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Comparative Incorporation of P^{32} into Lung Phosphatidyl Choline in Mammals with Different Metabolic and Pulmonary Morphologic Characteristics (38010)

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(Introduced by F. S. Vogel)

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Phosphatidyl choline (PC) is the major phospholipid found in lung washings or in normal, mammalian lung tissue extract (1). Previous studies indicate that the quantity of this surface active material increases during the development of the lung (2). The incorporation of P^{32} into PC by lung slices also increases during the developmental period and has been related to the biosynthesis of PC in the lung (3). In the present study, lung slices from mammals having different oxygen consumptions, lung surface areas, and alveolar diameters were incubated with P^{32} orthophosphate; determinations of percentage PC in the phospholipid fraction, concentration of PC, and incorporation of P^{32} into PC were performed to compare these parameters in various mammalian species.

Materials and Methods. Adult mice, rats, rabbits, cats, and a sloth, as well as newborn (3–5 days) mice, rats, rabbits, and cats were sacrificed by decapitation. Their hearts and lungs were carefully dissected from the chest and were rapidly chilled to 3–5°on 0.85% saline-soaked gauze. Duplicate (quadruple for the sloth) lung tissue samples of approximately 0.5 g were quickly obtained from the periphery (entire lungs were used in newborn mice and newborn rats) and weighed to the nearest 0.1 mg. On a Teflon block, the specimens were sliced finely (always by the same person) with a new razor blade which had been rinsed with chloroform:methanol (2:1). The sliced tissue was then placed in a 50 ml Erlenmeyer incubation flask containing 30 μCi carrier-free P^{32} orthophosphate (New England Nuclear Corp.) in H_2O and 4 ml modified Ringer's solution buffered to pH 7.38 at 38° with 0.01 *M* Tham (trishydroxyaminomethane). With a gentle flow of hydrated oxygen (100%), the tissue was incubated at 38° for 3 hr in an agitating water bath. The variables of temperature, time, substrate concentration, and oxygen concentration had been determined previously in this laboratory to be optimal for lung slice incorporation studies. In the species represented, the uptake of P^{32} orthophosphate into PC was linear for the 3 hr incubation period.

The reaction was terminated by the addition of 10 ml methanol, and the contents of the flask were homogenized at room temperature in a Teflon tissue grinder; 20 ml chloroform was added and the resultant chloroform:methanol (2:1) solvent was used to extract the lipids according to the method of Folch *et al.* (4). The tissue suspension was filtered through Whatman No. 1 filter paper into a volumetric cylinder, and the trapped tissue was washed with an additional 35 ml chloroform:methanol (2:1). Further extraction of selected samples of lung tissue for periods up to 24 hr revealed an initial extraction of at least 95% by this method. The chloroform:methanol extract was washed three times with 0.75% potassium chloride (0.2 ml wash/1.0 ml extract). The upper, methanol:water phase was discarded; and the lower, chloroform phase

was expanded to clearness with chloroform: methanol (2:1). The fluff formed at the interphase of this system readily dissolved upon addition of chloroform:methanol. Tissue samples and lipid extracts were stored at −10° until analyzed, and determinations were completed within 1 week.

Duplicate 0.5-ml samples of the washed chloroform:methanol extract were taken to dryness under nitrogen and analyzed for inorganic phosphorus content according to the colorimetric method of Bartlett (5). Duplicate analyses of lipid extracts differed by less than 10% and were averaged and compared to standards of known concentration of inorganic phosphorus. The lipid phosphorus content per gram fresh weight of lung tissue was multiplied by 25 to estimate the phospholipid content (6).

The component phospholipids were separated for phosphorus analysis and scintillation counting by thin-layer chromatography. Glass plates were prepared according to the method of Skipski *et al.* (7) with silica H, made slightly basic with 0.001 *M* sodium acetate. Duplicate 1.0 ml samples of chloroform:methanol extracts were partially dried under nitrogen and spotted on 3 cm lanes. Commercially available phospholipid standards were similarly applied, and the thin-layer plates were developed at room temperature in a solvent system of chloroform:methanol:glacial acetic acid:water (100:50:16:8). Seven areas were identified using Rhodamine 6G: the origin, lysophosphatidyl choline, sphingomyelin, phosphatidyl choline, phosphatidyl serine plus phosphatidyl inositol, phosphatidyl ethanolamine, and the solvent front. No attempt was made to identify other compounds.

Each zone was marked and scraped into a separate counting vial containing 10 ml scintillation fluid (15.6 g PPO, 17.1 g POPOP/3.79 liters toluene). Duplicate 0.5 ml samples of the chloroform:methanol extract were placed into vials, dried under nitrogen, and similarly prepared for scintillation counting. All samples were counted 2 min for P^{32} content with a Mark II Nuclear Chicago Scintillation Counter. Quench correction factors were derived by the external standard method, and the P^{32} counts were corrected for decay. Counts so derived were subsequently modified as to extract volume, tissue weight, and DNA content.

To determine the percent composition of the constituent phospholipids, duplicate 3.0 ml samples of the chloroform:methanol extract were separated by thin-layer chromatography as described above. After identification, each zone was scraped into a separate test tube for phosphorus determination as described by Parker and Peterson (8). The percent composition of each of the seven zones was calculated by dividing averaged duplicate optical densities for each zone by the averaged duplicate total optical density. Thin-layer chromatographic separations recovered approximately 90% of the phospholipid phosphorus applied to the plate.

Duplicate tissue samples contiguous to those for incubation were taken for DNA determinations by a modification of the colorimetric method of Schneider (9) and were compared to standards of known concentrations of DNA. DNA is an index of cell number using the constancy of 6.2 pg DNA per nucleus in lung (10). Some DNA and phospholipid analyses were performed on lungs not reacted with radioactive substrates.

Results. The results of these studies are presented in Table I. Adult lung DNA concentrations per gram fresh weight were greatest in the mouse and least in the sloth, but these values differed by a factor of only 1.5. The mean DNA content per gram fresh weight of lung of each of the newborn mammals was greater than that of the respective adult.

The quantity of PC/mg DNA in the adults was highest in the rabbit and was 1.8 times that of the sloth, which was lowest. All newborn values were equal to or less than their respective adults.

The incorporation of P^{32} into lung PC was 25 times greater in the mouse than in the sloth when expressed as per gram fresh weight of lung tissues; it was 15 times greater when expressed per mg DNA; and it was 11 times greater when expressed as specific activity. P^{32} incorporation in each

TABLE I. Lung Phosphatidyl Choline in Various Mammals.

Animal	mg DNA/g fresh wt	%PC/PL[a]	mg PC/mg DNA	(cpm P^{32} in PC/ g fresh wt) $\times 10^5$	(cpm P^{32} in PC/ mg DNA) $\times 10^5$	Specific activity $\times 10^5$
Adults						
Mouse	6.72 ± 0.54[b] (6, 9)[c]	48.0 ± 1.7 (6, 6)	1.63 ± 0.22 (6, 6)	103.9 ± 36.6 (5, 5)	15.0 ± 4.6 (5, 5)	9.12 ± 2.03 (5, 5)
Rat	5.98 ± 0.54 (6, 12)	43.4 ± 2.7 (6, 9)	1.61 ± 0.15 (6, 9)	74.4 ± 26.5 (3, 6)	11.7 ± 4.8 (3, 6)	7.10 ± 2.35 (3, 6)
Rabbit	5.60 ± 0.56 (6, 12)	48.7 ± 2.0 (6, 9)	2.19 ± 0.10 (6, 9)	44.9 ± 7.7 (3, 6)	6.84 ± 1.42 (3, 6)	2.98 ± 0.53 (3, 6)
Cat	5.16 ± 0.30 (3, 6)	39.7 ± 0.6 (3, 6)	1.38 ± 0.14 (3, 6)	27.1 ± 9.7 (2, 4)	4.94 ± 1.66 (2, 4)	4.10 ± 1.76 (2, 4)
Sloth	4.17 ± 0.24 (1, 4)	45.3 ± 1.9 (1, 4)	1.17 ± 0.07 (1, 4)	4.14 ± 0.12 (1, 4)	0.991 ± 0.028 (1, 4)	0.852 ± 0.034 (1, 4)
Newborns (3–5 days)						
Mouse	8.97 ± 0.41 (6, 9)	52.4 ± 1.8 (6, 6)	1.14 ± 0.05 (6, 6)	85.7 ± 23.3 (5, 5)	9.15 ± 2.41 (5, 5)	5.30 ± 1.62 (5, 5)
Rat	7.65 ± 0.35 (6, 12)	51.3 ± 0.7 (6, 9)	1.16 ± 0.08 (6, 9)	43.0 ± 15.2 (3, 6)	5.56 ± 1.93 (3, 6)	4.61 ± 1.69 (3, 6)
Rabbit	6.84 ± 0.31 (6, 11)	52.8 ± 1.5 (6, 8)	1.78 ± 0.18 (6, 8)	40.6 ± 5.4 (3, 5)	5.45 ± 0.65 (3, 5)	3.96 ± 1.06 (3, 5)
Cat	7.98 ± 1.10 (3, 6)	50.2 ± 0.9 (3, 6)	1.37 ± 0.17 (3, 6)	36.9 ± 16.8 (2, 4)	4.51 ± 2.50 (2, 4)	3.60 ± 1.80 (2, 4)

[a] PC = phosphatidyl choline; PL = phospholipid.

[b] Mean ± SE. In computing the mean ± SE, separate analyses from the same animal or litter were averaged and were considered as one sample except in the case of the sloth, where the mean ± SE represents four different tissue samples from the same animal.

[c] The first number in parentheses represents the number of adults or litters studied, and the second is the total number of different tissue samples analyzed.

newborn was equal to or less than that of the respective adult.

Discussion. In general, small mammals have a greater oxygen consumption per unit body weight than do large ones (11). In mammals with high oxygen consumption per unit body weight, the increased alveolar surface area required for adequate gaseous diffusion is effected by internal pulmonary partitioning, with a concomitant decrease in alveolar diameter and an increase in the number of alveoli per unit lung volume (12). Thus, body weight, metabolic rate, alveolar surface area, alveolar diameter, and lung volume are all interrelated. In addition, Clements *et al.* reported a direct relationship between the amount of surface active material present in different species and the total pulmonary surface area (13).

In this study, the greatest incorporation of P^{32} into lung PC is in mammals with the highest oxygen consumptions per kg, the most rapid respiratory rates, the largest pulmonary surface areas per unit lung volume, and the smallest alveoli. The incorporation of P^{32} into PC (cpm/mg DNA) is 15 times greater in the mouse than in the sloth, while the quantity of PC (mg/mg DNA) is only 1.4 times greater in the mouse than in the sloth (Fig. 1). Thus, the observed increase in isotope incorporation is not merely the result of an increase in PC pool size. Specific activity includes both these parameters (cpm P^{32} in PC/mg PC), and we interpret it to be an index of the rate of synthesis of PC. Viewed in this way, the present data indicate that the mouse synthesizes PC approximately ten times faster than does the sloth. It is of interest that dipalmitoyl PC is the largest single constituent of lung PC (1) and is an important part of the surface active phospholipid which presumably lines the alveoli and tends to stabilize them at low transpulmonary pressures by reducing alveolar surface tension (14).

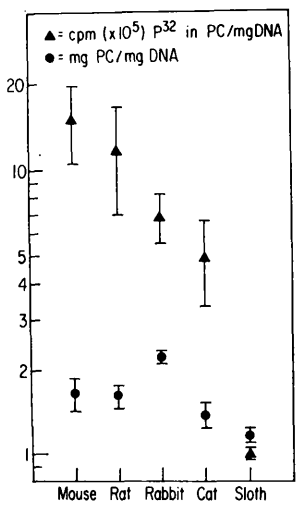

FIG. 1. P^{32} incorporation into lung phosphatidyl choline (PC) and concentration of PC in various adult mammals. Points represent mean ± SE.

The specific activities of P^{32} in lung PC do differ among the mammals tested; and it seems plausible that one or more anatomic, metabolic, or functional characteristics might influence the turnover of PC. Although the specific activity data from the present study correlates well (log–log plot) with such parameters (12) as alveolar surface area per unit lung volume ($r = 0.90$), alveolar diameter ($r = 0.94$), and oxygen consumption/kg/hr ($r = 0.97$), a specific relationship between structure, function, and the control of lung PC synthesis is unclear.

Summary. In the present study the incorporation of P^{32} into lung PC (cpm/mg DNA) was lowest in the sloth, was progressively higher in the cat, rabbit, and rat, and was highest in the mouse. It was 25 times greater in the adult mouse than in the sloth when expressed per gram fresh weight of lung tissue; it was 15 times greater when expressed as per mg DNA; and it was 11 times greater when expressed as specific activity. Lung PC specific activity from this study correlates well with alveolar surface area per unit lung volume, alveolar diameter, and oxygen consumption.

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