Pressure-permeability relationships in basement membrane: effects of static and dynamic pressures

KAREL KLAENTSCHI,1 J. ANNE BROWN,2 PHILIP G. NIBLETT,3 ANGELA C. SHORE,2 AND JOHN E. TOOKE3

1Department of Vascular Medicine, Postgraduate Medical School, Exeter EX2 5AX; 2Department of Biological Sciences, University of Exeter, Exeter EX4 4PS; and 3Department of Clinical Measurements, Royal Devon and Exeter Healthcare National Health Service Trust, Exeter EX2 5DW, United Kingdom

Klaentschi, Karel, J. Anne Brown, Philip G. Niblett, Angela C. Shore, and John E. Tooke. Pressure-permeability relationships in basement membrane: effects of static and dynamic pressures. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1327–H1334, 1998.—The glomerular basement membrane (GBM) is an important component of the filtration barrier that is the glomerular capillary wall. Previously GBM permeability has been investigated only under static pressures and often within a supraphysiological range. We used Matrigel as a model of GBM and formed membranes at the base of a filtration chamber. We measured membrane permeability under static and dynamic pressures. Matrigel membranes were size and charge selective toward neutrally and negatively charged dextrans. Their permeability (as measured by hydraulic conductivity) was found to decrease from 1.61 ± 0.06 to 0.75 ± 0.07 × 10−6 cm·s−1·cmH2O−1 as static pressure increased from 6 to 78 cmH2O, an effect attributed to membrane compression. In comparison to static pressure, sinusoidal pressure waves with a mean pressure of 50 cmH2O decreased membrane permeability, e.g., fluid flux was reduced by a maximum of 2% to a value of 5.47 ± 0.38 × 10−5 cm/s; albumin clearance was reduced by a maximum of 5.2% to a value of 9.63 ± 1.06 × 10−6 ml·cm−2·s−1. Such changes were affected by the frequency of pressure wave application and could be attributed to a switching on and off of the membrane compression effect.

THE ROLE of the basement membrane in capillary permeability is primarily determined by the continuity of the endothelium overlying it. In capillaries where the endothelium is continuous it is likely that the basement membrane functions as only a secondary barrier to filtration, whereas in the archetypal filtration capillaries of the glomeruli the basement membrane is directly exposed to the blood because of fenestrations in the endothelium. Indeed, the main permeselective function of the glomerular wall has been attributed to the glomerular basement membrane (GBM) (7). Unlike the endothelium the GBM is not known to rapidly change its permeability characteristics in response to endogenous chemical stimuli and therefore is often seen as a passive filter. However, there is evidence that the permeability of GBM is altered by mechanical forces such as pressure. Using films formed from fragments of GBM, it has been shown that the basement membrane compresses greatly under pressure and that such a compression leads to a decrease in membrane permeability to both water and macromolecules (13). The physiological significance of such findings is, however, uncertain because of the supraphysiological pressures used in this study.

Recently studies have been carried out at physiological pressures using Matrigel membranes (10). Matrigel is a solubilized basement membrane preparation isolated from a mouse tumor rich in extracellular matrix proteins. This study demonstrated that not only does basement membrane compression occur at physiological pressures, but its effects on membrane permeability were maximum at the lowest applied pressures. An increase in pressure from 12.5 to 15 cmH2O resulted in a 7% decrease in membrane hydraulic conductivity. Although not large, such a response to a small change in applied pressure raises the possibility that changes in mean glomerular capillary pressure or changes in glomerular capillary pulse pressure may serve to regulate glomerular filtration through their effects on basement membrane.

The primary aim of the present study was to investigate such a possibility by developing a system in which GBM permeability could be measured in vitro under pulsatile pressures. We chose to use Matrigel as a model of GBM. Although not glomerular in origin, Matrigel has the advantage of being very simple to use, and resulting membranes have been likened ultrastructurally to the basement membrane of amnion (18). Furthermore, Matrigel is biochemically similar to other basement membranes (11), and the structure of Engelbreth-Holm-Swarm (EHS) tumor matrix (the primary source of Matrigel) is comparable to basement membrane from a variety of sources including rat glomerulus (8). Although Matrigel has previously been used as a model for the investigation of basement membrane permeability (2, 9), one characteristic that is fundamental to the barrier function of basement membrane, i.e., size and charge selectivity, has as yet not been defined. For this reason a secondary aim of the present investigation was to determine the size- and charge-selective characteristics of Matrigel membranes.

MATERIALS AND METHODS

The filtration system. All filtration studies were carried out in an Amicon miniultrafiltration cell (model 3) (Fig. 1) on top of a magnetic stir table. Membranes were formed at the base of the chamber. Filtration buffer was introduced above the membrane via the pressure-release valve port. For static pressure studies the chamber was never filled with more than 3 ml of filtration buffer and was pressurized using nitrogen.
Pulsatile pressures and basement membrane permeability

**Gas**; pressure in the chamber was monitored using a water manometer.

For pulsatile pressure studies the chamber was filled to overflowing with filtration buffer and connected to the motor-driven piston via a rigid plastic tube also filled with filtration buffer and connected via pressure source connection to a nitrogen gas cylinder and water manometer.

Fig. 1. Diagram of ultrafiltration chamber connected to pulsatile pressure equipment. Membranes were formed at base of filtration chamber on top of the support filters. For pulsatile pressure studies, chamber was filled to overflowing with filtration buffer and connected to vibrator. For static pressure studies, chamber was partially filled with filtration buffer and connected via pressure source connection to a nitrogen gas cylinder and water manometer.

Alcohol (BSA) at the membrane surface had no significant effect on fluid and protein flux at this stir rate.

Formation of Matrigel membranes. Basement membranes were formed using Matrigel (Collaborative Biomedical Products, Bedford, MA), a commercially available preparation of EHS mouse sarcoma basement membrane containing 10–15 mg protein/ml. Frozen batches of Matrigel were thawed at 4°C, aliquoted, and refrozen for future use. On the day of the study an aliquot of Matrigel was thawed at 4°C. Meanwhile, the ultrafiltration cell was partially assembled such that a support filter (Whatman 50) overlaid by a prewet cellulose acetate filter (Sartorius, Goettingen, Germany) (0.45-µm pore size) was clamped between the base and the plastic sleeve of the filtration chamber (Fig. 1). This filter arrangement was freely permeable to blue dextran of molecular mass 1,000,000 Da. The cellulose acetate filter was prewet by submersion in filtration buffer [0.1 M tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.4], and any excess fluid was removed by shaking. Using a precooled pipette, we pipetted 80 µl of Matrigel onto the prewet cellulose acetate filter, and the filtration chamber was fully assembled. To aid even spreading of the Matrigel and to promote self-assembly of its components, a pressure of 50 cmH₂O (N₂ gas) was immediately applied to the chamber and sustained for 10 min. Gelling of the membrane was completed by warming the chamber to 37°C for 25 min. Membranes thus formed had a filtration surface area of 1.33 cm². After an equilibration period of 15 min at 22°C the membrane was ready for use. All filtration studies were carried out at 22°C, and at the completion of each study the confluence of each membrane was tested by use of a 1% solution of blue dextran (molecular mass 1,000,000 Da) with a pressure of 78 cmH₂O. Results obtained from any membrane allowing the passage of blue dextran were rejected; this was found in approximately one in every eight membranes prepared.

Membrane variability. Because batches of Matrigel varied in their protein concentration, we investigated what effect this may have on membrane permeability. Ten membranes were formed from each of four different batches of Matrigel containing 10.8, 11.5, 12.6, and 14.1 mg protein/ml. The chamber above each membrane was filled with 3 ml filtration buffer and a pressure of 78 cmH₂O was applied. Filtrate was collected for the determination of fluid flux (Jv).

Membrane thickness. Six Matrigel membranes (from a single batch of Matrigel) and their support filters were fixed in a 3% glutaraldehyde solution in filtration buffer and processed for light microscopy. Each membrane was sectioned in four randomly selected sites and stained with methylene blue. With the use of an eyepiece graticule in a light microscope, membrane thickness was measured at two randomly selected sites within each section.

Size and charge selectivity of Matrigel membranes. The size and charge selectivity of eight Matrigel membranes was assessed using neutral and negatively charged dextrans covering a range of molecular masses (10,000–160,000 Da). This range of dextrans was achieved by the hydrolysis of neutral dextran of molecular mass 2,000,000 Da (Sigma) and dextran sulfate of molecular mass 500,000 Da (Sigma). Neutral dextran was hydrolyzed (at 100°C in 0.1 M HCl) in two batches, one for 20 min and one for 60 min; by combination of these batches a quantitatively even distribution of molecular masses was obtained. Dextran sulfate was hydrolyzed under the same conditions for 20 min; this resulted in a quantitative loss of very low molecular mass fractions. This could not be compensated for by the addition of hydrolysates from a more prolonged hydrolysis because this may have resulted in a heterogeneity of negative charge (4).
was carried out with a dextran concentration of 5% (wt/vol), and the reaction was stopped by neutralization. After dialysis against phosphate-buffered saline and then distilled water, the hydrolysates were retrieved by evaporation to dryness at 40°C. A solution of either neutral or sulfated dextran (10 mg/ml in filtration buffer at pH 7.4) was pipetted into the filtration chamber above a freshly formed membrane. The chamber was pressurized to 50 cmH₂O (to approximate in vivo glomerular capillary pressure), and stirring was initiated at 400 revolutions/min. Filtrate was collected for periods of 8–10 h. Molecular mass fractions in samples of both the filtrate and the filtration buffer above the membrane were separated by gel-exclusion chromatography on Sephadex G-100. Columns (2.5 x 52 cm) were run at 4°C with 0.01 M Tris-HCl buffer (0.15 M NaCl, 0.02% sodium azide, pH 7.4) and were calibrated with blue dextran, albumin, carbonic anhydrase, cytochrome c, and aprotinin. The Stokes radius of molecular mass fractions was calculated using the data of Laurent and Granath (12).

Filtration studies under static pressures. Either filtration buffer alone or filtration buffer containing 40 mg/ml BSA was pipetted into the filtration chamber above a freshly formed membrane. Fluid filtration was determined at 11 different static pressures in the range of 6–78 cmH₂O: pressures were applied in a random order. At each pressure filtration was collected for a timed period until a volume of ~50 µl was obtained; the exact volume was considered to be equal to the weight change of the filtrate collection vial. When filtration for the determination of Jᵥ alone was collected, a 10-min equilibration period preceded collection. The pressure applied during equilibration was equal to that applied during collection.

For determination of protein filtration, a fresh membrane was formed and filtration buffer containing 40 mg/ml BSA was pipetted into the filtration chamber. Six pressures in the range 13–78 cmH₂O were applied to each membrane in a random order, and at each pressure filtration was collected for the determination of protein clearance. The filtration buffer containing protein was replaced after the application of three pressures, and samples of the filtration solution were taken at the beginning and at the end of a study as well as directly before and after application of filtration buffer containing protein. The change in membrane permeability were quantified by the measurement of membrane permeability under a static pressure of 50 cmH₂O before and after each application of a pulsatile pressure. The use of 16 membranes in the pulsatile pressure studies provided a power of 90% to detect a 1.8% change in Jᵥ when measured in the presence of protein.

Assays. Protein concentration in the filtrate and filtration buffer were measured by the Coomassie assay (3) using BSA (fraction V, Sigma) as the standard. Dextran concentration in collected eluent fractions was measured by the anthrone method (14). When neutral dextrans were quantified, a neutral dextran of molecular mass 2,000,000 Da was used as the standard; in the case of dextran sulfate, a dextran sulfate of molecular mass 500,000 Da was used as the standard. Preliminary studies showed that the molecular mass of the dextran did not influence its detection by the anthrone method.

Calculations. Jᵥ (cm/s) across membranes was calculated by dividing the fluid volume flow rate (cm³/s) by the membrane surface area (cm²). In the absence of protein, the hydraulic conductivity of the membrane (Kᵥ) was calculated as

\[ Kᵥ = \frac{Jᵥ \cdot L}{P} \]

where Jᵥ is the fluid flux (cm/s), L is the membrane thickness (cm), and P is the applied hydrostatic pressure (cmH₂O). The clearance of solute across the membrane was calculated by dividing the solute flux (mg·cm⁻²·s⁻¹) by the solute concentration of the filtration buffer in the chamber (mg/ml). Finally, the measured rejection of protein or dextran by the membrane was equal to 1 – φ, where φ is the ratio of the protein concentration of the filtrate to the protein concentration of the filtration buffer in the chamber. When 1 – φ was equal to 1, the membrane was completely impermeable to the solute under study.

Statistics. Selectivity curves and Jᵥ-pressure curves were analyzed using analysis of variance (ANOVA) with repeated measures; the probability level taken as significant was P < 0.05. When a significant difference was found, Fisher's least significant difference (LSD) between means was calculated.

Coefficients of variation (CVs) were calculated by dividing the SD by the mean and multiplying by 100%. All results are given as means ± SD.

RESULTS

Membrane variability and membrane thickness. No relationship existed between the amount of protein within a batch of Matrigel and the measured Jᵥ (Table 1). Because of this, we ensured that membranes within each study came from the same batch of Matrigel; the protein content of Matrigel is specified in the legends of Figs. 2–10. By light microscopy it could be seen that Matrigel membranes formed a distinct layer on the surface of the cellulose acetate filters. Membranes had a mean thickness of 66.33 ± 5.28 µm; the CV within

<table>
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<tr>
<th>Protein Content, mg/ml</th>
<th>Jᵥ × 10⁻⁵ cm/s</th>
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<tr>
<td>10.8</td>
<td>6.79 ± 0.42</td>
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<tr>
<td>11.5</td>
<td>5.33 ± 0.62</td>
</tr>
<tr>
<td>12.6</td>
<td>7.18 ± 0.39</td>
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<tr>
<td>14.1</td>
<td>5.85 ± 0.52</td>
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Values are means ± SD of 10 membranes. Fluid flux (Jᵥ) was measured at a pressure of 78 cmH₂O.
and between membranes was 8.0 and 8.6%, respectively.

Size and charge selectivity. At a static filtration pressure of 50 cmH₂O the measured rejection of dextrans (either neutrally or negatively charged) by Matrigel membranes increased progressively with increasing molecular radius (P = 0.001) (Fig. 2). The size selectivity of membranes to neutral and negatively charged dextrans was significantly different (P = 0.017); dextran sulfate molecules with molecular radii of 44.8, 54.1, 61.9, and 74.8 Å exhibited significantly greater measured rejection values than neutral dextrans of the same radius (Fig. 2).

Static pressure studies. Both in the presence (40 mg/ml BSA) and absence of protein, Jᵥ increased with increasing pressure (P = 0.001) (Fig. 3). This relationship deviated from linearity in that the rate of increase in Jᵥ appeared to show a deceleration as pressure was increased. This deviation from linearity was supported by the finding that a quadratic curve had a slightly improved fit to the data compared with that of a linear regression. For example, in the absence of protein, r² = 0.976 for a linear regression and r² = 0.996 for a quadratic curve. Such an improved fit was evident to a lesser degree in the presence of protein. As pressure increased from 6 to 78 cmH₂O, the Lₚ of the membrane, measured in the absence of protein, fell exponentially (Fig. 4). The greatest changes in Lₚ occurred at the lowest applied pressures; for example, a rise in pressure from 8 to 13 cmH₂O resulted in a decrease in Lₚ of ~13%. A change in pressure of similar magnitude at the highest applied pressures resulted in a change in Lₚ of <4%.

The clearance of BSA across membranes also increased with increasing pressures (P = 0.001) (Fig. 5) but did not appear to decelerate at higher pressures. The intercept with the ordinate of a straight line fitted to the clearance data was 1.88 ± 1.17 × 10⁻⁶ ml·cm⁻²·s⁻¹, this value was significantly different from zero (P = 0.003), suggesting that there was diffusive flux as well as convective flux of protein across Matrigel membranes. At the lower applied pressures, a rise in Jᵥ resulted in a rise in the measured rejection (Fig. 6), indicating an increasing predominance of convective over diffusive flux (17). At the highest Jᵥ values (result-
ing from the highest applied pressures: 48, 63, and 78 cmH$_2$O), measured rejection appeared to reach a constant value, and indeed no significant difference was found between measured rejection values at the highest applied pressures. When measured rejection becomes independent of $J_v$ in this manner, its value is a good estimate of the true membrane rejection (17), in this case $0.7$. The protein concentration of the buffer in the chamber did not change significantly during these studies.

Pulsatile pressure studies. The pressure wave parameters detected at the transducer were slightly higher than those set at the function generator. For example, the mean pressure measured by the transducer was $52.01 \pm 0.29$ cmH$_2$O, whereas the amplitude was $30.16 \pm 0.09$ cmH$_2$O. Despite this the SPWs detected at the transducer varied in amplitude and mean pressure by $<1\%$ both within and between studies. Stirring at rates up to 800 revolutions/min had no deleterious effects on the form of the SPWs when measured directly beneath the stir bar or at the pressure transducer.

$J_v$ at a static pressure of 50 cmH$_2$O, monitored at regular intervals throughout each pulsatile pressure study, remained constant over the duration of the study, whereas there was a small but nonsignificant increase in protein clearance (Fig. 7). The effects of pulsatile pressure on $J_v$ as measured in the absence of protein are shown in Fig. 8. At a static pressure of 50 cmH$_2$O, $J_v$ was $5.58 \pm 0.46 \times 10^{-5}$ cm/s. In comparison with this the application of pulsatile pressures of frequencies 1 and 2 Hz significantly reduced $J_v$ to values of $5.50 \pm 0.42 \times 10^{-5}$ cm/s ($P = 0.038$) and $5.47 \pm 0.38 \times 10^{-5}$ cm/s ($P = 0.006$), respectively. The trend of a reducing $J_v$ with increasing frequency was not significant. When protein was present in the filtration buffer, pulsatile pressures appeared to have no significant effect on mean $J_v$ (Fig. 9).

Under a static pressure of 50 cmH$_2$O, solute clearance was $10.16 \pm 1.31 \times 10^{-6}$ ml/cm$^2$ s$^{-1}$ (Fig. 10). There was no significant difference between the solute clearance measured under any of the three applied pressure conditions. When the measured protein rejections were compared between the three groups, a statistical difference between the two pulsatile pressure conditions was found ($P = 0.033$): measured rejection was similar under both a static pressure ($0.653 \pm 0.023$) and a pulsatile pressure of frequency 2 Hz ($0.657 \pm 0.026$) but was significantly increased.
under a pulsatile pressure of frequency 1 Hz (0.672 ± 0.026). The protein concentration of the buffer in the chamber did not change significantly during these studies.

**DISCUSSION**

Matrigel contains all the major components of basement membrane, including type IV collagen, laminin, and heparan sulfate proteoglycan (11), and it has recently been reported that membranes formed using Matrigel have hydraulic conductivity values similar to those reported for pig GBM (10). This, in combination with its ready availability and ease of use in the formation of membranes, suggests that Matrigel is a useful model for GBM. We have further validated Matrigel as an alternative to GBM fragments by demonstrating its size and charge selectivity toward neutral and negatively charged dextran fractions. In comparison to the rat glomerulus in vivo (1), Matrigel membranes had a much reduced size selectivity. For example, the passage of a neutral dextran of radius 40 Å across rat glomerulus was almost totally restricted, whereas its passage across Matrigel membranes was relatively free (1 - ϕ = 0.2). A similar, although less extensive, loss in size selectivity has also been shown in vitro (under an applied pressure of 68 cmH2O) using membranes prepared from rat GBM (6). Such differences between basement membrane and intact glomerulus imply an important role for cellular components in the size-selective function of the glomerulus. The difference in size selectivity between in vitro studies using GBM fragments or Matrigel may be explained by a structural difference resulting from the self assembly of Matrigel membranes. Such a difference has been observed by Yurchenco and Ruben (18), who showed that type IV collagen isolated from EHS tumor matrix, when allowed to self assemble, formed a network approximately one-half as tightly meshed as that occurring in vivo.

Within a single batch of Matrigel, membranes with similar thicknesses could reproducibly be formed. Furthermore, these membranes varied by only 8% in their Jv values as measured at 78 cmH2O. Variability in Jv...
rose to 13% when membranes between batches were compared. This, in combination with the finding that there was no relationship between membrane protein concentration and $J_v$, prompted us to use the same batch of Matrigel for each study. Variability between batches may have arisen from two sources: 1) the ratios of basement membrane components (collagen, laminin, and proteoglycans) in Matrigel, or 2) the reactivity or ability of the components to self assemble. Neither of these variables was quantified by either the producers of Matrigel or by ourselves.

We have shown that within a physiological pressure range, $J_v$ across basement membrane does not increase in a linear manner with increasing pressure; instead filtration rate tended to decrease with increasing pressure. Furthermore, membrane hydraulic conductivity fell by 13% when pressure was increased from 8 to 13 cmH$_2$O and continued to fall in an exponential manner. The pressure curve is dependent on membrane compression, it would appear that compression is switched on and off during a pressure wave with a mean compression effect greater than that seen under static pressure. The presence of physiological concentrations of protein in the filtration buffer appeared to abolish the effects of pulsatile pressure on $J_v$. However, the use of 16 membranes could not detect changes in $J_v$ of <1.8%, and so we cannot exclude the possibility of smaller changes. A loss or reduction in the effects of pulsatile pressure on $J_v$ in the presence of protein in the filtration buffer could have resulted either from a loss of membrane compliance due to the trapping of protein within the membrane structure or from the osmotic effects of the protein. A total loss in membrane compliance was not consistent with the nonlinear $J_v$-pressure curves described in the presence of protein. This does not, however, exclude the possibility that a small loss in compliance may contribute to the reduction in the effects of pulsatile pressure. In support of osmotic effects, it has been shown in theoretical analyses of the microcirculation (15) that fluctuating blood flow and pressure, due to the rhythmic contraction of arterioles and precapillary sphincters, results in fluctuating laminar protein concentrations, which lead to an increased net fluid filtration. On the basis of these analyses, it is tempting to conclude that in our system the compression effects, resulting in the decreased $J_v$ seen in the absence of protein, were canceled out or reduced in the presence of protein due to an increased $J_v$ resulting from fluctuating protein concentrations at the membrane-filtration solution interface.

In comparison to a static pressure and a pulsatile pressure of frequency 2 Hz, a pulsatile pressure of frequency 1 Hz appeared to decrease protein clearance, although this did not reach significance. However, a significant frequency effect was apparent in comparisons of measured rejection values made between groups. Because we have shown that convection is predominant at 50 cmH$_2$O, it is likely that measured rejection under pulsatile pressures reflects true rejection and hence membrane permeability. However, because pressure within the sine wave dropped below 50 cmH$_2$O, we cannot totally exclude changes in the relative contributions of diffusion and convection to the measured rejection values. If, as suggested previously, changes in membrane permeability are due to the compression effects of pressure, then our measured protein rejection results suggest that Matrigel membranes were viscoelastic in their behavior. Unlike elastic bodies their deformation, and therefore their permeability characteristics, were dependent not only on the force applied but also on the velocity of application, with a rapid application of force resulting in a smaller deformation than the same force applied more slowly. Our results regarding measured protein rejection under pulsatile pressure fit this theory in that measured rejection was
significantly greater at a pressure wave of 1 Hz than at 2 Hz. Such agreement could not be found in the results obtained for \( J_v \) in the absence of protein, for which the higher frequency of pressure wave application appeared to have the greatest effects. As with the static pressure studies, the effects of pulsatile pressures (which exceeded physiological limits) on membrane permeability were very small and therefore unlikely to have any major physiological significance.

In conclusion, we have further established Matrigel as a useful alternative to GBM films for in vitro permeability studies. As well as functioning as a size-selective and charge-selective filter, Matrigel membranes alter their permeability characteristics through compression effects in response to the application of both static and pulsatile pressures. These responses were, however, very small and would be unlikely to influence glomerular capillary filtration under physiological circumstances.

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Address for reprint requests: K. Klaentschi, Dept. of Vascular Medicine, Diabetes Research, Postgraduate Medical School, Barrack Rd., Exeter EX2 5AX, UK.

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