Further Studies on the Metabolism of the (+)-*anteiso*-Acids, (+)-12-Methyltetradecanoic Acid and (+)-14-Methylhexadecanoic Acid, in the Rat

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Recently, Livingston, Bell, Shorland, Gerson & Hansen (1957) found that Wistar rats on a fat-free diet supplemented with 0.1 g. of C₁₇ *anteiso*-[(+)-14-methylhexadecanoic] acid/rat/week excreted 10% and stored a similar amount which was partially converted into the C₁₅ *anteiso*-acid. The remaining 80% was metabolized to substances other than *anteiso*-acids.

In the present work the object was to study the storage, excretion and interconversion of (+)-*anteiso*-acids given at higher levels than previously. For this purpose the dosage of (+)-14-methylhexadecanoic acid was increased to as much as 3 g./rat/week, and other rats were fed with up to 0.6 g. of (+)-12-methyltetradecanoic acid/rat/week. It was hoped to show in this way the formation of (+)-14-methylhexadecanoic acid from (+)-12-methyltetradecanoic acid. In the course of these investigations the opportunity was taken to ascertain what effect, if any, the feeding of (+)-*anteiso*-acids had on the fatty acid composition of the carcass fat.

EXPERIMENTAL

Male weanling Wistar rats were fed *ad lib.* on a fat-free diet as previously described (Livingston *et al.* 1957). Table 1 shows the supplement given to the six groups of rats used in this work. The animals appeared to find these acids unpalatable, and to ensure ingestion they were given in the form of pellets, further food being withheld until the supplement was completely consumed. Twenty-four hours after the last supplement the rats were killed by decapitation, except those of group 5B, which were kept alive for a

further period of 9 days. The whole animals of group 4 (see Table 1) were used for fat extraction, but in the other groups the alimentary tract was discarded, as in the experiments already reported (Livingston *et al.* 1957).

The faeces of groups 1-4 were collected for 48 hr. after each supplement. Those of groups 5A and 5B were collected during the whole supplement period, and with group 5B for a further 9 days until the animals were killed. Faeces were freed from hair and food particles by blowing with a jet of air.

The tissues from each group were combined and minced after death and dropped into approximately 2 vol. of 95% ethanol and boiled for 20 min. The faeces were similarly dehydrated with boiling ethanol, the collections for each group being subsequently combined. The ethanol was distilled off and the tissues and faeces were dried *in vacuo*. The fat was extracted as described by Livingston *et al.* (1957).

Samples of the tissue fats, varying in weight from 30 to 50 g., were saponified, the unsaponifiable matter was extracted and the soaps were converted into fatty acids as described by Hilditch (1956*a*). The acids were crystallized three times from 15 vol. of acetone at -30° to separate them into 'solids' and 'liquids', which were then converted into methyl esters. As previously found (Hansen, Shorland & Cooke, 1954), the (+)-*anteiso*-acids remained in the liquid fraction. The esters were distilled in a fractionating column previously described (column E, Shorland, 1952).

The fatty acid composition of groups 1 and 5A were calculated as described by Hilditch (1956*b*), the necessary allowances for (+)-*anteiso*-acids present being made. The latter, when present in the methyl ester fractions from the 'liquids', were located from their saponification equivalents and quantitatively estimated from their optical rotations (cf. Livingston *et al.* 1957), the values reported by Milburn & Truter (1954) being used for the specific rotations of the pure substances. The (+)-*anteiso*-acids in the faecal lipids

Table 1. *Details of the dietary amounts of (+)-anteiso-acids given*

Group no.	No. of rats	Supplement			Duration of supplementary feeding (days)	Age at death (weeks)
		Acid	Wt. (g.)	Milli-equiv./rat		
1	11	—	—	—	—	21
2	5	C ₁₅	0.1	0.39 (weekly)	63	21
3	5	C ₁₅	0.6	2.34 (weekly)	63	21
4	5	C ₁₇	0.6	2.22 (weekly)	49	18
5A	3	C ₁₇	0.5	1.85 (daily)	6	20
5B	3	C ₁₇	0.5	1.85 (daily)	6	20

Table 2. *Storage, conversion and excretion of (+)-anteiso-acids expressed as a molar percentage of the dietary amounts given*

Group* no.	(+)- <i>anteiso</i> - Acids given	Total supplement per group (milli-equiv.)	Deposited in carcass (%)	Converted into other <i>anteiso</i> - acids (%)	Excreted in faeces (%)	Metabolized to substances other than <i>anteiso</i> -acids % (difference)
1	—	Nil	0.0	0.0	0.0	0.0
2	C ₁₅	17.55	2.1	0.7	9.5	87.8
3	C ₁₅	105.30	2.1	0.4	2.3	95.2
4	C ₁₇	77.70	2.1	0.6	2.8	94.5
5A	C ₁₇	33.30	7.8	2.7	1.0	88.5
5B†	C ₁₇	33.30	9.9	2.2	1.1	86.8

* See Table 1.

† Faeces were collected for 9 days after the last supplement but contained no detectable amounts of (+)-*anteiso*-acids.Table 3. *Comparison of fatty acid compositions (weight %) of rats fed on (+)-14-methylhexadecanoic acid and controls*

Group* no.	Saturated acids						Unsaturated acids			
	C ₁₄	C ₁₅ (+)- <i>anteiso</i>	C ₁₆	C ₁₇ (+)- <i>anteiso</i>	C ₁₈	C ₂₀	C ₁₄	C ₁₆	C ₁₈	C ₂₀₋₂₂
1	2.27	0.00	27.97	0.00	6.93	0.14	0.93 (-2.0, H)†	14.19 (-2.0, H)	45.70 (-2.1, H)	1.87 (-6.0, H)
5A	1.14	0.69	27.58	2.31	2.77	1.31	0.85 (-2.0, H)	15.39 (-2.0, H)	46.68 (-2.1, H)	1.28 (-6.0, H)

* See Table 1.

† Mean unsaturation in terms of hydrogen.

were determined after removal of the unsaponifiable matter and bleaching of the recovered acids with animal charcoal. It was assumed that the faecal (+)-*anteiso*-acids were the same as those administered.

RESULTS AND DISCUSSION

In Table 2 are shown the weights of (+)-*anteiso*-acids in the tissues and faeces, from which the extent of storage, conversion into other (+)-*anteiso*-acids and excretion are calculated. The results given show that the C₁₅ and C₁₇ (+)-*anteiso*-acids are deposited in similar amounts when given at the same level. The amounts of (+)-*anteiso*-acids found were similar in the whole animals of group 4 and in the animals of group 5 whose alimentary tracts had been removed. It would appear therefore that these acids are not stored to any appreciable extent in the alimentary tract. The amounts of C₁₅ acid converted into C₁₇ acid, and vice versa, are very similar. The behaviour of branched-chain fatty acids therefore does not resemble that of normal fatty acids, in which the lower members (below C₁₆) are less readily deposited than their higher homologues. Thus when decanoic acid is fed to pigs no appreciable amounts are incorporated into the depot fats, whereas dietary lauric acid is deposited to a much smaller extent than myristic acid (Shorland & de la Mare, 1945). It appears that the relative metabolic properties of fatty acids, such as the rate of synthesis and breakdown, determine to a large extent the composition of the depot fat. As storage and interconversion are similar for C₁₅ and C₁₇ (+)-

anteiso-acids, it is not surprising that these occur in similar amounts in natural fats, e.g. ewe-milk fat (Gerson, Shorland & Barnicoat, 1958) with 0.41 % of C₁₅ and 0.45 % of C₁₇ (+)-*anteiso*-acid and lamb-caul fat (F. B. Shorland, unpublished results), which contains 0.37 % of C₁₅ and 0.36 % of C₁₇ (+)-*anteiso*-acid.

Pihl, Bloch & Anker (1950) found that the carcass fatty acids of rats maintained on a fat-free diet had a half-life period of 18 days. As stated above, groups 5A and 5B received identical treatment except that group 5B was maintained for 9 days after the last supplement whereas the rats in group 5A were killed at this stage. A comparison of these groups thus provides a measure of the rate of disappearance of the (+)-*anteiso*-acids. This shows that the rate of turnover of (+)-*anteiso*-acids in rats is much slower than that of the normal fatty acids. In a recent experiment (T. Gerson, J. C. Hawke, F. B. Shorland & W. H. Melhuish, unpublished results) it was found that isotopically-labelled acetate is incorporated into *anteiso*-acids more slowly than into normal acids, thus further demonstrating the slow metabolism of (+)-*anteiso*-acids.

Table 3 shows the fatty acid compositions of groups 1 and 5A and indicates that the feeding of (+)-*anteiso*-acids does not affect appreciably the composition apart from the inclusion of (+)-*anteiso*-acids. However, the reduced amount of stearic acid in group 5A as compared with group 1 may be significant.

SUMMARY

1. Supplements of (+)-12-methyltetradecanoic acid and (+)-14-methylhexadecanoic acid to a fat-free diet did not materially alter the fatty acid composition of the depot fats of Wistar rats.

2. The weights of (+)-12-methyltetradecanoic acid and (+)-14-methylhexadecanoic acid deposited in the carcass under identical conditions of supplementation were proportional to the amounts of supplement given, and the degree of interconversion of these acids was similar, thus affording an explanation for their occurrence in the same proportions in animal fats.

3. The metabolic turnover of (+)-*anteiso*-acids was shown to be considerably slower than that of normal fatty acids.

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The N-Terminal Amino Acid Residues of Gelatin

4. CHAIN WEIGHT AND RIGIDITY RELATIONSHIP IN FRACTIONATED GELATINS*

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The relations between the macroscopic properties (solution viscosity, gel rigidity, melting point, etc.) of the gelatin–water system and the molecular size, weight, shape and chemical structure of the gelatin molecules remains as the central problem of research in this field.

Pouradier & Venet (1952) showed that an equation of the type $\eta = KM_n^\alpha$ exists between the intrinsic viscosity η (or the equivalent limiting viscosity number, LVN) and the number-average molecular weight, M_n , determined osmotically on fractions of gelatin. Whereas K and α were each the same for two alkali-processed gelatins, they were different for an acid-processed gelatin. The evidence for multichain gelatin molecules (Courts & Stainsby, 1958) introduces a further factor into considerations of molecular structure and suggests that the 'molecular-weight' values calculated from assay of N-terminal residues (Courts, 1954*a, b*; 1955) should be more correctly termed 'chain weight' (C_n).

Courts & Stainsby (1958) have shown that, in accordance with a multichain molecular model, there is no direct connexion between the following pairs of quantities for fractions of an alkali-

processed gelatin: (1) Weight-average molecular weight (M_w) and C_n . Thus C_n for high-grade gelatin fractions has the approximately constant value of 70 000, whereas M_w may vary from 70 000 to 350 000. (2) C_n and LVN. This was demonstrated experimentally but also must arise as an automatic consequence of the relation between M_n and LVN and (1) above, provided that reasonable homogeneity of fractions has been secured.

The picture for acid-processed gelatin fractions is not so readily interpreted. Whereas the value of C_n at 92 000 for the higher fraction is greater than in the corresponding alkali-processed series, M_w at 194 000 is somewhat lower. Since for single chains M_w is greater than C_n unless the fractions are completely homogeneous, branching must be regarded as probable rather than proved. In addition, for the lower fractions of the acid-processed gelatin, M_w is less than C_n , suggesting that some gelatin chains occur with no free α -amino groups. This could arise through blocking with some substance which the alkali process removes. The lack of end-groups in native collagen could be regarded as supporting this view. If a similar proportion of blocked groups occurred in the larger molecules it would explain the figure C_n 92 000

* Part 3: Courts (1955).