Glycerolipid biosynthesis in rat adipose tissue. I. Properties and distribution of glycerophosphate acyltransferase and effect of divalent cations on neutral lipid formation

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Abstract A sensitive radioactive assay of acyl CoA:*sn*-glycerol-3-phosphate-*O*-acyltransferase (EC 2.3.1.15) was developed to study the properties and subcellular distribution of this enzyme in rat epididymal adipose tissue. The esterification of *sn*-glycerol-3-phosphate was measured in the presence of palmitoyl CoA or palmitate, ATP, CoA, and Mg^{2+} at pH 7.5. The presence of glycerophosphate acyltransferase was detected in both mitochondria and microsomes. The product of this reaction was identified as phosphatidate by thin-layer chromatography and dual isotope incorporation studies. Several divalent cations reduced the activity of this enzyme. Although Mg^{2+} was not required for the activity of glycerophosphate acyltransferase, its addition to the incubation mixture resulted in an increased formation of neutral lipids at the expense of phosphatidate. This result is explained by an activation of microsomal phosphatidate phosphatase (EC 3.1.3.4). The effect of Mg^{2+} was completely abolished by Ni^{2+}, Co^{2+}, Mn^{2+}, and Zn^{2+}. These studies suggest that the balance between Mg^{2+} and several other divalent ions may be important in the regulation of neutral lipid synthesis in adipose tissue.

Supplementary key words phosphatidate formation · Mg^{2+} · phosphatidate phosphatase

The esterification of *sn*-glycerol-3-phosphate by CoA thioesters of long-chain fatty acids is the initial reaction in the major pathway in adipose tissue glycerolipid biosynthesis. This reaction is mediated by the enzyme acyl CoA:*sn*-glycerol-3-P acyltransferase (EC 2.3.1.15) (1). Previous studies have reported that this enzyme is found in both the mitochondria and microsomes of epididymal adipose tissue (2–5). However, the quantitative distribution pattern of this enzyme is controversial. Steinberg (2) and Tzur and Shapiro (3) reported the enzyme to be primarily in microsomes, whereas Daniel and Rubinstein (5) and Roncori and coworkers (4, 6) found the activity largely in mitochondria.

The product of the esterification reaction has been identified as phosphatidate (4, 7–10) in several studies of rat liver, intestine, adipose tissue, and mammary gland. Recently, evidence from this laboratory suggests that the initial product of the reaction in liver is lysophosphatidate rather than phosphatidate (11, 12). A similar result is reported in studies of the bacterial enzyme (13, 14).

The purposes of the present investigation were: (*1*) to establish the subcellular localization of glycerophosphate acyltransferase in adipose tissue; (*2*) to identify the product of the reaction catalyzed by the enzyme; and (*3*) to evaluate the effect of various cations on the reactions in the pathway of neutral lipid formation. A sensitive and reliable assay of glycerophosphate acyltransferase was developed to study its subcellular distribution.

EXPERIMENTAL PROCEDURE

Materials

Lipid standards were purchased from the Hormel Institute, Austin, Minn., or from Applied Science Laboratories, Inc., State College, Pa. The different acyl CoA derivatives were prepared according to the procedure of Seubert (15), and purity was determined by ultraviolet spectrophotometry, hydroxamate formation (16), and thin-layer chromatography on silica gel G plates (R_F 0.45–0.5) with the solvent system 1-butanol–acetic acid–water 5:2:3. Coenzyme A, ATP, and succinic acid were obtained from Sigma Chemical Co. Fatty acid-poor albumin

Abbreviations: *sn*-glycerol-3-P, *sn*-glycerol-3-phosphate; TLC, thin-layer chromatography.

TABLE 1. Intracellular distribution of marker enzymes, *sn*-glycerol-3-P acyltransferase, and protein

Subcellular Fraction	Succinate Dehydrogenase		Diglyceride Acyltransferase		α-Glycerophosphate Dehydrogenase		*sn*-Glycerol-3-phosphate Acyltransferase		Protein
	% of Total	Specific Activity	% of Total	Specific Activity	% of Total	Specific Activity	% of Total	Specific Activity	% of Total
Fat-free homogenate	100	1	100	1	100	1	100	1	100
Mitochondria	86	8.6	4	0.4	4.0	0.4	23	2.3	10
Microsomes	6	0.6	89	9.8	3.5	0.3	60	6.6	9
Cytosol	0	0	2	0.02	96	1.3	0	0	73

The enzymes were assayed as described in Methods. The results are expressed both as percentage of total activity of fat-free homogenate recovered in each subcellular fraction and as a relative specific activity with respect to protein using fat-free homogenate activity equal to 1.

(fraction V, Pentex) was obtained from Miles Laboratories, Kankakee, Ill., and other serum protein fractions were purchased from Mann Research Laboratories, New York. *sn*-[U-^{14}C]Glycerol-3-P was obtained from ICN Chemical and Radioisotope Division, Irvine, Calif. [9,10-^{3}H]Palmitic acid was purchased from Mann Research Laboratories. Diglyceride was prepared from purified egg lecithin (Sigma) by the action of phospholipase C (17). The purity of this preparation was determined by TLC. The amount of diglyceride recovered was measured by hydroxamate formation (16). Diglyceride was dispersed in 0.2% Tween 20 by sonication and this suspension was used for the assay. Phospholipase C was purchased from Boehringer Mannheim Corp., New York. Male Sprague-Dawley rats weighing 200–250 g were purchased from Zivic Miller, Inc., Philadelphia. All rats were fed laboratory chow for 1 wk before removal of adipose tissue.

METHODS

Preparation of subcellular fractions

Epididymal adipose tissue obtained from two or three rats was homogenized in a Teflon–glass homogenizer with 3 vol of cold buffer containing 0.25 M sucrose, 1 mM Tris, pH 7.4, and 1 mM EDTA. The homogenate was centrifuged at 600 *g* for 15 min and separated into an upper fat cake, a pellet containing nuclei, cell debris, and other tissue fragments, and an intermediate layer. This intermediate layer, called the fat-free homogenate, was used directly or was further fractionated into mitochondrial and microsomal fractions as described by McKeel and Jarett (18). The mitochondrial and microsomal pellets were resuspended in 10 ml of buffer and isolated by centrifugation. The washed pellets were dispersed by homogenization in 2–3 ml of buffer, and this final suspension was used in all assays.

The purity of these subcellular fractions was estimated by measuring appropriate marker enzymes. Succinic dehydrogenase was assayed according to Bachmann, Allmann, and Green (19), and the procedure of Young and Lynen (20) was followed to assay diglyceride acyltransferase. α-Glycerophosphate dehydrogenase was assayed according to Shonk and Boxer (21).

Table 1 shows the characteristic distribution of succinic dehydrogenase, diglyceride acyltransferase, and α-glycerophosphate dehydrogenase. Succinic dehydrogenase activity was present primarily in mitochondria, and diglyceride acyltransferase was in the microsomal fraction. 89% of diglyceride acyltransferase was found in the microsomal fraction, and rest of the activity was present in the mitochondrial and soluble fractions. Similar distribution of this enzyme has been reported for liver by Sarzala et al. (22). These workers suggested that diglyceride acyltransferase may be a better marker than glucose-6-phosphatase for microsomes. The distribution of diglyceride acyltransferase in the present study indicates that mitochondrial and soluble fractions were only slightly contaminated with the microsomal fraction. The studies of α-glycerophosphate dehydrogenase distribution suggest that mitochondria and microsomes were substantially free from contaminating soluble fractions.

Acyl CoA: *sn*-glycerol-3-phosphate acyltransferase

The standard assay was conducted in a final volume of 0.7 ml containing: 25 mM Tris buffer, pH 7.5; 50 mM KCl; 0.42 mM *sn*-glycerol-3-P; 0.1 μCi of *sn*-[U-^{14}C]glycerol-3-P; 0.7 mM dithiothreitol; 0.065 mM palmitoyl CoA; and 1.25 mg of fatty acid-poor albumin (system I). The reaction was initiated with 0.2 ml of particulate preparation; incubation was under air at 37°C in a shaking water bath. The reaction was terminated after 10 min by addition of 10 ml of chloroform–methanol 2:1. In some experiments, palmitoyl CoA was replaced by 0.21 mM ammonium palmitate, 1 mM ATP, 2.0 mM $MgCl_2$, and 0.01 mM coenzyme A (system II). Lipids were extracted and washed according to the procedure of Folch, Lees, and Sloane Stanley (23), dried under nitrogen, and stored in 1.0 ml of benzene at −40°C. Lipid products were fully soluble in benzene and were stable for at least 1 wk.

The product of the *sn*-glycerol-3-P acyltransferase reaction was identified by several techniques. First, the membrane-bound radioactive phospholipid product was isolat-

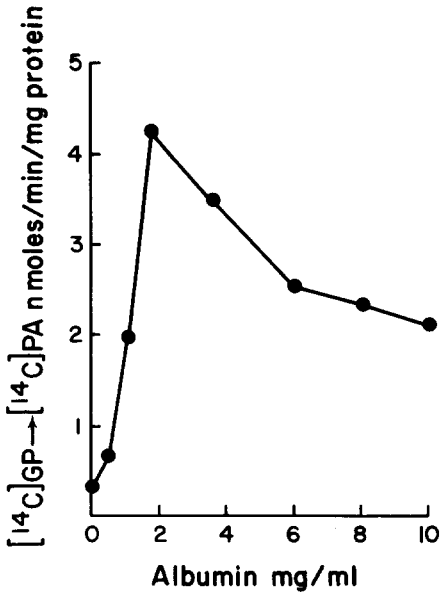

Fig. 1. Effect of albumin concentration on the incorporation of *sn*-[U-^{14}C]glycerol-3-P into phosphatidate. Incubation conditions were identical with the standard assay except for the different concentrations of albumin. GP, glycerophosphate; PA, phosphatidate.

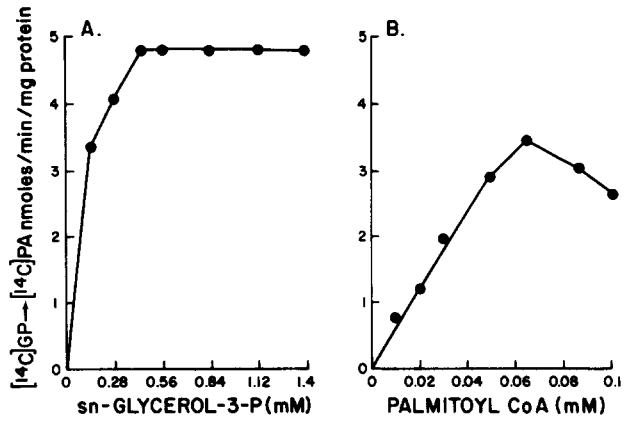

Fig. 2. *A*, effects of *sn*-glycerol-3-P concentration; and *B*, palmitoyl CoA concentration on phosphatidate formation. Experimental conditions were the same as described in Methods. GP, glycerophosphate; PA, phosphatidate.

ed and converted to diglyceride by addition of Mg^{2+}, which activates phosphatidate phosphatase. The phospholipid product was prepared by incubation using system I for 10 min. The reaction was stopped by addition of 5 ml of cold 0.05 M Tris, pH 7.5, at 4°C. The membrane-bound radioactive phospholipid was isolated by centrifugation at 160,000 *g* for 45 min, and the pellet was washed, resedimented, and used for identification.

The radioactive lipids formed during the reaction also were identified by TLC with appropriate standards. The TLC plates were coated with silica gel G (E. Merck) slurried in 0.1 M borate and activated at 110°C for 1 hr before the application of samples. Samples were applied in a volume of 0.1 ml, and the separated lipids were compared with appropriate standards in the adjacent lanes. The best separation of phospholipids was achieved with a solvent system of chloroform–methanol–3 N NH_4OH 130:70:16 and with chloroform–methanol–acetic acid–water 150:75:24:12 (24, 25). Neutral lipids were separated with hexane–ether–acetic acid 146:50:4 (26); they were visualized by exposure of the plates to iodine. Phospholipids were stained by spraying a mixture of 0.42% ammonium molybdate in 1 N H_2SO_4 and 10% ascorbic acid in water (6:1) on plates preheated at 80°C for 2 min. The phospholipids were stained blue but the neutral lipids remained unstained (27). This sensitive staining procedure detected as little as 5 μg of phospholipid.

Appropriate areas from the plates were scraped directly into scintillation vials containing 10 ml of Liquifluor (New England Nuclear) in toluene (160 ml of Liquifluor in 3.79 l of toluene). The radioactivity was counted in a Beckman LS-250 liquid scintillation counter at room temperature. The reaction rate was expressed as nmoles of *sn*-glycerol-3-P incorporated into lipid/min/mg protein.

Protein was determined by the procedure of Lowry et al. (28) with bovine crystalline albumin as the standard.

RESULTS

Characteristics of esterification reaction

Acyl CoA:*sn*-glycerol-3-P acyltransferase activity was present in both the mitochondrial and the microsomal fractions, as shown in Table 1. The major activity was located in the microsomal fraction. Therefore, the characteristics of microsomal glycerophosphate acyltransferase were studied in detail.

Reaction velocity was proportional to the concentration of microsomal protein over a limited range (0.06–0.24 mg of protein). Assays performed over this protein concentration range were linear for 10–12 min. Thereafter, a decline in the reaction velocity was observed.

The reaction was both substrate and enzyme dependent. Omissions of bovine albumin resulted in a 90% decrease in the reaction velocity. The maximum stimulation of reaction rate was achieved at an albumin concentration of 1.8 mg/ml. Concentrations of albumin above this level were inhibitory, as shown in Fig. 1. Other serum protein fractions could substitute for albumin, although none was as active. Fibrinogen and gamma globulin did not increase the reaction rate.

The effect of substrate concentration on reaction velocity was studied. Changes in *sn*-glycerol-3-P concentration are shown in Fig. 2*A* and palmitoyl CoA in Fig. 2*B*. A tentative Michaelis-Menten constant for *sn*-glycerol-3-P of K_m

TABLE 2. Incorporation of *sn*-[U-^{14}C]glycerol-3-phosphate and [^{3}H]palmitoyl CoA into phosphatidate

	Expt.	Substrates		Ratio of Palmitate to Glycerol
		[^{3}H]Palmitoyl CoA	*sn*-[^{14}C]Glycerol-3-P	
		nmoles/min/mg protein		
pH 7.5	1	8.9	4.3	2.1
	2	6.2	3.4	1.8
	3	7.2	3.6	2.0
pH 6.5	4	4.1	2.3	1.8
	5	3.8	2.0	1.9

The standard assay was conducted as described in Methods at pH 7.5 or 6.5. 0.1 μCi *sn*-[^{14}C]glycerol-3-P (300 nmoles) and 0.05 μCi [^{3}H]palmitoyl CoA (46 nmoles) were used as substrates. After a 10-min incubation at 37°C, lipids were extracted and separated by TLC. The molar ratio for glycerol and palmitate incorporated into phosphatidate was determined. Results of five experiments are recorded. Microsomes from individual rats were prepared and the results are mean values for duplicate determinations.

= 0.13 mM was calculated from these data. The reaction was stimulated by increasing palmitoyl CoA concentration up to 0.065 mM, as shown in Fig. 2*B*. At higher concentrations of palmitoyl CoA, the reaction rate declined. Palmitoyl CoA is largely protein-bound under these circumstances and therefore no kinetic constants were derived from these data. The reaction velocity was optimal at pH 7.5.

Identification of reaction products

The product of the esterification reaction was identified by TLC with a solvent system of chloroform–methanol–3 N NH_4OH (24). Radioactive products were compared with known phospholipid standards applied to adjacent lanes. In one experiment, a total of 4200 cpm was applied to the TLC plate. After separation, each fraction was counted. The origin contained 62 cpm, the lysophosphatidate area (R_F 0.15) 14 cpm, and the solvent front contained neutral lipids (R_F 0.91) 300 cpm. The major radioactive area contained 3700 cpm and corresponded to the phosphatidate standard (R_F 0.29). None of the other phospholipids, including lecithin (R_F 0.64), contained any radioactivity. In some studies the aqueous–methanol layer (upper phase of the lipid extract [23]) was extracted several times with $CHCl_3$–MeOH 17:3 as suggested by Long, Odavić, and Sargent (29) for complete recovery of lysophosphatidate. Chloroform layers were combined, dried, and counted. No radioactivity was detected in this fraction.

Further identification was obtained by measuring the simultaneous incorporation of [^{3}H]palmitoyl CoA and *sn*-[^{14}C]glycerol-3-P into the phospholipid product. The molar ratio of palmitate to glycerol was determined from isotope ratios in the major radioactive product after separation by TLC; results are shown in Table 2. Several experiments were conducted at different pH values. In each case, the molar ratio of the radioactive product was approximately 2. This provides further evidence that the product of this reaction in adipose tissue is phosphatidate rather than lysophosphatidate.

The reaction product was further confirmed as phosphatidate after activating microsomal phosphatidate phosphatase by addition of Mg^{2+}. In these experiments, membrane-bound radioactive phospholipid prepared as described above (Methods) was incubated in the presence of Tris buffer, pH 7.5, and 1 mM Mg^{2+} for various times at 37°C. Under these conditions, radioactive diglyceride was formed at the expense of the original radioactive phospholipid. This was a further indication that the initial product formed from palmitoyl CoA and *sn*-glycerol-3-P was phosphatidate.

Comparison of glycerolipid biosynthesis in subcellular fractions

Table 3 and Fig. 3 illustrate the rate of glycerolipid biosynthesis in various subcellular fractions. With the same amount of microsomal protein, the total lipid formation with palmitate, ATP, and CoA (system II) was three times greater than when palmitoyl CoA (system I) was used as substrate. The major lipids formed with these two systems also were different. When palmitoyl CoA was the substrate, the main lipid formed was phosphatidate. Considerable quantities of diglycerides and triglycerides were formed in addition to phosphatidate when palmitate, ATP, and CoA were used. Presumably, the presence of Mg^{2+} resulted in this increased formation of diglyceride and triglyceride.

In the presence of Mg^{2+}, the rate of total lipid formation was 8 nmoles/min/mg (Fig. 3). Nevertheless, the rate of total lipid formation in system I was usually lower than in system II (18 nmoles/min/mg protein, Table 3). The discrepancy in total lipid formation in these two systems may be related to inhibition caused by palmitoyl CoA (Fig. 2). In system I, palmitoyl CoA was added directly, whereas in system II it was generated from palmitate and rapidly esterified to *sn*-glycerol-3-P. When palmitoyl CoA concentration of system II was increased by addition of extra palmitoyl CoA or by preincubating system II with microsomes for 2 min before addition of *sn*-glycerol-3-P, the rate of total lipid formation decreased significantly. Under this condition the rate of lipid formation was 11.5 nmoles/min/mg protein (6.4 nmoles of phosphatidate and 5 nmoles of neutral lipids). A similar discrepancy between these two incubation systems has been reported for liver microsomes (12).

Although palmitoyl CoA serves as a substrate for *sn*-glycerol-3-P acyltransferase, it is inhibitory for this enzyme, as reported here, and also for phosphatidate phosphatase (30). Since palmitoyl CoA inhibits phosphatidate phosphatase, Brandes and Shapiro (30) suggested that the

TABLE 3. Comparison of glycerolipid biosynthesis in different subcellular fractions

Subcellular Fraction	Total Lipid	Phosphatidate	Diglyceride	Triglyceride
	nmoles sn-glycerol-3-P incorporated/min/mg protein			
		System I		
Mitochondria	2.73	2.3 ± 0.30	0.20 ± 0.08	0.23 ± 0.10
Microsomes	6.02	5.5 ± 0.45	0.30 ± 0.11	0.22 ± 0.11
		System II		
Homogenate	7.45	0.90 ± 0.085	0.99 ± 0.21	5.60 ± 0.58
Mitochondria	3.41	3.00 ± 0.21	0.22 ± 0.01	0.19 ± 0.02
Microsomes	18.71	10.50 ± 0.94	3.50 ± 0.33	4.67 ± 0.41

Assays were performed in the presence of *sn*-glycerol-3-P and palmitoyl CoA (system I) or with *sn*-glycerol-3-P, palmitate, ATP, CoA, and Mg^{2+} (system II) as described in Methods. Each value represents the mean ± SEM of assays using tissue fractions from five different rats and conducted in duplicate.

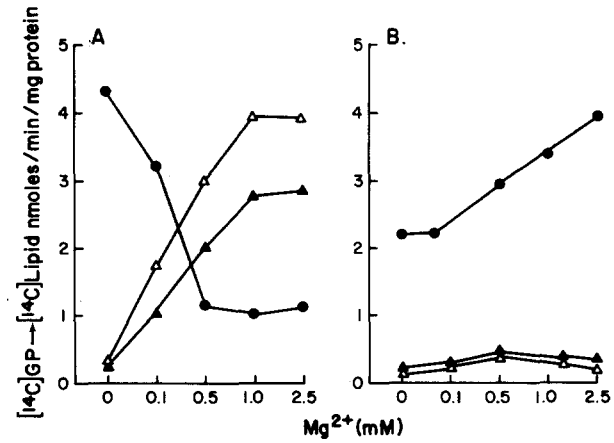

Fig. 3. Effect of Mg^{2+} on neutral lipid formation. The standard assay was conducted with increasing concentrations of Mg^{2+}. *A*, microsomal fraction; *B*, mitochondrial fraction; ●, phosphatidate; ▲, diglyceride; △, triglyceride. GP, glycerophosphate.

concentrations of palmitoyl CoA or other fatty acyl CoAs may have an influence on the nature of the lipids synthesized. In the absence of palmitate, when microsomes were incubated with system II, formation of phosphatidate and neutral lipids occurred. The rate of total lipid formation was 4 nmoles/min/mg protein (1.65 nmoles of phosphatidate and 2.4 nmoles of neutral lipids).

In contrast to microsomes, mitochondria did not show any significant difference in reaction rates with these two substrate systems. The major lipid formed was phosphatidate under both conditions. The formation of a small amount of neutral lipids by mitochondria was probably due to little contamination of this fraction by microsomes. When adipose tissue homogenate was used, the main lipid formed was triglyceride. This latter finding confirms earlier observations (1, 4, 6).

Effect of divalent ions and soluble fraction on esterification reaction

The addition of several divalent ions to the incubation mixture (system I) reduced the rate of phosphatidate formation, as shown in Table 4. A 25–40% decrease in the formation of phosphatidate was noted with addition of 1 mM Ca^{2+}, Fe^{2+}, Co^{2+}, Sn^{2+}, or Mn^{2+}. A 90% decrease was observed when 1 mM Zn^{2+} or Cu^{2+} was added to the reaction mixture. The effect of Mg^{2+} on the esterification reaction differed from the other divalent ions studied in increasing total glycerolipid formation.

Fig. 3 illustrates the effect of Mg^{2+} on neutral lipid formation by the microsomal and mitochondrial fractions. When Mg^{2+} was not present in the reaction mixture, the major lipid formed was phosphatidate. With addition of increasing concentrations of Mg^{2+} (0.1–2.5 mM), an increased formation of diglycerides and triglycerides was observed. This occurred with a simultaneous decrease in phosphatidate, indicating a probable activation of microsomal phosphatidate phosphatase. In contrast to the effect in microsomes, there was no increase in neutral lipid formation when Mg^{2+} was added to mitochondria. This suggests that mitochondrial phosphatidate phosphatase is not stimulated by Mg^{2+}.

Several of the divalent cations abolished the stimulatory effect of Mg^{2+} on neutral lipid formation, as shown in Fig. 4. In these experiments, the Mg^{2+} concentration was held constant at 1 mM while the concentration of various divalent ions was 0.1–1 mM. With increasing concentrations of Co^{2+}, Mn^{2+}, Zn^{2+}, and Ni^{2+}, there was a decrease in the formation of neutral lipids with concomitant accumulation of radioactivity in the phosphatidate fraction. This shift in the radioactive product from neutral lipids to phosphatidate as the concentration of divalent ions was increased indicates a probable reversal of the activating effect of Mg^{2+} on microsomal phosphatidate phosphatase. Other divalent ions, including Fe^{2+}, Ca^{2+} and Sn^{2+},[1] did not antagonize the stimulating effect of Mg^{2+} on neutral lipid synthesis.

When microsomes were prepared in the absence of EDTA, the effect of Mg^{2+} on neutral lipid formation was not detected. Presumably the presence of other divalent cations in the microsomal preparation prevented the Mg^{2+} effect under these conditions.

A stimulatory effect of the soluble fraction on neutral lipid synthesis has been reported in several studies with rat liver, intestine, and adipose tissue (4, 9, 31, 32). Fig. 5 illustrates the effect of the soluble fraction on neutral lipid formation in the presence and absence of Mg^{2+}. The soluble fraction was dialyzed against Tris buffer containing EDTA for several hours to remove Mg^{2+} and other divalent ions before addition to the mixture. However, dialysis

[1] Jamdar, S. C. Unpublished observations.

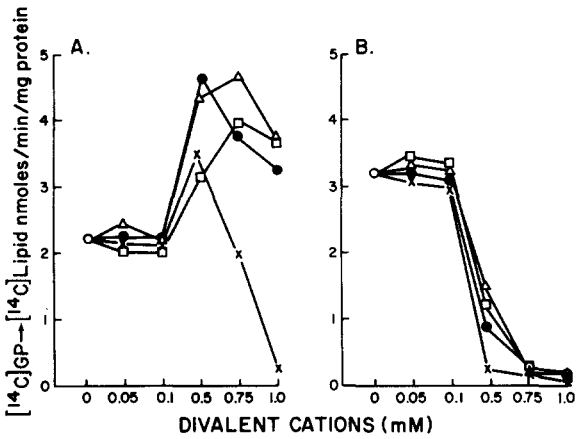

Fig. 4. Antagonism of the Mg^{2+} effect on neutral lipid formation by other divalent ions. The standard assay was performed in the presence of 1 mM Mg^{2+} with increasing concentrations of Co^{2+}, ●; Mn^{2+}, □; Ni^{2+} △; and Zn^{2+}, ×. After a 10-min incubation, lipids were extracted, separated, and identified by TLC. *A*, phosphatidate; *B*, neutral lipids; GP, glycerophosphate.

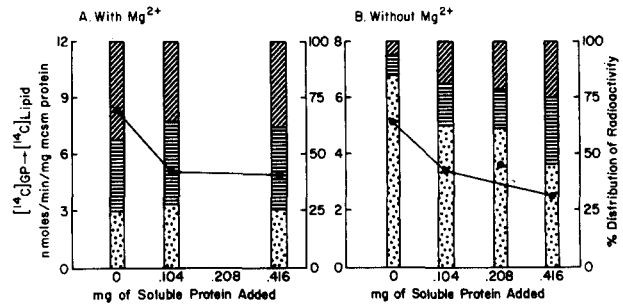

Fig. 5. Effect of Mg^{2+} and the soluble fraction on neutral lipid formation. The standard assay was conducted in the presence or absence of soluble fraction and with (*A*) and without (*B*) 1 mM Mg^{2+}. The soluble fraction was dialyzed at 4°C for 3 hr, first against Tris buffer, pH 7.5, containing 1 mM EDTA, and then against Tris buffer to remove Mg^{2+} and EDTA. After a 10-min incubation, lipids were extracted, separated, and identified by TLC. Total lipids, ●; phosphatidate, ⊡; diglyceride, ▤; triglyceride, ▨. mcsm, microsomal; GP, glycerophosphate.

did not remove Mg^{2+} completely, and the concentration of Mg^{2+} was 0.46 meq/l. Addition of soluble fraction to the incubation mixture (system I) resulted in an increased formation of neutral lipid at the expense of phosphatidate (Fig. 5B). Despite this increase in neutral lipid formation, the addition of soluble fraction caused a 30% decrease in glycerolipid synthesis in the absence of Mg^{2+}. In the presence of Mg^{2+}, neutral lipid formation was active and was not stimulated further by the addition of soluble fraction.

DISCUSSION

In this investigation, the presence of acyl CoA:*sn*-glycerol-3-P acyltransferase was detected both in the mitochondria and microsomes as observed by earlier workers (2–6). The major activity was located in the microsomal fraction. Therefore, the characteristics of the microsomal enzyme were studied in detail. Maximal reaction rates were observed at pH 7.5 in the presence of 0.42 mM *sn*-glycerol-3-P, 0.065 mM palmitoyl CoA, and 1.25 mg of albumin. The major radioactive product formed under these conditions was identified as phosphatidate by several techniques. The effect of omission of albumin was most striking and caused a 90% decrease in the reaction velocity. Other serum protein fractions also were effective, but none of them was as active as bovine serum albumin in stimulating the reaction velocity. Activation of the acyltransferase reaction by albumin was demonstrated previously with rat liver microsomes (33, 34).

TABLE 4. Effect of different cations on formation of phosphatidate and neutral lipid

Divalent Ion	Phosphatidate	Neutral Lipids
	nmoles [^{14}C]*GP*[a]*/min/mg protein*	
None	4.35	0.21
Fe^{2+}	2.08	0.20
Cu^{2+}	0.43	0
Co^{2+}	3.00	0.10
Sn^{2+}	2.53	0.18
Mn^{2+}	2.65	0.14
Ni^{2+}	2.82	0.14
Ca^{2+}	3.56	0.20
Zn^{2+}	0.48	0
Mg^{2+}	1.50	4.20

Assay conditions were the same as described for system I (*sn*-glycerol-3-P and palmitoyl CoA). All cations were added as the chloride salt at pH 7.5. The final concentration of each ion was 1 mM. Each value is the mean of duplicate assays.

[a] *sn*-[U-^{14}C]Glycerol-3-phosphate.

The subcellular distribution of *sn*-glycerol-3-P acyltransferase from adipose tissue has been studied previously by Daniel and Rubinstein (5). The enzyme was measured spectrophotometrically by the release of CoA from fatty acyl CoA in the presence or absence of *sn*-glycerol-3-P. In their studies, a major portion of the activity was found in the mitochondrial fraction. Substantial activity was also present in the soluble fraction, but microsomes did not contain any *sn*-glycerol-3-P acyltransferase. This subcellular distribution of *sn*-glycerol-3-P acyltransferase is contradictory to that reported here. Whether this discrepancy is due to the difference in assay system used or due to differences in the methods of preparation of subcellular fractions is not known. The studies of marker enzymes were not conducted by these workers (5).

The esterification of *sn*-glycerol-3-P also was studied in the presence of palmitate, ATP, CoA, and Mg^{2+}. This system was three times more active in the formation of lipids when compared with the palmitoyl CoA system. The radioactive products identified under these conditions were phosphatidate, diglyceride, and triglyceride. The ad-

dition of Mg^{2+} is an obligatory requirement for the formation of palmitoyl CoA when palmitate is used as a substrate (1, 35, 36). In the absence of Mg^{2+}, no esterification of *sn*-glycerol-3-P occurred.[1] The esterification of *sn*-glycerol-3-P with palmitoyl CoA occurred in the absence of Mg^{2+}, indicating that the acyltransferase reaction does not require Mg^{2+}. However, when Mg^{2+} was added to this latter reaction mixture, there was increased formation of neutral lipids with a concomitant decrease in the radioactivity of phosphatidate. The stimulatory effect of Mg^{2+} on neutral lipid formation is probably due to activation of microsomal phosphatidate phosphatase. Therefore, Mg^{2+} is important in two reactions for glycerolipid biosynthesis in adipose tissue. It acts as a cofactor for the thiokinase reaction and also activates microsomal phosphatidate phosphatase. This dual effect of Mg^{2+} may explain the observation that more neutral lipid was formed when palmitate, ATP, and CoA were used as substrates for the esterification reaction.

In the mitochondria, the rate of lipid synthesis was significantly lower than in the microsomal fraction. The major lipid formed by mitochondria was phosphatidate, and the addition of Mg^{2+} did not stimulate neutral lipid formation. The greater formation of glycerolipids by the microsomal fraction in comparison with the mitochondrial fraction may be explained by the increased glycerophosphate acyltransferase activity, the presence of Mg^{2+}-activated phosphatidate phosphatase, and diglyceride acyltransferase in microsomes. Although these studies suggest that mitochondria may not be involved in triglyceride formation, the presence of a significant amount of *sn*-glycerol-3-P acyltransferase in this fraction indicates that adipose mitochondria have the capacity to synthesize their own phospholipids in situ as do liver mitochondria (22, 37).

The stimulatory effect of Mg^{2+} on neutral lipid formation was quite specific. Most of the other divalent ions reduced the rate of phosphatidate formation and antagonized the stimulatory effect of Mg^{2+} on neutral lipid biosynthesis. The concentrations of Mn^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+}, which inhibit neutral lipid formation, were between 0.4 and 0.7 mM. At higher concentrations, these divalent cations also reduced the formation of phosphatidate. These concentrations are within the physiological range reported for several tissues (38, 39). These data emphasize the possible importance of various divalent cations in glycerolipid biosynthesis. The balance between Mg^{2+} and other divalent ions may be important in the regulation of neutral lipid formation in adipose tissue.

The stimulatory effect of the soluble fraction on neutral lipid biosynthesis has been demonstrated in several studies using rat liver, intestine, and adipose tissue (4, 9, 31, 32). This effect of the soluble fraction is attributed to phosphatidate phosphatase. Although the presence of this enzyme is also detected in mitochondria and microsomes from various tissues, recent studies of Hübscher et al. (31) and Johnston et al. (9) suggest that these particulate phosphatases are probably not involved in the formation of neutral lipids. These workers have emphasized the importance of soluble phosphatidate phosphatase in neutral lipid formation in liver and intestine. In contrast, these studies in adipose tissue indicate that the soluble fraction does not accelerate total lipid formation but rather reduces it below that observed under optimal conditions in the presence of Mg^{2+}. Therefore, the microsomal phosphatidate phosphatase is apparently more significant than the supernatant enzyme for neutral lipid formation in adipose tissue.

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