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Comparison of the fatty acid component in structural lipids from dolphins, zebra and giraffe: possible evolutionary implications

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Essential fatty acid compositional data on the structural lipids of mammals which predominantly eat n-3 fatty acids suggests preferential incorporation of n-6 essential fatty acids.

The structural lipids of liver, muscle and brain of five species of dolphin (*Tursiops truncatus, Stenella attenuata, Steno bredanesis, Delphinus delphis* and *Lagenorhynchus obliquidens*), obtained from the wild, contained substantial amounts of arachidonic acid and other n-6 long chain derivatives of linoleic acid. The n-6 to n-3 ratio was approximately 1:1.

Data for two species of leaf-eating land mammals, the zebra, *Equus burchelli* Gray and giraffe, *Giraffa camelopardalis* L., indicate that tissue phosphoglycerides were dominated by n-6 fatty acids.

Contents

Introduction

Studies on the evolutionary variation of amino acid sequences in proteins have shown that it is largely confined to minor alterations of one or, at the most, a few codons (Nolan & Margolaish, 1968), and the variability in terms of the component proportions of essential amino acids has been found to be small (Crawford, Gale, Somers & Hansen, 1970). Cell structural lipids, with the exception of the brain, exhibit wide variations in the essential fatty acid (EFA) component of cell structural lipids in such a manner that a contribution to evolutionary differences has been suggested (Crawford & Sinclair, 1972; Crawford, Casperd & Sinclair, 1976). There are particularly wide differences in the fatty acids in membrane lipids of marine compared to land animals.

The marine food chain commences with the photosynthetic phytoplankton, having an n-6:n-3 ratio of approximately 1:6 (Ackman, 1982). It progresses through the zooplankton to the fish with

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the proportions of the long chain, more unsaturated members of the n-3 fatty acids increasing and resulting in substantial amounts of eicosapentaenoic (C20:5, n-3) and docosahexaenoic (C22:6, n-3) acids becoming available to the dolphins. The fish which have been studied have been found to have an n-6:n-3 balance of between 1:20 to 1:50 (Ackman, Eaton & Jangaard, 1965; Ackman, 1982), and have an essential requirement for n-3 but not n-6 fatty acids for reproduction and growth (Nicolaides & Woodall, 1962; Cowey, 1976; Fujii & Yone, 1976; Yu & Sinnhuber, 1979).

We previously reported surprisingly large amounts of n-6 fatty acids in the tissues of two bottlenose dolphins (*T. truncatus*) which died in captivity, but could not know if this was natural or pathological (Williams, Davidson, Stevens & Crawford, 1977; Williams, 1981). Similarly, we had observed substantial amounts of linoleic (18:2, n-6) acid in the ethanolamine phosphoglyceride (EPG) from a zebra liver previously obtained from Karamoja, Uganda (Crawford *et al.*, 1976). The zebra is a grass-(n-3 rich)-eating animal. This evidence suggests that a powerful selection mechanism might be operating in the process of cell membrane construction in favour of the n-6 fatty acids in these mammals.

The terrestrial food chain, similar to the marine system, starts with photosynthesis in green leaves, in which the n-3 fatty acids dominate, mainly represented by the parent alpha-linolenic acid (18:3, n-3) (Hitchcock & Nichols, 1971). However, the largest concentrations of plant lipid are found stored in seeds which provide mainly linoleic acid (18:2, n-6). Land mammals have a nutritional requirement for n-6 essential fatty acids both for structural lipid and prostaglandin synthesis (Burr & Burr, 1930; Fiennes, Sinclair & Crawford, 1973; Holman, 1977; Crawford, 1983). In a study of 32 land mammals, the balance between the n-6 and n-3 fatty acids was found to be between 3:1 and 6:1 (Crawford *et al.*, 1976).

Evidence indicates that the Cetacea evolved on land and migrated to the marine environment (Gingerich, Wells, Russell & Ibrahim Shah, 1983). Marine lipids are generally considered to be rich in n-3 essential fatty acids. However, the land food chain is thought of as an n-6 rich source due to the high oil and linoleic acid content of seeds and the dominance of n-6 fatty acids in tissues of land mammals (Crawford *et al.*, 1976). The differential availabilities of n-6 and n-3 fatty acids in land and marine plants offers an interesting opportunity to establish whether the dolphins have developed a marine type nutritional system like that of the fishes or have retained a dependence on the land-based chemistry of their ancestors.

Published data on adipose fat from the fin whale, *Balaenoptera physalus*, (Ackman *et al.*, 1965) reflected its n-3 rich dietary fatty acids; however, the adipose fat would be expected to have a different fatty acid composition compared to the lipids of active tissues. We have taken the opportunity of comparing several species of dolphins with two land-based mammals which similarly eat an n-3 rich diet, the zebra (*E. burchelli* G.) which is a monogastric grazer and the giraffe (*G. camelopardalis* L.) representing the ruminant browsers.

Methods

Source of animals

Dolphin samples obtained from the Southwest Fisheries Center, California, were killed incident to tuna-seining in the Eastern Tropical Pacific (ETP). Five species of dolphin (*Tursiops truncatus*, *Stenella attenuata*, *Steno bredanensis*, *Delphinus delphis* and *Lagenorhynchus obliquidens*) were obtained from the wild in this manner.

The animals were chilled immediately after death and the organs stored at −20 °C until analysed. Two animals, one *T. truncatus* and one *L. obliquidens*, were the result of voluntary beaching; these animals were collected and frozen as quickly as possible. The adipose tissue, liver and muscle lipids of 14 dolphins from the 5 species and 2 brains from freshly caught dolphins (*T. truncatus*) were analysed for their fatty acid content. Although the habitats and the diets between the species will be different, for the purpose of this communication we have grouped the data from the 5 species. The individual species data will be the subject of a separate paper to be published elsewhere.

The liver, muscle and stomach contents from the zebra and giraffe were obtained from Karamoja, Uganda through the collaboration of the Uganda Game Department, Entebbe and Makerere University College, Kampala, as described previously (Crawford *et al.*, 1976). Tissues were excised within as short a time as possible after death during a cropping programme; this usually meant within 2–3 minutes. The tissues were frozen in dry ice and thereafter kept at −15 °C until being flown to London packed in dry ice. Because the results suggest a general n-6 selection principle, we have reported information on the capuchins (*Cebus capuchin*) which have been studied (Fiennes, Sinclair & Crawford, 1973). Recent data from the koala (*Phascolarctos cinereus*) and the grey kangaroo (*Macropus gigantius*) from eastern Australia (Naughton, 1981) have also been included (Table V).

Lipid extraction and fatty acid analysis

Total lipids were extracted from the tissues by the method of Folch, Lees & Stanley (1957). After solvent extraction (chloroform/methanol), the phosphoglycerides were separated from the tissue lipids and triglycerides from adipose tissue by thin-layer chromatography (TLC). Identification was made by developing alongside, known standards (Sigma Chemical Co. Ltd., Poole, Dorset, UK) using methods described previously (Sinclair, 1975). The fatty acids from the individual phosphoglyceride and triglyceride fractions were converted to methyl esters by transmethylation and determined by gas liquid chromatography as previously described (Sinclair & Crawford, 1972). Quantitation of the individual fatty acids was accomplished using an Infotronics 308 automatic digital integrator. A Pye Unicam series 104 gas chromatograph equipped with dual flame ionization detectors was used throughout. The column (1·5 M × 7 mm I.D.) was packed with 10% polyethylene-glycol adipate on Chromosorb W (60–70 mesh). The oven temperature was maintained at 190 °C and the carrier gas (nitrogen) flow was 40 ml per minute.

Results

Dolphin lipids

The dolphin results are expressed as a weight percentage of the total fatty acids with a chain length from C14–C22 carbons to facilitate comparison with the published data on land mammals. The skeletal muscle cholinephosphoglyceride (CPG) fatty acids of 14 dolphins are represented as the mean and standard error of the mean (SEM) (Table I). The mean and the SEM of the liver CPG fatty acids are also reported (Table II).

The fatty acid analysis of 14 wild dolphins confirmed our observations on captive *Tursiops truncatus* (Williams *et al.*, 1977) that a high proportion of n-6 fatty acids is natural to these marine mammals. The level of arachidonic acid (20:4, n-6) was found to be higher in the tissues of wild dolphins than in comparable tissue of dolphins which had died in captivity. The liver and muscle phosphoglycerides were especially rich in arachidonic acid and there were also significant amounts of the docosatetraenoic acid (22:4, n-6) in the ethanolamine phosphoglycerides (EPG).

TABLE I

Mean (weight %) plus or minus standard error (S.E.) of the fatty acids from skeletal muscle CPG from 14 wild dolphins of mixed species, EPG from two Tursiops truncatus and adipose tissue triglyceride TG fatty acids from three T. truncatus

Fatty acid	CPG $n=14$ mean	S.E.	EPG $n=2$	TG $n=3$
14:0	0·69	0·18	0·36	6·9
16:0	15·8	1·33	6·55	11·4
18:0	12·5	1·33	19·5	1·4
14:1	0·26	0·06	0·09	—
16:1	1·79	0·56	0·98	14·7
18:1	21·7	1·17	16·1	24·7
22:1	0·21	0·1	0·25	10·9
18:2, n-6	1·17	0·13	0·23	0·5
20:3, n-6	0·4	0·15	0·14	0·3
20:4, n-6	14·2	1·1	12·8	0·6
22:4, n-6	0·88	0·9	1·55	0·3
22:5, n-6	0·43	0·1	0·26	0·4
18:3, n-3	0·3	0·12	0·32	0·7
20:4, n-3	0·2	0·06	0·19	—
20:5, n-3	6·4	0·9	2·92	2·2
22:5, n-3	1·2	0·12	1·25	1·8
22:6, n-3	7·6	0·6	8·84	9·2
Tot. sat.	30·3	1·9	26	20·0
Tot. mono.	29·5	2·1	18	50·3
Tot. PUFA	34·8	2·3	29	17·0
Tot. n-3	16·3	1·6	13	13·2
Tot. n-6	18·6	1·2	15	2·1
n-6:n-3	1·14	0·1	1·1	0·16
P/S ratio	1·1	0·1	1·1	0·9

The n-6:n-3 ratio in the tissues of dolphins was found to be about 1:1 to 2:1, which is closer to the ratio found in the tissues of land mammals than that reported for fish. Arachidonic acid (20:4, n-6) was quantitatively the most important of the n-6 fatty acids in the dolphins' tissue. The mean value for liver cholinephosphoglyceride linoleic acid (18:2, n-6) for the 14 wild animals was 1·2%.

Comparative data from zebra and giraffe

The previous data which we published on a variety of land animal lipids described the ethanolamine phosphoglyceride fatty acids. The data on the choline phosphoglycerides indicated that they generally held high proportions of linoleic but relatively lower amounts of arachidonic acid by comparison with the ethanolamine phosphoglycerides (Crawford *et al.*, 1976). In this respect, the differences between the two phosphoglyceride fractions were not especially marked in the zebra but were in the giraffe (Tables III and IV).

Tables III and IV present data from adipose tissue, liver, muscle and brain of two species of land mammals for comparison. Only the data for the main essential fatty acids are represented as these

TABLE II

Mean (weight %) plus or minus standard error (S.E.) from liver tissue CPG from 14 wild dolphins of mixed species and EPG from four ***T. truncatus***

Fatty acid	CPG ($n = 14$)	S.E.	EPG ($n = 4$)
16:0	18·0	1·1	5·2
18:0	22·9	1·2	29·7
16:1	3·3	0·6	1·88
18:1	20·0	1·0	14·5
22:1	0·1	0·01	0·2
24:1	0·3	0·01	0·4
18:2, n-6	1·2	0·13	0·94
20:3, n-6	0·6	0·07	0·54
20:4, n-6	10·6	1·2	16·8
22:4, n-6	2·1	0·4	2·6
22:5, n-6	0·85	0·1	0·9
18:3, n-3	0·8	0·3	0·22
20:5, n-3	2·9	0·3	3·67
22:5, n-3	1·4	0·2	3·55
22:6, n-3	5·4	0·9	11·2
Tot. sat.	46·4	1·8	35
Tot. mono.	26·4	0·8	17
PUFA	23·7	2·1	40
Tot. n-3	10·7	1·2	19
Tot. n-6	15·4	1·3	22
n-6:n-3	1·4	0·1	1·2
P/S ratio	0·5	0·1	1·1

illustrate the principal points. As with the dolphins, the adipose tissue fatty acids bore little resemblance to that found in the cell membrane phosphoglycerides. Zebra adipose tissue had an n-6:n-3 ratio in favour of the n-3 fatty acids (1:5·8), but the cell membrane fatty acids were in favour of the n-6 family. The zebra phosphoglycerides contained the highest levels of linoleic acid so far recorded (Crawford *et al.*, 1976). The intake of large amounts of n-3 fatty acids in the diet, and the reflection of this in the adipose tissue of the zebra, bore no significance to the level of this essential fatty acid in the structural lipids.

The giraffe was selected as a ruminant in which a high proportion of the dietary essential fatty acids would be hydrogenated in the rumen. This was reflected in a low content of n-6 essential fatty acids in the adipose tissue but substantial amounts in the structural lipids.

Because of the extreme contrast of the dolphins' dietary lipids, one might have expected any significant response of mammalian brain lipids to diet to be observed in this species; however, this was not the case. Despite the differences in adipose and liver fatty acids in the species described, the composition of the brain choline and ethanolamine phosphoglycerides of the different species was similar (Table IV). Although large proportions of linoleic acid was detected in the liver phosphoglycerides of the zebra, there was little of this essential fatty acid in the brain. In the giraffe and the dolphin, the profile was similar, with arachidonic acid (20:4, n-6) and docosatetraenoic acid (22:4, n-6) being the principal metabolites of linoleic acid (18:2, n-6) and docosahexaenoic acid (22:6, n-3) being the main derivative of alpha-linolenic acid (18:3, n-3).

TABLE IIIa

*Mean (weight %) plus or minus standard error (S.E.) of the essential fatty acids from liver CPG and EPG and adipose tissue from two large land mammals, zebra (**Equus burchelli**) and giraffe (**Giraffa camelopardalis**)*

	Zebra			Giraffe		
	Liver		Adipose tissue	Liver		Adipose tissue
	CPG	EPG		CPG	EPG	
18:2, n-6	42·2	31·6	7·88	25·4	9·69	3·67
	(2·9)	(3·0)	(3·0)	(2·5)	(3·3)	(0·5)
20:2, n-6	0·22	0·10	0·04	0·36	0·28	0·20
	(0·05)	(0·06)	(0·01)	(0·09)	(0·05)	(0·02)
20:3, n-6	0·63	0·29	0·11	0·89	0·30	0·10
	(0·09)	(0·08)	(0·01)	(0·09)	(0·04)	(0·06)
20:4, n-6	4·10	4·83	0·02	8·33	14·2	0·11
	(0·11)	(0·09)	(0·08)	(0·10)	(0·22)	(0·03)
22:4, n-6	0·12	0·22	—	0·86	2·12	—
	(0·05)	(0·05)	—	(0·04)	(0·03)	—
22:5, n-6	0·02	0·06	—	0·22	1·19	—
	(0·01)	(0·02)	—	(0·06)	(0·09)	—
Total n-6	47·3	37·1	8·0	36·1	27·8	4·1
18:3, n-3	3·44	1·65	47·5	3·70	2·11	2·30
	(0·87)	(0·25)	(5·22)	(0·55)	(0·31)	(0·19)
20:5, n-3	0·33	0·82	0·03	0·44	0·12	0·02
	(0·01)	(0·03)	(0·01)	(0·05)	(0·03)	(0·01)
22:5, n-3	1·22	2·21	—	1·82	9·34	—
	(0·20)	(0·24)	—	(0·94)	(0·75)	—
22:6, n-3	0·34	0·46	—	0·35	1·45	—
	(0·04)	(0·06)	—	(0·04)	(0·08)	—
Total n-3	5·33	5·14	47·5	6·31	13·0	2·32
Ratio n-6:n-3	8·9	7·2	0·17	5·7	2·1	1·8

Data on the chemical composition of East African grasses and browse materials have been published (Dougall, Drysdale & Glover, 1964). There are few published data on the lipid chemistry of these plants and such a study would be a substantial undertaking. To obtain an index of average food intakes, the stomach contents were analysed from the giraffe and the zebra. It should be accepted that these data only refer to single time points. The animals were obtained in December and in June, examples of the dry and the end of the rainy season. There was approximately 35% more linoleic acid (18:2, n-6) in the stomach contents of three zebra taken in the dry season compared to three zebra in the wet season but the sample numbers were too small for a statistical comparison. For the purpose of this analysis, the data have been pooled.

Preliminary analysis of lipids from plants commonly eaten by zebra and giraffe indicate that the leaf and seed material of the plants studied had the expected composition. The seeds from the Acacia (*Acacia sinagalensis*) varieties contained significant amounts (5–18%) of alpha-linolenic acid (18:3, n-3), whilst the balenites (*Balenites aegyptiaca*), which has a very hard protective shell, had a high proportion (70%) of its fatty acids as linoleic acid and only 0·2% alpha-linolenic acid. Examination of the stomach contents of giraffe demonstrated that approximately one-third of the balenite kernels were broken open, presumably by mastication, but the remainder were intact and would have passed through the animal.

TABLE IIIb

*Mean (weight %) plus or minus standard error (S.E.) of the essential fatty acids from muscle CPG and EPG from two large land mammals, zebra (**Equus burchelli**) and giraffe (**Giraffa camelopardalis**)*

	Zebra		Giraffe	
	CPG	EPG	CPG	EPG
18:2, n-6	40·2	31·6	24·4	11·3
	(3·6)	(3·4)	(2·1)	(1·8)
20:2, n-6	0·42	0·22	0·31	0·11
	(0·06)	(0·02)	(0·04)	(0·03)
20:3, n-6	2·14	1·90	2·66	1·30
	(0·15)	(0·16)	(0·23)	(0·22)
20:4, n-6	3·00	4·51	7·41	12·8
	(0·40)	(0·39)	(0·85)	(1·05)
22:4, n-6	0·10	1·10	0·55	3·84
	(0·02)	(0·08)	(0·03)	(0·18)
22:5, n-6	0·02	0·27	0·32	1·24
	(0·01)	(0·03)	(0·04)	(0·11)
Total n-6	45·9	39·6	35·6	30·6
18:3, n-3	3·67	2·88	3·45	2·74
	(0·65)	(0·27)	(0·35)	(0·30)
20:5, n-3	1·54	1·32	1·39	0·75
	(0·20)	(0·09)	(0·14)	(0·04)
22:5, n-3	1·20	2·25	4·52	7·84
	(0·22)	(0·21)	(0·51)	(0·66)
22:6, n-3	0·23	0·58	0·25	1·26
	(0·02)	(0·03)	(0·02)	(0·02)
Total n-3	6·64	7·03	9·61	12·6
Ratio n-6:n-3	6·91	5·6	3·7	2·4

Discussion

The data demonstrate that, despite the high proportion of n-3 fatty acids in the marine environment, the free-living dolphins have remarkably high concentrations of arachidonic and other n-6 fatty acids in their tissue or membrane phosphoglycerides. In this respect, they are similar to land mammals, where the balance between the n-6 and n-3 families of fatty acids in membrane systems (Crawford *et al.*, 1976) is distinctly different from that of fish (Ackman, 1982). The high proportion of arachidonic acid is of particular interest as it is a principle precursor for the n-6 series of eicosanoids synthesized by land mammals.

The high levels of arachidonic acid and other long chain n-6 fatty acids in wild-caught dolphins raises the question as to their source. The sea-weed, *Chrondrus crispis*, contains 24% arachidonic acid (Ackman, 1982) and Sargent (1976) has reported at least one phytoplankton to contain similarly high proportions of arachidonic acid which, when consumed by the zooplankton, would enter the food chain. Squid and perhaps other cephalopods could provide significant amounts of arachidonic acid as they have been estimated to represent about 30% of the diet of some species of dolphin in the wild (Fitch & Brownell, 1968). Lilly & Bottino (1981) have identified and characterized arachidonic acid in the muscle and organs of the Gulf of Mexico shrimp (*Penaeus*

TABLE IV

*Mean (weight %) plus or minus standard error (S.E.) of the essential fatty acids from zebra (n = 6) (**Equus burchelli**) and giraffe (**Giraffa camelopardalis**) (n = 6) brain EPG and CPG compared with dolphin brain (**Tursiops truncatus** n = 2) phosphoglycerides*

	Zebra		Giraffe		Dolphin	
	CPG	EPG	CPG	EPG	CPG	EPG
18:2, *n*-6	1·22	0·14	1·18	1·07	1·8	0·2
	(0·22)	(0·01)	(0·2)	(0·03)		
20:2, *n*-6	0·11	0·10	0·25	0·23	0·1	0·1
	(0·02)	(0·02)	(0·06)	(0·04)		
20:3, *n*-6	1·15	0·89	0·55	0·40	0·1	0·1
	(0·07)	(0·09)	(0·04)	(0·04)		
20:4, *n*-6	5·33	10·6	6·01	13·2	4·5	8·6
	(0·62)	(2·06)	(0·10)	(1·88)		
22:4, *n*-6	1·15	4·28	1·43	5·12	0·8	2·7
	(0·65)	(0·76)	(0·55)	(0·73)		
22:5, *n*-6	0·84	2·11	0·52	2·09	0·4	1·3
	(0·08)	(0·02)	(0·07)	(0·1)		
Total *n*-6	9·8	19·1	8·9	22·1	7·7	13·0
18:3, *n*-3	1·14	1·55	0·80	0·41	0·4	0·3
	(0·23)	(0·25)	(0·05)	(0·06)		
20:5, *n*-3	0·24	0·26	0·14	0·13	0·2	0·8
	(0·01)	(0·03)	(0·04)	(0·04)		
22:5, *n*-3	1·94	0·95	1·82	0·61	0·9	1·5
	(0·32)	(0·08)	(0·94)	(0·08)		
22:6, *n*-3	3·84	19·4	4·59	22·4	4·3	29·0
	(0·26)	(0·06)	(0·04)	(2·01)		
Total *n*-3	7·16	22·2	7·35	23·5	5·8	31·6
Ratio *n*-6:*n*-3	1·4:1	1:1·2	1·3:1	1:1	1·3:1	1:2·4

setiferus). They also report that the arachidonic acid found would probably have been obtained via the diet. This data suggests a significant source in specialized aspects of the marine food chain, probably with coastal or shallow water origins. High levels of arachidonic acid (20:4, n-6) have also been reported in fish obtained from Australia (Sinclair *et al.*, 1983), suggesting a temperature effect. We have made similar observations on the liver oils of fish caught off the Tanzanian coast (Munhambo & Crawford, unpubl.). Most of the general data on fish lipids comes from cold water regions where the most highly unsaturated fatty acids (i.e. docosahexaenoic acid 22:6, n-3) become concentrated in the food chain. In warmer waters, this requirement for highly unsaturated n-3 fatty acids might be diminished.

The data on the zebra and dolphins illustrate the same principle for selection of n-6 fatty acids against an opposite concentration gradient. It is interesting that the balance of n-6:n-3 fatty acids in the adipose tissue of the zebra (0·17) was of the same order as that of the dolphin (0·22): the difference was in the chain length. The zebra n-3 fatty acid content was mainly alpha-linolenic acid, whereas in the case of the dolphin, the n-3 component consisted of 20 and 22 carbon chain-length fatty acids.

Certain seeds, such as those of balenites and the acacia, would provide larger quantities of oil and of linoleic acid than would be available from grass seeds. None the less, the giraffe

TABLE V

The ratio of n-6 to n-3 fatty acids determined in the stomach content, adipose fat, liver and brain phosphoglycerides of five land mammals and five species of dolphin. The data on koala and kangaroos were obtained from Naughton (1981), and that of the other species from our own study

Origin	Capuchin	Koala	Zebra	Giraffe	Grey kangaroo	Dolphin
Stomach	6:1	1:3	1:4	1:2	1:1	1:40
Fat	4:1	1:1·7	1:5·8	1·8:1	2:1	1:4·8
Liver						
CPG	5:1	13:1	8·9:1	5·7:1	2:1	1·1:1
EPG	2·1:1	9:1	7·2:1	2·1:1	3:1	1·2:1
Brain						
CPG	1·2:1	1·7:1	1·4:1	1·3:1	1·4:1	1·3:1
EPG	1:1	1:1	1:1·2	1:1	—	1:2·4

phosphoglycerides contained less linoleic and more arachidonic acid than those of the zebra. Despite the fact that the giraffe is a ruminant and eats green leaves, its tissue lipids were still dominated by the n-6 essential fatty acids. Naughton's (1981) information on leaf-eating marsupials (Table V) is of interest because it is the first data on marsupials and illustrates a similar selective phenomenon in favour of n-6 fatty acids, despite an n-3 rich food structure.

The evidence in the literature suggests that fish require n-3 fatty acids (Nicolaides & Woodall, 1962; Cowey, 1976; Fujii & Yone, 1976; Yu & Sinnhuber, 1979) and that mammals require n-6 fatty acids. It is interesting that a concentrated source of linoleic acid was added to the land food chain following the evolution of flowering and seed-bearing plants toward the end of the Cretaceous period. Mammals evolved a requirement for linoleic acid and its derivatives and we found no evidence to suggest that dolphins need have lost that requirement. Evolution of the fishes would assumedly have taken place in the presence of a preponderance of the n-3 fatty acids: linoleic acid and its derivatives would have been relatively scarce during the earlier evolution of the fishes.

The zebra data offered no evidence that this species had adapted to an n-3 biology like the fish. Despite eating n-3 rich food, the n-6 fatty acids were found to dominate the tissue structural lipids. Naughton's (1981) data on the koala similarly suggests that dependence on n-6 fatty acids might have been established before the marsupials diverged from the eutherians.

Our data imply that dolphins are similar to land mammals in respect of their structural lipids. Hence, it is possible that dolphins may have a similar requirement for n-6 fatty acids and their eicosanoid derivatives, which are known to be associated with mammalian reproduction (Karim & Hillier, 1973) and regulation of several biological functions (Bunting, Moncada & Vane, 1983). It has been reported that alpha-linolenic acid (18:3, n-3) in high dietary amounts suppresses the metabolism of linoleic acid (18:2, n-6) (Holman, 1977), which makes it all the more surprising to find high concentrations of n-6 fatty acids in the dolphin structural lipids. We suggest that dolphins have adapted a specialized mechanism for concentrating the n-6 fatty acids; this could be through preferential selection or differences in rates of utilization. Similar mechanisms enable the rat to hold on tenaciously to small amounts of dietary n-3 fatty acids (Tinoco, Williams, Hincenbergs & Lyman, 1971) and differences in the efficiency of incorporation of individual essential fatty acids have been reported (Sinclair & Crawford, 1972). There are also species differences in essential fatty acid metabolism (Rivers, Sinclair & Crawford, 1975; Cunnane, Keeling, Thompson & Crawford, 1984). These mechanisms, combined with behavioural selection of foods containing significant amounts of n-6 fatty acids, could explain the high proportions of arachidonic acid in dolphin tissue lipids.

The data suggests a consistent mammalian preference of n-6 essential fatty acids in the phosphoglycerides of structural tissue. The conclusion that there might be specific behavioural and biochemical selection for fatty acid types disposes of the conventional view that fats simply reflect the diet of an animal. This generalization may contain some truth for adipose tissue but not for cell membrane structural lipids. The contrasting food and tissue data in the giraffe, zebra and other species reported here suggest that the selection of n-6 fatty acids for structural purposes may be a general rule for mammalian species.

The fact that dolphins do not appear to have changed their allegiance to n-6 fatty acids on migration to the sea is of interest to evolutionary theory. We established that the lion, *Panthera leo* L. and the cat, *Felis catus* L. lacked significant, delta-6-desaturase (Rivers *et al.*, 1975; Rivers *et al.*, 1976; Frankel & Rivers, 1978; Frankel, 1980), which is the first step in the synthesis of arachidonic acid from linoleic acid. The animal focuses on preformed arachidonic acid from its diet rather than the parent n-6 fatty acid, linoleic acid and also requires preformed vitamin A as opposed to its vegetable precursor (Scott, 1968). This dependence on preformed derivatives could make it difficult for the cat to change evolutionary direction to a herbivorous diet. The data on dolphins offers similar evidence, suggesting that once locked in to a set of nutrient requirements, it is difficult to change.

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