Formation of 1-*O*-2′-hydroxyalkyl glycerophosphatides from 1,2-heptadecanediol in myelinating brain

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ABSTRACT 1,2-Heptadecanediol-2-^{14}C was administered intracerebrally to 18-day-old rats, and its incorporation, after 8 hr, into the individual aliphatic moieties of the ethanolamine glycerophosphatides was determined.

Much of the radioactivity was found in a lipid fraction identified as 1-*O*-2′-hydroxyheptadecyl glycerol. Evidence is presented that a major portion of the precursor was incorporated into 1-*O*-2′-hydroxyheptadecyl-2-acyl ethanolamine phosphatides.

Some of the diol administered was degraded to palmitic acid. The palmitic acid-1-^{14}C derived from 1,2-heptadecanediol-2-^{14}C apparently served as precursor for stearic and oleic acids, which were found as acyl groups, and for the biosynthesis of the corresponding *O*-alkyl and *O*-alk-1-enyl glycerols.

The data presented prove that biological dehydration of 1-*O*-2′-hydroxyalkyl glycerophosphatides to the corresponding plasmalogens does not occur in myelinating brain.

SUPPLEMENTARY KEY WORDS ethanolamine phosphatides · alkyl acyl glycerols · alk-1-enyl acyl glycerols · diacyl glycerols · intracerebral injection · 1,2-heptadecanediol-2-^{14}C

PREVIOUS WORK from this laboratory has shown that long-chain alcohols occur in mammalian brain, that they may be produced from long-chain fatty acids, and that they are rapidly incorporated into both alkyl and alk-1-enyl moieties of the ethanolamine phosphatides, as well as into their acyl moieties (1, 2). The presence of long-chain alcohols in mammalian tissues has also been demonstrated by Blank and Snyder (3), and very recent experiments by Wood and his colleagues (4, 5) have also established the role of long-chain alcohols in the biosynthesis of both alkyl and alk-1-enyl glycerol ether lipids in Ehrlich ascites cells. Stoffel, LeKim, and Heyn (6) have shown that palmitaldehyde released in the course of sphinganine degradation can be incorporated into the alk-1-enyl moieties of the phosphatides of myelinating rat brain, presumably via hexadecanol. Palmitaldehyde, doubly labeled at carbon 1, administered to Ehrlich ascites cells (4, 5) or derived biologically from doubly labeled sphinganine in myelinating brain (6) was found to lose about half of its ^{3}H activity relative to its ^{14}C activity upon incorporation into alk-1-enyl glycerol ethers.

Therefore, most of the current evidence supports the view that long-chain alcohols are precursors in the biosynthesis of both the alkyl glycerol ethers and alk-1-enyl glycerol ethers of mammalian tissues, and a biodehydrogenation of alkyl glycerophosphatides (7, 8) to the corresponding alk-1-enyl derivatives appears to be a plausible pathway (4–8).

However, from the work of Joffe (9) one may conclude that in myelinating brain the alkyl moieties of the ethanolamine phosphatides are cleaved considerably faster than the alk-1-enyl moieties are formed. From a recent report by Debuch, Friedemann, and Müller (10), it follows that alkyl acyl ethanolamine phosphatides administered to brain are not very effective precursors of the corresponding plasmalogens.

Therefore, both a direct conversion of alkyl to alk-1-enyl glycerol ether lipids and the possibility of an alternative pathway from long-chain alcohols to alk-1-enyl glycerol ethers involving neither alkyl glycerol ethers

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

nor aldehydes deserve further study. For example, structures such as hydroxyalkyl glycerol ethers could serve as intermediates which could possibly be dehydrated to the corresponding plasmalogens. Lipids having the hydroxyalkyl glycerol ether backbone have not been identified as natural products, but the corresponding methoxyalkyl glycerol ethers have been found in shark liver oils (11).

Long-chain 1,2-alkanediols, which were found as natural wax constituents of mammalian skin (12, 13), could serve as the precursor of the hydroxyalkyl moieties in analogy to the alkyl glycerol ether biosynthesis. In this communication we report studies on the metabolism of 1,2-heptadecanediol administered to myelinating brain, with special emphasis on its possible role in the biosynthesis of the alk-1-enyl moieties of plasmalogens.

EXPERIMENTAL

Fatty acids, alcohols, and palmitoyl chloride were obtained from The Hormel Institute Lipids Preparation Laboratory, Austin, Minn. Glycerol ethers were synthesized according to Baumann and Mangold (14). Palmitic acid-1-^{14}C (58 mCi/mmole) was purchased from New England Nuclear, Boston, Mass. Male albino rats of the Sprague-Dawley strain, 18 days old at the day of the experiment, were purchased from the Dan Rolfsmeyer Co., Madison, Wis.

1,2-Heptadecanediol was synthesized from palmitic acid as described by Mangold (15). From 60 mg of starting material, the final product, purified by preparative TLC (hexane–diethyl ether 20:80), mp 74.5°C, was obtained in 85% yield and was characterized by its infrared spectrum. The diacetate and the isopropylidene derivatives were prepared, and they were identified by their IR spectra, by their migration rates on TLC, and by their retention times on GLC. When 1,2-heptadecanediol was prepared on a smaller scale (6 mg), the yield was considerably lower (41%), and the principal side product was hexadecanol.

1,2-Heptadecanediol-2-^{14}C was prepared in the same manner from palmitic acid-1-^{14}C (0.5 mCi; specific activity, 58 mCi/mmole). A thin-layer chromatogram of the final reaction products showed that about half of the radioactivity was in a fraction corresponding to 1,2-heptadecanediol and that the other half was in a fraction resembling hexadecanol. The 1,2-heptadecanediol was purified twice by TLC, using hexane–diethyl ether 20:80, to a radiopurity of more than 99%. To the preparation, which was virtually free of labeled hexadecanol, was added 1,2-heptadecanediol as carrier, and the diacetate and the isopropylidene derivative were prepared. Analysis by GLC showed that more than 99% of the radioactivity recovered was associated with the appropriate fractions.

1-*O*-2′-Hydroxyheptadecyl glycerol, mp 80°C, was synthesized from 1,2-heptadecanediol via the following intermediates: 1-trityloxy-2-heptadecanol, 1-trityloxy-2-benzyloxyheptadecane, 2-benzyloxy-1-heptadecanol, 2-benzyloxy-1-heptadecyl methanesulfonate, and 1-*O*-(2′-benzyloxy)-heptadecyl-2,3-isopropylideneglycerol. Detailed procedures for the preparation of 1-*O*-2′-hydroxyalkyl glycerols and their physical characteristics will be the subject of a forthcoming publication.[1]

Emulsification and administration of precursor, extraction of lipids, and preparation of ethanolamine phosphatides were as described by Schmid and Takahashi (1). Adsorption chromatography was carried out on layers of silica gel H, 0.3 mm thick, in tanks lined with filter paper or, for the relatively large amounts of synthetic products, on layers 2 mm thick in unlined tanks (16); solvent systems are listed throughout the text. Fractions were eluted from the adsorbent with diethyl ether saturated with water, or with chloroform–methanol 1:1 if compounds with two or more hydroxy groups were involved (1). GLC was performed with a Victoreen 4000 instrument, using ethylene glycol succinate as stationary phase as described previously (1). In addition, a copper column, 190 cm long, 0.2 cm I. D., filled with silicone gum rubber SE-30 (6%) on Anachrom ABS 90–100 mesh (Analabs, Inc., North Haven, Conn.), was used at 270°C for the analysis of the acetylated 1-*O*-2′-hydroxyheptadecyl glycerol. Collection of radioactive fractions was as described previously (1). In each case an amount of sample equal to that injected into the gas chromatograph was counted to check the recovery. Total recoveries of radioactivity were between 70 and 80%. Radioactivity was measured in a Packard Tri-Carb scintillation spectrometer (counting efficiency 80–81%) as described previously (1).

Infrared spectra were recorded with a Perkin-Elmer spectrophotometer, model 21, using carbon disulfide as solvent except in the ranges 2400–2000 cm^{-1} and 1650–1400 cm^{-1}, where tetrachloroethylene was used. Melting points were determined on a Kofler hot stage and are corrected.

Acetylations were carried out by reactions with acetic anhydride in pyridine at 80°C for 2 hr, and methanolyses by reactions with methanol–HCl (5%) at 80°C for 2 hr. Isopropylidene derivatives were prepared with anhydrous acetone in the presence of catalytic amounts of perchloric acid (17). Periodate cleavage was carried out as described by Baumann, Schmid, and Mangold (18). Phospholipase C (*Clos-*

[1] Muramatsu, T., and H. H. O. Schmid. Unpublished data.

tridium welchii) hydrolysis was as described by Takahashi and Schmid (19).

The ethanolamine phosphatides were reduced with $LiAlH_4$ as follows. The sample was suspended in diethyl ether and added slowly to a solution of $LiAlH_4$ in diethyl ether at reflux temperature. The reaction mixture was held at reflux temperature for 2 hr and was then cooled to 4°C. 10% HCl was added until the reaction mixture was acidic; the mixture was then held at room temperature for 1 hr. The products of reduction and hydrolysis were extracted in the conventional manner. The use of HCl for the decomposition of the lithium–aluminum complex proved advantageous because of its solubility in diethyl ether, thus allowing a complete hydrolysis of the alk-1-enyl ethers with only minimal formation of cyclic acetals.

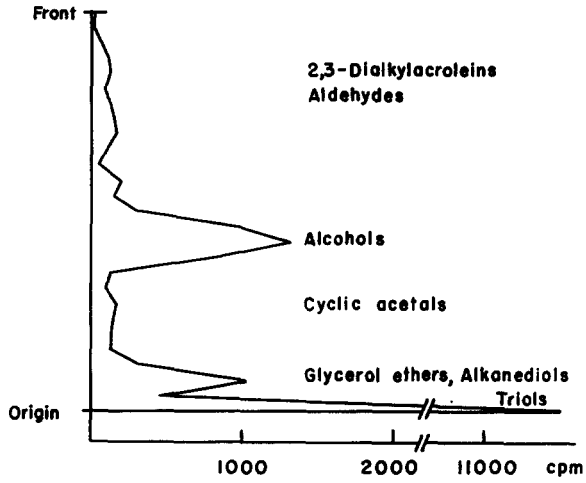

FIG. 1. Distribution of radioactivity recovered from TLC fractions of the products of $LiAlH_4$ reduction of the ethanolamine phosphatides. Silica gel H; hexane–diethyl ether 50:50.

RESULTS

1,2-Heptadecanediol-2-^{14}C (58 mCi/mmole) was injected intracerebrally into four 18-day-old rats. The rats were killed after 8 hr, the brains were pooled, and the incorporation of radioactivity into the brain lipids was determined. Of a total radioactivity of 5.96×10^6 cpm administered per brain, 1.78×10^6 cpm per brain were recovered in the lipid extract.

Preliminary fractionations of small aliquots by TLC showed that about 60% of the radioactivity recovered was associated with unchanged precursor, a total of about 10% was in various fractions of neutral lipids, 12% was in a crude ethanolamine phosphatide fraction, and 13% was in a fraction containing choline phosphatides and sphingomyelin. Since the ethanolamine phosphatides are known to consist of alkyl acyl, alk-1-enyl acyl, and diacyl species, they were purified by preparative TLC, and the distribution of radioactivity among their aliphatic moieties was determined.

Part of the ethanolamine phosphatide fraction was reduced with $LiAlH_4$ and the reaction mixture was treated with HCl. A mixture of hexadecyl and octadecyl glycerol ethers (5 mg each) and 1,2-heptadecanediol (5 mg) was added to the reaction mixture as carrier. An aliquot of the reaction products was fractionated by TLC using hexane–diethyl ether 50:50. The distribution of radioactivity in the chromatogram is demonstrated in Fig. 1.

The highest amount (>60%) of radioactivity originally present in the phosphatide was found at the origin in a fraction more polar than glycerol ethers and was tentatively identified as "triols"; significant radioactivity was also present in the alcohol and "glycerol ether" fractions. Very little radioactivity was found in fractions that could have been derived from alk-1-enyl ether moieties such as aldehydes, cyclic acetals (20), and 2,3-dialkyl acroleins (21). The reaction products were, therefore, separated by TLC, using diethyl ether as solvent, into fractions corresponding to standard alcohols (R_F = 0.62) and glycerol ethers (R_F = 0.33); the triol fraction was recovered at the origin.

Alcohol Fraction (*2.77×10^4 cpm per brain*). The sample was acetylated and an aliquot was checked by TLC, using hexane–diethyl ether 90:10. Essentially all of the radioactivity was found in the alkyl acetates. The alkyl acetates were fractionated by preparative GLC, and most of the radioactivity was recovered with the 16:0, 18:0, and 18:1 acetates (49%, 18%, and 8.5%, respectively).

"Glycerol Ether" Fraction (*1.33×10^4 cpm per brain*). The sample was acetylated and an aliquot was fractionated by TLC, using hexane–diethyl ether 90:10 (developed three times). Most of the radioactivity was found in the fractions corresponding to the standard 1,2-heptadecanediol diacetate (65%), R_F = 0.52, and glycerol ether diacetates (13%), R_F = 0.40; a small amount of radioactivity (12%) was found in an unidentified fraction at R_F = 0.20. Preparative GLC showed that essentially all radioactivity of the 1,2-alkanediol diacetate fraction was in the 1,2-heptadecanediol diacetate, whereas in the glycerol ether diacetates most of the radioactivity was in the 16:0 fraction, with small amounts also in the 18:0 and 18:1 fractions. The total radioactivity of the glycerol ether diacetates was too low to permit an accurate determination of its chain-length distribution.

Triol Fraction (*31.1×10^5 cpm per brain*). Small aliquots of the sample were acetylated or reacted with acetone–perchloric acid. TLC with hexane–diethyl ether 50:50 showed that all radioactivity after acety-

lation was present in an area with R_F = 0.44 and, for the isopropylidene derivative, in an area with R_F = 0.25. In view of the fact that the acetylated product was significantly less polar than the acetone adduct, the triol fraction was tentatively identified as a hydroxyalkyl glycerol ether. A synthetic standard of 1-*O*-2′-hydroxyheptadecyl glycerol was, therefore, prepared and added to an aliquot of the triol fraction.

TLC, using chloroform–methanol–water 65:25:4 as solvent, showed that almost all radioactivity was associated with the 1-*O*-2′-hydroxyheptadecyl glycerol standard. The triacetate and the isopropylidene derivative were prepared and fractionated by TLC using hexane–diethyl ether 50:50. Again, essentially all radioactivity was recovered with the appropriate fractions.

To ascertain the position of the ether linkage at the 1 position of glycerol, the mixture of triol and 1-*O*-2′-hydroxyheptadecyl glycerol was reacted with sodium metaperiodate in pyridine (18), and the reaction products were checked by TLC, using hexane–diethyl ether 20:80. As all radioactivity was recovered with the *O*-hydroxyheptadecyl glycolaldehyde (R_F = 0.55) derived from the standard, a 2-*O*-2′-hydroxyheptadecyl glycerol structure for the triol was ruled out.

Fig. 2 shows the separation of 1-*O*-2′-hydroxyheptadecyl glycerol from its isopropylidene derivative and triacetate. The thin-layer chromatogram of Fig. 3 proves that in a polar solvent 1-*O*-2′-hydroxyheptadecyl glycerol is clearly separable from 1,2-heptadecanediol and from the ethanolamine phosphatides.

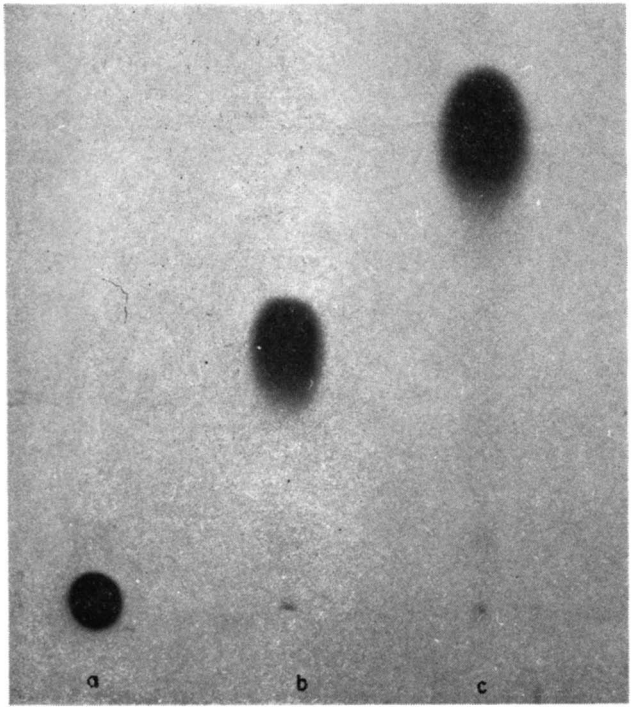

FIG. 2. Thin-layer chromatogram of 1-*O*-2′-hydroxyheptadecyl glycerol (*a*), its isopropylidene derivative (*b*), and its triacetate (*c*). Silica gel H; hexane–diethyl ether 50:50.

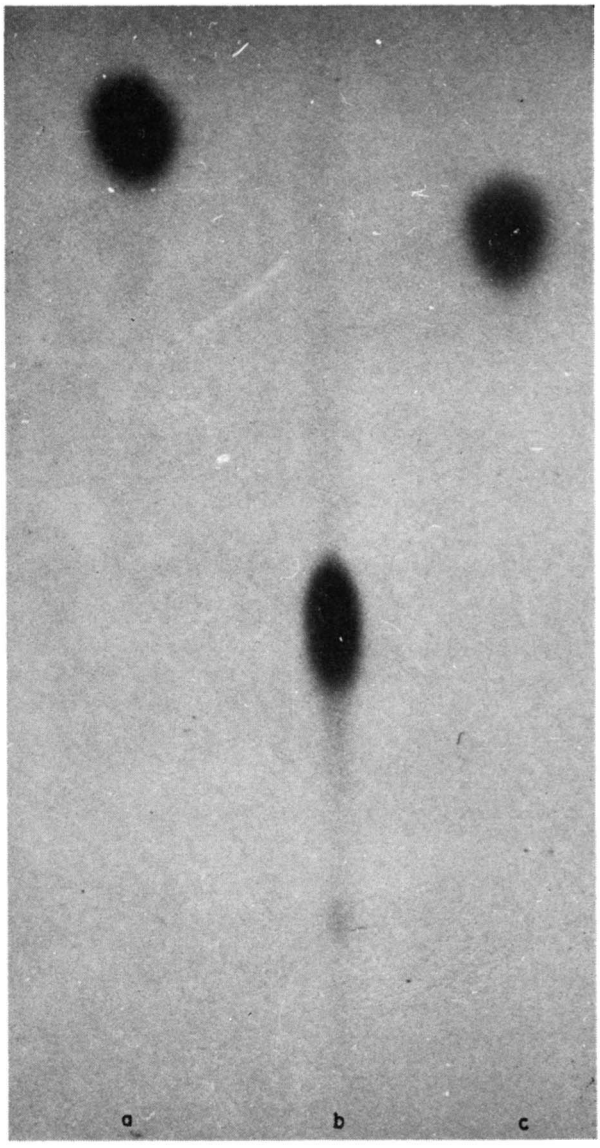

FIG. 3. Thin-layer chromatogram of 1,2-heptadecanediol (*a*), ethanolamine phosphatides (*b*), and 1-*O*-2′-hydroxyheptadecyl glycerol (*c*). Silica gel H; chloroform–methanol–water 65:25:4.

The triacetate was also fractionated by preparative GLC, and the result is shown in Fig. 4. Thus, comparison of the chromatographic properties of the triol fraction and its derivatives with those of synthetic standards led to its identification as 1-*O*-hydroxyheptadecyl glycerol, presumably with the hydroxy group in the 2′ position. Since it was derived from a purified fraction of ethanolamine phosphatides through $LiAlH_4$ reduction, one could assume that the original product had been a 1-*O*-2′-hydroxyheptadecyl 2-acyl ethanolamine glycerophosphatide.

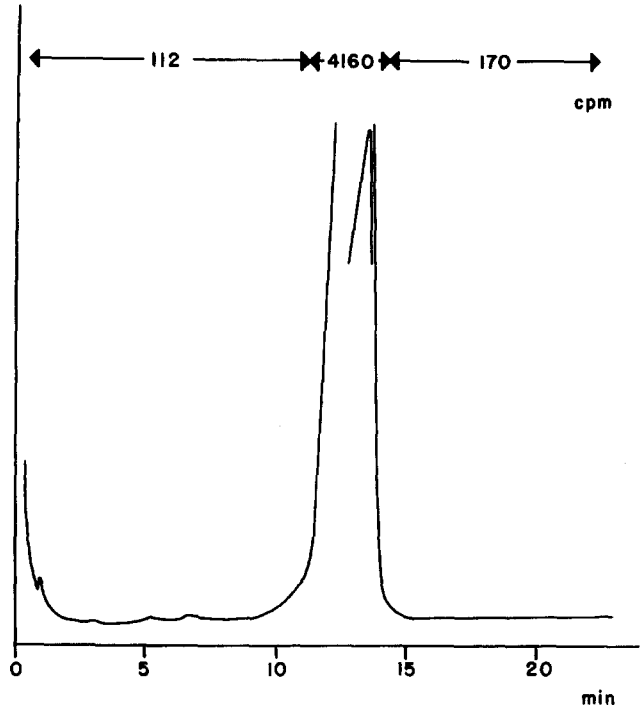

FIG. 4. Gas–liquid chromatogram of a mixture of radioactive triol–triacetate and 1-*O*-2′-hydroxyheptadecyl glycerol triacetate standard (SE-30, 270°C.). Fractions were collected in glass tubing fitted with conical ground glass joints to the heated outlet tube and were transferred to counting vials. Total recovery was 70% of that injected into the gas chromatograph.

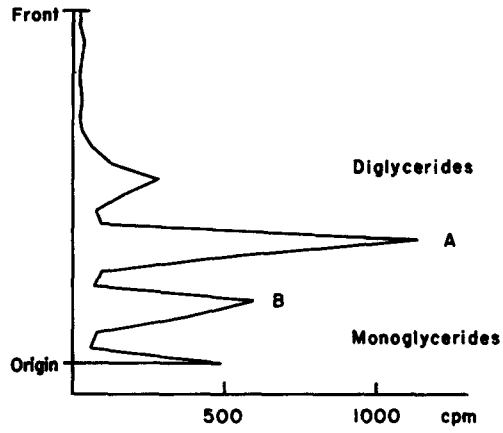

FIG. 5. Distribution of radioactivity recovered from TLC fractions of the products of phospholipase C hydrolysis of the ethanolamine phosphatides. Silica gel H; hexane–diethyl ether 40:60.

This assumption was supported by the fact that ethanolamine phosphatides were found to be more polar than and clearly separable from 1-*O*-2′-hydroxyheptadecyl glycerol, as shown in Fig. 2. However, an acyloxy or keto group originally present at the 2′ position could also have yielded the 2′-hydroxy derivatives. Therefore, the ethanolamine phosphatide fraction was hydrolyzed with phospholipase C in the presence of 2-acyl choline lysophosphatides (19).

Analysis of the reaction products by TLC (hexane–diethyl ether 40:60) showed most of the radioactivity to be in two fractions more polar than diglycerides but less polar than monoglycerides, as demonstrated in Fig. 5. Only 15% of the radioactivity was recovered with the fraction representing alkyl acyl, alk-1-enyl acyl, and diacyl glycerols; 13% was found as nonhydrolyzed phospholipid. The major fractions, *A* (43%) and *B* (20%), were isolated by preparative TLC and were subjected to methanolysis. Fraction A yielded several unidentified radioactive products, but fraction B yielded a product identical on TLC to the standard 1-*O*-2′-hydroxyheptadecyl glycerol. Also, GLC of its triacetate yielded all radioactivity in the appropriate fraction.

The structure of fraction A is presently under investigation. Catalytic hydrogenation of fraction A yielded a product whose migration rate in TLC was identical to that of fraction B, and reduction of fraction A with $LiAlH_4$ produced a radioactive compound tentatively identified as 1-*O*-2′-hydroxyheptadecyl glycerol. These preliminary results indicate the presence of 1-*O*-2′-hydroxyheptadec-1′-enyl glycerol or its tautomeric 2′-keto derivative as a constituent of fraction A. It also follows that the major portion of the radioactive 1-*O*-2′-hydroxyalkyl glycerol obtained by reduction of the ethanolamine phosphatides with $LiAlH_4$ may have been derived from a 2′-hydroxyenol ether or 2′-keto ether analog.

However, the fact that phospholipase C hydrolysis produced a fraction that yielded radioactive hydroxyalkyl glycerol upon methanolysis confirmed the presence of a 1-*O*-2′-hydroxyalkyl 2-acyl ethanolamine glycerophosphatide as one of the major products derived from 1,2-heptadecanediol-2-^{14}C.

Incorporation of radioactivity into the alk-1-enyl moieties of the ethanolamine phosphatides was determined after acidic hydrolysis of the ethanolamine phosphatides. An aliquot was treated with hydrochloric acid (22), and the resulting aldehydes (2.0×10^3 cpm per brain) were isolated by TLC. Gas–liquid chromatographic fractionation of the aldehydes yielded most of the radioactivity in the 16:0, 18:0, and 18:1 fractions, with relative amounts of 26, 55, and 19%, respectively, but with no measurable radioactivity in the 17:0 fraction.

DISCUSSION

The results presented here show that 1,2-heptadecanediol-2-^{14}C administered to myelinating rat brain was incorporated into glycerophosphatides, in part under formation of an ether linkage. Preliminary analysis of the

choline phosphatides showed that, as in the ethanolamine phosphatides, much of the radioactivity was in a fraction corresponding to 1-*O*-2′-hydroxyheptadecyl glycerol. Apparently, the effect of the additional hydroxy group on the migration rates on TLC was not strong enough to achieve a subfractionation of the ethanolamine or choline phosphatides.

The presence of a substance resembling labeled 1,2-heptadecanediol among the products of $LiAlH_4$ reduction of the ethanolamine phosphatides remains unexplained. It appeared reasonable to assume that it was produced from labeled 2′-hydroxyacyl groups. However, we could not ascertain the presence of labeled hydroxyacyl groups in the ethanolamine phosphatides or in the total lipids.

Our results show also that 1-*O*-2′-hydroxyalkyl glycerophosphatides derived from racemic 1,2-alkanediols cannot be dehydrated to the corresponding plasmalogens, as the small amount of radioactivity found in the alk-1-enyl moieties was not associated with the corresponding chain length.

The 1,2-heptadecanediol-2-^{14}C administered to the brain was also degraded to some extent, yielding labeled palmitic acid. It appears possible that after the loss of carbon 1, palmitic acid-1-^{14}C and its elongation and dehydrogenation products were incorporated into the acyl moieties. Apparently, some of these fatty acids were also reduced and, thus, utilized for the synthesis of the labeled alkyl and alk-1-enyl moieties (1). No appreciable amounts of radioactivity were found in aliphatic moieties having less than 16 carbon atoms.

Very recently, Blank et al. (23) reported that a glycerol ether, doubly labeled in the glycerol moiety and in the aliphatic chain administered to Ehrlich ascites cells, yielded doubly labeled plasmalogens. This report confirmed Thompson's earlier conclusion (8) that alkyl glycerol ether lipids can be direct precursors of alk-1-enyl ether lipids. Blank et al. (23) also reported the occurrence of an unknown material exhibiting many, but not all, of the characteristics of 1-*O*-2′-hydroxyalkyl glycerols. This material, obtained by $LiAlH_4$ reduction of the total lipids and of the phospholipids, had the same $^3H/^{14}C$ ratio as the alkyl and alk-1-enyl glycerol ethers and was, therefore, proposed as an intermediate in the suggested biological conversion. The results of our experiments rule out the participation of 1-*O*-2′-hydroxyalkyl glycerols as intermediates in such a pathway in the developing brain.

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