Incorporation of Radioactive Polyunsaturated Fatty Acids into Liver and Brain of Developing Rat

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ABSTRACT

The incorporation of radioactivity from orally administered linoleic acid-1-^{14}C, linolenic acid-1-^{14}C, arachidonic acid-$^{3}H_8$, and docosahexaenoic acid-^{14}C into the liver and brain lipids of suckling rats was studied. In both tissues, 22 hr after dosing, 2 distinct levels of incorporation were observed: a low uptake (from 18:2-1-^{14}C and 18:3-1-^{14}C) and a high uptake (from 20:4-$^{3}H_8$ and 22:6-^{14}C). In adult rats, the incorporation of radioactivity into brain lipids from 18:2-1-^{14}C and 20:4-^{3}H was considerably lower than the incorporation into the brains of the young rats. In the livers of the suckling rats, the activity from the 18 carbon acids was associated mostly with the triglyceride fraction, whereas the activity from the 20:4-$^{3}H_8$ and 22:6-^{14}C was concentrated in the phospholipid fraction. In the brain lipids, the activity from the different fatty acids was associated predominantly with the phospholipids. In the liver and brain phospholipid fatty acids, some of the activity in the 18:2-1-^{14}C and 18:3-1-^{14}C experiments was associated with 20 and 22 carbon polyunsaturated fatty acids; however, radioactivity from orally administered 20:4-$^{3}H_8$ and 22:6-^{14}C was incorporated intact into the tissue phospholipid to a much greater extent compared with the incorporation of radioactivity into 20:4 and 22:6 in the experiments where 18:2-1-^{14}C and 18:3-1-^{14}C, respectively, were administered. Possible reasons for these differences are discussed. Rat milk contains a wide spectrum of polyunsaturated fatty acids, including linoleate, linolenate, arachidonate, and docosahexaenoate. During the suckling period in the rat, there is a rapid deposition of 20:4 and 22:6 in the brain. The results of the present experiments suggested that dietary 20:4 and 22:6 were important sources of brain 20:4 and 22:6 in the developing rat.

INTRODUCTION

Long chain polyenoic fatty acids are of particular interest in relation to the mammalian brain, since, in a wide variety of mammals, the brain grey matter phosphoglycerides are characterized by the presence of large amounts of 20:4ω6, 22:4ω6, and 22:6ω3 and by low levels of 18:2ω6 and 18:3ω3 (1). In the laboratory rat, a significant proportion of brain development occurs during the suckling period (2) and more than 70% of the long chain polyenoic fatty acids (20:4 and 22:6) in the rat brain are laid down by the end of the suckling period (3,4). Rat milk contains both linoleate and linolenate, as well as their longer chain metabolic products, such as 20:3ω6, 20:4ω6, 22:4ω6, 20:5ω3, 22:5ω3, and 22:6ω3 (4,5).

To investigate the possible role of dietary polyenoic fatty acids in contributing to brain lipids, we have examined the tissue uptake of a series of radioactive fatty acids which were administered orally to suckling rat pups. Some preliminary results of these experiments have been published elsewhere (5,6).

METHODS AND MATERIALS

Animals and Diets

Rat pups, 16-17 days old, were used in these experiments. They were bred from female rats of the Wistar strain which were maintained on a semisynthetic diet (7). The diet contained 14.4% of its calories as fat, which was a mixture of soybean oil and linseed oil (SBOL) (5:1, v/v) and the linoleic:linolenic ratio in the diet was 3.3:1. The animals were mated when ca. 4 months old. During the first 24 hr after birth, large litters were reduced to 9 pups, and, if the size of the litter fell below 6 during the suckling period, the litter was not used. Owing to the association between litter size, body growth, and the extent of brain development in rats (2), the litter size was controlled in the above manner so that results from different litters could be compared. Three adult female rats (300 g) also were used after they had been on the above diet for 12 months.

Radioactive Experiments

Linoleic acid-1-^{14}C (61 mCi/mmole), α-linolenic acid (60 mCi/mmole), and arachidonic

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TABLE I

Incorporation of Radioactivity from Labeled Fatty Acids into Liver and Brain Lipids of Suckling and Adult Rats

Time	Isotope	Liver lipids: Percent dose	Liver lipids: Specific[a] activity	Brain lipids: Percent dose	Brain lipids: Specific activity
Suckling rats[b]					
22 hr	18:2-1-^{14}C (6)[c]	2.69 ± 0.24	155 ± 14.5	0.44 ± 0.05	29.1 ± 3.17
	20:4-$^{3}H_8$ (9)	14.9 ± 1.58	1050 ± 120	2.06 ± 0.36	157 ± 28.0
	18:3-1-^{14}C (3)	3.29 ± 0.32	151 ± 8.89	0.29 ± 0.03	18.5 ± 2.74
	22:6-^{14}C (4)	19.8 ± 1.32	1360 ± 153	2.71 ± 0.79	197 ± 57.0
48 hr	18:2-1-^{14}C (4)	1.84 ± 0.08	81.9 ± 4.74	0.42 ± 0.02	23.7 ± 1.84
	20:4-$^{3}H_8$ (3)	7.84 ± 0.68	341 ± 37.7	1.66 ± 0.19	88.9 ± 5.33
	18:3-1-^{14}C (3)	0.85 ± 0.15	60.6 ± 10.9	0.36 ± 0.05	26.1 ± 4.71
Adult rats[d]					
22 hr	18:2-1-^{14}C (3)	4.17 ± 0.17	54 ± 2.6	0.039 ± 0.003	1.2 ± 0.3
	20:4-$^{3}H_8$ (3)	19.3 ± 0.10	222 ± 0.4	0.134 ± 0.004	3.4 ± 0.1

[a]Specific activity = dpm/mg lipid/μCi dose.

[b]18:2-1-^{14}C And 20:4-$^{3}H_8$ were injected simultaneously in 6 and 3 rats at 22 and 48 hr, respectively. The dose ratio ($^{3}H/^{14}C$) was 1.2:1 at 22 hr and 1.3:1 at 48 hr.

[c]The numbers in parenthesis represent the numbers of rats used. The results are shown as the mean ± standard error of mean.

[d]Isotopes injected simultaneously; the dose ratio ($^{3}H/^{14}C$) was 0.4:1.

acid-1-^{14}C (54 mCi/mmole) were obtained from the Radiochemical Centre, Amersham, U.K. Arachidonic acid-$^{3}H_8$, methyl ester (32 mCi/mmole) was a gift from Unilever Research, Vlaardingen, The Netherlands, and docosahexaenoic acid-^{14}C, methyl ester (1.3 mCi/mmole) was prepared biosynthetically in this laboratory (8). Between 1-4 μCi each isotope was given to each rat, except in the 22:6-^{14}C experiments where 0.03 μCi was used. A known amount of a radioactive fatty acid (in solvent) was introduced into a vial containing 0.3 ml olive oil, and the solvent was evaporated with a stream of N_2. The oil and isotope mixture then was drawn into a glass syringe (with a blunt 18 gauge needle) and administered orally to unanesthetized animals. The isotope content of the residue in the syringe and vial was estimated, giving by difference the actual dose. This usually amounted to ca. 70% of the activity introduced into the vial (see above). In some experiments, linoleic-1-^{14}C and arachidonic-$^{3}H_8$ were administered simultaneously. The radiochemical purity of the isotopes, determined by gas liquid chromatography (GLC) of the methyl esters, was better than 98%, except for the 22:6-^{14}C where 7% of the activity was associated with 22:5ω3.

Lipid Extraction and Liquid Scintillation Counting

After dosing, the pups were returned to their mothers and sampled at 22 and 48 hr. Animals were killed by decapitation and the liver and brain quickly removed, washed in ice-cold saline, blotted dry, and weighed. These tissues then were extracted in chloroform-methanol (2:1, containing 10 mg/liter 2:6-di-*tert*-butyl-*p*-cresol as an antioxidant). The lipid extracts were washed according to the Folch procedure (9), and the total lipid wt was estimated by weighing dried aliquots of the lipid extract. Aliquots of the total lipids also were assayed for radioactivity by liquid scintillation counting using a Packard Tri-Carb model 3000 scintillation spectrometer. The scintillation solution consisted of 4 g diphenyloxazole (PPO) and 0.2 g diphenyloxozole-benzene (POPOP)/liter toluene. The counting efficiency for carbon 14 was normally ca. 75% and for double-label experiments (^{14}C and ^{3}H), ca. 23% for ^{3}H and ^{14}C in the mixed channel, and 25% for ^{14}C in the carbon only channel. The efficiency of counting was determined by use of internal standards of n-hexadecane-1-^{14}C and n-hexadecane-1,2-^{3}H (The Radiochemical Centre, Amersham, U.K.).

Aliquots of tissue lipids were separated by thin layer chromatography (TLC) using Silica Gel G as the adsorbent and light petroleum (bp 40-60)-diethyl ether-glacial acetic acid (85-15-5 or 90-10-1) as the solvents. The fractions were detected under UV light after spraying the TLC

TABLE II

Percentage Distribution[a] of Isotopes in Liver and Brain Lipid Fractions[b]

Isotope	18:2-1-^{14}C	20:4-$^{3}H_8$	18:3-1-^{14}C	22:6-^{14}C
Liver lipid fraction				
Triglyceride	69 ± 3	20 ± 2	82 ± 2	28 ± 3
Phospholipid	25 ± 3	78 ± 2	14 ± 2	70 ± 3
n[c]	6	6	4	4
Brain lipid fraction				
Cholesterol	14 ± 1	1 ± 0.3	26 ± 1	ND[d]
Phospholipid	84 ± 1	96 ± 1	72 ± 1	ND
n	6	4	4	

[a]The results are expressed as a percentage of the total isotope recovered from five different lipid fractions of the liver and brain (cholesteryl ester, triglyceride, free fatty acids, cholesterol + diglyceride, and phospholipid). The distribution was checked each time using two solvent systems (see "Methods and Materials"). The results are shown as mean ± standard error of mean.

[b]The pups were killed 22 hr after dosing with the different fatty acids.

[c]The number of separate experiments.

[d]ND = not determined.

plates with a methanolic solution (0.2%) of dichlorofluorescein. The radioactivity in the different fractions separated by TLC (phospholipids [PL], cholesterol, free fatty acids, triglycerides [TG], and cholesteryl esters) was measured by the elution of the samples from silica gel with 1 ml hyamine hydroxide (1 M in methanol) and 10 ml scintillation solution (5). This method proved unsatisfactory when estimating the distribution of ^{14}C and ^{3}H together in double-label experiments because of the quenching of ^{3}H by the hyamine. In these experiments, the TLC fractions were eluted from the silica gel with 40 ml solvent (chloroform-acetone-methanol-water, 10-1-5-0.1), and the samples were counted following evaporation of the solvents. Recoveries of 95.3 ± 0.2% for ^{14}C and 94.1 ± 0.2% for ^{3}H (mean ± standard error of mean for 12 determinations) were obtained.

Brain lipids also were treated with chloroform-0.2 N methanolic sodium hydroxide (2:1) (10) to convert ester-bound fatty acids to methyl esters. The lipid extract from this reaction was separated on TLC using light petroleum (bp 40-60)-diethyl ether-glacial acetic acid (90-10-1) as the solvent, and the fractions (fatty acid methyl esters, cholesterol, and alkali-stable lipids) were eluted from the silica gel and assayed for radioactivity.

The distribution of the radioactivity in the fatty acids of tissue TG and PL was determined by the separation and fraction collection of the methyl esters of these lipids using a preparative GLC (5). The methyl esters were prepared as described previously (4), and the preparative GLC was carried out using a glass column 2.1 m in length x 7 mm inside diameter packed with 10% ethylene glycol succinate methyl silicone polymer (EGSS-X) on Diatomite C-AW 60-70 mesh (W.G. Pye and Co., Cambridge, U.K.) at 190 C. The carrier gas flow rate was 170 ml/min.

Fatty acid fractions were decarboxylated by the Schmidt procedure as described by Goldfine and Bloch (11).

RESULTS

Incorporation of Activity from Radioactive Fatty Acids into Total Lipids of Liver and Brain

In the 16-17 day old rat pups (22 and 48 hr after dosing), there was a substantially greater incorporation of radioactivity from 20:4-$^{3}H_8$ and 22:6-^{14}C into the total lipids of liver and brain by comparison with the incorporation of activity from 18:2-1-^{14}C and 18:3-1-^{14}C (Table I). With all fatty acids, there was a greater recovery of activity in the liver lipids compared with brain lipids.

In adult rats, there was also a greater recovery of activity from 20:4-^{3}H in liver and brain lipids by comparison with the uptake of ^{14}C from linoleic acid-1-^{14}C (Table I).

The percentage of the dose of 18:2-1-^{14}C and 20:4-$^{3}H_8$ recovered in the brain lipids of the adult rats was less than 10% of the values obtained in the suckling rats, whereas the recovery of the dose in the liver lipids was of the same order of magnitude in adult and suckling rats. The values obtained for the adult rats are in close agreement with the results published in the literature for the recovery of activity from orally administered 18:2-1-^{14}C

TABLE III

Percentage Distribution[a] of Radioactivity in Fatty Acids of Liver Triglycerides

Fatty acid fraction[b]	Wt%[c]	18:2-1-^{14}C 22 hr	18:3-1-^{14}C 22 hr	20:4-$^{3}H_8$ 22 hr	22:6-^{14}C 22 hr
Solv.-14:0	5.7	---[d]	---	---	---
16:0-16:1	21.6	---	---	---	---
18:0-18:1	22.0	---	---	---	---
18:2	19.4	93 ± 1.2[e]	---	---	---
18:3-20:1	3.0	3.6 ± 0.4	80 ± 1.7	---	2
20:2-20:3	1.5	1.6 ± 0.3	---	1.4 ± 0.2	---
20:4	4.5	---	2.5 ± 0.4	79 ± 0.7	1
22:1-20:5	2.4	---	7.5 ± 0.8	3.1 ± 0.4	3
22:4-22:5	1.2	---	---	15 ± 0.6	1
22:5	5.6	---	5.6 ± 0.9	1.5 ± 0.5	8
22:6	8.0		1.8 ± 0.3		86
n		(3)	(3)	(3)	(1)[f]

[a]The results are expressed as the percentage of radioactivity in a fraction relative to the total radioactivity collected for all fractions.

[b]Under the gas liquid chromatographic conditions used the 18:3-20:1 fraction would include 18:3ω6, 18:3ω3, 20:0, and 20:1; the 20:2-20:3 fraction would include 20:2ω9, ω6, 18:4ω3, and 20:3ω6; fraction 20:4 includes 20:4ω6, 20:3ω3, and 22:0; fraction 22:1-20:5 includes 22:1, 20:4ω3, and 20:5ω3; and fraction 22:4-22:5 includes 22:4ω6, 22:5ω6, 24:0, and 24:1.

[c]Fatty acid composition (wt %) of liver triglycerides from 16-17 day old rats.

[d]Less than 1%.

[e]The number of separate analyses is shown in parenthesis and the mean value ± standard error of mean is shown. Fractions with greater than 10% of the total activity are underlined.

[f]In the 22:6-^{14}C experiment the liver TG from four animals were pooled prior to analysis.

and 20:4-1-^{14}C in liver and brain lipids of adult rats (12-14).

Recovery of Radioactivity in Whole-Body Lipids of 16-17 Day Old Suckling Rats

The recovery of radioactivity from the whole body lipids, 22 hr after dosing of the pups with 20:4-1-^{14}C, 22:6-^{14}C, 18:2-1-^{14}C, and 18:3-1-^{14}C, was (as percent of administered dose): 80.4 ± 2.1, 65.4 ± 3.2, 46.8 ± 2.0, and 51.1 ± 2.4, respectively (mean ± standard error of mean for 3 animals in each group, except the 22:6-^{14}C group where 4 animals were used). The arachidonic acid value was significantly greater ($p<0.05$) than the other three values.

Distribution of Radioactivity in Liver and Brain Lipid Fractions

In the liver lipids, radioactivity from the 4 different fatty acids was found in either the TG or PL with less than 5% of the activity being associated with the free fatty acids, cholesterol, or cholesteryl esters. Twenty-two hr after dosing, the activity from the 18 carbon acids (18:2 and 18:3) was concentrated in the TG, whereas, for the longer chain acids (20:4 and 22:6), the activity was associated predominantly with the PL (Table II).

In the brain lipids, the radioactivity from the different fatty acids mostly was found in the PL fraction, but, with the carboxyl-labeled fatty acids (18:2 and 18:3), some 14-26% of the activity also was found in the TLC-fraction corresponding to cholesterol (Table II). Diglycerides have similar R_f values to cholesterol; however, cleavage of the brain glyceride-ester lipids (see "Methods and Materials") showed that this activity was still associated with cholesterol. Although not reported in Table II, the distribution in the liver and brain lipids at 48 hr was very similar to the distribution shown for 22 hr.

Distribution of Radioactivity in Fatty Acids of TG and PL

There were differences in the distribution of the isotope in the individual fatty acids of the three fractions examined (liver TG, PL, and brain PL).

In the liver TG and PL, the majority of the radioactivity was associated with the fatty acid which had been administered (Tables III and IV). An exception to this occurred in the liver PL in the linolenic acid-1-^{14}C experiment where most of the activity was associated with the 22:6, 22:5, and 20:5 fractions. In the brain PL fatty acids in the linoleic acid-1-^{14}C experi-

TABLE IV

Percentage Distribution[a] of Radioactivity in Fatty Acids of Liver Phospholipids

Fatty acid fraction[b]	Wt %[c]	18:2-1-^{14}C		18:3-1-^{14}C		20:4-$^{3}H_8$		22:6-^{14}C
		22 hr	48 hr	22 hr	48 hr	22 hr	48 hr	22 hr
Solv.-14:0	0.6	---[d]	---	---	---	---	---	---
16:0-16:1	24.4	---	---	3.5 ± 0.6	4.4 ± 0.3	---	---	1
18:0-18:1	24.4	1.1 ± 0.3[e]	1.1 ± 0.3	3.7 ± 0.5	7.0 ± 0.3	---	---	1
18:2	9.3	83 ± 1.5	65 ± 3.7	---		---	---	---
18:3-20:1	0.1	2.3 ± 0.5	2.8 ± 0.8	8.7 ± 0.8	8.1 ± 0.8	---	---	1
20:2-20:3	0.9	2.1 ± 0.2	2.5 ± 0.6	1.0 ± 0.1	---	---	---	---
20:4	19.4	7.4 ± 1.0	23 ± 3.8	5.9 ± 0.3	5.4 ± 0.4	94 ± 1.1	93 ± 1.7	---
22:1-20:5	---	---	1.1 ± 0.3	11 ± 0.7	4.5 ± 0.4	4.4 ± 0.9	4.8 ± 1.4	1
22:4-22:5	0.5	---	---	2.9 ± 0.7	2.1 ± 0.2	1.3 ± 0.1	1.4 ± 0.3	2
22:5	3.0	1.5 ± 0.3	1.9 ± 0.4	26 ± 1.5	19 ± 2.4	---	---	3
22:6	14.7			36± 3.7	44 ± 3.1	---	---	90
n		(6)	(4)	(3)	(3)	(8)	(3)	(1)[f]

[a,b,d,e,f]See footnotes Table III.

[c]Fatty acid composition (wt %) of liver phospholipids from 16-17 day old rats.

ment, the radioactivity was associated with the 16:0 + 16:1, 18:0 + 18:1, 18:2, and 20:4 fractions (Table V), whereas, in the linolenic acid-1-^{14}C experiment, the activity mostly was found in the saturated and monounsaturated fatty acids and a little in the 22:6 fraction. In the arachidonic acid-$^{3}H_8$ and docosahexaenoic acid-^{14}C experiments, most of the activity was associated with the 20:4 and 22:6 fractions, respectively.

In the linolenic acid-1-^{14}C experiment, very little radioactivity was associated with the linolenate fraction of the brain PL (Table V). However, in the brain PL, 18:3ω3 amounts to only 0.1% of the total fatty acids. The calculated relative specific activity (RSA) of the brain linolenate was 0.09. This value was similar to the calculated RSA for brain 18:2, 20:4, and 22:6 in the 18:2-1-^{14}C, 20:4-$^{3}H_8$, and 22:6-^{14}C experiments. In these experiments, the RSA were found to be 0.10, 0.13, and 0.13, respectively. (The RSA was calculated as follows: for example, in the 18:2-1-^{14}C experiment, 22 hr after dosing, percent dose in brain lipids = 0.44% (Table I); activity in brain PL as percent of total brain lipids = 84% (Table II); activity in brain PL 18:2 as percent of total PL fatty acids = 31% (Table V); and the wt percent of 18:2 in brain PL fatty acids = 1.2%. Therefore, RSA = 0.44 x 0.84 x 0.31 ÷ 1.2 = 0.10).

Incorporation of Radioactivity from 18:2-1-^{14}C, 18:3-1-^{14}C, and 20:4-1-^{14}C into Brain Lipids

In view of the difference in the incorporation of radioactivity from 1-^{14}C fatty acids (18:2 and 18:3) and ^{3}H-labeled arachidonic acid into brain cholesterol (Table II) and saturated and monounsaturated fatty acids (Table V), it was decided to study the incorporation of radioactivity from 20:4-1-^{14}C into brain lipids. In this experiment, 3 animals were used, and it was found that 3% of the activity in the brain lipids was associated with cholesterol (1% in the 20:4-$^{3}H_8$ experiment, Table II), and ca. 6% of the ^{14}C in brain PL was found in saturated and monounsaturated fatty acids (0.2% in ^{3}H experiment). These differences may, in part, be accounted for by the use of the different isotopes (20:4-^{3}H vs 20:4-^{14}C) which could result in a selective loss of ^{3}H to body water relative to ^{14}C during the oxidation of arachidonic acid to acetyl CoA and CO_2 and during the recyclization of the isotope to other compounds, e.g. fatty acids and cholesterol.

When the results for three different carboxyl-carbon labeled fatty acids (18:2, 18:3, and 20:4) were calculated as a percentage of the dose appearing in different fractions of the brain lipids, the incorporation of ^{14}C into cholesterol and saturated plus monounsaturated fatty acids was the same for each fatty acid (Table VI). The differences in the incorporation of radioactivity from these three 1-^{14}C fatty acids into the total lipids of the brain could be accounted for entirely by the differences in the incorporation of the ^{14}C into the polyunsaturated acids of the brain.

Decarboxylation Studies

In both the linoleic acid-1-^{14}C and linolenic acid-1-^{14}C experiments, the low activity in the carboxyl carbon atoms of the 20:4ω6, 20:5ω3, and 22:5 + 22:6ω3 fractions (Table VII)

TABLE V

Percentage Distribution[a] of Radioactivity in Fatty Acids of Brain Phospholipids

Fatty acid fraction[b]	Wt %[c]	18:2-1-^{14}C		18:3-1-^{14}C		20:4-$^{3}H_8$		22:6-^{14}C
		22 hr	48 hr	22 hr	48 hr	22 hr	48 hr	22 hr
Solv.-14:0	1.5	---[d]	---	---	---	---	---	---
16:0-16:1	28.8	21 ± 1.0[e]	21 ± 1.8	39 ± 1.8	36 ± 0.6	---	---	---
18:0-18:1	32.4	14 ± 0.6	17 ± 0.8	26 ± 1.1	32 ± 2.6	---	---	7
18:2	1.2	31 ± 0.8	18 ± 1.4	1.8 ± 0.2	2.0 ± 0.2	---	---	
18:3-20:1	0.4	2.0 ± 0.4	2.1 ± 0.3	4.4 ± 0.5	2.5 ± 0.1	---	---	
20:2-20:3	0.5	4.7 ± 0.5	4.2 ± 0.4	---	---	---	---	3
20:4	13.1	24 ± 0.9	31 ± 2.8	3.0 ± 0.4	1.6 ± 0.6	85 ± 1.1	83 ± 3.1	
22:1-20:5	0.1	1.2 ± 0.2	1.2 ± 0.2	3.1 ± 0.2	1.3 ± 0.1	2.3 ± 0.4	3.3 ± 0.7	2
22:4-22:5	4.0	2.9 ± 0.2	4.3 ± 0.3	2.4 ± 0.3	2.7 ± 0.2	11 ± 1.1	12 ± 1.0	6
22:5	0.3	1.0 ± 0.2	1.0 ± 0.1	15 ± 1.9	2.3 ± 0.1	1.0 ± 0.2	1.1 ± 0.2	4
22:6	14.3				15 ± 1.1			78
n		(6)	(4)	(3)	(3)	(5)	(3)	(1)[f]

[a,b,d,e,f]See footnote Table III.

[c]Fatty acid composition (wt %) of brain phospholipids from 16-17 day old rats.

TABLE VI

Incorporation of Radioactivity from Carboxyl-Labeled Fatty Acids into Total Lipids, Cholesterol, and Fatty Acids of Brain

	Percentage of dose recovered in brain lipids			
			Phospholipid fatty acids[a]	
Isotope	Total lipid	Cholesterol[b]	16:0 to 18:1	18:2 to 22:6
18:2-1-^{14}C	0.47 ± 0.12[c]	0.07 ± 0.002	0.16 ± 0.001	0.25 ± 0.01
18:3-1-^{14}C	0.29 ± 0.03	0.07 ± 0.007	0.19 ± 0.03	0.03 ± .004
20:4-1-^{14}C	2.58 ± 0.14	0.07 ± 0.003	0.16 ± 0.02	2.35 ± 0.12

[a]The fatty acid methyl esters from brain phospholipids were fractionated using preparative gas liquid chromatography (see "Methods and Materials"), and two fractions were collected: from 16:0-18:1 and from 18:2-22:6.

[b]Separated from total lipids by TLC (see "Methods and Materials").

[c]The results are from 3 separate experiments with each isotope (t = 22 hr) and are presented as the mean ± standard error of mean.

suggested that the ingested fatty acids were incorporated intact into their respective longer chain metabolites.

In the brain, the carboxyl-carbon activity in the 16 carbon fatty acids was very close to the value predicted for de novo synthesis from an acetate molecule with only one of its two carbon atoms labeled. Such a molecule would be produced by β-oxidation of an even chain fatty acid labeled in the carboxyl position. In the 18 carbon saturated and monounsaturated acids of the brain, the result for the carboxyl-carbon activity suggested that a combination of de novo synthesis from acetate and chain elongation of 16 carbon acids was responsible for the labeling of these acids.

Incorporation of Radioactivity from Linoleic Acid-1-^{14}C and Arachidonic Acid-$^{3}H_8$ into Tissue 20:4

There was a greater incorporation of radioactivity from the exogenous arachidonate (-$^{3}H_8$) into tissue 20:4 compared with the endogenous formation of arachidonate from linoleic acid-1-^{14}C (Table VIII). Similarly, by calculation of the amount of activity in the 22:6 of liver and brain PL from 22:6-^{14}C compared with the incorporation of ^{14}C into 22:6 from linolenic acid-1-^{14}C, it was shown that the former compound yielded 95 times and 59 times as much activity in the 22:6 of liver and brain, respectively.

Linoleic acid is the chief precursor of arachidonate, and a possible reason for the difference

TABLE VII

Decarboxylation Studies on Fatty Acids Collected from Preparative Gas Liquid Chromatography

Fatty acid fraction	Relative carboxyl activity[a] 18:2-1-^{14}C (3)[b] Liver phospholipid 22 hr	Brain phospholipid 22 hr	18:3-1-^{14}C (3) Liver phospholipid 22 hr	Brain phospholipid 22 hr
16:0 + 16:1	---[c]	0.13	---	0.13
18:0 + 18:1	---	0.17	---	0.19
18:2	0.91	0.99	---	---
18:3	---	---	0.96	---
20:4	0.08	0.03	---	---
20:5	---	---	0.05	---
22:5+22:6	---	---	0.03	0.03

[a]Radioactivity in the -COOH group/activity in total fatty acid.

[b]The number of separate experiments is shown in parenthesis, and the results are presented as the mean. The relative carboxyl activity also was determined for the liver and brain fatty acids at 11 and 48 hr after dosing rats with linoleic acid-1-^{14}C, and the results were almost identical to those shown for 22 hr.

[c]Not determined.

in the $^{3}H/^{14}C$ ratio in the tissue arachidonate could be the dilution of the linoleic-1-^{14}C during dosing.

In the preceding double-label experiments, the 18:2-1-^{14}C and 20:4-$^{3}H_8$ were given to the pups in 0.3 ml olive oil. This oil contained 8% of its fatty acids as 18:2, and, therefore, 0.3 ml oil would provide ca. 23 mg linoleate. This would dilute the specific activity of the 18:2-1-^{14}C in the dose relative to the 20:4-$^{3}H_8$. To test the effect of the specific activity of the dose upon the uptake of ^{3}H-20:4 and ^{14}C-18:2 into tissue lipids, pups were dosed with the olive oil-isotope mixture to which was added 14, 43, or 86 mg methyl arachidonate (Table IX). The addition of the cold arachidonate was associated with a small decrease of the $^{3}H/^{14}C$ ratio in the total lipids of liver and brain; however, it had little effect upon the $^{3}H/^{14}C$ ratio in the arachidonate fraction of the liver and brain PL. Therefore, even when the specific activity of the 20:4-$^{3}H_8$ was lower than the specific activity of the 18:2-1-^{14}C (experiments 3 and 4, Table IX), the ^{3}H incorporation was still significantly greater than the ^{14}C incorporation into tissue 20:4.

In these experiments, the diet fed to the dams had a linoleic to linolenic ratio of 3.3 to 1. The incorporation of radioactivity from linoleic acid-1-^{14}C and arachidonic acid-$^{3}H_8$ into tissue lipids also was studied in pups whose mothers were fed a diet in which the linoleic to linolenic ratio was 48:1 (Table X). The feeding of this diet (which was almost completely devoid of linolenic acid) was associated with an increased conversion of linoleic to arachidonic acid in the liver PL (decreased $^{3}H/^{14}C$ in 20:4). However, there was no change in the $^{3}H/^{14}C$ ratio in the 20:4 of the brain PL.

TABLE VIII

Incorporation of Radioactivity from 20:4-$^{3}H_8$ and 18:2-1-^{14}C into Liver and Brain Arachidonate[a]

Time (hr)	n[c]	Ratio of $^{3}H/^{14}C$[b] Liver phospholipid 20:4	Brain phospholipid 20:4
22	6	140 ± 11[d]	22 ± 2.1
48	3	73 ± 10	14 ± 2.3

[a]Methyl arachidoante was isolated from the total phospholipid methyl esters by preparative gas liquid chromatography (see "Methods and Materials").

[b]This ratio = $^{3}H/^{14}C$ of collected 20:4 ÷ $^{3}H/^{14}C$ of dose. The dose ratio was 1.2:1 in the 22 hr experiment and 1.3:1 in the 48 hr experiment.

[c]Number of separate experiments.

[d]Results are presented as the mean ± standard error of mean.

DISCUSSION

In the developing rat, the incorporation of radioactivity from 20:4-$^{3}H_8$ and 22:6-^{14}C into liver and brain total lipids was very much greater than the incorporation of radioactivity from 18:2-1-^{14}C and 18:3-1-^{14}C. In the liver, this difference could be accounted for by the faster incorporation of radioactivity from the longer chain acids into PL and TG. In the brain, the differences could be explained entirely by a faster incorporation of radioactivity from the longer chain acids (20:4 and 22:6) into the PL fraction.

Previous workers have demonstrated that radioactivity from 18:2-1-^{14}C, 18:3-1-^{14}C, and 20:4-1-^{14}C is incorporated into the adult rat brain (12-15). This finding was confirmed in

TABLE IX

Incorporation of Radioactivity from 20:4-$^{3}H_8$ and 18:2-1-^{14}C into Tissue Lipids and Tissue Arachidonate: Effect of Dilution of Dose with Carrier Methyl Arachidonate

Experiment	Isotopes	Carrier oil: Olive oil	Carrier oil: Me 20:4	Ratio of $^{3}H/^{14}C$[a]: Liver total lipid	Liver phospholipid 20:4	Brain total lipid	Brain phospholipid 20:4
1[b]	18:2-1-^{14}C plus 20:4-$^{3}H_8$	0.3 ml[c]	0 mg	4.6	140	3.1	22
2[d]		0.3	14	4.3	78	2.9	18
3		0.3	43	4.6	140	2.4	19
4		0.3	86	4.0	150	1.9	20

[a]This ratio = $^{3}H/^{14}C$ in fraction ÷ $^{3}H/^{14}C$ in dose.

[b]Six animals in this experiment (dose ratio $^{3}H/^{14}C$ = 1.2:1). Data from Table VIII.

[c]Estimated 18:2 content of 0.3 ml oil = 23 mg.

[d]In experiments 2-4, there was one pup/experiment (dose ratio $^{3}H/^{14}C$ = 1.3:1). In these experiments, the olive oil-isotope mixture contained three different levels of methyl arachidonate (Me 20:4, >99% pure, NuChek Prep.).

the present experiments (Table I) where it also was demonstrated that there was a greater uptake of radioactivity from 20:4-$^{3}H_8$ by comparison with 18:2-1-^{14}C in the adult brain.

For all fatty acids, the uptake of radioactivity by the adult rat brain was markedly lower than the uptake in the developing rat brain (Table I). Although this difference might be accounted for by a difference in the permeability of brain membranes in rats of different ages, it also could be a reflection of the rapid rate of deposition of 20:4ω6, 22:4ω6, and 22:6ω3 in the developing rat brain (3,4).

Although radioactive 20:4 and 22:6 were isolated from the liver and brain lipids following the dosing of the pups with 18:2-1-^{14}C and 18:3-1-^{14}C, respectively, in the liver, at least, the bulk of the radioactivity was still present as the administered fatty acid. Thus, the comparison of the uptake of the radioactivity from orally administered 20:4-$^{3}H_8$ with the appearance of radioactivity in 20:4 from orally administered 18:2-1-^{14}C showed that, in both liver and brain, most of the radioactivity in the 20:4 fraction was derived from the orally administered 20:4 (Table VIII). This pattern also was observed when the 22:6 formation from 18:3-1-^{14}C was compared with the direct incorporation of 22:6-^{14}C.

Several factors may have influenced the different uptake of radioactivity from linoleic acid-1-^{14}C compared with arachidonic acid-$^{3}H_8$. First, in the initial experiments, the specific activity of the 18:2-1-^{14}C was lower than that of the 20:4-$^{3}H_8$ during dosing. When the arachidonic acid-$^{3}H_8$ specific activity was reduced below that of the linoleic acid-1-^{14}C, the radioactivity in the liver and brain 20:4 was still mostly derived from the arachidonic acid-$^{3}H_8$. Second, the SBOL diet ("Methods and Materials") contained both linoleic and linolenic acids, and it is known that linolenic acid can reduce the conversion of linoleic to arachidonic acid (16-18). In the absence of dietary linolenate (SSO diet, Table X), there was an increased formation of arachidonate (^{14}C) from linoleic acid-1-^{14}C in the liver PL; however, despite this, the majority of the radioactivity in the liver and brain PL arachidonate was derived from the orally administered 20:4-$^{3}H_8$.

Although linoleic acid is converted to arachidonic acid in the body, to explain the consistently greater uptake of dietary (exogenous) arachidonate by liver and brain lipids compared with the endogenous formation of 20:4 from dietary linoleate, the factors discussed below should be taken into account:

Linoleic acid is oxidized to CO_2 faster than arachidonic acid (19), and this may explain the

TABLE X

Effect of Dietary Fatty Acids upon Incorporation[a] of Radioactivity from 18:2-1-^{14}C and 20:4-$^{3}H_8$ into Liver and Brain Fatty Acids

Fatty acid fraction	Liver phospholipid		Brain phospholipid	
	Diet SBOL[b]	Diet SSO[c]	Diet SBOL	Diet SSO
18:2	83[d]	44	31	24
18:3-20:1	2.3	3.3	2.0	1.7
20:2-20:3	2.1	2.6	4.7	6.6
20:4	7.4	35	24	16
$^{3}H/^{14}C$ in 20:4	140 ± 11[e]	28 ± 3	22 ± 2	22 ± 1

[a]The pups were killed 22 hr after dosing with 18:2-1-^{14}C and 20:4-$^{3}H_8$. The dose ratio ($^{3}H/^{14}C$) in the soybean oil and linseed oil (SBOL) experiment was 1.2:1 and 0.7:1 in the safflower seed oil (SSO) experiment.

[b]SBOL diet (see "Methods and Mateials"), results from Tables IV, V, and VIII.

[c]SSO diet = same gross composition as SBOL diet, except that fat was supplied as safflower seed oil. Linoleic to linolenic ratio in this diet was 48:1 and in the SBOL diet, 3.3:1. Mean of results from three animals.

[d]The results are expressed as the percentage of radioactivity in a fraction relative to the total radioactivity collected for all fractions.

[e]This ratio = $^{3}H/^{14}C$ in collected 20:4 ÷ $^{3}H/^{14}C$ in dose.

retention of 80% of the ^{14}C in the whole body lipids following the dosing of pups with 20:4-1-^{14}C compared with a retention of only 47% of the ^{14}C when pups were dosed with 18:2-1-^{14}C.

The turnover of linoleic acid is faster than arachidonic acid in tissue lipids (20,21); this may mean that linoleic acid is more frequently exposed to the β-oxidation enzymes than arachidonic acid.

In the liver, linoleic acid-1-^{14}C was preferentially incorporated into TG, whereas arachidonic acid-$^{3}H_8$ was concentrated in the PL. If it is assumed that this distribution applied to all tissues and organs, then it would result in a dilution of the linoleic acid-1-^{14}C relative to arachidonic acid-$^{3}H_8$ owing to the different pool sizes of TG and PL in the whole animal.

The slow rate of conversion of linoleic acid to arachidonic acid may, in part, be accounted for by the substrate competition and end-product inhibition of the enzymes involved in this process (16,17). In the present experiments, substrates and end-products are found in the pups' diet (4,5).

The greater uptake of ^{14}C from 22:6-^{14}C compared with 18:3-1-^{14}C into the tissue lipids of developing rats may be accounted for by similar processes to those described above.

In view of these observations, it would be wrong to equate 1 molecule of dietary linoleic and linolenic acids with 1 molecule of dietary arachidonic and docosahexaenoic acids, respectively. Thus, although only relatively small amounts of these longer chain acids (20:4ω6 and 22:6ω3) are present in the pups' diet (milk) during this period of brain development, quantitatively they may be of considerable importance in supplying tissues with longer chain fatty acids.

A significant amount of the radioactivity in the brain lipids was associated with cholesterol and the saturated and monounsaturated fatty acids following the administration of carboxyl-labeled fatty acids (Tables II, V, and VI). It was calculated that, as a proportion of the administered dose of 18:2, 18:3, and 20:4 (all 1-^{14}C), the extent of labeling of cholesterol, saturated, and monounsaturated fatty acids in the brain was 8, 4, and 5 times, respectively, greater than the labeling of the same compounds in the liver. In these experiments, the radioactivity in the carboxyl-carbon of brain 16:0 and 16:1 suggested that these fatty acids were being synthesized de novo from acetate. This acetate was most probably derived by the β-oxidation of the fed carboxyl-labeled fatty acid. Acetate is an efficient precursor of cholesterol and saturated and monounsaturated fatty acids, and, in suckling rats, the brain has a marked preference over the liver for incorporating carboxyl-labeled acetate into lipids (22,23). This preference may be explained by the observations that, during the suckling period, the rate of synthesis and chain elongation of fatty acids in the brain is greater than that of the liver (24,25).

The high content of 20:4, 22:4, and 22:6 relative to 18:2 and 18:3 in rat brain is well recognized, and, indeed, this pattern has been observed in the brains of a number of different mammalian species (1). This pattern could originate because of a rapid desaturation and

chain elongation of 18:2 and 18:3 in the brain. Alternatively, it may be a result of a specific uptake of longer chain polyunsaturated fatty acids by the brain. The results of the present experiments demonstrate that the polyunsaturated fatty acids of the developing rat brain are derived from two sources, one endogenous and the other exogenous. The endogenous source is that formed in the liver from dietary precursors (18:2ω6 and 18:3ω3) and supplemented in part by that formed in the brain in situ. A larger source (exogenous) is the preformed dietary polyunsaturated acids (20:4ω6 and 22:6ω3).

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