Acetate Incorporation into Lipids of Canine Lung Cells[1] (35995)

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Various type of cells are found in mammalian lungs (1). Of the cells found in the area of terminal air passages, type-II alveolar lining cells are currently thought to synthesize pulmonary surfactant. Said *et al.* (2), in 1966, reported that the alveolar cells lavaged from lungs of normal dogs had considerable metabolic activity consistent with a biosynthetic function. Alveolar cells (great alveolar cells, septal cells, corner cells, granulopneumocytes, or type-II cells) were tested for the presence of several enzymes in oxidative and synthetic pathways; they showed strong to moderately strong reactions. They found that these large enzyme-rich alveolar cells were lacking in species with a deficiency of pulmonary surfactant. The correlation was made that the large alveolar cells were responsible for surfactant production. Autoradiographs of lung tissues from rabbits after an injection of tritium-labeled acetate showed radioactivity predominantly localized in the giant alveolar cells, as well as in a few alveolar macrophages at 30 min after the injection (3). From autoradiographs and chemical analyses of the lipid fractions at various time intervals, Buckingham *et al.* (3) concluded that the cytoplasm of the giant alveolar cells represented the active sites of phospholipid synthesis and turnover in the lung. To date, no direct evidence has shown the exact site of synthesis of the surface-active material found in the alveoli. The present study was undertaken to investigate the lipid synthesizing ability of cells washed from the lungs of dogs by bronchopulmonary lavage (4). Sodium acetate-1-^{14}C was incubated with these cells and their incorporation of the label into lipids was observed.

Materials and Methods. Three beagle dogs (3.5 years old) from the Lovelace Foundation beagle dog colony were used. Dogs anesthetized with pentobarbital sodium were intubated with a double lumen Carlens catheter and the pulmonary lavage was accomplished by techniques previously documented (5). The lavage fluid was removed from one lung (the other lung sustained the dog), stored at 5° until all lavages were completed and the fluid thus obtained was centrifuged for 6000*g*/min. The supernatant fluid was discarded, the cellular button was resuspended in Hanks' balanced salt solution, and the cell concentration was determined in a Model F Coulter counter ($\sim 1.4 \times 10^6$ cells/ml). Counting vials, thoroughly cleaned and sterilized, were used as the incubation containers. A 150 μl aliquot of Hanks' solution (containing 500 units of penicillin and 500 μg of streptomycin sulfate) was pipetted into each of six vials, followed by 250 μl of the cellular suspension and finally 50 μl of a sodium acetate-1-^{14}C solution (1.136 μCi and 52 nmoles) was added. The cellular reactions were allowed to take place in a Dubnoff metabolic incubator at 37°, with gentle shaking (100 strokes/min), and a vial was removed every 30 min. After incubation, the metabolic reaction was terminated by the addition of CH_3OH (the first step of the Bligh and Dyer (6) lipid extraction procedure). Addition of the proper proportions of $CHCl_3$ and H_2O allowed the separation of the $CHCl_3$ phase containing most of the lipid-like materials. Reextraction of the CH_3OH–H_2O phase yielded a second $CHCl_3$ extract that was combined with the first. The $CHCl_3$ fraction was evaporated to dryness under N_2 and the lipid residue was redissolved in 60 μl of $CHCl_3$.

Thin-layer (250 μm) chromatograms (2 ×

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TABLE I. Lipids[a] Synthesized from NaOAc-^{14}C After Incubation with Lung Cells Derived from a Pulmonary Lavage.

Incubation time (min)	Percentage of total lipid								Percentage of ^{14}C incorporated into lipids
	CHOL	FFA	TG	SE	PI + PS + SPHI	PC	PG + PDME	PE	
30	11	32	21	5	2	16	5	2	3.1 ± 0.1
60	16	27	21	6	2	17	8	2	4.7 ± 0.2
90	23	24	15	9	1	15	8	2	5.2 ± 0.5
120	27	21	20	4	2	15	8	2	5.4 ± 0.4
150	33	21	17	3	1	13	8	2	5.9 ± 0.2
180	36	18	17	2	1	12	9	2	5.7 ± 0.2

[a] Other lipid solvent-soluble material contained a few percent of the ^{14}C radioactivity, but the components listed are the principle identifiable compounds: CHOL, cholesterol; FFA, free fatty acids; TG, triglycerides; SE, sterol esters; PI, phosphatidyl inositol; PS, phosphatidyl serine; SPHI, sphingomyelin; PC, phosphatidyl choline; PG, phosphatidyl glycerol; PDME, phosphatidyl *N*-dimethylethanolamine; PE, phosphatidyl ethanolamine.

20 cm) on silica gel G and HR were prepared and developed as previously described (4). Each sample from the lung cell–NaOAc incubation and mixtures of standard lipids were chromatographed on the same plate in four solvent systems: two neutral and two phospholipid resolving systems (4). The chromatograms were developed (13 cm), air dried, and exposed to I_2 vapor. The standard lane was marked to identify a particular area with a particular lipid. Zonal scan analysis (7) of the radioactivity distributed on thin layers was used to achieve high counting efficiencies and high resolution of the separated lipid components. Zones (2 mm wide) of silica gel along the chromatograms were scraped into counting vials. A liquid scintillation spectrometer with a toluene – naphthalene – methylcellosolve – water cocktail was used to assay for carbon-14 at a counting efficiency of approximately 46%. The total activity recovered from each plate was compared to the activity recorded from a direct pipetting of an aliquot of the sample into a counting vial which was used as the standard. This provided a check on quenching, instrument, and technical errors involved in the procedures. The total activity in each lipid class was compared to the total activity on the plate and the percentages of the activity were calculated. The values are averages from three incubation reactions at each time period and from four TLC plates from each $CHCl_3$ extract.

Results and Discussion. The data in Table I show that the cells obtained from a lung lavage can actively synthesize phospholipids and neutral lipids from acetate. The cells obtained from the lungs of beagle dogs by lavage and identified by their morphologic appearance represented a variety of cell types including alveolar cells, macrophages, lymphocytes, neutrophils, and bronchial epithelial cells (4). Although maximal incorporation of ^{14}C into lipids occurred at 2.5 hr of incubation, active cellular metabolism was still evident after 3 hr of incubation (5 hr after removal from the lung). Unfortunately, the techniques do not permit the identification of the cell type or types responsible for lipid synthesis. It is quite possible that cell death occurred during the 5 hr time period and that only a select group of cells were the active participants. Penicillin and streptomycin were used in the incubation media to prevent the development of bacteria, which could have contributed to total acetate utilization. However, in other studies in this laboratory, lung lining cells cultured in Eagle's basal medium containing 10% serum and antibiotics did not show a pH shift (color change) until 96 hr postlavage. At this point Gram stains revealed bacterial overgrowth.

One of the principle routes for acetate incorporation into lung lipids is in the synthesis of fatty acid, which quickly become esterified and form triglycerides and phospholipids (8, 9). The data in Table I show that

after a 30-min incubation most of the total lipid ^{14}C was in the free fatty acid fraction (32% of total lipid ^{14}C). This is a greater incorporation of acetate into lipids than has been reported with lung slices (9). Phosphatidyl choline (PC) contained the highest amount of ^{14}C among the phospholipids. This was not unexpected since PC, allegedly the most active component of pulmonary surfactant, has been shown (4) to be the predominant phosphatide in both pulmonary surfactant and alveolar lining cells.

The amount of ^{14}C, however, appeared to increase throughout the time of incubation in the phosphatidyl glycerol + dimethylethanolamine and cholesterol fractions. The increase in ^{14}C in these phospholipids probably indicates that the component(s) are synthesized at a relatively slow rate and that they have not reached an equilibrium concentration. Also, with ^{14}C-labeled fatty acids in the system, a net increase in ^{14}C due to esterification would be entirely possible. A linear increase in cholesterol-^{14}C for 3 hr was observed and was probably related to a net synthesis from ^{14}C-acetate. Cholesterol, a necessary component of biological membranes, is associated with both both alveolar lining cells and pulmonary surfactant (4), the membranous layer at the air–tissue interface within the alveoli.

The amount of ^{14}C in other lipid components (TG, SE, PC) appeared to increase and then decrease with time of incubation (Table I). This is probably a real phenomenon, since most of the ^{14}C would be in the fatty acids which, with time, would be synthesized, esterified, and then catabolized to acetate (beta oxidation).

Although Buckingham *et al.* (3) demonstrated the uptake of ^{3}H-acetate into palmitate in the "special" alveolar cells *in vivo*, their studies did not rule out the possibility of extrapulmonary synthesis. Chida and Adams (10) incubated acetate-1-^{14}C with lung slices and showed the radiolabel incorporated predominantly into lecithin, diglycerides, and triglycerides with some ^{14}C in cholesterol and cholesterol esters. These studies (3, 11), although useful in showing the active metabolism of lung tissue, do not specifically address the question of the site of synthesis of the pulmonary surfactant lipids.

This work demonstrated that the lung cells removed by pulmonary lavage and principally derived from that region where surfactant is found were metabolically active in incorporating acetate into lipid compounds such as those found in pulmonary surfactant. Since these cells are able to synthesize the major lipid components of pulmonary surfactant, they may be considered as one possible site of the synthesis of the surface-active material.

Summary. Cells principally derived from the alveolar portion of the lungs of beagle dogs were obtained by pulmonary lavage and incubated with 1-^{14}C-acetate. Labeled lipids were isolated and quantitated by zonal profile thin-layer chromatographic scanning and liquid scintillation counting. The cells actively incorporated the acetate into both neutral lipids and phospholipids. Phosphatidyl choline was the predominantly labeled phosphatide and cholesterol was the predominantly labeled neutral lipid. The results show that the alveolar lining cells may be considered as a site of the synthesis of the lipid components of pulmonary surfactant.

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