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Protein Transport by Lung (37767)

KENNETH DICKIE AND DONALD MASSARO

Pulmonary Division, V. A. Hospital and the Department of Medicine, George Washington University School of Medicine, Washington, D. C. 20422

Lung alveoli are lined by a duplex extracellular layer (1, 2) which is believed to contain a surface-active lipoprotein (3), commonly referred to as "pulmonary surfactant." If the protein moiety of this molecule originates in the lung, the lung must be able to synthesize and secrete proteins. In this regard it has been shown that after the intravenous administration of radioactive leucine, the kinetics of the *in vivo* distribution of acid-insoluble radioactivity in lung tissue is consistent with the intracellular transport of protein from microsomes to particles that sediment at 15,000*g* (4). Electron microscopic radioautography has provided further support for the intracellular transport of protein by lung and has shown that at least some of this transport takes place from the rough endoplasmic reticulum to the lamellar bodies of the granular pneumocytes (5, 6). The present work was undertaken to investigate the time course of the transport of radioactive protein from lung tissue into pulmonary air spaces after the intravenous administration of radioactive leucine.

Methods. In vivo *radioisotopic studies.* Twenty-three New Zealand male white rabbits weighing about 2.6 kg were given 0.4 ml of 2.7×10^{-5} *M* L-leucine 4,5-^{3}H (sp act 36 .2 Ci/mmole) intravenously followed 4 min later by 2.0 ml of 1×10^{-3} *M* L-leucine-^{12}C. The animals were anesthetized with intravenously injected sodium pentobarbital (30 mg/kg), and sacrificed by severing the large abdominal blood vessels. They were sacrificed at selected intervals up to 17 hr after the leucine-^{12}C injection.

Isolation of surface-active fraction. Immediately after sacrifice, the intact lungs were removed from the thorax. The lungs' internal surface was lavaged via the trachea with 0.15 *M* NaCl at 40° using six lavages of 50 ml of saline for each animal. After lavage the lungs were minced and then homogenized with 30 passes of a Teflon pestle in a glass homogenizer. The lung tissue homogenate and the lavage material were centrifuged at 300*g* for 10 min forming a cellular sediment and a supernatant fluid termed the "crude extract." The surface-active fraction was isolated as previously described (7) from the lung lavage material and the tissue homogenate.

Assay for protein and radioactivity. Proteins were precipitated with trichloroacetic acid (TCA), extracted with lipid solvents and hot TCA, and assayed for radioactivity and protein content as previously described (8, 9). Crystalized bovine albumin was used as standard for protein determinations.

Results. The specific radioactivity of tissue proteins rises more rapidly than that of protein in lung lavage material (Fig. 1). The specific radioactivity of protein in the lavage fluid rises above that of the tissue proteins after 4 hr but begins to decline after 8 hr. It becomes equal to that of tissue protein by 17 hr. The cellular pellet fraction revealed a slower increase in specific radioactivity which does not achieve a value comparable to the other two fractions.

The specific radioactivity of protein in the surface-active fraction obtained from the lung tissue rises more rapidly than that present in the surface-active fraction obtained from lung lavage returns (Fig. 2). Unlike the results obtained in the crude material these curves do not cross.

Discussion. The kinetics of the distribution of palmitate-1-^{14}C between lung tissue

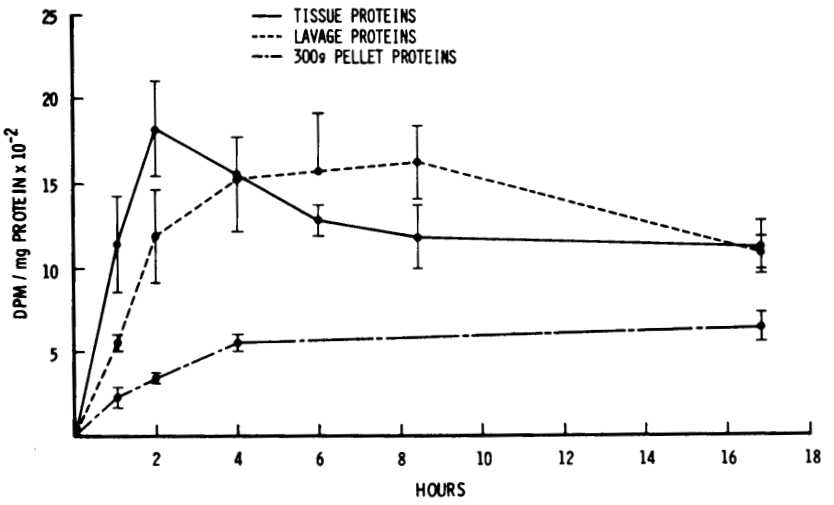

FIG. 1. Time course of the protein specific radioactivity in various fractions of lung lavage material and lung homogenate after the intravenous injection of L-lucine 4,5-^{3}H followed in 4 min by a "chase" dose of L-leucine-^{12}C.

lecithins and lecithins in lung lavage returns are consistent with a precursor–successor relationship between lecithin in the lung and in the lung lavage returns (10, 11). This is also true for dipalmitoyl lecithin (12). In both of these circumstances the specific radioactivity of the lipid in the lung lavage material became higher than in the lung tissue about 6 hr after administration of the labeled fatty acid. In the present study the protein specific radioactivity of the tissue crude extract and lavage crude extract show a similar cross-over at about 6 hr. Thus, the time course of the initial distribution of radioactive phospholipids and proteins between lung tissue and material obtained by lung lavage are virtually

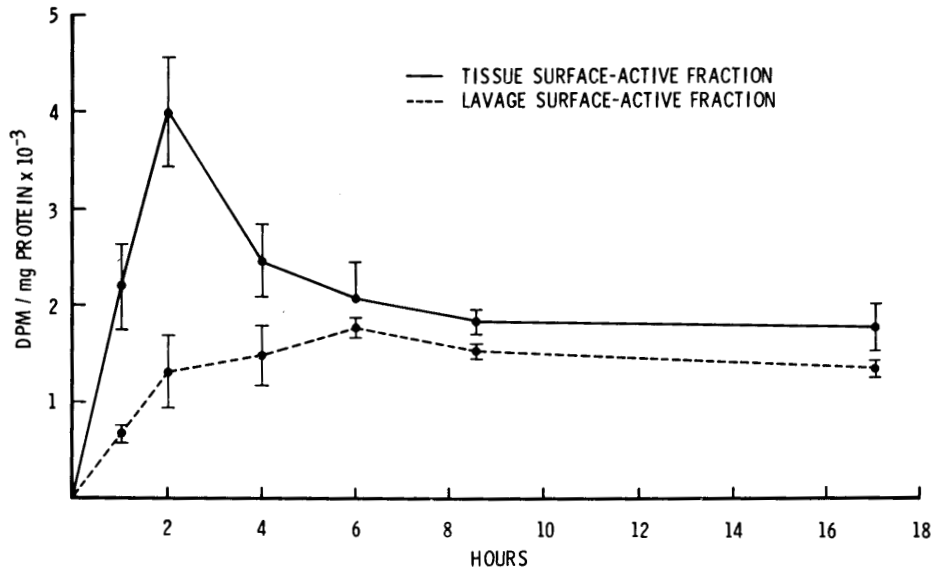

FIG. 2. Time course of the protein specific radioactivity in the surface-active fraction of lavage material and lung homogenate after the intravenous administration of radioactive and nonradioactive leucine as described in Fig. 1.

identical.

Young and Tierney (12) found that between 24 and 48 hr after the administration of radioactive palmitate the specific radioactivity of dipalmitoyl lecithin was again higher in the tissue than in the lavage returns. We observed a similar decrease in the protein specific radioactivity of lung lavage material occurring about 17 hr after injection of radioactive leucine. The reason for this decrease is not clear. It could represent breakdown of macromolecules on the surface with subsequent resorption and reutilization of these radioactive moieties in biosynthetic processes. Since cell surfaces in general contain degradative enzymes, this could represent normal utilization of precursor molecules by the lung. Indeed, during times of hypoperfusion material on the alveolar surface might be a source of substrate for alveolar lining cells to be used for various metabolic processes.

The time course of protein specific radioactivity in the surface-active fractions from lung tissue and lung lavage returns differ from those obtained from the crude extracts and from the studies on lipids cited; the protein specific activity in the surface-active lung lavage fraction does not attain values higher than that of the tissue fraction. However, this is very similar to the kinetics of the distribution of radioactive palmitate into surface-active material isolated from lung tissue and from lung lavage returns (10–12). In both cases, *i.e.*, crude lung tissue or lung lavage returns, or a more purified surface-active fraction, the kinetics of the distribution of radioactive phospholipids and proteins between lung tissue and lung lavage material (presumably alveolar material) are remarkably similar.

Summary. After the intravenous injection of radioactive leucine the time course of the protein specific activity of lung tissue protein and protein obtained by lung lavage are consistent with a precursor–successor relationship between these compartments.

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