Journal of Neurochemistry
Raven Press, New York

Biosynthesis of Triglyceride and Other Fatty Acyl Esters by Developing Rat Brain

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Abstract: The biosynthesis of triglyceride from 1,2-diglyceride and long-chain acyl coenzyme A (CoA) was studied in developing rat brain. Diglyceride acyltransferase activity was highest in a microsomal fraction, had a neutral pH optimum, and was stimulated by $MgCl_2$. Palmitoyl CoA and oleoyl CoA served equally well as acyl donors. The enzyme catalyzed the acylation of both endogenous diglyceride and several naturally occurring and synthetic exogenous diglycerides. In addition, short-chain primary and secondary alcohols were found to be acylated under these conditions. A second acylation system, active at low pH, was found to catalyze esterification of ethanol and cholesterol, but not diglyceride, with free fatty acid. These results demonstrate that brain has the capacity to acylate a wide variety of physiological and nonphysiological hydroxyl compounds. **Key Words:** Diglyceride acyltransferase—Ethanol acyltransferase—Trigylceride. **Bishop J. E. and Hajra A. K.** Biosynthesis of triglyceride and other fatty acyl esters by developing rat brain. *J. Neurochem.* **43**, 1046–1051 (1984).

A number of workers have reported that brain contains small but measurable amounts of triglycerides (Rowe, 1969; Cook, 1981; Horrocks and Harder, 1983). Previously, while studying reduction of [1-^{14}C]palmitoyl coenzyme A (CoA) to [1-^{14}C]-hexadecanol by a rat brain microsomal preparation it was noted (on TLC analysis) that a radioactive compound less polar than hexadecanol was formed (Bishop and Hajra, 1978; Bishop, 1981). This compound was tentatively identified as triglyceride. However, Jagannatha and Sastry (1981) reported that under identical conditions cholesterol esters, not triglycerides, are formed in brain microsomal preparations. The identity of this compound was reexamined and current investigations confirm our previous characterization of this nonpolar lipid as triglyceride. An enzymatic system is demonstrated to be present in brain that catalyzes the acylation of endogenous and exogenous diglycerides by long-chain acyl CoAs to triglycerides. In addition, we found that a number of other alcohols and thiols are acylated in brain microsomes to form the corresponding palmitate esters, showing the presence, in brain, of a nonspecific enzymatic acylation system.

MATERIALS AND METHODS

ATP, CoA, Tween-20, cholesteryl palmitate, cholesteryl oleate, and phosphatidyl serine were obtained from Sigma Chemical (St. Louis, MO). All diglycerides and 1-monoolein were supplied by Serdary Research Laboratories (London, Ontario, Canada). Bovine serum albumin (fatty-acid-free) was obtained from Miles Laboratories (Kankakee, IL). New England Nuclear (Boston, MA) supplied [1-^{14}C]oleic acid and [1-^{14}C]palmitic acid. E. Merck silica gel 60 TLC plates were supplied by Brinkmann Instruments (Des Plaines, IL).

[1-^{14}C]Palmitoyl CoA was prepared by the condensation of [1-^{14}C]palmitoyl chloride with CoA (Bishop and Hajra, 1980). Methyl [1-^{14}C]palmitate was prepared by acid-catalyzed esterification of [1-^{14}C]palmitic acid and methanol in the presence of dimethoxypropane (Davis and Hajra, 1981). Ethyl [1-^{14}C]palmitate was prepared by the pyridine-catalyzed condensation of [1-^{14}C]palmitoyl chloride (Bishop and Hajra, 1980) with ethanol. A brain microsomal fraction was isolated by differential centrifugation of 10% homogenate in 0.25 *M* sucrose from 12-day-old Sprague-Dawley rats as the particulate preparation sedimented between 18,000 *g*, 30 min and 105,000 *g*, 30 min centrifugation (Bishop and Hajra, 1978). Diglycerides (and other alcoholic lipids) were emulsified at a constant mole ratio of lipid to detergent by a modification of the method of Vance and Burke (1974). Sufficient

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Abbreviation used: CoA, Coenzyme A.

diglyceride[1] (or other lipid) to give a final concentration of 400 μ*M* was dried down under a stream of N_2. Tween-20 (0.015% wt/vol) and 75 m*M* phosphate (K^+ salt, pH 7.4) were then added. The test tube was flushed with N_2, capped, sonicated in an ultrasonic bath for sufficient time to bring the lipid into a cloudy suspension (1–5 min), and then vortexed vigorously to clarity (usually 5–10 min).

Palmitoyl CoA:1,2-diglyceride *O*-acyltransferase (EC 2.3.1.20; diglyceride acyltransferase) was assayed by the [^{14}C]palmitoyl CoA-dependent conversion of endogenous diglyceride to ^{14}C-triglyceride. Assay mixtures in a total volume of 500 μl contained the following: 75 m*M* phosphate (K^+ salt, pH 7.4), 10 m*M* $MgCl_2$, 100 μg brain microsomal protein, 15 μ*M* bovine serum albumin, and 15 μ*M* [^{14}C]palmitoyl CoA (125 dpm/pmol). In indicated experiments, the mixture was supplemented with exogenous 1,2-diglyceride (from egg phosphatidyl choline) emulsified in Tween-20 as detailed above. All reagents were added, except for albumin and acyl CoA, and the mixture was vortexed for 10 s, then warmed to 37°C. Reaction was started by addition of a mixture of albumin and acyl CoA, and incubation was for 5 min at 37°C. The lipids were extracted (Bligh and Dyer, 1959) and separated by TLC (plates were activated at 100°C for 1 h before use) using either solvent system A (petroleum ether-ethyl ether-8.4 *M* NH_4OH, 90:10:1.2; triglyceride R_f = 0.19) or solvent system B (petroleum ether-ethyl ether-8.4 *M* NH_4OH, 80:20:1.2; triglyceride R_f = 0.42). Radioactive lipids were visualized by autoradiography, scraped out, and the radioactivity was quantified by liquid scintillation counting (LaBelle and Hajra, 1972).

Three different assays described by other authors for the biosynthesis of cholesteryl ester were utilized. That of Choi and Suzuki (1978) contained 660 μ*M* [1-^{14}C]oleic acid, 533 μ*M* cholesterol, 660 μg brain phosphatidyl serine, 83 μ*M* sodium citrate-phosphate (pH 5.2), and 1 mg microsomal protein in a volume of 1.2 ml. The low-pH assay of Jagannatha and Sastry (1981) contained 100 μ*M* [1-^{14}C]oleic acid, 2.5% (vol/vol)ethanol, 50 m*M* sodium citrate-phosphate (pH 5.2), and 1 mg brain protein. The neutral pH assay of Jagannatha and Sastry (1981) contained 100 μ*M* [1-^{14}C]oleic acid, 2.5% (vol/vol)ethanol, 25 m*M* phosphate (sodium salt, pH 7.4), 20 m*M* ATP, 250 μ*M* CoASH, 20 m*M* $MgCl_2$, and 1 mg brain protein. For all three assays, incubation was for 1 h at 37°C. The lipids were extracted by the method of Bligh and Dyer (1959) and separated by TLC (see Fig. 1). Protein was determined by the method of Lowry et al. (1951). All other methods are the same as previously described (Bishop and Hajra, 1981).

All data presented are the averages of, or representative of, two to four separate experiments.

RESULTS

It was shown previously that on incubation of rat microsomes with [1-^{14}C]palmitoyl CoA, a number of radioactive lipids are formed due to enzymatic acylation of endogenous hydroxyl compounds. Whereas the major amount of radioactivity is incorporated into phosphatidyl choline, the major nonpolar lipid labeled in this system was tentatively identified as triglyceride (Bishop and Hajra, 1978; Bishop, 1981). This study confirms the identification of that compound as triglyceride by comparing its migration rate on thin-layer chromatograms with authentic standard triglyceride (Fig. 1, lane E). Addition of exogenous diglyceride enhanced the formation of this compound (Fig. 1, lane F). This compound was also found to comigrate with the triglyceride standard on TLC using the following four different solvent systems: benzene, R_f = 0.08; petroleum ether-ethyl ether-8.4 *M* NH_4OH, 80:20:1.2, R_f = 0.42; benzene-ethyl ether-ethyl acetate-17.5 *M* acetic acid, 80:10:10:0.2 (Jagannatha and Sastry, 1981), R_f = 0.80; and chloroform-methanol-8.4 NH_4OH, 92:2:1 (Bishop and Hajra, 1978), R_f = 0.84. This ^{14}C-triglyceride was destroyed by alkaline methanolysis (Hajra, 1969) with a resultant conversion to ^{14}C-fatty acid methyl ester.

Jagannatha and Sastry (1981) have reported the biosynthesis of cholesteryl ester with little or no concomitant triglyceride formation using a similar esterification system. However, using their assay system, we found formation of significant amounts of triglyceride and only traces of cholesteryl ester (Fig. 1, lane C). In addition, we found formation of another radioactive nonpolar lipid (Fig. 1, lane C; R_f = 0.56). This compound has been identified as the ethyl ester of ^{14}C-fatty acid, formed by the enzyme-catalyzed esterification of [^{14}C]palmitoyl CoA or [^{14}C]palmitic acid with ethanol, which was included in this assay to solubilize the labeled fatty acid (see Materials and Methods). Identification was based on its comigration with a synthetic standard (Fig. 1, lane C, standard 4), by its absence when ethanol was deleted from the assay of Jagannatha and Sastry (1981) (Fig. 1, lane D), and by its presence when ethanol was added to the assay methods employed in the present study (Fig. 1, lane G). Small amounts of fatty acid methyl ester are also formed in some assays (Fig. 1, standard 3) and were found to be the result of nonenzymatic reaction of radioactive lipid (probably [^{14}C]acyl CoA) with methanol during the Bligh and Dyer lipid extraction procedure (see Materials and Methods).

Formation of cholesteryl ester in brain from free fatty acid at low pH in the absence of ATP and CoASH has also been reported (Choi and Suzuki, 1978; Jagannatha and Sastry, 1981), a finding that we confirm using their assays (Fig. 1, lanes A and B, respectively). Exogenous cholesterol was not added to the assay shown in lane B (and also lane C) of Fig. 1, as the brain fraction used contains large amounts of endogenous cholesterol (detected by TLC analysis; data not shown) and also because exogenous cholesterol was not found to accelerate these esterification reactions significantly (Jagannatha and Sastry, 1981). Free fatty acid was not a

[1] Only fresh batches of 1,2-diglyceride were used. Batches that were a few months old contained a degradation product slightly more polar than diglyceride (on thin-layer chromatogram) and were completely inactive in diglyceride acyltransferase assay in both brain and liver.

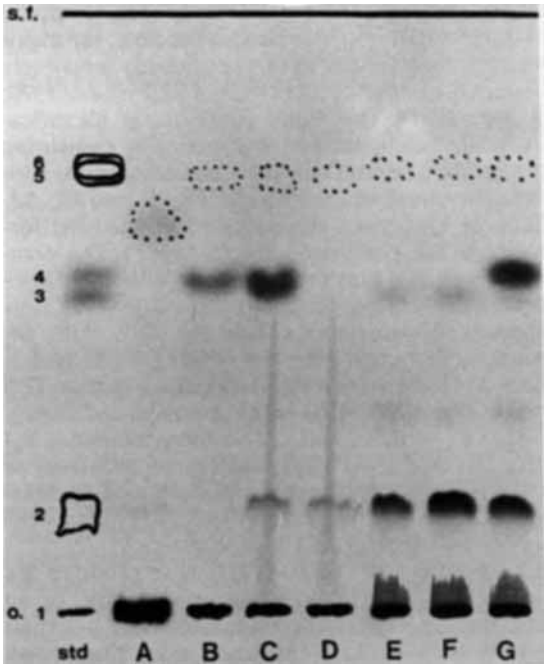

FIG. 1. Lipid products of various assays for ester formation. Three assays for formation of cholesteryl ester were utilized (see text for details): (A) that of Choi and Suzuki (1978); (B) the low-pH assay of Jagannatha and Sastry (1981); (C) the neutral pH assay of Jagannatha and Sastry (1981) (see Materials and Methods); (D) the same assay as in (C), except that the ethanol was omitted; (E) the diglyceride acyltransferase assay used in this study utilizing endogenous diglyceride substrate (see Materials and Methods), except that the protein was increased to 200 μg; (F) the same assay as (E) with the inclusion of 80 μ*M* 1,2-diglyceride and 0.003% Tween-20; (G) the same assay as (E) with the inclusion of 2.5% (vol/vol) ethanol. In all of the above assays, postnuclear total particulate protein (8×10^3 g/min to 8.4×10^6 g/min) was used. Isotope specific activities of ^{14}C-fatty acids (2.08 dpm/pmol) and [^{14}C]palmitoyl CoA (125 dpm/pmol) were used such that, assuming time and protein linearity of all assays, equal enzyme specific activities would result in equal spot densities. Carrier triglyceride (50 nmol) and either cholesteryl oleate (50 nmol, tubes A–D) or cholesteryl palmitate (50 nmol, tubes E–G) was added to the lipid extraction. Lipids were separated by TLC (solvent system A), and film exposure was for 3.6 days. Nonradioactive compounds were visualized by spraying with 2% I_2 in methanol. std, Standards, identified by comparison with the same compounds in adjacent lanes (not shown), are: (1) [^{14}C]oleic acid; (2) triglyceride; (3) methyl [^{14}C]palmitate; (4) ethyl [^{14}C]palmitate; (5) cholesteryl oleate; and (6) cholesteryl palmitate. The dotted areas show the positions of I_2-positive carrier cholesteryl esters. The presence of high lipid concentration was responsible for the retardation of the migration of cholesteryl ester in lane (A). The radioactive triglycerides comigrated with the leading edge of the carrier triglyceride (not marked for clarity).

substrate for triglyceride synthesis under these conditions (Fig. 1, lanes A and B). However, ethyl ester was formed when the incubation mixture contained ethanol (Fig. 1, lane B). Although [^{14}C]oleic acid was used as substrate in the assays shown in lanes A through D of Fig. 1, use of [^{14}C]palmitic acid gave similar results (data not shown).

Reaction conditions for the formation of triglyceride from [^{14}C]palmitoyl CoA and 1,2-diglyceride were optimized. Activity (measured with exogenous diglyceride) was highest in a microsomal fraction (see Materials and Methods) as compared to the nuclear, crude mitochondrial/synaptosomal, and soluble fractions. $MgCl_2$, at a concentration of 10 m*M* and above, was found to stimulate activity nearly twofold. Triglyceride accumulation was linear with time for only 5–10 min, and using 5 min incubation time, the reaction was linear with microsomal protein to at least 400 μg in the absence of exogenous diglyceride and to 40 μg in its presence (Fig. 2). The basal reaction rate (utilizing endogenous diglyceride) varied between 30 and 85 pmol/min/mg protein. Maximal activity with exogenous 1,2-digylceride was 6- to 10-fold higher and was attained by concentrations in the 100 μ*M* range (Figs. 2 and 3). Use of increasing concentrations of diglyceride (Fig. 3) was accompanied by increasing concentrations of Tween-20, as the diglyceride was emulsified at a constant ratio to the amount of Tween-20 (see Materials and Methods). Tween-20 alone was found to inhibit acylation of endogenous substrate (Fig. 3).

Concentrations of palmitoyl CoA of at least 15 μ*M* were found to give maximal rates of triglyceride formation when measured in the presence of bovine serum albumin (Fig. 4). Albumin was included to inhibit the active acyl CoA hydrolase found in brain (Brophy and Vance, 1976). Although higher activities could be obtained with lower amounts of albumin (perhaps due to the detergent properties of unbound acyl CoA), earlier work (Bishop, 1981; Bishop and Hajra, 1981) suggested that more reproducible assays and more reliable kinetics of acyl

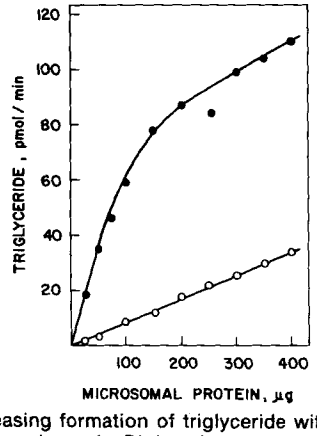

FIG. 2. Increasing formation of triglyceride with increasing brain microsomal protein. Diglyceride acyltransferase assays (see Materials and Methods) were conducted with the indicated microsomal protein amounts either in the absence (○) or in the presence (●) of 80 μ*M* diglyceride (plus 0.003% Tween-20).

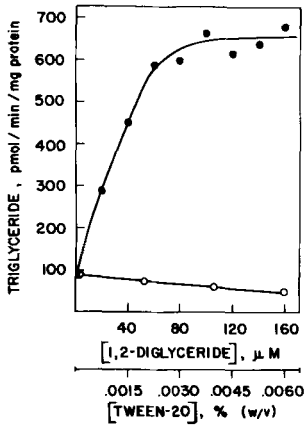

FIG. 3. Effect of exogenous diglyceride on triglyceride formation. 1,2-Digylceride was emulsified in Tween-20 as described in Materials and Methods and added to diglyceride acyltransferase assays to give the indicated diglyceride and Tween-20 concentrations (●). (○), Tween-20 alone was added to assays.

CoA utilization are obtained with approximately equimolar amounts of albumin and acyl CoA.

A variety of exogenous 1,2-diacylglycerols were found to be readily acylated to form the corresponding triglyceride (Table 1). Although all lipid substrates seemed to be well-emulsified, the more readily soluble 1,2-dicaprin was most actively acylated, and the more insoluble 1,2-dioleoylglyceryl ether was least active (Table 1). Both of the glycerides with a free *sn*-2-hydroxyl, 1,3-diolein and 1-monoolein, were converted to triglyceride at a low rate (Table 1).

Ethanol and other short-chain primary alcohols were also found to be acylated by palmitoyl CoA (Fig. 1 and Table 1, column 3). The secondary alcohol isopropanol was acylated, but at a much lower rate (Table 1, column 3). These short-chain

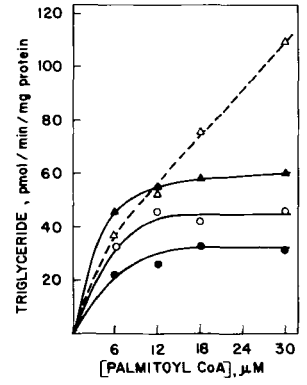

FIG. 4. Effects of varying concentrations of palmitoyl CoA and bovine serum albumin on triglyceride formation. Diglyceride acyltransferase assays were conducted as described in Materials and Methods at the given palmitoyl CoA concentrations, either in the absence of albumin (△) or in the presence of constant mole ratios of albumin to palmitoyl CoA of 0.5 (▲), 1.0 (○) or 2.0 (●).

TABLE 1. *Esterification of glycerides and aliphatic alcohols by palmitoyl CoA*

Additions	Triglyceride (pmol/min/mg)	Alkyl palmitate (pmol/min/mg)	Alkyl palmitate R_f (solvent system)
None	45		(B)
(*sn*)-1,2-Diglyceride from egg phosphatidyl choline	299		(B)
DL-1,2-Dioleoyl glyceryl ether	71		(B)
DL-1,2-Dicaprin	45/752[a]		(B)
(*sn*)-1,2-Diolein	266		(B)
DL-1,3-Diolein	70		(B)
DL-1-Monoolein	89	40[b]	0.03 (B)
Methanol	64	21[c]	0.44 (A)
Ethanol	89	25[d]	0.48 (A)
n-Propanol	107	65[d]	0.53 (A)
i-Propanol	113	15[d]	0.53 (A)
n-Butanol	14	61[d]	0.54 (A)
n-Hexanol	0	0[d]	0.56 (A)
n-Decanol	0	0[d]	0.58 (A)
n-Hexadecanol	61	0[d]	0.62 (A)
Cholesterol	50	0[e]	0.65 (A)

Indicated substrates were added to the diglyceride acyltransferase assay described in Materials and Methods, utilizing 0.002% Tween-20 but excluding diglyceride. All of the diradylglycerols, hexadecanol, and cholesterol were emulsified in Tween-20 as described in Materials and Methods and were added to assay mixtures to give final concentrations of 50 μ*M* lipid and 0.002% detergent. The aliphatic alcohols were added to a concentration of 2.0% (vol/vol).

Extracted lipids were separated by either solvent system A (triglyceride R_f = 0.13) or B (triglyceride R_f = 0.42). Esterification products were identified by use of standards and by the dependence of formation on the presence of the particular alcohol.

[a] 1,2-Didecanoyl-3-palmitoyl-*sn*-glycerol (R_f = 0.37, 752 pmol/min/mg) was slightly separated from the esterification product of endogenous diglyceride (R_f = 0.42, 45 pmol/min/mg).

[b] Diglyceride.

[c] Methyl palmitate. The small amounts formed nonenzymatically during the Bligh and Dyer extraction were subtracted off.

[d] Alkyl palmitate.

[e] Cholesteryl palmitate.

alcohols (through C_3) all stimulated acylation of diglyceride to triglyceride (Table 1, column 2). Hexanol and decanol were at least partially insoluble at the concentration used and caused entire losses of enzyme activities (Table 1). Neither hexadecanol nor cholesterol, emulsified in Tween-20, was acylated under these assay conditions (Table 1, column 3).

The effects of ethanol and butanol were explored further. Increasing amounts of both alcohols caused a parallel enhancement of both triglyceride and alkyl palmitate formation (Fig. 5). The more hydrophobic butanol peaked in its effect at a much lower concentration than did ethanol (Fig. 5).

The data of Fig. 1 showed that both free fatty acid and acyl CoA could serve as acyl donors for esterification in brain. This was found to be the case for ethyl palmitate biosynthesis in that both substrates could be utilized for its synthesis (Table 2). In contrast, it was found that, at higher pH values, triglyceride was formed exclusively from acyl CoA (Table 2). This was also true for the acylation of added 1,2-dicaprin, 1,3-diolein, and diglyceride obtained from the hydrolysis of egg phosphatidyl cho-

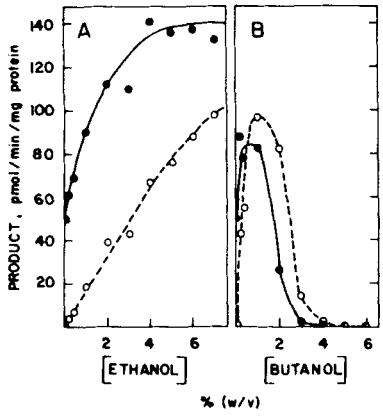

FIG. 5. Effects of increasing amount of ethanol (**A**) and butanol (**B**) on triglyceride and alkyl palmitate formation. Diglyceride acyltransferase assays were conducted as described in Materials and Methods with the inclusion of indicated amounts of alcohol. The extracted lipids were separated using solvent system A: triglyceride, R_f = 0.13 (●); ethyl palmitate, R_f = 0.48 (○, A); butyl palmitate, R_f = 0.54 (○, B).

line (data not shown). At the other extreme, cholesteryl palmitate was formed only from free fatty acid, most actively at the low pH (Table 2). The effect of $MgCl_2$ was tested under all the conditions listed in Table 2. With the exception of its twofold stimulation of triglyceride synthesis (pH 7.4), its presence caused changes of no more than ± 30% in other esterification rates (data not shown).

DISCUSSION

Although triglyceride is present in brain as a quantitatively minor lipid (0.15–0.25μmol/g wet weight; Rowe, 1969; Cook, 1981), it is an active metabolic entity (Sun and Horrocks, 1971; MacDonald et al., 1975; Horrocks and Harder, 1983). Studies with whole brain or brain slices have demonstrated a rapid, high-specific-activity labeling of triglyceride using labeled fatty acid. This has led to the hypothesis that triglyceride acts either as a fatty acid buffer or as a reservoir for phospholipid synthesis, perhaps by controlling the availability of particular diglyceride species for phospholipid synthesis (Yavin and Menkes, 1973; Mizobuchi et al., 1982).

TABLE 2. *Comparison of esterification with free fatty acid vs. acyl CoA*

		Products (pmol/min/mg protein)		
^{14}C Substrate	pH	Triglyceride	Ethyl palmitate	Cholesteryl palmitate
Palmitic acid	5.2	0	24	5–30
Palmitic acid	7.5	0	10	1–3
Palmitoyl CoA	5.2	19	48	0
Palmitoyl CoA	7.5	73	20	0

Assays for diglyceride acyltransferase were employed as described in Materials and Methods with the inclusion of 2.5% (vol/vol) ethanol. In the indicated experiments, 40 μ*M* [^{14}C]palmitic acid was substituted for the [^{14}C]palmitoyl CoA and albumin, and 75 m*M* citrate-phosphate buffer (pH 5.2) was substituted for the pH 7.5 phosphate buffer. The extracted lipids were separated by TLC as shown in Fig. 1.

Little *in vitro* work is available describing the enzymology of triglyceride synthesis in brain. This study demonstrates the presence of a Mg^{2+}-stimulated diglyceride acyltransferase having a neutral pH optimum in rat brain microsomal preparations. That both oleoyl and palmitoyl CoA served as substrates (Fig. 1) is consistent with the observation that brain triglyceride is composed mainly of palmitoyl, oleoyl, and stearoyl residues (Rowe, 1969). Free fatty acid could not serve as an acyl donor for this reaction (Table 2). The endogenous substrate appears to be 1,2-diglyceride, as exogenously added 1,2-diglyceride stimulated triglyceride formation several-fold. The results indicate that the exogenous substrate does not compete for the enzyme with the endogenous substrate. For example, although added dicaprin was actively acylated, acylation of endogenous diglyceride was not inhibited, perhaps due to a greater accessibility of the latter to the enzyme (Table 1). The variability in enzyme activities reported in this study using endogenous substrate (see Results) may be due to variability in the amount of diglyceride present in the membrane fraction caused by postmortem increases (Banschbach and Geison, 1974). These properties of triglyceride synthesis in brain are very similar to those found for triglyceride synthesis in other tissues (Bell and Coleman, 1980).

It was found that brain microsomes would also catalyze the acylation of the *sn*-2-hydroxyl of glycerides in that both 1-monoolein and 1,3-diolein were converted to triglyceride (Table 1). Whether or not the same enzyme is responsible for both *sn*-2 and *sn*-3 acylation is not known.

As mentioned before, in our earlier work on the NADPH-dependent reduction of [^{14}C]palmitoyl CoA to [^{14}C]hexadecanol in brain, we reported a concomitant active formation of a more nonpolar lipid by this assay system, which was identified as triglyceride (Bishop and Hajra, 1978). Natarajan and Sastry (1976) had earlier identified this compound as palmitaldehyde, a proposed intermediate in the reduction of palmitoyl CoA to hexadecanol, and more recently as cholesteryl ester (Jagannatha and Sastry, 1981). In this study, we have confirmed our original identification of the nonpolar lipid as triglyceride (see Results). We have found that use of the assay of Jagannatha and Sastry (1981), which uses ethanol to solubilize the labeled fatty acid, results in the enzymatic formation of the ethyl ester of fatty acid (Fig. 1). It is possible that a mistaken identification could have resulted from the close migration of ethyl ester to cholesteryl ester on the TLC plate (Fig. 1). However, we have confirmed the formation of cholesteryl ester at low pH from

free fatty acid (Fig. 1; Table 2) reported by both Jagannatha and Sastry (1981) and by Choi and Suzuki (1978).

In addition to the acylation of glycerides, a number of other alcohols were found to be acylated by palmitoyl CoA by brain microsomal preparations. Water-soluble straight-chain primary alcohols were actively acylated, as was also the secondary alcohol isopropanol (Table 1; Fig. 5). This confirms the report of Polokoff and Bell (1978) of the presence of an ethanol acyltransferase in brain and other organs. Another route of ethanol esterification, from free fatty acid, was also found (Table 2). This reaction could be catalyzed by the same enzyme that is responsible for cholesterol esterification by this mechanism (Choi and Suzuki, 1978; Jagannatha and Sastry, 1981). This esterification by free fatty acid at low pH may be due to the reverse reaction catalyzed by a nonspecific (lysosomal?) esterase. Earlier acylation studies from this laboratory utilizing [^{14}C]palmitoyl CoA as substrate showed that the hydroxyl group of Triton X-100 and both the sulfhydryl and hydroxyl groups of β-mercaptoethanol and dithiothreitol, but not glutathione, were also acylated (Bishop, 1981). The lack of formation of hexadecyl palmitate (Table 1) is consistent with the absence of wax esters in brain (Mukherjee et al., 1980). Substrate competition experiments to determine whether or not these palmitoyl CoA-dependent acylations were catalyzed by diglyceride acyltransferase were inconclusive, as it was found that alcohols caused a stimulation of triglyceride formation (Table 1; Fig. 5).

This report clearly demonstrates that brain has the capacity to esterify a wide variety of primary and secondary alcohols. Whether one or many enzymes are responsible is not known. It may well be that the enzyme responsible for the biosynthesis of the naturally occurring triglyceride has a sufficiently broad alcohol acceptor specificity to allow esterification of artificial substrates such as aliphatic alcohols, dithiothreitol, Triton X-100, etc. *in vitro*, but may act exclusively, *in vivo*, to esterify diglycerides.

Acknowledgment: This work was supported by the National Institutes of Health Grant NS 08841.

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