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On the Existence of Stretchable Pores in the Exchange Vessels of the Isolated Rabbit Lung Preparation

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Summary

In the present work our aim has been to seek evidence for or against the existence of stretchable pores in the exchange vessels of the lungs. In isolated rabbit lungs ventilated by positive pressure and perfused with homologous blood we performed repeated tests with fluid filtration from the exchange vessels. In these tests the outflow pressure was elevated to specific values for periods of 6 min. The rate of weight gain of the preparation during the last 2 min of each test period was taken as the rate of fluid filtration from the exchange vessels. We found a linear relationship between rate of filtration and outflow pressure in the range from 5 to 20 mm Hg. This indicates that the hydraulic conductivity of the exchange vessels did not change with outflow pressure and thus that no pore stretching occurred within this pressure range. An abrupt increase in filtration rate took place when the outflow pressure was set at 25 or 30 mm Hg. The hydraulic conductivity of the exchange vessels was therefore probably increased at these high pressures. Since in 3 lungs this increase in filtration rate was fully reversible we suggest that a stretching of pores in the exchange vessels of the lungs contributed to the increase in hydraulic conductivity. This stretching of pores occurred only when vascular pressures were at or above the upper level of the physiological pressure range for the lungs.

Introduction

The "stretched pore" phenomenon in capillaries has been debated since introduced by *Wasserman, Loeb* and *Mayerson* (1). It implies that the radius of the functional pores in the capillary walls depends upon the capillary transmural hydrostatic pressure. *Wasserman* et al. (1) studied the concentrations of dextrans of different molecular weights in plasma and in thoracic duct lymph during graded volume expansion. They found that the lymph/plasma ratios for the individual test molecules increased with increasing degree of volume expansion. This observation was later confirmed in a similar investigation in which lymph from the right duct was studied (2). The lymph in the right duct stems mainly from the heart and the lungs, and thus from a much more homogenous capillary bed than the lymph in the thoracic duct. Some later studies in which other methods were used have been taken to support the notion of "stretched pores" at least in the lungs (3, 4). Observations from other studies seem to contradict the existence of pore stretching. *Erdman* et al. (5) found nearly linear increase in lung lymph flow with increasing lung microvascular hydrostatic pressure and at the same time decreasing lymph/plasma ratios for plasma proteins. In the dog paw *Garlick* and *Renkin* (6) found no increase in capillary permeability to dextran or serum albumin upon elevations in venous pressure.

As pointed out by *Michel* (7) measurements of the hydraulic conductivity of the exchange vessels would be the most sensitive method for detection of pore stretching. We have performed a series of experiments along this line using an isolated perfused and ventilated rabbit lung preparation. We could repeatedly measure the capillary filtration rate and thus in the same preparation obtain values for several levels of vascular pressure.

Methods

Rabbits of either sex weighing 2.8–3.5 kg were used. They were anesthetized with Nembutal® (Abbott) 30–40 mg/kg i.v. The heart and lungs were removed after the animal had received heparin (3 mg/kg) intracardially. Cannulas were placed in the left auricle and in the pulmonary artery.

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The preparation was suspended underneath a force transducer (Sanborn FTA 100-1) within a thermostated (37 °C) chamber. Perfusion was carried out with a variable speed peristaltic pump (Harvard Model 1210). The flow was kept constant throughout each experiment. Papaverin (0.1 mg/ml as chloride) was added to the perfusate to counteract spontaneous vasoconstriction. The pulmonary arterial pressure was recorded by connecting a sidearm of the pulmonary artery cannula with a Statham P23De transducer. The left atrial pressure could be varied by leading the perfusate coming from the left atrium through the desired connection in a vertical ladder of plastic and rubber tubings.

The lungs were ventilated by positive pressure at constant pressure, using a Starling "Ideal" pump (C.F. Palmer, London). End expiratory pressure was set at 1.5–2 cm H_2O and peak inspiratory pressure at 6–7 cm H_2O. The ventilation gas was 5% CO_2 in air.

The lungs were perfused with homologous blood (0.1 mg heparin/ml). The pH of the circulating blood was within 7.32–7.48. The perfusate flow was measured at the end of each experiment and was within the range 99–156 ml/min. The pulmonary arterial pressure was between 11 and 18 mm Hg when the left atrial pressure (P_{LA}) was 0 ± 1 mm Hg.

Determination of capillary filtration rate. At the start of perfusion the P_{LA} was set at 0 ± 1 mm Hg. The capillary filtration rate was determined in tests in which the P_{LA} was increased to a specific level for periods of six minutes. The difference between the rate of weight change during the last 2 min before the elevation of P_{LA} and during the last 2 min of the period with elevated P_{LA} was taken as the capillary filtration rate caused by this P_{LA} elevation. In the following such a period of increased P_{LA} is referred to as a filtration test. In most cases the rate of weight change in the period before the P_{LA} elevation was negligible. In each experiment the two first P_{LA} elevations were to the same level, either 10 or 15 mm Hg. We accepted only experiments in which the filtration rate in these 2 tests differed by less than 20%. In the subsequent tests P_{LA} was elevated to values in the range 5 to 30 mm Hg. Between tests the P_{LA} was kept at 0 ± 1 mm Hg for 14 min. In this period the weight of the preparation decreased and in most cases reached the pretest level.

Intravascular volume in periods of elevated P_{LA}. In 9 experiments we determined the intravascular volume repeatedly during periods of increased P_{LA} using an indicator-dilution technique. The lungs were perfused with heparinized (0.1 mg/ml) horse plasma. We had to resort to this type of perfusate due to the large volume required. After an initial perfusion period at low P_{LA} the P_{LA} was increased to 10 or 15 mm Hg and kept at this level for the remainder of the experiment. The rate of weight change was recorded. The intravascular volume was determined as follows: A bolus injection of about 20 μCi ^{125}I human plasma albumin (Institutt for atomenergi, Kjeller, Norway) in 0.1–0.2 ml plasma was given in the pulmonary artery tubing. The total venous outflow was sampled for 45 sec with 1 sample per 0.67 sec as described by *Nicolaysen* (8). The perfusate flow was estimated from the added weight of the samples. The radioactivity of each sample was counted in a Packard scintillation counter. The mean transit time ($\bar{t}$) was calculated according to the formula:

$$\bar{t} = \sum_{i=o}^{n} (C_i \cdot t_i) / \sum_{i=o}^{n} C_i$$

where C_i denotes radioactivity in a sample and t_i the time from injection to that sample. Flow times $\bar{t}$ then gave the volume in the perfusion system from site of injection to the site of sampling. For technical reasons (see Discussion) the bolus injections were performed at about 5 min intervals. This implies that in these experiments the period of increased P_{LA} was longer than 6 min.

Regression lines were obtained using the method of least square deviation.

Results

The capillary filtration rate was evaluated from the rate of weight gain during periods

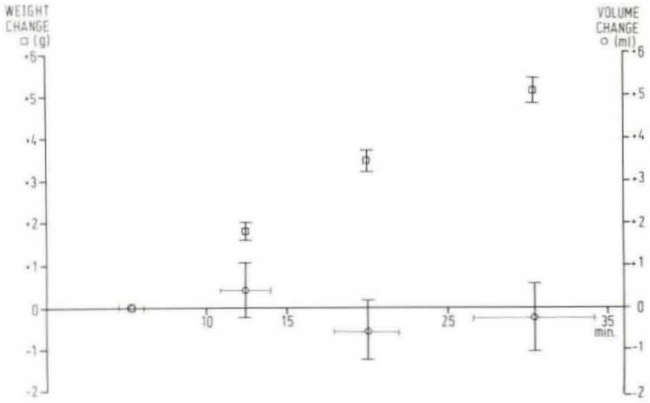

Fig. 1 Changes in intravascular volume (○) and in weight (□) of isolated perfused rabbit lungs subsequent to a step increase in outflow pressure (left atrial pressure) to 10 or 15 mm Hg. Values are mean ± SEM (n = 9)

with increased P_{LA}. This requires evidence for the intravascular volume being constant in these test periods. We therefore in a separate series of experiments determined the intravascular volume during periods of increased P_{LA} using the indicator dilution technique. The results from these experiments are shown in Fig. 1. We normalized the data using the intravascular volume and weight of the preparation at the first bolus injection as zero. On an average no change in intravascular volume took place in the period 2–30 min subsequent to a step increase in P_{LA}. During the same period the weight of the preparation increased steadily. This weight gain must represent a continuous increase in extravascular fluid. Since there was no observable lymph drainage from the preparations this increase must have equalled the rate of net filtration from the exchange vessels.

In Fig. 2 are shown the critical parts of 6 filtration tests in one experiment. Five different levels of P_{LA} are represented. The rate of weight gain was nearly constant during the last 2 min of each 6 min test period. In Fig. 3 the filtration rates obtained in this same experiment have been plotted as a function of P_{LA}. The rate of filtration increased linearly with increasing P_{LA} values up to 25 mm Hg. With P_{LA} at 30 mm Hg the filtration rate was considerably above this line of linear relationship. When tests at low P_{LA} were again

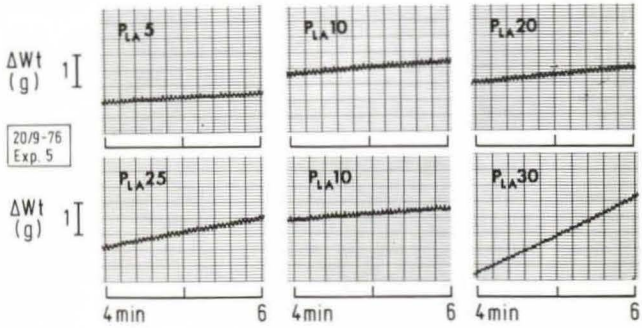

Fig. 2 The effect of different levels of left atrial pressure (P_{LA}) on the fluid filtration rate in an isolated rabbit lung preparation. Weight recordings from 6 different tests at 5 different levels of P_{LA} are shown. The level of P_{LA} in mm Hg is indicated on the individual recording. Each recording starts 4 min after the P_{LA} was set at the desired leve. The rate of fluid filtration was obtained from the rate of weight gain in the period 4 to 6 min after adjustment of P_{LA}. The tests depicted are tests no 4, 5, 6, 8, 9 and 10 in this particular experiment. Note the linearity of the weight increase within each test. Also note the very rapid weight increase at P_{LA} 30 mm Hg

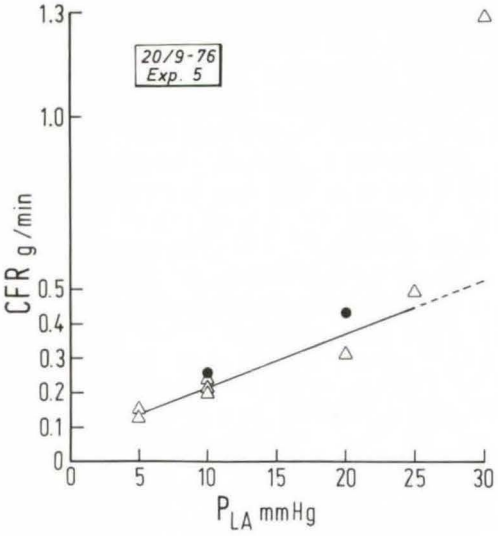

Fig. 3 The relation between left atrial pressure (P_{LA}) and capillary filtration rate (CFR) in an isolated perfused rabbit lung. The P_{LA} was increased for periods of 6 min, the CFR was obtained from the rate of weight gain of the preparation during the last 2 min of this period. The tests were performed in the sequence: P_{LA} 10, 10, 5, 5, 10, 20, 10, 25, 10, 30, 20 and 10 mm Hg. The CFRs obtained at the last 2 tests are shown with filled circles. The line is the regression line (y = 0.0154x + 0.063, r = 0.96) calculated from the CFRs in the first 9 tests

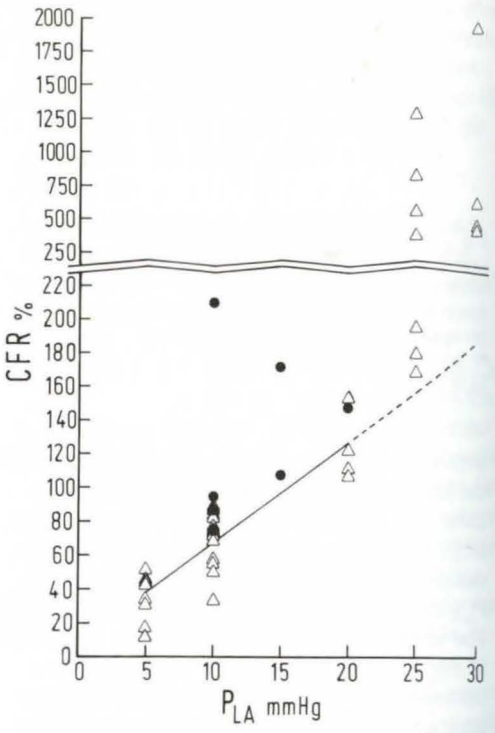

Fig. 4 Relationship of capillary filtration rate (CFR) (normalized as per cent of CFR at P_{LA} 15 mm Hg [see Results]) to P_{LA} in all experiments. In each experiment we found an abrupt increase in CFR obtained in tests at P_{LA} either 25 or 30 mm Hg. CFRs obtained in tests subsequent to these are denoted by filled circles. The line is the regression line (y = 5.9x + 8.5) calculated from the CFRs denoted by open triangles in the P_{LA} range 5–20 mm Hg

performed after the test at P_{LA} 30 mm Hg we found filtration rates about as expected from the first part of the experiment (Fig. 3).

The filtration rate at P_{LA} 15 mm Hg was not the same in all lungs (Table 1). To allow comparison between experiments we normalized the results in each experiment using the mean filtration rate obtained at P_{LA} 15 mm Hg as the 100% value. In 4 experiments tests at P_{LA} 15 mm Hg were not carried out. In each of these experiments we derived a filtration rate at P_{LA} 15 mm Hg from the regression line given by the filtration rates at 5, 10 and 20 mm Hg.

Fig. 4 shows the normalized data from the 8 experiments in this series. In all experiments we found that a P_{LA} of either 25 or 30 mm Hg caused filtration rates considerably higher than predicted from the linearly related filtration rates found at lower P_{LA}. In 6 of the

Tab. 1 Capillary filtration rate (CFR) in isolated rabbit lungs. CFR is measured as the rate of weight gain of the preparation at P_{LA} 15 mm Hg

Experiment	1	2	3	4	5	6	7	8
CFR (g/min)	0.36	0.23	0.35	0.39	0.29	0.17	0.35	0.32

experiments we performed 1 or 2 tests at lower P_{LA} subsequent to tests at 25 or 30 mm Hg. In 3 of these experiments we found values within the range expected from the first part of the experiment, while in 3 experiments we obtained values 25–150% higher (Fig. 3 and 4).

In 3 of the experiments we performed tests at P_{LA} 25 or 30 mm Hg after only 2 or 3 preceding tests at lower P_{LA}. These experiments gave the same results as those in which we applied several tests at low P_{LA} first.

Discussion

The rate of weight gain of the lung preparation in the interval 4 to 6 min after the P_{LA} had been raised to a new level, was taken to represent net rate of transvascular fluid filtration. In order to exclude changes in the intravascular volume during the filtration period we should ideally have measured intravascular volume at the beginning and at the end of this 2 min interval. This was not possible, however, since the procedure for one volume determination lasted several min. We therefore had to extend the filtration period to obtain several volume determinations in each experiment. The indicator bolus injections and thus volume determinations, were performed at about 5 min intervals. As Fig. 1 shows there was some scatter in the observations of intravascular volume. This stems at least in part from the problem of exact timing of bolus injection and sampling. Since the plasma flow was at least 140 ml/min and the time per sample 0.67 sec an error of at least 1.5 ml in volume easily arises. The results strongly support the assumption that changes of intravascular volume do not take place in the period when the "filtration rate" is measured. *Lunde* and *Waaler* (9) reached the same conclusion in experiments in which they used radioactively labelled erythrocytes and measured the radioactivity of the preparation by external counting. In skeletal muscle *Mellander, Öberg* and *Odelram* (10) found that the intravascular volume resetting took place during the first 10–20 sec after a step increase in outflow pressure, thereafter the volume remained constant. Taken together all these observations validate our method for evaluation of fluid filtration rate.

In the filtration experiments several filtration tests were performed. One important point is then the reproducibility of the filtration rate at one level of P_{LA}. Previous studies with the same preparation have shown a high degree of reproducibility (11, 12). This was also the case in the present study as shown in Fig. 2 and 3 for one pair of lungs in which several filtration tests at P_{LA} 10 mm Hg gave nearly identical filtration rates.

We found a linear relationship between P_{LA} and rate of filtration when the P_{LA} was 20 or 25 mm Hg or less (Fig. 3 and Table 2). At P_{LA} 25 mm Hg (in some experiments) or 30 mm Hg the rate of filtration was much higher than expected from this linear relationship obtained at lower P_{LA} levels (Figs. 3 and 4).

The rate of filtration is dependent on the area available for filtration, the net filtration pressure and the hydraulic conductivity of the exchange vessels. During the experimental periods of the present experiments the whole lung was in zone III (13). It has been found that the surface area of the pulmonary capillaries increases with capillary pressure also in zone III (14). It is unlikely, however, that a huge step increase in area should take place at P_{LA} 25 or 30 mm Hg. The net filtration pressure is the resultant of the hydrostatic and colloid osmotic forces across the walls of the exchange vessels. If the net filtration pressure increased proportionally with P_{LA} throughout the range of P_{LA} explored the hydraulic conductivity most probably was constant at the lower levels of P_{LA} but increased at P_{LA} 25 or 30 mm Hg. The question therefore arises whether the net filtration pressure is overesti-

Tab. 2 Coefficients of correlation (r) for the straight lines of best fit for the relation between the capillary filtration rate and the left atrial pressure (P_{LA}) in experiments with at least 5 observations at 3 or more levels of P_{LA} in the range 5–20 mm Hg

Experiment	2	3	5	6	8
r	0.96	0.93	0.92	0.94	0.86

mated at the lower levels of P_{LA} or underestimated at high P_{LA}. At constant flow the hydrostatic pressure in the exchange vessels will increase nearly in proportion to increases in P_{LA} although the expected distention of the vessels will result in microvascular pressure increasing slightly less than the induced increases in P_{LA}. Due to the large volume of circulating blood (about 200 ml) the colloid osmotic pressure of the plasma must be nearly constant regardless of the protein concentration in the filtrate from the exchange vessels. An increasing interstitial hydrostatic pressure and/or a decreasing interstitial colloid osmotic pressure during the filtration tests would imply a decreasing net filtration pressure. Such changes would be particularly important at low levels of P_{LA} at which a few mm Hg change in the sum of the interstitial forces could change the net filtration pressure by a large fraction. At high P_{LA} and thus high filtration pressure the same change in interstitial forces would be of minor importance. However, as shown in Fig. 2, the rate of filtration did not change during the last 2 min of each test. Since the intravascular forces were constant (see above) this constant rate of filtration must imply that also the sum of interstitial forces was constant. It is unlikely that this sum of extravascular forces should be different at different levels of P_{LA}. On this basis we conclude that the net filtration pressure in these experiments must have been close to a linear function of P_{LA}. The results obtained then give strong evidence that the hydraulic conductivity of the microvessels was independent of the absolute intravascular (i.e. transmural) pressure except that it increased at high levels of vascular pressures, about 30 mm Hg. This increase in hydraulic conductivity observed at high P_{LA} could be explained by real "pore stretching" or by capillary wall disruption. In 3 lungs the filtration rate at P_{LA} 10 or 15 mm Hg was increased by about 25–150% in tests performed subsequent to tests at P_{LA} 25 or 30 mm Hg. This could indicate that disruption of capillary walls had occurred. We will, however, emphasize the fact that in 3 lungs the change in hydraulic conductivity was fully reversible (Fig. 4). This finding would be improbable if capillary walls had ruptured. We therefore think that the present experiments indicate that stretching of pores in the exchange vessels can take place at high intravascular pressures in this preparation.

Erdman et al. (5) found no direct evidence of capillary pore stretching in observing a linear correlation between lung microvascular pressure up to about 30 mm Hg and lung lymph flow in sheep. They found that at about this microvascular pressure the extravascular water volume started to increase. Therefore the possibility exists that at the higher microvascular pressure part of the capillary filtrate was not drained by the lymphatics. It is thus feasible that the net transvascular flux of fluid increased more than linearly at the higher levels of microvascular pressure. Pore stretching could then be the explanation of this nonlinearity of net transvascular fluid flux. The observation by the same authors of a linearly increasing lymph protein flux with increasing microvascular pressure does not necessarily contradict pore stretching: if fluid with the same protein concentration as the lymph accumulated in the tissue at the higher pressure, this means that the protein flux from the exchange vessels increased more than linearly with microvascular pressure. Such an alinear total protein flux could easily be explained by pore stretching.

The physiological implications of the present findings are not completely clear. We found evidence for increased hydraulic conductivity only at levels of P_{LA} which were well above normal capillary mean pressure and also above the pulmonary arterial systolic peak pressure during resting conditions. During exercise or other stress conditions, however, the peak pressure might conceivably exceed this threshold.

In conclusion, we found no evidence in the isolated rabbit lung preparation for increase in hydraulic conductivity of the exchange vessels with increase in intravascular pressure except at high pressures. At left atrial pressures of 25 or 30 mm Hg increased hydraulic conductivity was observed and this increase

might be due to pore stretching in the walls of the exchange vessels.

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