612. *The Component Acids of a Hippopotamus Fat.*

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The chief component acids of the fat of a hippopotamus have been found to be palmitic (27·1%), stearic (22·3%), and oleic (39·3%), with minor proportions of other saturated acids (myristic 2·3, arachidic 1·1%) and other unsaturated acids [tetra- and hexa-decenoic acids 2·6%, octadecadienoic 3·4%, octadecatrienoic (linolenic) 1·5%, and unsaturated acids of the C_{20} series 0·4%]. The C_{18} diene-acids included comparable proportions of ordinary linoleic acid, a conjugated diene-acid, and other acids in which the double bonds are separated by more than one methylene group.

Apart from the minor amounts of polyethenoid acids present the hippopotamus fat is typically a stearic-rich fat similar to that of some of the larger land animals (such as the ox or sheep). This is somewhat surprising in view of the amphibious character of the animal and the extent to which its food may be derived from aquatic sources. The lack of a comprehensive range of data for the composition of the fats of diverse species of animals is emphasised.

Our knowledge of the composition of fats laid down in the adipose tissues of land animals is very much less comprehensive than that of vegetable seed fats or of the fats of aquatic species. Although far from complete, survey of vegetable fat composition has covered a sufficiently wide range of species to define in some measure the specific mixtures of fatty acids which characterise the seed fats of different kinds of plants, and to indicate marked correlation in many instances between seed-fat composition and the families into which plants have been divided from considerations of morphology and physiology. This statement holds, although to a less extent, for the fats of aquatic organisms, including fish and marine mammalia. In the land animals, attention has been concentrated mainly on the fats of a very few common animals, notably those of oxen, sheep, pigs, and rats; no systematic attempt has yet been made to study in detail fats from a range of animal species sufficiently broad and diverse, and comparable with that now available in the vegetable kingdom (although the latter, of course, covers only a small fraction of the total number of plant species involved). It is only within the past decade that accurate knowledge of the composition of human depot or milk fats has been acquired. Similarly, although the component fatty acids of fats of some few species of domestic animals have been given in detail, little information is yet available for fats of wild animals: apart from a few isolated observations, the detailed data in this field are almost wholly restricted to those for fats of the lion, Ceylon bear, giant panda, baboon, emu, and kangaroo (Hilditch, Sime, and Maddison, *Biochem. J.*, 1942, **36**, 98), tiger (Pathak and Godbole, *Indian Soap J.*, 1945, **11**, 68), puma (Giral, *J.*, 1945, 112), black bear (Sell, Taylor, and Miller, *J. Amer. Oil Chem. Soc.*, 1948, **25**, 416), and some species of deer (Baugham, Jamieson, and McKinney, *Oil and Fat Ind.*, 1929, **6**, No. 8, 11; Treadwell and Eckstein, *J. Biol. Chem.*, 1939, **128**, 373). It is clearly necessary to obtain detailed knowledge of the fatty acid composition of fats from many more animal species before adequate understanding in due perspective can be gained as to similarities or differences between fats deposited by animals of different biological origin and habits.

Recently we have had an opportunity, through the kindness of Professor F. L. Engledow, F.R.S., to examine a specimen of the fat of a hippopotamus killed in Uganda. The results have been interesting, since the depot fat of this large amphibian has proved to resemble those of oxen, sheep, and some other land animals much more closely than those of smaller amphibia which have so far been investigated. The component acids of the hippopotamus fat were separated into groups of more or less unsaturated character by crystallisation from acetone at low temperatures, and by submitting the more saturated part of the acids deposited from acetone to a lead salt separation, as described in the Experimental section of this paper. Each group of acids was converted into methyl esters, which were distilled in a vacuum through a fractionating column and separated into a number of ester fractions the composition of each of which was determined.

The component acids of the hippopotamus fat were thus found to be of the following nature. The saturated acids present were largely palmitic (27%) and stearic (22%), with minor proportions of myristic (2%) and arachidic (1%) acids. Of the unsaturated acids, oleic acid (39%) was the only major component, but the following were present in very small proportions: hexadecenoic (accompanied by traces of tetradecenoic) 2%, octadecadienoic acids 3·5%, octadecatrienoic (linolenic) acid 1·5%, and small amounts (less than 1%) of highly unsaturated acids of the C_{20} and possibly C_{22} series.

It is thus seen that palmitic, stearic, and oleic acids together form nearly 90% of the total acids in the hippopotamus fat. This composition is closely similar to that of " stearic-rich animal depot fats," of which those of oxen and sheep are the most familiar and typical examples. The content of 27% (wt.), or 29% (mol.), of palmitic acid lies within the range of $30 \pm 3\%$

(mol.) which Banks and Hilditch (*Biochem. J.*, 1931, **25**, 1168) and Hilditch and Longenecker (*ibid.*, 1937, **31**, 1805) showed to be characteristic for the depot fats of most land animals. The high proportion of stearic acid and the relatively low proportion of oleic acid places hippopotamus fat definitely in the group of " stearic-rich " depot fats.

On the other hand, the components of the unsaturated acids other than oleic (which form only 8% of the total acids) present features of interest. The amounts of tetradecenoic, hexadecenoic, and unsaturated C_{20} acids are small, even in comparison with those of animals such as the ox or sheep; but the polyethenoid C_{18} acids appear to be unusual in comparison with those in a land animal fat such as beef or mutton tallow. The C_{18} triene-acid is the linolenic acid of vegetable fats, whilst the C_{18} diene-acids appear to consist of roughly equal proportions of the linoleic acid of vegetable fats, a conjugated diene-acid, and an acid or acids in which the two ethenoid bonds are separated by more than one methylene group. It is probable that these polyethenoid C_{18} acids are derived directly from dietary fat assimilated by the animal, since there is adequate evidence for the view that the forms of linoleic and linolenic acids met in vegetable fats are not producible by synthesis from carbohydrate in the animal.

In contrast, the bulk of the fatty acids (palmitic, stearic, and oleic, in which the molar ratio of palmitic to the two C_{18} acids is 1 : 2·05) has the general composition which has been shown to be characteristic of fat synthesised in an animal from carbohydrate (Hilditch, Lea, and Pedelty, *ibid.*, 1939, **33**, 493; Hilditch and Pedelty, *ibid.*, 1941, **35**, 932). Hippopotami are herbivorous animals, reputed to feed mainly on water vegetation, although also resorting to dry land where they are liable to ravage herbage and growing grain. The composition of the fat suggests that it may be derived for the most part from a carbohydrate diet, whilst the nature of the polyethenoid acids present is in harmony with the view that these are derived from the fats of aquatic vegetation. Lovern (*ibid.*, 1936, **30**, 387) has shown that freshwater algæ and pondweeds contain fats with low saturated acid contents (15—20%, chiefly palmitic) and unsaturated acids in which C_{18} acids with an average unsaturation of $-5{\cdot}0H$ * predominate, accompanied by smaller proportions of unsaturated acids of the C_{16} and C_{20} series.

The hippopotamus fat further stands in marked contrast to the fats of some smaller amphibians (frog, tortoise, lizard), the compositions of which have been determined (Klenk, *Z. physiol. Chem.*, 1933, **221**, 67, 259, 264; Klenk, Ditt, and Diebold, *ibid.*, 1935, **232**, 54; Hilditch and Paul, *Biochem. J.*, 1937, **31**, 227), as illustrated in Table I. It will be seen that the fats of the smaller amphibia have more resemblance to those of typical aquatic flora, suggesting that fat assimilated from the latter forms a much greater proportion of their depot fat than it does in the hippopotamus fat.

TABLE I.

Component fatty acids of amphibia fats (and pondweed fat).

	Saturated.			Unsaturated.				
	C_{14}. %	C_{16}. %	C_{18}. %	C_{16}. %	C_{18}. %	C_{18}. Unsatn.	C_{20}. %	C_{20}. Unsatn.
Hippopotamus (present work)	2	27	22	2	44	−2·3H	0·4	?
Frog (Klenk)	4	11	3	15	52	−2·5H	15	−6H
Tortoise (Klenk *et al.*)	1	14	4	9	65	−2·4H	7	−4H
Lizard (Klenk *et al.*)	4	18	7	10	56	−2·4H	5	−5H
Lizard (Hilditch and Paul)	4	29	10	12	40	−2·7H	5	−6H
Pondweed (Lovern)	1	15	5	25	39	−4·9H	12	−6H

In the hippopotamus fat, the palmitic content (approaching 30%) and the very high stearic content, as already mentioned, are the most distinctive features. The class of " stearic-rich animal depot fats " to which it clearly belongs includes depot fats from a diversity of animal species. The depot fats of ruminants (ox, sheep, goats, deer) are typically stearic-rich, but so also are those of some other herbivorous non-ruminant animals (camel, kangaroo) and also those of (carnivorous) feline species (lion, tiger, puma, cat); on the other hand, other herbivora (horse, panda, baboon), bears, and man elaborate depot fats with low stearic acid contents (usually not exceeding 7—8% of the total fatty acids).

Attention is drawn to these interesting similarities and differences in animal-fat composition in order to stress the need for very many more data on this subject from a wide variety of species, and in the hope that others may be attracted to engage in similar studies with the aid of the experimental methods which are now available for determination of the component acids of natural fats with considerable precision.

* *I.e.*, the number of hydrogen atoms necessary to bring 1 mol. of acid to the saturated state.

Experimental.

The hippopotamus fat was pale yellow in colour and solid at the ordinary temperature. It had a saponification equivalent of 283·7, an iodine value of 46·2, and contained 3·1% of free fatty acid (as oleic) and 0·5% of unsaponifiable matter.

Determination of the Component Acids.—The mixed fatty acids (174·2 g.) obtained by hydrolysis of about 190 g. of the fat were crystallised from acetone (10 ml. per g. of acids) at −60°; 18·7 g. (iodine value 117·8) were left in solution; the deposited solids (155·5 g.) were recrystallised at −60° from acetone, a further 11·2 g. (iodine value 104·0) remaining in solution. These soluble portions were combined (group *D*; 29·9 g.) for further analysis.

The deposited acids (144·3 g.) from the second crystallisation at −60° were next crystallised from acetone (10 ml. per g. of acids) at −40°, this operation being again repeated at −40°. Two fractions of acids soluble in acetone at −40° were thus obtained (26·5 g. of iodine value 82·4, and 13·5 g. of iodine value 80·3) which were united for further examination (group *C*; 40·0 g.).

The acids insoluble in acetone at −40° (104·3 g.; iodine value 17·5) were then converted into lead salts in alcohol solution (cf. Hilditch, " Chemical Constitution of Natural Fats," 2nd Edn., 1947, p. 469), and the solution set aside at room temperature. There were thus obtained finally the following four groups of fatty acids (corrected weights):

Group.		G.	%.	Iodine value.
A	From insoluble lead salts	71·8	41·2	4·1
B	,, soluble lead salts	32·5	18·7	46·7
C	Soluble in acetone at −40°	40·0	22·9	81·7
D	,, ,, −60°	29·9	17·2	112·5

Each group of acids was separately converted into methyl esters, which were distilled in a vacuum through an electrically-heated and packed column. Table II is a summary of the ester-fractionation data, showing the fractions obtained from each group, with their equivalents and iodine values.

Table II.

Fractionation of methyl esters of fatty acids of groups A, B, C, *and* D.

No.	Wt. (g.).	Sap. equiv.	Iodine value.	No.	Wt. (g.).	Sap. equiv.	Iodine value.
	Methyl esters from group A.				*Methyl esters from group* B.		
1	1·82	267·6	0·3	1	1·79	262·8	3·1
2—5	11·18	270·1—270·9	0·1—0·5	2	2·07	273·4	6·8
6	2·64	283·1	1·5	3	2·35	285·0	38·4
7	2·25	287·8	5·3	4	2·78	293·2	64·7
8—10	8·45	295·1—297·4	6·2—6·5	5	1·95	295·3	73·5
11	2·67	298·2	6·6	6	2·09	297·7	71·0
12	2·71	301·7	6·7	7	1·61	310·9 *	57·0 *
13	1·94	310·4 *	8·9 *				
	33·66				14·64		
	Methyl esters from group C.				*Methyl esters from group* D.		
1	2·53	251·3	14·4	1	2·83	255·9	36·8
2	2·85	281·3	53·7	2	2·96	271·5	77·0
3	3·13	292·9	80·3	3	2·49	289·5	105·7
4	3·64	295·0	83·3	4	3·50	294·6	119·2
5	4·15	295·5	85·9	5	3·49	295·0	121·3
6	4·50	296·0	87·0	6	3·02	294·9	122·2
7	4·47	295·5	87·3	7	2·59	294·7	122·7
8	2·91	296·1	87·7	8	2·94	299·1	123·2
9	3·29	296·4	89·9	9	1·89	378·2 *	137·1 *
10	1·76	305·8 *	87·3 *				
	33·23				25·71		

* Residual esters, freed from unsaponifiable matter:

*A*13	Sap. equiv. 304·3;	iodine value	6·6.	*C*10	Sap. equiv. 294·5;	iodine value	87·9.
*B*7	,, 296·1;	,,	59·5.	*D*9	,, 298·9;	,,	142·4.

The following unsaturated acids were definitely identified in the fractions named: hexadecenoic acid (fraction *D*1) by conversion into dihydroxypalmitic acid (m. p. and mixed m. p., 126·5—127°); oleic acid (fraction *C*7) as dihydroxystearic acid (m. p. and mixed m. p., 129·5—130°); and linolenic acid (fraction *D*6) as crystalline hexabromostearic acid (m. p. and mixed m. p., 180·5—181°). There was evidence (fractions *C*8—10, and *D*8, 9) of very small amounts of esters of unsaturated C_{20} acids, in quantity too small for definite identification; these were allowed for in the subsequent calculations in the usual manner (cf. Hilditch, *op. cit.*, p. 509).

The proportions of polyethenoid acids, when present (groups *C* and *D* only), were determined by spectrophotometric analysis (fractions *C*1, *C*7, *D*2, *D*6, and *D*9). The acids in fractions *C*1 and *D*2 belong wholly to the C_{16} series, those in *C*7 and *D*6 are wholly C_{18} acids, and those in *D*9 contain polyethenoid

C_{20} (and possibly C_{22}) acids in addition to those of the C_{18} acids (which still predominate). The extinction coefficients of the unisomerised acids at 234, 268, 300, and 315 mμ. were determined in order to detect any conjugated diene-, triene-, or polyene-acids respectively, whilst each acid was isomerised with alkali under standardised conditions (Hilditch, Morton, and Riley, *Analyst*, 1945, **70**, 68), the extinction coefficients being measured at 268 mμ. (for " linolenic " unsaturation, after isomerisation at 170° for 15 minutes) or at 234 mμ. (for " linoleic " unsaturation after isomerisation at 180° for 60 minutes). The results are given in Table III.

TABLE III.

Spectrophotometric analyses of acids in selected ester fractions.

		Mixed acids of fractions.				
Ester fraction.		Iodine value.	Extinction-coefficients ($E^{1\%}_{1\,cm.}$) at 234 mμ.	268 mμ.	300 mμ.	315 mμ.
*C*1	Isomerised (180°/60 mins.)	15·8	negligible	—	—	—
*D*2	,, (170°/15 mins.)	18·4	—	negligible	—	—
	,, (180°/60 mins.)	,,	44	—	—	—
*C*7	Unisomerised	91·5	negligible	—	—	—
	Isomerised (170°/15 mins.)	,,	—	negligible	—	—
	,, (180°/60 mins.)	,,	17	—	—	—
*D*6	Unisomerised	128·6	75	—	—	—
	Isomerised (170°/15 mins.)	,,	—	66	—	—
	,, (180°/60 mins.)	,,	229	—	—	—
*D*9	Unisomerised	149·4	188	28	9	6
	Isomerised (180°/60 mins.)	,,	Not determined		32	28

TABLE IV.

Component fatty acids of the hippopotamus fat.

	Acid groups:				Total fatty acids (excluding unsaponifiable).	
	A. % (wt.).	*B*. % (wt.).	*C*. % (wt.).	*D*. % wt.).	% (wt.).	% (mol.).
Myristic	0·4	3·2	4·1	3·3	2·3	2·8
Palmitic	44·6	29·6	7·2	8·3	27·1	28·9
Stearic	47·9	13·0	—	—	22·2	21·4
Arachidic	2·7	—	—	—	1·1	1·0
Tetradecenoic	—	—	0·6	1·6	0·4	0·5
Hexadecenoic	—	—	1·7	10·2	2·2	2·3
Oleic	4·3	53·6	84·5	46·3	39·3	38·0
Octadecadienoic :						
Linoleic	—	—	1·5	7·1	1·6	1·5
Conjugated	—	—	—	4·6	0·8	0·8
Other forms	—	—	Traces	6·3	1·1	1·1
Linolenic	—	—	—	8·6	1·5	1·4
Unsaturated C_{20} (and C_{22}) ...	—	—	0·2	2·1	0·4	0·3
Unsaponifiable	0·1	0·6	0·2	1·6	—	—

The data in Table III lead to the following composition of the unsaturated acids in the respective fractions: *C*1, hexadecenoic only; *D*2, hexadecenoic 94·3%, hexadecadienoic 5·7%; *C*7, oleic 98·1%, octadecadienoic 1·9%; *D*6, oleic 63·5%, linoleic 9·8%, conjugated dienoic 6·3%, other forms of octadecadienoic 8·6%, linolenic 11·8%. [After allowance for the increments of iodine value, in the acids of *D*6, due to the linolenic, conjugated diene, and linoleic acids as determined spectrophotometrically, the remaining acids were found to have an iodine value (101·0) still in excess of monoethenoid unsaturation (90·1). This indicates the presence of acids not measurable by the spectrographic analysis, *i.e.*, other octadecadienoic acids in which more than one methylene group occurs between the two double bonds. The proportion of these was calculated from the residual iodine value of 101·0. Acids of this nature were also apparently present, but only in traces, in fraction *C*7.]

Thc unsaturated C_{16} and C_{18} acids present in the ester fractions of groups *C* and *D* were calculated on the basis of these compositions.

The spectra of the acids of the residual ester fraction *D*9 show slight absorption bands with heads at 300 and 315 mμ. as well as at the lower wave-lengths, thus confirming the presence of tetra- or penta-ethenoid acids (of the C_{20} or higher series).

The components of each ester fraction were then calculated (by using the compositions of unsaturated C_{16} or C_{18} acids indicated by the analyses in Table III) by the procedures which have been described at length elsewhere (Hilditch, *op. cit.*, pp. 505—509). The percentages of the component acids in each of the four groups, and therefrom those for the total fatty acids of the original hippopotamus fat, then follow (Table IV).

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