Essential fatty acid metabolism in the feline: relationship between liver and brain production of long-chain polyunsaturated fatty acids

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Abstract A comparison was made between the liver and brain conversion of linoleic acid, 18:2n-6, and linolenic acid, 18:3n-3, to long chain polyunsaturated fatty acids in domestic felines. This report demonstrates that 6-desaturase activity does exist in the feline. The liver produced deuterium-labeled polyunsaturated fatty acids up to 22:4n-6 and 22:5n-3. The brain was found to accumulate the deuterium-labeled polyunsaturated fatty acids, 22:5n-6, 22:6n-3, 24:4n-6, 24:5n-6, 24:5n-3, and 24:6n-3. Adult felines were provided a diet consisting of either 10% fat (hydrogenated coconut oil-corn oil 9:1) containing no 20- or 22-carbon n-6 or n-3 fatty acids or a chow diet with meat and meat by-products that contained these long chain polyunsaturated fatty acids for a 6-month period. During this time, the in vivo production of long chain polyunsaturated fatty acids was evaluated in these animals. The cats were given oral doses of both [17,17,18,18,18-^{2}H]18:3n-3 and [9,10,12,13-^{2}H]18:2n-6 and the deuterium-labeled fatty acid metabolites were measured in the blood, liver, and brain using a highly sensitive and specific gas chromatography-mass spectrometry technique. Contrary to previous claims, 6-desaturase activity was shown to exist in the feline. The evidence for this was the detection of [9,10,12,13-^{2}H] 18:3n-6 which was converted from [9,10,12,13-^{2}H]18:2n-6 and observed in the plasma. For the first time, direct evidence for the metabolism of n-3 fatty acids in cats was obtained by the detection of deuterium-labeled metabolites including the polyunsaturated fatty acid, 22:5n-3, in the plasma, following an oral dose of deuterium-labeled 18:3n-3. The more highly unsaturated deuterium-labeled 22- and 24-carbon fatty acids including: 22:6n-3, 24:5n-3, 24:6n-3, 22:5n-6, 24:4n-6, and 24:5n-6 accumulated in the nervous system. These deuterium-labeled fatty acids were not detected in either the liver or plasma. As the liver was found to produce and export into the blood the deuterium-labeled 22:5n-3 and 22:4n-6, it is suggested that these intermediates are then transported to the brain and retina where they are converted to 22:6n-3 and 22:5n-6, respectively. This route for the accretion of 22:6n-3 in the nervous system has not been previously proposed. In the feline, it appears that both the liver and the brain are involved in biosynthesizing long-chain polyunsaturated fatty acids when no preformed 20- and 22-carbon essential fatty acids are present in the diet.—**Pawlosky, R., A. Barnes, and N. Salem, Jr.** Essential fatty acid metabolism in the feline: relationship between liver and brain production of long-chain polyunsaturated fatty acids. *J. Lipid Res.* 1994. **35:** 2032-2040.

Supplementary key words domestic felines • docosahexaenoic acid • arachidonic acid • deuterium-labeled linoleic and linolenic acids • GC-MS • desaturation

In 1975, Rivers, Sinclair, and Crawford (1) observed that cats developed clinical signs of essential fatty acid (EFA) deficiency and expressed low levels of arachidonic acid, 20:4n-6, in their plasma phospholipids when fed purified diets containing safflower oil as the fat source. This led to the view that carnivores were incapable of synthesizing 20:4n-6 from 18:2n-6 (1). An apparent lack of 6-desaturase in cats was suggested based on evidence that no desaturated products could be found in plasma when radiolabeled 18:2n-6 was given orally (2). However, 5-desaturase activity was implied when it was noted that 20:4n-6 increased in erythrocyte membranes in felines that were given a diet supplemented with 18:3n-6. Research carried out by MacDonald, Rogers, and Morris (3) showed that the level of 20:3n-9 increased in the plasma and in erythrocyte membranes in felines that were maintained on a diet containing hydrogenated beef tallow as the source of fat. This indicated that felines were capable of synthesizing a portion of their polyunsaturated fatty acids from 18-carbon precursors (3).

The view that most long-chain polyunsaturated fatty acids are made in the liver of mammals and transported to other organs has been well documented. However, it remains uncertain to what degree the brain produces its own docosahexaenoic acid, 22:6n-3. Although Cook (4) showed that 6-desaturase activity was present in the neonatal rat brain using an in vitro assay, Moore, Yoder,

Abbreviations: CO, corn oil; HCO, hydrogenated corn oil; EFA, essential fatty acid; NCI, negative chemical ionization; PFB, pentafluorobenzyl; ME, methyl ester; GC-MS, gas chromatography-mass spectrometry.

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and Spector (5) observed that cultured endothelial cells from rat brain microvessels desaturated fatty acids. Several studies by Chen et al. (6), Wetzel et al. (7), and Stinson, Weigand, and Anderson (8) have shown that the retina has the capability to synthesize 22:6n-3 from 18:3n-3, but the degree to which adult mammals synthesize polyunsaturated fatty acids in the brain in vivo is unknown.

This report gives evidence of essential fatty acid metabolism in the feline liver and brain using a highly sensitive and specific gas chromatography-mass spectrometry technique with deuterium-labeled compounds. Animals that were maintained on diets that did not contain long-chain essential fatty acids converted deuterium-labeled 18:2n-6 and 18:3n-3 to desaturated products that were detected in the blood, liver, and brain. Desaturation of 18:2n-6 to 18:3n-6 via a 6-desaturase was clearly demonstrated in felines when they were required to do so because the diet was lacking in long-chain polyunsaturates. It was further demonstrated that felines were able to convert 18:3n-3 to long-chain polyunsaturates in the liver.

TABLE 1. Fatty acid composition of commercial cat food (chow diet) and a diet composed of 9% hydrogenated coconut oil with 1% corn oil (HCO/CO diet)

Fatty Acids	Chow Diet	HCO/CO
Nonessential fatty acids		
12:0	1.5	46.0
14:0	1.1	14.8
16:0	21.4	9.2
16:1	3.9	-
18:0	9.6	6.3
18:1	40.5	4.8
N-6 polyunsaturated fatty acids		
18:2	17.1	5.8
18:3	0.11	0.02
20:3	0.10	-
20:4	0.40	-
22:4	0.11	-
22:5	0.02	-
N-3 polyunsaturated fatty acids		
18:3	0.70	0.09
20:5	0.03	-
22:5	0.06	-
22:6	0.05	-

Data are expressed as weight percent of fatty acids as determined by capillary gas chromatography. A blank indicates that the fatty acids were not detected or were less than 0.01% of the total fatty acids.

MATERIALS AND METHODS

Materials

A standard, nutritionally balanced feline diet was obtained from ICN Biochemical (Cleveland, OH) and fed to four animals. The fat content of the diet consisted of hydrogenated coconut oil (9 wt%) and corn oil (1 wt%) (designated as HCO/CO diet). A separate group of animals (n = 3) was maintained on commercial cat food (Purina cat chow, Formula One) (designated as Chow diet). The fat composition of both diets is given in **Table 1.** Two-year-old, male, domestic short hair felines weighing 4-6 kg were obtained from Liberty Research Inc. (Waverly, NY). The deuterium-labeled fatty acid ethyl ester, [17,17,18,18,18-^{2}H]18:3n-3, was obtained from Cambridge Isotope Labs, (Andover, MA) and [9,10,12,13-^{2}H]18:2n-6 ethyl ester was from Medical Isotopes (Concord, NH). The chemical purity of the fatty acids were 97% or greater as determined by GC-FID (flame ionization detection) analysis. Minor impurities were identified by GC-MS analyses. The impurities consisted of small amounts of the deuterium-labeled *trans* isomers (the level of any single impurity was less than 1.2% of the major component) and methyl esters of the principle labeled fatty acids.

Animal care and sample collection

Animals were maintained on their respective diets for 2 months. They were then fasted overnight and given a 100 mg oral dose of each deuterium-labeled fatty acid in a gelatin capsule prior to the morning feeding. After dosing, 2 ml of blood from the jugular vein was collected at 0, 8, 24, 48, 72, 96, 168, 196, and 244 h. The animals were maintained on these diets for an additional 4 months and then given 10 mg each of [17,17,18,18,18-^{2}H]18:3n-3 and [9,10,12,13-^{2}H]18:2n-6 in capsules once per day for 10 days. Blood was collected from the jugular vein at 48, 72, 96 h and at termination. On the tenth day, the animals were killed with a lethal injection of sodium pentobarbital and the liver and brains were removed.

The plasma was separated from the red blood cells by centrifuging at 5000 *g* and then transferred to 13 × 100 mm glass screw-cap test tubes. The plasma lipids were extracted into chloroform using the Bligh and Dyer total lipid extraction method (9). One half of the chloroform layer (approximately 0.5 ml) was transferred to 13 × 100 mm glass screw-cap test tubes and evaporated under a nitrogen stream. The residue was dissolved in 1 ml of a solution of 10% KOH in methanol and heated for 1 h at 75°C to saponify the lipids. The samples were acidified with 12 N HCl to a pH of 1 and lipids were extracted twice with 3 ml of hexane. The hexane extracts were transferred to half-dram screw-cap vials and the solvent was evaporated under a stream of nitrogen. Seventy μl of a pentafluorobenzyl derivatizing reagent (acetonitrile-diisopropylamine-pentafluorobenzyl bromide; 1000:100:1 (v/v)) was added to the vials and heated to 65°C for 12 min. The excess reagent was evaporated under nitrogen and the samples were redissolved in 100 μl of hexane.

TABLE 2. Fatty acid composition of cat plasma after 6 months maintenance on a commercial food (Chow diet) or a diet containing 1% corn oil/9% hydrogenated coconut oil (HCO/CO diet) as the fat source

Fatty Acids	Chow Diet (n = 3)	HCO/CO Diet (n = 4)
Nonessential fatty acids		
14:0	0.3	0.5
16:0	12.4	13.2
16:1	3.9	5.1
18:0	20.6	17.3
18:1	20.0	24.0
N-6 polyunsaturated fatty acids		
18:2	36.4	25.1
18:3	0.06	0.4
20:3	0.7	0.2
20:4	4.1	1.8
22:4	0.35	0.1
22:5	0.30	-
N-3 polyunsaturated fatty acids		
18:3	0.7	0.10
20:5	0.34	0.05
22:5	0.35	0.02
22:6	0.41	0.12

Data are expressed as weight percent of fatty acids as determined by capillary gas chromatography. A blank indicates that the fatty acids were not detected or were less than 0.01% of the total fatty acids.

Two hundred mg each of liver and brain tissue were homogenized in 1 ml of water using a ground-glass hand homogenizer. The tissues were extracted using the Bligh and Dyer method (9) and samples were derivatized as described above.

One half of the sample extracts was used to determine fatty acid composition. The lipids were converted to their methyl esters following the procedure of Morrison and Smith (10) and analyzed using a Hewlett-Packard 5890 GC with flame ionization detection as previously described (11). One hundred nanograms of 23:0 was added to the samples to quantify the fatty acids.

GC-MS samples were analyzed on a Hewlett-Packard 5989 GC-MS in the negative ion mode using methane as the reagent gas as previously described (12). The PFB derivatives were injected using the splitless technique on a 30 m × 0.25 mm FFAP capillary column (J & W Scientific, Las Palmas, CA) using an oven temperature program of 80° to 185°C at 20°C/min followed by heating to 240°C at 10°C/min. Selected ion monitoring of the base peak (M-PFB ion) for the analytes of interest was carried out with continuous monitoring.

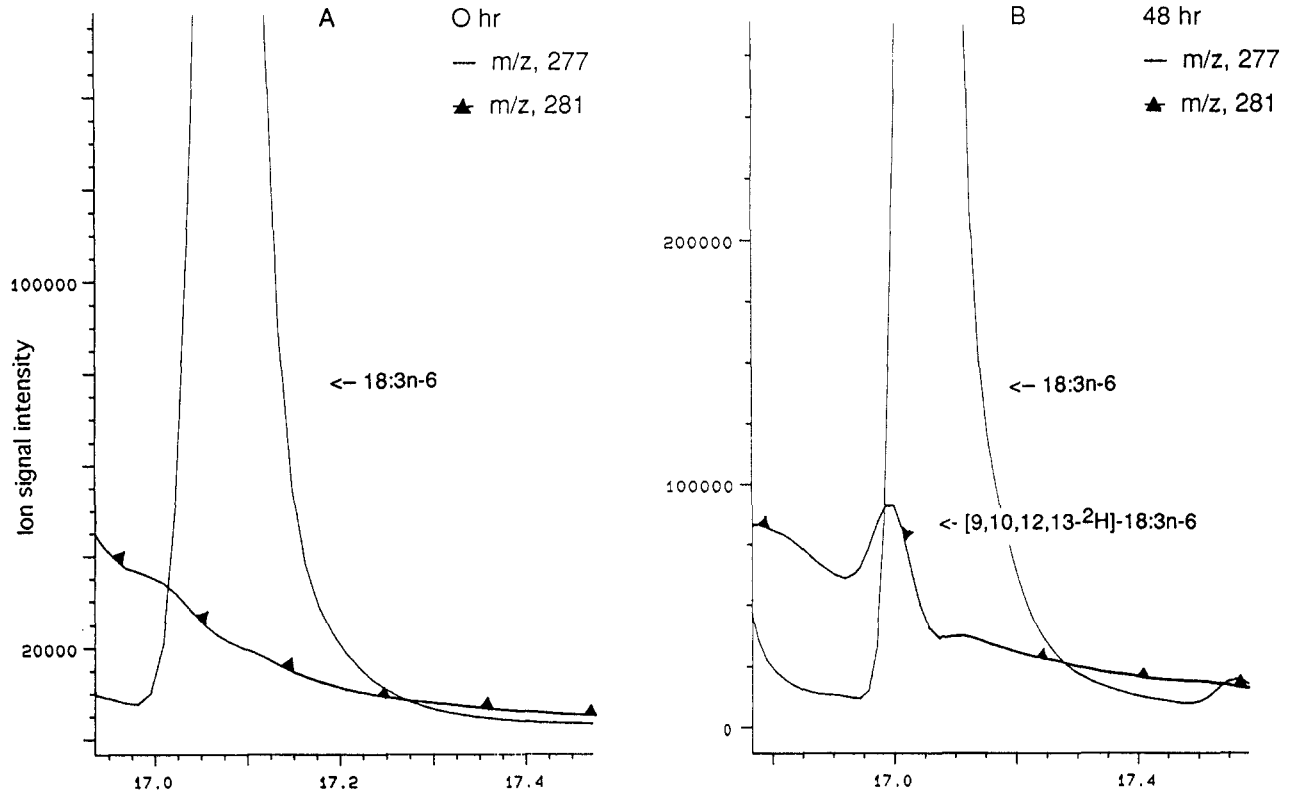

Fig. 1. The GC-MS selected ion chromatograms of the fatty acid PFB (pentafluorobenzyl) esters from plasma obtained from a cat maintained on the HCO/CO diet for 2 months prior to being given a single oral dose of [9,10,12,13-^{2}H]18:2n-6 ethyl ester. The ions chosen are *m/z* 277 for the detection of the M-PFB ion of 18:3n-6 and *m/z* 281 for detecting the M-PFB ion of [9,10,12,13-^{2}H]18:3n-6. Panel A depicts the chromatograms from a plasma sample at time 0 and panel B shows chromatographic tracings from a plasma sample taken 48 h after dosing.

TABLE 3. Negative ion GC-MS analyses of the products of the conversion of deuterium-labeled 18:2n-6 in feline plasma in two diets

Fatty Acid-PFB	HCO/CO Diet (n = 4)	Chow Diet (n = 3)
	ng/ml	
[9,10,12,13-^{2}H]18:2n-6	2956 ± 311	3085 ± 212
[9,10,12,13-^{2}H]18:3n-6	17 ± 6	0.06 ± 0.01
[11,12,14,15-^{2}H]20:2n-6	260 ± 32	38 ± 11
[11,12,14,15-^{2}H]20:3n-6	81 ± 14	-
[11,12,14,15-^{2}H]20:4n-6	162 ± 8	-
Total conversion	520	38

The deuterium-labeled metabolites were formed from the conversion of a 100 mg oral dose of [9,10,12,13-^{2}H]18:2n-6 and observed in the plasma 48 h after dosing the animals. A blank indicates that levels were below detection limits of 100 fg.

RESULTS

Plasma fatty acyl composition

The plasma fatty acid composition from felines on the chow diet and those on the HCO/CO diets is given in **Table 2.** The 20:4n-6 content of the plasma from animals on the chow diet was 4.1% of the total fat. In comparison, 20:4n-6 content of plasma from animals on the HCO/CO diet was about 1.8%. The amount of plasma 22:6n-3 in animals on the HCO/CO diet was also much lower than in animals on the chow diet. This indicated that the felines on the HCO/CO diet had a reduced capacity to maintain long-chain polyunsaturated fatty acids when it was necessary to synthesize them from 18-carbon precursors.

6-Desaturase in the domestic feline and production of arachidonic acid

The existence of 6-desaturase in felines was demonstrated by the conversion of the deuterium-labeled 18:2n-6 to 18:3n-6. **Figure 1** presents GC-MS selected ion chromatograms of plasma samples taken from a cat that had been maintained for 2 months on the HCO/CO diet just prior to and 48 h after being given a single oral dose of the labeled 18:2n-6. The PFB ester of the fatty acid, [9,10,12,13-^{2}H]18:3n-6, eluted slightly earlier, within the expected retention time window, than that of the PFB ester of 18:3n-6. The appearance of the labeled 18:3n-6 indicated that cats maintained on a diet that was devoid of long-chain polyunsaturated fatty acids converted 18:2n-6 to 18:3n-6 via 6-desaturase. In chow-fed animals, production of labeled 18:3n-6 occurred at levels well below those observed in animals on the HCO/CO diet (the level of 18:3n-6 production in chow-fed animals was about three orders of magnitude lower than in animals maintained on the HCO/CO diet).

The metabolites occurring from the [9,10,12,13-^{2}H]18:2n-6 at the 48-h time point and present in the plasma are given in **Table 3.** On average, 0.06% of the labeled 18:2n-6 detected at 48 h was found in the form of [9,10,12,13-^{2}H]18:3n-6. Roughly 15 times that amount or 0.8% of the [9,10,12,13-^{2}H]18:2n-6 was in the form of the elongated product, [11,12,14,15-^{2}H]20:2n-6. About 0.21% was in the form of [11,12,14,15-^{2}H]20:3n-6 and approximately 0.5% of the [9,10,12,13-^{2}H]18:2n-6 was in the form of [11,12,14,15-^{2}H]20:4n-6. A total of 1.7% of the

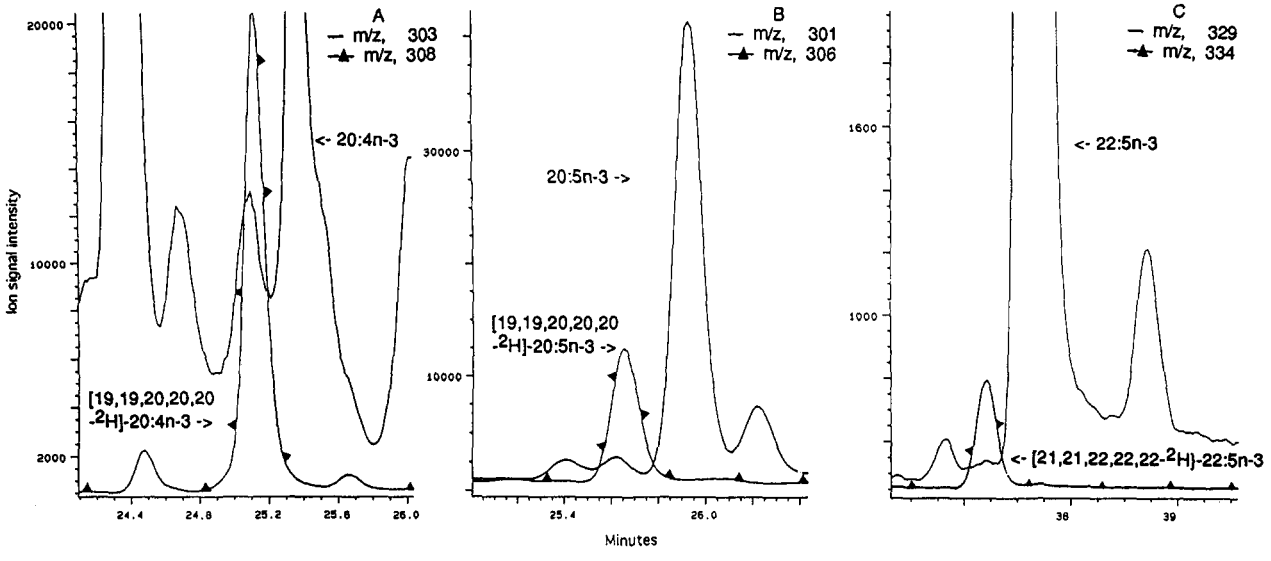

Fig. 2. GC-MS selected ion chromatograms of the n-3 fatty acid PFB esters from feline liver after the metabolism of an oral dose of 100 mg of [17,17,18,18,18-^{2}H]18:3n-3 ethyl ester. The animal was maintained on the HCO/CO diet for 2 months prior to being given the label. Panel A depicts selected ion tracings for the fatty acids 20:4n-3 and deuterium-labeled 20:4n-3. The ions, *m/z* 303 and 308, were used to detect the M-PFB ions of 20:4n-3 and the labeled 20:4n-3, respectively. Panel B shows the selected ion chromatograms for 20:5n-3 and deuterium-labeled 20:5n-3. The ions, *m/z* 301 and *m/z* 306, are used to detect the M-PFB ions of 20:5n-3 and the labeled 20:5n-3, respectively. Panel C shows the selected ion chromatograms of 22:5n-3 and labeled 22:5n-3. The ions, *m/z* 329 and 334, are used to detect the M-PFB ions of 22:5n-3 and labeled 22:5n-3, respectively.

[9,10,12,13-^{2}H]18:2n-6 was found in the desaturated and elongated products in the 48-h plasma.

N-3 metabolites from 18:3n-3 in the plasma

The products of 18:3n-3 metabolism subsequent to a single dose of [17,17,18,18,18-^{2}H]18:3n-3 were determined in the plasma. [17,17,18,18,18-^{2}H]18:3n-3 was converted to [19,19,20,20,20-^{2}H]20:4n-3, [19,19,20,20,20-^{2}H]20:5n-3, and [21,21,22,22,22-^{2}H]22:5n-3 and these products were detected in the plasma (**Fig. 2**). The 6-desaturase product, [17,17,18,18,18-^{2}H]18:4n-3 was not detected in significant amounts and this is consistent with the low level of 18:4n-3 in the plasma. The disappearance of the labeled 18:3n-3 and the increase in 20:5n-3 and 22:5n-3 over a period of 244 h was monitored in the plasma (**Fig. 3**). The maximum amounts of [19,19,20,20,20-^{2}H]20:5n-3 and [21,21,22,22,22-^{2}H]22:5n-3 were observed at 48 and 96 h, respectively. At 48 h, the amount of [19,19,20,20,20-^{2}H]20:5n-3 found in the plasma was about 8 μg/ml and at 96 h, the amount of [21,21,22,22,22-^{2}H]22:5n-3 was about 1.5 μg/ml. Significantly, the fatty acid, [21,21,22,22,22-^{2}H]22:6n-3 was not detected in the plasma during any of the sampling times.

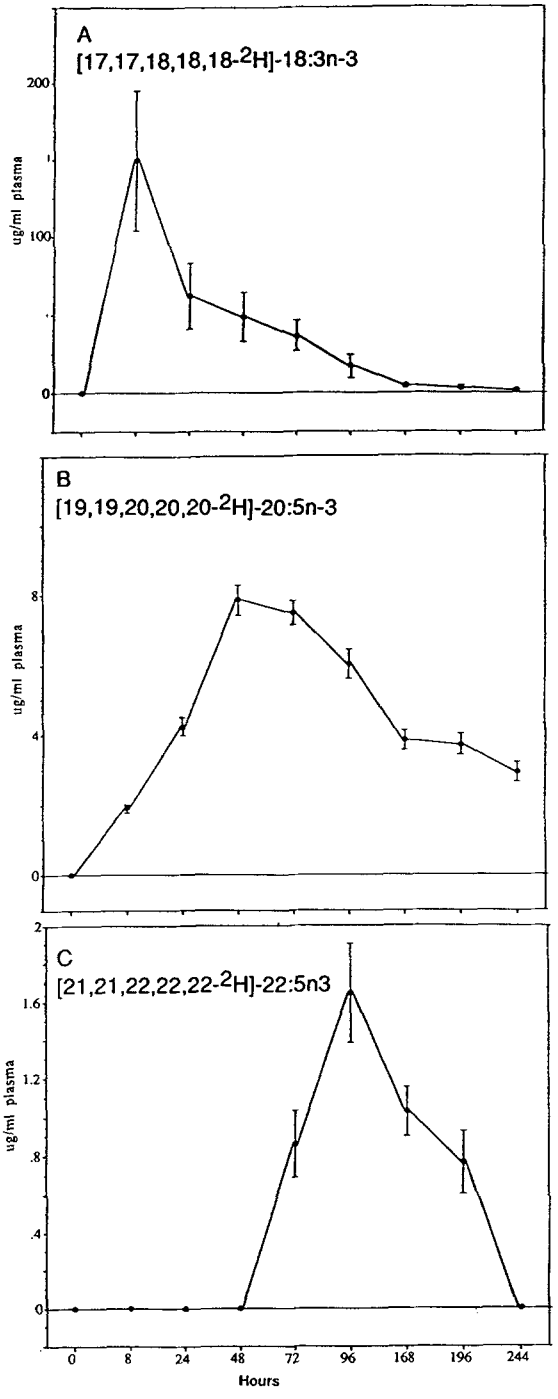

Fig. 3. The accumulated products from the conversion of the deuterium-labeled 18:3n-3 to 20:5n-3 and 22:5n-3 as determined in feline plasma over time. The plots represent the averages of four animals that had been maintained on the HCO/CO diet for 2 months before being given 100 mg of labeled fatty acid ethyl ester.

TABLE 4. Brain and liver GC-MS analyses of some deuterium-labeled polyunsaturated fatty acids

Metabolite	Liver	Brain
	μg/g wet weight	
N-6 polyunsaturated fatty acid		
18:2	3.80 ± 1.31	0.7 ± 0.31
18:3	0.07 ± 0.02	-
20:4	0.18 ± 0.03	0.3 ± 0.06
22:4	0.007 ± 0.002	0.13 ± 0.04
22:5	-	0.15 ± 0.1
24:4	-	0.03 ± 0.02
24:5	-	0.06 ± 0.04
N-3 polyunsaturated fatty acids		
18:3	4.3 ± 0.8	0.03 ± 0.01
20:5	0.82 ± 0.4	0.01 ± 0.004
22:5	0.08 ± 0.03	0.05 ± 0.01
22:6	-	0.28 ± 0.11
24:5	-	0.009 ± 0.005
24:6	-	0.002 ± 0.001

Deuterium-labeled polyunsaturated fatty acids resulted from the conversion of 10 mg each of [9,10,12,13-^{2}H]18:2n-6 and [17,17,18,18,18-^{2}H]18:3n-3 that were given once per day for 10 days to cats (n = 4) maintained on the HCO/CO diet for 6 months. Results are given as mean ± SE. A blank indicates that levels were below detection limits of 100 fg.

Liver and brain accumulation of deuterium-labeled 22- and 24-carbon polyunsaturated fatty acids

After the animals had been on the HCO/CO diet for 6 months, they were given 10 mg each of [17,17,18,18,18-^{2}H]18:3n-3 and [9,10,12,13-^{2}H]18:2n-6 for 10 days prior to their termination. The deuterium-labeled n-6 and n-3 metabolites found in the liver are given in **Table 4.** The deuterium-labeled n-3 fatty acid, [21,21,22,22,22-^{2}H]22:5n-3, and the n-6 polyunsaturate, [13,14,16,17-^{2}H]22:4n-6, were detected in the liver (**Fig. 4**). However, the n-3 and n-6 fatty acids, [21,21,22,22,22-^{2}H]22:6n-3 and [13,14,16,17-^{2}H]22:5n-6, were not found in

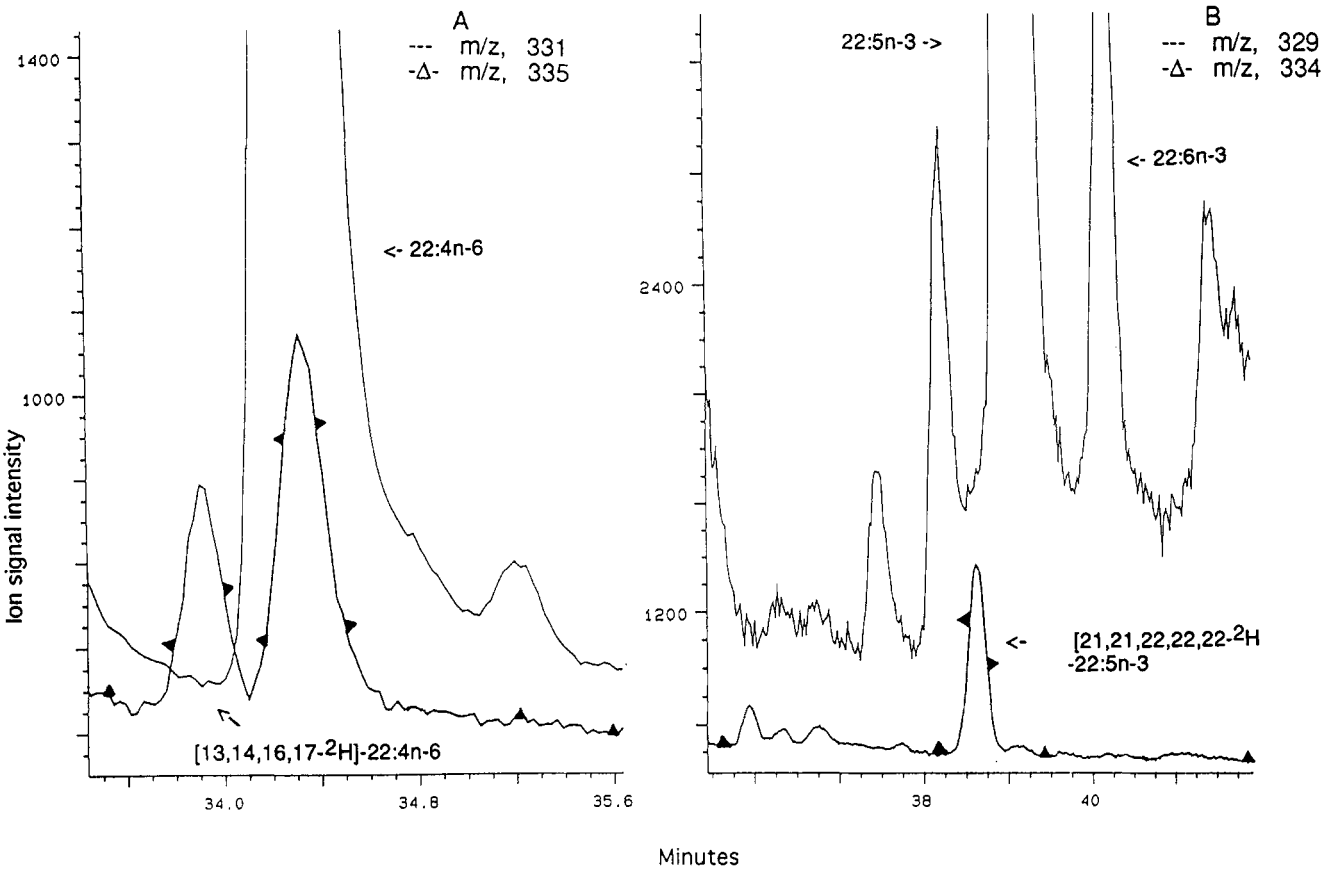

Fig. 4. GC-MS selected ion chromatograms of some fatty acid PFB esters obtained from feline liver. The liver sample was obtained from a cat that had been maintained on the HCO/CO diet for 6 months prior to being given an oral dose of 10 mg each of the deuterium-labeled 18:2n-6 and 18:3n-3 each day for 10 days. Panel A shows the selected ions for 22:4n-6 and labeled 22:4n-6. The ions, *m/z* 331 and 335, are used to detect the M-PFB ions of 22:4n-6 and labeled 22:4n-6, respectively. Panel B depicts the selected ions for 22:5n-3 and deuterium-labeled 22:5n-3. The ions, *m/z* 329 and 334, are used to detect the M-PFB ion of 22:5n-3 and deuterium-labeled 22:5n-3, respectively.

the liver. Also, no deuterium-labeled 24-carbon polyunsaturated fatty acids were detected in the liver.

GC-MS analyses of brain tissue taken at termination revealed that the brain contained deuterium-labeled polyunsaturated fatty acids not detected in the liver or blood. The labeled fatty acids found in the brain were [21,21,22,22,22-^{2}H]22:6n-3, [23,23,24,24,24-^{2}H]24:5n-3, [23,23,24,24,24-^{2}H]24:6n-3, [13,14,16,17-^{2}H]22:5n-6, [15,16,18,19-^{2}H]24:4n-6, and [15,16,18,19-^{2}H]24:5n-6 (**Fig. 5** and **Fig. 6**). The fatty acyl compositional analyses of the feline liver and brain confirmed that only insignificant amounts of the 24-carbon polyunsaturated fatty acids could be detected in the liver compared to the brain (**Table 5**).

DISCUSSION

It has previously been claimed and subsequently accepted that cats lack 6-desaturase. The present work demonstrates that when domestic felines are maintained on a diet devoid of long-chain n-3 and n-6 fatty acids, 6-desaturase activity can clearly be demonstrated. Although both the livers and brains of cats on such diets accumulated deuterium-labeled long-chain polyunsaturated fatty acids, the brain and not the liver appears to produce 22:6n-3 and 22:5n-6.

The present findings, which demonstrated that only 0.06% of the labeled 18:2n-6 was converted to 18:3n-6 at 48 h, do not necessarily contradict the results of Sinclair et al. (2). In that study, using radiolabeled 18:2n-6 and thin-layer chromatography, they did not attempt to identify products at low concentrations. The present methodology is more definitive and clearly shows that 18:2n-6 is converted to 18:3n-6. Our findings confirm their report that a higher proportion of the 18:2n-6 is chain elongated to 20:2n-6 than is desaturated to 18:3n-6. This leaves open the question as to whether an 8-desaturase contributes to 20:4n-6 production as they proposed (2). It is possible that the 6- and 8-desaturase both function in the feline as routes of 20:4n-6 and 20:5n-3 biosynthesis.

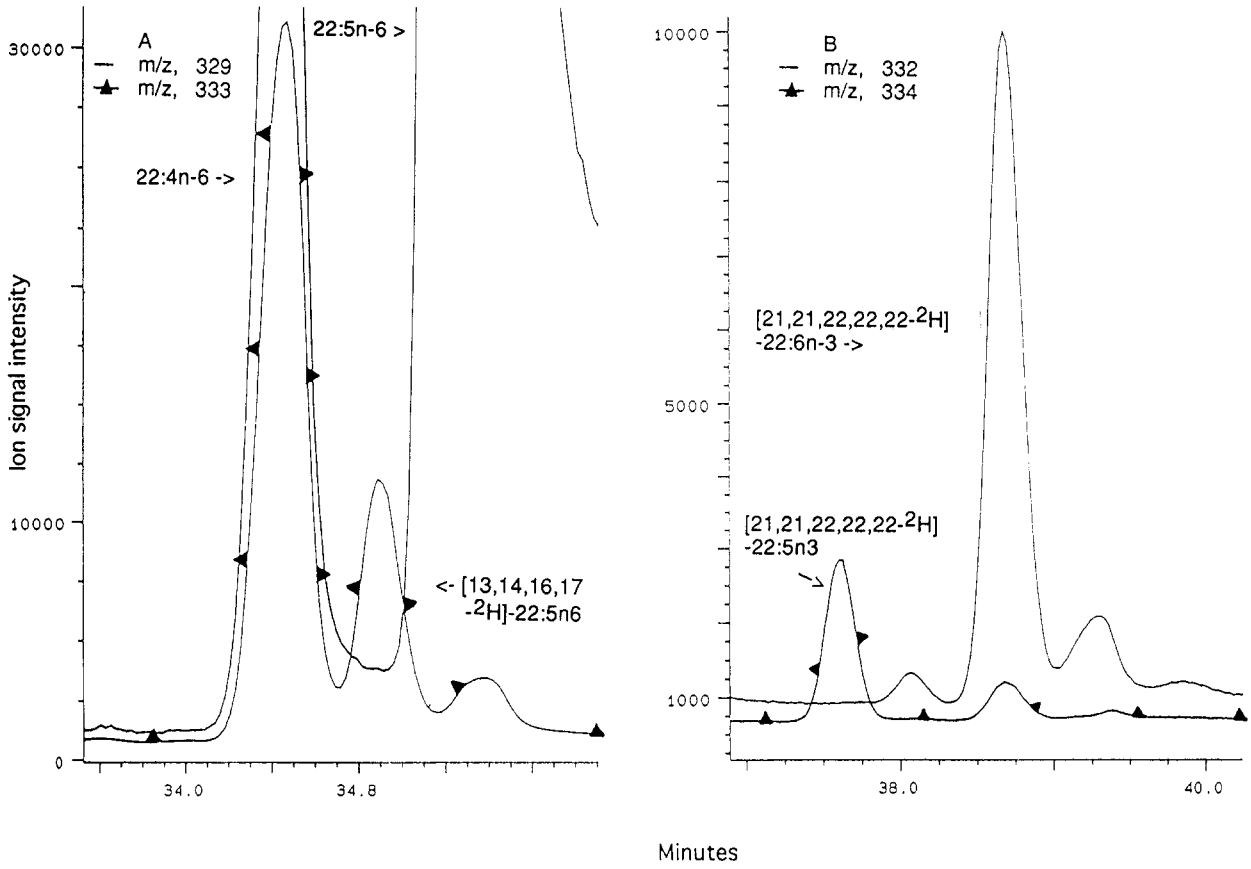

Fig. 5. GC-MS selected ion chromatograms of some fatty acid PFB esters obtained from a feline brain. The animal had been maintained on the HCO/CO diet for 6 months prior to being given an oral dose of 10 mg each of deuterium-labeled 18:2n-6 and 18:3n-3 each day for 10 days. Panel A depicts the selected ions for 22:5n-6 and deuterium-labeled 22:5n-6. The ions, *m/z* 329 and 333, are used to detect the M-PFB ions of 22:5n-6 and labeled 22:5n-6, respectively. Panel B shows the selected ion chromatograms for the labeled 22:5n-3 and deuterium-labeled 22:6n-3. The ions, *m/z* 334 and 332, are used to detect the M-PFB ions of labeled 22:5n-3 and deuterium-labeled 22:6n-3, respectively.

Cats that were maintained on the HCO/CO diet exhibited a decline in the amount of plasma 20:4n-6. The minimum level of 20:4n-6 that is sufficient to meet their requirements is not known; however, the animals in both groups appeared no different in so far as their general health, body weights, and coat condition were concerned. This is consistent with a report by McLean and Sinclair (13) where, when felines were fed diets composed of safflower oil (containing about 75% 18:2n-6) as the only lipid source for 8 years, they reported that animals appeared normal except for a slight dulling of their coats. However, one study has suggested the need for preformed 20:4n-6 in feline reproduction (14).

The apparent greater conversion of labeled 18:3n-3 to 20:5n-3 observed in the cat in 48-h plasma samples compared to that of 18:2n-6 to 20:4n-6 may be due, in part, to the different enzyme affinities for these two substrates as suggested by Brenner and Peluffo (15). However, the isotope dilution must be taken into account. The 270:1 ratio of 18:2n-6 to 18:3n-3 in the feline plasma and the 20:1 ratio in the liver would be expected to be primary factors contributing to the apparent greater amount of deuterium-labeled n-3 fatty acid metabolites.

To our knowledge, there have been no previous reports that have documented the conversion of n-3 fatty acids in the feline liver or brain. Our study provides conclusive evidence for the conversion of 18:3n-3 to 22:5n-3 in the liver. The feline's inability to convert 22:5n-3 to 22:6n-3 in the liver contrasts with the demonstrated ability of the rodent liver to synthesize 22:6n-3. This suggested to us that an alternate site for 22:6n-3 formation existed.

Although desaturase activity has been demonstrated in the rodent brain and conversion of 18:3n-3 to 22:6n-3 has been observed in the retina (7), this study demonstrates, for the first time, that the brain plays a necessary role in the synthesis of 22:6n-3 in a mammal. As neither 22:5n-6 nor 22:6n-3 were detected in the liver or plasma in felines when maintained on a low essential fat diet devoid of 20- or 22-carbon polyunsaturated fatty acids, it is highly likely that the deuterium-labeled 22- and 24-carbon polyunsaturated fatty acids with five and six double bonds that were found in the brain were synthesized there. This

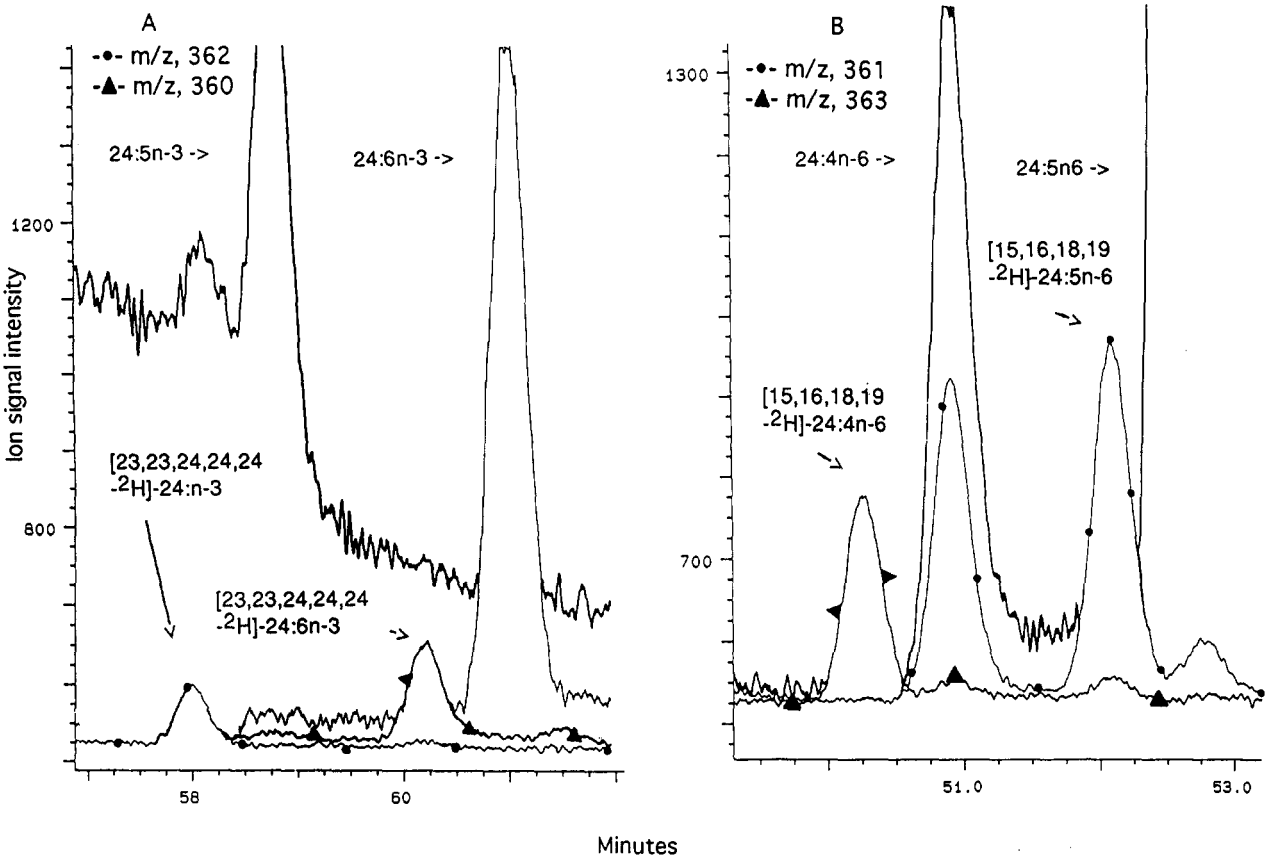

Fig. 6. GC-MS selected ion chromatograms of some fatty acid PFB esters obtained from a feline brain. The animal had been maintained on the HCO/CO diet for 6 months prior to being given an oral dose of 10 mg each of deuterium-labeled 18:2n-6 and 18:3n-3 each day for 10 days. Panel A depicts the selected ion tracings of 24:5n-3, 24:6n-3, labeled 24:5n-3, and labeled 24:6n-3. The ions, *m/z* 360 and 362, are used to detect the M-PFB ions of deuterium-labeled 24:5n-3 and deuterium-labeled 24:6n-3, respectively. Panel B show the selected ion chromatographs of 24:4n-6, 24:5n-6, deuterium-labeled 24:4n-6, and deuterium-labeled 24:5n-6. The ions, *m/z* 363 and 361, are used to detect the M-PFB ions of deuterium-labeled 24:4n-6 and deuterium-labeled 24:5n-6, respectively.

suggests that the elongation and desaturation system in the brain that produces 22:6n-3 from 22:5n-3 is not operable in the liver.

TABLE 5. Long-chain polyunsaturated fatty acyl composition of feline livers and brains

Fatty Acids	Liver	Brain
	μg/g	
N-6 polyunsaturated fatty acid		
20:4	35 ± 3.4	532 ± 11
22:4	4.2 ± 1.3	310 ± 13
22:5	2.2 ± 0.5	340 ± 7
24:4	0.01 ± 0.002	14.2 ± 2.2
24:5	0.01 ± 0.003	7.5 ± 1.1
N-3 polyunsaturated fatty acid		
22:5	12.3 ± 1.0	3.9 ± 1.1
22:6	6.3 ± 1.1	680 ± 42
24:5	<0.005	0.31 ± 0.2
24:6	<0.005	0.34 ± 0.4

Animals (n = 4) were maintained on HCO/CO diet, consisting of hydrogenated coconut oil (9 wt%) and corn oil (1 wt%) for 6 months. Values are given as mean ± SE.

It has been suggested by Voss et al. (16) that the 24-carbon n-6 and n-3 polyunsaturated fatty acids are intermediates in the production of 22:6n-3 and 22:5n-6. They proposed, for example, that the 6-desaturase introduces a double bond at the 6 position of 24:5n-3 which is then partially oxidized to form 22:6n-3. We found that all 22- and 24-carbon n-3 fatty acids became deuterium-labeled in the feline brain. The only labeled 22-carbon n-3 fatty acid incorporating deuterium from 18:3n-3 in the liver was 22:5n-3. Similarly, the 22- and 24-carbon n-6 fatty acid homologues were also found labeled in the brain where 22:5n-6 was produced. These findings, therefore, support their hypothesis as the site where 22:6n-3 and 22:5n-6 are made is also the location where the labeled 24-carbon n-3 and n-6 fatty acids of 4, 5, and 6 double bonds are made. Conversely, no labeled 24-carbon n-3 and n-6 fatty acids are found in the liver or plasma where 22:6n-3 and 22:5n-6 are not made. However, it should be noted that elongation of 22:5n-3 to 24:5n-3 could not be demonstrated in the liver in this study. This at least raises a possibility that cats lack a 22-carbon elongase activity needed to form, for example, the 24:5n-3

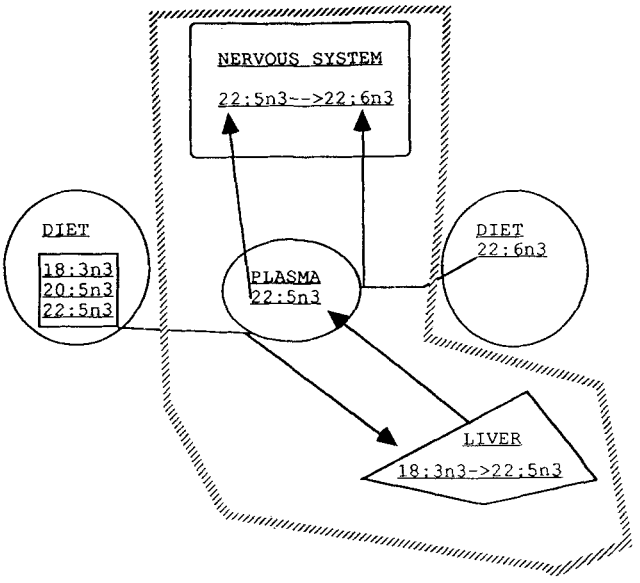

Fig. 7. A schematic indicating possible sources of 22:6n-3 in the cat brain.

substrate for 6-desaturase. Another interesting aspect of our results is the possibility that there are different 6-desaturase isozymes in the cat liver and brain having different chain length selectivities.

It is suggested from these observations that a portion of the docosahexaenoic acid in mammalian brain is derived from the uptake of 22:5n-3 which is then locally converted to 22:6n-3, (**Fig. 7**). The quantitative importance of this pathway is unknown but is expected to vary across species, with age, and be strongly influenced by the supply of 22:6n-3 in the diet (17). It is suggested that this is an alternate method of synthesis that may be used to supply the nervous system with 22:6n-3 during periods of nutritional stress and/or brain growth periods. The 22:5n-3 is, in turn, derived from liver metabolism of dietary 18:3n-3, 20:5n-3, or other n-3 intermediates. This is supported by the observation that the only other n-3 fatty acid that normally accumulates in the mammalian brain is 22:5n-3 (18). Also consistent with this view are studies carried out in rodents and rhesus monkeys, maintained on diets where the sole n-3 fatty acid was 18:3n-3, where we observed that deuterium-labeled 22:5n-3 and 22:6n-3 were made in comparable quantities in the liver and exported to the blood after a single dose of deuterium-labeled 18:3n-3 (R. Pawlosky, A. Barnes, and N. Salem, unpublished data).

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