3. *Crystallization of neutral panther fat from acetone*

The figures in parentheses represent ml. of acetone/g. of fat.

Fraction	Description	Weight		Iodine value
		(g.)	(%)	
A	Material insoluble in acetone (10 ×) at 0° and -12°	56.1	28.8	47.5
B	Material soluble in acetone (10 ×) at -12° together with insoluble in acetone (5 ×) at -12°	56.9	29.2	62.1
C	Material soluble in acetone (5 ×) at -12°	82.0	42.0	66.4

Table 4. *Separation of the mixed acids of glyceride fractions A, B and C*

For preparation of fractions see text.

Fraction A	Solubility of lead salt in ethanol at 15°	Weight		Iodine value
		(g.)	(%)	
AA	Insoluble	20.6	41.6	4.7
AB	Soluble	28.9	58.4	78.2
Fraction B				
BA	Insoluble	13.4	26.3	4.9
BB	Soluble	37.6	73.7	79.3
Fraction C				
CA	Insoluble	14.0	20.0	5.6
CB	Soluble	56.0	80.0	84.6

Table 5. *Component acids of glyceride fractions A, B and C*

Component acids	A	B	C	Total
Myristic	0.6	0.7	0.7	2.0
Palmitic	8.8	6.1	6.5	21.4
Stearic	5.2	4.3	6.0	15.5
Arachidic	Trace	Trace	0.2	0.2
Tetradecenoic	0.3	0.5	0.7	1.5
Hexadecenoic	2.7	4.8	4.8	12.3
Octadecenoic	10.3	11.9	21.2	43.4
Eicosenoic	1.0	1.0	1.7	3.7

Table 6. *Component glycerides of panther fat*

Calculations in this table are based on the suggestions of Hilditch & Meara (1942).

	A	B	C	Whole fat
Component acid groups (% mol.)				
Myristic and palmitic	9.4	6.9	7.1	23.4
Stearic and arachidic	5.2	4.3	6.2	15.7
Tetra- and hexa-decenoic	3.0	5.3	5.5	13.8
Octadec- and eicos-enoic	11.3	12.9	22.9	47.1
Component glyceride groups (% mol.)				
(a) (i) Disaturated-mono-unsaturated	14.9	4.2	—	19.1
(ii) Monosaturated-diunsaturated	14.0	25.1	40.0	79.1
(iii) Triunsaturated	—	—	1.8	1.8
(b) (i) Dipalmito-mono-others	8.3	—	—	8.3
(ii) Monopalmito-di-others	11.5	16.4	21.1	49.0
(iii) Tri-others	9.0	13.0	20.7	42.7
(c) (i) Monostearo-di-others	15.7	13.0	18.5	47.2
(ii) Tri-others	13.2	16.4	23.2	52.8
(d) (i) Dioleo-mono-others	4.9	9.3	27.1	41.3
(ii) Mono-oleo-di-others	24.0	20.0	14.7	58.7
(e) (i) Hexadeceno-di-others	9.0	15.8	16.5	41.3
(ii) Tri-others	19.9	13.5	25.3	58.7
Possible component glycerides (% mol.)				
Mono-unsaturated-disaturated (19.2 %)				
Dipalmito-olein	8.3	—	—	8.3
Palmitostearo-olein	6.7	4.2	—	10.9
Diunsaturated-monosaturated (79.0 %)				
Dioleopalmitin	4.9	9.3	6.7	20.9
Dioleostearin	—	—	18.5	18.5
Mono-oleohexadecanostearin	9.1	8.8	—	17.9
Mono-oleohexadecenopalmitin	—	7.0	14.7	21.7
Triunsaturated (1.8 %)				
Dioleohexadenoic	—	—	1.8	1.8

Table 7. *Component acids (% by wt.) of the fats of some carnivorous animals and of English ox and Indian sheep*

Acids	Lion [Hilditch, Sime & Maddison (1942)]	Tiger [Pathak & Agarwal (1952)]	Panther (Present work)	Ox [Hilditch & Longenecker (1937)]	Sheep [Hilditch & Srivastava (1949)]	Puma [Giral (1945)]
Decanoic	1.4	—	—	—	—	—
Lauric	1.1	—	—	0.2	—	—
Myristic	4.9	1.0	2.3	3.1	2.9	1.3
Palmitic	28.9	22.4	20.1	24.9	27.8	22.4
Stearic	17.8	24.6	13.7	24.1	27.7	26.9
Arachidic	0.1	1.2	1.7	0.8	1.5	3.7
Tetradecenoic	0.6	0.6	1.8	0.4	0.4	—
Hexadecenoic	1.9	7.1	10.8	2.4	2.7	12.6
Oleic	40.3	39.0	39.2	41.8	33.0	26.2
Octadecadienoic	—	4.1	2.1	1.8	3.4	2.3
C ₂₀₋₂₂ unsaturated	3.0	—	8.3	0.5	0.6	4.6

Table 8. *Distribution of acyl groups in the glyceride of panther fat*

	Actual	Computed	
		Even [Hilditch (1956), pp. 327-332]	Random [Kartha (1949, 1953)]
Fully saturated glyceride	0	0	6.0
Disaturated-mono-unsaturated	19.1	17.4	28.0
Monosaturated-diunsaturated	79.0	82.6	43.5
Triunsaturated	1.8	0	22.5

unsaturated components tetra- and hexa-decenoic acids are present in lion, tiger and panther fat as well as in ox and sheep fat, though in the lion, ox and sheep fat they are only minor constituents, whereas in tiger and panther fat they occur in appreciable quantities (7.1 and 10.8%, by wt., respectively). The relatively high content of hexadecenoic acid in tiger and panther fat is noteworthy, and suggests that unsaturation increases from lion to tiger fat and tiger to panther fat. In this respect tiger and panther fat resembles the puma body fat studied by Giral (1945) (Table 7).

Component glycerides. Panther fat contains 19.2% of mono-unsaturated-disaturated glycerides (dipalmito-olein 8.3%, palmitostearo-olein 10.9%), 79.0% of diunsaturated-monosaturated glycerides (dioleopalmitin 20.9%, dioleostearin 18.5%, mono-oleohexadecenopalmitin 21.7%, mono-oleohexadecenostearin 17.9%) and 1.8% of triunsaturated glycerides (hexadecenodioleoin 1.8%).

The distribution of acyl groups in the glycerides of panther fat follows the rule of 'even' distribution, according to which the component fatty acids tend to be distributed as widely as possible among all the triglyceride molecules. The actual values shown in Table 8 are nearly identical with the values computed on the assumption that this rule is valid. In this respect panther fat does not align itself with the general land-animal type.

According to the 'rule of even (widest) distribution' of Hilditch (1956), which appears to hold

good for vegetable oils, fully saturated glycerides do not occur in significant proportions unless the unsaturated acid content is below that (about 35%) necessary to permit of each mixed triglyceride molecule containing one unsaturated acyl group. On this assumption panther fat, which contains about 61% of unsaturated acids, should not contain any trisaturated glycerides. Land-animal fats in general do not follow this rule, but probably conform to the 'random-distribution' pattern advanced by earlier workers and supported by Kartha (1949, 1953), according to which the fatty acids distribute themselves indiscriminately throughout the glyceride molecules. This pattern, however, has not been found with panther fat.

SUMMARY

1. The composition of the depot fat from a panther (*Panthera pardus fusca*) has been studied. The lead salt-ethanol method was adopted for preliminary separation of the mixed fatty acids into groups differing in unsaturation. The compositions of the resulting fractions were studied by the ester-fractionation procedure.

2. The panther fat aligns itself with other carnivorous-animal fats in its fatty acid composition with some differences in the proportion of saturated and unsaturated acids.

3. The glycerides have been fractionated by crystallization of the neutral fat from acetone. The

composition of each of the three fractions thus obtained has been studied separately and the final possible glyceride composition computed from the resulting data.

4. The glyceride composition of panther fat, unlike that of the fat of certain other land animals, closely follows Hilditch's (1956) rule of 'even' distribution.

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5-Formylfuroic Acid and the Carbazole Reaction for Uronic Acids and Acidic Polysaccharides

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It has been shown (Bowness, 1957) that furfuraldehyde and 5-hydroxymethylfurfuraldehyde in sulphuric acid do not give any substantial coloration with carbazole under the conditions of the procedure (Dische, 1947) for estimating uronic acids.

In the present work it was found that two other derivatives of furan, 5-formylfuroic acid (I) and 2:5-diformylfuran (II), both give intense colorations under these conditions, and also under the conditions of the modified carbazole reaction which Dische (1950) showed would distinguish between various polyuronides. It appears probable that formylfuroic acid is the principal agent responsible for the formation of colour from uronic acids in these two procedures.

MATERIALS AND METHODS

Materials

5-Formylfuroic acid (m.p. 195°) and 2:5-diformylfuran (m.p. 107°) were prepared and purified by the methods of Cooper & Nuttall (1912). Methyl β -D-glucofuranurono-

lactone (m.p. 135°) and 1:2-O-isopropylidene- α -D-glucofuranuronolactone (m.p. 116°) were prepared by the methods of Owen, Peat & Jones (1941). The sulphuric acid for the reaction at 60° (Dische, 1950) was obtained by adding 6 vol. of H_2SO_4 (A.R., Hopkin and Williams Ltd.) cooled to 0°, to 1 vol. of water in an ice bath. Heparin (Pularin) was obtained from Evans Medical Supplies Ltd. The sources or methods of preparation of all other reagents have been described previously (Bowness, 1957).

Methods

Reaction at 100°. Details of this procedure have been described in a previous paper (Bowness, 1957).

The absorption spectra recorded in Fig. 1 and the absorption values in Table 1 were obtained with a Hilger Uvispek (H. 700) spectrophotometer. Measurements were made 2.0–2.75 hr. after removing the sulphuric acid solutions from the boiling water. The solutions without carbazole were read against a sulphuric acid blank; those with carbazole were read against the blank containing carbazole.

Reaction at 60°. The procedure of Dische (1950) was employed, except that 0.2 ml. of a 0.3% (instead of a 0.1%) (w/v) ethanolic solution of carbazole was used. Absorption spectra (Fig. 2 and Table 2) were obtained as described above for the reaction at 100°. Measurements were made 0.5–1.25 hr. after removal of the solutions from the water bath.

RESULTS

Reaction at 100°

In the presence of carbazole. Table 1 shows that furfuraldehyde and 5-hydroxymethylfurfuraldehyde do not give appreciable colours in the reaction

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