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THE CONTENT OF DIACYLGLYCEROL, TRIACYLGLYCEROL AND MONOACYLGLYCEROL AND A COMPARISON OF THE STRUCTURAL AND METABOLIC HETEROGENEITY OF DIACYLGLYCEROLS AND PHOSPHATIDYLCHOLINE DURING RAT LUNG DEVELOPMENT

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Summary

The content of diacylglycerol in fetal rat lung is approx. 36% of the adult and rapidly increases to adult levels by 1 day after birth. Triacylglycerol content is also low (23%) and increases to adult levels between 1 and 2 days following birth. Monoacylglycerol content is relatively low at all stages of development. The analysis of the molecular species of diacylglycerols showed that the disaturated species accounted for 30—40% of the diacylglycerols and the monoene species 20—28%. Phosphatidylcholine contained 40—45% disaturated and approx. 30% monoene species. The overall pattern of molecular species of phosphatidylcholine was similar to the pattern for diacylglycerol. The in vivo incorporation of [2-^{3}H]glycerol into molecular species of diacylglycerol and phosphatidylcholine in —1-day-fetal (i.e., 1 day before birth) lung showed that the disaturated species of diacylglycerol had the highest incorporation and appeared to have a higher rate of turnover. In contrast, [2-^{3}H]glycerol was incorporated by fetal liver most actively in the monoenoic and dienoic species of diacylglycerol. The relative incorporation of radioactivity into disaturated, monoene and diene species of phosphatidylcholine in fetal lung was very similar to that for the corresponding diacylglycerol species. The rate of the reaction from the disaturated species of diacylglycerol to the disaturated species of phosphatidylcholine, calculated from the in vivo data, was one of the higher rates and indicated considerable potential for the synthesis of disatu-

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rated phosphatidylcholine via this route. The overall results suggests that de novo synthesis of disaturated phosphatidylcholine from the disaturated species of diacylglycerol can be a major route for the synthesis of dipalmitoylphosphatidylcholine in fetal lung.

Introduction

1,2-Dipalmitoyl-*sn*-glycerol 3-phosphorylcholine (dipalmitoylphosphatidylcholine) is the principal component of lung surfactant which is secreted into the alveolar spaces and functions to prevent collapse of the alveoli during expiration [1,2]. The putative role of dipalmitoylphosphatidylcholine in the maintenance of alveolar structural integrity, its known high concentration in lung [3—6], and the need for the production of relatively large amounts, suggest that the pathway for the synthesis of phosphatidylcholine by lung is specially adapted to synthesize this rather unique phospholipid. Consequently, an important aspect of pulmonary biochemistry is the determination of the point or points in the metabolic pathways leading to the synthesis of phosphatidylcholine that are responsible for imparting the unique fatty acid distribution of dipalmitoylphosphatidylcholine [7].

There are at least two major mechanisms by which dipalmitoylphosphatidylcholine could be synthesized in the lung: (1) The initial acylations of *sn*-glycerol 3-phosphate may be highly non-random, thereby producing large amounts of 1,2-dipalmitoyl-*sn*-glycerol 3-phosphate, the acyl distribution pattern of which would be maintained through subsequent steps to the phosphatidylcholine level. Dipalmitoylphosphatidylcholine would, then, be produced as a consequence of the substrate and positional specificity of *sn*-glycerol 3-phosphate acyltransferase(s) [8]. (2) The initial acylations of *sn*-glycerol 3-phosphate may produce phosphatidic acid and, subsequently, phosphatidylcholine species with varying degrees of randomness and asymmetry. The nonspecific fatty acid distribution pattern of these de novo produced phosphatidylcholine species would then be modified to give dipalmitoylphosphatidylcholine through the interaction of other enzymatic reacylation systems; i.e., lysophosphatidylcholine acyltransferase(s) [9—12] and/or lysophosphatidylcholine:lysophosphatidylcholine acyltransferase [6,13—17].

Which of these mechanisms is the major route for the synthesis of dipalmitoylphosphatidylcholine has not been clearly elucidated. However, the CDP-choline pathway is known to be the major route for lung phosphatidylcholine synthesis [18—21] and the activity of this pathway increases rapidly during the late stages of prenatal development [18,20,22,23]. The dipalmitoylphosphatidylcholine concentration in the lung also increases rapidly during the last few days of prenatal development [16,24].

Since 1,2-diacylglycerols are the immediate precursors for the de novo synthesis of phosphatidylcholine as well as phosphatidylethanolamine, triacylglycerols, and possibly monoacylglycerols, developmental changes in the pool size, molecular composition and dynamics of diacylglycerols may influence the synthesis of phosphatidylcholine during lung development. Comparisons between the molecular composition of diacylglycerols to that of phosphatidyl-

choline during development also would greatly assist in deciding which reactions in the biosynthetic pathways are important in regulating the formation of dipalmitoylphosphatidylcholine.

In the present study, we measured the pool sizes of diacylglycerols, triacylglycerols and monoacylglycerol in rat lung during development and the relative concentration of the molecular species of diacylglycerols and phosphatidylcholine. We also examined the dynamics of the molecular species of diacylglycerols relative to the synthesis of the corresponding phosphatidylcholine molecular species by measuring the rate of incorporation of in vivo injected [2-^{3}H]glycerol into diacylglycerols and phosphatidylcholine molecular species in 21-day fetal rats.

Experimental procedures

Materials. Pregnant rats were obtained from Holtzman Company, Madison. The age of fetuses (±12 h) was determined by considering the sperm-positive date as day zero. [^{3}H]Acetic anhydride (spec. act. 500 Ci/mol) and [2-^{3}H]glycerol (spec. act. 200 Ci/mol) were purchased from Amersham and New England Nuclear, respectively. Triglyceride C-37 Rapid Stat Kit from Pierce was used for the determination of glycerol. Acetylation kit (acetic anhydride and pyridine) for the preparation of diacylglycerol acetate and sodium methoxide/methanol kit for the preparation of fatty acid methyl ester were purchased from Applied Science. Trioleoylglycerol, 1,2-dipalmitoylglycerol, 1,2-dioleoylglycerol, 1,3-dioleoylglycerol and 2-monoacylglycerol were obtained from Sigma Chemical Company and used without further purification.

Determination of triacylglycerol, diacylglycerol, and monoacylglycerol levels. Lung from fetal, newborn and adult (female) rats were rapidly removed from the animals and frozen on solid CO_2. The lipid from the lung tissue was extracted according to the Folch method [25]. The crude $CHCl_3$ extract was washed twice with saline and the $CHCl_3$ was removed by evaporation under a stream of N_2. The total lipids were weighed and dissolved in $CHCl_3/CH_3OH$ (1 : 1). The solution was divided into several portions and stored under N_2 at —40°C. A portion (4 mg lipid) of the lipid extract was chromatographed on Anasil H TLC plates in $C_6H_6/CHCl_3/CH_3OH$ (80 : 15 : 5) [26]. The plate was briefly exposed to I_2 vapor. The triacylglycerols, diacylglycerols, monoacylglycerols and phospholipid areas on the chromatograph were removed. The neutral lipids were extracted from the silica gel with chloroform/methanol (2 : 1) and the phospholipid with chloroform/methanol/acetic acid/water (50 : 39 : 1 : 10) [27]. The solvent was evaporated with a stream of N_2 and the glycerol content was determined using the Pierce C-37 Rapid Stat Kit for triacylglycerol determinations. Phospholipid was determined by measuring the lipid phosphorus content [28]. Standards of trioleoylglycerol, 1,2- and 1,3-dioleoylglycerol and 2-monooleoylglycerol were used to verify the chromatographic and analytical procedures.

Determination of diacylglycerol molecular species. A second portion of the total lipid extract (12 mg) was applied to an Anasil-H TLC plate and the plate was developed in the C_6H_6/chloroform/methanol solvent system. The area corresponding to the 1,2-diacylglycerol was scraped into tubes. Small amounts

of 1,3-diacylglycerol were also detected and combined with the 1,2-diacylglycerol species. The combined 1,2- and 1,3-diacylglycerol fractions were extracted three times with 4 ml of chloroform/methanol (2 : 1) and the extract was washed as described by Arvidson [27]. The chloroform was evaporated from the extract with a stream of N_2 and the lipid residue was thorougly dried under vacuum. The dried diacylglycerol samples were acetylated with [^{3}H]-acetic anhydride by a slight modification of the methods by Banschbach et al. [29,30]. The diacylglycerols (100—150 nmol) were dissolved in 125 μl dry pyridine and 50 μl [^{3}H]acetic anhydride (125 μCi) was added followed by 1.75 μl 70% $HClO_4$. The addition of perchloric acid improved the yield of diacylglycerol acetates without affecting the molecular species analysis. The reaction was allowed to stand for 1 h at room temperature and then for 30 min at 37°C. After cooling the reaction on ice, 1.0 ml water was added and the tubes were returned to the 37°C bath for 20 min. The reaction mixture was extracted three times with 1.5 ml hexane. The hexane extract that contained the diacylglycerol acetates was washed once with 1.5 ml 50% methanol. The hexane was evaporated with a stream of N_2 and the diacylglycerol acetates were dissolved in a small volume of chloroform/methanol (2 : 1). 100 μg non-radioactive diacylglycerol acetates (prepared from lung phosphatidylcholine) were added as carrier and the diacylglycerol acetates were purified on Anasil H plates using the solvent system *n*-heptane/isopropyl ether/acetic acid (60 : 40 : 4) [31]. The diacylglycerol acetates were separated according to the degree of unsaturation by TLC on Silica gel G-$AgNO_3$ plates [31,32]. The diacylglycerol acetate bands were scraped into tubes and a solution of 1% NaCl in 90% CH_3OH was added in portions (approx. 0.5 ml) with vigorous mixing until the characteristic red color of the silver-dichlorofluorescein complex was destroyed. The silica was then extracted three times with chloroform/methanol (2 : 1). The extracts were dried in a scintillation vial and the amount of radioactivity was determined. In several experiments, portions of the extracts were removed and subjected to methanolysis [33]. The fatty acyl methyl esters were analyzed by gas chromatography using 10% EEGS (Supelco) at 190°C column temperature. The fatty acid analysis indicated that the major diacylglycerol species were saturated, monounsaturated, diunsaturated and tetraunsaturated. The percent distribution of the radioactivity was used to calculate the relative amount of each molecular species.

In the initial separation of the lipid extract, 1,2-diacylglycerol was separated from 1,3-diacylglycerol. Lipid extracts from lung and liver contained predominantly 1,2-diacylglycerol with only trace amounts of the 1,3-diacylglycerol species. However, where diacylglycerol was formed from phosphatidylcholine via the phospholipase C reaction, a larger and variable amount of 1,3-diacylglycerol was present; probably due to acyl migration from the 1,2-diacylglycerol species. We decided to combine the 1,2- and 1,3-diacylglycerol species into a single sample for subsequent analysis of both tissue diacylglycerols and diacylglycerols produced from phosphatidylcholine. In order to verify that this would not lead to difficulties in the subsequent analysis, we analyzed, in several instances, 1,3-diacylglycerol acetate and 1,2-diacylglycerol acetate separately. The 1,3-diacylglycerol acetate and 1,2-diacylglycerol acetate migrated as a single spot on thin-layer chromatograms in all subsequent solvent systems used

to purify the acetates. The molecular species composition and fatty acid content of the molecular species were the same for both the 1,3- and 1,2-diacylglycerols.

Since the final assessment of the percent composition of the molecular species of diacylglycerol was dependent upon the extent of the acetylation of the diacylglycerols with [^{3}H]acetic anhydride, several preliminary experiments were performed to examine this reaction in some detail. The amount of diacylglycerol acetate formed, calculated from the specific activity of the [^{3}H]acetic anhydride, was the same as the amount of diacylglycerol added to the reaction. The molecular composition and fatty acid analysis of the diacylglycerol acetates formed with or without the addition of perchloric acid were identical. Furthermore, the fatty acid analysis of diacylglycerols before and after acetylation were the same. The total recovery of radioactive diacylglycerol acetate after separation into molecular species ranged from 80 to 85%. The above observations, coupled with the fact that the molecular species distribution of phosphatidylcholine obtained with this method agrees with previously reported values obtained with other methods [3,4,6,10,32] indicates that the [^{3}H]acetic anhydride method is a valid way to determine the molecular species composition of diacylglycerols.

Determination of the molecular species of phosphatidylcholine. A third portion of the total lipid extract (10 mg lipid) was chromatographed on Anasil H plates in the solvent system chloroform/methanol/acetic acid/water (25 : 15 : 4 : 2) [34] to separate the phospholipids. The phosphatidylcholine area was detected with 2,7-dichlorofluorescein and scraped into tubes. Phosphatidylcholine was extracted from the silica gel as described by Arvidson [27]. The isolated phosphatidylcholine was hydrolyzed with phospholipase C (*Clostridium welchii*, type I from Sigma) [35]. The resulting diacylglycerols were purified by TLC using the benzene/chloroform/methanol solvent system and analyzed exactly as described previously.

In vivo incorporation of [^{3}H]glycerol. Pregnant rats (21 day of gestation) were lightly anesthesized with ether and the uterus exposed through a small incision in the abdomen. Each fetus was injected intraperitoneally with [2-^{3}H]-glycerol (50 μCi in 50 μl saline). After the injections the fetuses were returned to the abdominal cavity and the incision closed with a hemostat. Two fetuses were removed at each time point and the lung and liver rapidly removed and frozen on solid CO_2. The mother was maintained under light anesthesia throughout the experiment. Lipids were extracted from the lungs and livers. Lipids extracts were washed once with 0.2 vol. of 0.1 M $CaCl_2$ containing 1% glycerol and once with a 0.2 vol. of 0.1 M $CaCl_2$ in order to convert phosphatidic acid to its Ca^{2+} salt [36]. The lipid was analyzed as described above except that non-radioactive acetic anhydride was used for converting diacylglycerol acetates. Phospholipid was analyzed by two-dimensional TLC on Redi-Coat-2D plates (Supelco) using the solvent systems chloroform/methanol/28% ammonia (65 : 25 : 5) in the first direction followed by chloroform/acetone/methanol/acetic acid/water (3 : 4 : 1 : 1 : 0.5) in the second direction [37]. Carrier phosphatidic acid (dioleoyl-, from Serdary Research Laboratories, 30—50 μg) was added to each lipid sample before TLC. The plate was dried after development in the first direction in a vacuum oven with a continuous flow of N_2.

Results

Triacylglycerols, diacylglycerols, monoacylglycerols and phospholipid content

The concentration of triacylglycerols, diacylglycerols and monoacylglycerols are 23%, 36% and 52%, respectively, of adult in −2 day fetal lung (Fig. 1). The diacylglycerol content remains at 0.14—0.16 μmol/g fresh lung until birth and rapidly increases to adult levels by 1 day following birth. The values for the diacylglycerol content of adult lung agree with those reported by others [32, 38] and are similar to values reported for rat liver [36]. The triacylglycerol content slowly increases from −2 days to 1 day after birth and then increases rapidly between 1 and 2 days, at a time after the diacylglycerol content had reached adult levels. The triacylglycerol content was 8—12-times higher than the diacylglycerol levels at all stages of development. The monoacylglycerol content was relatively low at all stages of development and tended to increase parallel with the diacylglycerol content. These data are presented on a wet tissue weight basis. Subsequently we measured the water content of lung during prenatal and postnatal development. The water content of fetal lung is relatively constant during the last 3 days of prenatal development (87—88%). The water content decreases approx. 6% within 3—8 h following birth. Thus, only a small part of the rapid rise in diacylglycerol content results from the decrease in water content. The pattern of changes represented in Fig. 1 are the same when expressed on a dry weight basis.

The disaturated species of diacylglycerols was the highest at all stages of development and accounted for 30—40% of all diacylglycerols (Fig. 2A). The highest relative content of the disaturated species occurred shortly before birth and the lowest content was found in the adult. The most prominent difference in relative composition of diacylglycerol species between fetus and adult occurred in the tetraene species, which accounted for 23% in the adult but only 12—14% in the fetus. Our results for the relative composition of diacylglycerols in fetal and adult lung do not agree with those reported by Okano and coworkers

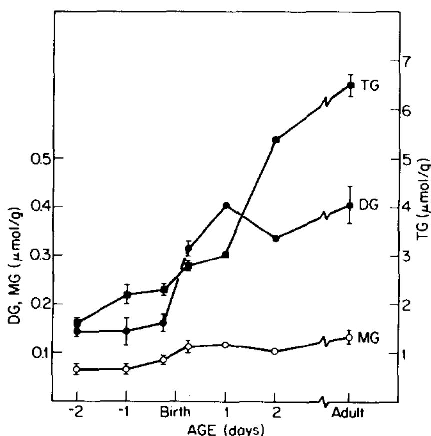

Fig. 1. Changes in the concentration of triacylglycerol (TG) diacylglycerol (DG) and monoacylglycerol (MG) during lung development. The values are averages based on gram wet weight for 3—4 separate litters and for six adults. Points without bars are averages from two litters. The bars represent ±1 S.E.

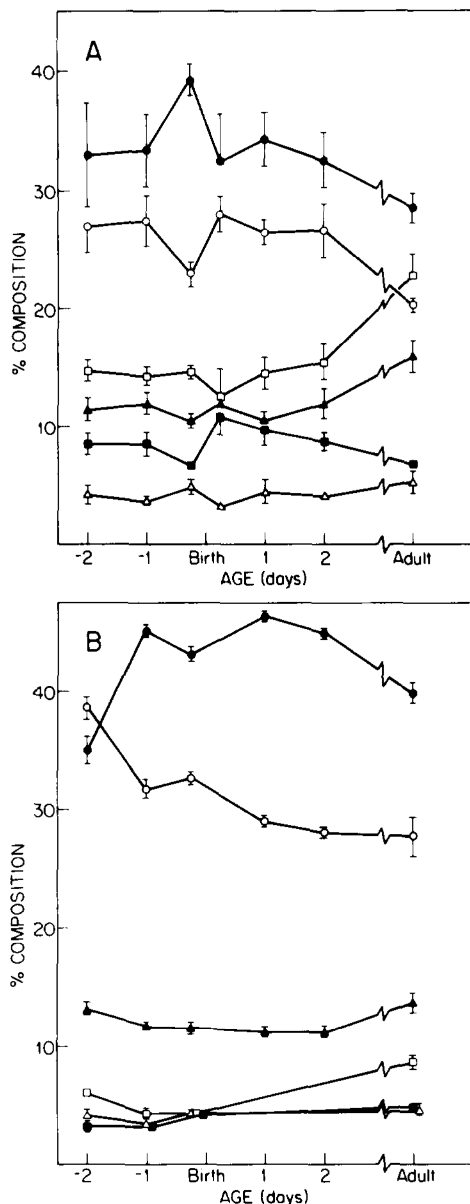

Fig. 2. Changes in the molecular species of diacylglycerol (A) and phosphatidylcholine (B) during lung development. Each point is the average of three litters ±S.E. The adult values are the average of six animals. ●, disaturated; ○, monoene; ▲, diene; △, triene; □, tetraene, and ■, polyene.

[32,39] or Moriya and Kanoh [38]. They reported much lower amounts of disaturated species and considerably higher polyene species. Both of these groups used the chemical determinations of glycerol content to quantify the various species. Our method, which adapted the use of [^{3}H]acetic anhydride to measure the relative distribution of molecular species, is considerably more sensitive and is more suitable for assessing the relative distribution with small amounts of diacylglycerol.

The relative distribution of molecular species of phosphatidylcholine during development is shown in Fig. 2B. The disaturated and monoene fraction accounted for over 70% of the total phosphatidylcholine. The relative amounts of disaturated species increased with development while the monoene species decreased. The overall pattern for phosphatidylcholine was very similar to the distribution pattern for diacylglycerols, except that in the adult lung, the relative amount of tetraene phosphatidylcholine (8.7%) was lower than the relative amount of the tetraene species of diacylglycerol (23%). Our results for the molecular species of phosphatidylcholine in fetal lung generally agree with those reported by Okano and Akino [16] except that we found relatively more disaturated species and somewhat less monoene species. Our values for the composition of phosphatidylcholine in adult lung are consistent with results obtained by others [3,4,6,10,32].

In vivo incorporation of [2-^{3}H]glycerol

The incorporation of [2-^{3}H]glycerol into total lipid from lung and liver from 21-day gestation (—1 day) fetuses is shown in Fig. 3. The incorporation increased linearly for 30 min. Liver lipids were labeled approx. 5—10-times more than lung lipids.

The distribution of radioactivity in specific glycerolipids indicated that the [2-^{3}H]glycerol was rapidly incorporated into phosphatidic acid in both lung and liver (Fig. 4). The relative amount of radioactivity in phosphatidic acid and diacylglycerols decreased rapidly as the radioactivity in phosphatidylcholine and triacylglycerols increased. These results are consistent with the precursor-product relationships expected for the de novo synthesis of phosphatidylcholine from glycerol 3-phosphate. However, since the [2-^{3}H]glycerol was given by intraperitoneal injection a true pulse chase experiment was not accomplished. Thus, an ideal precursor-product relationship may not be apparent. The diffi-

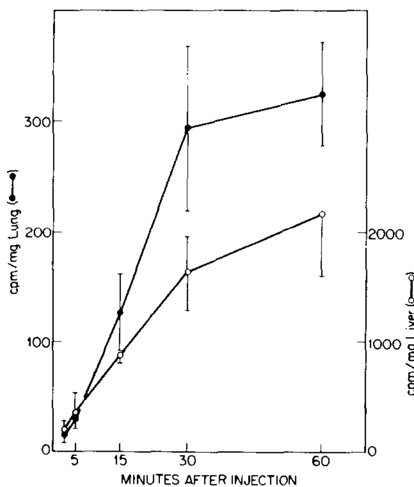

Fig. 3. The in vivo incorporation of [2-^{3}H]glycerol into total lipid in —1 day fetal lung and liver. Each point represents the average of three experiments ±S.E. Each experiment involved a separate pregnant rat with two fetuses for each time. The incorporation is expressed as cpm/mg fresh tissue.

culties associated with experiments with fetal animals dictated the present experimental design. Since we found 97% of the radioactivity was in the glycerol portion of the lipid molecules, no correction was made for label in the fatty acids.

The distribution of radioactivity among the molecular species of diacylglycerol and the relative specific activities 15 and 60 min after the injection of [2-^{3}H]glycerol are presented in Table I. The amount of radioactivity incorpo-

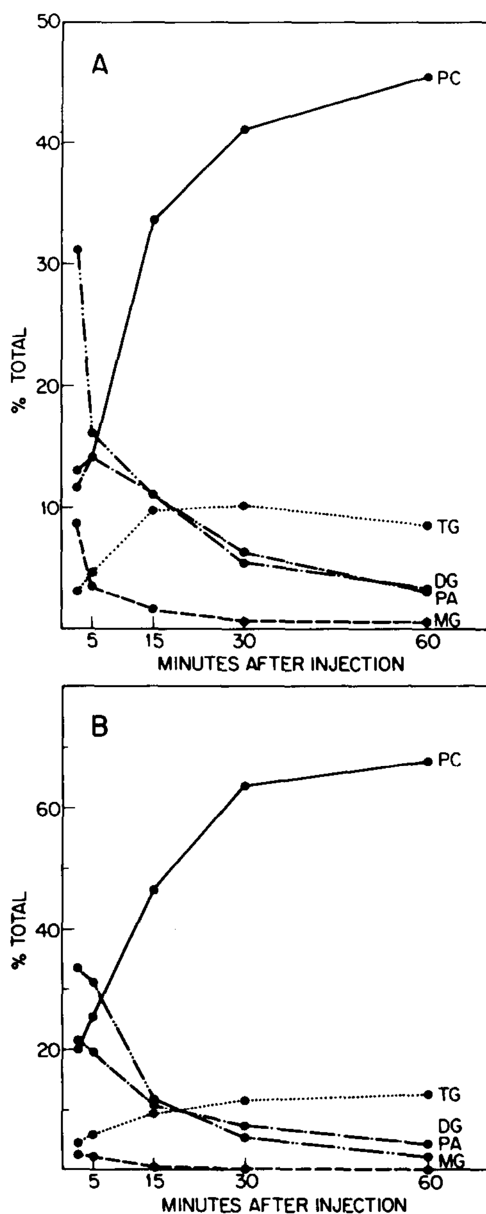

Fig. 4. The in vivo incorporation of [2-^{3}H]glycerol into specific lipids from −1 day fetal lung (A) and liver (B).

TABLE I

DISTRIBUTION OF RADIOACTIVITY AMONG DIACYLGLYCEROL MOLECULAR SPECIES FROM 21-DAY GESTATION FETAL LUNG AFTER INTRAPERITONEAL INJECTION OF [2-^{3}H]-GLYCEROL

Each value is the mean ± S.E. for three experiments. Relative specific activity = % distribution of radioactivity/% composition.

Molecular species	% Distribution of ^{3}H		% composition	Relative spec. act.	
	15 min	60 min		15 min	60 min
Saturated	40.0 ± 1.9	35.6 ± 2.9	33.5	1.31	1.06
Monoene	28.7 ± 1.5	28.9 ± 1.5	27.5	1.04	1.05
Diene	12.6 ± 1.3	15.0 ± 0.6	11.9	1.06	1.26
Triene	2.9 ± 0.2	3.2 ± 0.7	3.7	0.78	0.86
Tetraene	2.6 ± 0.6	5.1 ± 0.9	14.2	0.18	0.36
Polyene	9.0 ± 0.9	12.0 ± 2.3	8.5	1.06	1.41

rated into diacylglycerol at 2 and 5 min was too small to allow accurate analysis of molecular species distribution. The saturated species incorporated the most radioactivity at both time points and it had the highest relative specific activity at 15 min. The relative specific activity of the disaturated acylglycerol decreased at 60 min, while the relative specific activity of all other species either increased or remained the same. Thus, the disaturated species has the highest initial incorporation of glycerol and appears to have a higher rate of turnover. In contrast, the tetraene species had the lowest initial incorporation and the lowest rate of turnover. The results from the same experiments for fetal liver diacylglycerol are shown in Table II. In sharp contrast to the results in lung, monoenoic and dienoic species were most actively labeled with radioactivity. These results with fetal liver are similar to results reported by Akesson et al. [36] for adult liver. The saturated species showed both low initial incorporation and apparently a low rate of turnover. Since we did not determine the mass distribution of diacylglycerol species for fetal liver, the relative specific activities cannot be calculated.

The relative incorporation of radioactivity into the disaturated, monoene

TABLE II

DISTRIBUTION OF RADIOACTIVITY AMONG DIACYLGLYCEROL MOLECULAR SPECIES FROM 21-DAY GESTATION FETAL LIVER AFTER INTRAPERITONEAL INJECTION OF [2-^{3}H]-GLYCEROL

The percent composition data for adult liver are taken from Akesson et al. [36].

Molecular species	% distribution of radioactivity			% composition of adult
	5 min	15 min	60 min	
Saturated	4.5 ± 0.1	6.0 ± 0.4	7.5	6.9
Monoene	36.0 ± 4.7	40.7 ± 1.0	38.1	24.8
Diene	32.4 ± 0.6	31.0 ± 1.3	34.0	29.9
Triene	3.6 ± 0.4	2.9 ± 0.1	3.5	10.6
Tetraene	2.3 ± 0.6	2.1 ± 0.5	3.1	20.7
Polyene	21.3 ± 3.4	17.2 ± 1.5	14.0	7.1

TABLE III

DISTRIBUTION OF RADIOACTIVITY AMONG PHOSPHATIDYLCHOLINE MOLECULAR SPECIES FROM 21-DAY GESTATION FETAL LUNG AFTER INJECTION OF [2-^{3}H]GLYCEROL

The values are means ± S.E. for three experiments. Relative specific activity = % distribution of radioactivity/% composition.

Molecular species	% distribution of ^{3}H			% composition	Relative spec. act.		
	15 min	30 min	60 min		15 min	30 min	60 min
Saturated	34.7 ± 1.0	32.6 ± 1.9	31.6 ± 1.1	45.2	0.77	0.72	0.70
Monoene	32.5 ± 0.5	31.7 ± 1.0	34.2 ± 1.9	31.8	1.02	1.00	1.08
Diene	15.8 ± 2.4	14.9 ± 2.0	15.7 ± 0.6	11.7	1.35	1.27	1.34
Triene	3.6 ± 0.4	3.6 ± 0.2	3.5 ± 0.6	3.9	0.92	0.92	0.90
Tetraene	6.0 ± 0.4	9.5 ± 0.7	8.7 ± 0.6	4.3	1.40	2.20	2.02
Polyene	7.5 ± 0.9	7.7 ± 0.7	6.3 ± 0.4	3.2	2.34	2.41	1.97

and diene species of phosphatidylcholine from fetal lung was very similar to that found for the diacylglycerol (Table III). Relatively higher incorporations occurred in the tetraene species of phosphatidylcholine than observed in the tetraene species of diacylglycerols. This may be a reflection of the formation of the tetraene species by the phospholipase A-acyltransferase remodeling of more saturated species of phosphatidylcholine. Since the pool size of saturated species of phosphatidylcholine in fetal lung accounts for 45% of all the molecular species, the relative specific activity of the disaturated species were lower than other species. The disaturated species may, however, have a higher rate of turnover, since the relative specific activity of the disaturated species tends to decrease with time, whereas other species either increase or remain the same. The analysis of the distribution of radioactivity among the molecular species at times earlier than 15 min might reveal rapid remodeling processess. However, the fact that the radioactive label is in the glycerol portion of the molecule and the incorporation of radioactivity continues to increase from 15 to 30 min suggests that if rapid and continuous formation of disaturated species from unsaturated species was occurring it should also be detected by comparing the 15 min distribution to the 60 min distribution.

TABLE IV

THE APPROXIMATED RATE OF SYNTHESIS OF PHOSPHATIDYLCHOLINE MOLECULAR SPECIES FROM THE CORRESPONDING DIACYLGLYCEROL SPECIES IN 21-DAY GESTATION FETAL LUNG

nmol/min = CPM in phosphatidylcholine species at 30 min — CPM in phosphatidylcholine species at 15 min/average CPM/nmol of diacylglycerol species between 15 and 30 min : 15.

Molecular species	nmol/min	Relative rate
Sataurated	7.0 ± 1.4	1.00
Monoene	8.8 ± 2.0	1.27
Diene	4.1 ± 1.0	0.58
Triene	1.3 ± 0.1	0.18
Tetraene	19.1 ± 6.0	2.75
Polyene	2.3 ± 0.7	0.33

Discussion

The concentration of diacylglycerols is considerably lower in fetal lung than in adult. Furthermore, the concentration does not increase significantly until after birth even though the content of phosphatidylcholine approaches adult levels prior to birth [16]. The rapid increase in the amount of diacylglycerol following birth coincides with an increase in the activity of phosphatidic acid phosphohydrolase [40] and thus may reflect an increased rate of diacylglycerol formation via phosphatidic acid. Interestingly, although both diacylglycerol and phosphatidylcholine concentrations reach adult levels by 1 day following birth, the triacylglycerol content does not increase for another 24 h. Thus, either the triacylglycerol formation occurs in a different cell type than phosphatidylcholine synthesis or the acyl-CoA:diacylglycerol acyltransferase activity limits the formation of triacylglycerols.

Clearly, the increase in concentration of diacylglycerol content is not the primary trigger for the increased synthesis of phosphatidylcholine which occurs between 20 and 21 days of gestation in the rat [18]. However, the increase following birth is consistent with the requirements of newborn animals to synthesize larger amounts of dipalmitoylphosphatidylcholine coincident with the need for continued secretion into the alveolar spaces. Some caution in this interpretation must be made since an increase in the content of diacylglycerol could occur by either a decreased utilization, an increased synthesis or an increase in supply via blood lipids. Serum triacylglycerol concentrations [41] and lipoprotein lipase activity in lung [42] increase within hours following birth. Whether the combination of these events could lead to an increase in lung diacylglycerol content is unknown.

The high concentration of dipalmitoylglycerol in lung is consistent with the observed properties of the glycerol phosphate acyltransferase system. While in liver 1-acylglycerol phosphate acyltransferase shows a specificity for unsaturated fatty acids, the lung system does not exhibit this specificity [8] and at low concentrations of 1-acylglycerol phosphate the enzyme from lung exhibits a relatively high specificity for palmitoyl-CoA [43]. This specificity, coupled with the fact that palmitic acid apparently is the predominant fatty acid synthesized by lung [44—47] could explain the formation of large amounts of dipalmitoylglycerol.

The relative molecular composition of diacylglycerols does not change appreciably during prenatal and postnatal development. Furthermore, the molecular composition of phosphatidylcholine closely parallels that of the diacylglycerol precursor at all stages of development and suggests that the formation of the dipalmitoyl species is a reflection of the composition of the diacylglycerols. This is in sharp contrast to the results for liver where the molecular species of phosphatidylcholine differs greatly from that of the diacylglycerol precursors [26]. Nevertheless, a definite conclusion cannot be made from this type of data. The heterogeneous cell population of the lung and the occurrence of multiple metabolic pools of diacylglycerols and phosphatidylcholine dictate that caution must be taken in the interpretation of composition data obtained from whole lung samples. Nevertheless, our results in developing lung suggest that the increased synthesis of dipalmitoylphosphatidylcholine prior to birth

reflects an increase in the overall pathway and not a shift in specificity of the enzymes involved or an increase in fatty acid redistribution via either the deacylation-reacylation system [9,10,48] or the deacylation-transacylation process [13,14,17]. The observations by Oldenburg and Van Golde [17] that the activity of acyl-CoA:lysophosphatidylcholine acyltransferase did not change during the period of prenatal development when the content of dipalmitoylphosphatidylcholine increased also suggest that the deacylation-reacylation system is not responsible for the increased synthesis of dipalmitoylphosphatidylcholine during development. On the other hand, the observations that the activity of lysophosphatidylcholine:lysophosphatidylcholine acyltransferase increases 1 day before birth in the mouse [17], rat [16] and rabbit [49], support a possible role for deacylation-transacylation in the developmental increase in dipalmitoylphosphatidylcholine. However, since the primary activity of this enzyme appears to be as a lysophosphatidylcholine hydrolase [50—52], the increase in activity during development may be related more to an increase in the breakdown of tissue phosphatidylcholine than to a function in dipalmitoylphosphatidylcholine synthesis.

The results from the incorporation of [2-^{3}H]glycerol into diacylglycerol species further support the idea that the synthesis of dipalmitoylphosphatidylcholine in fetal lung may occur via de novo synthesis from dipalmitoylglycerol. A rough approximation of the in vivo rate of formation of the different molecular species of phosphatidylcholine can be made by dividing the increases in radioactivity incorporated into the phosphatidylcholine species during the period between 15 min and 30 min by the average specific activity of the diacylglycerol species during the same time period. This estimate assumes that the molecular species of phosphatidylcholine was formed directly from the corresponding species of diacylglycerol and that a single pool of each species of diacylglycerol exists. These estimated rates of formation are shown in Table IV. The formation of disaturated species and monoene species are similar whereas the rate of formation of tetraene is apparently 2—3-times faster and the formation of diene, triene and polyene is considerably slower than that of the disaturated species. Thus, not only is [2-^{3}H]glycerol extensively incorporated into the disaturated species of diacylglycerol but the relative rates of the reaction forming dipalmitoylphosphatidylcholine from dipalmitoylglycerol could be sufficiently active to account for a major portion of the synthesized dipalmitoylphosphatidylcholine. The type of data generated in these experiments cannot indicate whether fatty acid redistribution occurs on the diacylglycerols or phosphatidylcholine. However, such a redistribution mechanism need not be invoked in order to explain the synthesis of dipalmitoylphoshatidylcholine, at least in fetal lung.

Studies from several laboratories have been interpreted to indicate that dipalmitoylphosphatidylcholine is not synthesized by the CDPcholine pathway but rather by modification of unsaturated phosphatidylcholine [10,38,53]. However, the data upon which this conclusion is based are in some cases circumstancial and in other cases contradictory. For example, Vereyken et al. [10] studied the in vivo incorporation of [^{3}H]glycerol into molecular species of phosphatidylcholine over short periods following the injection of radioactivity and concluded that dienoic and trienoic species had higher turnover rates than

the other species. However, Tierney et al. [3], in earlier experiments, found similar turnover rates for the different molecular species. Several studies have indicated that more radioactive palmitic acid is incorporated into the 2-acyl position of dipalmitoylphosphatidylcholine than into the 1-acyl position [38, 43,53–56]. However, both Moriya and Kanoh [38] and Jobe [54] observed this unequal labeling only at short time intervals after injection and the labeling of 1-acyl and 2-acyl positions became equal at longer time periods. Moriya and Kanoh [38] suggested that the unequal labeling pattern was more likely caused by an initial incorporation of palmitoyl-CoA from a pool of high specific activity which had not equilibrated with the cellular pool of palmitoyl-CoA. Furthermore, Yamada and Okuyama [43] have suggested that the inconsistencies in labeling patterns may result from changes in the specificity of 1-acylglycerol phosphate acyltransferase which occurs with changing concentrations of 1-acylglycerol phosphate substrate. The inability of CDP choline: diacylglycerol cholinephosphotransferase from lung to utilize dipalmitoylglycerol as a substrate in vitro [57,58] has lead to the conclusion that dipalmitoylphosphatidylcholine cannot be synthesized by the cholinephosphotransferase reaction. However, our data as well as others [6,10] strongly suggest that dipalmitoylphosphatidylcholine can be made in vivo by the cholinephosphotransferase reaction. While the evidence is compelling that the lysophosphatidylcholine acyltransferase cycle occurs in lung as it does in most other tissues, we believe the conclusion that this process is the major avenue for dipalmitoylphosphatidylcholine synthesis in lung is unwarranted.

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