Int. J. Biochem., 1977, Vol. 8, pp. 537 to 544 Pergamon Press. Printed in Great Britain

PULMONARY LIPID BIOSYNTHESIS: SIMULTANEOUS DOUBLE-LABEL IN LIPIDS FROM PLASMA, LUNG TISSUE AND SURFACTANT USING A DOG HEART–LUNG PREPARATION

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(*Received* 11 *October* 1976)

Abstract—1. The incorporation of [^{14}C]glycerol-3-phosphate and [^{3}H]palmitic acid into different classes of lipids from plasma, lung tissue and surfactant was studied simultaneously using a dog heart–lung preparation.

2. Isotope incorporation into total lipids indicated the tissue production of surfactant and its excretion to the alveolar light in a certain periodicity. Total lipids were fractionated into free fatty acids, total phosphoglycerides, diacylglycerols and triacylglycerols. Phosphoglycerides and triacylglycerols from surfactant exhibited also a rhythmic incorporation of ^{14}C and ^{3}H. Labelling of phosphoglycerides was 10-fold that of triacylglycerols in both surfactant and lung tissue.

3. Total phosphoglycerides were fractionated into phosphatidic acid, phosphatidylcholine, lyso-phosphatidylcholine and phosphatidylethanolamine from lung tissue, and into phosphatidylcholine and phosphatidylethanolamine from pulmonary surfactant.

4. A *de novo* pathway accounted for the synthesis of phosphatidic acid and phosphatidylethanolamine in the lung tissue.

5. Both *de novo* and deacylation–acylation mechanisms contributed to the synthesis of phosphatidylcholine; in the first part of the experiment, endogenous phosphatidylcholine was used for a deacylation–acylation pathway using labelled palmitic acid, whereas in the second half of the experiment a *de novo* pathway was the predominant mechanism of synthesis. Isotope incorporation into lyso-phosphatidylcholine was in agreement with these results.

6. Patterns of isotope incorporation into phosphatidylethanolamine and phosphatidylcholine from pulmonary surfactant agree with the different biosynthesis mechanisms for both phosphoglycerides and with their rhythmic excretion to the alveolar lumen.

7. An optical and micrographic study has been carried out during the whole experiment. It showed the maintenance of the alveolar integrity of the preparation as well as the lamellar bodies moving to the alveolar surface in which macrophages acted as scavengers for the surfactant.

INTRODUCTION

Interest in lung metabolism has received special attention in the last years from several groups of investigators. In most instances lung metabolism study has been approached by different *in vitro* experiments (Barron *et al.*, 1947; Faridy & Naimark, 1971; Fee & Teitelbaum, 1972; Felts, 1964; Fridovich, 1972; Katz & Wood, 1963; Krebs, 1970; Levy & Harvey, 1974; O'Neil & Tierney, 1974; Gregory & Fridovich, 1973; Kirkman, 1971; Yeager & Hicks, 1972. It is however apparent that most methods satisfactorily used with other tissues cannot be extrapolated to the lung. As in other organs, we should obtain ideally our information about lung metabolism by *in vivo* studies, and according to Tierney (1974) the isolated perfused lung would seem to be most similar to the lung *in vivo*.

The lung is actively engaged in phosphoglyceride synthesis (Wang & Meng, 1972). Various substrates, including glucose, acetate and palmitate, were used for lipid biosynthesis in the lung tissue after administration (Gassenheimer *et al.*, 1972), during incubation of lung slices and homogenates (Felts, 1964; Wang & Meng, 1972; Salisbury *et al.*, 1966) and during perfusion of isolated lungs (Longmore *et al.*, 1973). Palmitate incorporation into lung lipids occurs by esterification of triose phosphates (Felts, 1964; Wolfe *et al.*, 1970) but may also occur through acylation of lysophosphatidylcholine (Akino *et al.*, 1971; Kyei-Aboagye *et al.*, 1973). The incorporation of products of glucose catabolism (Gassenheimer *et al.*, 1972; Salisbury *et al.*, 1966) into triacylglycerols and phosphatidylcholine is partially dependent on energy derived from oxidative metabolism (Bassett *et al.*, 1974).

On the other hand, the sudden appearance of the pulmonary function at birth and its consideration as a metabolic organ in adults, are related to the so-called lung surfactant that exhibits a surface tension-lowering activity (Klaus *et al.*, 1962) and it is manufactured and secreted by lung type II epithelial cells (Macklin, 1954). Within these cells, multi-lamellar bodies seem to act as storehouses of surfactant (Hoffman, 1972; Page-Roberts, 1972; Gil & Reiss, 1973; Littman *et al.*, 1974; Spitzer *et al.*, 1975). In fact, the very methods employed to obtain lung surfactant will determine the nature and chemical composition of this functionally active mixture; however, alveolar washes are now commonly used (King & Clements, 1972; Stein *et al.*, 1969; Pawlowski *et al.*, 1971; Colacicco *et al.*, 1973). The surfactant activity is connected with mechanical lung function and its deficiency or inadequacy has been related to lung diseases.