precise methods of analysis now available will reveal the widespread occurrence of γ -aminobutyric acid in small amounts.

SUMMARY

1. Aqueous extracts from barley roots contain an active L-glutamic acid decarboxylase, but do not decarboxylate L-aspartic acid, L-arginine, L-histidine, L-tryptophan or L-tyrosine at measurable rates.

2. Active precipitates can be obtained from aqueous extracts by treatment with ammonium sulphate, and the enzyme is stable in this state.

3. Almost quantitative decarboxylation can be achieved using redissolved precipitates and the

product has been identified by chromatography as γ -aminobutyric acid.

4. A calculation of the Michaelis constant has given a value of 9.6×0.001 M, allowance being made for inhibition by high substrate concentrations.

5. The pH relationships and reactions to inhibitors of the barley enzyme are similar to those previously described.

6. It is suggested that the natural occurrence of γ -aminobutyric acid may be ascribed to the functioning of the enzyme *in vivo*.

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The Component Acids and Glycerides of a Horse Mesenteric Fat

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Until recently no comprehensive account of the component acids of fats from the horse has been recorded. Nevertheless, the general studies of Heiduschka & Steinruck (1921) and of Schuette, Garvin & Schwoegler (1934) showed that horse body fats differed strikingly from those of oxen or sheep in containing relatively small proportions of stearic acid and definite amounts of the linoleic (octadeca-9-12-dienoic) and linolenic (octadeca-9:12:15-trienoic) acids which are present in grass fats (and indeed in many other vegetable fats):

acids of lower molecular weight than C_{16} acids, and 11% of unsaturated acids of lower molecular weight than C_{18} acids): the low proportion of stearic acid, and the presence of linoleic and linolenic acids, are again noteworthy and contrast with the higher proportions of the former acid, and the substantial absence of linoleic and linolenic acids, in cow or sheep milk fats.

It was thus felt desirable to undertake a further detailed examination of a horse body fat, not only in relation to its component fatty acids, but especially

	% (w/w)				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Horse body fat (Heiduschka & Steinruck, 1921)	29	7	55	7	2
Horse abdominal fat (Schuette <i>et al.</i> 1934)	28	5	50	13	4

A detailed analysis of horse milk fat (Hilditch & Jasperson, 1944) showed its acids to contain 16% palmitic, 3% stearic, 19% oleic, 8% linoleic and 16% linolenic acids (in addition to 22% of saturated

in regard to its component glycerides. Whilst this work was in progress two further important studies of horse fat component acids have appeared, in both of which the more recent techniques of separa-

tion of the fatty acids by low-temperature crystallization, ester fractionation, and spectrophotometric analysis of mixed oleic, linoleic and linolenic acids have been utilized. Holmberg & Rosenqvist (1949) found that Swedish horse kidney and bone fats contained fairly large proportions of linoleic and 2–4% of linolenic acids, with significant proportions of myristic (tetradecanoic), palmitoleic (hexadec-9-enoic) and unsaturated C_{20} acids. Brooker & Shorland (1950), in the case of offal, bone and hoof fats from New Zealand horses, also observed the presence of myristic, palmitoleic and unsaturated C_{20} acids, but recorded only about 5% of linoleic acid and about 16% of linolenic acid. Both groups of workers reported oleic (34–42%) and palmitic (18–28%) as the main component acids of the fats. Their data are discussed more fully later in this paper (Table 6).

The present study was based on a specimen of adipose tissue from the mesentery of a normal healthy horse, which was obtained for us by the Department of Veterinary Science of the University of Liverpool. The mesenteric fat, extracted from the tissues by acetone, was a clear yellow material, semi-liquid at the ordinary temperature; it had an iodine value of 95.6, saponification equivalent 283.1, and contained 0.2% of free fatty acid (calculated as oleic acid).

METHODS

Component fatty acids. The mixed fatty acids from the oil were first divided into four groups of differing mean unsaturation (*A*, *B*, *C* and *D*) by crystallization from acetone and then from ether at low temperatures. Each group of acids finally obtained was separately converted into methyl esters, which were fractionally distilled in a vacuum through an electrically heated and packed column. The composition of the mixtures of mono-, di- and tri-ethylenic acids present was determined, after alkali-isomerization under standard conditions (Hilditch, Morton & Riley, 1945) from the extinction coefficients of the ultraviolet absorption bands developed at 268 and 234 $m\mu$, and from the I_2 value of the mixture under examination. When only oleic, linoleic and linolenic acids are present, this is most conveniently done by spectrophotometric analysis of each group of acids as a

The requisite data were then available for calculation of the composition of each ester fraction, therefrom the composition of each group of separated acids, and thence, finally, the component acids of the original horse mesenteric fat.

Component glycerides. The mesenteric fat was crystallized systematically from solutions in acetone or in ether at a range of temperatures from -65 to 0° until little further separation could be observed (by I_2 value). The original fat of I_2 value 95.6 was thus resolved into nine groups of glycerides (*A*–*I*), the I_2 values of which ranged from 13.8 to 184.0.

Seven of the nine groups of glycerides were hydrolysed and their mixed fatty acids separately analysed by methods similar to those outlined above; the preliminary resolution by solvent separation was, however, limited to crystallization of the acids from ether or acetone at about -40° , and omitted altogether in the case of the glyceride groups *A*, *B*, *F* and *G* (Fig. 2, Table 4). The amounts of the two most unsaturated groups were too small for complete analysis by these methods, and their examination was confined to spectrophotometric analysis.

Each individual group of glycerides being relatively simple in composition, it becomes possible to deduce from the component fatty acids present the general composition of the mixed glycerides in each group, and therefrom in the whole fat.

RESULTS

Component fatty acids of horse mesenteric fat

The mixed fatty acids (136.5 g.) obtained by hydrolysis of about 145 g. of the horse fat were systematically crystallized from 10% solutions in acetone or

Fig. 1. Crystallization of mixed fatty acids of horse mesenteric fat. *I.V.* means iodine value.

ether at low temperatures according to the scheme in Fig. 1. Four groups of acids were finally obtained:

Group	Description	Weight		Iodine value
		(g.)	(% of total)	
<i>A</i>	Insoluble in ether at -30°	38.9	28.5	10.8
<i>B</i>	Soluble in ether at -30°	43.8	32.1	82.5
<i>C</i>	Insoluble in acetone at -70°	30.9	22.6	153.5
<i>D</i>	Soluble in acetone at -70°	22.9	16.8	205.6

whole; in the present instance unsaturated acids of the C_{18} series were present in addition to the unsaturated C_{18} acids, and it was therefore necessary to carry out the spectrophotometric analyses on the acids from selected ester fractions in which the unsaturated components were seen to belong wholly to either the C_{18} or the C_{16} series of acids. The presence of natural ('seed fat') linoleic and linolenic acids was confirmed by the isolation of their respective characteristic crystalline tetrabromo- and hexabromo-adducts.

The ester-fractionation data for the methyl esters of each of the four groups of acids are summarized in Table 1.

The acids from selected ester fractions from groups *B*, *C* and *D*, in each of which the unsaturation was clearly due to acids of the C_{18} series, and one from group *D* in which the unsaturated acids were wholly of the C_{16} series, were analysed spectrophoto-

metrically after isomerization with alkali under the standard conditions described by Hilditch *et al.* (1945), with the results summarized in Table 2.

Table 1. *Fractionation of methyl esters of horse mesenteric fatty acids A, B, C, D*

Fraction	Weight (g.)	Saponification equivalent	I ₂ value
Methyl esters of acids A			
A1	1.03	247.0	0.9
A2	2.62	265.0	1.3
A3	3.57	269.4	1.4
A4	4.10	269.5	1.1
A5	4.40	270.3	1.7
A6	3.00	271.4	1.7
A7	4.19	287.3	31.1
A8	3.16	303.9*	33.4
Total	26.07		
Methyl esters of acids B			
B1	1.49	245.1	5.8
B2	1.95	270.4	32.1
B3	3.18	280.9	61.1
B4	3.80	294.4	84.7
B5	3.63	294.1	90.4
B6	5.86	296.2	91.0
B7	4.33	296.2	90.4
B8	2.04	296.1	86.8
B9	1.70	310.5*	84.3
Total	27.98		
Methyl esters of acids C			
C1	0.97	240.0	38.2
C2	2.98	262.0	89.4
C3	2.97	276.5	133.2
C4	2.79	286.1	160.5
C5	3.25	294.2	162.8
C6	3.25	294.3	167.5
C7	2.29	294.6	165.8
C8	2.31	294.1	168.8
C9	2.39	294.9	167.3
C10	2.30	326.8*	146.3
Total	25.50		
Methyl esters of acids D			
D1	1.27	250.5	84.4
D2	1.04	266.6	131.7
D3	2.52	286.1	190.4
D4	2.36	288.9	209.5
D5	3.02	291.7	212.4
D6	3.39	293.0	217.0
D7	2.57	317.1*	202.7
Total	16.17		

* Equivalents of esters (freed from unsaponifiable matter): A8, 298.8; B9, 297.6; C10, 315.4; D7, 306.3.

In the C₁₈ series, spectrophotometric evidence thus obtained for the presence of linolenic and linoleic acids was confirmed by the production from the acid of ester fractions C7, C8, D5 and D6 of typical bromo-additive products: (a) hexabromostearic acid, m.p. 179–180° (unchanged on admixture with a specimen of authentic hexabromostearic acid from the acids of linseed oil), and (b) a crystalline specimen of (impure) tetrabromostearic acid which

melted at 95–100°, this melting point being somewhat raised when the acid was mixed with authentic tetrabromostearic acid (m.p. 114.5°) from vegetable sources. (The effective separation of pure tetrabromostearic acid from a mixture of tetra- and hexabromostearic acids is very difficult, and the low melting point of the specimen finally obtained is almost certainly caused by traces of the hexabromo-acid still being present.)

From the analytical results in Table 2 the compositions of the respective unsaturated C₁₈ or C₁₆ acids present in the four fractions analysed, and from these the iodine values and equivalents of the corresponding unsaturated methyl esters, were calculated. These data were employed in calculating the composition of all the ester fractions in the respective groups B, C and D, it being assumed that the composition of the C₁₈ (or C₁₆) unsaturated esters was approximately constant in all the distilled fractions concerned (this assumption is not completely justified, but introduces no significant error into the analyses).

The composition of each ester fraction was then evaluated by methods which have been described by Hilditch (1947, pp. 498–509), and the percentage composition (by weight) of each group of acids thus calculated, and hence the composition of the total fatty acids of the horse mesenteric fat (Table 3).

Component glycerides of horse mesenteric fat

The fat (268 g.) was submitted to systematic crystallizations from 10% solutions in acetone at temperatures successively rising from –65 to 0°. Certain intermediate fractions left in solution were combined and subjected to further crystallization from acetone at appropriate temperatures. The glycerides, which were finally insoluble in acetone at 0°, were given a further separation from solution in ether at 0°. Each solution was left at the selected temperature for 4.5–5 hr., with the exception of the crystallizations at 0°, which were left overnight in a refrigerator. A complete scheme of the separations used is given in Fig. 2.

The mixed acids in the groups A–G of the glycerides were determined by fractionation of their methyl esters, those in groups C, D and E being first separated into relatively saturated and unsaturated portions by crystallization from ether or acetone at –35°. The acids in the small groups H and I of greatest solubility and unsaturation were determined from spectrophotometric analyses alone, the small proportion of saturated acids present therein being taken as palmitic acid. Each of these analyses thus involved a series of determinations and consequently calculations of the same nature as those described above, and illustrated in Tables 1–3, for the component acids of the whole horse fat. The details of each component acid analysis are therefore

Table 2. Spectrophotometric analyses of unsaturated C_{18} and unsaturated C_{18} acids

Acids from fractions	I_2 value	$E_{1\text{ cm.}}^{1\%}$ (after alkali-isomerization)		Composition of acids (%)				
				Unsaturated				
		At 268 $m\mu$.	At 234 $m\mu$.	Series	Monoene	Diene	Triene	Saturated
B6	95.5	23	37	C_{18}	90.1	1.3	4.3	4.3
C8	177.2	225	369	C_{18}	39.5	14.1	42.3	4.1
D6	228.0	358	570	C_{18}	6.7	20.6	67.9	5.4
D2	138.9	119	211	C_{18}	60.9	8.3	20.1	10.7

Reference values ($E_{1\text{ cm.}}^{1\%}$) used for pure di- and tri-enoic acids:

	λ ($m\mu$)	C_{18} acids* ($E_{1\text{ cm.}}^{1\%}$)	C_{18} acids† ($E_{1\text{ cm.}}^{1\%}$)
Trienoic acid, alkali-isomerized at 170° for 15 min.	268	532	592
Trienoic acid, alkali-isomerized at 180° for 60 min.	234	569	633
Dienoic acid, alkali-isomerized at 180° for 60 min.	234	906	1006

* Hilditch *et al.* (1945).† Calculated from molar extinction coefficients ϵ , derived from C_{18} acid data.

Table 3. Component acids in groups A, B, C and D, and in the whole horse mesenteric fat

Acid	Component acids in the groups				Fatty acids in the whole fat	
	A (28.5%)*	B (32.1%)*	C (22.6%)*	D (16.8%)*	(%, w/w)	(%, by mol.)
	(%)†	(%)†	(%)†	(%)†		
Lauric (dodecanoic)	—	—	0.9	1.3	0.4	0.6
Myristic	5.8	4.6	4.4	2.2	4.5	5.3
Palmitic	69.7	10.2	7.8	5.7	25.9	27.3
Stearic	11.9	3.9	—	—	4.7	4.4
Arachidic (eicosanoic)	0.8	—	—	—	0.2	0.2
Hexadecenoic	—	4.4	9.3	9.2	5.1	5.4
Hexadecadienoic	—	—	1.2	1.2	0.5	0.5
Hexadecatienoic	—	—	3.1	3.1	1.2	1.5
Oleic	11.6	72.1	27.9	5.0	33.7	32.2
Linoleic	—	1.1	9.9	15.4	5.2	5.0
Linolenic	—	3.4	29.8	50.3	16.3	15.8
Unsaturated C_{20-22}	—	—	5.4	6.0	2.3	2.0
Unsaponifiable	0.2	0.3	0.3	0.6	—	—

* Groups as % (w/w) of total acids.

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

not recorded here, owing to the space that would be required. Table 4, however, shows the proportions and characteristics of each of the groups A-I of glycerides obtained by crystallization, and also the percentage composition (by weight and by molecular proportions) determined from the detailed analysis of the fatty acids in each group of glycerides.

In considering the glyceride composition of each of the individual groups A-I, the minor proportions of lauric acid present may be taken with myristic acid, and those of arachidic with stearic acid. The increment (by mol.) of each fatty acid category in each glyceride group is shown in Table 5. Considering, in the first place, the relative amounts of saturated and unsaturated acids in the glycerides of the different groups, it is clear that the most saturated glycerides have been concentrated in the least soluble fractions A and B, which indeed contain some trisaturated glycerides in addition to mono-unsaturated disaturated glycerides. In fraction C, di- as well as mono-unsaturated glycerides are present;

in view of the very small proportion of trisaturated glycerides in the preceding fraction B, it is reasonable to suppose that the latter are not present in C. In the remaining fractions, tri-unsaturated glycerides are evidently present in increasing proportions; it is possible that fraction D may also contain some mono-unsaturated glycerides, but in view of the drastic crystallization procedure applied, and of the fact that glycerides such as oleopalmitostearin are relatively insoluble in comparison with the di- and tri-unsaturated glycerides present, it is very unlikely that any appreciable proportion of mono-unsaturated glycerides have been left in fraction D, and still less so in the later fractions. Similarly, it is almost certain that no di-unsaturated glycerides have been retained in the least soluble fractions A and B. Thus, when care has been taken to pursue the original crystallization of the fat to a point at which little or no change takes place on further recrystallization of intermediate glyceride fractions, it can be assumed with considerable certainty that any one

glyceride group contains only binary mixtures of tri- and di-saturated, di- and mono-saturated, or mono-saturated and tri-unsaturated glycerides. The proportions of such binary mixtures can be calculated from the component acid data (Table 5, i).

to indicate that each will contribute only one acyl group to any triglyceride in which it is present.

By summation of the increments of acids or of glyceride categories in each of the nine groups of glycerides, the amount of each in the whole fat

Fig. 2. Crystallization of mixed glycerides of horse mesenteric fat. All crystallizations were from 10 % solutions in acetone, except in one instance (ether) indicated. Figures in parentheses are iodine values (I.V.).

The probable proportions, in each glyceride group, of mono-, di-, and tri-palmitoglycerides (Table 5, ii), mono- and di-oleoglycerides (Table 5, iii), and mono- and di-linolenoglycerides (Table 5, iv), can be deduced on similar lines, although it should be noted that the degree of segregation of the unsaturated glycerides, at all events, cannot be postulated with so much certainty as in the case of the various categories of saturated-unsaturated triglycerides.

The proportions of the remaining acids (myristic, stearic, unsaturated C_{16} , linoleic, unsaturated C_{20-22} acids) in all the groups of glycerides are small enough

(Table 5, final column) is computed. Comparison of the fatty acid figures (mol. %) thus found for the whole fat with those for the direct analysis of its total fatty acids (Table 3) is a partial check on the degree of analytical accuracy of the component glyceride analysis: the figures are in close accordance except in the cases of palmitic and oleic acids, where the differences are respectively 1.4 and 1.8 units (mol. %).

When, in any of the groups of glycerides, the number of different fatty acids present is not more than three, it is possible by consideration of the

Table 4. *Horse fat glyceride groups obtained by low-temperature crystallization*

	A	B	C	D	E	F	G	H	I	Total
Weight (g.)	9.4	26.0	85.0	70.1	40.8	14.3	13.6	5.9	2.9	268.0
Iodine value	13.8	39.5	72.6	104.3	115.2	136.6	156.7	184.0	144.1	95.6
Saponification equivalent	270.2	276.8	281.2	287.4	290.2	287.6	288.8	295.5	350.6	283.1
Unsaponifiable (% w/w)	—	0.2	0.1	0.5	1.4	0.6	0.8	2.4	18.5	—
Glycerides (% w/w)	3.5	9.7	31.7	26.2	15.2	5.3	5.1	2.2	1.1	100.0
Glycerides (% by mol.)	3.7	10.0	32.1	25.9	15.0	5.3	5.0	2.1	0.9	100.0
Component acids (% w/w)										
Lauric	—	0.6	0.5	0.3	—	—	—	—	—	0.3
Myristic	16.9	8.0	4.2	3.3	2.1	3.9	2.4	—	—	4.2
Palmitic	55.2	44.3	32.6	17.0	16.3	6.2	5.2	9.5	17.2	24.5
Stearic	12.1	13.9	5.4	4.0	1.2	1.1	2.0	—	—	4.9
Arachidic	—	—	0.4	0.3	0.5	—	—	—	—	0.2
Unsaturated C ₁₆	—	2.9	6.3	6.9	4.8	10.3	10.7	—	—	6.0
Oleic	15.8	23.5	34.1	40.4	43.4	40.3	30.4	24.6	32.5	35.5
Linoleic	—	1.7	5.3	6.3	8.5	9.8	9.3	12.7	9.9	6.2
Linolenic	—	5.0	9.8	16.9	23.2	28.4	39.0	53.2	40.4	16.6
Unsaturated C ₂₀₋₂₂	—	—	1.4	4.4	—	—	—	—	—	1.6
Component acids (% by mol.)										
Lauric	—	0.8	0.6	0.4	—	—	—	—	—	0.4
Myristic	19.1	9.2	5.0	4.0	2.6	4.7	2.8	—	—	5.0
Palmitic	55.5	45.6	34.1	18.2	17.4	6.6	5.5	10.3	18.5	25.9
Stearic	10.9	12.9	5.1	3.8	1.2	1.1	1.9	—	—	4.7
Arachidic	—	—	0.3	0.3	0.4	—	—	—	—	0.2
Unsaturated C ₁₆	—	3.0	6.6	7.4	5.2	11.0	11.7	—	—	6.3
Oleic	14.5	22.1	32.5	39.3	42.1	39.1	29.5	24.1	31.8	34.0
Linoleic	—	1.6	5.1	6.2	8.3	9.6	9.2	12.6	9.7	6.0
Linolenic	—	4.8	9.5	16.6	22.8	27.9	38.4	53.0	40.0	16.1
Unsaturated C ₂₀₋₂₂	—	—	1.2	3.8	—	—	—	—	—	1.4

Table 5. *Component acid and glyceride categories in the glyceride groups A to I*

	A	B	C	D	E	F	G	H	I	Total
Glycerides % (by mol.)	3.7	10.0	32.1	25.9	15.0	5.3	5.0	2.1	0.9	100.0
Component acid categories (increments, % by mol.)										
Myristic (with C ₁₂)	0.7	1.0	1.8	1.1	0.4	0.2	0.1	—	—	5.3
Palmitic	2.1	4.6	10.9	4.7	2.6	0.4	0.3	0.2	0.2	26.0
Stearic (with C ₂₀)	0.4	1.3	1.7	1.1	0.2	0.1	0.1	—	—	4.9
Unsaturated C ₁₆	—	0.3	2.1	1.9	0.8	0.5	0.7	—	—	6.3
Oleic	0.5	2.2	10.4	10.2	6.3	2.1	1.5	0.5	0.3	34.0
Linoleic	—	0.1	1.7	1.6	1.3	0.5	0.4	0.3	0.1	6.0
Linolenic	—	0.5	3.1	4.3	3.4	1.5	1.9	1.1	0.3	16.1
Unsaturated C ₂₀₋₂₂	—	—	0.4	1.0	—	—	—	—	—	1.4
Component glyceride categories (increments, % by mol.)										
(i) Trisaturated	2.1	0.5	—	—	—	—	—	—	—	2.6
Disaturated-mono-unsaturated	1.6	9.5	11.3	—	—	—	—	—	—	22.4
Monosaturated-di-unsaturated	—	—	20.8	20.7	9.7	2.0	1.5	0.7	0.5	55.9
Tri-unsaturated	—	—	—	5.2	5.3	3.3	3.5	1.4	0.4	19.1
(ii) Dipalmito-	2.5	3.7	0.7	—	—	—	—	—	—	6.9
Monopalmito-	1.2	6.3	31.4	14.1	7.8	1.1	0.8	0.7	0.5	63.9
No palmito-	—	—	—	11.8	7.2	4.2	4.2	1.4	0.4	29.2
(iii) Dioleo-	—	—	—	4.6	4.0	0.9	—	—	—	9.5
Mono-oleo-	1.6	6.6	31.3	21.3	11.0	4.4	4.4	1.5	0.8	82.9
No oleo	2.1	3.4	0.8	—	—	—	0.6	0.6	0.1	7.6
(iv) Dilinolenic-	—	—	—	—	—	—	0.8	1.2	0.2	2.2
Monolinolenic-	—	1.4	9.2	12.9	10.2	4.4	5.2	0.9	0.7	43.9
No linolenic-	3.7	8.6	22.9	13.0	4.8	0.9	—	—	—	53.9

glyceride categories i-iv (Table 5) to give a fairly close estimate of the individual mixed glycerides present in each group, and thus to give an approximate statement of the probable component glycerides present in the whole fat (Barker & Hilditch, 1950). In the horse fat glycerides, however, there are more than three acids present, often in fairly high proportions, in each group of separated glycerides, and consequently there are several combinations of mixed triglycerides each of which would satisfy the conditions i-iv in Table 5. It is therefore not practicable to suggest any detailed statement for all the mixed glycerides present. The major components of the horse fat studied are, however, undoubtedly palmito-oleo-linolenins (approx. 30 %), and glycerides with one oleic and two other (usually different) unsaturated acyl groups (approx. 18 %); the rest of the fat is made up of oleodipalmitin, oleo-myristopalmitin and oleopalmitostearin (approx. 6-7 % each), with lesser proportions (approx. 5 % each) of palmitodiolein, palmito-hexadeceno-olein, and palmito-oleo-linolein, and still smaller amounts of a number of other mixed glycerides, including about 2 % of trisaturated glycerides.

DISCUSSION

Component fatty acids of horse mesenteric fat

The composition of the mixed fatty acids (Table 3) shows that the horse fat resembles other animal body fats in many respects, but stands apart in its high content of linolenic acid and in the presence of more linoleic and unsaturated C_{16} acids than is usual in such fats. Its saturated acids are typical of those animal depot fats which are not rich in stearic acid. Palmitic acid is the main saturated acid and falls within the range of 30 ± 3 % (by mol.) of the total

fatty acids, which Banks & Hilditch (1931) considered to be characteristic for the fats of land animals. Myristic and stearic are the only other saturated acids present in appreciable amounts in the horse fat, but each forms only about 5 % of the total fatty acids.

The unsaturated acids, apart from very small proportions of acids of the C_{20} (and possibly C_{22}) series, belong entirely to the C_{16} or C_{18} series. In the C_{16} group there are small but definite amounts of a hexadecatrienoic acid of the linolenic type, as shown by the spectrophotometric evidence after alkali-isomerization. The latter also suggests the presence of still smaller proportions of a hexadecadienoic acid, but in the absence of more conclusive evidence it is perhaps better merely to record the presence of the triene acid and to note that the mean unsaturation of the unsaturated acids is $-2.8H$ (i.e. 2.8 atoms of hydrogen/molecule short of saturation). The total amount of unsaturated C_{16} acids is definitely greater in the horse fat than in corresponding fats from, for instance, the ox, sheep or pig.

The characteristic presence of relatively large proportions of linolenic and linoleic acids (identical with these acids as found in vegetable fats) has been fully confirmed. In the present instance, linolenic acid formed 16 % and linoleic acid 5 % of the total fatty acids. At this point, however, it is useful to compare our present data with the other recently recorded detailed studies of horse fats to which reference was made in the introduction to this paper. The relevant data are given in Table 6.

Whilst the offal fat of Brooker & Shortland (1950) and the kidney fat of Holmberg & Rosenqvist (1949) may be considered most analogous to the mesenteric horse fat which we have examined, it will be noticed that the linolenic acid (17 %) and also the linoleic

Table 6. *Component fatty acids of horse fats*

	Mesenteric*	Offal†	Bone‡	Hoof‡	Milk‡	Kidney§	Marrow§	
							(a)	(b)
Iodine value of fat	95.6	97.9	99.0	109.3	84.3	84.4	90.2	84.9
Component acids (% w/w)								
Lauric	0.4	—	0.2	—	5.6	0.6	—	—
Myristic	4.5	1.1	3.1	0.8	7.0	2.8	2.7	4.4
Palmitic	25.9	27.4	25.3	17.9	16.1	20.3	22.0	20.0
Stearic	4.7	1.7	4.1	2.5	2.9	7.2	1.6	2.2
Arachidic	0.2	—	—	0.7	0.3	—	—	0.3
Unsaturated C_{16} (and C_{14})	6.8	11.3	8.9	19.4	9.3¶	4.3	10.9	10.9
Oleic	33.7	34.7	35.1	34.3	18.7	38.8	36.3	42.0
Linoleic	5.2	5.1	5.2	5.1	7.6	22.1	14.6	14.6
Linolenic	16.3	17.0	17.3	16.9	16.1	2.2	2.4	3.6
Unsaturated C_{20-22}	2.3	1.7	0.7	2.4	5.1	1.5	8.4	1.9

(a) Gelatinous marrow.

(b) From porous bone.

* Present work.

† Brooker & Shortland (1950).

‡ Hilditch & Jasperson (1944).

§ Holmberg & Rosenqvist (1949).

|| Also 5.5 % decanoic (capric), 2.6 % octanoic (caprylic), 0.9 % hexanoic (caproic) and 0.4 % butyric acids.

¶ Also 1.9 % unsaturated C_{12} and C_{10} acids.

acid (5%) contents of all three New Zealand fats (offal, bone and hoof) closely resemble that observed by us, whereas the kidney and bone marrow fats of the Swedish horse contained much smaller proportions (2–4%) of linolenic acid, but much more linoleic acid (15–22%). On the other hand, the mare milk fat, studied by Hilditch & Jasperson (1944), also contained 16% of linolenic acid. It may be suggested that these consistent differences may depend upon the diet of the animals. Holmberg & Rosenqvist (1949) state that the fats which they examined were from a 19-year-old gelding fed entirely on hay and oats during the last months before slaughtering. The milk fat (Hilditch & Jasperson, 1949) was from a mare fed on pasture in summer and on hay with a small amount of crushed oats in winter. The New Zealand fats studied by Brooker & Shorland (1950) were from horses which were invariably pasture fed. Unfortunately we have not been able to ascertain the feed of the animal from which the fat used in the present work was derived. The data as a whole, however, are consistent with the probability that the grass fats (rich in linolenic acid) are the source of the linolenic glycerides of the horse depot fats: it is not known whether these linolenic glycerides are still present in comparable proportions in hay, but they are reported to be absent from the fatty oil of oats which, however, contains high proportions of linoleic glycerides (Amberger & Hill, 1927).

The palmitic acid content of the Swedish horse depot fats is a few per cent lower than that of the New Zealand or English fats, but apart from the differences in linolenic and linoleic acid content mentioned the mesenteric, offal, kidney and bone fats all exhibit fairly close resemblance in fatty acid composition. The content of unsaturated C_{18} acids in the English mesenteric fat is, however, somewhat lower than that in most of the other horse fats. All six fats are characterized by the presence of 34–40% of the chief component, oleic acid, and by the absence of more than minor proportions of stearic acid.

The two other fats quoted in Table 6, mare milk fat and horse hoof oil, show not only the characteristics peculiar to horse fat, but also those of the particular organ fats concerned. Thus, in linolenic and linoleic acid contents both fats are very closely similar to the intestinal depot fats from English or New Zealand horses. At the same time, the horse hoof fat has the high hexadecenoic acid and lower palmitic acid content which Hilditch & Shrivastava (1948) observed in specimens of neat's foot oil of Irish and Australian origin. Similarly, the mare milk fat has a much reduced proportion of oleic acid and, to a less degree, of palmitic acid, with a corresponding introduction of shorter chain unsaturated and saturated acids (the latter, however, not extending much below the C_{10} series). In connexion with the

suggestion that the short-chain acids of milk fats may be derived from the oleoglycerides of the blood fat (Hilditch, 1947, pp. 306–310), it may be significant that, whilst the oleic acid content of the mare milk fat is only about half that of the horse depot fats, its linoleic and linolenic acid contents are almost exactly the same as those in the latter fats.

We are of the opinion that horse depot fat (and, doubtless, many other animal fats) may well represent a mixture of synthesized and assimilated dietary fat, in which polyethenoid acids present in the dietary fats have undergone little or no transformation prior to their deposition in the adipose tissues. This is a relatively simple instance of assimilation, whereas, with Brooker & Shorland (1950), we regard the ingestion of fat by ruminant animals as a particular case in which polyethenoid C_{18} glycerides may be altered during prolonged digestion, and, moreover, where oleoglycerides are also converted in part into stearglycerides before storage in the fat depots. The fats, depot or milk, of ruminant species are indeed to be looked upon as a special instance of a more complicated character than the processes of fat assimilation and transformation in many non-ruminant animals. For the rest, our conclusions on these points are in entire agreement with the arguments put forward in some detail by Brooker & Shorland. Moreover, on the basis of different dietary conditions in the two cases, we consider that the apparently divergent results of Holmberg & Rosenqvist (1949) are quite reconcilable with the observations of the New Zealand workers and ourselves.

Component glycerides of horse mesenteric fat

Besides the three major component acids—oleic, palmitic and linolenic—which together make up 75% of the mixed fatty acids of the horse fat, five other acids each contribute about 5% to the total, with the result that a complicated mixture of mixed glycerides is present. Consideration of Table 5 shows, however, that the fat exhibits the usual tendency for its constituent fatty acids to be distributed on the whole evenly throughout the triglyceride molecules. In this respect it stands in contrast to animal fats which are rich in stearic acid and which are characterized by much more trisaturated glycerides (palmitostearins) than is consistent with the simple operation of the usual principle of 'even distribution' (Hilditch, 1949).

This principle was first observed by Collin & Hilditch (1929) in vegetable seed fats, and expressed by these authors as a 'pronounced tendency to even distribution of the fatty acids throughout the glycerides'. Subsequently it has been defined (Hilditch, 1947, p. 16) more explicitly by stating that when a given fatty acid forms about 35% or more of the total fatty acids in a fat, it will occur at least once

in practically all the triglyceride molecules of the fat; but that a simple triglyceride containing three identical acid groups does not appear until the acid in question forms about 65 % or more of the total fatty acids. Owing to the comparative simplicity of the fatty acid mixtures in many vegetable fats, the latter lend themselves more readily to investigation of glyceride constitution than the more complicated fats. Recently (Barker & Hilditch, 1950; Hilditch & Seavell, 1950) study of a large number of vegetable fatty oils has provided data which show that, for a given individual acid, the proportion of glycerides with one group of the acid reaches a maximum when the acid forms 33 % of the total acids, and the proportion of glycerides with two groups of the acids reaches a maximum when the acid forms 67 % of the total acids; but the maxima are about 80–90 % of the total glycerides in the fat, whereas rigid adherence to the 'rule of even distribution' would, of course, imply that the whole of the fat would be constituted entirely at these points of glycerides containing either one or two of the acyl groups in question. In other words, some of the glycerides commence to contain two acyl groups somewhat before the acid concerned forms one-third of the total fatty acids, and some simple triglycerides with three acyl groups of the acid concerned appear before the latter forms two-thirds of the total fatty acids. This recent work has served to define more rigidly, and to confirm, the more general statement first given by Collin & Hilditch in 1929.

The general constitution of the horse fat glycerides, in which eight or nine component acids have to be considered, can be followed by consideration of the distribution of the individual acids in the mixed glycerides, especially that of the major component acids oleic, palmitic and linolenic. Oleic forms 34 % (by mol.) of the total fatty acids, i.e. if it were wholly evenly distributed it would be present (once) in all the triglyceride molecules. Table 5 (iii) shows that actually it is absent from 8 % of the latter, and that 9 % of glycerides with two oleic groups are present in the horse fat. This is exactly similar to the proportions of dilinoleo- or dilinolenoglycerides observed in vegetable fatty oils (Hilditch & Seavell, 1950) when the proportion of linoleic or linolenic acid is of a similar order. Similar data for stearic-rich animal fats, such as those of the ox or sheep, show that oleic acid is absent from considerably more of their triglycerides than in the present instance, and illustrate the manner in which such fats differ constitutively from the more usual type:

Depot fat	Glycerides containing no	
	Oleic acid % (by mol.)	oleic groups % (by mol.)
Sheep (Hilditch & Zaky, 1941)	40.4	14
Ox (Hilditch & Paul, 1938)	38.7	17
Ox (Hilditch & Murti, 1940)	30.5	28

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In the fats mentioned, the proportion of trisaturated glycerides is thus from 14 to 28 % of the whole, whereas the horse fat now studied contained only 2.5 % of trisaturated glycerides.

Palmitic acid, the next most prominent acid in the horse fat (of the acids of which it forms 26 % by mol.), would if strictly evenly distributed be present in all but 22 % of the triglyceride molecules. Actually it is absent from 29 % of the triglycerides, 7 % of the fat consisting of dipalmitoglycerides. Linolenic acid, which forms 16 % of the total fatty acids, occurs as dilinolenoglycerides in an extremely small proportion (2 %) of the fat. All the other acids present only occur once in any triglyceride molecule, so far as can be ascertained from our investigation of the horse fat component glycerides.

The horse fat glycerides are thus a somewhat complicated mixture of mixed glycerides, constituted on the usual lines of approximation to even distribution of each fatty acid amongst the glycerol molecules.

SUMMARY

1. The component acids of mesenteric fat from a horse consisted mainly of oleic (34 %), palmitic (26 %) and linolenic (octadeca-9:12:15-trienoic) (16 %) acids, with about 5–6 % each of myristic (tetradecanoic), stearic, hexadecenoic, and linoleic (octadeca-9:12-dienoic) acids, 2 % of higher unsaturated acids and traces of lauric (dodecanoic) and arachidic (eicosanoic) acids.

2. The presence of ordinary linolenic and linoleic acids in some quantity in horse fats is confirmed by the present study. The proportion of these acids observed is the same as in a group of New Zealand horse fats recently examined by Brooker & Shorland (1950), but both results differ from the experience of Holmberg & Rosenqvist (1949), who found more linoleic and much less linolenic acid in a Swedish horse fat. It is probable that the variations are connected with differences in the diet of the animals.

3. The component glycerides of the horse fat have been examined in some detail and found to consist of a complex mixture of mixed glycerides, each individual acid being for the most part distributed evenly throughout the triglyceride molecules. In general structure, therefore, the horse fat glycerides resemble those of vegetable fats, marine animal oils, etc., rather than those of other land animals, such as oxen and sheep, which are rich in stearic acid and which also contain an abnormal proportion of trisaturated glycerides (palmitostearins). The chief individual components of the horse fat include palmito-oleo-linolenins (approx. 30 %) and glycerides with one oleic acid and two other (usually different) unsaturated acyl groups (approx. 18 %), with lesser proportions of oleodisaturated glycerides, palmitodiolein, palmito-hexadeceno-olein and pal-

mito-oleo-linolein, and very small amounts of many other mixed glycerides. Trisaturated glycerides form little more than 2% of the whole fat.

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Fish Tropomyosin and Fish Nucleotropomyosin

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Tropomyosin has been prepared from muscles of several animals (rabbit, pig, horse and whiting) by dehydrating the minced muscle with organic solvents and extracting with m-potassium chloride (Bailey, 1948). A detailed account of its isolation and properties has also been published (Bailey, 1948; Bailey, Gutfreund & Ogston, 1948; Astbury, Reed & Spark, 1948).

In the course of an extensive ultracentrifugal study on fish myosins, it has been found that two proteins showing the same solubility properties as tropomyosin can be isolated from fish muscles without previous treatment with organic solvents. Both tropomyosins have been obtained in crystalline form and their ultracentrifugal and electrophoretic behaviours have been investigated. One of these proteins is practically identical with Bailey's tropomyosin. Dr K. Bailey kindly suggested that the other, which had the larger sedimentation constant, might be an association of the first compound and nucleic acid. This was completely confirmed by phosphorus and ribose determinations and by the ultraviolet absorption. The name 'nucleotropomyosin' is proposed for this compound.

EXPERIMENTAL

Isolation

Extraction of fish muscles with salt solutions (mixtures of phosphate and potassium chloride) of varying pH between 5 and 7 shows that the pro-

portion of tropomyosin in the extract increases when the pH of the extraction fluid decreases and when the time of extraction is short. The tropomyosins are still very extractable when the duration of the extraction is 10 min. and the pH of the extract 5.5, but the amount of myosins going into solution under such conditions becomes much smaller. The proportion of the tropomyosins in the extracts can be further increased if the extraction is carried out with muscles kept for several months in the frozen state. Such treatment decreases the extractability of the myosins (Reay & Kuchel, 1936) and does not change the behaviour of the tropomyosins. Separation of nucleotropomyosin from tropomyosin in such extracts is easy. On dilution with water the former coprecipitates with the myosins while the latter remains in solution and precipitates only on acidifying the supernatant to pH 4.6. A final purification of the substances is carried out by ammonium sulphate fractionation. The details of the method are given below.

The preparation has been carried out throughout in the cold and all the separations have been done by centrifugation. Carp muscles cut with a freezing microtome into slices 40 μ . thick (Dubuisson, 1947) are extracted for 20 min. with 3 vol. of a KCl-phosphate solution (0.15 M-KCl and 0.1 M-KH₂PO₄ brought to pH 5). This extract is diluted with 3.5 vol. of cold water; the precipitate is discarded and the supernatant (I) kept.

The residue from this first extraction is re-extracted for 10 min. with 3 vol. of 0.5 M-phosphate solution of pH 5.5 containing 0.3% sodium adenosinetriphosphate. The