6. The experimental observations, discussed in the light of model surfaces of known structure, are consistent with the hypothesis that the cells of *Aero. aerogenes* trained to crystal violet have a surface which is lipid in nature, in contrast to that of the normal cells, which is polysaccharide.

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The Metabolism in the Rat of Naturally Occurring (+)-14-Methylhexadecanoic Acid

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The recent isolation of branched-chain fatty acids from animal fats (Hansen, Shorland & Cooke, 1952) has aroused interest in the fate of such acids as dietary components. Hitherto, feeding experiments with branched-chain acids were carried out mainly in relation to the effects of such acids present in synthetic fat. Thus Appel, Böhm, Keil & Schiller (1947) fed glycerides of the higher branched-chain fatty acids with methyl, ethyl or propyl side chains to goats, whereas Weitzel (1951) fed dogs with α -substituted myristic and stearic acid triglycerides. The metabolism of the acids was followed by determining the nature of the ether-soluble breakdown products in the urine. In addition, without giving experimental evidence, Appel *et al.* (1947) stated that 13.6% of the branched-chain acids fed in synthetic fat to goats was found in the depot fats. Weitzel (1951) estimated that 90% of the α -substituted myristic and stearic acids fed to dogs was metabolized, but he did not determine the storage of these acids in the body.

In the present study the branched-chain acid (+)-14-methylhexadecanoic acid, naturally occurring up to approximately 0.5% in ruminant fat, has been fed to rats to determine its storage, excretion, breakdown and effect on growth.

Because of the difficulty of isolation of traces of branched-chain fatty acids from animal fats, metabolic studies without the use of isotopically labelled compounds are not normally feasible. However, as the acid used in this work is optically active it has been possible to utilize this property as a sensitive method of estimation.

EXPERIMENTAL

Rats of the Wistar strain maintained as an inbred colony for the past 22 years were used. They were fed on the experimental diet from weaning (40–50 g.) until they reached 160 g.

The 'fat-free' basal diet used was similar to that described by Longenecker (1939). It consisted of (%): casein, 20; corn starch, 60; sucrose, 9; salt mixture, 4; agar-agar, 2; dried yeast, 5. The diet was computed to contain not more than 1.36% of fat.

The animals were divided into groups, each with two sub-groups consisting of five bucks and five does. Group A (controls) were given the basal 'fat-free' diet; group B received the same diet but with a supplement of 0.1 g. of C_{17} anteiso-acid (from a preparation obtained by Hansen *et al.* 1952)/rat/week. Altogether seven such supplements were given, after which the bucks returned to the basal diet for 3 weeks, and the does for 7 weeks. The animals were then killed. The faeces of bucks and does were collected for 48 hr.

after each dose. By the use of a jet of air they were freed from hairs and food particles, and were then stored in alcohol at 0°, and combined, giving a total weight of 54.8 g. of dry matter.

In the second week of the experiment, it was found that owing to scattering of the 'fat-free' food, the weights began to decline. To prevent this complication, the 24 hr. supply of food was made into a paste. The average time required to attain the target weight of 160 g. was taken as an indication of the growth rate. The group A bucks required 9½ weeks and the group B bucks 10 weeks. The does in both groups required over 14 weeks.

At the conclusion of the feeding period the animals were decapitated and the blood was drained out. The alimentary canal was discarded and the bodies were minced and then boiled for 20 min. in 2 vol. of 95% ethanol. After distilling off the alcohol *in vacuo* the tissues were exhaustively extracted with boiling acetone, and then with 95% ethanol followed by peroxide-free ether. The extracts were combined and the solvents evaporated. The fat was taken up in light petroleum (b.p. 50–60°), filtered, and the solvent removed at 100° *in vacuo*. The faecal lipids were prepared in the same way.

The fats were saponified and the unsaponifiable matter was extracted as described by Hilditch (1947). The soaps were acidified and the fatty acids crystallized five times from 10 vol. of acetone at –33°. The liquid, high-iodine value fractions were bulked and converted into methyl esters. Previous experience (Hansen, Shorland & Cooke, 1954) has

shown that the *anteiso*-acids occur in these fractions when this method of crystallization is used. The methyl esters were fractionally distilled at 0.1 mm. in a spiral fractionating column based on column E (Shorland, 1952). The optical activity of the fractions was determined and the proportions of (+)-14-methylhexadecanoic and (+)-12-methyltetradecanoic acids were calculated from the values given by Weitkamp (1945) for the pure acids. No optical activity was observed apart from that shown by fractions corresponding to methyl esters of C₁₅ and C₁₇ acids (see Tables 1 and 2). No activity was found in the fractions from the control group A.

The amount of buck fat fractionated represents 75.0 g. out of a total of 110.0 g. The total fat, therefore, contained 0.003 g. of C₁₅ *anteiso*- and 0.33 g. of C₁₇ *anteiso*-acids.

Out of a total of 91.3 g. of doe fat 76.2 g. was used, so that the total C₁₅ *anteiso*-acid present was 0.014 g., whereas the C₁₇ *anteiso*-acid amounted to 0.28 g.

Since insufficient group B combined faecal fat was available for fractionation, 20.42 g. of fat from group A does, devoid of optical activity, and 0.95 g. of group B faecal fat were combined. After removal of unsaponifiable matter as described by Hilditch (1947) the fatty acids were crystallized as outlined above. The liquid fractions were combined and subjected to ester fractionation, with column E (Shorland, 1952) at 0.1 mm., and 0.16 g. of optically active acid was obtained (Table 3). The C₁₇ *anteiso*-acid present in the total faecal fat (3.89 g.) was therefore 0.66 g.

Table 1. Fractional distillation of the methyl esters of the acetone-soluble fatty acids of the bucks of group B

Fraction	Wt. (g.)	Saponification equiv.	Iodine value	α_D^{18} *	<i>anteiso</i> -Acid† (g.)
1	0.20	237.5	18.0	+0.00°	Nil
2	0.17	252.7	14.6	0.06	0.002 (C ₁₅)
3	6.19	267.1	84.0	0.00	Nil
4	1.89	270.8	79.4	0.10	0.038 } (C ₁₇)
5	4.99	293.5	83.5	0.19	0.189 }
6	9.09	296.4	96.7	0.00	Nil
7	13.33	296.6	87.2	0.00	Nil
8	2.41	297.6	85.0	0.00	Nil
Residue	5.24	353.1	95.3	—†	—
Total	43.51				

* Rotation measured in a 1 dm. cell without dilution.

† Calculated from $[\alpha]_D^{18}$ C₁₅ *anteiso* = +4.7° and $[\alpha]_D^{18}$ C₁₇ *anteiso* = +5.0° (Weitkamp, 1945).

‡ Rotation not measurable because of dark colour.

Table 2. Fractional distillation of the methyl esters of the acetone-soluble fatty acids of the does of group B

Fraction	Wt. (g.)	Saponification equiv.	Iodine value	α_D^{18} *	<i>anteiso</i> -Acid† (g.)
1	0.61	241.7	25.6	+0.00°	Nil
2	0.85	257.4	54.3	0.07	0.012 (C ₁₅)
3	7.60	269.2	92.4	0.00	Nil
4	0.92	272.1	64.8	0.00	Nil
5	0.85	290.2	71.8	0.24	0.041 } (C ₁₇)
6	1.59	292.9	88.8	0.16	0.051 }
7	3.72	295.1	90.4	0.14	0.104 }
8	7.01	296.8	87.4	0.03	0.042 }
9	8.38	296.9	87.9	0.00	Nil
10	7.23	294.9	86.2	0.00	Nil
Residue	5.07	327.2	105.1	—†	—
Total	42.83				

* † ‡ See Table 1.

Table 3. *Fractional distillation of the methyl esters of the acetone-soluble fatty acids of the combined faeces of group B*

Fraction	Wt. (g.)	Saponification equiv.	α_D^{18} *	C ₁₇ anteiso-Acid† (g.)
1	1.56	263.1	+0.00°	Nil
2	1.38	266.4	0.00	Nil
3	1.61	283.6	0.11	0.03
4	2.56	291.7	0.22	0.11
5	1.61	296.7	0.06	0.02
Residue	4.09	313.7	0.00	Nil
Non-sap.	0.90	—	—†	—
Total	13.71			

* † ‡ See Table 1.

Table 4. *Metabolism of C₁₇ anteiso-acid in the rat*

C ₁₇ anteiso [(+)-14-Methylhexadecanoic]-acid	Bucks		Does	
	Wt. (g.)	%	Wt. (g.)	%
Found in total fat	0.28	8.0	0.34	9.7
Found in faecal fat*	0.33	9.4	0.33	9.4
Metabolized†	2.89	82.6	2.83	80.9
Total administered	3.50	100.0	3.50	100.0
Approximate amount of C ₁₅ anteiso-acid found	0.003	0.09	0.014	0.40

* It has been assumed that equal weights of acid were present in the buck and doe faeces.

† By difference.

DISCUSSION

It will be seen from Table 4 that some 8–10 % of the dietary branched-chain acid was stored in the depot fat and that a similar amount was excreted, whereas the remainder, comprising approximately 80 %, was metabolized. It should be borne in mind, however, that the animals were not killed immediately after the last dose was administered. Although the period involved was 7 weeks for the does and 3 weeks for the bucks, the amount of (+)-anteiso-acids remaining in the depot fats in each case was practically identical, which suggests that the mobilization of these acids from the depots is very slow, or else that there is a large difference in the storage of these acids in does and bucks. The latter possibility, however, appears less likely. In addition, a small amount of the C₁₇ anteiso-acid (0.1–0.4 %) was broken down to (+)-12-methyltetradecanoic acid. The presence of this latter substance is consistent with the operation of a Lynen (1954) fatty acid cycle, in which the branched-chain acids are degraded or synthesized by the successive removal or addition of acetate units.

As there were no differences in growth rates of the experimental and control groups it appears that (+)-14-methylhexadecanoic acid does not influence growth at the level used and behaves similarly to saturated *n*-fatty acids.

SUMMARY

1. Rats were fed on a 'fat-free' diet and a similar diet augmented by (+)-14-methylhexadecanoic acid at the rate of 0.1 g./rat/week, seven supplements being given.

2. It was found that 8–10 % of the acid was stored, about 10 % was excreted and approximately 80 % was metabolized. Furthermore, the presence of traces of the C₁₅ anteiso-acid in the experimental animals, but not in the control animals, affords evidence for degradation of the C₁₇ anteiso-acid included in the diet.

3. The addition of (+)-14-methylhexadecanoic acid to the 'fat-free' diet at the rate of 0.1 g./rat/week was without influence on the growth rate of the rat.

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