Reductive and oxidative biosynthesis of plasmalogens in myelinating brain

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ABSTRACT Palmitic acid-1-^{14}C and hexadecanol-1-^{14}C were administered intracerebrally to 18-day-old rats. Incorporation of radioactivity into the constituent alkyl, alk-1-enyl, and 1-acyl moieties, as well as into the 2-acyl moieties, of the ethanolamine phosphatides of brain was determined after 1, 2, 3, 6, and 22 hr. Incorporation of radioactivity from hexadecanol into both alkyl ethers and alk-1-enyl ethers proceeded at a rate more than 10 times higher than from palmitic acid. Hexadecanol was rapidly oxidized to fatty acids which were incorporated into the acyl moieties of the ethanolamine phosphatides. When palmitic acid was used as a precursor, labeled long-chain alcohols could be isolated from the lipid extract. As labeled long-chain aldehydes could not be detected in any of the lipid extracts, alcohols appear to be key intermediates for the biosynthesis of both alkyl and alk-1-enyl glycerophosphatides.

SUPPLEMENTARY KEY WORDS ethanolamine phosphatides · alkyl acyl glycerols · alk-1-enyl acyl glycerols · diacyl glycerols · alcohols · aldehydes · fatty acids · intracerebral injection · palmitic acid-1-^{14}C · hexadecanol-1-^{14}C

It has been shown that long-chain fatty acids (1–3), aldehydes (4, 5), and alcohols (2) are incorporated by mammalian tissues into the alk-1-enyl moieties of plasmalogens, yet the identity of the direct precursor is unknown, and the pathway leading to the alk-1-enyl glycerol ethers is still the subject of controversy.

Myelinating brain is an organ especially suited for studies of plasmalogen biosynthesis. In the rat brain, the rate of phospholipid biosynthesis as well as the formation of plasmalogens reaches highest levels when the rat is 10–20 days old (1). Thus, plasmalogen biosynthesis has been studied in the developing brain by intracerebral (6, 7) or intraperitoneal (8) injection of acetate-^{14}C, and by intracerebral injection of differently labeled palmitaldehydes (4, 5).

Bickerstaffe and Mead (4) reported that long-chain aldehydes are more rapidly incorporated in acyl moieties than into alk-1-enyl moieties, and they suggested a metabolic conversion of esters to alk-1-enyl ethers. However, studies by Bell and White (5) showed that doubly-labeled long-chain aldehydes administered to the developing brain are not oxidized to fatty acids prior to their incorporation into alk-1-enyl ethers.

We have recently demonstrated the presence of very small amounts of long-chain alcohols in mammalian brain (9). As the role of alcohols as intermediates in the biosynthesis (10–12) and biocleavage (13) of alkyl glycerol ethers by mammalian cells appears to be established, their role as precursors in the biosynthesis of the alk-1-enyl ether lipids of brain should be of interest. Keenan, Brown, and Marks (2) have shown that alcohols were more rapidly incorporated into the alk-1-enyl ethers of heart muscle than were fatty acids when these compounds were administered to heart-lung preparations. Systematic studies of the origin or the metabolic role of free long-chain alcohols in mammalian tissues have not been reported.

In this investigation we used the developing rat brain for experiments designed to produce labeled alk-1-enyl ethers from long-chain precursors within short periods of time. Palmitic acid or hexadecanol was administered through intracerebral injection, and incorporation into alk-1-enyl, alkyl, and acyl moieties of the ethanolamine phosphatides was determined. Attempts were made to isolate and analyze products of initial reduction or oxidation, such as long-chain aldehydes.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

EXPERIMENTAL METHODS

Materials

Palmitic acid-1-^{14}C (30 mCi/mmole) was purchased from Applied Science Laboratories Inc., State College, Pa. It was found to be better than 99% pure as judged by TLC, GLC, and radioautography.

Hexadecanol-1-^{14}C (30 mCi/mmole) was prepared from palmitic acid-1-^{14}C via the methyl ester by reduction with lithium aluminum hydride. It was purified by preparative TLC (hexane–ether 50:50) to a radiopurity of better than 99%.

Unlabeled fatty acids and alcohols were obtained from The Hormel Institute Lipids Preparation Laboratory, Austin, Minn. Glycerol ethers were synthesized according to the procedure of Baumann and Mangold (14). Aldehydes were prepared from alcohols via mesylates (14) by oxidation with dimethyl sulfoxide (15).

Male, albino rats of the Sprague-Dawley strain, 18 days old at the day of the experiment, were purchased from the Dan Rolfsmeyer Company, Madison, Wisc.

Administration of Precursors

Palmitic acid (100 μCi) was dissolved in a drop of chloroform, 0.02 ml of a 1% aqueous sodium hydroxide solution was added, and the mixture was homogenized by vibration. The solvents were removed in a stream of nitrogen, and an aqueous solution, 0.2 ml, of sodium choleate (10 mg/ml) was added. The mixture was emulsified by vibration and repeated heating (to about 40°C) and cooling. Of this emulsion, 6 or 10 μl (3 or 5 μCi) were injected into each brain through the sutura sagittalis using a 50 μl Hamilton syringe in the manner described by Bickerstaffe and Mead (4). The needle was inserted about 5 mm deep at a 45° angle, the solution was injected slowly, and, after about 15 sec the needle was withdrawn. A slight pressure applied to the point of injection prevented bleeding in most cases. Hexadecanol was emulsified directly with sodium choleate in the same amounts as palmitic acid, and was administered in the same manner.

Extraction of Lipids

The animals were killed by decapitation, 1–22 hr after injection, and the brains were removed immediately. For each experiment the brains from four animals were pooled. They were homogenized with chloroform–methanol 2:1, and standard mixtures of alcohols and aldehydes (10 mg each) consisting of equal amounts of 16:0, 18:0, and 18:1 compounds were added immediately. The homogenates were extracted with a total of 300 ml of chloroform–methanol 2:1, and the solution was washed with 75 ml of a 0.5% aqueous solution of NaCl (16). The upper phase was reextracted with chloroform–methanol–water 86:14:1, in a volume equal to the lower phase. The combined lipid extracts were brought to dryness in a rotary evaporator at room temperature. Residual water was removed by azeotropic distillation with several portions of chloroform–methanol 85:15. The lipids were redissolved in chloroform; 1% of the solution was used to determine total radioactivity of the extract.

Preparation of Lipids

Lipid classes and their derivatives were isolated and purified by preparative TLC. The ethanolamine phosphatides were prepared as described previously (17), using chloroform–methanol–water 65:25:4 and, in a second purification, chloroform–methanol–acetic acid–water 50:25:8:4 (18). Fractions were made visible by spraying the plates lightly with a 0.1% ethanolic solution of 2′,7′-dichlorofluorescein. All fractions were eluted from the adsorbent in a standard manner which assured the complete recovery of the lipids, that is, bands were scraped off into columns, 20 × 200 mm, and the volumes of silica gel were estimated. The lipids were eluted immediately with volumes of solvent 20 times that of the adsorbents. All phospholipids were eluted with chloroform–methanol–water 30:50:20. Then, appropriate amounts of chloroform and water were added to achieve a 8:4:3 ratio of chloroform–methanol–water (16). The lower phase was removed, and the upper phase was reextracted with chloroform–methanol–water 86:14:1, in a volume equal to the lower phase. Alkyl glycerol ethers were eluted with chloroform–methanol 1:1; all other neutral lipids were eluted with diethyl ether which had been saturated with water.

Free aldehydes and their condensation products (19) were isolated from the lipid extract by TLC using hexane–diethyl ether 95:5 as developing solvent. Free alcohols were isolated together with diglycerides using hexane–diethyl ether 50:50, and were then purified as alkyl acetates as described previously (9).

Chemical and Enzymic Reactions

Alcohols were converted to alkyl acetates by reaction with acetic anhydride in the presence of pyridine. Ethanolamine phosphatides were treated with 90% acetic acid to hydrolyze the constituent alk-1-enyl ethers (17). The resulting aldehydes, 2,3-dialkylacroleins (19) and (2-acyl) lysophosphatides as well as the unchanged alkyl acyl and diacyl phosphatides were isolated. Part of the acid-stable ethanolamine phosphatide was hydrolyzed with phospholipase A[1] (EC 3.1.1.4) in yields of better than 90%, as described previously (17). Nonhydrolyzed material was isolated, and its radioactivity was taken into account. Reduction of the acid stable ethanolamine phosphatides

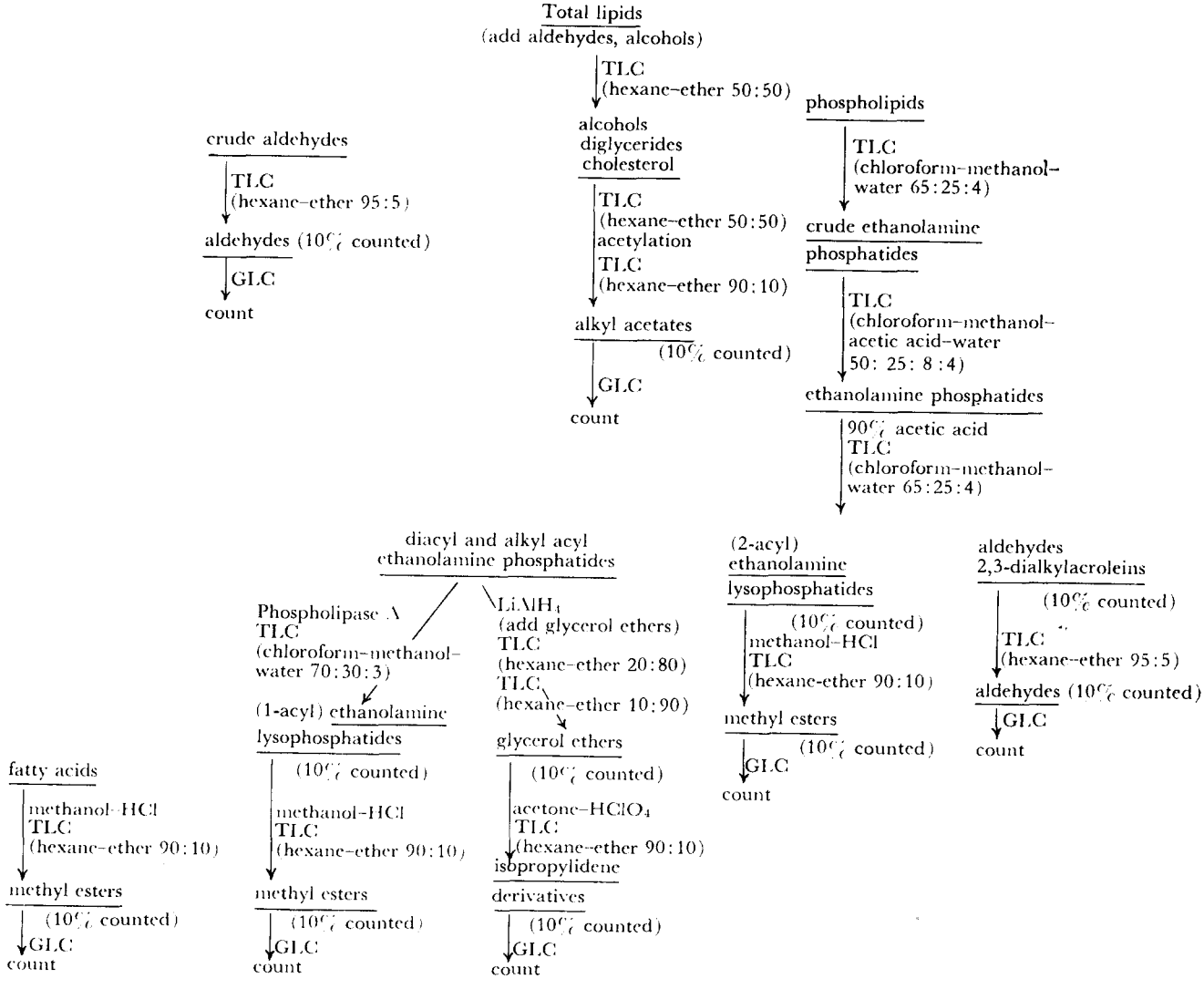
[1] Lyophilized venom of *Crotalus adamanteus*, Ross Allen's Reptile Institute, Inc., Silver Springs, Fla.

with lithium aluminum hydride yielded alkyl glycerol ethers and alcohols. Standard mixtures of alkyl glycerol ethers (10 mg) consisting of equal amounts of 16:0, 18:0, and 18:1 compounds, were added to the reaction mixture to facilitate the isolation of the glycerol ethers and the subsequent fractionation of their isopropylidene derivatives (20). Fatty acids and (1-acyl) lysophosphatides derived through phospholipase A hydrolysis as well as (2-acyl) lysophosphatides derived through acidic hydrolysis were converted to methyl esters by reacting them with methanol–HCl (5%) in a sealed ampule at 80°C for 2 hr. The methyl esters were isolated by TLC. Thus, all lipids analyzed were converted into derivatives suitable for preparative gas chromatography. The fractionation and degradation are outlined in Scheme I.

Gas Chromatography

A Victoreen 4000 instrument equipped with both a flame ionization detector and a thermoconductivity detector was used for gas chromatography. An aluminum column, 180 × 0.4 cm. (I.D.) filled with ethylene glycol succinate (18%Hi-EFF-2BP) on Gas Chrom P, 80–100 mesh, (Applied Science Laboratories Inc.) was operated alternatively at 160, 190, 205, and 220°C. Peaks on the chromatograms were identified by comparison with standards and were quantified by triangulation.

For preparative gas chromatography a collecting device consisting of a conical stainless steel tube in a heating block was attached to the thermoconductivity detector. Samples were collected in glass tubes fitted with ground joints to the outlet tube. Each glass tube was blown into a series of bulbs to optimize condensation. Each fraction was washed from the tube into a scintillation vial with diethyl ether, and the solvent was removed under a stream of nitrogen. Counting solution was added, and the radioactivity was determined. In each preparative fractionation an amount of sample equal to that injected into the gas chromatograph was counted in order



SCHEME I

to determine the recovery. Total recoveries of radioactivity were in all cases between 70 and 80%.

Determination of Radioactivity

Each sample was transferred to a scintillation vial, the solvent was removed under nitrogen, and 15 ml of a solution of 4.55 g of 2,5-diphenyloxazole and 0.45 g of 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene per liter of toluene were added. The radioactivity was then measured in a Packard Tri-Carb scintillation spectrometer (counting efficiency 80–81%). The samples were counted for 1, 2, or 10 min.

Radioactivity was calculated as cpm per brain; this allowed convenient comparisons. One may assume that variations in the lipid composition were minimal and that they were sufficiently averaged by pooling the brains of four animals. To insure that the aldehydes, alkyl acetates, methyl esters, and isopropylidene derivatives counted were free of contaminants, determinations of radioactivity were carried out in each case before and after GLC fractionation.

RESULTS

The biosynthesis of plasmalogens in the myelinating rat brain was studied after intracerebral injection of labeled palmitic acid or hexadecanol. Three series of experiments were performed. The first series was designed to demonstrate an incorporation of radioactivity from palmitic acid-1-^{14}C into the constituent alk-1-enyl ethers of the ethanolamine phosphatides over relatively short periods of time, and to compare the rate of this reaction with the rates of acylation. A second series of experiments was aimed at comparing the rates of formation of alk-1-enyl ethers and alkyl ethers of the ethanolamine phosphatides with the formation of free long-chain aldehydes and alcohols using palmitic acid-1-^{14}C as precursor. In the third series, hexadecanol-1-^{14}C was administered under identical conditions, and its incorporation into the constituent alk-1-enyl, alkyl, and individual acyl moieties of the ethanolamine phosphatides was determined.

Analyses of brain lipids agreed well with published reports (4, 8). The average weight of a brain was 1.2 g; the extracted lipids weighed 56.2 mg, and contained 15 mg (30%) of ethanolamine phosphatides. These consisted of the following three classes: alk-1-enyl acyl (45%), alkyl acyl (5%), and diacyl (50%) ethanolamine phosphatides. Aliphatic moieties at the 1-position were mainly C_{16}- and C_{18}-saturated, and C_{18}-monounsaturated constituents, whereas at the 2-position about 80% of the constituents were long-chain polyunsaturated fatty acids.

Incorporation of Palmitic Acid-1-^{14}C into Alk-1-enyl and Acyl Moieties

Results of the first experiment are shown in Table 1. Palmitic acid-1-^{14}C, about 3 μCi, was injected into each brain. The animals, in groups of four, were killed after 3, 6, or 22 hr.

As is evident from Table 1, the constituent alk-1-enyl ethers of the ethanolamine phosphatides showed a significant amount of radioactivity, but incorporation proceeded at a rate considerably lower than that observed for the constituent fatty acids.

A fraction of the neutral lipids, the "free aldehydes," became labeled at approximately the same rate as the alk-1-enyl ethers reaching a total activity of 15.2×10^3 cpm per brain after 22 hr. However, when this material was fractionated by GLC, no radioactivity was recovered with any of the carrier aldehydes.

Fractionation of the aldehydes derived from alk-1-enyl moieties and of the methyl esters derived from the 1-acyl moieties of the ethanolamine phosphatides into individual compounds showed that considerable amounts of radioactivity had been incorporated into the 18:0 and 18:1 compounds. Radioactivity in the alk-1-enyl ethers was too low after 3 hr to permit an accurate determination of individual fractions. Distribution of radioactivity between the C_{16} and C_{18} moieties was almost identical after 6 and 22 hr. In contrast, the percentage of radioactivity incorporated into the C_{18} fatty acids increased over the whole period of time. These data are shown in Table 2.

The fatty acids derived from the 2-acyl moieties of the ethanolamine phosphatides contained very little C_{16} and C_{18} compounds (8) which contained, however, most of the radioactivity. To avoid an error due to differences in recovery between the small amount of 18:0 and the larger fraction of 18:1 methyl esters, they were collected in a single fraction.

When the long-chain polyunsaturated fatty acids comprising the major fractions of the 2-acyl moieties were isolated individually, they had essentially no radio-

TABLE 1 INCORPORATION OF RADIOACTIVITY FROM PALMITIC ACID-1-^{14}C INTO ETHANOLAMINE PHOSPHATIDES

	3 hr	6 hr	22 hr
Total radioactivity recovered per brain [cpm $\times 10^{-6}$]	2.93	2.74	2.11
Radioactivity of ethanolamine phosphatides [% of total]	2.5	4.5	6.0
Radioactivity per brain of aliphatic moieties [cpm $\times 10^{-3}$]			
1-alk-1'-enyl	1.23	5.39	15.13
2-acyl	6.22	8.16	8.54
1-acyl	54.50	89.87	90.50
2-acyl*	9.07	14.56	8.90

* Including 2-acyl of 1-alkyl-2-acyl ethanolamine phosphatides.

TABLE 2 INCORPORATION OF RADIOACTIVITY FROM PALMITIC ACID-1-^{14}C INTO INDIVIDUAL ALIPHATIC MOIETIES OF ETHANOLAMINE PHOSPHATIDES

	1-Alk-1′-enyl				1-Acyl			
		3 hr	6 hr	22 hr		3 hr	6 hr	22 hr
	% weight		*% cpm*		*% weight*		*% cpm*	
16:0*	38.5	—	73.0	73.0	15.6	75.0	67.2	51.6
18:0	44.2	—	22.4	23.0	71.2	19.2	26.2	39.9
18:1	17.3	—	4.6	4.0	13.2	5.8	6.6	8.5
	2-Acyl				2-Acyl†			
16:0	3.1	82	75	60	2.1	74	61	51
18:0	1.1				1.3			
		18	25	40		26	39	49
18:1	13.7				12.9			
Others	82.1	—	—	—	83.7	—	—	—

* Number of carbon atoms: number of double bonds.
† Including 2-acyl of 1-alkyl-2-acyl ethanolamine phosphatides.

activity. However, when all fractions emerging from the GLC column after methyl octadecenoate were collected together, their radioactivity amounted to approximately 10% of the total. This may have been due to traces of saturated and monounsaturated fatty acids with more than 18 carbon atoms. No radioactivity was ever found in any fraction containing fatty acids with less than 16 carbon atoms.

Incorporation of Palmitic Acid-1-^{14}C into Alkyl Moieties

Radioactivity from palmitic acid-1-^{14}C (8.05×10^6 cpm per brain) was incorporated into alkyl ethers somewhat faster than into alk-1-enyl ethers as shown in Table 3. Although the alkyl ethers comprised only about $^1/_{10}$ of the alk-1-enyl ethers their total radioactivity after 1 hr was higher. However, the further increase in the radioactivity of the alkyl ethers was slow, and after 22 hr their specific activity was similar to that of the alk-1-enyl ethers.

80% of all radioactivity in the isopropylidene derivatives of the glycerol ethers was recovered in the 16:0 fraction after 3 and 6 hr; the remainder was in the 18:0 and 18:1 fractions. As standard mixtures of unlabeled carrier containing equal amounts of 16:0, 18:0, and 18:1 compounds were used, the specific activity of the isolated material was quite low, and an accurate determination of the radioactivity of individual fractions was not always possible.

In contrast to the free aldehydes, the free long-chain alcohols added as carriers to the lipid extract and isolated as alkyl acetates contained appreciable amounts of radioactivity. After fractionation of the alkyl acetates by GLC, about 80% of the radioactivity injected into the gas chromatograph was recovered with the C_{16} and C_{18} fractions.

The total radioactivities of the free alcohols reached their maxima after 3 hr. Total radioactivities per brain of alk-1-enyl ethers, alkyl ethers, and alcohols after different periods of time are shown in Fig. 1.

TABLE 3 INCORPORATION OF RADIOACTIVITY FROM PALMITIC ACID-1-^{14}C INTO ALK-1-ENYL ETHERS AND ALKYL ETHERS OF ETHANOLAMINE PHOSPHATIDES

	1 hr	2 hr	3 hr	6 hr	22 hr
Total radioactivity recovered per brain [cpm $\times 10^{-6}$]	3.21	3.67	4.36	3.53	2.43
Radioactivity of ethanolamine phosphatides [% of total]	1.5	2.3	2.6	4.7	6.3
Alk-1-enyl per brain [cpm $\times 10^{-3}$]	0.99	2.62	4.20	9.98	25.80
Alkyl per brain [cpm $\times 10^{-3}$]	1.21	1.41	2.09	2.34	4.90

Fractionation by GLC of the alkyl acetates, derived from the free alcohols, showed that almost all the radioactivity after 1 and 2 hr was associated with hexadecanol. After 3 hr 87%, and after 6 hr 73% of the radioactivity were recovered with the 16:0 fraction, and the rest was with the 18:0 and 18:1 fractions.

The fact that the individual alkyl acetates contained some radioactivity indicated that labeled alcohols were present in the tissue extract, yet their amounts were too small to be determined. Thus, the specific activity of the free alcohols could not be estimated. We have recently found free alcohols in bovine and porcine brain at a level of about 0.002% of the total lipids (9). If alcohols occur in the developing rat brain in similarly small amounts, their high specific activity would characterize them as intermediates having a very rapid turnover.

Incorporation of Hexadecanol-1-^{14}C into Alkyl, Alk-1-enyl, and Acyl Moieties

When hexadecanol-1-^{14}C (8.52×10^6 cpm per brain) was used as precursor, the incorporation of radioactivity into both alkyl ethers and alk-1-enyl ethers was vastly greater than from palmitic acid-1-^{14}C. Incorporation of

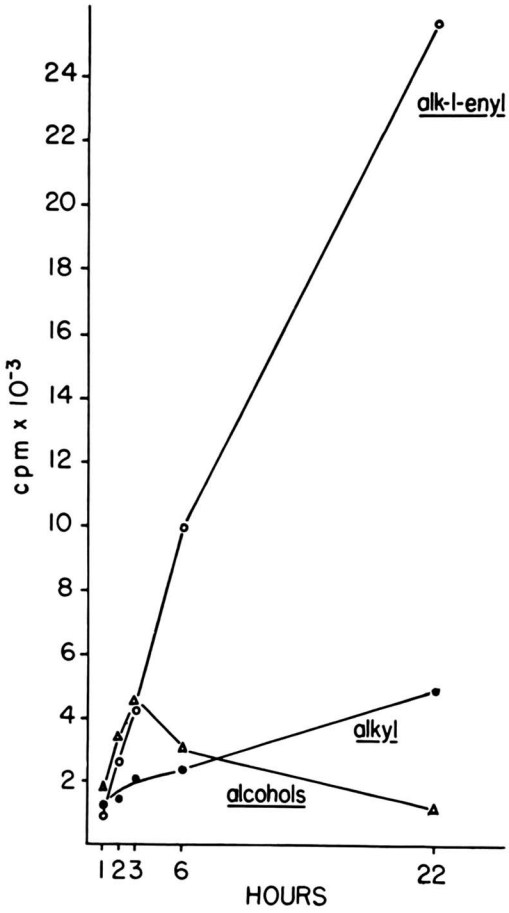


FIG. 1. Total radioactivities (cpm per brain) of long-chain alcohols and of the constituent alkyl and alk-1-enyl glycerol ethers of the ethanolamine phosphatides, 1–22 hr after injection of palmitic acid-1-^{14}C.

radioactivity from hexadecanol into ethanolamine phosphatides and their individual aliphatic moieties is shown in Table 4.

The total radioactivities recovered from the brains after 1 and 3 hr were low (by factors of 2.5 and 2.1), which was probably caused by loss of some precursor through bleeding immediately after injection. However, the relative amounts of radioactivity in the ethanolamine phosphatides and in their aliphatic moieties can be directly compared among all five groups of animals as demonstrated in Fig. 2.

Fig. 2*A* shows that after 1 hr the total radioactivities in the three aliphatic moieties at the 1-position of the ethanolamine phosphatides were almost equal; therefore, the alkyl glycerol ethers had the highest specific activity. The relative amount of radioactivity in the alk-1-enyl ethers increased at the expense of both alkyl ethers and fatty acids, a fact also observed with palmitic acid as a precursor (Fig. 2*B*).

If one assumes that the differences in the radioactivities recovered after the different time periods (Table 4) were due to differences in radioactivity effectively administered, one can use correction factors (2.5 and 2.1) to compare absolute values. Thus, all values of the radioactivities after 1 and 3 hr were corrected, and the results are shown in Fig. 3.

TABLE 4 INCORPORATION OF RADIOACTIVITY FROM HEXADECANOL-1-^{14}C INTO ETHANOLAMINE PHOSPHATIDES

	1 hr	2 hr	3 hr	6 hr	22 hr
Total radioactivity recovered per brain [cpm × 10^{-6}]	1.22	2.97	1.32	2.86	2.22
Radioactivity of ethanolamine phosphatides [% of total]	1.9	3.1	6.7	9.7	20.4
Radioactivity per brain of aliphatic moieties [cpm × 10^{-3}]					
1-alk-1′-enyl	6.09	40.60	45.20	167.80	330.00
2-acyl	1.86	4.79	3.12	9.73	12.70
1-alkyl	5.48	19.70	16.00	43.42	35.00
1-acyl	7.91	24.61	21.90	50.00	65.00
2-acyl*	1.43	3.44	3.12	6.92	10.50

* Of 1,2-diacyl and 1-alkyl-2-acyl ethanolamine phosphatides.

Although a large portion of the administered hexadecanol was incorporated into alkyl and, especially, alk-1-enyl ethers, a significant amount was found in the 1-acyl and 2-acyl moieties of the ethanolamine phosphatides. Thus, oxidation of alcohol to the corresponding fatty acids and their elongation, dehydrogenation and incorporation into the ethanolamine phosphatides must have proceeded quite rapidly. The distribution of radioactivity among individual alk-1-enyl ethers and fatty acids is listed in Table 5. The alkyl glycerol ethers contained all radioactivity, even after 22 hr, exclusively in 16:0 the homologue.

The data listed in Table 5 show that incorporation of radioactivity from hexadecanol into individual fatty acids at the 1-position of the ethanolamine phosphatides was similar to that from palmitic acid (compare Table 2).

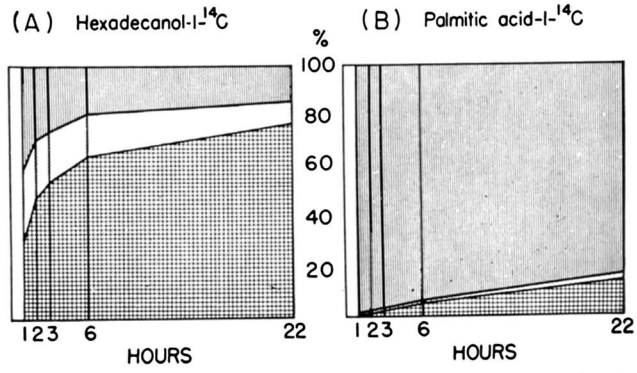


FIG. 2. Percentage of total radioactivity in the alk-1-enyl (▦), alkyl (▭), and 1-acyl (▥) moieties of the ethanolamine phosphatides, 1–22 hr after injection of hexadecanol-1-^{14}C (A) and palmitic acid-1-^{14}C (B).

TABLE 5 INCORPORATION OF RADIOACTIVITY FROM HEXADECANOL-1-^{14}C INTO INDIVIDUAL ALIPHATIC MOIETIES OF ETHANOLAMINE PHOSPHATIDES

	1 hr	2 hr	3 hr	6 hr	22 hr
			% *cpm*		
1-Alk-1′-enyl					
16:0	100.0	99.1	99.2	99.0	95.0
18:0	—	0.9	0.8	1.0	3.9
18:1	—	—	—	—	1.1
1-Acyl					
16:0	78.5	73.5	68.6	54.6	39.6
18:0	18.0	18.9	25.1	87.7	52.5
18:1	3.5	7.6	6.8	7.7	7.9

However, the radioactivities of the C_{18} acids relative to the C_{16} acids were somewhat higher. Similarly, the fatty acids at the 2-position showed slightly higher relative radioactivities in the C_{18} moieties than those listed in Table 2. As with palmitic acid as precursor, no radioactivity was found in the long-chain polyunsaturated fatty acids at the 2-position of the ethanolamine phosphatides.

Table 5 also shows that, with hexadecanol as precursor, only very little radioactivity was incorporated into the C_{18} chains of the alk-1-enyl ethers relative to the C_{16} chains. However, as the C_{18}-alk-1-enyl ethers did contain some radioactivity after 22 hr one can assume that the fatty acids produced from hexadecanol contributed measurably to the alk-1-enyl ether synthesis.

DISCUSSION

Our experiments show that the developing brain is able to incorporate radioactivity from long-chain fatty acids as well as from alcohols into both the alk-1-enyl glycerol ethers and alkyl glycerol ethers of the ethanolamine phosphatides without prior degradation of either precursor. Although hexadecanol is a much better precursor for the biosynthesis of these lipids than is palmitic acid, the rates of incorporation have some common characteristics. In each case the alk-1-enyl glycerol ethers show a relatively slow and steady increase in labeling over the whole 22 hr, whereas the constituent fatty acids of the ethanolamine phosphatides become labeled more rapidly and reach a plateau after about 6 hr. Also, with either precursor, the alkyl glycerol ethers become labeled more rapidly than the alk-1-enyl ethers over a short time, but their radioactivity does not increase at the same rate.

Joffe has shown (8) that, after intraperitoneal injection of acetate-1-^{14}C, the constituent alkyl glycerol ethers of the ethanolamine phosphatides of developing rat brain exhibit almost the same rapid rate of turnover as the constituent fatty acids at the 1-position. In contrast, the slow turnover of plasmalogens in the brain has long been recognized (6–8).

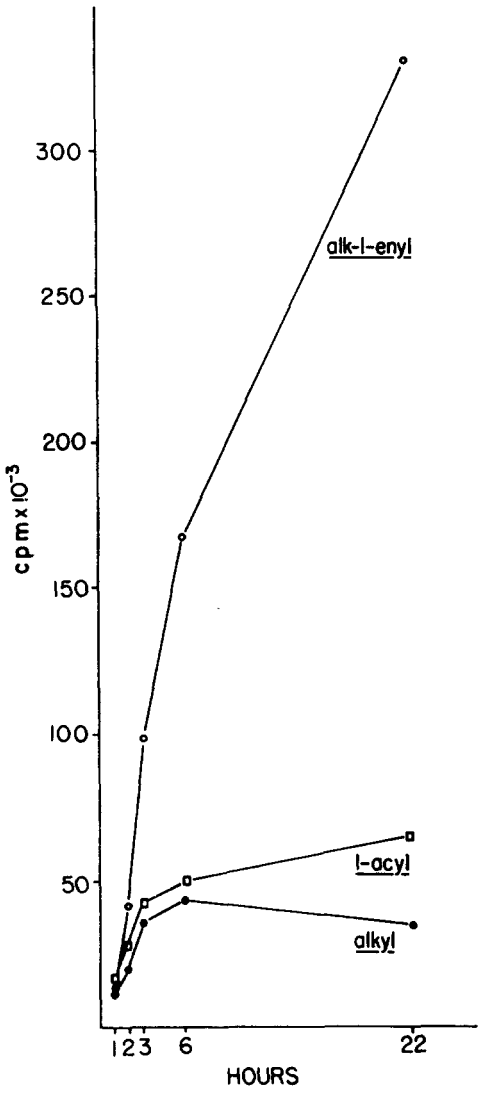


FIG. 3. Total radioactivities (cpm per brain, corrected) of the alkyl, alk-1-enyl, and 1-acyl moieties of the ethanolamine phosphatides, 1–22 hr after injection of hexadecanol-1-^{14}C.

In our experiments alcohols were quite rapidly oxidized to fatty acids which were incorporated into all lipid classes in the usual manner. Conversely, we found that fatty acids can be reduced to alcohols. The question arises, however, whether long-chain aldehydes play any role as precursors in the biosynthesis of alk-1-enyl ethers or, of more importance, whether long-chain aldehydes occur at all in brain tissue. In contrast to Gilbertson, Ferrell, and Gelman (21), who demonstrated the presence of long-chain aldehydes in heart muscle, we have not been able to confirm the presence of free long-chain aldehydes in brain. Despite the relative lability of aldehydes, we did not prepare the more stable acetals or hydrazones in order to avoid errors caused by reacting aldehydogenic compounds rather than aldehydes. By immediately adding nonlabeled aldehydes to the homogenates before ex-

tracting the lipids, even trace amounts of radioactive aldehydes should have become accessible to isolation and fractionation. There is no obvious reason why naturally occurring long-chain aldehydes should be preferentially lost through condensation or polymerization reactions. However, the standard mixtures of long-chain aldehydes recovered from the lipid extracts did not contain any radioactive material when fractionated into individual compounds. In contrast, the aldehydes released from the alk-1-enyl ethers could be isolated by TLC and GLC quite easily. In both cases, the only apparent side reaction was some condensation to form 2,3-dialkylacroleins as observed earlier (19).

From the experiments of Bickerstaffe and Mead (4), it appears that long-chain aldehydes are not particularly good precursors of alk-1-enyl ethers. By comparing the rates of incorporation of radioactivity into fatty acids and alk-1-enyl ethers, it seems that alcohols are much better precursors of alk-1-enyl ethers than are aldehydes. Carr, Haerle, and Eiler (22) showed that aldehydes could not be incorporated into the alk-1-enyl ethers of rat brain homogenates. Bell and White (5), who used palmitaldehyde-1-^{14}C, 1-^{3}H as the precursor, proved that much of the tritium is retained in the alk-1-enyl ethers, but the ratio of ^{3}H/^{14}C is changed. One may speculate that an aldehyde administered to brain tissue is first reduced to the corresponding alcohol before being utilized for the biosynthesis of plasmalogen. Such a reduction would explain the loss of ^{3}H relative to ^{14}C.

Our results exclude the possibility that the 1-acyl moieties could be the precursors of 1-alk-1′-enyl moieties because of their lower specific activities and because the apparent chain elongation in the former is not reflected in the latter. These differences in the labeling pattern of acyl and alk-1-enyl moieties are so pronounced that they appear to be significant even for a heterogeneous organ such as the brain, whose sections show different concentrations of alk-1-enyl acyl and diacyl ethanolamine phosphatides.

The fact that some radioactivity was found in the C_{18}-alk-1-enyl ethers but none in the corresponding alkyl ethers is more difficult to interpret. As exact margins of error for the recovery of radioactive fractions from the gas chromatograph have not been established, the significance of a 5% "chain elongation" of the alk-1-enyl ethers is questionable.

However, if both the chain elongation in the alk-1-enyl ethers and the lack of it in the alkyl ethers are more than an experimental error, this would cast some doubt on the role of the alkyl ethers as direct precursors in plasmalogen biosynthesis. It is quite conceivable that the oxidative process from alcohol to alk-1-enyl ether lipid involves one or more yet unidentified intermediates. This process appears to be sufficiently pronounced to encourage further study.

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