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Linoleic Acid and Linolenic Acid Elongation Products in Muscle Tissue of *Syncerus caffer* and other Ruminant Species

By M. A. CRAWFORD and MURIEL M. GALE
Nuffield Institute of Comparative Medicine, The Zoological Society of London, Regent's Park, London N.W.1

and M. H. WOODFORD
Nuffield Unit of Tropical Animal Ecology, Queen Elizabeth Park, Uganda

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The metabolic elongation products of both linoleic acid and linolenic acid were found in muscle tissues of *Syncerus caffer* and other ruminants. The acids with four double bonds were predominantly in the linoleic acid series, whereas the higher degrees of unsaturation, mainly five double bonds, were in the linolenic acid series. The total linoleic acid and linolenic acid groups were present in the relative proportions of about 4:1, in contrast with the fish oils, where the acids are mainly in the linolenic acid series. The consistent occurrence of members of both groups of acids in the animals studied here suggests to us that both may be important for structural purposes.

We previously reported on the fatty acids of bovid muscle tissue (Crawford, 1968*a*; Gale, Crawford & Woodford, 1969). We described the distribution of the fatty acids in terms of the balance between polyenoic, monounsaturated and saturated acids. In previous reports we grouped the polyenoic acids together in order of their carbon number and degree of unsaturation. This method of presentation was adopted through the use of Apiezon columns. We now report reanalyses on polyester columns, which separate the polyenoic acids on the basis of positioning of the double bonds. This enables a description of the parent polyenoic molecule on the basis of the length of the saturated end chain.

MATERIALS AND METHODS

Animal material was obtained as described by Crawford (1968*a*). In this work the African animals were obtained from East Africa through co-operation with the Nuffield Unit of Tropical Animal Ecology and the Uganda Game Department.

The g.l.c. apparatus used was an S6 Research Chromatogram with 6ft. steel columns of 3mm. internal diameter. Columns were packed with either 10% diethylene glycol succinate on 100–120-mesh Celite or 3% EGSP-Z organosilicone polyester on 100–120-mesh Gas-Chrom Q. Operating conditions were: injection port, 250°; chromatography oven, 170° for EGSP-Z, and 190° for diethylene glycol succinate; detector oven, 230°. Analyses were carried out isothermally, and the argon entry flow rate was set at 12ml./min. A Pye Argon Gas Chromatogram (series 104) was also used with 5ft. glass columns, 4mm. internal diameter, and columns were packed with 10% polyethylene glycol adipate and 10% Apiezon L, both on 100–120-mesh Celite. For routine analysis the Apiezon column was run at 210° and the polyethylene glycol adipate column at 184·5°. Flash heaters and detector ovens were maintained at 240° and 230° respectively. The argon flow rate emerging from the column on the Pye instrument was adjusted to 52ml./min. Flame-ionization detectors were used in both cases.

The identification procedure used linear logarithmic plots (James, 1960) and the techniques of Ackman & Burgher (1963, 1964, 1965) for fish oils. Standards were obtained from the Hormel Laboratory, Austin, Minn., U.S.A., for the eicosatetraenoic acid, the eicosapentaenoic acid, the docosahexaenoic acid and methylated cod liver oil. The structural assignment of the individual fatty acids was made by calculation of separation factors on the three polyester columns (Ackman & Burgher, 1963, 1965; Ackman, 1966). For confirmation, the methyl esters were separated by t.l.c. with $AgNO_3$-impregnated silica gel and ether as solvent. Numerical description of the fatty acids used here follows the convention of $C_{x:y\omega n}$ where x refers to the length of the carbon chain, y to the number of double bonds and n to the saturated end carbon chain.

Quantitative determination was carried out by measurement of the peak areas (Keulemans, 1957) in relation to the known response obtained from individual standards where these were available. Samples were hydrogenated with platinum dioxide (Koch–Light Laboratories Ltd., Colnbrook, Bucks.) as catalyst for confirmation and to distinguish the saturated fatty acids.

RESULTS

The distribution of the main linoleic acid and linolenic acid elongation products in muscle tissue of *Syncerus caffer* are described in Table 1. It is

Table 1. *Percentage composition of linoleic acid and linolenic acid elongation products in muscle tissue from* Syncerus caffer *compared with a fish oil*

The results obtained in the present work are given as means ± S.E.M. for seven animals.

Fatty acid assignment	Positions of double bonds	Percentage composition: Extracted fatty acids (present work)	Percentage composition: Cod liver oil (Ackman & Burgher, 1964)
$C_{18:2\omega6}$	9, 12	17·3 ± 1·1	0·8
$C_{18:3\omega3}$	9, 12, 15	5·4 ± 0·8	0·1
$C_{20:2\omega6}$	11, 14	0·27 ± 0·07	0·2
$C_{20:3\omega6}$	8, 11, 14	0·42 ± 0·14	0·1
$C_{20:4\omega6}$	5, 8, 11, 14	7·5 ± 0·67	1·2
$C_{20:4\omega3}$	8, 11, 14, 17	0·3 ± 0·06	0·5
$C_{20:5\omega3}$	5, 8, 11, 14, 17	1·5 ± 0·31	5·0
$C_{22:5\omega3}$	7, 10, 13, 16, 19	3·0 ± 0·18	1·9
$C_{22:6\omega3}$	4, 7, 10, 13, 16, 19	0·26 ± 0·05	10·5
Total $\omega6$ acids	25		2·3
Total $\omega3$ acids	10		18

Table 2. *Linoleic acid and linolenic acid elongation products in muscle tissue from different ruminants*

Fatty acid assignment	Species ...	*Giraffa camelopardalis*	*Taurotragus oryx*	*Connochaetes taurinus*	*Kobus defassa*	*Acephalus buselaphus*	*Damaliscus korrigum*	*Bos taurus*
	Location ...	Nabiswa	Kelim	Sereri	Nabiswa	Kiryandongo	Karamoja	U.K.
		Percentage composition of extracted fatty acids						
$C_{18:2\omega6}$		27	23	18	21	21	24	7·6
$C_{18:3\omega3}$		2·4	4·0	3·2	4·0	6·0	5·0	2·1
$C_{20:2\omega6}$		0·9	0·2	0·1	0·1	0·09	0·1	0·5
$C_{20:3\omega3}$		0·1	0·03	—	—	—	0·05	—
$C_{20:3\omega6}$		0·8	0·1	0·2	0·1	0·09	0·1	0·7
$C_{20:4\omega6}$		7·4	6·0	5·0	6·6	4·0	7·2	3·2
$C_{20:4\omega3}$		0·4	0·6	0·09	0·1	Trace	0·1	0·03
$C_{20:5\omega3}$		2·1	1·0	1·05	1·2	0·9	1·2	0·9
$C_{22:4\omega6}$		0·06	Trace	Trace	0·2	0·08	0·03	—
$C_{22:4\omega3}$		Trace	Trace	0·07	—	0·09	—	—
$C_{22:5\omega6}$		0·08	Trace	0·1	—	—	—	—
$C_{22:5\omega3}$		4·1	3·2	2·6	1·2	3·0	2·7	0·6
$C_{22:6\omega3}$		0·4	0·7	0·5	0·1	0·1	0·3	0·1
Total $\omega6$ acids		36	29	23	28	25	31	12
Total $\omega3$ acids		9	9	7	7	10	9	4

clear that both linoleic acid and linolenic acid and their elongation products are present in muscle tissue. We have already shown (Gale *et al*, 1969; M. A. Crawford, M. M. Gale & M. H. Woodford, unpublished work) that only traces of the elongation products appear in adipose tissue. In muscle tissue the ratio of acids in the linoleic acid to those in the linolenic acid series is about 4:1 in the species studied here. Small amounts of eicosadienoic acid and eicosatrienoic acid appear. Separation on t.l.c. and rechromatography on diethylene glycol succinate suggests that these acids are predominantly those with a C_6 end chain.

Of the C_{20} acids, eicosatetraenoic acid was always the major constituent and again possessed a C_6 end chain. In contrast, C_{22} polyenoic acids were predominantly of C_3 end chains. As the eicosapentaenoic acid possessed a C_3 end chain and the major component of the C_{22} acids was the docosapentaenoic acid ($C_{22:5\omega3}$), it is evident that the acids with four double bonds are mostly in the linoleic acid series whereas those with five double bonds are in the linolenic acid series.

Table 2 shows that the distribution of the acids obeyed a similar pattern regardless of species, including *Bos taurus*. It was calculated that every gram of dry protein in meat from the wild animals described here will be accompanied by approx.

20mg. of linoleic acid, 5mg. of linolenic acid, 7mg. of eicosatetraenoic acid, 1–2mg. of eicosapentaenoic acid and 3mg. of docosapentaenoic acid.

It is difficult to analyse the polyenoic fatty acids in the muscle tissue of domestic cattle (*Bos taurus*) with any degree of accuracy because they are present in such small amounts relative to the other acids, mostly $C_{16:0}$, $C_{16:1}$, $C_{18:0}$ and $C_{18:1}$ (Hubbard & Pocklington, 1968; Gale *et al.* 1969; M. A. Crawford, M. M. Gale & M. H. Woodford, unpublished work). The sample analysed here was deliberately selected as unmarbled tissue with a low fat content of 3·6% to try to avoid this difficulty. It may not be representative, but it demonstrates the potential reversibility of the high-fat marbled state (M. A. Crawford, M. M. Gale & M. H. Woodford, unpublished work). In this sample of muscle the total polyenoic content was about half that of the wild ruminants on a protein basis. There appeared to be more eicosatrienoic acid and less eicosatetraenoic acid in the sample of *Bos taurus* compared with the wild animals.

DISCUSSION

As the African buffalo is a true ruminant it is unlikely to have access to polyenoic acids higher than $C_{18:2_{\omega 6}}$ and $C_{18:3_{\omega 3}}$ in its food. The source of polyenoic acids of C_{20} and longer chain length is most likely a result of animal metabolism. Of the parent C_{18} fatty acids the $C_{18:2_{\omega 6}}$ acid is the most abundant in the muscle tissue. We suggest that the source of $C_{18:2_{\omega 6}}$ acid could be seed material that might act as a capsule or, being rich in oil, provide a surface interphase, hence bypassing the hydrogenating influence of the rumen. *Syncerus caffer* will graze during the wet season but will increase its browsing habits in the dry season; it is also found in woodland habitats (Lamprey, 1963; Field, 1968; Crawford, 1968*a*,*b*). It will have access to seed material both from the grasses and the larger vegetation. The main fatty acid of grass pasture is the $C_{18:3_{\omega 3}}$ (Garton, 1960) whereas seed material is usually a rich source of $C_{18:2_{\omega 6}}$. It is noteworthy that acids in the $\omega 6$ series are more prevalent in the tissues of both buffalo and domestic ox than are those in the $\omega 3$ series.

As the C_{18} and C_{20} acids were predominantly of the $\omega 6$ series it might have been expected that the C_{22} acids would also have the same parental origin. This was not the case, as the major acid in this group was the $C_{22:5_{\omega 3}}$ acid. Only small amounts of $C_{22:4_{\omega 3}}$ acid and $C_{22:4_{\omega 6}}$ acid were apparent. No $C_{18:4_{\omega 3}}$ acid was detectable, but small amounts of $C_{20:4_{\omega 3}}$ acid appeared, followed by larger amounts of $C_{20:5_{\omega 3}}$ acid and then $C_{22:5_{\omega 3}}$ acid. This pattern was consistent in all buffalo and was also true in other ruminants studied here (Table 2).

The consistency of the polyenoic pattern among the different species studied is remarkable. In this context it is worth noting that, whereas linoleic acid is predominantly incorporated into elongation products with four double bonds, it is linolenic acid that is incorporated into products with five double bonds. Such a consistent finding, regardless of species so far studied, suggests that the acids of both series may be important for structural purposes.

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