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Pulmonary Extravascular Chloride Space and Albumin Content in Adult Dogs and Puppies

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Extract

The extravascular chloride space and albumin content of the lungs were determined in 6 adult dogs and 10 puppies. ^{125}I-labeled human serum albumin was administered and its concentration in lung tissue was determined after equilibration with the total body albumin pool. The blood content of the lung was determined by ^{51}Cr erythrocyte tagging, and the extravascular fluid volume by ^{36}Cl dilution. Total water in the lung was measured by drying the lungs to constant weight. The interstitial albumin concentration, assuming uniform distribution in the chloride space, averaged 1.0 g/100 ml interstitial fluid in the adult dogs and 0.62 in the puppies; the difference between these means is statistically significant. These interstitial albumin concentrations are considerably lower than lymph protein concentrations in lung reported previously.

Speculation

These data support the notion that a large net absorptive gradient of colloid osmotic pressure is present across the fluid-exchanging vessels of the lung. In view of the relatively low level of pulmonary capillary hydrostatic pressure, the application of Starling's law of capillary exchange to these data indicates that interstitial hydrostatic pressure is substantially subatmospheric [12]. Although consistent with recent measurements of interstitial pressure using perforated capsules [9], this line of argument must be considered speculative because of uncertainty as to the distribution of protein in the interstitial fluid.

Information is lacking as to the mechanism whereby the protein concentration of lymph is increased relative to that of capillary filtrate or the interstitial fluid. This could occur if the hydrostatic pressure within the lymph channels were significantly higher than the surrounding interstitial pressure. The maintenance of a low protein concentration in the interstitial fluid, by virtue of limited protein filtration and efficient lymphatic removal, would constitute an important barrier against pulmonary edema formation. The present study suggests that this barrier may be more effectively maintained in the immature than in the mature animal, thus counterbalancing the increased tendency for fluid filtration in the immature lung reported previously [13].

Introduction

Divergent views have been expressed as to the balance of forces governing fluid movement across the fluid-exchanging vessels of the lung and other tissues, based on conflicting estimates of interstitial hydrostatic and colloid osmotic pressures [9, 16]. Because interstitial fluid is normally unavailable for direct sampling, its protein composition has been estimated from analysis of lymph, or of fluid obtained from capsules implanted previously [9, 16]. However, in the systemic circulation, the protein content of lymph exceeds that of the capillary filtrate, which implies that the lymphatic apparatus is designed primarily for protein removal [11].

The purpose of the present study was to determine the volume and albumin content of pulmonary interstitial fluid in order to compare these data with the protein concentration of lung lymph. Measurements of lung lymph flow and protein content (4, 17) reported previously suggest that the pulmonary capillaries are more permeable in immature than in mature animals. In order to determine whether comparable differences exist in pulmonary interstitial fluid volume and albumin content, the present experiments were carried out in adult dogs and in puppies.

Methods

Seven to 10 days before the experiment, 5 μCi/kg body wt ^{125}I-labeled human serum albumin was administered to two groups of animals: (*I*) 6 adult mongrel dogs, average weight 12.6 kg (range 9.6–15.6), injected intravenously; (*II*) 10 mongrel puppies, 1–3 weeks old, average weight 0.85 kg (range 0.6–1.1), injected intraperitoneally.

On the day of the experiment the dogs and puppies were anesthetized with pentobarbital, 30 mg/kg body wt and injected intravenously with 0.5 μCi/kg Na^{36}Cl- and 30 μCi/kg ^{51}Cr-labeled erythrocytes. The erythrocytes were tagged by techniques described previously [7]. After 1.5–2 hr of equilibration, a blood sample was drawn for isotopic analysis and the lungs were removed. Two 1-g portions of each lung were ground in 5 ml distilled water with a glass tissue grinder and the resulting homogenates were analyzed for ^{125}I, ^{51}Cr, and ^{36}Cl. The remainder of the lung was weighed and dried to constant weight as described previously [12] to determine total lung water.

One milliliter whole blood and 1 ml lung homogenate were counted in an automatic, 2-channel, well-type crystal scintillation counter for ^{125}I and ^{51}Cr. *Channel 1* was set to count ^{51}Cr alone, the counts in *channel 2* (^{125}I) were corrected for ^{51}Cr counts by determining the *channel 2* to *channel 1* ratio from a ^{51}Cr standard. Plasma and lung homogenate were analyzed for ^{36}Cl in a liquid scintillation counter by techniques already reported from this laboratory [7]. The plasma albumin fraction was determined by protein electrophoresis and the total plasma protein concentration by means of a Hitachi hand refractometer.

Calculations

The ^{125}I content of whole blood retained in the lung was subtracted from the total ^{125}I content of the lung and divided by the specific activity of plasma albumin to yield albumin content in lung interstitial fluid. This was then divided by the interstitial fluid volume, here defined as the extravascular chloride space, to give the albumin concentration of the lung. The equations used were:

$$(\text{Alb})_{\text{ISF}} = \frac{(\text{RI Alb})_{\text{L}} - [\text{RI Alb}]_{\text{B}}(\text{Cr})_{\text{L}}/[\text{Cr}]_{\text{B}}}{\text{Sp act}}$$

$$V_{\text{ISF}} = \frac{(\text{Cl})_{\text{L}} - [\text{Cl}]_{\text{B}}(\text{Cr})_{\text{L}}/[\text{Cr}]_{\text{B}}}{[\text{Cl}]_{\text{P}}}$$

$$[\text{Alb}]_{\text{ISF}} = (\text{Alb})_{\text{ISF}}/V_{\text{ISF}}$$

where $(\text{Alb})_{\text{ISF}}$ is lung interstitial fluid albumin content in grams of albumin per 100 g lung; $(\text{RI Alb})_{\text{L}}$ is lung radioiodinated albumin content in counts per minute per 100 g lung; $[\text{RI Alb}]_{\text{B}}$ is blood concentration of radioiodinated albumin in counts per min per milliliter of whole blood; $(\text{Cr})_{\text{L}}$ is lung ^{51}Cr content in counts per minute per 100 g lung; $[\text{Cr}]_{\text{B}}$ is blood concentration of ^{51}Cr in counts per minute per milliliter of whole blood; sp act is plasma albumin specific activity in counts per minute per gram of albumin; V_{ISF} is lung interstitial fluid volume in milliliters per 100 g lung; $(\text{Cl})_{\text{L}}$ is lung content of ^{36}Cl in counts per minute per 100 g lung; $[\text{Cl}]_{\text{B}}$ is blood concentration of ^{36}Cl in counts per minute per milliliter of blood; $[\text{Cl}]_{\text{P}}$ is plasma concentration of ^{36}Cl in counts per minute per milliliter of water in plasma; and $[\text{Alb}]_{\text{ISF}}$ is lung interstitial fluid albumin concentration in grams of albumin per 100 ml ISF.

The concentration of ^{36}Cl in the blood was calculated from the plasma concentration, packed cell volume, and appropriate Gibbs-Donnan factor for the erythrocyte [8].

Table I. Volume and albumin concentration of lung interstitial fluid (ISF)[1]

	V_{ISF}, ml/100 g lung	$(Alb)_{ISF}$, g/100 g lung	$[Alb]_{ISF}$, g/100 g ISF	$[Alb]_{ISF}$, % of plasma
Adult dogs, n = 6				
Mean	33.4	0.33	1.0	40
SD	10.2	0.11	0.23	
Puppies, n = 10				
Mean	43.9	0.26	0.62	32
SD	9.3	0.11	0.25	

[1] V_{ISF}: lung interstitial fluid volume = extravascular chloride space; $(Alb)_{ISF}$: lung interstitial fluid albumin content; $[Alb]_{ISF}$ concentration of albumin in the extravascular chloride space.

Table II. Pulmonary interstitial fluid volume: values from the literature

Species	Indicator	Volume, ml/100 g lung[1]	Reference
Dog lobe	^{24}Na	25.3 (12.5)	[19]
Dog lobe	^{24}Na	28 (6)	[18]
Rabbit	(^{131}I)albumin	26.4 (1.0)	[15]
Rabbit	(^{14}C)sucrose	31.6	[1]
Adult sheep	(^{14}C)sucrose	28.8	[20]
Rabbit	(^{14}C)sucrose	23.8	[21]

[1] Mean (SD).

Results and Discussion

The results are shown in Table I. Using the t test, the interstitial fluid volume of the puppy lung was significantly larger than that of the adult dog lung ($P < 0.05$). The interstitial albumin content of the puppy lung was not significantly different from that of the adult dog ($P > 0.05$). The pulmonary interstitial albumin concentration, assuming uniform distribution in the chloride space, was significantly lower in the puppy than in the adult dog ($P < 0.01$).

Evaluation of Method

Because the animals were injected with (^{125}I)albumin at least 1 week before the experiment, it is likely that the total body albumin pool had a uniform specific activity [2], and that the intraperitoneal route of administration was equivalent to the intravenous.

The ^{36}Cl space may be somewhat larger than the albumin space, as Cl penetrates dense interstitial tissue more readily than albumin.

The value for pulmonary extravascular chloride space in the present study, 33.4 ml/100 g lung, is somewhat higher than that reported in the literature. Representative values range from 23.8 to 31.6 ml/100 g lung (Table II). This variation may be related to species differences, the use of isolated lungs *versus* intact animals, or to the different indicators employed for the measurement of extracellular fluid volume. If differences in distribution volumes existed on the basis of molecular size of the indicators, these volumes would vary inversely and systematically with molecular size. Such a relation is not evident in the published data (Table II). Therefore, it is unlikely that the extravascular chloride space measured in the present study contains a compartment from which albumin was excluded on the basis of its larger molecular size. Rather, the published data indicate that the concept of an excluded volume, whether extra- or intracellular in location, is inappropriate for lung tissue [15].

The use of the ^{51}Cr erythrocyte label provides a correction for blood retained in lung tissue. An error may be incurred in assuming that the packed cell volume of peripheral blood is applicable to residual blood within the lung. This variable has been disregarded on the basis that venous and total body hematocrit differ by less than 10% [5].

Interstitial Fluid Volume of Lung

The value for extravascular chloride space of the adult dogs in the present experiments is somewhat higher than those reported in the literature (Table II). The interstitial fluid volume of the lung is larger in the puppies than in the adult dogs. This suggests that the lung participates in the relative loss of total extracellular fluid volume that accompanies normal maturation [6].

Interstitial Albumin Concentration

The distribution of albumin in the extravascular fluid compartment of the lung is unknown. Based on the assumption of uniform distribution, the present studies suggest that interstital fluid albumin concentration is significantly lower in immature than mature animals. The ISF albumin concentration, expressed as a percentage of the plasma value, also decreases with increasing immaturity. These results may be interpreted in two ways: (*1*) the exchanging vessels of the lung are less permeable to albumin in the immature animal, or (*2*) the exchanging vessels of the immature lung are as permeable as, or more permeable to albumin than those of the mature lung, but lymphatic drainage suffices to maintain a lower interstitial albumin concentration.

Two lines of evidence support the latter hypothesis,

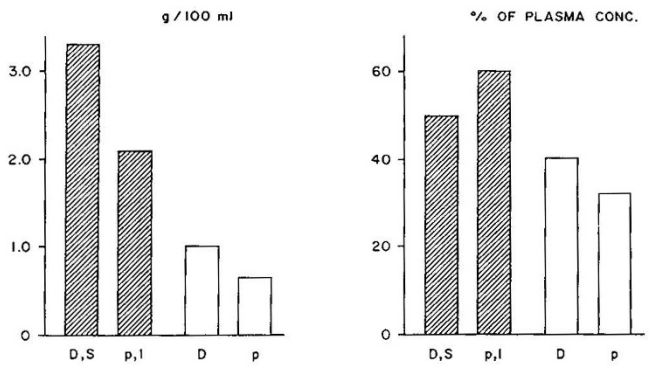

Fig. 1. Comparison of published values for total protein concentration of lung lymph (*cross-hatched bars*) in adult dogs and sheep (*D,S*) and in puppies and fetal lambs (*p,l*) with interstitial albumin concentration (*open bars*) in adult dogs and puppies (present study).

namely that exchanging vessels of the lung are more permeable in the immature animal. (*1*) Studies in this laboratory have demonstrated that the filtration coefficient for water in the puppy is 6–17 times that for the adult dog [13]. (*2*) Both the rate of lymph flow and the rate of protein removal by lung lymph are greater relative to body weight in immature than in mature animals [3, 4, 10, 17].

Published data indicate that the total protein concentration of lung lymph averages 3.3 g/100 ml in adult dogs and sheep, and 2 g/100 ml in puppies and fetal lambs [3, 4, 10, 17]. These values are approximately 3 times our values for interstitial albumin concentration (Fig. 1).

Because the interstitial fluid of the lung is inaccessible to direct sampling, the colloid osmotic pressure of interstital fluid (π_{if}) may be calculated from the empiric equations of Landis and Pappenheimer [11]. Utilizing our interstitial albumin concentrations, and assuming an interstitial A/G ratio of 1.5 [11], π_{if} is 4 mm Hg in adult dogs and 2.3 mm Hg in puppies. As plasma colloid osmotic pressure (π_{pl}) in adult mammals is 21–25 mm Hg [11] and that in fetal lambs at term 15–20 mm Hg [14], the relatively low values for π_{if} could provide a substantial net absorptive gradient of colloid osmotic pressure across the fluid-exchanging vessels of the lung. In view of the uncertainty as to the distribution of albumin in the extravascular chloride space, these calculations of π_{if} must be considered tentative. Maintenance of a net absorptive gradient of colloid osmotic pressure may contribute importantly to the protective barrier against pulmonary edema formation [9].

Summary

The extravascular chloride space and albumin content of the lungs were determined in 6 adult dogs and 10 puppies. The albumin concentration in the interstitial fluid of the lung, assuming uniform distribution in the chloride space, is considerably lower than that of lung lymph, and is significantly less in the puppy than in the adult dog.

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