Indirect Estimation of Filtration Variables in Rat Lungs Calculated by Protein Concentration or Osmotic Pressure Method

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TANITA, T., KOIKE, K. and FUJIMURA, S. Indirect Estimation of Filtration Variables in Rat Lungs Calculated by Protein Concentration or Osmotic Pressure Method. Tohoku J. Exp. Med., 1991, 163 (2), 77-83 — We reported a new way for estimating filtration variables: $K$ (filtration coefficient), $P_{pmv}$ (perimicrovascular pressure) and $\sigma$ (protein reflection coefficient), from 3 different measurements of weight gain at two protein concentrations of perfusate ($C_{mv}$) and at two vascular pressures ($P_{vasc}$). We used the Starling equation for calculating those variables using two different formulas which expressed that all protein moves by convection. In this report, we compared those two formulas: 1) $1 - \sigma = \frac{H_{pmv}}{H_{mv}}$, which is already reported (Tanita et al. 1990), and 2) $1 - \sigma = \frac{C_{pmv}}{C_{mv}}$. We measured filtration rate ($Q$) by a gravimetric method in isolated rat lung lobes in zone 1 conditions (alveolar pressure = 20 cmH$_2$O) at two vascular pressures, $P_{vasc}$ = 15 or 8 cmH$_2$O and perfused the lobes with plasma containing a low or a high concentration of protein. By extrapolating the log of the rate of weight gain to $t = 0$, we obtain the initial filtration rate. $C_{mv}$ was measured and $H_{mv}$ was estimated by Yamada's equation (Yamada et al. 1985). The reflection coefficient calculated in $1 - \sigma = \frac{H_{pmv}}{H_{mv}}$ method is computed 10% higher than that in $1 - \sigma = \frac{C_{pmv}}{C_{mv}}$ method. However, the filtration coefficient or the perimicrovascular pressure calculated in either method was identical. We conclude that filtration variables can be estimated easily by $\Pi$ method and those variables are comparable to those estimated by $C$ method which are theoretically more accurate but more complicated in calculation. —— filtration coefficient; perimicrovascular pressure; reflection coefficient; isolated rat lungs; the Starling equation.

The filtration variables, filtration coefficient ($K$), perimicrovascular pressure ($P_{pmv}$), and osmotic reflection coefficient for total proteins ($\sigma$), were estimated independently in previous reports using the Starling equation or the micropuncture method, mainly in isolated dog lungs (Goldberg 1980; Bhattacharya et al. 1984; Townsley et al. 1986), or in isolated rat lungs (Fox et al. 1986). The indirect simultaneous estimation of filtration variables in dog lungs was undertaken recently, and was improved in the methods and theories (Tanita 1987, 1988, 1990; revision accepted for publication January 9, 1991.)
1990). For the assumption in which all protein moves by convection, they introduced a formula that $1 - \sigma = \Pi \text{pmv}/\Pi \text{mv}$. Using this assumption, filtration variables in rat lungs were calculated simultaneously under the Starling equation at any given situations. However, this formula contains an error especially at the higher portion of protein concentration, since calculation equations for osmotic pressure from protein concentration contain square and cubic terms of protein concentration. In this report, we calculated filtration variables using assumptions 1) $1 - \sigma = \Pi \text{pmv}/\Pi \text{mv}$, which is already reported (Tanita et al. 1990), and 2) $1 - \sigma = \text{Cpmv}/\text{Cmv}$, which is theoretically more accurate.

**Theory**

We write the Starling equation in our standard nomenclature as,

$$\dot{Q} = K[(P\text{mv} - P\text{pmv}) - \sigma(\Pi\text{mv} - \Pi\text{pmv})],$$

where $\dot{Q} =$ net filtration rate, $K =$ filtration coefficient, $P =$ hydrostatic pressure in the microvascular (mv) and perimicrovascular (pmv) components, respectively, $\sigma =$ the osmotic reflection coefficient for total protein, and $\Pi =$ protein osmotic pressure. The equation is equally valid under zone 1 or zone 3 conditions.

The only dependent variable directly measured during lung filtration experiments is the rate of weight gain. The standard interpretation of the weight gain curve is that there is an initial vascular volume increment plus a continuous filtration (Lunde and Waaler 1969). The former can be eliminated by making a semilog plot of weight gain over time and determining the slow component (Drake 1978; Tanita 1987).

The independent variables in lung filtration experiments are microvascular hydrostatic and osmotic pressures ($P\text{mv}$ and $\Pi\text{mv}$). That leaves four unknowns requiring four independent pieces of information. Then we assumed that all protein moves by convection. Thus,

$$1 - \sigma = \text{Cpmv}/\text{Cmv}$$

where Cpmv and Cmv are total protein concentration in either perimicrovascular (pmv) or microvascular (mv) compartment, respectively. In our previous report, we used:

$$1 - \sigma = \Pi \text{pmv}/\Pi \text{mv}$$

Although we assumed that relations of total protein concentration and osmotic pressure were linear, they do not obey Van't Hoff's law. The relations are nonlinear and of the form

$$\Pi = a(2.98C^2 + 0.23C + 0.005C^3) + (1 - a)(1.06C + 0.083C^2)$$

where $\Pi$ is osmotic pressure in mmHg and at 39°C, $a$ is albumin fraction, and $C$ is protein concentration (Yamada et al. 1985) (Fig. 1). Now we can start to compare two formulas.

1) $1 - \sigma = \Pi \text{mv}/\Pi \text{pmv}$ (Π method)

Using this formula, the Starling equation becomes:

$$\dot{Q} = K[(P\text{mv} - P\text{pmv}) - \sigma^2(\Pi\text{mv})].$$

This is an equation with three unknowns ($K$, $P\text{pmv}$, $\sigma$) and can readily be solved by matrix algebra, if three pieces of independent data are obtained (Tanita et al. 1987). When we apply microvascular pressures $P\text{mv}_1$, and $P\text{mv}_2$, the observed net filtration rates will be $Q_1$ and $Q_2$, while $\Pi \text{mv}$ does not change. Then we apply microvascular pressure $P\text{mv}_1$, while the osmotic pressure of perfusate is changed ($\Pi \text{mv}'$), the net filtration rate will be $Q_3$. The filtration variables are:
Indirect Estimation of Filtration Variables

\[ \text{K} = \frac{Q_1 - Q_2}{P_{mv1} - P_{mv2}} \]
\[ \sigma^2 = \frac{Q_1 - Q_3}{K (P_{mv} - P_{mv})} \]
\[ P_{pmv} = P_{mv1} - \sigma^2 P_{mv} - \frac{Q_1}{K} \]

(6) (7) (8)

2) \( I - \sigma = C_{mv}/C_{pmv} \) (C method)

When we write osmotic pressure in either microvascular or perimicrovascular compartment, using (4) and (2):

\[ \Pi_{mv} = a(2.98 C_{mv} + 0.23 C_{mv}^2 + 0.005 C_{mv}^3) \]
\[ + (1-a)(1.06 C_{mv} + 0.083 C_{mv}^2) \]

(9)

and:

\[ \Pi_{pmv} = a(2.98 C_{pm} + 0.23 C_{pm}^2 + 0.005 C_{pm}^3) \]
\[ + (1-a)(1.06 C_{pm} + 0.083 C_{pm}^2) \]

(10)

Then what we need to calculate in the Starling equation is,

\[ \Delta \Pi = \Pi_{mv} - \Pi_{pmv} \]
\[ = (1.92a + 1.06) C_{mv} + (0.147a + 0.083) C_{mv}^2 (2 - \sigma) \]
\[ + 0.005a C_{mv}^3 (\sigma^2 - 3\sigma + 3) \]

(11)

For the adjustment of the pressure at 25°C and in cmH\(_2\)O, using Van’t Hoff’s low, the constant A = 1.36 × 298/312 was employed. Then the Starling equation is written as:

\[ \hat{Q} = K [(P_{mv} - P_{pmv}) - \sigma \Delta \Pi] \]

(12)

This is a non-linear cubic equation with three unknowns (K, P_{pmv}, \sigma) and can be solved, if three pieces of independent data are obtained. In this study, we assumed a value of \( \sigma \) first which was between 0 and 1, and calculated K and P_{pmv}, and then recalculated \( \sigma \) .

Fig. 1. Relationship between total protein concentration (abscissa) and osmotic pressure (ordinate), calculated by Yamada’s equation. Three lines are drawn by different albumin fractions (0.3, 0.5 and 0.7).
again. We performed these circulatory calculation until K, Ppmv and o became identical. For these calculation, we used IBM personal computer (model 5550; IBM Japan, Tokyo) and Multiplan (Microsoft Corp., Tokyo).

MATERIALS AND METHODS

Preparation of animals

We anesthetized adult male Sprague-Dawley rats (12 rats, 125.4±32.6 g body weight) with pentobarbital sodium (65 mg/kg ip) and heparinized (500 unit/kg, ia). After exsanguination, we made a sternum-splitting incision, and opened the pericardium. We inserted plastic tubes (PE 200, Clay Adams, Parsippany, NJ, USA) connected with silastic tubes (OD 4.65 mm, ID 3.35 mm, Dow Corning, Midland, MI, USA) into pulmonary artery via right ventricle and left atrium via left auricle. Then we ligated the venae cavae via left auricle. Then we ligated the venae cavae and removed the heart and lungs en bloc.

We wrapped the heart and lungs by waterproof plastic film. To measure the weight gain of the lungs we suspended the heart and lungs in a plexiglass box at room temperature (25±1°C) from a counter-balancing bar which was pivoted opposite the force displacement strain gauge (FTO3C; Grass, Quincy, MA, USA). We connected the trachea to a constant pressure gas source containing 30% O₂, 5% CO₂ and 65% N₂, and the vascular catheters to a reservoir of perfusate. The reservoir could be individually set at various heights to give any desired vascular pressures. To measure the vascular (Pvasc) and alveolar pressures (Palv) we made sidearms by T tube in each catheter which connected to the reservoir or the gas sturce. The sidearms were connected to pressure transducers (P23 ID; Gould-Statham, Hato Rey, Puerto Rico). All pressures were measured relative to the lobe base and pleural surface (atmospheric) pressure. The lobe weight gain and all pressures were recorded continuously on a direct writing recorder (Model 7; Grass, Quincy, MA, USA). Initial lung weight was determined as follows; the initial weight of lungs and heart with catheters minus the weight of heart with catheters from which the lungs were removed after the experiment.

Preparation of perfusate

We used sheep plasma for the perfusate. After heparinized sheep (500 unit/kg) we collected blood and centrifuged blood at 2,300×g, 10 min.

Experimental protocol

After connecting the heart and lungs to the measuring system, we inflated the lungs Palv at 25 cmH₂O so that there were no collapsed area in the lungs, and then decreased Palv at 20 cmH₂O. We raised the reservoir to 15 cmH₂O, so that pulmonary artery pressure=15 cmH₂O, while the catheter inserted into left atrium was opened and the pulmonary venous pressure was kept at 0 cmH₂O. The perfusate in the reservoir was randomly selected either whole or diluted plasma depended upon which plasma we started first. We measured the protein concentration of plasma in the reservoir on an automated analyzer (AAII; Technicon, Tarrytown, NY, USA) in order to calculate the colloid osmotic pressure for perfusate (Pmv). Then the reservoir was set at 0 cmH₂O and Palv=20 cmH₂O, until the lobe weight reached stable. We raised the reservoir to increase the vascular pressure to either 8 or 15 cmH₂O and recorded the weight gain for 10 min under zone 1 conditions (Palv> Pvasc). After each measurement of the weight gain we changed alveolar, pulmonary artery and venous pressures 10, 15 and 0 cmH₂O, respectively, in order to washout the perfusate.

Data expression

The data on lung weight gain and the filtration variables are expressed as mean±S.D.
RESULTS

The weight changes in the lungs are expressed as two phases in the gravimetric method. When we plot the changes in weight of the lungs semilogarithmically as a function of time, two phases of weight change are seen. By fitting a line to the slow phase (data obtained from last 7 min) by the least square method and extrapolating to $t=0$, we obtained the initial filtration rate. Data including the initial filtration rates, applied microvascular pressure increment and the osmotic pressure for the protein were substituted into the Equation (5). Showing an example, weight of rat lung was 1.21 g, the initial filtration rates were 175.4, 21.4 and 54.8 (mg/min x g), using diluted plasma at the vascular pressure 15 and 8 cmH$_2$O and whole plasma 15 cmH$_2$O, respectively. The total protein concentrations and the osmotic pressures for the protein were 0.4, 6.2 g/100 ml and 0.9, 23.8 cmH$_2$O for the diluted and whole plasma, respectively in the room temperature 25°C. Using these data we computed the filtration coefficient ($K$), perimicrovascular pressure ($P_{pmv}$) and reflection coefficient ($\sigma$) as 22.0 mg/ (min x cmH$_2$O x g), 6.9 cmH$_2$O and 0.46, respectively, in the $\Pi$ method ($1-\sigma = \Pi_{pmv}/\Pi_{mv}$), and 22.0 mg/(min x cmH$_2$O x g), 6.9 cmH$_2$O and 0.43, respectively, in the C method ($1-\sigma = C_{pmv}/C_{mv}$).

We computed the filtration variables in each isolated lungs (1.01±0.22 g). Data were used from our formar paper (Tanita et al. 1990). The total protein concentrations and osmotic pressures for protein were 0.5±0.3, 6.5±0.4 g/100 ml and 1.1±0.7, 24.0±1.5 cmH$_2$O for the diluted and whole plasma, respectively. The initial filtration rates for diluted plasma with high (15 cmH$_2$O) vascular pressure were higher than those with low (8 cmH$_2$O) vascular pressure, and those

<table>
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<tr>
<th>TABLE 1. Summary of initial filtration rates in 12 rats</th>
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<tr>
<td>Filtration rate [mg/(min x g wet weight)]</td>
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<tr>
<td>P$_{vasc}$ (cmH$_2$O)</td>
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<tr>
<td>15</td>
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<td>8</td>
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*mean±s.d.

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<th>TABLE 2. Estimated filtration variables</th>
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<td>$II$ method</td>
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<td>$K$</td>
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*mean±s.d.
for concentrated plasma (Table 1). We computed the filtration coefficient (K), perimicrovascular pressure (Ppmv) and reflection coefficient (σ) for either Π or C method are listed on the Table 2.

**Discussion**

Since the fluid movement across the microvascular barrier is governed by hydrostatic and protein osmotic pressures under the Starling equation. The filtration coefficient (K), the perimicrovascular pressure (Ppmv) or the reflection coefficient was estimated or determined independently (Goldberg 1980; Bhattacharya et al. 1984; Parker et al. 1984; Fox et al. 1986; Perry and Taylor 1988). Only a little reports were found in which the filtration variables were determined simultaneously (Tanita et al. 1989, 1988, 1990; Townsley et al. 1990). Although, this simultaneous estimation method was simple to calculate the filtration variables, the assuming formul was pointed out to contain an error especially at the higher portion of protein concentration. In this report, we calculated filtration variables using assumptions 1) \( 1 - \sigma = \frac{\Pi_{pmv}}{\Pi_{mv}} \), which is already reported (Tanita et al. 1990), and 2) \( 1 - \sigma = \frac{C_{pmv}}{C_{mv}} \), which is theoretically more accurate. In 1) \( 1 - \sigma = \frac{\Pi_{pmv}}{\Pi_{mv}} \), the Starling equation was simplified and readily solved using matrix algebra. By contrast, in 2) \( 1 - \sigma = \frac{C_{pmv}}{C_{mv}} \), the substituted Starling equation has cubic terms of σ and is non-linear to solve. Although, 2) \( 1 - \sigma = \frac{C_{pmv}}{C_{mv}} \) is much theoretical, it took a lot of time to calculate. For the calculation, we used spreadsheet which has recalculating programs, and took more than 20 min to calculate. In the calculation results, there was only 10% difference in σ. It is because that calculation equations for osmotic pressure from protein concentration contain square and cubic terms of protein concentration. For the calculation of σ, Townsley et al. showed a formula which was originated from the Starling equation:

\[
\sigma = \frac{\Delta W/\Delta t}{\Delta \Pi p} K
\]

(13)

where \( \Delta W/\Delta t \) is the initial filtration rate, \( \Delta \Pi p \) is a step decrease in plasma osmotic pressure. This formula still has an error, because she neglected term of \( \Pi_{pmv} \) when this was derived. In conclusion, the assumption formula 1) \( 1 - \sigma = \frac{\Pi_{pmv}}{\Pi_{mv}} \) is expedient for simultaneous calculation of filtration variables, as compared with 2) \( 1 - \sigma = \frac{C_{pmv}}{C_{mv}} \), which is theoretically more accurate.

**References**


