Metabolism of Glycerophospholipids of Myelin and Microsomes in Rat Brain

REUTILIZATION OF PRECURSORS*

(Received for publication, September 13, 1976, and in revised form, January 17, 1977)

SHELDON L. MILLER,† JOYCE A. BENJAMINS,§ and PIERRE MORELL¶

From the Biological Sciences Research Center and Department of Biochemistry and Nutrition, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

The metabolic turnover of phospholipids of rat brain myelin and microsomes was investigated after 17-day-old animals received intracranial injections of [2-3H]glycerol and either [1,3-14C]glycerol, [1-14C]choline, [1-14C]acetate, [1-14C]ethanolamine, [methyl-14C]choline, or [3-32P]orthophosphate. The turnover rate, with respect to the 3H at the C-2 position of the glycerol moiety of individual lipids, was calculated for the first 15 days after injection (rapid phase) and for the time period between 15 and 80 days following injection (slow phase). The results for the half-life of phosphatidylethanolamine plasmalogen and phosphatidylethanolamine in microsomes were similar (3 to 4 days in the fast phase and 13 to 14 days in the slow phase, respectively). In myelin, the corresponding values were 6 to 10 days in the fast phase and 25 days in the slow phase.

The isotope ratio (3H/14C or 3H/32P) was determined for each lipid of microsomes and myelin at each time point studied. In each case, the ratios declined as a function of time, indicating a preferential recycling of the 3C- or 3P-labeled precursor relative to [2-3H]glycerol. Reference to the data for the turnover rate of [3H]glycerol in these phospholipids (see above), together with the isotope ratio data, made possible a calculation of the apparent half-life of the other radioactive moiety (incorporated as base, phosphate group, fatty acid, or vinyl ether) with many fewer data points than would be the case if experiments involving only a single radioactive label were carried out.

When [14C]choline or [14C]ethanolamine was a precursor, the half-lives of the base moieties of phosphatidylcholine and phosphatidylethanolamine were 26 days in the microsomes and 39 and 33 days, respectively, for myelin. The half-life of ethanolamine in the plasmalogen was significantly longer, 40 and 58 days, in microsomes and myelin, respectively. The half-lives for [32P]phosphate in each of the individual lipid classes were slightly shorter or the same as that of the labeled base.

With respect to [14C]acetate as a precursor, the half-lives of phosphatidylcholine and phosphatidylethanolamine were 28 and 63 days, respectively. The corresponding values for the myelin fraction were 54 and 125 days. Therefore, although the glycerol moieties of phosphatidylcholine and phosphatidylethanolamine are metabolized at about the same rate, acyl moieties are preferentially reutilized for synthesis of phosphatidylethanolamine.

Since the work of Smith (see Ref. 1 for a review), it has been generally accepted that the lipids in myelin are not metabolically stable. Values reported for the half-life of phospholipids from rat brain subcellular fractions vary considerably (e.g. the reported half-life of phosphatidylcholine in myelin varies from less than 10 days (2) to 167 days (3). Sources of experimental variation that might explain these differences within a given species include injection routes, age of animals, and the use of different labeled precursors, which may be reutilized to different extents. The suggestion that the use of different labeled precursors can lead to differences in observed half-life for a given membrane phospholipid is supported by previous results in diverse membrane systems (4, 5), including brain (6-10).

This investigation was designed to test the hypothesis that brain membrane phospholipids are catabolized to their constituent moieties during the normal course of metabolic turnover and that, to varying degrees, these constituents are reutilized for de novo synthesis of phospholipids. We took advantage of the fact that [2-3H]glycerol represents a good pulse label for brain phospholipid (11). After incorporation into phospholipids, reutilization of the label from this precursor is an inefficient process because, once the phospholipid is catabolized to the level of glycerol 3-phosphate, the isotope is likely to be lost.

1 The abbreviations used are: phosphatidylcholine, 1,2-diacyl-sn-glycero-3-phosphorylcholine; phosphatidylethanolamine, 1-alkenyl-2-acyl-sn-glycero-3-phosphorylcholine; phosphatidylethanolamine plasmalogen, 1-alkenyl-2-acyl-sn-glycero-3-phosphorylethanolamine; phosphatidylethanolamine plasmalogen, 1-alkenyl-2-acyl-sn-glycero-3-phosphorylethanolamine.
by a rapid interchange of glycerol 3-phosphate with dihydroxyacetone phosphate, with accompanying loss of \[^{3}H\](via \[^{3}H\]NADH) to water (11), a reaction catalyzed by glycerol phosphate dehydrogenase (EC 1.1.1.8). The \[^{3}H\] may also be lost to the respiratory chain by a mitochondrial glycerophosphate dehydrogenase (EC 1.1.99.5) and transferred to water without the mediation of a reduced pyridine nucleotide.

The experimental approach consisted of giving injections to young rats of \[^{2}H\]glycerol and a \[^{14}C\] or \[^{32}P\]-labeled precursor of another phospholipid constituent (e.g. \[^{14}C\]acetate to label acyl or alkyl moieties, \[^{32}P\]phosphate to label the phosphoryl moiety, or \[^{14}C\]choline to label the base of phosphatidylcholine). The change in \[^{3}H\]/\[^{14}C\] (or \[^{3}H\]/\[^{32}P\]) isotope ratio with time was used to determine the extent of reutilization of the \[^{3}H\] or \[^{32}P\]-labeled moiety for de novo synthesis of phospholipids relative to the turnover of \[^{2}H\]glycerol.

These experiments were carried out simultaneously with lipids of the microsomal and myelin fractions to clarify the possible role of subcellular compartmentation with regards to reutilization of phospholipid precursors. The data obtained in this study also made possible certain conclusions about the relative roles of the glycerol 3-phosphate and dihydroxyacetone phosphate pathways in the synthesis of diacyl phospholipids and alkyl-acyl phospholipids.

**EXPERIMENTAL PROCEDURES**

**Materials** – \[^{2}H\]Glycerol (200 mCi/mmol), \[^{3}H\]Acetate (100 mCi/mmol), \[^{14}C\]Choline (2.3 Ci/mmol), \[^{13}C\]Glycerol (57 mCi/mmol), \[^{1,2-14}C\]Cholesterol (6.3 mCi/mmol), \[^{14}C\]Glycerol (44 mCi/mmol), \[^{14}C\]Acetate (2.2 mCi/mmol), \[^{3}H\]Glucose (247 mCi/mmol), and \[^{32}P\]Orthophosphate (carrier free) were purchased from New England Nuclear, Boston, Mass. All other chemicals were of reagent grade or as specified in the appropriate references.

Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Wilmington, Mass., and were bred in our own animal care facilities. Litters were reduced to 8 to 10 animals at birth and weaned at 21 days of age.

**Labeling Protocol** – Litters of rats, at 17 days of age, were given intracranial injections (12) of 20 \mu l of a solution of 0.9% NaCl solution (saline) buffered to a pH of 7.4 with 0.01 M sodium hydroxide when necessary and reduced in volume under a stream of nitrogen to give the appropriate injection volume.

Thin layer chromatography was carried out on precoated (250 \mu m thick) Analtech Silica Gel G plates (20 x 20 cm) (Fisher Scientific Co., Raleigh, N. C.). Lipid standards were obtained from Serdary Research Laboratories (London, Ontario, Canada) Phosphatidylcholine (cabbage) and alkaline phosphatase (type III, Escherichia coli) were purchased from Sigma Chemical Co., St. Louis, Mo. All other chemicals were of reagent grade or as specified in the appropriate references.

**Sprague-Dawley rats** were obtained from Charles River Breeding Laboratories, Wilmington, Mass., and were bred in our own animal care facilities. Litters were reduced to 8 to 10 animals at birth and weaned at 21 days of age.

**Labeling Protocol** – Litters of rats, at 17 days of age, were given intracranial injections (12) of 20 \mu l of a solution of 0.9% NaCl solution (saline) buffered to a pH of 7.4 with 0.01 M sodium hydroxide containing the radioactive compounds specified in the figure legends (the \[^{32}P\]-containing solutions did not have phosphatase buffer added).

**Preparation of Microsomes and Myelin** – The rats given injections were maintained in our animal colony, killed at intervals over the next 80 days, and processed individually. After decapitation, the brain was rapidly removed. The forebrain (12) was homogenized in 9 volumes of 0.32 M sucrose and then diluted with an additional 10 volumes of 0.32 M sucrose. Half of the homogenate was used to isolate a microsomal fraction (13). The remaining homogenate was diluted to 18 ml with 0.32 M sucrose and used to isolate myelin by a slight modification of the method of Norton and Poduslo (14). Crude myelin was collected at the interface between 0.32 and 0.85 M sucrose in the following centrifugation step. chill and buffer solution was added to a final solution of 0.85 M sucrose that contained 0.5 volumes (w/w) of cold 0.32 M sucrose following centrifugation in a Beckman SW-27 rotor at 82,500 \times g for 15 min. The myelin was resuspended with ice-cold water, collected by centrifugation at 82,500 \times g for 30 min. The myelin was resuspended in ice-cold water, collected by centrifugation at 82,500 \times g for 15 min. The microsomal and myelin membranes were resuspended in small amounts of water and lyophilized.

**Extraction and Separation of Lipids** – Lyophilized membrane fractions were resuspended in 0.6 M sucrose and then washed using a modification (13) of the procedure of Folch et al. (15). The organic phase that contained the phospholipids was taken to dryness in a warm water bath under a stream of nitrogen.

**Lipid Extraction** – Half of each membrane preparation was subjected to two-dimensional thin layer chromatography (16) for separation of individual lipids. This procedure utilized chloroform/methanol/water (65:25:4 by volume) in the first direction, an acid hydrolysis step, and chromatography in the second direction with chloroform/methanol/acetone/acetic acid/water (75:15:30:15:7.5 by volume). The intermediate bands were scraped off, eluted with chloroform/methanol/water (7:7:1 by volume). Extracts were combined and taken to dryness under a stream of nitrogen.

**Distribution of Label between Acyl and Glycerol Moieties** – Individual lipids, collected after thin layer chromatography as outlined above, were suspended for 1 h in 3 ml of chloroform, 0.2 M methanol, and 0.2 M sodium carbonate (pH 10.5), and were boiled with the original aqueous phase. Radioactivity was measured as described below, and the proportion of total \(^{3}H\) radioactivity or \(^{14}C\) radioactivity in glycerophospholipid base moieties was calculated.

**Distribution of \(^{3}H\) between the Glycerol Carbons** – Individual lipids were collected from thin layer chromatography plates and subjected to alkaline methanolysis followed by reaction with periodate and Dimedone (Eastman Kodak Co., Rochester, N. Y.) (18). This procedure separates the C-1 carbon of glycerol (precipitate) from the C-2 and C-3 carbons (soluble). The \(^{3}H\) in the precipitate and the soluble fraction were determined as described below, and the proportion of the label in the soluble fraction was calculated. This corresponded to radioactivity in the C-2 position. These experiments were carried out simultaneously with lipids of the microsomal and myelin fractions to clarify the possible role of subcellular compartmentation with regards to reutilization of phospholipid precursors. The data obtained in this study also made possible certain conclusions about the relative roles of the glycerol 3-phosphate and dihydroxyacetone phosphate pathways in the synthesis of diacyl phospholipids and alkyl-acyl phospholipids.

**Measurement of Radioactivity** – For determination of radioactivity in individual lipids following thin layer chromatography, the silica gel containing the phospholipid was scraped into a scintillation vial, the silica gel was deactivated by addition of 0.4 M of water, and 10 ml of Triton X-100 solution (1.4 by volume) scintillation solvent (16) was added. Samples from alkaline methanolysis or from the glycerol degradation procedure were also counted in this solvent (samples in organic solvents were first evaporated to dryness). Radioactivity was quantitated in a Packard Tri-Carb model 3800 liquid scintillation spectrometer. Individual times after injection with \[^{2}H\]glycerol, were subjected to phospholipase D treatment (19). The phosphatidic acid product was degraded as described by Agronoff and Hajar (18). This procedure, which distinguishes between \[^{3}H\] label on each of the glycerol carbons, showed no label at the C-3 position.

**Autoradiography** – The specificity of incorporation of \[^{14}C\]choline into phosphatidylcholine or \[^{3}H\]choline into phosphatidylethanolamine was confirmed by Beckman scanning microdensitometry on a layer chromatography plates using Kodak No-Screen x-ray film (only the \(^{14}C\)-labeled lipids darken the film under these conditions) (20).
was determined by taking 1 S.D. of the slope and adding it to and subtracting it from the calculated slope. These slopes were divided into log 2 to give the extreme values for half-lives. Because this is a logarithmic function, the range, in days, is not symmetrical on both sides of the calculated half-life.

The smooth curve fit to the experimental points of Fig. 3 is a linear regression to the exponential function:

\[ Y = P_0 e^{r_1 x} + P_1 e^{r_2 x} + P_2 \]

where \( Y \) equals log dpm and \( X \) equals time after injection in days. This function gave a better fit than either a hyperbolic or a simple exponential equation.

**RESULTS**

**Experimental Plan**

Litters of 8 to 10 animals were given injections of \(^{[3]H}\)glycerol and one of the other isotopes, and individual animals were killed at various times over the subsequent 80-day period. Myelin and microsomes were isolated, and lipids were extracted. The total lipid extract obtained from each subcellular fraction was divided and subjected to thin layer chromatography in duplicate. Individual lipid classes from one chromatography plate were scraped directly into scintillation vials, radioactivity was quantitated, and the absolute \(^1H\) radioactivity as well as the \(^{14}C/^{3}H\) or \(^{31}P/^{3}H\) ratio was tabulated. Lipid classes isolated from the second plate were collected and subjected to chemical degradation to determine label distribution within the lipid class. Despite the fact that a different \(^{14}C\) or \(^{31}P\) compound was injected in each experimental protocol, each litter represented a replicate experiment with regards to \(^{[3]H}\)glycerol. Since some of the experiments were repeated, approximately 70 experimental points were available to determine half-lives of the glycerol moiety of each lipid in the two subcellular fractions. Results regarding phosphatidylcholine, phosphatidylethanolamine, and phosphatidylethanolamine plasmalogen are given in detail.

Results, but not individual data points, are described for phosphatidylcholine plasmalogen. Although statistical analysis of the phosphatidylcholine plasmalogen data indicates a high degree of significance, it is possible that the results were systematically biased during the collection of samples from the chromatography plates by inclusion of small amounts of phosphatidylcholine. Because of the much greater amount of phosphatidylcholine relative to phosphatidylcholine plasmalogen (22) and the concomitant greater levels of radioactivity, a trailing of 2% of the phosphatidylcholine into the phosphatidylcholine plasmalogen region can introduce a possible systematic error of up to 50% for phosphatidylcholine plasmalogen. Thus, the results, although briefly reported, may simply reflect contamination of the phosphatidylcholine plasmalogen by phosphatidylcholine. This was potentially more of a problem in the present work (since we were interested in quantitative recovery and therefore scraped a large area of the silica gel) than in a previous study (10) (in which we were interested only in isotope ratios and could selectively scrape only the phosphatidylcholine plasmalogen area not likely to be contaminated by phosphatidylcholine). The possibility that a small fraction of one of the ester-linked fatty acids of phosphatidylcholine is hydrolyzed, leading to contamination of the phosphatidylcholine plasmalogen, also has not been eliminated.

Data for phosphatidylserine were also subject to sampling errors because, under our chromatography conditions, this lipid trailed severely. The data for this lipid in myelin was so scattered that it was not significant and is not reported. The statistically significant data for phosphatidylserine in microsomes are briefly indicated.

**Metabolism of the Glycerol Moiety**

Relative Distribution of \(^{3}H\) between Glycerol and Acyl Moieties—Fig. 1 shows the percentage of lipid \(^{3}H\) in glycerol for phosphatidylcholine, phosphatidylethanolamine, and phosphatidylethanolamine plasmalogen in microsomes and myelin. The results, with respect to initial incorporation of \(^{3}H\) into

![Fig. 1. Distribution of radioactivity between glycerol and acyl moieties of phospholipids following intracranial injection of 200 to 400 \(\mu\)Ci of [2-\(^{3}H\)]glycerol into 17-day-old rats. Individual animals were killed at the indicated times, the myelin and microsomal fractions were isolated, total lipids were extracted, and a portion (50%) was fractionated by thin layer chromatography. Individual lipid classes were eluted and subjected to alkaline methanolysis. The proportion of \(^{3}H\) in the aqueous phase (presumably in the glycerol moiety of glycerophosphoryl base) and organic phase (acyl methyl esters) was quantitated. Lines were fit to the data points by a linear regression program (\(p < 0.001\) in each case), with the exception of the myelin phosphatidylethanolamine plasmalogen (PE PI) data, which was fit by visual inspection. In this and subsequent figures, the data for phosphatidylethanolamine data does not include the radioactivity in the alkenyl moiety, which is cleaved during the thin layer chromatography procedure. In these experiments, the animals received either a \(^{14}C\)lipid precursor or \(^{31}P\)orthophosphate in addition to the [2-\(^{3}H\)]glycerol. In this (and subsequent figures), the \(^{3}H\) radioactivity levels studied can be back-calculated from data in Fig. 3. Even at the later \(t\)ime points, radioactivity levels were still above those necessary for statistically significant results. The average \(^{3}H\) in glycerol at the last time point for phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylethanolamine plasmalogen of microsomes were 1657, 534, and 228 dpm, respectively. For myelin, the equivalent values were 4488, 965, and 1085. These numbers represent radioactivity on the thin layer plate from one-half of the total lipid extracted from the subcellular fractions. Material remaining after a portion was taken for this label distribution study was used for quantitation of \(^{3}H\) radioactivity (Fig. 3) and for determination of isotope ratios (following figures).
the glycerol portion of the phospholipid relative to acyl moieties (80 to 95%) and with respect to decrease with time of percentage of lipid $^3$H in glycerol, do not vary markedly for any of the lipids, except for the initially more severe drop in the case of myelin phosphatidylethanolamine plasmalogen. For phosphatidylethanolamine plasmalogen, the amount of label in the long chain hydrocarbons would be approximately doubled if the cleaved alkenyl residue was accounted for. Results were also obtained for microsomal phosphatidylserine (y intercept = 89, slope = -0.14), microsomal phosphatidylcholine plasmalogen (y intercept = 95, slope = -0.28), and myelin phosphatidylcholine plasmalogen (y intercept = 94, slope = -0.30).

Percentage of $^3$H Glycerol at the C-2 Position—Phosphatidylcholine and phosphatidylethanolamine in myelin and microsomes initially have all of the $^3$Hglycerol in the C-2 position (Fig. 2). Even by 80 days following injection, most of the label is retained at the C-2 position, and in the extreme case, that of phosphatidylethanolamine in microsomes, less than 20% of the label is in the C-1 position at 80 days following injection. The phosphatidylethanolamine plasmalogen data show a contrast between the two subcellular fractions. In microsomes, phosphatidylethanolamine plasmalogen behaves in a manner analogous to the other lipids, whereas in the myelin lipid a large percentage of the label is found at the C-1 position even at early time points. Randomization of the label with time probably indicates a certain degree of recycling of the label from $[2-^3$H]glycerol (see "Discussion").

In analogous plots, phosphatidylserine and phosphatidylcholine plasmalogen in microsomes and phosphatidylethanolamine plasmalogen in myelin also showed some randomization of the $^3$H at the C-2 position of glycerol: phosphatidylserine microsomes (y intercept = 101, slope = -0.21), phosphatidylethanolamine plasmalogen microsomes (y intercept = 102, slope = -0.29), and phosphatidylcholine plasmalogen myelin (y intercept = 100, slope = -0.18).

Turnover of $[2-^3$H]Glycerol—$^4$C radioactivity in individual lipids was calculated, on the basis of the fraction of the original homogenate analyzed, to give the value expected if the entire preparation of microsomes or myelin had been used for analysis. Since the actual amount of $[^3$H]glycerol varied from experiment to experiment, this value was normalized to that expected if 100 μCi of $[^3$H]glycerol had been injected. These values were further corrected to include only the $^3$H in the C-2 position of glycerol using the data in Figs. 1 and 2. The resultant data were plotted as log disintegrations per min versus time after injection (Fig. 3). There is clearly more than one component of decay; straight lines were arbitrarily fitted separately for the first 15 days and for the later time period (dashed lines in Fig. 3), and the half-lives were calculated from these lines.

In each case, the half-life for the first 15 days following injection was significantly shorter than the half-life for the subsequent 2-month period. Half-lives for individual lipids in each membrane fraction and the statistical significance of the linear approximations (dashed line) are indicated in Fig. 3. Although the data points are not shown, the half-life of phosphatidylserine in microsomes in the first 14 days was 4.5 days (range, 3.5 to 6.0 days), and for the subsequent 2-month period it was 18 days (range, 15 to 23 days). Microsomal phosphatidylethanolamine plasmalogen turnover for the first 14 days was 5.0 days (range, 4.0 to 7.0 days), and for the next 2 months it was 11 days (range, 10 to 12 days). Myelin phosphatidylcholine plasmalogen turnover for the first 15 days was 8.5 days (range, 6.5 to 12), and for the subsequent 2-month period it was 31 days (range, 27 to 38 days).

A smooth curve (solid lines on Fig. 3) was also fit to the experimental points. No biological interpretation of the complex equation (see "Experimental Procedures") that fits the curve is feasible. However, readings from this curve were used to normalize the isotope ratio data (see below).

Comparison of $[2-^3$H]- and $[1,3-$¹⁴C]Glycerol Turnover—Animals were given injections of $[2-^3$H]glycerol and $[1,3-$¹⁴C]glycerol, individual lipids were isolated from brain microsomes and myelin as described above, and the $^3$H/¹⁴C ratio was determined. For establishment of the proportion of lipid $¹⁴C$ that could be attributed to glycerol, an aliquot of the lipids was subjected to alkaline methanolysis, and the percentage of lipid $¹⁴C$ in glycerol was plotted as a function of time (Fig. 4).

The isotope ratio data were corrected to include only the $^3$H at the C-2 position of glycerol (using Figs. 1 and 2) and only the $¹⁴C$ in glycerol (using Fig. 4), and the $^3$H/$¹⁴C$ ratio for each lipid was plotted as a function of time (Fig. 5). The initial $^3$H/$¹⁴C$ ratio was less than 3 for myelin phosphatidylethanolamine plasmalogen, relative to the $^3$H/$¹⁴C$ ratio of over 30 for myelin phosphatidylethanolamine and phosphatidylcholine. This marked difference is most easily accounted for by the assumption that dihydroxyacetone phosphate is an intermediate in the synthesis of the plasmalogen. This effect is not as marked in microsomal phosphatidylethanolamine plasmalogen, although the differences in its isotope ratio as compared to the microsomal diacyl lipids are still highly significant. The contrast between the long term stability of the isotope ratio for...
Metabolism of Glycerophospholipids in Rat Brain

Metabolism of Phosphate and Base Moieties

Ethanolamine Turnover - Microsomes and myelin were isolated at various times after young rats were given injections of a mixture of [2-3H]glycerol and [1,2-14C]ethanolamine. Following separation from other lipids by thin layer chromatography, phosphatidylethanolamine and phosphatidylethanolamine plasmalogen (actually lyso phosphatidylethanolamine plasmalogen because of the acid hydrolysis during chromatography) were eluted, and an aliquot of each sample was subjected to alkaline methanolysis. Radioactivity in the organic and aqueous fraction was determined. The percentage of 14C radioactivity, converted to an aqueous-soluble form, presumably in ethanolamine (23), was constant as a function of time after injection for both phosphatidylethanolamine and phosphatidylethanolamine plasmalogen in both membrane fractions. The values are 82 and 75% for phosphatidylethanolamine of microsomes and myelin, respectively, and 93 and 96% for phosphatidylethanolamine plasmalogen of these same subcellular fractions.

A second aliquot from each of the unhydrolyzed ethanolamine-containing lipid samples was used to quantitate 3H and 14C radioactivity. After correction of the 3H radioactivity to include only the label at the C-2 position of glycerol (Figs. 1 and 2) and correction of 14C radioactivity to include only label in the base, the 3H/14C ratio was plotted as a function of time after injection (Fig. 6).

The decrease of the 3H/14C ratio indicated that [3H]glycerol is being metabolized much more rapidly than [14C]ethanolamine or, conversely, after catabolism of the lipid, ethanolamine is reutilized preferentially for synthesis of phospholipids. For quantitation of the decay of the ethanolamine moieties, the 3H/14C ratio of each sample was divided into the absolute whole brain [3H]glycerol radioactivity present at the same time point for that particular phospholipid in the membrane fraction under consideration. As an example, consider the isotope ratio of 0.51 for phosphatidylethanolamine in microsomes at 32 days (Fig. 6). This was divided into the average 3H radioactivity in that same lipid at the same time point, 1.27 × 10^3 cpm, obtained from the smooth curve fit to the phosphatidylethanolamine microsome portion of Fig. 3. This data treatment gave a normalized value of 2.5 × 10^2 for [14C]ethanolamine radioactivity (in essence, each single etha-
Metabolism of Glycerophospholipids in Rat Brain

Radioactivity in the resultant aqueous fraction (presumably in the glycerol portion) was quantitated. Lines were fit to the data points by a linear regression. For determination of the decay curves of choline in microsomal and myelin fractions (Fig. 4). The isotope ratio was calculated for this time period (e.g. it is clear that the phosphatidylethanolamine plasmalogen in microsomes was 220, 35, and 34 dpm, respectively, whereas for myelin the equivalent values were 481, 95, and 147 dpm. The $^3$H values recorded were higher, as can be determined from the $^{3}$H/$^{14}$C ratio values.

Fig. 4. Distribution of radioactivity between glycerol and acyl moieties of phospholipid following intracranial injection of 17 $\mu$Ci of [1,2,3$^{14}$C]glycerol into 17-day-old rats. Animals were killed at the indicated times following injection with both radioactive precursors, and lipids were extracted from microsomal and myelin fractions (Fig. 4). The isotope ratio was constant with time, indicating that the radioactivity was corrected to include only that present in the glycerol moiety (Fig. 4). Lines were fit by linear regression. For microsomal phosphatidylethanolamine (PE) and phosphatidylethanolamine plasmalogen (PE PI) and myelin phosphatidylethanolamine, $p < 0.05$. For microsomal phosphatidylcholine (PC) and myelin phosphatidylethanolamine plasmalogen and phosphatidylcholine, $p$ is large (see Fig. 2 for interpretation of $p$ value). The levels of radioactivity determined declined with time, but even at the last time point the $^{14}$C values for phosphatidylcholine, phosphatidylethanolamine, and phosphatidylethanolamine plasmalogen in microsomes were 220, 35, and 34 dpm, respectively, whereas for myelin the equivalent values were 481, 95, and 147 dpm. The $^3$H values recorded were higher, as can be determined from the $^{3}$H/$^{14}$C ratio values.

Fig. 5. Isotope ratios in phospholipids following intracranial injection of 250 $\mu$Ci of [2-$^3$H]glycerol and 17 $\mu$Ci of [1,3-$^{14}$C]glycerol into 17-day-old rats. Animals were killed at the indicated times following injection with both radioactive precursors, and lipids were extracted from microsomal and myelin fractions (Fig. 4). The $^3$H/$^{14}$C ratio was corrected to include only that present in the C-2 position of glycerol (Figs. 1 and 2), and the $^{14}$C radioactivity was corrected to include only that present in the glycerol moiety (Fig. 4). Lines were fit by linear regression. For microsomal phosphatidylethanolamine (PE) and phosphatidylethanolamine plasmalogen (PE PI) and myelin phosphatidylethanolamine, $p < 0.05$. For microsomal phosphatidylcholine (PC) and myelin phosphatidylethanolamine plasmalogen and phosphatidylcholine, $p$ is large (see Fig. 2 for interpretation of $p$ value). The levels of radioactivity determined declined with time, but even at the last time point the $^{14}$C values for phosphatidylcholine, phosphatidylethanolamine, and phosphatidylethanolamine plasmalogen in microsomes were 220, 35, and 34 dpm, respectively, whereas for myelin the equivalent values were 481, 95, and 147 dpm. The $^3$H values recorded were higher, as can be determined from the $^{3}$H/$^{14}$C ratio values.
phosphatidylethanolamine plasmalogen of microsomes (isolated from rats 2 days after injection) were 1.6, 1.1, and 0.13, respectively. The corresponding ratios for the myelin lipids were 2.1, 1.6, and 0.16, respectively.

**Metabolism of Acyl and Alkenyl Moieties**

**Turnover of Acyl Moieties Labeled with Acetate**—Rats, 17 days of age, were given injections of a mixture of [2-"H]glycerol and [1-14C]acetate. After subcellular fractionation, lipid extraction, and thin layer chromatography, an aliquot of each lipid was subjected to alkaline methanolysis and the radioactivity in the aqueous phase (glycerophosphoryl base) and the organic phase (acyl methyl esters) was quantitated. Over 97% of the 14C present in phosphatidylcholine and phosphatidylethanolamine were located in the acyl moiety, and the distribution remained constant with time (see Ref. 25). In the case of phosphatidylethanolamine plasmalogen, the aldehyde released from the vinyl ether by acid hydrolysis was also collected after chromatography, and radioactivity was quantitated. Radioactivity in the 2 hydrophobic residues accounted for 91 and 97% of the total 14C of microsomes and myelin, respectively.

The initial 14H/14C isotope ratios (2 days after injection) for microsomal phosphatidylcholine, phosphatidylethanolamine, and phosphatidylethanolamine plasmalogen were 11, 12, and 10, respectively. For myelin lipids the ratios were 11, 12, and 3.8, respectively. In each case, the isotope ratio decreased as a function of time. After correction of the 14H/14C ratios to include only that present in the C-2 position of glycerol, the 14C radioactivity of each phospholipid in both subcellular fractions was calculated by dividing the 14H/14C ratio into the absolute [14H]glycerol radioactivity (Fig. 3), as described previously. The log of these values was plotted as a function of time (Fig. 12). The apparent metabolic stability of microsomal phosphatidylethanolamine plasmalogen (after 15 days following injection), as well as the continuous increase of label in myelin phosphatidylethanolamine plasmalogen, are of interest (see "Discussion").

**Relative Turnover Rate of Acyl and Alkenyl Moieties of Phosphatidylethanolamine Plasmalogen**—In the experiment described above, the ratio of 14C radioactivity in the acyl moiety of phosphatidylethanolamine plasmalogen relative to that in the acyl moiety (i.e. radioactivity in the remaining 2-lyso derivative) was determined and plotted as a function of time. The isotope ratio was constant with time, indicating that both long chain hydrocarbon groups have the same or similar turnover rates. For both microsomes and myelin, the 14C fatty acid/14C vinyl ether ratios were less than 1 (0.25 for the phosphatidylethanolamine plasmalogen of microsomes and 0.62 for the phosphatidylethanolamine plasmalogen of myelin), indicating in each case a preferential labeling of the vinyl ether moieties.

**Mechanism of Reutilization of Acyl and Alkenyl Moieties**—Young rats were given simultaneous injections of [14H]acetate and [2-"H]glycerol and 10 µCi of [1,2-14C]ethanolamine into 17-day-old rats. Animals were killed at various times following injection, and radioactivity in individual lipid classes obtained from the microsomal and myelin fractions was determined. The 1H radioactivity was corrected to include only that present at the C-2 position of glycerol (Figs. 1 and 2), and the 14C radioactivity was corrected to include only that present in the base (see text). The curves were fit by visual inspection. At 70 days of age, the 14C radioactivity values determined for phosphatidylethanolamine (PE) and phosphatidylethanolamine plasmalogen (PE P1) of microsomes were 915 and 1210 dpm, respectively, whereas for myelin the equivalent values were 1282 and 6942 dpm.

**Fig. 6.** Isotope ratios in phospholipids isolated following intracranial injection of 250 µCi of [2-"H]glycerol and 10 µCi of [1,2-14C]ethanolamine into 17-day-old rats. Animals were killed at various times following injection, and radioactivity in individual lipid classes obtained from the microsomal and myelin fractions was determined. The 1H radioactivity was corrected to include only that present at the C-2 position of glycerol (Figs. 1 and 2), and the 14C radioactivity was corrected to include only that present in the base (see text). The curves were fit by visual inspection. At 70 days of age, the 14C radioactivity values determined for phosphatidylethanolamine (PE) and phosphatidylethanolamine plasmalogen (PE P1) of microsomes were 915 and 1210 dpm, respectively, whereas for myelin the equivalent values were 1282 and 6942 dpm.

**Fig. 7.** Decline in radioactivity in the ethanolamine moiety of phospholipids with time. Individual phospholipid classes from microsomes and myelin were isolated at various times following injection of 250 µCi of [2-"H]glycerol and 10 µCi of [1,2-14C]ethanolamine. The data points (●) were obtained by dividing the 1H/14C ratio (corrected as described in Fig. 6) into the absolute [14C]glycerol radioactivity given for that subcellular fraction at the same time (see text and Fig. 3). The data points corresponding to times equal to or greater than 15 days after injection were fit by a linear regression program, and half-lives and ranges were calculated. Earlier time points were fit by visual inspection. The uncorrected data, observed 14C radioactivity, is also indicated (△). PE P1, phosphatidylethanolamine plasmalogen.
FIG. 8. Autoradiograph of myelin lipids at 55 days following injection of 10 μCi of [methyl-3H]choline into a 17-day-old rat. Myelin was isolated, total lipids were extracted, and phospholipid classes were separated by thin layer chromatography. Lipids were visualized with iodine, and this pattern was superimposed on the autoradiograph (see "Experimental Procedures"). **Chol**, origin; **PS**, phosphatidylserine; **PE Pl**, phosphatidylethanolamine plasmalogen; **S**, sulfatide; **Cer**, cerebroside; **PC Ald** and **PC AId**, aldehydes liberated by the cleavage of the alkanyl group of phosphatidylethanolamine plasmalogen (**PE Pl**) and phosphatidylcholine plasmalogen, respectively; **Chol**, cholesterol.

FIG. 9. Isotope ratios in phosphatidylcholine (**PC**) following intracranial injection of 300 μCi of [2-3H]glycerol and 10 μCi of [methyl-3H]choline (---) or 100 μCi of [methyl-3H]choline and 35 μCi of [1,3-14C]glycerol (----) into 17-day-old rats. Animals were killed at the indicated times, myelin and microsomal subfractions were isolated, total lipids were extracted, and phosphatidylcholine was separated from other lipids by thin layer chromatography. The **H/14C** ratios were corrected to include only the **H** at the C-2 position of glycerol and only the 14C in glycerol (Fig. 4). The curves were fit by visual inspection. The 14C radioactivity values for phosphatidylcholine at the last time point were 1891 and 5337 dpm for microsomes and myelin, respectively.

FIG. 10. Decline in radioactivity in the choline moiety of phosphatidylcholine (**PC**) with time. Individual phospholipid classes from microsomes and myelin were isolated at various times following injection of 400 μCi of [2-3H]glycerol and 10 μCi of [methyl-3H]choline. The **H/14C** ratio (corrected to account only for the **H** at the C-2 position of glycerol) was divided into the absolute [1H]glycerol radioactivity obtained from a number of experiments (see text). A half-life was calculated from data points corresponding to the time period equal to or greater than 15 days after injection.

Turnover of Acyl Moieties Labeled with [14C]Glucose—Rats given injections of [2-3H]glycerol and [U-14C]glucose were killed at various times after injection, and microsomal and myelin lipids were isolated. An aliquot was subjected to alkaline methanolysis to determine the percentage of lipid 14C in the acyl moiety (Fig. 14). Due to the relatively low levels of radioactivity and the limited material available, distribution of 14C could accurately be determined only in microsomal and myelin phosphatidylethanolamine and phosphatidylethanolamine plasmalogen. The high proportion of label initially in the glycerol backbone is as expected from production of three carbon products by the glycolytic pathway. With time the more efficiently reutilized hydrophobic moieties account for an increasing percentage of the total radioactivity.

The remaining lipid was used for determination of the **H/14C** ratio, which was corrected to include only the **H** at the C-2 position of glycerol and only the 14C in the acyl moiety. The microsomal phosphatidylethanolamine and phosphatidylethanolamine plasmalogen and myelin phosphatidylethanolamine plasmalogen ratios were corrected assuming the same distribution of 14C radioactivity as that observed for phosphatidylcholine (Fig. 14) for the appropriate fractions. The initial **H/14C** ratios (4 days after injection) for microsomal phosphatidylcholine,
Metabolism of Glycerophospholipids in Rat Brain

FIG. 11. Decline with time of radioactivity in the phosphate moiety of phospholipids. Rats, 17 days of age, were given injections of 400 μCi of [3H]glycerol and 200 μCi of [32P]orthophosphate. Animals were killed at the indicated times, myelin and microsomal fractions were isolated, total lipids were extracted, and aliquots were subjected to thin layer chromatography for separation of individual lipid classes. The data plotted was obtained by dividing the \(^{3}H/^{32}P\) ratio (corrected to include the \(^{3}H\) only in the C-2 position of glycerol) into the absolute \(^{3}H\)glycerol radioactivity obtained from a number of experiments (see text). A half-life was calculated from data points corresponding to the time period following 15 days after injection. Data points corresponding to the time period before 15 days following injection were fit by visual inspection. The \(^{32}P\) radioactivity values for phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylethanolamine plasmalogen (PE PI) of microsomes were 396, 144, and 142 dpm, respectively; the equivalent values for the myelin lipids were 187, 228, and 80 dpm.

FIG. 12. Decline with time of radioactivity in the \(^{14}C\)acetate-labeled acyl moieties of phospholipids. Individual phospholipid classes from microsomes and myelin were isolated at various times following injection of 300 μCi of [1-\(^{3}H\)glycerol and 100 μCi of [1-\(^{14}C\)acetate (Fig. 2). The data plotted were obtained by dividing the \(^{3}H/^{14}C\) ratio (corrected to include only the \(^{3}H\) at the C-2 position of glycerol) into the absolute \(^{3}H\)glycerol radioactivity obtained for that same phospholipid from a number of experiments (see text). For the diacyl lipids, a half-life was calculated from the data points corresponding to times equal to or greater than 15 days after injection. For microsomal and myelin phosphatidylethanolamine plasmalogen (PE PI), the half-life could not be calculated. Data corresponding to times before 15 days after injection were fit by visual inspection. The \(^{14}C\) radioactivity values for phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylethanolamine plasmalogen (PE PI) of microsomes were 1035, 324, and 181 dpm, respectively; the equivalent values for myelin were 1990, 468, and 438 dpm.

FIG. 13. Isotope ratios in phospholipids isolated following intracranial injection of 320 μCi of [\(^{3}H\)]acetate and 80 μCi of [1-\(^{14}C\)acetate into 17-day-old rats. Animals were killed at various times following injection, myelin and microsomal fractions were isolated, total lipids were extracted, and aliquots were subjected to thin layer chromatography for separation of individual lipid classes. Phospholipid samples were eluted and subjected to alkaline methanolysis. The myelin (- - -) and microsomal (-----) \(^{3}H/^{14}C\) ratios of material in the organic phase were determined. The aldehyde obtained from the cleavage of phosphatidylethanolamine plasmalogen (PE PI Ald) was collected directly from the thin layer chromatography plate (Fig. 8) PC, phosphatidylcholine; PE, phosphatidylethanolamine; PE PI, phosphatidylethanolamine plasmalogen.
Fig. 14. Distribution of radioactivity between acyl and base moieties of phospholipids following intracranial injection of 30 μCi of \( ^{14} \text{C} \)-labeled \( \text{U} \)-glucose into 17-day-old rats. Animals were killed at the indicated times, myelin and microsomal fractions were isolated, total lipids were extracted, and aliquots were subjected to thin layer chromatography for separation of the individual lipid classes. Lipids were eluted and subjected to alkaline methanolysis, and the proportion of the total radioactivity present in the organic phase was determined. The fact that these animals, which received \( ^{14} \text{C} \)-labeled \( \text{U} \)-glucose, were simultaneously given injections of 400 μCi of \( ^{3} \text{H} \)-glycerol is relevant not to this figure but to interpretation of the results of Fig. 15. The \( ^{14} \text{C} \) radioactivity values determined for phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylethanolamine plasmalogen (PE PI) of microsomes were 48, 18, and 17 dpm, respectively; for myelin the equivalent values were 57, 25, and 68 dpm.

order of magnitude more efficient than glucose for labeling acyl groups.

**DISCUSSION**

**Experimental Strategy**

The double label protocol provides direct evidence for preferential recycling of phosphate, base, and hydrophobic moieties relative to glycerol, without the experimental error involved in the calculation of half-lives from two different precursors. Furthermore, the ratio data allow for a calculation of the absolute turnover rate of the \( ^{14} \text{C} \)- or \( ^{3} \text{H} \)-labeled precursor by reference to the turnover of \( ^{3} \text{H} \)-glycerol. The results obtained in this manner represent a level of significance that could be achieved in single isotope experiments only by the use of a much greater number of animals. The intracranial injection procedure was a major source of variability in the present study. However, a more uniform systematic route, such as intraproteroncal injection, was not feasible because \( ^{3} \text{H} \)-glycerol injected by this route is incorporated into brain lipid 2 orders of magnitude less efficiently than intracranially injected \( ^{3} \text{H} \)-glycerol (11). In interpreting the results the authors should also be kept in mind that myelin is a relatively homogeneous membrane produced by oligodendroglial cells, whereas the microsomal fraction is heterogeneous, with respect to both the type of membrane and the cells of origin.

\( ^{2} \text{H} \)-Glycerol as Label for Brain Phospholipids

Following the injection of \( ^{2} \text{H} \)-glycerol, most of the radioactivity in the glycerol phospholipids was initially present in the C-2 position (Figs. 1 and 2). However, the proportion of \( ^{3} \text{H} \) randomized to the acyl moieties or to the C-1 carbon of glycerol increased as a function of time after injection. The extent to which this took place was unexpected, emphasizing the need for chemical localization of the label in long term experiments. The most probable explanation for the data in Fig. 1 involves utilization of the \( ^{3} \text{H} \) from C-2, possibly by a pathway where label lost from glycerol 3-phosphate to \( ^{3} \text{H} \)-NADH is conserved for synthetic purposes, via \( ^{3} \text{H} \)-NADPH, which might be formed by a transhydrogenase.

Alternatively, contamination of the \( ^{2} \text{H} \)-glycerol by a fraction of a per cent of a substrate that labels fatty acids efficiently might account for the small amount of label initially present in the hydrophobic moieties. Due to the relative metabolic stability of the acyl and alkenyl side chains (see below), they would rapidly account for an increasing percentage of the total lipid label.

With respect to Fig. 2, the results are best explained by an argument presented by Agranoff and Hajra (18), that label lost from the C-2 position of glycerol 3-phosphate is partially recovered in the C-1 position of glycerol 3-phosphate via \( ^{3} \text{H} \)-NADH (and glyceraldehyde 3-phosphate) by reversal of the glycolysis pathway.

Another possible drawback to the use of \( ^{2} \text{H} \)-glycerol for studies of phospholipid synthesis concerns the isotope effect that has been shown with the mitochondrial glycerophosphate dehydrogenase (EC 1.1.99.5); \( ^{2} \text{H} \)-glycerol is not a good substrate and is enriched in the tissue relative to \( ^{14} \text{C} \)-glycerol.
Metabolism of Glycerophospholipids in Rat Brain

(26). That this enrichment also occurs in brain is suggested by the high $^{3}H/^{14}C$ ratio in phosphatidylcholine and phosphatidylethanolamine relative to the injected isotope ratio (Fig. 5). However, there was this effect very significant with respect to reutilization of label for phospholipid synthesis by the extramitochondrial enzyme (EC 1.1.1.81), one would expect that $^{14}C$glycerol would be recycled less efficiently than $[2-3]$Hglycerol, which is not the case (Fig. 5 and Table I).

Fig. 5 does, however, illustrate an interesting difference in the metabolism of phosphatidylethanolamine as compared to phosphatidylcholine. In the ethanolamine-containing lipids (with the exception of the more metabolically stable myelin phosphatidylethanolamine plasmalogen), the glycerol backbone was recycled more efficiently than the $^{3}H$ at the C-2 position. This is not true of phosphatidylcholine, a result analogous to that reported by Benjamins and McKhann (11). One interpretation of this result is that both the $[2-^{3}H]$- and the $[^{14}C]$glycerol undergo some recycling and that there is a contribution of the dihydroxyacetone phosphate pathway (see last section of the “Discussion”) to the synthesis of phosphatidylethanolamine. This would result in preferential loss of the $^{3}H$, relative to $^{14}C$, with time.

Turnover of $[2-^{3}H]$Glycerol in Phospholipids

Microsomal and myelin lipids showed a decrease in $^{3}H$ radioactivity with time, which we have approximated as a biphasic decay, although the decay scheme is almost certainly more complicated than that. A similar biphasic decay assumption has been utilized by others (9, 27, 28). The half-lives of microsomal phosphatidylcholine, phosphatidylethanolamine, and phosphatidylethanolamine plasmalogen were equal (about 3 days) in the first 14 days following injection. The half-lives of microsomal phosphatidylcholine and phosphatidylethanolamine in the slower turnover phase (13 to 14 days) were equal and probably shorter than that of phosphatidylethanolamine plasmalogen.

During the first 14 days, myelin phosphatidylcholine, phosphatidylethanolamine, and phosphatidylethanolamine plasmalogen turn over less rapidly than the corresponding microsomal lipids, and there is some indication of greater metabolic stability for phosphatidylethanolamine plasmalogen than for phosphatidylethanolamine. In the slow phase, phosphatidylcholine and phosphatidylethanolamine had equivalent half-lives of 25 days, somewhat shorter than the 34-day half-life of phosphatidylethanolamine plasmalogen (see Table I). Since rats were given injections at 17 days of age, rapid accumulation of myelin was still occurring for a number of days after injection (29). Therefore, to the extent that this new myelin was formed from previously labeled lipids in a membrane precursor fraction (30) (e.g. myelin-like material (31)), the data from early time points may represent an overestimation of the half-life of myelin lipids (32).

For both microsomal and myelin lipids, the apparent half-lives are overestimations of the true half-lives to the extent that: (a) phospholipids were metabolized to glycerol 3-phosphate and recycled without interconversion with dihydroxyacetone phosphate; (b) $^{3}H$ removed from $[2-{3}H]$glycerol during the step of equilibration with dihydroxyacetone phosphate was returned stereospecifically during a subsequent reduction; or (c) $[2-{3}H]$glycerol was enriched at each reutilization step due to an isotope effect (26).

Metabolism of Base Moieties

The isotope ratio data for ethanolamine (Fig. 6) and choline (Fig. 9) (and similar data for phosphate) indicate that these moieties are preferentially reutilized for synthesis of phospholipids, resulting in apparent lipid half-lives longer than those calculated using $[2-^{3}H]$glycerol (Table I). Therefore, differences in half-lives reported for particular brain phospholipids by various laboratories can, in part, be attributed to the use of different precursors, as suggested by others (4, 7, 33). Choline is reutilized more efficiently than the phosphate moiety for resynthesis of phosphatidylcholine, possibly reflecting a relatively small pool of choline because of the limited capacity of

| Precurser | Half-life of microsomal and myelin lipid |
|-----------|----------------------------------------|
|           | Phosphatidylcholine | Phosphatidylethanolamine | Phosphatidylethanolamine plasmalogen |
| MIC | MY | MIC | MY | MIC | MY |
| $^{3}H$Glycerol | 4.0 | 10 | 3.0 | 6.6 | 4.0 | 11 |
| (3.5-5.0) | (8.0-13) | (2.5-4.5) | (5.5-8.0) | (3.0-5.0) | (8.5-17) |
| $[^{14}C]$Choline | 26 | 39 | (22-31) | (33-47) |
| $[^{14}C]$Ethanolamine | 26 | 39 | (22-31) | (33-47) |
| $[^{38}P]$Phosphate | 17 | 30 | (16-19) | (26-35) |
| $[^{14}C]$Glucose | 35 | 56 | (27-49) | (45-73) |
| $[^{14}C]$Acetate | 28 | 54 | (23-35) | (45-67) |

MIC, microsomes; MY, myelin.

Based on data obtained less than 15 days after injection.

Based on data obtained 15 or more days after injection.

Half-lives were also calculated for $[^{14}C]$glycerol in phosphatidyl-
ethanolamine of microsomes and myelin and phosphatidylethanolami-
plasmalogen of microsomes using Figs. 3 and 5. These values were 21, 32, and 33 days, respectively. For phosphatidylethanolamine of microsomes and myelin and phosphatidylethanolamine plasmalogen of myelin, the data in Fig. 5 indicate that $[2-^{3}H]$glycerol and $[1,3-
$^{14}C]$glycerol have identical turnover rates.
brain for synthesis of this compound (34). Jungalwala and Dawson (35) gave adult rats injections of $^{14}$Cethanolamine and followed the decrease of radioactivity in microsomal and myelin phosphatidylethanolamine for 128 days. Horrocks et al. (28) used these data (from 21 to 98 days after injection) to calculate a half-life for microsomal and myelin phosphatidylethanolamine of 20 and 27 days, respectively, results of the same order as the values we have obtained. In another set of experiments, Jungalwala (9) gave adult rats injections of $^{14}$Ccholine and followed the decrease in radioactivity of myelin phosphatidylcholine for 145 days. Between 15 and 145 days after injection, the calculated half-life of myelin phosphatidylcholine was 42 days, in close agreement with the value we obtained with choline as a substrate.

Although other reports have been published in which labeled base and phosphate precursors have been used to follow the turnover of microsomal and myelin lipids (7, 27, 33), these results are not readily comparable with ours due to experimental differences (e.g., differences in techniques or time period of the study). Another critical variable is animal age, since Horrocks (36) has shown, at least for the short turnover phase, that the half-life of ethanolamine lipids in mouse brain subcellular fractions increases with increasing age at time of isotope administration.

**Turnover of Acyl and Alkenyl Moieties**—The results relating to the utilization of the hydrophilic moieties are not exactly analogous to the reutilization data reported for ethanolamine and choline. In the case of the bases, ethanolamine and choline are presumably reutilized intact without any metabolic alteration. The acyl moieties, however, can undergo chain elongation and desaturation before reincorporation into phospholipids. This has been demonstrated and used as evidence for recycling of acyl moieties of phospholipids (8, 37).

The reutilization of essential fatty acids for synthesis of brain phospholipids has also been investigated (38). Our $[^{14}]$Cacetate/$[^{14}]$Clactate experiment (Fig. 13) supplements this work by demonstrating that the reutilization of the acyl precursor does not involve recycling through the acetate pool. In addition, this experiment demonstrates the suitability of the $[^{14}]$Cacetate as a precursor for labeling of phospholipids.

The decrease in the $[^{3}H/^{14}C$ ratio in each phospholipid as a function of time (not illustrated, since it can be calculated from data in Fig. 3 and in Fig. 12, for acetate, and in Fig. 15, for glucose) indicates that radioactivity from acyl groups is reutilized more efficiently than is radioactivity from phosphate or base moieties (Table I). The same presumably holds true of the alkenyl groups of phosphatidylethanolamine plasmalogen since, as indicated in the results, their reutilization appears to be the same as that of the acyl moiety. Although the turnover time for the glycerol moiety of phosphatidylethanolamine and phosphatidylethanolamine in myelin is the same, radioactivity in acyl groups is much more stable in phosphatidylethanolamine than in phosphatidylethanolamine (Table I). The same observation holds with respect to the microsomal lipids. This indicates that labeled acyl groups were even more efficiently reutilized for the resynthesis of phosphatidylethanolamine than for the resynthesis of phosphatidylcholine.

Our reported half-lives for the hydrophobic moiety of phosphatidylcholine in myelin with acetate or glucose as a precursor, 54 or 56 days, are almost the same as the 2-month half-life reported by Smith and Eng (39), who used acetate as a precursor, and are similar to the 42-day value later reported by Smith (1) using glucose as a precursor. In both of these studies, it was reported that ethanolamine phospholipids (a mixture of the diacyl and the plasmalogen compounds) were more stable than phosphatidylcholine. We are now in a position to confirm and interpret these earlier data of Smith and Eng in greater detail and to emphasize that phosphatidylcholine and phosphatidylethanolamine in myelin really have the same turnover rate of 25 days with regard to the $[2-{^3}H]glycerol, but that the acyl and alkenyl moieties are reutilized more efficiently for the synthesis of phosphatidylethanolamine and phosphatidylethanolamine plasmalogen than for phosphatidylcholine synthesis. This difference between the metabolism of ethanolamine- and choline-containing lipids (demonstrated by the use of either $[^{14}]$Cacetate or $[^{14}]$Glucose as precursor) is reminiscent of metabolic differences between phosphatidylethanolamine and phosphatidylcholine with regards to short term isotope experiments studying the assembly of myelin (13). As discussed in that reference, the metabolic differences between ethanolamine- and choline-containing lipids may be related to preferential localization of ethanolamine lipids in the inner half of the bilayer and choline lipids on the outer surface, in analogy to the architecture of the red cell membrane (40).

**Involvement of Dihydroxyacetone Phosphate in the Synthesis of Phosphatidylethanolamine Plasmalogen**—Following simultaneous injection of $[2-{^3}H]glycerol and $[1,3-{^3}H]glycerol, myelin phosphatidylethanolamine plasmalogen had a 10-fold lower initial $[^{3}H]/[^{14}C$ ratio than did phosphatidylethanolamine or phosphatidylcholine in the same subcellular fraction (Fig. 5). This observation is consistent with the hypothesis that myelin phosphatidylethanolamine plasmalogen is primarily synthesized by a route in which dihydroxyacetone phosphate is an obligatory intermediate (41, 42) with consequent loss of the $[^{3}H]$ at the C-2 position. The significance of this pathway in brain has been demonstrated (43, 44). This result is in contrast to the preferential involvement of the glycerol 3-phosphate pathway (45, 46) for synthesis of phosphatidylcholine and phosphatidylethanolamine. The preferential loss of the C-2 tritium for synthesis of phosphatidylethanolamine plasmalogen of myelin relative to synthesis of phosphatidylethanolamine of myelin can also be ascertained from examination of the $[^{3}H]/[^{14}C$ ratios for the various substrate pairs (Fig. 6 for $[^{14}]$Cethanolamine; see "Results" for data obtained with $[^{14}]$Clactate or $[^{14}]$Glucose).

The $[^{3}H]/[^{14}F$ ratio data for the individual myelin lipids supports the hypothesis of involvement of the dihydroxyacetone phosphate pathway for synthesis of phosphatidylethanolamine plasmalogen of myelin.

The results with regard to phosphatidylethanolamine plasmalogen of microsomes were not as clear cut as for the myelin data. There was a preferential loss of the C-2 tritium during synthesis of phosphatidylethanolamine of myelin relative to that observed for synthesis of phosphatidylethanolamine plasmalogen in microsomes. However, the magnitude of this effect was not always as prominent in microsomes as in myelin (Figs. 5 and 6). This might be due to the more rapid turnover of the microsomal lipids, resulting in a greater loss of C-2 tritium from glycero before the first time point was taken. Another possible complication is the heterogeneous cellular origin of the microsomal fraction.

**Acknowledgments**—We thank Dr. Robert Elston and Mr. Charles Chase for assistance in the statistical analysis. Mr. Chase also modified an existing curve-fitting program to handle our data. Miss Nina Siegler and Mr. James Hattaway provided technical assistance.
REFERENCES

1. Smith, M. E. (1967) Adv. Lipid Res. 5, 241-278
2. Sun, G. Y. (1973) J. Neurochem. 21, 1083-1082
3. Lapetina, E. G., Lunt, G. G., and De Robertis, E. (1970) J. Biol. Chem. 242, 2389-2396
4. Chlapowski, F. J., and Band, R. N. (1971) J. Cell Biol. 50, 625-633
5. Lapetina, E. G., Rodriguez de Lores Arnaiz, G., and De Robertis, E. (1969) Biochim. Biophys. Acta 176, 643-646
6. Manning, R., and Brindley, D. N. (1972) Biochem. J. 130, 1003-1012
7. Pasquini, J. M., Krawiec, L., and Soto, E. F. (1973) J. Neurochem. 21, 647-653
8. Horrocks, L. A., Toews, A. D., Thompson, D. K., and Chin, J. Y. (1976) in Function and Metabolism of Phospholipids in the Central and Peripheral Nervous System (Porcellati, G., Anaducci, L., and Galli, C., eds) pp. 37-54, Plenum Publishing Corp., New York
9. Norton, W. T., and Poduslo, S. E. (1973) J. Neurochem. 21, 759-773
10. Hofteig, J. H., and Druse, M. J. (1976) Life Sci. 18, 543-552
11. Agrawal, H. C., Banik, N. L., Bone, A. H., Davison, A. N., Mitchell, R. F., and Spohn, M. (1970) Biochem. J. 120, 635-642
12. T. J., Lees, M., and Sloane Stanley, G. H. (1957) J. Biol. Chem. 226, 497-509
13. Horrocks, L. A., Toews, A. D., Thompson, D. K., and Chin, J. Y. (1976) in Function and Metabolism of Phospholipids in the Central and Peripheral Nervous System (Porcellati, G., Anaducci, L., and Galli, C., eds) pp. 37-54, Plenum Publishing Corp., New York
14. Norton, W. T., and Poduslo, S. E. (1973) J. Neurochem. 21, 749-757
15. Folch, J., Lees, M., and Sloane Stanley, G. H. (1957) J. Biol. Chem. 226, 497-509
16. Horrocks, L. A., and Sun, G. Y. (1972) in Research Methods in Neurochemistry (Marks, N., and Rodnight, R., eds) Vol. 1, pp. 293-391, Plenum Press, New York
17. Kishimoto, Y., Davies, W. E., and Radin, N. S. (1965) J. Lipid Res. 6, 525-531
18. Agaroff, B. W., and Hajra, A. K. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 413-415
19. Kates, M., and Sastry, P. S. (1969) Methods Enzymol. 14, 197-203
20. Morell, P., and Radin, N. S. (1970) J. Biol. Chem. 245, 342-350
21. Dixon, W. J., and Massey, F. J. (1957) Introduction to Statistical Analysis, 2nd Ed, pp. 199-200, McGraw-Hill Book Co., Inc., New York
22. Ansell, G. B. (1973) in Form and Function of Phospholipids (Ansell, G. B., Hawthorne, J. N., Dawson, R. M. C., eds) pp. 377-419, Elsevier Scientific Publishing Co., New York
23. Ansell, G. B., and Spanner, S. (1967) J. Neurochem. 14, 873-885
24. Ansell, G. B., and Spanner, S. (1968) Biochem. J. 110, 201-206
25. Lapetina, E. G., Rodriguez de Lores Arnaiz, G., and De Robertis, E. (1969) Biochim. Biophys. Acta 176, 643-646
26. Manning, R., and Brindley, D. N. (1972) Biochem. J. 130, 1003-1012
27. Pasquini, J. M., Krawiec, L., and Soto, E. F. (1973) J. Neurochem. 21, 647-653
28. Horrocks, L. A., Toews, A. D., Thompson, D. K., and Chin, J. Y. (1976) in Function and Metabolism of Phospholipids in the Central and Peripheral Nervous System (Porcellati, G., Anaducci, L., and Galli, C., eds) pp. 37-54, Plenum Publishing Corp., New York
29. Norton, W. T., and Poduslo, S. E. (1973) J. Neurochem. 21, 759-773
30. Hofteig, J. H., and Druse, M. J. (1976) Life Sci. 18, 543-552
31. Agrawal, H. C., Banik, N. L., Bone, A. H., Davison, A. N., Mitchell, R. F., and Spohn, M. (1970) Biochem. J. 120, 635-642
32. Davison, A. N., and Dobbing, J. (1961) Nature 191, 844-848
33. Lapetina, E. G., Lunt, G. G., and De Robertis, E. (1970) J. Neurobiol. 25, 913-914
34. Ansell, G. B. (1973) in Form and Function of Phospholipids (Ansell, G. B., Hawthorne, J. N., Dawson, R. M. C., eds) pp. 377-419, Elsevier Scientific Publishing Co., New York
35. Bretschger, M. S. (1973) Science 181, 622-629
36. Hajra, A. K. (1968) Biochem. Biophys. Res. Commun. 37, 496-492
37. Wykle, R. L., and Snyder, F. (1969) Biochem. Biophys. Res. Commun. 37, 658-662
38. Hajra, A. K. (1968) Biochem. Biophys. Res. Commun. 33, 929-935
39. T. B. A., Plantadosi, C., and Snyder, F. (1975) Biochim. Biophys. Acta 388, 5-11
40. Kornberg, A., and Price, W. E., Jr. (1968) J. Biol. Chem. 244, 345-357
41. Kennedy, E. P., and Weiss, S. B. (1956) J. Biol. Chem. 222, 193-214
Metabolism of glycerophospholipids of myelin and microsomes in rat brain.

Reutilization of precursors.

S L Miller, J A Benjamins and P Morell

J. Biol. Chem. 1977, 252:4025-4037.

Access the most updated version of this article at http://www.jbc.org/content/252/12/4025.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/12/4025.citation.full.html#ref-list-1