Metabolism of palmitic acid in the subcellular fractions of mouse brain

Grace Y. Sun and Lloyd A. Horrocks

Laboratory of Neurochemistry, Cleveland Psychiatric Institute, Cleveland Ohio 44109, and Department of Physiological Chemistry, The Ohio State University, Columbus, Ohio 43210

Abstract After an intracerebral injection of [^{14}C]palmitic acid to C57BL/10J mice, the radioactivity in the brains decreased rapidly with time. The incorporated radioactivity was primarily in the 16:0 acyl groups of the diacyl phosphoglycerides at 1 and 3 days after injection. At longer times, increasing proportions of the radioactivity were found in cerebrosides, alkenyl groups, and other acyl groups. The specific radioactivities of the phosphoglycerides were highest in the microsomal fraction at 1 day after injection. The exchange of the diacyl glycerophosphorylcholines and diacyl glycerophosphorylethanolamines between the microsomes and the myelin required 8–14 days. When calculated on the basis of the radioactivity in the 16:0 acyl groups, the half-lives for both of these phosphoglycerides were 6–8 days in all subcellular fractions during the period from 14 to 30 days after injection. The radioactivity in the total lipids from the purified myelin fraction did not decline until more than 14 days after injection because of the reutilization of labeled 16:0 acyl groups for lipid biosynthesis. Recycling of the acyl groups explains the long half-lives reported for myelin phosphoglycerides after injection of [^{14}C]acetic acid. Lipids with a relatively high specific radioactivity were lost from the myelin fraction during the purification procedure. The most likely source of these lipids is the most recently formed myelin that is not consolidated into the myelin sheath.

Supplementary key words phosphoglycerides · turnover · acyl groups · microsomes · myelin

THE DIFFERENCES and similarities in the composition and metabolism of cerebral membranes are important for an understanding of phospholipid metabolism in the brain. Intracerebrally injected [^{14}C]ethanolamine is incorporated rapidly into the EPG of all subcellular fractions (1). A rapid equilibration and turnover of the EPG of brain microsomes and myelin has been observed in vivo (2–5), but much longer half-lives have been reported for the acyl groups of myelin phospholipids after intraperitoneal injections of [^{14}C]acetate (6–8). In the brain of the adult mouse, intracerebrally injected palmitic acid was metabolized rapidly and incorporated into all major lipids (9). The half-life of the free palmitic acid in the brain was less than 5 min. Between 1 and 8 days after injection, an increasing amount of radioactivity was found in the alkenyl groups, the monounsaturated acyl groups, and the galactolipid fraction (10). This apparent recycling of the incorporated palmitic acid suggested that the much longer half-lives of the acyl groups from myelin phosphoglycerides labeled with [^{14}C]acetic acid might also be due to recycling of labeled acyl groups. In order to determine if the palmitoyl groups of myelin phosphoglycerides turn over as rapidly as the ethanolamine moiety of the EPG, we have studied the phosphoglycerides of subcellular fractions of mice kept for up to 3 months after intracerebral injections of [^{14}C]palmitic acid.

MATERIALS AND METHODS

Animals and mode of administration

24 female C57BL/10J mice at 3 months of age were given intracerebral injections of [U-^{14}C]palmitic acid (5, 10) (sp act 560 Ci/mole, Applied Science Laboratories, State College, Pa.). Each mouse received 25 μl (0.5 μCi) of a solution of [^{14}C]palmitate dissolved in a 15% (w/v) solution of bovine serum albumin. At each time interval after injection, three mice were killed. Individual mouse brains were dispersed immediately in

Presented in part at the first meeting of the American Society for Neurochemistry, Albuquerque, N.M., 1970.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; CPG, choline phosphoglycerides; EPG, ethanolamine phosphoglycerides; GPC, *sn*-glycero-3-phosphorylcholine; GPE, *sn*-glycero-3-phosphorylethanolamine; IPG, inositol phosphoglycerides; SPG, serine phosphoglycerides; Sph, sphingomyelin; PA, phosphatidic acid; CL, cardiolipin; CB, cerebroside.

Downloaded from www.jlr.org by guest, on June 19, 2012

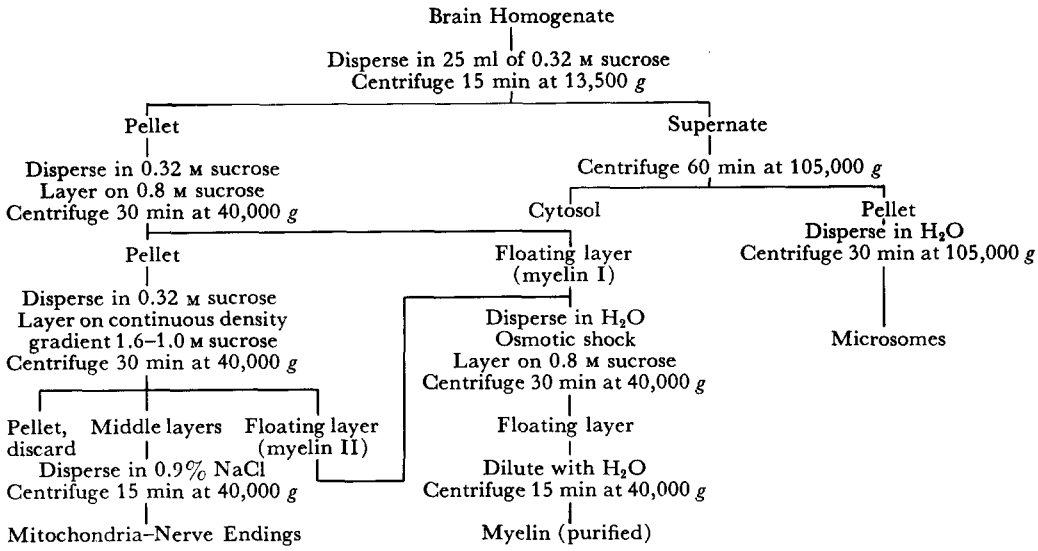

FIG. 1. Outline of the isolation procedure for the mouse brain cytosol, myelin, nerve endings plus mitochondria, and microsomal fractions.

20 vol of 0.32 M sucrose solution containing 1 mM EDTA and 3 mM Na_2HPO_4 with the aid of a Potter-Elvehjem tissue grinder equipped with a Teflon pestle.

Subcellular fractionation

At each time interval after injection, microsomal and cytosol fractions from three subjects were obtained separately by high-speed centrifugation of the brain dispersions (Fig. 1). The crude mitochondrial pellets obtained after the flotation of the first myelin layer were resuspended (using a syringe) in 0.32 M sucrose, and the suspension was layered on top of a continuous density gradient made from 1.0 M and 1.6 M sucrose. Centrifugation gave a broad mitochondria–nerve ending band in the lower half of the tube, a small pellet containing mainly cell nuclei and debris, and a thin floating layer containing mostly myelin (myelin II). The mitochondria–nerve ending layer was resuspended (in 0.9% NaCl) and centrifuged. All myelin fractions were purified by osmotic shock, reflotation, and water washing for the removal of sucrose. Similar subcellular fractions have previously been characterized by electron microscopy, enzymic assays, and lipid analysis (5, 11–15). The purified myelin fractions were not contaminated by other types of membranous material as judged by their lipid compositions. The mitochondria–nerve ending fraction contained mostly intact nerve endings with only a small proportion of free mitochondria. We frequently saw profiles of nerve endings with a single mitochondrion surrounded by a plasma membrane. A few small mitochondria were found in the microsomal fraction.

Lipid extraction, separation, and radioactivity assay

Final pellets from the microsomal fractions were suspended in 3 ml of water, and pellets from myelin and mitochondria–nerve ending fractions were suspended in 6 ml of water. A portion of each fraction was taken for the measurement of radioactivity. The lipids were extracted from the remainder of each suspension with chloroform–methanol 2:1 (v/v) and the phosphorus content was determined (5). Lipids from brain subcellular fractions were separated by separation–reaction–separation TLC with silica gel G plates (16, 17). Exposure of the TLC plates to HCl fumes after the first dimension quantitatively cleaved the alk-1-enyl groups from the respective phosphoglycerides. Lipid spots on the TLC plates were visualized by exposing the plates to iodine vapor. The lipid spots were recovered from eight plates for each subcellular fraction for phosphorus determination according to Gottfried (18). The results in Table 1 are quite similar to those reported previously (15). Other TLC plates were used for the determination of the distribution of radioactivity in the phospholipids, cerebrosides, and lipids at the solvent front. Radioactive samples from subcellular fractions and lipid samples recovered from TLC plates were assayed for radioactivity in vials containing 10 ml of XDC scintillation fluid (5). Lipid samples in chloroform were evaporated to dryness before adding the XDC scintillation fluid, which was prepared with 600 ml of 1,4-dioxane, 200 ml of xylene, 600 ml of 2-ethoxyethanol, 112 g of naphthalene, and 4 g of 2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]thiophene.

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 1. Composition of the phospholipids from subcellular fractions of mouse brain

	Purified Myelin	Microsomes	Nerve Endings + Mitochondria
		μg-atoms/brain	
Lipid phosphorus[a]	4.16 ± 0.14 (n = 21)	1.43 ± 0.09 (n = 20)	7.73 ± 0.44 (n = 22)
		% of lipid phosphorus (*n* = 8)	
SPG	13.8 ± 0.4	10.8 ± 0.7	9.7 ± 0.6
IPG	3.1 ± 0.3	4.5 ± 0.3	4.8 ± 0.3
Sphingomyelin	4.2 ± 0.5	4.9 ± 0.3	5.3 ± 0.3
CPG	26.9 ± 0.6	42.4 ± 0.8	36.2 ± 0.8
Acid-labile EPG	32.4 ± 0.5	17.9 ± 0.4	18.6 ± 0.5
Acid-stable EPG	16.2 ± 0.3	17.1 ± 0.6	20.1 ± 0.3
PA	4.0 ± 0.3	3.9 ± 0.3	2.8 ± 0.2
CL			2.3 ± 0.2

Results are given as means ± SEM.

[a] The total homogenate contained 25.5 μg-atoms of lipid phosphorus/brain.

Acyl group derivatives and GLC

Portions of the total lipid extracts from subcellular fractions were subjected to alkaline methanolysis for transesterification of the acyl groups (19). The purified fatty acid methyl esters contained 50–60% of the radioactivity in the total lipids. The methyl esters were separated by GLC (Aerograph model 1740) (20). For the measurement of the radioactivity of individual peaks, a preparative column, 6 ft by 0.25 inch, packed with 10% EGSS-X on 100–120 mesh Gas-Chrom P (Applied Science Laboratories) was used for the separation, and a Packard model 852 fraction collector at 275°C was used for the collection. The effluent was trapped in 4.5-cm-long glass cartridges packed with siliconized glass wool (21). After collection of the effluent material, the cartridges were placed in vials containing 10 ml of XDC scintillation fluid and the radioactivity of the samples was measured. Some samples of the methyl esters were also separated on TLC plates impregnated with $AgNO_3$ (10).

By using two or three samples of fatty acid methyl esters prepared from whole brain or from individual subcellular fractions for each time period, we determined the proportion of acyl group radioactivity in the 16:0 acyl groups. These proportions were 1.00 at 1 hr and 1 day, 0.79 at 3 days, 0.51 at 8 days, 0.40 at 14 days, and 0.22 at 30 days after injection. The remainder of the acyl group radioactivity was in 18:0 and 18:1 acyl groups. The amounts of radioactivity in the 20:1 acyl groups and in the polyunsaturated acyl groups were not different from the background radioactivity. The number of these determinations was limited by the amount of material available. Similar distributions of radioactivity were found for different subcellular fractions from the same time period.

Calculation of specific radioactivities

The specific radioactivities of the diacyl GPE were calculated as dpm/μmole of 16:0 acyl groups according to the following equation:

$$\text{dpm}/\mu\text{mole} = \frac{(\text{dpm in acyl groups})\quad(\text{correction factor})}{(2)\,\dfrac{(16{:}0\text{ acyl groups})}{(\text{total acyl groups})}\,(\mu\text{moles of diacyl GPE})}$$

The amount of radioactivity in the acyl groups was determined by two-dimensional TLC. The correction factor, the proportion of radioactivity remaining in 16:0 acyl groups, was determined as described above. In the denominator, the proportion of 16:0 acyl groups in a specific lipid fraction was obtained from a previous publication (15). The amount of diacyl GPE in a subcellular fraction was calculated by multiplying the proportion of diacyl GPE in a subcellular fraction (Table 1) by the amount of lipid phosphorus in that fraction. The same procedure was used for the calculation of the specific radioactivity of the diacyl GPC.

The specific radioactivities of the alkenyl groups from the alkenyl acyl GPE were calculated as dpm/μmole of 16:0 alkenyl groups according to the following equation:

$$\text{dpm}/\mu\text{mole} = \frac{\text{dpm in alkenyl groups}}{\dfrac{(16{:}0\text{ alkenyl groups})}{(\text{total alkenyl groups})}\,(\mu\text{moles of alkenyl acyl GPE})}$$

The amount of radioactivity in the EPG aldehydes as separated by two-dimensional TLC was divided by the product of the amount of alkenyl acyl GPE and the proportion of 16:0 alkenyl groups in the EPG alkenyl groups (15).

The specific radioactivities of the 18:1 acyl groups from the alkenyl acyl GPE were calculated as the dpm/μmole of 18:1 acyl groups as follows:

$$\text{dpm}/\mu\text{mole} = \frac{\text{dpm in alkenyl groups}}{\dfrac{(18{:}1\text{ acyl groups})}{(\text{total acyl groups})}\,(\mu\text{moles of alkenyl acyl GPE})}$$

The amount of radioactivity in the monoacyl GPE, separated by two-dimensional TLC, was divided by the product of the amount of alkenyl acyl GPE and the proportion of 18:1 acyl groups in the alkenyl acyl GPE (15).

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 2. Content and subcellular distribution of radioactivity in mouse brains after intracerebral injections of [^{14}C]palmitate

Time after Injection	Radioactivity per Brain[a]			Cytosol	Microsomes	Crude Myelin[b]	Nerve Endings + Mitochondria
	A	B	C				
days	*dpm* × 10^{-3}			*% distribution of radioactivity (mean ± SEM)*			
1 hr	224.0	143.0	74.0	17.3 ± 0.5	14.6 ± 2.9	24.0 ± 0.4	44.1 ± 3.8
1	148.0	143.0	14.9	8.8 ± 1.3	22.5 ± 1.0	22.3 ± 0.8	50.2 ± 3.8
3	88.7	79.0	63.5	7.4 ± 0.4	18.7 ± 2.1	22.3 ± 0.8	51.6 ± 1.9
8	57.5	25.6	–	5.1 ± 0.1	19.5 ± 1.3	27.0 ± 1.0	48.4 ± 0.3
14	47.3	20.0	–	3.4 ± 0.5	12.9 ± 0.0	29.5 ± 0.9	54.1 ± 0.5
30	13.6	13.0	11.5	2.8 ± 0.2	14.2 ± 0.9	35.5 ± 1.8	47.4 ± 2.5
60	9.9	8.8	25.0	2.5 ± 0.6	8.4 ± 0.6	38.3 ± 1.2	50.8 ± 1.9
90	6.1	4.6	–	2.0 ± 0.1	7.1 ± 1.0	42.9 ± 0.3	47.9 ± 0.5

[a] The letter designations for individual brains are also used in Table 3. Because of the variance between injections, for further processing we used the subcellular fractions from the two brains (A and B) that were in closest overall agreement. A plot of the radioactivity per brain A plus brain B vs. time after injection gave a smooth curve. The dashes indicate that very little radioactivity was found.

[b] The crude myelin values are the sums of myelin I and myelin II (Fig. 1).

Specific radioactivity ratios were calculated from specific radioactivity values for different lipids from the same animals.

RESULTS

The radioactivity of the brain lipids decreased so rapidly with time that at 90 days after injection, the lipids contained less than one-twentieth of the radioactivity found at 1 day after injection (Table 2). The percentage distribution of radioactivity among the subcellular fractions was based on the amount of radioactivity recovered from the fractions. Between 30 and 40% of the original radioactivity and phospholipids in the dispersion was not recovered in the four subcellular fractions. Approximately one-half of the recovered radioactivity was found in the mitochondria–nerve ending fraction at all time intervals after injection. The crude myelin fraction contained from 22 to 43% of the radioactivity. Especially at the earlier time intervals, a large part of the radioactivity in the crude myelin fraction was not recovered after purification.

The radioactivity of the microsomal and mitochondrial fractions was highest at 1 day after injection and gradually decreased with time (Table 3). However, the radioactivity of the purified myelin fraction did not decrease until after 14 days and then the decrease was at a relatively slow rate. Most of the radioactivity in the lipids was found in the CPG and diacyl GPE (Table 4). With time, the proportion of radioactivity in the CPG from the microsomal and mitochondria–nerve ending fractions and the acid-stable EPG from the myelin and mitochondria–nerve ending fractions decreased rapidly. The proportions of the radioactivity in the alkenyl acyl GPE and cerebrosides increased throughout the first 60 days. In the alkenyl acyl GPE, the incorporation into the alkenyl groups was greater than the incorporation into the acyl groups, particularly in the microsomal fraction. After 60 days, the alkenyl acyl GPE and the cerebrosides contained 22 and 28%, respectively, of the radioactivity in the myelin lipids. The myelin lipids exhibited a pattern of labeling different from the microsomal and mitochondria–nerve ending fractions. Between 1 hr and 1 day after injection, over 50% of the radioactivity in the myelin fraction was found in the acid-stable EPG.

At all time periods and for all three subcellular fractions, the specific radioactivities calculated for the diacyl GPE were greater than those for the diacyl GPC (Figs. 2 and 3). Specific radioactivities of the alkenyl acyl GPE were plotted for the 16:0 alkenyl groups (Fig. 4) and the 18:1 acyl groups (Fig. 5). For this lipid, the specific radioactivities of the myelin fraction were highest at 14 days after injection.

TABLE 3. Radioactivity in the total lipids of subcellular fractions from mouse brain

Time after Injection	Purified Myelin		Microsomes		Nerve Endings + Mitochondria	
	A	B	A	B	A	B
days	*dpm/brain*					
1 hr	3330	2320	19640	7750	58170	38160
1	2100	2870	20230	17760	34970	36710
3	2520	2020	4630	10860	23710	21750
8	5670	1960	6260	3470	16940	7430
14	6020	2740	3920	1920	17560	4740
30	2610	2260	1480	1130	3630	3520
60	1590	1380	212	375	2610	2380
90	924	478	242	200	1430	1380

The values were not corrected for the amounts taken for the measurements given in Table 2.

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 4. Percentage distribution of radioactivity in the lipid classes from subcellular fractions

Time after Injection	SPG and IPG	Sph	CPG	Alkenyl Acyl GPE		Acid-stable EPG	LPL[a]	CB	CL
				Acyl	Alkenyl				
days				Microsomes					
1 hr	5.7	2.3	46.5	0.9	1.0	23.9	19.6		
1	8.7	6.9	47.3	0.8	3.3	20.3	13.0		
3	10.1	13.6	38.0	1.3	7.5	19.8	9.9		
8	13.0	11.2	38.5	1.1	10.8	18.9	7.3		
14	15.2	9.4	35.3	1.9	12.9	16.9	8.5		
30	10.3	8.7	35.3	1.7	13.1	19.5	11.7		
60	13.8	7.0	27.5	2.6	13.0	21.6	13.7		
				Purified Myelin					
1 hr	4.7	1.2	22.7	0.7	0.7	59.4	9.3	1.0	
1	7.5	2.4	23.8	0.8	2.3	45.1	11.1	3.5	
3	18.9	2.6	33.2	0.8	5.0	34.3	11.3	4.7	
8	17.0	4.0	25.8	4.7	8.2	21.0	8.6	9.5	
14	17.6	2.7	24.2	7.0	9.9	22.2	6.5	10.2	
30	15.5	2.6	19.2	9.9	10.9	13.2	7.5	18.9	
60	13.0	2.8	17.8	8.2	13.5	9.4	7.4	28.0	
				Nerve Endings + Mitochondria					
1 hr	5.9	1.2	43.3	0.4	0.6	39.8	11.0		
1	6.8	3.4	43.6	0.7	1.8	32.1	9.0		2.6
3	9.6	4.8	44.0	1.1	3.7	25.6	10.0		1.1
8	11.9	3.2	39.1	2.2	7.6	24.2	7.4		5.0
14	14.9	4.5	38.4	2.3	6.6	20.9	7.2		6.0
30	12.5	4.4	35.7	4.5	8.5	19.3	9.1		7.4
60	13.3	5.7	26.4	5.4	13.7	17.5	9.5		8.8

The lipids were separated in the first dimension with chloroform–methanol–15 N NH_4OH 65:25:4 (by volume). After exposure to HCl fumes, the thin-layer plates were developed in the second dimension with chloroform–methanol–15 N NH_4OH 100:50:12 (by volume). The mean values from two samples are given. The individual values for each time period did not differ by more than 10% of the value for major components. The data from 90 days after injection were not included because the amounts of radioactivity were very low. No cardiolipin was found in the myelin or microsomes and very little cerebroside was found in the nerve endings plus mitochondria fraction.

[a] LPL are the less polar lipids from the intersection of the two solvent fronts.

The half-lives of several lipid components (Table 5) were calculated for the period between 14 and 30 days after injection. By these times the transfer of lipids among subcellular fractions had reached an apparent equilibrium. The values for the same phosphoglyceride class from different membranes are not significantly different. Also, the half-lives of the diacyl GPC and the diacyl GPE are quite similar. Although the calculated values for the alkenyl acyl GPE are somewhat larger than for the diacyl GPE, they cannot be compared directly because the palmitate was not a direct precursor of the alkenyl groups and pulse labeling was not achieved.

TABLE 5. Half-lives of the ethanolamine and choline phosphoglycerides from subcellular fractions

Nonpolar Side Chains	Phosphoglycerides	Purified Myelin	Microsomes	Nerve Endings + Mitochondria
			days	
16:0 acyl groups	Diacyl GPC	8	8	6
16:0 acyl groups	Diacyl GPE	6	6	6
16:0 alkenyl groups	Alkenyl acyl GPE	38	11	22
18:1 acyl groups	Alkenyl acyl GPE	20	13	12

The half-lives were calculated from the means of the specific activities for 14 and 30 days using the equation: $\text{half-life} = \frac{(16)\,(0.301)}{(\log \text{sp act at 14 days}) - (\log \text{sp act at 30 days})}$.

DISCUSSION

Palmitic acid was incorporated into the lipids of all subcellular fractions, but at longer times after injection increasing proportions of radioactivity were found in alkenyl groups and in 18:0 and 18:1 acyl groups. Because of this conversion of 16:0 acyl groups to other acyl groups and alkenyl groups, the amount of radioactivity was corrected before calculating the values of specific activities for the different phosphoglycerides. Since very little of the injected free palmitic acid was available at the time these metabolites were formed (9, 10), part of the conversion must have been due to recycling of the 16:0 acyl groups.

Downloaded from www.jlr.org by guest, on June 19, 2012

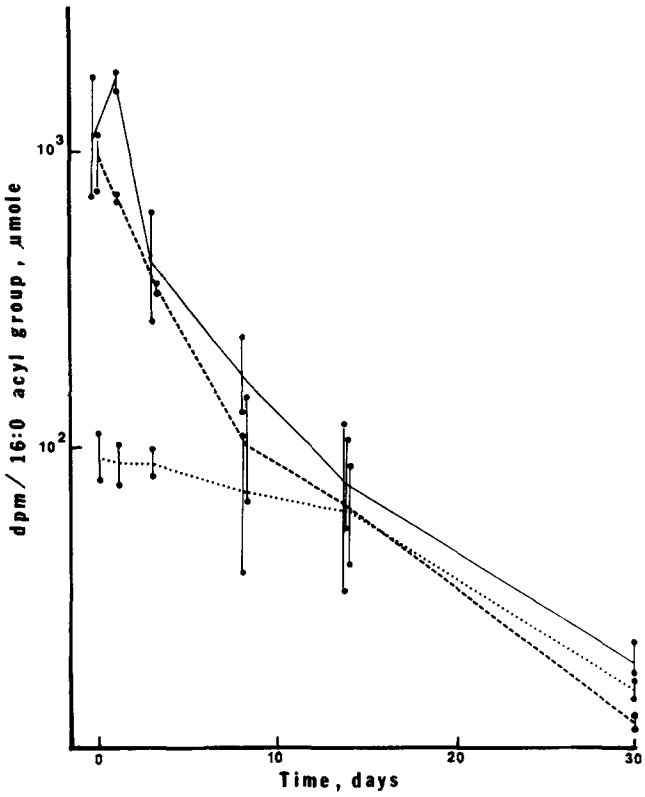

FIG. 2. Graph of the logarithms of the specific radioactivity of the palmitoyl groups from the diacyl GPC as a function of time after injection of [^{14}C]palmitate. The specific activities were calculated as described in the text. At each time period, the values for samples A and B (see Table 2) were plotted and lines were drawn through the means of the samples: (·····), purified myelin; (———), microsomes; (---------), nerve endings plus mitochondria fraction.

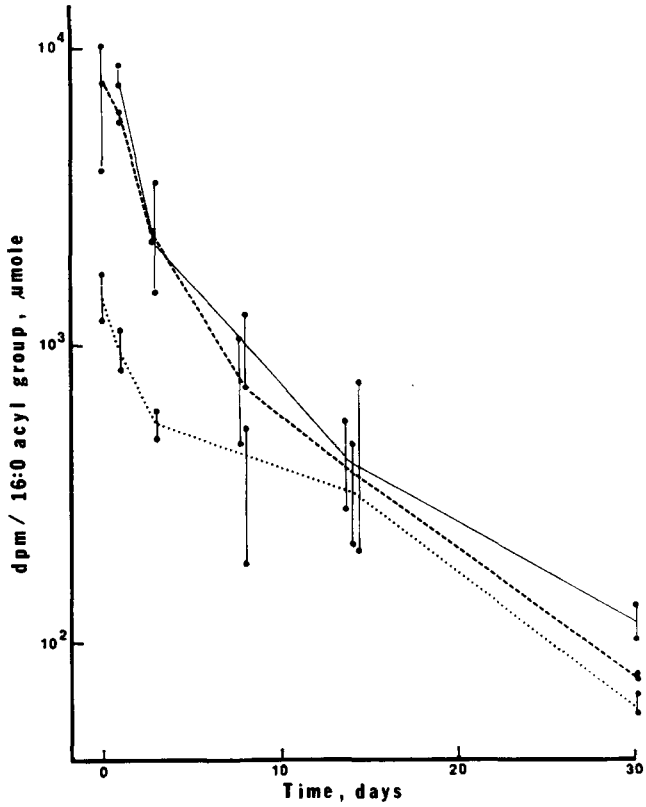

FIG. 3. Graph of the logarithms of the specific radioactivity of the palmitoyl groups from the diacyl GPE as a function of time after injection of [^{14}C]palmitate. Details were the same as in Fig. 2.

At 1 day, the specific radioactivity (dpm/μmole of 16:0 acyl groups) was at or near the peak for the diacyl GPE and diacyl GPC in the microsomes, mitochondria–nerve endings, and myelin (Figs. 2 and 3). At longer time intervals after the intracerebral injection of [^{14}C]palmitic acid, decreasing specific radioactivities were found for both of these lipids in all subcellular fractions, including myelin. If these lipids were formed from the same pool of diacyl glycerols in the endoplasmic reticulum, then the ratio of the specific radioactivities of the microsomal lipids should be constant with time. Indeed, the ratio of the specific radioactivity of the microsomal diacyl GPE was constant (0.16–0.19) during the period from 1 hr to 30 days after injection. As judged by ratios of the specific radioactivities, the microsomal diacyl GPC exchanged with the myelin diacyl GPC quite slowly, since the myelin-to-microsome ratio did not reach 0.75 until 14 days after injection. At most times, the specific radioactivities of the diacyl GPC and diacyl GPE in the mitochondria–nerve ending fraction were similar to those of the microsomal fraction, indicating that the exchange is quite rapid; this was also found by Miller and Dawson (22, 23) with other precursors.

For the alkenyl acyl GPE in the myelin fraction (Figs. 4 and 5), the specific radioactivity of the 18:1 acyl groups increased more slowly than that of the 16:0 alkenyl groups. Apparently, the 16:0 acyl groups were not a good precursor for the 18:1 acyl groups. The microsomal alkenyl acyl GPE exchanged with the myelin alkenyl acyl GPE very slowly, requiring 14–30 days for equilibration. A precursor–product relationship was evident for the microsomal and myelin alkenyl acyl GPE.

The specific radioactivity data are consistent with a synthesis of the CPG and EPG in the endoplasmic reticulum followed by a transfer to the other membranes. A similar relationship between liver microsomes and mitochondria (24, 25), brain microsomes and myelin (3), and brain microsomes and mitochondria (22, 23), has been shown previously with labeling of the polar portions of the molecules. In the central nervous system, the myelin sheath is in contact with the cytosol only at the nodes of Ranvier. The rate of exchange of myelin phosphoglycerides with microsomal phosphoglycerides

Downloaded from www.jlr.org by guest, on June 19, 2012

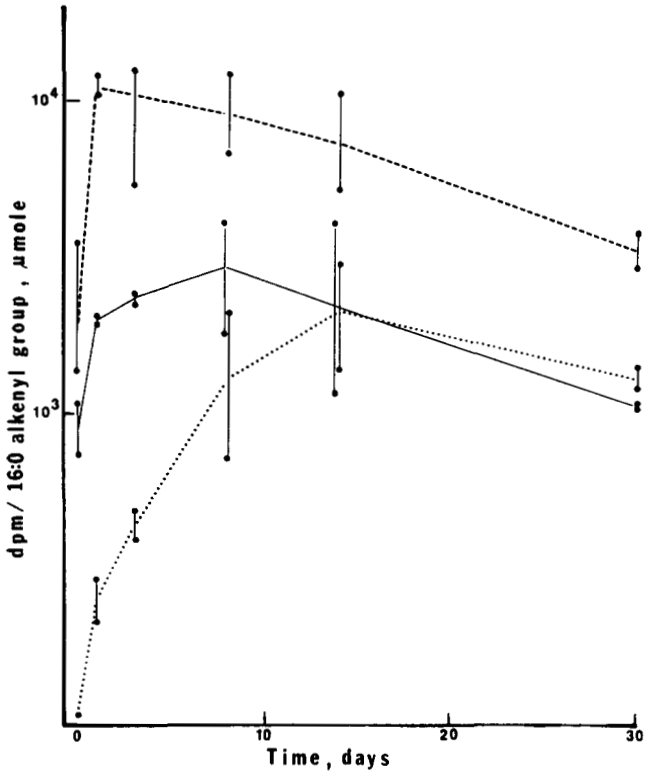

FIG. 4. Graph of the logarithms of the specific radioactivity of the hexadec-1-enyl groups from the alk-1-enyl acyl GPE as a function of time after injection of [^{14}C]palmitate. Details were the same as in Fig. 2, but no conversion factor was used (see text).

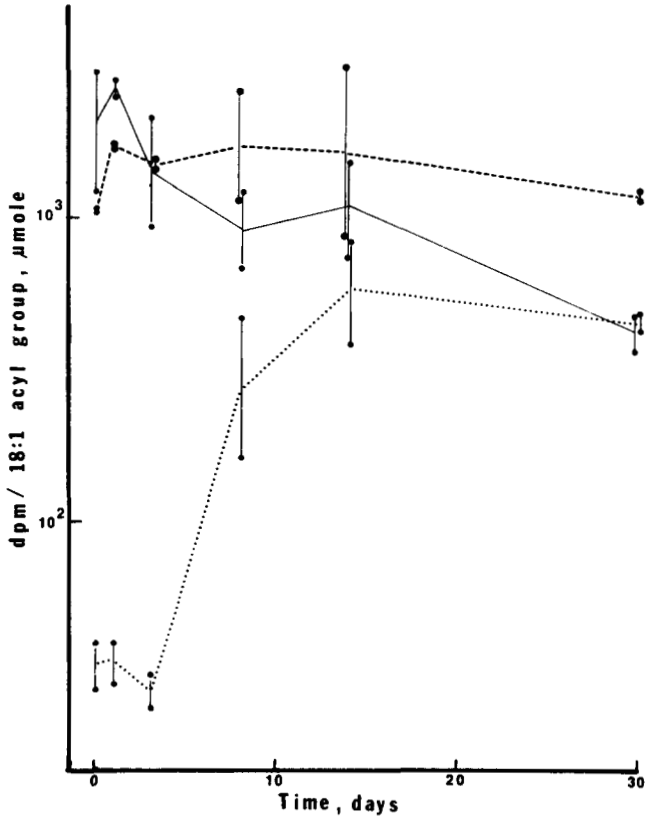

FIG. 5. Graph of the logarithms of the specific radioactivity of the octadecenoyl groups from the alk-1-enyl acyl GPE as a function of time after injection of [^{14}C]palmitate. Details were the same as in Fig. 2, but no conversion factor was used (see text).

may be influenced by the proportion of the myelin in contact with the cytosol, by the rate of lateral diffusion of myelin phosphoglycerides, and by the concentration of exchange proteins within the oligodendroglial cytosol.

The amount of radioactivity in the *total lipids* from the purified myelin did not decline until more than 2 wk after injection. The apparent delay may have been due to the time required for an equilibration of microsomal and myelin phospholipids, the greater proportion in the myelin of 18:1 acyl groups and 16:0 alkenyl groups that require additional synthetic steps, and recycling of radioactivity. The loss of a substantial amount of radioactivity during the purification of the crude myelin sample obtained at 1 hr after injection and the decreased specific radioactivity of the purified myelin suggest that the lost material had a relatively high specific radioactivity. The most likely source of this material is the "nascent myelin," i.e., the most recently formed myelin that had not been consolidated into the myelin sheath (15, 26, 27). Another possibility is that axoplasmic or glial membranes with a high specific radioactivity are removed from the crude myelin by the purification procedure.

The half-lives calculated for the 16:0 acyl groups of the diacyl GPC and diacyl GPE were 6–8 days (Table 5). The half-lives of the EPG from subcellular fractions of mouse brains from 1 to 7 days after intracerebral injections of [^{14}C]ethanolamine were 1.9–3.0 days for diacyl GPE and 2.4–3.2 days for alkenyl acyl GPE (28). The data from a similar experiment (29) for the small myelin fraction from rats gave half-lives of about 5 days for both the diacyl and alkenyl acyl GPE between 1 and 7 days after injection. For the myelin diacyl GPE, it is clear that the hypothesis of inert myelin is no longer tenable (30). The turnover of the alkenyl acyl GPE has been discussed in detail (28).

Previous investigations of the turnover of the hydrophobic moieties of brain lipids have been carried out using [^{14}C]acetate. Since the primary product of fatty acid biosynthesis from acetate is palmitic acid, the results should be comparable with those from experiments in which acetate was the radioactive precursor. For the present experiment, a plot of the logarithm of the total radioactivity in the brain lipids vs. time gave a curve that could be approximated by two straight lines. The half-lives taken from this plot were 5–7 days from 1 to

Downloaded from www.jlr.org by guest, on June 19, 2012

14 days after injection and 49–50 days from 30 to 90 days after injection. These values are much larger than those for the individual lipids after correction for the conversion of palmitoyl groups into other groups because the recycling is not counted as turnover.

This reutilization of palmitoyl groups also accounts for a disparity in the half-lives of the lipids from rat cerebral cortex (31). These half-lives were 5.1 days with glycerol as the precursor and 38 days with acetate as the precursor. The latter value compares well with our uncorrected value of 49–50 days for mouse whole brain lipids, but we have shown that this value is an apparent turnover rate that does not include the recycling of hydrophobic moieties. The CPG from rat brain mitochondria, microsomes, and synaptic vesicles have measured half-lives of 27–34 days with choline as the precursor (32), but with glycerol as the precursor the half-life of the CPG from cerebral microsomes of young rats was 2.7 days (33). The half-lives found for the 16:0 acyl groups in the brains of mature mice are slightly longer than the half-lives of phosphoglycerides labeled with glycerol (3, 31, 33) or ethanolamine (3, 5, 29). A longer half-life with palmitic acid as the precursor may be due to a greater degree of recycling of the palmitoyl groups released from the phosphoglycerides by hydrolytic catabolism because we would expect turnover of the palmitoyl groups when the glycerol or ethanolamine turn over.

The technical assistance of Mrs. Hildegarda Winniczek is gratefully acknowledged. This investigation was supported in part by PHS research grants NS-08291 and NS-09338 from the National Institute of Neurological Diseases and Stroke and PHS grant GRS-05563 from the National Institutes of Health.

Manuscript received 30 May 1972; accepted 16 November 1972.

REFERENCES

1. Ansell, G. B., and S. Spanner. 1967. The metabolism of labelled ethanolamine in the brain of the rat *in vivo*. *J. Neurochem.* **14:** 873–885.
2. Abdel-Latif, A. A., and L. L. Abood. 1965. Incorporation of ortho [^{32}P]phosphate into the subcellular fractions of developing rat brain. *J. Neurochem.* **12:** 157–166.
3. Lapetina, E. G., G. Rodriguez de Lores Arnaiz, and E. DeRobertis. 1969. ^{32}P incorporation into different membranous structures separated from rat cerebral cortex. *J. Neurochem.* **16:** 101–106.
4. Jungalwala, F. B., and R. M. C. Dawson. 1971. The turnover of myelin phospholipids in the adult and developing rat brain. *Biochem. J.* **123:** 683–693.
5. Horrocks, L. A. 1969. Metabolism of the ethanolamine phosphoglycerides of mouse brain myelin and microsomes. *J. Neurochem.* **16:** 13–18.
6. Smith, M. E., and L. F. Eng. 1965. The turnover of the lipid components of myelin. *J. Amer. Oil Chem. Soc.* **42:** 1013–1018.
7. Cuzner, M. L., A. N. Davison, and N. A. Gregson. 1966. Turnover of brain mitochondrial membrane lipids. *Biochem. J.* **101:** 618–626.
8. Smith, M. E. 1967. The metabolism of myelin lipids. *Advan. Lipid Res.* **5:** 241–278.
9. Sun, G. Y., and L. A. Horrocks. 1971. The incorporation of [^{14}C]palmitic acid into the lipids of mouse brain *in vivo*. *J. Neurochem.* **18:** 1963–1969.
10. Sun, G. Y., and L. A. Horrocks. 1969. The metabolism of palmitic acid in the phospholipids, neutral glycerides and galactolipids of mouse brain. *J. Neurochem.* **16:** 181–189.
11. Horrocks, L. A. 1967. Composition of myelin from peripheral and central nervous systems of the squirrel monkey. *J. Lipid Res.* **8:** 569–576.
12. Sun, A. Y., and T. Samorajski. 1970. Effects of ethanol on the activity of adenosine triphosphatase and acetylcholinesterase in synaptosomes isolated from guinea-pig brain. *J. Neurochem.* **17:** 1365–1372.
13. Sun, A. Y., G. Y. Sun, and T. Samorajski. 1971. The effect of phospholipase C on the activity of adenosine triphosphatase and acetylcholinesterase in synaptic membranes isolated from the cerebral cortex of squirrel monkey. *J. Neurochem.* **18:** 1711–1718.
14. Whittaker, V. P. 1968. The morphology of fractions of rat forebrain synaptosomes separated on continuous sucrose density gradients. *Biochem. J.* **106:** 412–417.
15. Sun, G. Y., and L. A. Horrocks. 1970. The acyl and alk-1-enyl groups of the major phosphoglycerides from ox brain myelin and mouse brain microsomal, mitochondrial and myelin fractions. *Lipids.* **5:** 1006–1012.
16. Horrocks, L. A. 1968. The alk-1-enyl group content of mammalian myelin phosphoglycerides by quantitative two-dimensional thin-layer chromatography. *J. Lipid Res.* **9:** 469–472.
17. Horrocks, L. A., and G. Y. Sun. 1972. Ethanolamine plasmalogens. *In* Research Methods in Neurochemistry. R. Rodnight and N. Marks, editors. Plenum Press, New York. 223–231.
18. Gottfried, E. L. 1967. Lipids of human leukocytes: relation to cell type. *J. Lipid Res.* **8:** 321–327.
19. Sun, G. Y., and L. A. Horrocks. 1968. The fatty acid and aldehyde composition of the major phospholipids of mouse brain. *Lipids.* **3:** 79–83.
20. Sun, G. Y., and L. A. Horrocks. 1969. Acyl and alk-1-enyl group compositions of the alk-1′-enyl and the diacyl glycerophosphoryl ethanolamines of mouse and ox brain. *J. Lipid Res.* **10:** 153–157.
21. Bennett, M., and E. Coon. 1966. Inexpensive cartridge for the collection of radioactive methyl esters from gas–liquid chromatographs. *J. Lipid Res.* **7:** 448–449.
22. Miller, E. K., and R. M. C. Dawson. 1972. Can mitochondria and synaptosomes of guinea-pig brain synthesize phospholipids? *Biochem. J.* **126:** 805–821.
23. Miller, E. K., and R. M. C. Dawson. 1972. Exchange of phospholipids between brain membranes *in vitro*. *Biochem. J.* **126:** 823–835.
24. Wirtz, K. W. A., and D. B. Zilversmit. 1968. Exchange of phospholipids between liver mitochondria and microsomes *in vitro*. *J. Biol. Chem.* **243:** 3596–3602.
25. McMurray, W. C., and R. M. C. Dawson. 1969. Phospholipid exchange reactions within the liver cell. *Biochem. J.* **112:** 91–108.
26. Eng, L. F., and E. P. Noble. 1968. The maturation of rat brain myelin. *Lipids.* **3:** 157–162.
27. Cuzner, M. L., and A. N. Davison. 1968. The lipid composition of rat brain myelin and subcellular fractions during development. *Biochem. J.* **106:** 29–40.

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 19, 2012

28. Horrocks, L. A. 1972. Content, composition and metabolism of mammalian and avian lipids with ether groups. *In* Ether Lipids: Chemistry and Biology. F. Snyder, editor. Academic Press, New York. 177–272.
29. Ansell, G. B., and S. Spanner. 1968. The long-term metabolism of the ethanolamine moiety of rat brain myelin phospholipids. *J. Neurochem.* **15:** 1371–1373.
30. Davison, A. N. 1964. Myelin metabolism. *In* Metabolism and Physiological Significance of Lipids. R. M. C. Dawson and D. N. Rhodes, editors. John Wiley & Sons, London. 527–540.
31. Lapetina, E. G., G. Rodriguez de Lores Arnaiz, and E. DeRobertis. 1969. Turnover rates for glycerol, acetate, and orthophosphate in phospholipids of the rat cerebral cortex. *Biochim. Biophys. Acta.* **176:** 643–646.
32. Lapetina, E. G., G. G. Lunt, and E. DeRobertis. 1970. The turnover of phosphatidyl choline in rat cerebral cortex membranes in vivo. *J. Neurobiol.* **1:** 295–302.
33. Abdel-Latif, A. A., and J. P. Smith. 1970. *In vivo* incorporation of choline, glycerol and orthophosphate into lecithin and other phospholipids of subcellular fractions of rat cerebrum. *Biochim. Biophys. Acta.* **218:** 134–140.

ASBMB JOURNAL OF LIPID RESEARCH JLR

Downloaded from www.jlr.org by guest, on June 19, 2012