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Stereologic Evaluation of Granular Pneumocyte Lamellar Bodies in Different Species (38801)

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Physiological studies indicate that respiratory frequency may be an important determinant of the rate at which surface-active material is lost from alveolar surfaces (1, 2). Animals with small alveoli and rapid respiratory rates might, therefore, be expected to have different rates of synthesis and secretion of components of surface active material. To this end Goldner and Brumley have recently shown that the incorporation of radioactive phosphorus into phospholipids by lung tissue is inversely proportional to the species alveolar diameter and hence directly proportional to the species respiratory rate (3). The secretion of radioactive protein into a surface-active fraction isolated from lung lavage returns also shows an interspecies variation proportional to respiratory rate and inversely proportional to alveolar diameter (Massaro, D., and Massaro, G. D., unpublished observations).

These considerations led to an examination of interspecies differences in lung oxygen consumption ($\dot{Q}O_2$) since the biosynthetic and secretory processes involved are energy dependent. We found that interspecies lung $\dot{Q}O_2$ varied directly with the species respiratory rate and inversely with the species alveolar diameter (4). Furthermore, the mitochondrial volume density of the granular pneumocyte, currently thought to be the cell responsible for the synthesis and secretion of surface-active material, was related in a direct linear fashion with the species lung $\dot{Q}O_2$.

These considerations led to the present study in which we wished to examine the lamellar bodies of pulmonary granular pneumocytes because of the current well-founded belief that they represent the intracellular storage site (secretory granules) of pulmonary surfactant (for review see Ref. 5). We specifically sought to measure the volume density and surface to volume ratio of granular pneumocyte lamellar bodies in the mouse, rat, rabbit, and dog.

Methods. Animals. We used the following animals: (1) male black mice, strain C57BL/6J (The Jackson Laboratory, Bar Harbor, ME), (2) male Long–Evans descent hooded rats (Blue Spruce Farms, Inc., Altamont, NY), (3) male New Zealand rabbits (Ginrich Animal Supply, Fredericksburg, PA), and (4) mongrel dogs of either sex (Leach Kennels, Chase City, Va). The animal weights (mean ± SD) were: mice 0.024 ± 0.003 kg, rats 0.166 ± 0.046 kg, rabbits 2.67 ± 0.4 kg, dogs 14.6 ± 1.29 kg. The mice and rats were fed Wayne Lab-Blox (Allied Mills, Inc., Chicago, IL). The rabbits were fed Wayne Rabbit ration (Allied Mills, Inc.) and the dogs were fed Wayne Dog Food (Allied Mills, Inc.).

All animals were allowed food and water ad lib. until the time of sacrifice. The animals were sacrificed by cutting the abdominal aorta after the intraperitoneal (mice and rats) or intravenous (rabbits and dogs) injection of sodium pentobarbital (30 mg/kg).

Preparation of tissue for electron microscopy. A portion of the right lower lobe from each animal was removed, diced into tissue cubes, and prepared for electron microscopic examination as previously described (6).

Sampling procedures. The primary sample consisted of some 40 tissue cubes from each animal (7). From these 40 tissue blocks we randomly selected the following number of blocks from each animal in the species studied for electron microscopic examination: mice, five blocks; rats five blocks; rabbits six blocks; and dogs nine blocks. From each block we took the following number of electron microscopic photographs of granular pneumocytes (8): mice four, rats four, rabbits five, dogs four. The photographs were analyzed at a magnification of 18,500 ×.

Stereological procedures. Stereological

TABLE I. VOLUME DENSITY AND SURFACE-TO-VOLUME RATIO OF GRANULAR PNEUMOCYTE LAMELLAR BODIES[a]

Species	No of animals	Volume density (%)	Surface-to-volume ratio ($\mu m^2/\mu m^3$)
Mouse	5	22.7 ± 1.6	4.36 ± 0.17
Rat	9	18.6 ± 0.6	4.26 ± 0.16
Rabbit	7	24.4 ± 2.3	4.23 ± 0.26
Dog	4	22.7 ± 1.5	4.14 ± 0.08

[a] The values represent the mean ± one standard error of the mean.

analysis was performed using the methods of Weibel (7, 9). A multipurpose test system (7) 17.6 × 17.6 cm having 168 test points 1.5 cm apart was used to estimate the volume density and surface-to-volume ratio of lamellar bodies. We counted a sufficient number of points in each animal to attain a relative error in each instance of less than 5% (9).

Results. Table I reveals that the volume density of granular pneumocyte lamellar bodies exhibits little interspecies variation. Although the difference between the rat and rabbit is statistically significant ($P < 0.025$), there is a poor correlation ($r = -0.23$) between these values and the species respiratory rate as culled from the literature (10). The surface-to-volume ratio is the same for the species studied.

In a point-counting system, lamellar body volume density is determined by counting the number of points falling over these organelles and dividing this figure by the number of points falling on the cell's cytoplasm and its other organelles including lamellar bodies but exclusive of the nucleus. It is thus possible that interspecies differences in cytoplasmic volume in relation to the point counting system might account for the lack of difference between species in the volume density of lamellar bodies. We, therefore, examined the cytoplasmic volume relative to the point counting system and found it was not responsible for the lack of species differences in lamellar body volume density.

Discussion. The present study has shown that there is virtually no difference in the volume density of lamellar bodies in individual granular pneumocytes. In addition, the ratio of lamellar body envelope to lamellar body (surface-to-volume ratio) indicates that the lamellar bodies are of equal size in the species studied. Since we have shown that granular pneumocyte cytoplasmic volume relative to our stereologic test system shows little interspecies variation, it appears that each granular pneumocyte has the same volumetric content of lamellar bodies in the species studied. Because we have not measured the total volume of granular pneumocyte cytoplasm relative to lung tissue we cannot assess the total volume of lamellar bodies in the lung.

If we assume that lamellar bodies contain only material destined to be secreted onto the the alveolar surface, our study indicates few interspecies differences per granular pneumocyte of this material and certainly no correlation per granular pneumocyte with the species respiratory rate. This suggests two possible means by which species with rapid respiratory rates might provide for more rapid rates of secretion of surface-active material than species with slow respiratory rates. First, species with high respiratory frequency might have more granular pneumocytes per unit of alveolar surface than species with lower respiratory rates. That this may be the case is suggested by the relationship between the phosphytidyl choline content of lungs of different species and their alveolar surface area (11). However, that the granular pneumocytes may show interspecies variation in their rates of synthesis and secretion of surface-active material is suggested by the direct linear relation between the species respiratory rate and the granular pneumocyte mitochondrial volume density and the lung's oxygen consumption (4), oxidative metabolism being required for synthetic and secretory activity. We, therefore, postulate that the demands for increased synthesis and secretion of surface-active material, imposed on species with rapid respiratory rates, is met by an increased number of lamellar bodies per alveolar surface area (more granular pneumocytes) and an increased rate of synthesis and secretion by individual granular pneumocytes in the species with more rapid respiratory rates.

Summary. Lamellar bodies in individual pulmonary granular pneumocytes in the

species examined (mouse, rat, rabbit, and dog) have virtually the same volume density with respect to the cytoplasmic volume as estimated by stereological techniques. The surface-to-volume ratio for these structures also fail to show any interspecies variation in these species.

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