Use of the isolated perfused rat lung in studies on lung lipid metabolism

Rodolfo I. Godinez and William J. Longmore

Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63104

Abstract A procedure for the use of the isolated perfused rat lung in studies on metabolic regulation has been developed. The procedure, reasonably uncomplicated, yet physiological, maintains the lung so that edema is not observed. The phospholipid content remains normal, and incorporation of [1-^{14}C]-palmitate, [2-^{14}C]acetate, and [U-^{14}C]glucose is linear with time for a minimum of 2 hr. The incorporation of [1-^{14}C]-palmitate and [2-^{14}C]acetate into the total lung phospholipid fraction and into the phosphatidylcholine and phospatidyl-ethanolamine fractions has been studied. Increasing the concentration of palmitate in the medium from 0.14 to 0.51 mM increased by 60% the incorporation of [1-^{14}C]palmitate into the total lung phospholipid fraction at 2 hr. When the palmitate concentration of the medium was 0.14 mM, addition of 0.11 and 0.79 mM oleate to the medium decreased [1-^{14}C]palmitate incorporation into the total lung phospholipid fraction at 2 hr by 37 and 49%, respectively.

The results suggest that the incorporation of exogenous fatty acids, present in the medium perfusing the lung, into lung phospholipids may depend upon the fatty acid composition of the medium. Known specific acyltransferase activities may be responsible for the ordered incorporation of available fatty acids into lung phospholipids.

Supplementary key words   [1-^{14}C]palmitate · [2-^{14}C]acetate · [U-^{14}C]glucose · phosphatidylcholine · phosphatidylethanolamine

$\mathbf{T}$HE STUDY of the metabolic activities of the lung and of the regulation of these activities has not kept pace with similar biochemical investigation in many other organs. Several recent reports have appeared which contain data on the in vivo incorporation of lipid precursors into lung lipids (1–3) and on the incorporation of similar precursors into the lipids of lung slices (4–9). However, in vivo incorporation experiments do not permit the isolated study of lung metabolism. Experiments utilizing lung slices suffer greatly from the lack of penetration of substrates to all but the cells nearest the cut surface of the slice. As in the case of the study of other organs, convenient physiological tissue preparation for studies on metabolic regulation is that of the isolated perfused organ. Prior reports of procedures for the isolated perfusion of lungs from the rat (10, 11), rabbit (12, 13), and dog (14) have appeared. However, in each case, the preparation has suffered from lack of concern for physiological conditions, appearance of obvious edema during perfusion, or techniques too elaborate to be useful in normal laboratory experimental use. Our interest in utilizing the isolated perfused rat lung as a physiological model for the biochemical study of metabolic regulation has led to the development of a procedure which appears suitable for such studies. Experiments have been carried out to establish rates of incorporation of acetate, palmitate, and glucose into the phospholipids of the isolated rat lung and to study the effect of oleate concentration on palmitate incorporation.

EXPERIMENTAL PROCEDURE

Earlier attempts in our laboratory to perfuse isolated rat lungs with medium containing washed bovine erythrocytes and certain commercially available bovine serum albumin preparations, as had been used in the perfusion of isolated rat liver (15), resulted in inconsistent results associated with a decreased total lung lipid/protein ratio and often an increase in water retention of the tissue. Continued investigation indicated that these problems could be overcome by deleting erythrocytes from the medium and by careful selection of the bovine serum albumin used. It was established that oxygen consumption of the lung tissue was sufficiently low so that normal oxygenation of the tissue was achieved without addition of erythrocytes to the medium. A normal value

of 130.0 mg of protein/g (wet wt) of lung tissue was maintained throughout the 2-hr perfusion period used in these studies.

Animals

Male rats of the St. Louis University strain fed Purina laboratory chow ad lib. were used in these studies. Animals weighing 180–250 g were injected with heparin (1000 units/kg) and pentobarbital (50 mg/kg). A tracheotomy was performed and positive pressure ventilation with room air (volume approximately 2.5 ml, rate 32 strokes/min) was begun. The chest cavity was opened along the midline, and a cannula attached to the circulating medium supply of the perfusion system was inserted into the pulmonary artery through an incision in the right ventricle. The pulmonary veins were severed and the flow rate of the medium was adjusted to 10 ml/min. The lung was quickly removed and inspected for absence of atelectasis and for total perfusion as observed by uniform blanching of all lobes. The lung was then carefully suspended in the perfusion cabinet and the flow rate of medium perfusing the lung was readjusted to approximately 10 ml/min with a hydrostatic pressure of 20 cm H_2O. The elapsed time between the beginning of the tracheotomy and placement of the lung in the perfusion cabinet was approximately 10 min.

Perfusion technique

The perfusion medium consisted of Krebs-Ringer bicarbonate medium (16), containing 3% bovine serum albumin (fraction V, Miles Laboratories) and, unless otherwise indicated, 5.6 mM D-glucose. The bicarbonate concentration of the perfusion medium was adjusted to provide a pH of 7.35–7.40 when the medium was equilibrated with a gas mixture containing 95% O_2–5% CO_2.

The perfusions were carried out in a water-saturated atmosphere in a cabinet maintained at 37.5°C. The perfusion medium (150 ml) was pumped from a lower reservoir (mixed by a magnetic stirrer) to the top of a glass "lung" in which the O_2–CO_2 gas mixture was passed over the medium, which coated the glass surface of the "lung." It then flowed by gravity either to the rat lung at a controlled flow rate or out through an overflow tube to the reservoir. Two in-line filters were used. One was a silk filter cloth (Joymar Scientific, Inc., New York) at the exit of the lower reservoir, and the other was a metal screen, pore size 0.2 mm, in the line supplying the rat lung. Perfusate was pumped by means of a roller pump (Masterflex pump system, Cole-Parmer) set to deliver a total flow (to lung plus overflow) of 90 ml/min. The pH of the medium was monitored continuously during the

perfusion, and no adjustment of pH was found to be necessary during a 2-hr perfusion to maintain the range of 7.35–7.40.

Additions of free fatty acids to the medium were made prior to addition of the bovine serum albumin and were dispersed by sonication. Bovine serum albumin was then added, and the medium was allowed to stand at 37°C until clear. The medium was then filtered through a very fine glass fiber filter pad (H. Reeve Angel, Clifton, N.J.). Lungs were perfused for 5 min prior to addition of ¹⁴C-labeled substrates (purchased from New England Nuclear Corp., Boston, Mass.) to the lower reservoir. Fatty acids labeled with ¹⁴C were bound to bovine serum albumin in a small aliquot (10 ml) of medium before addition to the circulating medium.

At intervals during the perfusion, aliquots of perfusion medium were taken from the lower reservoir for assay of substrate concentrations and determination of specific activities. Lung lobes were obtained at either 60 and 120 min, or 40, 80, and 120 min. The lobes taken at 40-, 60-, and 80-min intervals were obtained by placing a suture loop around the base of the hilus, drawing it tight, and removing the lobe. All lung samples were immediately placed in liquid nitrogen. Flow rate of the medium and the respiratory volume were readjusted to the smaller lung mass remaining.

Chemical analyses

Samples of medium were assayed for glucose content (17, 18), and the pH of samples obtained under oil was determined to confirm those obtained by monitoring during the perfusion. To determine concentrations of palmitic and oleic acid in the medium, the medium was acidified and extracted two times with petroleum ether–ethanol 9:1; the methyl esters were prepared (19) and then analyzed by gas–liquid chromatography on EGSS-X (10% on Gas-Chrom P) (Applied Science Laboratories, State College, Pa.) at 180°C using a hydrogen flame detector. Fatty acid methyl ester standards were used as references (Applied Science Laboratories). For determination of the specific activities of [1-¹⁴C]-palmitate in the medium, a specially designed effluent splitter and trapping device on the gas chromatograph was used. The specific activities of the radioactive substrates, [2-¹⁴C]acetate (5 μCi/mmole), [1-¹⁴C]palmitate (73 μCi/mmole), and [U-¹⁴C]glucose (9.0 μCi/mmole), in the medium were found not to vary significantly with time during the 2-hr perfusions. Radioactivity was measured by liquid scintillation counting methods.

Samples of lung tissue were stored in liquid nitrogen for a maximum of 30 min. Samples were then weighed, a small portion was taken for protein determination (20), and the remainder of each lobe was extracted by homogenization in chloroform–methanol 2:1. Nonlipid com-
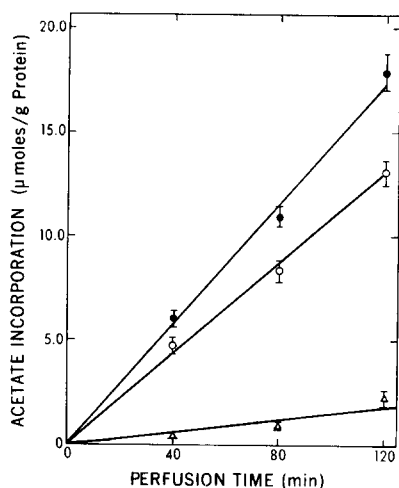
FIG. 1.   Incorporation of [2-¹⁴C]acetate into rat lung phospholipids. The perfusion medium contained initially 5.6 mM glucose and 4.0 mM acetate. All other conditions were as described under Experimental Procedure. ●, incorporation into total phospholipid fraction; O, incorporation into phosphatidylcholine fraction; △, incorporation into phosphatidylethanolamine fraction. Values are averages of eight experiments; vertical bars indicate SE.
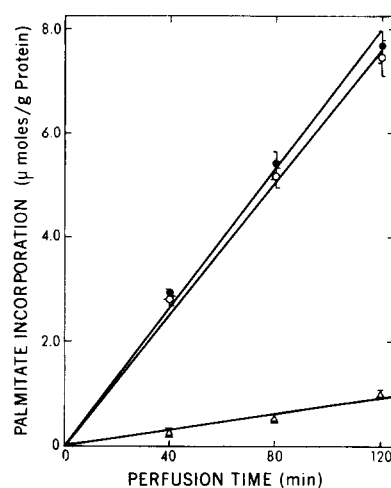
FIG. 2.   Incorporation of [1-¹⁴C]palmitate into rat lung phospholipids. The perfusion medium contained initially 5.6 mM glucose and 0.1 mM palmitate. All other conditions were as described under Experimental Procedure. ●, incorporation into total phospholipid fraction; O, incorporation into phosphatidylcholine fraction; △, incorporation into phosphatidylethanolamine fraction. Values are averages of four experiments; vertical bars indicate SE.

ponents were removed from the lipid extract by washing (21). The lung lipids were then placed on a column containing 1 g of silicic acid (Unisil, Clarkson Chemical Co., Williamsport, Pa.). The column was eluted with 40 ml of chloroform to obtain the neutral lipid fraction, and then with 40 ml of methanol to obtain the phospholipid fraction. The phospholipid fraction was then rechromatographed on a second silicic acid column (2 g), which was eluted in sequence with 25 ml of chloroform–methanol 19:1, 80 ml of chloroform–methanol–water

11:1:0.06, 40 ml of chloroform–methanol–water 3:1: 0.08, and 25 ml of methanol. Thin-layer chromatography (22) established that the chloroform–methanol–water 11:1:0.06 fraction contained greater than 90% phosphatidylethanolamine and is referred to as the phosphatidylethanolamine fraction; the 3:1:0.08 fraction contained greater than 90% phosphatidylcholine and is referred to as the phosphatidylcholine fraction. Phospholipid concentration was determined by a modifica-

TABLE 1.   Concentrations of phospholipids in perfused rat lung

Fatty Acid Concentration of Perfusion Medium		Total Phospholipid			Phosphatidylcholine Fraction			Phosphatidylethanolamine Fraction		
		Perfusion Time Elapsed (min)			Perfusion Time Elapsed (min)			Perfusion Time Elapsed (min)		
		0	60	120	0	60	120	0	60	120
	mM					μmoles P_i/mg protein				
Palmitate	0.103	0.146 ± 0.003[a] (6)	0.154 ± 0.016 (6)	0.142 ± 0.015 (5)	0.088 ± 0.004 (6)	0.103 ± 0.07 (6)	0.101 ± 0.004 (5)	0.036 ± 0.002 (6)	0.034 ± 0.004 (6)	0.034 ± 0.003 (5)
Palmitate	0.506		0.130 ± 0.018 (6)	0.097 ± 0.015[b] (5)		0.093 ± 0.011 (6)	0.065 ± 0.009 (4)		0.032 ± 0.006 (6)	0.026 ± 0.003[c] (4)
Palmitate Oleate	0.143 0.112		0.145 ± 0.090 (5)	0.103 ± 0.004 (5)		0.090 ± 0.006 (5)	0.088 ± 0.006 (5)		0.033 ± 0.002 (5)	0.031 ± 0.002 (5)
Palmitate Oleate	0.143 0.788		0.145 ± 0.008 (4)	0.135 ± 0.009 (4)		0.099 ± 0.008 (4)	0.100 ± 0.005 (4)		0.031 ± 0.003 (4)	0.032 ± 0.002 (4)

[a] Zero-time phospholipid content was obtained from a separate series of animals in which the normal procedure of surgery and perfusion with medium (containing no added fatty acids) was followed up to the time the lungs were placed in the perfusion cabinet. At that time, lung samples were taken for assay. Values are averages ± SE of number of experiments in parentheses.
[b] Value significantly less than zero time at 0.05 > P > 0.01.
[c] Value significantly less than zero time at P < 0.01.

TABLE 2. Incorporation of [1-¹⁴C]palmitate into phospholipids of isolated perfused rat lung; effect of palmitate concentration in the perfusion medium

Palmitate Concentration of Perfusion Medium	Total Phospholipid		Phosphatidylcholine Fraction		Phosphatidylethanolamine Fraction	
	Perfusion Time Elapsed (min)		Perfusion Time Elapsed (min)		Perfusion Time Elapsed (min)	
	60	120	60	120	60	120
mM	μmoles/g protein					
(A) 0.143	3.96 ± 0.41 (6)[a]	8.89 ± 1.26 (5)	3.40 ± 0.21 (6)	7.95 ± 0.64 (5)	0.27 ± 0.03 (5)	0.80 ± 0.12 (4)
(B) 0.506	9.33 ± 1.22 (5)	14.18 ± 3.28 (3)	8.16 ± 0.77 (5)	12.04 ± 2.58 (3)	0.80 ± 0.14 (5)	2.35 ± 0.88 (4)
Ratio B/A	2.36	1.60	2.40	1.52	2.97	2.94
	$P < 0.01$[b]	$P > 0.05$	$P < 0.01$	$P > 0.05$	$0.05 > P > 0.01$	$P > 0.05$

[a] Values are averages ± SE of number of experiments in parentheses. The data represent μmoles of [1-¹⁴C]palmitate incorporated.
[b] Probability that values in line A are not significantly different from those in line B.

tion (23) of the Bartlett phosphorus analysis, and specific radioactivities were obtained by counting appropriate aliquots.

The amount of acetate, palmitate, or glucose incorporated into the phospholipid fractions was calculated from the specific activity of acetate, palmitate, or glucose and the pool size and specific activity of each phospholipid fraction at the time of the sample.

RESULTS

To determine the usefulness of the perfusion procedure in biochemical studies, several series of experiments were performed to establish the linearity with time of the incorporation of phospholipid precursors into the phospholipids of the perfused rat lung. The incorporation of [2-¹⁴C]acetate and [1-¹⁴C]palmitate into the total phospholipid, phosphatidylcholine, and phosphatidylethanolamine fractions was determined (Figs. 1 and 2, respectively). The rates of incorporation of both radioactive precursors were essentially constant. It was observed that more [1-¹⁴C]palmitate than [2-¹⁴C]acetate was incorporated into phosphatidylcholine. Incorporation of [U-¹⁴C]glucose (5.8 mM) in the presence of 0.3 mM palmitic acid into the total phospholipid fraction was found to be linear for 2 hr, and amounted to 6.0 μmoles of glucose/g of protein.

As a result of observing a decreased phospholipid/protein ratio during perfusions in which the tissue was known to have been in a deteriorating physiological condition, a comparison was made of the concentrations of the total phospholipid, phosphatidylcholine, and phosphatidylethanolamine fractions of lung at three time intervals during several series of perfusions (Table 1). The results of these comparisons indicated that there were no significant changes (from zero-time values) during the 2-hr perfusion period. The only exception observed was in the series of perfusions in which palmitic acid was present in the medium at a concentration of 0.506 mM. At the 2-hr interval of the perfusion, a significant de-

TABLE 3. Incorporation of [1-¹⁴C]palmitate[a] into phospholipids of isolated perfused rat lung; effect of oleate concentration in the perfusion medium on palmitate incorporation

Oleate Concentration of Perfusion Medium	Total Phospholipid		Phosphatidylcholine Fraction		Phosphatidylethanolamine Fraction	
	Perfusion Time Elapsed (min)		Perfusion Time Elapsed (min)		Perfusion Time Elapsed (min)	
	60	120	60	120	60	120
mM	μmoles/g protein					
(A) 0.000	3.96 ± 0.41 (6)[b]	8.89 ± 1.26 (5)	3.40 ± 0.21 (6)	7.95 ± 0.61 (5)	0.27 ± 0.03 (5)	0.80 ± 0.12 (4)
(B) 0.112	2.89 ± 0.23 (5)	5.62 ± 0.41 (5)	2.67 ± 0.27 (5)	5.05 ± 0.48 (5)	0.22 ± 0.02 (5)	0.56 ± 0.11 (5)
(C) 0.788	2.71 ± 0.41 (4)	4.51 ± 0.43 (4)	2.52 ± 0.37 (4)	4.60 ± 0.45 (4)	0.16 ± 0.05 (4)	0.40 ± 0.15 (4)
Ratio B/A	0.73	0.63	0.79	0.64	0.81	0.70
	$0.05 > P > 0.01$[c]	$0.05 > P > 0.01$	$P > 0.05$	$P < 0.01$	$P > 0.05$	$P > 0.03$
Ratio C/A	0.68	0.51	0.74	0.58	0.59	0.50
	$P > 0.05$[d]	$P < 0.01$	$P > 0.05$	$P < 0.01$	$P > 0.05$	$P > 0.05$

[a] The palmitate concentration was 0.143 mM.
[b] Values are averages ± SE of number of experiments in parentheses. The data represent μmoles of [1-¹⁴C]palmitate incorporated.
[c] Probability that values in line A are not significantly different from those in line B.
[d] Probability that values in line A are not significantly different from those in line C.

crease in total phospholipids and in phosphatidylethanolamine had occurred.

A study of the effect of medium palmitate concentration on [1-^{14}C]palmitate incorporation into the total phospholipid, phosphatidylcholine, and phosphatidylethanolamine fractions was also carried out. Data presented in Table 2 indicate that increasing the medium concentration of palmitic acid from 0.143 to 0.506 mM resulted in 136, 140, and 197% increases in the incorporation of [1-^{14}C]palmitate into the total phospholipid, phosphatidylcholine, and phosphatidylethanolamine fractions, respectively, after 60 min of perfusion. After 120 min of perfusion, the increase was reduced to 60 and 52% for the total phospholipids and phosphatidylcholine, respectively, but the increase in incorporation into the phosphatidylethanolamine fraction remained unchanged.

Studies designed to determine the effect of the presence of an unsaturated fatty acid, oleic acid, on [1-^{14}C]palmitate incorporation were performed; the concentration of palmitate in the medium was 0.143 mM and oleate was either 0, 0.112, or 0.788 mM. The data (Table 3) indicate that the presence of oleate at 0.112 mM decreased the incorporation of [1-^{14}C]palmitate into the phospholipids between 19 and 27% after 60 min and between 30 and 37% after 120 min of perfusion. When the concentration of oleate in the medium was 0.788 mM, the percentage decrease in [1-^{14}C]palmitate incorporation after 60 min of perfusion was between 26 and 41%, and after 120 min was between 42 and 50%, for each of the phospholipid fractions studied. The significance of the changes observed in Tables 2 and 3 is given in the tables. In general, lack of significance ($P > 0.05$) occurred only where a small number of perfusions were carried out.

DISCUSSION

The production of dipalmitoyl glycerophosphorylcholine by the lung as a major component of the surface-active material necessary for pulmonary function has been established (24–26). Studies concerned with lung lipid, and particularly phospholipid, metabolism and factors which regulate this metabolism have been fragmentary and in some cases contradictory. In vivo studies have been limited primarily to developmental studies (2), estimations of turnover times (3), and the effect of altered lung ventilation on metabolism (1). Early studies performed with rabbit lung slices established the incorporation of palmitate, acetate, and glucose into lung phospholipids (4, 6, 7). More recent reports of experiments utilizing lung slices have dealt with delineation of the pathway of dipalmitoyl glycerophosphorylcholine biosynthesis (5, 8).

Reports of the use of the isolated perfused lung in biochemical studies of lung metabolism have been limited perhaps because of difficulties encountered in establishing a suitable procedure. Levey and Gast (10) developed an isolated perfused rat lung preparation for use in such studies, but the lung reportedly gained about 12% weight during a 3-hr perfusion due to observed edema. We found the preparation of Levey and Gast (10) to be unsatisfactory both because of the variable results obtained and because of a decrease in the lipid/protein ratio during perfusion. Several differences exist between the preparation described by Levey and Gast (10) and that reported here. The use of positive pressure ventilation prior to and during removal of the lung from the rat and continuous ventilation during perfusion of the lung was necessary to provide a constant perfusate flow rate. The requirement of continued ventilation to preclude an increase in pulmonary vascular resistance has been reported (11). In our procedure, flow of blood or perfusate to the lung was continuous, as the cannula from the perfusate was inserted into the pulmonary artery and flow was begun before removal of the lung from the rat. Thus, tissue oxygenation was continuous. Care in the selection of the bovine serum albumin preparation used in the medium was found to be essential. Bovine serum albumin obtained from commercial sources was tested and one source (see Experimental Procedure) proved to be satisfactory, producing only occasional problems of water retention after longer periods of perfusion. Usually, a failure was associated with a particular new lot and its use was discontinued. It has not been possible to establish the property (or properties) of the bovine serum albumins that proved unsatisfactory, although attempts were made to remove possible contaminants by conventional methods of extraction and adsorption. We chose to omit erythrocytes from the perfusion medium to avoid substrate utilization and metabolism by the erythrocytes and contamination of lung tissue samples with erythrocyte phospholipids.

The incorporation of [2-^{14}C]acetate, [1-^{14}C]palmitate and [U-^{14}C]glucose into lung phospholipids is presented to demonstrate linear incorporation with time of these precursors. However, it is of interest that, when compared with incorporation of each isotope into the total phospholipid fraction, [1-^{14}C]palmitate served as a better precursor of the phosphatidylcholine fraction than did [2-^{14}C]acetate (see Figs. 1 and 2). Incorporation of both precursors into the phosphatidylethanolamine fraction appeared similar. These results indicate that exogenous [1-^{14}C]palmitate is incorporated to a greater extent into the phosphatidylcholine fraction than are fatty acids synthesized de novo from [2-^{14}C]acetate or by elongation of available fatty acids by [2-^{14}C]acetate. Presumably this incorporation would occur via acyl-

transferase activity, which is believed to be responsible for the ordered distribution of fatty acids in phospholipids (27) rather than for the acylation of glycerol-3-phosphate. Acyltransferase activity in lung was first suggested by Lands (28). More recently it has been reported that 1-acyl-2-lysophosphatidylcholine served as a much better substrate for acyltransferase activity than 1-acyl-2-lysophosphatidylethanolamine in dog lung (29).

The observed increase in the incorporation of medium [1-^{14}C]palmitate with increased medium palmitate concentration indicates the ability of the lung to utilize circulating fatty acids for phospholipid synthesis. A previous report has demonstrated such a concentration dependence on palmitate incorporation into the phospholipids of rabbit lung slices (7). The demonstration that increased medium oleate concentration markedly decreases [1-^{14}C]palmitate incorporation into phospholipid may be interpreted to indicate that incorporation of exogenous fatty acids from the medium is somewhat dependent upon the relative concentration of available fatty acids. Evidence has been presented from studies with Ehrlich ascites tumor cells that the presence of one fatty acid does affect the uptake of a second fatty acid and that the total fatty acid uptake is essentially the same as if only one fatty acid were present (30). It has been observed (29) that specific acyltransferases are present in the lung microsomal fraction and that a preference for unsaturated fatty acids at the 2-position of the phospholipid exists while at the 1-position a lack of preference between saturated and unsaturated fatty acids is seen. Thus, the composition of the lung phospholipid fatty acid component at the 1-position may be dependent upon the relative concentration of fatty acids available. Although our studies have not been concerned with the synthesis of dipalmitoyl glycerophosphorylcholine associated with the surfactant material, it is possible that the medium (or blood) fatty acid composition may be of importance in the synthesis of this and other lung lipids.

REFERENCES

1. Newman, D., and A. Naimark. 1968. Palmitate-^{14}C uptake by rat lung: effect of altered gas tensions. *Amer. J. Physiol.* **214:** 305–312.

2. Weinhold, P. A. 1968. Biosynthesis of phosphatidyl choline during prenatal development of the rat lung. *J. Lipid Res.* **9:** 262–269.

3. Thomas, T., Jr., and R. A. Rhoades. 1970. Incorporation of palmitate-1-^{14}C into lung tissue and "alveolar" lecithin. *Amer. J. Physiol.* **219:** 1535–1538.

4. Felts, J. M. 1965. Carbohydrate and lipid metabolism of lung tissue *in vitro. Med. Thorac.* **22:** 89–99.

5. DiAugustine, R. P. 1971. Lung phospholipids. I. *In vivo* studies of the incorporation of ^{32}P, [methyl-^{14}C]choline, [1-^{14}C]palmitic acid and [1-^{14}C]oleic acid into phosphatidylethanolamine, phosphatidyl-N,N-dimethylethanolamine and phosphatidylcholine. *Biochem. Biophys. Res. Commun.* **43:** 311–317.

6. Chida, N., and F. H. Adams. 1967. Incorporation of acetate into fatty acids and lecithin by lung slices from fetal and newborn lambs. *J. Lipid Res.* **8:** 335–341.

7. Wolfe, B. M. J., B. Anhalt, J. C. Beck, and D. Rubinstein. 1970. Lipid metabolism in rabbit lungs. *Can. J. Biochem.* **48:** 170–177.

8. Akino, T., M. Abe, and T. Arai. 1971. Studies on the biosynthetic pathways of molecular species of lecithin by rat lung slices. *Biochim. Biophys. Acta.* **248:** 274–281.

9. Tierney, D. F. 1971. Lactate metabolism in rat lung tissue. *Arch. Int. Med.* **127:** 848–860.

10. Levey, S., and R. Gast. 1966. Isolated perfused rat lung preparation. *J. Appl. Physiol.* **21:** 313–316.

11. Hauge, A. 1968. Conditions governing the pressor response to ventilation hypoxia in isolated perfused rat lung. *Acta Physiol. Scand.* **72:** 33–44.

12. Koga, H. 1954. Studies on the function of isolated perfused mammalian lung. I. Method of perfusing isolated lung of rabbit *in vitro. Kumamoto Med. J.* **7:** 67–72.

13. Heinemann, H. O. 1961. Free fatty acid production by rabbit lung tissue *in vitro. Amer. J. Physiol.* **201:** 607–610.

14. Evans, C. L., and E. H. Starling. 1913. The part played by the lungs in the oxidative processes of the body. *J. Physiol.* (London). **46:** 413–434.

15. Longmore, W. J., C. M. Niethe, and M. L. McDaniel. 1969. Effect of CO_2 concentration on intracellular pH and on glycogen synthesis from glycerol and glucose in isolated perfused rat liver. *J. Biol. Chem.* **244:** 6451–6457.

16. Krebs, H. A., and K. Henseleit. 1932. Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Z. Physiol. Chem.* **210:** 33–66.

17. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153:** 375–380.

18. Somogyi, M. 1945. Determination of blood sugar. *J. Biol. Chem.* **160:** 69–73.

19. Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. *J. Lipid Res.* **5:** 600–608.

20. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

21. Radin, N. S. 1969. Preparation of lipid extracts. *Methods Enzymol.* **14:** 245–254.

22. Skipski, V. P., M. Barclay, E. S. Reichman, and J. J. Good. 1967. Separation of acidic phospholipids by one-dimensional thin-layer chromatography. *Biochim. Biophys. Acta.* **137:** 80–89.

23. Dittmer, J. C., and M. A. Wells. 1969. Quantitative and

qualitative analysis of lipids and lipid components. *Methods Enzymol.* **14:** 482–530.

24. Pattle, R. E., and L. C. Thomas. 1961. Lipoprotein composition of the film lining the lung. *Nature.* **189:** 844.

25. Buckingham, S., and M. E. Avery. 1962. Time of appearance of lung surfactant in the foetal mouse. *Nature.* **193:** 688–689.

26. Frosolono, M. F., B. L. Charms, R. Pawlowski, and S. Slivka. 1970. Isolation, characterization, and surface chemistry of a surface-active fraction from dog lung. *J. Lipid Res.* **11:** 439–457.

27. Hill, E. E., D. R. Husbands, and W. E. M. Lands. 1968. The selective incorporation of ¹⁴C-glycerol into different species of phosphatidic acid, phosphatidylethanolamine, and phosphatidylcholine. *J. Biol. Chem.* **243:** 4440–4451.

28. Lands, W. E. M. 1958. Metabolism of glycerolipides: a comparison of lecithin and triglyceride synthesis. *J. Biol. Chem.* **231:** 883–888.

29. Frosolono, M. F., S. Slivka, and B. L. Charms. 1971. Acyl transferase activities in dog lung microsomes. *J. Lipid Res.* **12:** 96–103.

30. Spector, A. A. 1970. Free fatty acid utilization by mammalian cell suspensions. Comparison between individual fatty acids and fatty acid mixtures. *Biochim. Biophys. Acta.* **218:** 36–43.