2. A peptide appears in the fibrinogen-thrombin system.

3. It is suggested that this hitherto unknown substance should be called 'fibrino-peptide', since it is believed to be derived by splitting off part of the fibrinogen molecule.

4. A method for isolating apparently pure fibrino-peptide is given.

5. The release of fibrino-peptide is correlated with previous results, and the clotting of fibrinogen is discussed in the light of the new finding.

I wish to thank Prof. W. T. Astbury, F.R.S., for the benefit of discussions, and Dr W. R. Middlebrook for his valuable help in the chromatographic experiments.

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The Branched-Chain Fatty Acids of Mutton Fat

1. THE ISOLATION OF (+)-14-METHYLHEXADECANOIC ACID

By R. P. HANSEN, F. B. SHORLAND AND N. J. COOKE

*Fats Research Laboratory, Department of Scientific and Industrial Research,
Wellington, New Zealand*

(Received 24 January 1952)

Recent investigations in this laboratory have revealed the presence of branched-chain fatty acids as minor constituents of butterfat (Hansen & Shorland, 1950, 1951, 1952; Hansen, Shorland & Cooke, 1951a) and of beef fat (Hansen, Shorland & Cooke, 1952). The branched-chain acids isolated from butterfat comprised two C_{17} and one C_{18} saturated mono-methyl acids, and a saturated multi-methyl acid. From beef fat was derived a C_{17} methyl-substituted acid corresponding with one of the C_{17} acids isolated from butterfat.

Prior to the above findings, branched-chain acids had been located in the lipids of a number of bacilli (Anderson, 1927, 1941; Anderson & Chargaff, 1929; Velick, 1944a, b; Hofmann & Lucas, 1950) and in wool grease (Weitkamp, 1945), while the short-

chain acid *isovaleric* was found by Chevreul in 1817 (cf. Hilditch, 1947) in the head and jaw oil of the dolphin.

The purpose of this paper is to describe in detail the isolation of (+)-14-methylhexadecanoic acid from the external tissue fat of ewes. The occurrence of this acid in ewe fat was briefly reported last year (Hansen, Shorland & Cooke, 1951b). Although (+)-14-methylhexadecanoic acid has been shown by Weitkamp (1945) to be present in wool grease, where it occurs as a sterol ester, and although Velick & English (1945) synthesized this acid and confirmed its structure, it has not hitherto been reported as a constituent of natural fats.

Since this manuscript has been prepared Dr G. Weitzel and Dr K. Thomas have, in a private com-

munication, referred to their discovery of optically active branched-chain higher fatty acids in the cecygeal glands of ducks and geese.

EXPERIMENTAL

The fat used in this investigation (saponification equiv. 286.9, iodine value 46.6, unsaponifiable matter 0.56% and free fatty acids 0.4%) was obtained by mincing and then

jecting it to chromatographic separation using 320 g. of activated alumina in a column of 3.5 cm. diam. (cf. Table 4).

Fractions H10S2Lc to H10S2Lg were combined (5.03 g.) and denoted H15, while fraction H10S2Lb (7.62 g.) was submitted to further chromatographic separation. The characteristics of fraction H15 were as follows:

Saponification equivalent 284.7, iodine value 1.4, m.p. 2.5°; m.p. (acid) 37.5°. The acids were crystallized as follows:

steam-rendering the external tissue fats of old overweight ewes. In accordance with the procedure earlier outlined (Hansen & Shorland, 1951), methyl esters (7.69 kg.) were prepared, and after hydrogenation were crystallized repeatedly from acetone at -30° to yield 332.6 g. of 'liquid' esters which were distilled in a 22-plate Vigreux column at about 0.2 mm. (cf. Table 1). Fractions HL10, HL11 and HL12 were bulked together, denoted H1, and fractionated *in vacuo* in a 40-plate column of a modified Lecky & Ewell (1940) type (cf. Table 2). Details of this column will be published elsewhere.

X-ray diffraction measurements reported in this work were made using a Philips Geiger X-ray spectrometer. Samples were melted on a glass slide and quickly cooled.

All melting points were determined in closed capillaries and are uncorrected.

Table 1. Fractionation of 'liquid' methyl esters from hydrogenated mutton fat

(Iodine value 7.0.)

Fraction	Wt. (g.)	Saponification equiv.	Iodine value (Wijs)	
HL1	17.00	234.9	1.7	Liquid
HL2	22.64	240.5	0.7	Semi-solid
HL3	21.64	241.8	0.1	Solid
HL4	25.23	242.5	0.9	Solid
HL5	22.27	246.9	0.5	Liquid
HL6	21.48	256.0	1.1	Liquid
HL7	23.76	261.2	2.1	Liquid
HL8	21.74	265.1	2.4	Liquid
HL9	19.35	273.1	1.4	Liquid
HL10	22.65	279.6	5.8	Liquid
HL11	21.99	284.9	7.3	Liquid
HL12	25.87	287.8	3.3	Liquid
HL13	16.94	297.9	9.5	Solid
HLR	47.50	319.6	28.9	Viscous liquid
Total	330.06			

Methyl esters H1L2, H1L3, H1L4 from the above fractionation were combined, denoted H4, and crystallized as shown in Table 3.

Fraction H10S2L was further purified by dissolving the fraction in 300 ml. of light petroleum b.p. 50-60°, and sub-

Table 2. Refractionation of fractions HL10, HL11 and HL12 combined and denoted H1

Fraction	Wt. (g.)	Saponification equiv.	Iodine value (Wijs)	
H1L1	12.39	276.6	1.2	Liquid
H1L2	11.34	283.5	0.8	Liquid
H1L3	11.57	284.1	1.4	Liquid
H1L4	6.26	284.7	0.6	Liquid
H1L5	11.16	282.6	1.5	Liquid
H1LR	12.22	301.1	11.7	Liquid
Total	64.94			

DISCUSSION

The saponification equivalent and the combustion analysis of fraction H15S2S agree closely with the theoretical values for a saturated fatty acid with empirical formula $C_{17}H_{34}O_2$. The *C*-methyl value, however, which is equivalent to 1.55 molecules of acetic acid, affords evidence of the presence of one side-chain methyl group (cf. Ginger, 1944). With an X-ray long spacing of 30.0 Å. and a melting point of 38° this acid is further established as being distinct from normal heptadecanoic acid, m.p. 59.2-60.5 (Cason & Winans, 1950), X-ray long spacing 40.45 Å. (Francis & Piper, 1939). From the melting point evidence adduced by Cason & Winans (1950) in their investigations on the methyl-substituted isomers of octadecanoic acid, the relatively high melting point of H15S2S (38°) suggests that the

Table 3. *Low-temperature crystallization of concentrate (H 4) of methyl esters of C₁₇ acids*

side chain will not be located at the centre of the molecule, for in this position minimum melting points are shown to occur.

Table 4. *Chromatographic separation of fraction H 10S 2L*

Fraction	Wt. (g.)	Eluant	n_D^{20}
H 10S 2La	0.03	300 ml. light petroleum (each fraction)	Solid
b	7.62		1.4421
c	2.38		1.4417
d	1.12		1.4419
e	0.73		1.4417
f	0.52		1.4418
g	0.40		1.4418
h	0.28		1.4423
i	0.40	600 ml. light petroleum	1.4423
j	0.33	900 ml. light petroleum	1.4424
k	0.03	300 ml. light petroleum containing 5% ether	—
l	0.12	300 ml. light petroleum containing 10% ether	1.4431
m	0.01	300 ml. containing 50% light petroleum and 50% ether	—

Comparison of the properties of this optically active branched-chain C_{17} saturated acid fraction with those of the C_{17} dextrorotatory *ante-iso* acid isolated from wool grease by Weitkamp (1945) and

subsequently synthesized by Velick & English (1945) identifies H 15S 2S as (+)-14-methylhexadecanoic acid (cf. Table 6).

Although Velick (1947) reported a value of 33.4 Å. for the X-ray long spacing of (+)-14-methylhexadecanoic acid from wool grease, Nunn (1951), when analysing a sample of the same acid, also supplied by Weitkamp, observed an X-ray long spacing of 29.9 Å. This latter figure corresponds closely with that reported in this paper. Nunn was unable to confirm Velick's (1947) value, but stated: 'It is possible that the method of preparation of the samples (for X-ray examination) used by us gave crystals in a metastable state.' The accuracy of the measurement recorded here (30.0 Å) was established by determining the X-ray long spacing of a standard sample of palmitic acid which gave a value of 35.6 Å., this figure being identical with the accepted value of Francis & Piper (1939).

The main fraction reported in this paper (H 15S 2S) comprises 3.71 g., but this quantity represents only a portion of the total content of this acid. Other fractions derived from H 10S 2L by chromatographic separation (cf. Table 4) also consisted mainly of this acid. For example, chromatography and fractional crystallization of H 10S 2Lb (7.62 g.) yielded a further 3.45 g. of acids, m.p.

Table 5. *Characteristics of fraction H 15S 2S*

Fraction	M.p.	Saponification equiv.	Combustion analysis*	Iodine value (Wijs)	C-Me*	X-ray long spacing	Specific rotation [α_D^{25}] + 3.83° (CHCl ₃)	Refractive index
H 15S 2S acid	38.0°	270.5 (Calc. for $C_{17}H_{34}O_2$: 270.4)	C, 75.7; H, 12.7% (Calc. for $C_{17}H_{34}O_2$: C, 75.5; H, 12.7%)	0.9	8.6% (equiv. to 1.55 mol. acetic acid)	30.0 Å.	—	—
H 15S 2S methyl ester	2.5°	283.1 (Calc. for $C_{18}H_{36}O_2$: 284.5)	—	—	—	—	—	n_D^{20} 1.4424

* Analysis by Weiler and Strauss, Oxford, England.

Table 6. *Comparison of properties of fraction H 15S 2S with those of natural and synthetic forms of 14-methylhexadecanoic acid*

Sample	M.p.	X-ray long spacing (Å.)	Optical rotation	
H 15S 2S	38.0°*	30.0	$[\alpha]_D^{25}$ (in chloroform) + 3.83°	This work
(+)-14-Methylhexadecanoic acid	—	33.4c, 29.9d	$[\alpha]_D^{26}$ (in acetone) + 5.0°a	Isolated by Weitkamp (1945) from wool grease
	36.8a, 37.3–37.6b	—	$[\alpha]_D^{26}$ (in acetone) + 5.23°b	—
(+)-14-Methylhexadecanoic acid	36–36.8°b	—	$[\alpha]_D^{26}$ (in acetone) + 5.16°	Synthesized by Velick & English (1945)
(±)-14-Methylhexadecanoic acid	39.5–40.1°d	29.7d	—	Synthesized by Nunn (1951)

* Melting point uncorrected.

a, determined by Weitkamp (1945); b, determined by Velick & English (1945); c, determined by Velick (1947); d, determined by Nunn (1951).

37.5°. In the sample of external tissue fat analysed, (+)-14-methylhexadecanoic acid constitutes not less than 0.2% of the total fatty acids. Examination of other fractions from mutton fat is in progress, and the evidence already accumulated suggests that appreciable quantities of branched-chain acids are present.

The work now reported establishes that certain branched-chain acids isolated from wool grease (Weitkamp, 1945) and presumably secreted by the sebaceous glands of the sheep are also present in the external tissue fat, and possibly in the other fatty tissues of this animal.

SUMMARY

(+)-14-Methylhexadecanoic acid has been shown to occur as a minor constituent of mutton fat.

We wish to acknowledge the assistance given in this work by Mr M. Fieldes, Soil Bureau, Department of Scientific and Industrial Research, Wellington, who kindly carried out all X-ray measurements.

To Mr M. S. Carrie, Chemist to The Hawkes Bay Farmers Freezing Company Ltd., and to the management of that Company, we are indebted for arranging supplies of suitable material for this investigation.

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Seasonal Variations in Fatty Acid Composition of New Zealand Butterfat

BY R. P. HANSEN AND F. B. SHORLAND

*Fats Research Laboratory, Department of Scientific and Industrial Research,
Wellington, New Zealand*

(Received 6 February 1952)

New Zealand butterfat has been shown by Cox & McDowall (1948) to exhibit seasonal variations in softening points, iodine values, saponification values and Reichert values. The characteristic trend of variation of each of these properties was observed to be remarkably uniform in the four different years reviewed, and in butterfats from different localities. Interpretation of the precise nature of these changes, however, requires a knowledge of the fatty acid composition and the glyceride structure of the different butterfats.

Many workers have determined the fatty acid composition of butterfat to elucidate its nature (Hilditch & Jones, 1929; Jack, Henderson & Hinshaw, 1946), to study the effect of diet on the

milk fat (Hilditch & Sleightholme, 1930; Hilditch & Thompson, 1936; Hilditch & Jasperson, 1943) or to find the factors responsible for seasonal changes (Hilditch & Jasperson, 1941). A great deal of information has thus been accumulated, but so far as we are aware no systematic investigations have been made similar to the work now reported. It comprises both triplicate analyses of butterfat samples taken from the same locality at regular intervals over a whole year, and statistical evaluation of the results.

The purpose of this investigation was to measure statistically at regular intervals the fluctuations in the fatty acid composition of New Zealand butterfat and to attempt correlation of these changes in