Phosphatidylglycerol in Rat Lung. II. Comparison of Occurrence, Composition, and Metabolism in Surfactant and Residual Lung Fractions†

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ABSTRACT: A comparison of the occurrence, fatty acid composition, and metabolism of phosphatidylglycerol and phosphatidylcholine in the surfactant and residual fraction of rat lung has been carried out. The surfactant and residual fractions were separated by discontinuous sucrose density gradient centrifugation. The surfactant fraction was found to contain 69% phosphatidylcholine and 7% phosphatidylglycerol. The residual fraction contained 46% phosphatidylcholine and 3% phosphatidylglycerol. Phosphatidylcholine and phosphatidylglycerol were found to contain 85 and 79% palmitate in the surfactant fraction and 67 and 68% in the residual fraction, respectively. Isolated rat lungs were perfused with medium containing [U-^{14}C]glucose, [9,10-^{3}H]palmitate, and [1-^{14}C]acetate and the incorporation into palmitate isolated from the α and β position of phosphatidylcholine and phosphatidylglycerol was determined. Each radioactive substrate was found to be incorporated into palmitate of phosphatidylcholine equally at the α and β position of the surfactant fraction. In the residual fraction the specific activity of the β position palmitate was found to be twice that of the α position. The incorporation of [9,10-^{3}H]palmitate and [1-^{14}C]acetate into palmitate at the α and β positions of phosphatidylglycerol was similar in both the surfactant and residual fractions. In each case palmitate at the α position had approximately twice the specific activity of that at the β position. The incorporation of [U-^{14}C]glucose into phosphatidylglycerol of the surfactant fraction was, however, greater in palmitate at the β position than at the α. The results show that phosphatidylglycerol is associated with the lung surfactant fraction and suggest that palmitate esterified to the α and β positions of phosphatidylglycerol and phosphatidylcholine occurs at different rates and is dependent upon the precursor source of palmitate.

Identification of phosphatidylglycerol as a metabolically active phospholipid in rat lung (Godinez *et al.*, 1975) and its presence as a component of pulmonary surfactant obtained by lavage of dog lung (Pfleger and Thomas, 1971) have been established. Phosphatidylglycerol is present in lung in unusually high concentration for a mammalian tissue and its occurrence in surfactant suggests that it may be specifically related to surfactant synthesis or function. Lung tissue contains many cell types, but surfactant is reported to be synthesized and secreted by one specific cell type, the type II pneumocyte (Buckingham and Avery, 1962; Bensch *et al.*, 1964; Askin and Kuhn, 1971; Petrik and Collet, 1974). The suggestion (Niden, 1967) that Clara cells were responsible for surfactant synthesis has been repudiated by the more recent references stated above. Within the type II pneumocyte, evidence indicates that surfactant is stored in the "lamellar body" prior to secretion (Kikkawa *et al.*, 1965; Brumley *et al.*, 1967). The major phospholipid of surfactant is phosphatidylcholine containing a high percentage of saturated fatty acids both in the α and β position (Brown, 1964).

An investigation of the relationship of phosphatidylglycerol to the surface-active lipoprotein complex synthesized in lung is presented. In order to separate the surfactant lipoprotein complex of lung from whole lung tissue a procedure using discontinuous sucrose gradient centrifugation has been employed (Frosolano *et al.*, 1970). This surfactant fraction, unlike the surface active fraction obtained by lavage of lung, contains the total surfactant pool of the lung, both intracellular and extracellular components. These two pools of surfactant material have been found to have a similar chemical composition and surface activity (Pawlowksi *et al.*, 1971). The present communication compares the fatty acid composition of, and incorporation of radioactive substrates into, phosphatidylglycerol and phosphatidylcholine of the surfactant complex to that of phosphatidylglycerol and phosphatidylcholine otherwise present in the lung.

Experimental Procedures

Lungs from fed adult Wistar strain male rats (National Laboratory Animal Company, O'Fallon, Mo.) were used in all experiments. All ^{14}C-labeled compounds were purchased from New England Nuclear Corp., Boston, Mass. Procedures related to the isolated lung perfusions and preparation of perfusion medium as well as conditions during perfusion were as previously reported (Godinez and Longmore, 1973). Separation of the surfactant fraction from the residual fraction of lung tissue was carried out by the following modification of the method of Frosolano *et al.* (1970) immediately upon completion of the 2-hr perfusion. Tissue was homogenized (Tenbroeck tissue grinder, 0.004-0.006 in. clearance) at 4° for approximately 2 min at which time all but a small amount of connective tissue was disrupted. Medium for homogenization (10 ml/g of tissue) contained 154 mM NaCl, 10 mM Tris-Cl (pH 7.4), and 1 mM EDTA. All centrifugations were performed in a SW-41 rotor (Beckman Instrument Co.). Referring to the procedure of Frosolano *et al.* (1970), the first centrifugation was over 3 ml of 0.75 M sucrose and of 1 hr duration at 40,000*g*. The

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Table I: Phospholipid Composition of the Surfactant and Residual Fraction of Rat Lung.[a]

Phospholipid	Surfactant[b] Fraction (%)	Residual Fraction (%)
Phosphatidylcholine	68.8 ± 1.3[c]	46.2 ± 1.4
Phosphatidylethanolamine	9.8 ± 0.4	23.6 ± 0.7
Phosphatidylserine + phosphatidylinositol	6.1 ± 0.5	10.5 ± 0.7
Sphingomyelin	5.0 ± 0.6	12.5 ± 0.5
Phosphatidic acid and cardiolipin	3.6 ± 0.2	4.3 ± 0.5
Phosphatidylglycerol	6.7 ± 0.3	2.8 ± 0.2

[a] Total phospholipid concentration (μmoles/mg of protein); surfactant fraction, 2.15, residual fraction, 0.42. [b] See experimental procedures for definition of surfactant and residual fractions. [c] Mean ±S.E. of 30 determinations.

band (I) over 0.75 M sucrose was diluted to 8.0 ml with the medium for homogenization and layered over a discontinuous gradient of 2 ml each of 0.25 and 0.68 M sucrose and centrifuged 1 hr at 70,000*g*. The surfactant band (IB) was found between the 0.25 and 0.68 M sucrose layers. All material from both centrifugations not recovered in the surfactant band was pooled and is referred to as the residual fraction. Omitted from our procedure were two additional centrifugations used as a washing procedure. The washes were found to be unnecessary as RBC's are not used in the medium perfusing the lungs. Volumes of the surfactant and residual fractions were reduced by thin film evaporation to approximately 2 ml. Lipids were extracted from these fractions with 45 ml of $CHCl_3$-CH_3OH (2:1 v/v). Procedures for the separation of lipids are those previously reported with the procedure designated method 2 being used for isolation of phosphatidylglycerol (Godinez *et al.*, 1975). Fatty acids were isolated from the β position of phosphatidylglycerol and phosphatidylcholine using phospholipase A_2 (from *Crotalus adamateus*) (Wells and Hanahan, 1969). Under the conditions of this procedure, approximately 80% of the phospholipids were hydrolyzed. The phospholipase A_2 treated mixtures of phosphatidylglycerol and phosphatidylcholine, their respective lyso compounds, and free fatty acids were separated by thin-layer chromatography. The lyso compounds were extracted from the silicic acid scraped from the thin-layer chromatogram and the α position fatty acids were isolated as their methyl esters by transesterification (Morrison and Smith, 1964). Fatty acids hydrolyzed by phospholipase A_2 were similarly extracted and methyl esters prepared. Methyl esters of the fatty acids of isolated phosphatidylglycerol and phosphatidylcholine not subjected to phospholipase A_2 hydrolysis were prepared directly by transacylation (Morrison and Smith, 1964). Methyl esters of fatty acids were separated and quantitated by gas-liquid chromatography (Godinez and Longmore, 1973). All radioactive counting was performed by scintillation methods. In all cases the specific activities of radioactive substrates present in the perfusion medium were determined from medium samples taken at 10, 60, and 120 min after isotope addition and the values averaged. Approximately 50 μCi of [U-^{14}C]glucose, 40 μCi of [9,10-^{3}H]palmitate, and 5 μCi of [1-^{14}C]acetate were added to the recirculating perfusion medium. The volume of the perfusion medium was adjusted to approximately 100 ml following a 10-min equilibration of the lung in the perfusion cabinet prior to isotope addition. So that all lung phospholipid specific activities from the same series of experiments could be compared and the mean values obtained, all phospholipid specific activities were corrected to a substrate specific activity of 1×10^5 dpm/μmol.

The percentage of total lung phospholipids isolated in the surfactant band (IB) was determined by isotope dilution as follows. A rat lung was perfused with medium containing [1-^{14}C]palmitate. The surfactant fraction (IB) was isolated and the specific activity of the total phospholipid in that fraction was determined. A known quantity of this material was then added to a rat lung homogenate prepared for isolation of surfactant band (IB) and total lung phospholipid determination. From that preparation the percentage of surfactant phospholipid was determined from the surfactant pool size (as determined by the dilution of the specific activity of the added radioactive surfactant material) and the total phospholipid content of the lung tissue.

Results

Comparison of the phospholipid composition of the surfactant and residual fractions (Table I) indicates that the surfactant fraction contains significantly greater quantities of phosphatidylcholine and phosphatidylglycerol and less phosphatidylethanolamine than the residual fraction. These results agree with results reported for surfactant obtained by lavage and for lung tissue from the beagle dog (Pfleger and Thomas, 1971). The surfactant fraction obtained from isolated perfused rat lung tissue was found by isotopic dilution to contain 18.7% of the total lung phospholipids. The fatty acid composition of phosphatidylcholine and phosphatidylglycerol of the surfactant and residual fractions is given in Table II. The surfactant fraction phosphatidylcholine and phosphatidylglycerol were both found to contain a higher percentage of saturated fatty acids, 93 and 95%, respectively, than in the residual fraction, 81 and 88%, respectively. Palmitic acid accounted for 85 and 67% of the saturated fatty acids in the surfactant and residual fraction of phosphatidylcholine and 79 and 68%, respectively, of phosphatidylglycerol. Composition of the α and β positional fatty acids isolated from phosphatidylcholine and phosphatidylglycerol of the surfactant and residual fractions is given in Table III. As stated under Experimental Procedures, the procedure used for the isolation of the α and β fatty acids from phosphatidylcholine and phosphatidylglycerol involved the use of phospholipase A_2 from *Crotalus atrox*. It has previously been reported (Frosolono *et al.*, 1973) that this enzyme possesses a higher V_{max} for phospholipids which possess saturated fatty acids in both the α and β positions. As the hydrolysis proceeds to about 80% completion under the conditions employed, phospholipids which contain unsaturated fatty acids are hydrolyzed to a lesser extent. Thus the percentage composition of the fatty acids in phosphatidylcholine and phosphatidylglycerol of the residual fraction differs markedly from that given in Table III. The fatty acid composition of the surfactant fraction phospholipids, because of their higher degree of saturation, is more nearly the same as that given in Table III. Considering these limitations the data do indicate that of the phosphatidylcholine hydrolyzed by phospholipase A_2, palmitate was equally abundant in both the α and β positions of both fractions. In contrast, the fatty acid composition of phosphatidylglycerol isolated from the two fractions

Table II: Fatty Acid Composition of Phosphatidylcholine and Phosphatidylglycerol in the Surfactant and Residual Fraction of Rat Lung.[a]

Fatty Acid	Phosphatidylcholine		Phosphatidylglycerol	
	Surfactant Fraction (%)	Residual Fraction (%)	Surfactant Fraction (%)	Residual Fraction (%)
Myristic	2.5 ± 0.2[b]	2.4 ± 0.1[b]	5.6 ± 1.3[c]	3.7 ± 0.4[d]
Palmitic	85.2 ± 0.7	66.9 ± 1.3	78.6 ± 1.4	68.4 ± 2.0
Palmitoleic	2.4 ± 0.5	3.4 ± 0.2	1.0 ± 0.1	1.9 ± 0.2
Stearic	5.2 ± 0.5	12.1 ± 1.0	11.1 ± 0.4	16.3 ± 0.7
Oleic	2.4 ± 0.4	8.7 ± 0.6	2.0 ± 0.4	6.0 ± 1.2
Linoleic		2.6 ± 0.4	0.9 ± 0.2	2.3 ± 0.4
Others	4.7 ± 0.5	4.5 ± 1.8	1.0 ± 0.1	1.5 ± 0.2

[a] See Experimental Procedures for definition of surfactant and residual fractions. [b] Values given are the means ±S.E. of 30 determinations. [c] Values given are the means ±S.E. of 5 determinations. [d] Values given are the means ±S.E. of 6 determinations.

Table III: Fatty Acid Composition (α and β Position) of Phosphatidylcholine and Phosphatidylglycerol in the Surfactant and Residual Fraction of Rat Lung.[a]

	Phosphatidylcholine[b]				Phosphatidylglycerol[c]			
	Surfactant Fraction (%)		Residual Fraction (%)		Surfactant Fraction (%)		Residual Fraction (%)	
Fatty Acid[d]	α	β	α	β	α	β	α	β
Myristic	3.8 ± 1.0	7.2 ± 1.3	3.3 ± 0.8	5.6 ± 0.9	1.3 ± 0.3	15.1 ± 1.2	2.2 ± 0.4	14.0 ± 1.4
Palmitic	84.4 ± 2.3	85.0 ± 1.2	83.0 ± 1.7	84.8 ± 1.9	84.5 ± 1.4	68.7 ± 4.3	75.1 ± 1.1	57.9 ± 2.4
Stearic	9.2 ± 1.6	3.7 ± 1.2	10.9 ± 1.9	2.1 ± 0.4	11.6 ± 0.7	10.0 ± 2.1	19.1 ± 1.8	16.6 ± 1.4
Oleic	1.5 ± 0.6	1.1 ± 0.4	Trace	Trace	Trace	1.6 ± 0.4	1.6 ± 0.4	3.4 ± 1.4

[a] See Experimental Procedures for definition of surfactant and residual fractions. [b] Values given are the means ±S.E. of seven determinations. [c] Values given are the means ±S.E. of four determinations. [d] Fatty acids present at <1% are not reported except where indicated by "trace."

differed markedly in the two fractions and in the α and β positions. The α position of both fractions contained approximately 84% palmitic acid but the β position contained 69 and 58% in the surfactant and residual fractions, respectively. In both fractions, myristic acid accounted for 15% of the fatty acids in the β position compared to 2% in the α position. Approximately twice the percentage of stearic acid (18%) was found in the α and β position of the residual fraction as compared to the α and β position (10%) of the surfactant fraction.

Incorporation of [U-^{14}C]glucose, [9,10-^{3}H]palmitate, and [1-^{14}C]acetate into phosphatidylcholine and phosphatidylglycerol of the two fractions was determined in the isolated perfused lung. Comparison of the specific activities of phosphatidylcholine and phosphatidylglycerol of both the surfactant and residual fraction is given in Table IV. [U-^{14}C]Glucose incorporation was similar into each fraction of phosphatidylcholine while the specific activity of phosphatidylglycerol was twice that of phosphatidylcholine in the surfactant fraction and nine times that of phosphatidylcholine in the residual fraction. The incorporation of [9,10-^{3}H]palmitate into both phosphatidylcholine and phosphatidylglycerol was twice as great into the residual as the surfactant fraction, with the specific activity of phosphatidylglycerol being half that of phosphatidylcholine in each fraction. The incorporation of [1-^{14}C]acetate into both fractions of phosphatidylcholine and the surfactant fraction of phosphatidylglycerol was similar with the specific activity of phosphatidylglycerol of the residual fraction being twice that of the other values.

To determine whether the relative incorporation of [U-^{14}C]glucose into phosphatidylcholine and phosphatidylglycerol of each fraction was similarly dependent upon or independent of glucose concentration, a series of perfusions were carried out with the perfusion medium containing either 1.5, 3.0, or 6.7 mM glucose and 0.5 mM palmitic acid (Table V). Since the phospholipid specific activities were corrected to a constant medium glucose specific activity of 1×10^5 dpm/μmol of glucose (see Experimental Procedures), the phospholipid specific activities may be compared directly to observe the effect of glucose concentration on [U-^{14}C]glucose incorporation. Incorporation of [U-^{14}C]glucose was similar into phosphatidylcholine of both fractions at each of the three glucose concentrations. The specific activities of phosphatidylglycerol approximately doubled as glucose concentration rose from 1.5 to 6.7 mM in both the surfactant and residual fractions. The specific activity of the residual phosphatidylglycerol was again twice that in the surfactant fraction at each glucose concentration.

As the β position fatty acids of lung phospholipids are unique in that they possess a higher degree of saturation than phospholipids found in other tissues, it was of interest to determine if [U-^{14}C]glucose, [9,10-^{3}H]palmitate, or [1-

Table IV: Incorporation of [U-^{14}C]Glucose, [9,10-^{3}H]Palmitate, and [1-^{14}C]Acetate into Phosphatidylcholine and Phosphatidylglycerol of the Surfactant (S) and Residual (R) Fraction of Isolated Perfused Lung.[a]

Substrate	Phosphatidylcholine (dpm/nmol)		Phosphatidylglycerol (dpm/nmol)	
	S	R	S	R
[U-^{14}C]Glucose (5.6 mM) and palmitate (0.5 mM)	7.6 ± 0.7 (4)	10.5 ± 0.3 (4)	16.7 ± 0.8 (4)	89.7 ± 9.2 (4)
[9,10-^{3}H]Palmitate (0.5 mM) and glucose (5.6 mM)	88.4 ± 6.4 (6)	170.7 ± 16.3 (5)	40.5 ± 6.1 (6)	85.2 ± 16.2 (6)
[1-^{14}C]Acetate (3.0 mM), palmitate (0.5 mM), and glucose (1.5 mM)	7.3 ± 0.3 (2)	8.8 ± 0.5 (2)	6.0 ± 0.4 (2)	15 ± 1 (2)

[a] Values are obtained from lungs perfused 2 hr; see Experimental Procedures for definition of surfactant and residual lung fractions. Values given are the means ±S.E. of number of determination in parentheses.

Table V: Concentration Dependence of [U-^{14}C]Glucose Incorporation into Phosphatidylcholine and Phosphatidylglycerol of the Surfactant (S) and Residual (R) Fraction of Isolated Perfused Lung.[a]

Perfusate Glucose Concn (mM)	Specific Activity (dpm/nmol)			
	Phosphatidylcholine		Phosphatidylglycerol	
	S	R	S	R
1.5	2.51 ± 0.19 (6)	3.67 ± 0.10 (6)	12.93 ± 1.74 (5)	18.34 ± 0.59 (3)
3.0	4.19 ± 0.45 (3)	4.15 ± 0.32 (3)	13.54 ± 1.71 (3)	21.43 ± 0.86 (3)
6.7	4.49 ± 0.91 (3)	4.90 ± 0.45 (3)	20.55 ± 2.26 (3)	44.27 ± 0.46 (3)

[a] Perfusions were of 2 hr duration; see Experimental Procedures for definition of surfactant and residual fractions. Present in the perfusate at each glucose concentration was 0.5 mM palmitic acid. Values given are the means ±S.E. of number of determination in parentheses.

^{14}C]acetate might be specifically incorporated into fatty acids of either the α and β position of phosphatidylcholine and phosphatidylglycerol of each fraction. The data, Table VI, are presented as relative specific activities of palmitic acid isolated from the α and β position of phosphatidylcholine and phosphatidylglycerol in each fraction. For each phospholipid, the specific activity of the α position palmitate in the surfactant fraction is set to 1.0 and the specific activity of the β position palmitate of the surfactant fraction and the α and β position palmitate of the residual fraction are compared to it. Incorporation of [U-^{14}C]glucose, [9,10-^{3}H]palmitate, and [1-^{14}C]acetate into phosphatidylcholine yielded in both the surfactant and residual fraction a similar pattern of specific activities in the α and β position palmitic acid. In the surfactant fraction, the β position palmitic acid had a slightly greater specific activity than the α. In the residual fraction, the specific activity of the β position palmitic acid was approximately twice that of the α. In contrast to this, incorporation of [9,10-^{3}H]palmitate and [1-^{14}C]acetate into phosphatidylglycerol yielded palmitic acid with twice the specific activity in the α position than in the β position. [U-^{14}C]Glucose incorporation into palmitic acid residues of surfactant phosphatidylglycerol was nearly twice as great in the β than α position in the surfactant but nearly equal in both positions of the residual fraction.

Discussion

One of the major metabolic functions of lung tissue is the synthesis of the surfactant lipoprotein complex and the secretion of this material onto the alveolar surface (Heinemann and Fishman, 1969). The unusually high concentration of phosphatidylglycerol found in lung and its rapid labeling with radioactive lipid precursors (Godinez *et al.*, 1975) raise the possibility that phosphatidylglycerol might somehow be involved in this process or present as a functional component of surfactant. Separation of the surfactant component synthesized by the type II pneumocyte from the remaining tissue of the lung has been carried out. The method used (Frosolano *et al.*, 1970) was originally applied to dog lung. It should be emphasized that the phospholipids isolated from the surfactant fraction are thought to come from one cell type, the type II pneumocyte, while the residual fraction phospholipids are from all other cell types in lung as well as those phospholipids of the type II cell which are not components of the surfactant lipoprotein complex. The surfactant fraction as isolated actually represents two pools of material, the extracellular and intracellular pool as identified by Pawlowski *et al.* (1971).

Data concerning the phospholipid composition of the surfactant fraction are consistent with those reported for this fraction from dog lung (Frosolano *et al.*, 1970) with the important exception that no phosphatidyl-*N*,*N*-dimethylethanolamine was found. However, a similar percentage of phosphatidylglycerol was present and as suggested previously (Body, 1971; Pfleger *et al.*, 1972; Rooney *et al.*, 1974; Godinez *et al.*, 1975) phosphatidylglycerol may have

Table VI: Incorporation of [U-^{14}C]Glucose, [9,10-^{3}H]Palmitate, and [1-^{14}C]Acetate into Palmitic Acid Isolated from the α and β Position of Phosphatidylcholine and Phosphatidylglycerol of the Surfactant (S) and Residual (R) Fraction of Isolated Perfused Lung.[a]

	Comparison of Relative Specific Activities[b] of Palmitate Isolated from							
	Phosphatidylcholine				Phosphatidylglycerol			
	S		R		S		R	
Substrate	α	β	α	β	α	β	α	β
[U-^{14}C]Glucose (5.6 mM) and palmitate (0.5 mM)	1.0	1.4	0.5	1.0	1.0	1.8	1.6	1.3
[9,10-^{3}H]Palmitate (0.5 mM) and glucose (5.6 mM)	1.0	1.2	2.4	4.6	1.0	0.3	2.5	1.4
[1-^{14}C]Acetate (3.0 mM), palmitate (0.5 mM), and glucose (5.6 mM)	1.0	1.3	1.6	2.9	1.0	0.5	2.5	1.4

[a] Perfusions were of 2 hr duration; see Experimental Procedures for definition of surfactant and residual lung fractions. [b] The experimentally determined specific activity of palmitic acid in the α position of the surfactant fraction phosphatidylcholine or phosphatidylglycerol has been set to 1. The specific activities of the surfactant β and residual α and β positional palmitic acid are then compared to it. All comparisons of specific activities derived are from mean values obtained from two perfusions with each substrate combination. Relative specific activities may be compared only within each substrate combination.

been previously misidentified as phosphatidyl-*N,N*-dimethylethanolamine. Also, a comparison of the fatty acid composition of phosphatidylcholine in the isolated surfactant fraction in dog lung to that reported here in rat lung can be made. A greater percentage of palmitic acid is found to be present (85%) in rat lung than in dog lung (63%).

The occurrence of 6.7% phosphatidylglycerol in the surfactant fraction and only 2.8% in the residual lung fraction suggests an association of phosphatidylglycerol specifically with the surfactant lipoprotein complex. However, as the total surfactant fraction, intracellular and extracellular, represents only 19% of the total lung phospholipids, only approximately 35% of the total phosphatidylglycerol in rat lung is associated with the surfactant fraction. This finding does not seriously detract from a possible role of phosphatidylglycerol in surfactant synthesis or as a necessary component of surfactant. Dipalmitoylphosphatidylcholine, the major surface-active component of surfactant, has been found to be distributed about equally between the surfactant fraction and remaining lung tissue (King *et al.*, 1973, 1974). The phosphatidylglycerol isolated from the surfactant fraction was found to contain a higher percentage of palmitic acid while phosphatidylglycerol of the residual fraction contained relatively more stearic acid. In both fractions phosphatidylglycerol contained a lower percentage of palmitic acid in the β position than did phosphatidylcholine.

Following incorporation of [U-^{14}C]glucose, [9,10-^{3}H]palmitate, and [1-^{14}C]acetate, the specific activities of phosphatidylcholine and phosphatidylglycerol isolated from the surfactant and residual fractions were found to differ. While the incorporation of [9,10-^{3}H]palmitate and [1-^{14}C]acetate into the surfactant and residual fraction and into the α and β positional palmitate moieties of phosphatidylcholine and into phosphatidylglycerol followed a similar pattern, one difference is observed. As noted previously (Godinez *et al.*, 1975), [1-^{14}C]acetate, like [U-^{14}C]glucose and [2-^{14}C]lactate, was incorporated into whole lung phosphatidylglycerol to give a higher specific activity than in phosphatidylcholine. [9,10-^{3}H]Palmitate, however, does not yield higher whole lung specific activity phosphatidylglycerol as compared to phosphatidylcholine. This is seen in Table IV where, while the specific activity of the residual fraction is consistently twice that of the surfactant fraction in both phosphatidylcholine and phosphatidylglycerol, the specific activity of phosphatidylcholine in both fractions is twice that of phosphatidylglycerol. While no explicit explanation may be offered, it should be noted that all precursors which yield palmitic acid *via de novo* synthesis yield phosphatidylglycerol with a higher specific activity than that found in phosphatidylcholine from incorporation of exogeneous palmitate.

The slightly greater incorporation of [9,10-^{3}H]palmitate into the β position palmitate as compared to the α position of surfactant phosphatidylcholine agrees with the results obtained following intravenous injection of [1-^{14}C]palmitate in dogs (Frosolano *et al.*, 1970). More significantly palmitic acid isolated from the β position of the residual fraction had twice the specific activity of the α positional palmitic acid.

The incorporation of [U-^{14}C]glucose into phosphatidylglycerol is observed to differ markedly from incorporation into phosphatidylcholine. The incorporation of [U-^{14}C]glucose into phosphatidylglycerol of both fractions was observed to be dependent upon glucose concentration (Table V). Incorporation into phosphatidylcholine was not significantly affected by glucose concentration. Study of the relative specific activities of both the α and β positional palmitate of phosphatidylcholine isolated from the surfactant and residual fractions following [U-^{14}C]glucose incorporation was observed to be similar to that following [9,10-^{3}H]palmitate and [1-^{14}C]acetate incorporation. However, [U-^{14}C]glucose incorporation into phosphatidylglycerol differs markedly. In the surfactant fraction the specific activity of the β position palmitate derived from [U-^{14}C]glucose is nearly twice that of the α position and is in sharp contrast to the specific activity pattern with [9,10-^{3}H]palmitate and

[1-^{14}C]acetate. In the residual fraction, the specific activity of the α and β palmitate is similar following [U-^{14}C]glucose incorporation while the specific activity of the α position was nearly twice that of the β following incorporation of [9,10-^{3}H]palmitate or [1-^{14}C]acetate. A comparison of the relative specific activities of the α and β positional palmitate of phosphatidylglycerol from [U-^{14}C]glucose does not reflect the much higher specific activity of the residual fraction phosphatidylcholine as compared to the surfactant fraction measured as a whole molecule (Table IV). Thus, the increased specific activity of the residual fraction phosphatidylglycerol must reside in the glycerol moieties of the molecule.

The significance of the observed differences in phosphatidylglycerol and phosphatidylcholine metabolism reported here as they relate to surfactant synthesis is not clear. The precursor source of palmitic acid and the rate of incorporation of palmitate into the α and β position of phosphatidylcholine and phosphatidylglycerol are different in the two fractions. The association of a higher percentage of phosphatidylglycerol with the surfactant fraction than the residual fraction raises the question of its function in the surfactant fraction. One possible role that has been suggested (Godinez *et al.*, 1975) is that phosphatidylglycerol, because of its physical properties, may play a role in stabilization of the surfactant lipoprotein complex.

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