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Size-selectivity of a synthetic high-flux and a high cut-off dialyzing membrane compared to that of the rat glomerular filtration barrier

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Abstract

The aim of the present study was to investigate the size-selectivity of two different synthetic dialyzing membranes, having widely differing sieving properties, with respect to their handling of polydispersed fluorescein isothiocyanate (FITC)-Ficoll, FITC-dextran and of proteins, i.e. ^{125}I-human serum albumin (RISA) and ^{125}I-myoglobin (Myo). Are Ficoll and dextran, compared to proteins, "hyperpermeable" across synthetic dialyzing membranes, similar to their behavior across the glomerular filtration barrier (GFB)? A high-flux membrane (HF-Revaclear®; n=12) and a high cut-off membrane (HCO; n=14) in capillary mini-dialyzers were perfused with diluted horse serum. The perfusate contained polydisperse FITC-Ficoll 70/400 or FITC-dextran (mol.radius 13-80Å), FITC-Inulin, and, in some experiments, RISA/Myo. After a priming period, sampling of filtrate occurred, and a midpoint plasma sample taken. Filtrate-to-plasma concentration ratios (θ) *vs.* molecular radius (a_e) were assessed using HPLC for Ficoll and dextran. Size-selectivity for Ficoll increased in the order: HF-Revaclear®<rat glomerulus<HCO. Although the HCO filter showed the highest cut-off, this occurred at the expense of a high permeability to albumin and large Ficoll molecules and a high degree of dispersity of (small) pore radii, as assessed using a log-normal + shunt distributed pore model. According to a two-pore model, the fractional hydraulic conductance accounted for by large pores (α_L) was 6.54±0.70 x 10^{-3} and 1.82±0.69 x 10^{-3} for the HCO and the HF-Revaclear®, respectively, compared to 4.1±0.80 x 10^{-5} for the rat glomerulus. In conclusion, the HCO filter investigated showed a high θ for myoglobin, similar to that of the GFB. However, the number of large pores was markedly higher and the pore size heterogeneity markedly larger than for the GFB. Membrane permeability was dependent on molecular species and increased in the order: proteins<Ficoll<dextran.

Keywords: Ficoll, dextran, light chains, pore-modeling, log-normal distributed pore model

Introduction

It is well established that the polysaccharide dextran, due to its random coil conformation, is "hyperpermeable" across the glomerular filtration barrier (GFB) [1-3]. Lately, also Ficoll, a highly cross-linked copolymer of sucrose and epichlorohydrine, has been shown to be more permeable than proteins across the GFB for molecules of radius approaching the radius of the membrane pores [3-6]. This phenomenon has been attributed to an increased flexibility and/or "molecular extension" of polysaccharides *vs.* proteins [3]. On this background, the present study aimed at investigating the size-selectivity of two different synthetic dialyzing membranes, showing widely differing sieving properties, with respect to their handling of FITC-Ficoll, FITC-dextran and of proteins, namely radiolabeled myoglobin (Myo) and albumin (RISA). Special interest was devoted towards a high cut-off (HCO) dialyzing membrane, representing a further development of current artificial membrane technology in a direction to mimic the GFB, both with respect to sieving properties (high cut-off properties) and biocompatibility. Hence, it was deemed of interest to make a direct comparison of the sieving characteristics among the GFB and the synthetic membranes investigated.

The sieving characteristics of the two artificial membranes chosen were investigated using techniques that we have previously extensively employed in our laboratory for investigating the rat GFB *in vivo*. A recently developed HCO filter (Gambro HCO 1100 dialyzer; Gambro Dialysatoren GmbH, Hechingen, Germany) apparently share many properties with the GFB in that it is highly permeable to small proteins and has a high surface area, making it suitable for removing free light chains and myoglobin from the circulation [7-9]. How does this HCO dialyzer compare to the GFB? In turn, how do the GFB and the HCO dialyzers, highly permeable to small proteins, compare with newly developed high-flux filters, in this case a Polyflux Revaclear® dialyzer (HF-Revaclear®; Gambro Dialysatoren GmbH, Hechingen, Germany). Furthermore, do the artificial membranes treat polysaccharides and proteins in a fashion similar to the GFB, i.e. do they show a higher permeability to molecules that are "extended" in shape, i.e. molecules with an increased "frictional ratio" [3]?

To study the functional behavior of the synthetic membranes selected we performed hemofiltration experiments *in vitro* using polydisperse FITC-dextran, FITC-Ficoll 70/400 and two proteins, Myo and RISA, as size probes and diluted plasma as perfusate. The filtrate-to-plasma concentration ratios (θ) were assessed using high performance size-exclusion chromatography (HPSEC), for a broad spectrum of polysaccharide molecular radii (a_e ~13-80Å) with emphasis on the sieving patterns of molecules of high molecular mass (MW ~400 kDa). Data were analyzed in terms of a two-pore model [10] and using a log-normal distributed + shunt pore size model [4].

Methods

Filter devices

Two different mini-capillary dialyzer devices were obtained from Gambro Dialysatoren GmbH (Hechingen, Germany): 1) A high-flux membrane (Polyflux Revaclear®; HF-Revaclear®; n=12) with a total surface area of 360 cm^2 and having a total number of 355 fibers, each of which with an inner diameter 190 µm, and a wall thickness of 35 µm (total intracapillary volume: 1.70 mL) and a total nominal filtration coefficient (hydraulic conductance; L_pS) of 0.15-0.20 ml/min/mmHg; 2) A high-cut-off membrane (HCO 1100; n=14) showing the same total surface area as the HF-Revaclear® filter, but having 314 fibers, each of which with an inner diameter of 215 µm and a wall thickness of 50 µm (total intracapillary volume: 1.93 mL) and a nominal L_pS of 0.77-0.99 ml/min/mmHg.

Experimental setup

The experimental setup is shown in Figure 1. The capillary filters were perfused using a peristaltic pump (ALITEA-XV, Tc Instrument AB, Gothenburg, Sweden). The perfusion fluid consisted of equal amounts of inactivated horse serum (SVA, Uppsala, Sweden) and 0.9 % saline and had a colloid osmotic pressure of 7.1±0.06 mmHg, determined with a colloid osmometer (Gonotec Osmomat 050, Colloid Osmometer). To this fluid mixture, FITC-Inulin (TdB Consultancy, Uppsala, Sweden) (23.31 µg/mL) and FITC-Ficoll (TdB Consultancy Sweden) (70 and 400 kDa; 0.932 µg/mL and 22.37 µg/mL, respectively) or FITC-Dextran (Sigma) (5 kDa: M_w/M_n ~1.6, 9.0 µg/mL; 25 kDa: M_w/M_n ~1.30, 7.2 µg/mL and 150 kDa: M_w/M_n ~1.47, 6.3 µg/mL, respectively) were added. In different experimental series radioactively iodinated (^{125}I; 0.1MBq) myoglobin (Myo) (from horse skeletal muscle, M0630, lot nr 039K7004, Sigma, St Louis, MO) or radiolabeled native human serum albumin (^{125}I-HSA; 0.15 MBq; RISA) (gift from Prof. Olav Tenstad, University of Bergen, Norway) were included in the perfusate. The proteins were radioiodinated at the Department of Medical Radiation Physics, Lund University at Lund University Hospital, Lund, Sweden (by Dr. Thomas Olsson) using the iodogen method [11, 12].

The inflow pressure just before the dialyzer was set at approximately 25-30 mmHg (29.7±0.6 and 26.9±0.4 mmHg for the HF-Revaclear® and HCO dialyzers, respectively) and outflow pressure at approximately 15 mmHg (15.7±0.67 and 15.6±0.3 mmHg for the HF-Revaclear® and HCO dialyzers, respectively), yielding an average hydrostatic filter capillary pressure of 22.6±0.23 mmHg and 20.8±0.14 mmHg, respectively. The pump rate was set at ~8 mL/min (7.8±0.2 mL/min), to yield a (plasma) flow per fiber averaging 24.8 μl/min and 22.2 μl/min for the HCO and the HF-Revaclear®, respectively, according to recommendations for clinical use (17.4-43.8 μl/min per fiber and 20.8-52.1 μl/min per fiber, respectively). Dead-space volume, i.e. the volume filtered from start of perfusion until the filtrate could be collected, was similar for the two filters, 7.4±0.3 and 8.1±0.4 mL for the HCO and HF-Revaclear® dialyzer, respectively. Filtration was allowed for at least 5 min to prime the filter before sampling began. Then filtrate sampling took place during 5 min, with a mid-point perfusate sample taken for assessments of concentrations of FITC-Ficoll/FITC-dextran or of radioactivity (Myo and RISA), and also for colloid osmometry. Samples were processed using high performance size exclusion chromatography (HPSEC) to assess plasma and urine concentrations of FITC Ficoll and FITC-dextran, respectively. In the protein sieving experiments radioactivity (^{125}I) was performed in a gamma scintillation counter (Wizard 1480, LKP Wallac, Turku, Finland), and appropriate corrections for radioactive decay was performed. To reduce the influence of free ^{125}I on the measurements of protein radioactivity, all samples were precipitated with 10% TCA, centrifuged and the supernatant (free ^{125}I) discarded. θ for ^{125}I-HSA and ^{125}I-myoglobin were calculated by dividing the filtrate concentration of each protein by its average perfusate concentration during the period of measurement (5 min) .

Binding of protein tracer to the membrane (pores) of the filter devices

To investigate the extent of binding of proteins to the filter membranes, a set of separate experiments was carried out. Both initial adsorption (“binding”) and continual adsorption over time (“clogging”)

were investigated for the two filters and for the two radiolabeled (iodinated) proteins investigated. The experiments were run as filter perfusions and sieving experiments as described above (see "Experimental setup") and carried out for either 5 min or 30 min, to be able to study both initial protein binding and gradual protein clogging of the membranes over time. To remove unbound tracer at the end of the experiments, the filters were rinsed with a solution containing equal amounts of horse serum and 0.9% saline, but no radioactivity, until the final rinse solution contained less than 0.3% of initial radioactivity. After this washout, the housing of dialyzer was removed and the fibers of the filter collected *in toto* and assessed for radioactivity in the gamma scintillation counter (see above). Both the HF-Revaclear® and HCO filters showed low grade initial binding of albumin to the filter membrane, but there were no significant differences in tracer distribution space at 30 min vs. 5 min. Tracer distribution space is defined as the total intrafiber radioactivity (CPM) over "plasma" radioactivity (CMP/mL). The total fraction of ^{125}I-albumin (RISA) binding to the HF-Revaclear® membrane at 5 min was 1.61±0.11% (n=3) of the total initial "plasma" radioactivity present in the (total) capillary fiber volume and 2.03±0.09% (n=3) at 30 min. The corresponding figures for the HCO filter was 3.44±0.72% (n=3) at 5 min and 3.51±0.23% (n=3) at 30 min. Thus there was no evidence of significant clogging of any of the membranes with albumin during the short time span of the experiments. In these RISA experiments the upconcentration of RISA at the "venous" end of the fibers was found to be directly proportional to the filtration fraction of the membrane (i.e. ~20% for the HCO membrane, and ~10% for the HF-Revaclear® membrane). With Myo there was an instantaneous binding of the tracer protein to the filter membranes, but also a gradual adsorption, which increased equally over time for the two synthetic filters investigated. For the HF-Revaclear® the fractional membrane adsorption of tracer at 5 min for Myo was 4.48±0.43% (n=3) of total initial capillary fiber volume radioactivity and 18.92±2.09% (n=3) after 30 min, with a zero time retropolated value of 1.59%. The total rate of increment in the apparent Myo distribution space over time was 0.58%/min. For the HCO filter the 5 min fractional Myo space was 18.59±2.96% (n=3) in excess of the total intrafiber radioactivity, and increased to 35.69±3.35% (n=3) after 30 min, with a zero time retropolated value of 15.17%. The rate of space increment over time was 0.68%/min. For the sieving experiments the calculated fractional "trapping" of tracer in the membrane was found to be 4% of

filtered radioactivity for the HF-Revaclear® and 1.2% of filtered radioactivity for the HCO. Due to the low trapping of albumin in both filters and the low relative trapping of Myo in the HCO filter, only the sieving coefficients for HF-Revaclear® were mathematically corrected for tracer trapping.

High performance size exclusion chromatography (HPSEC)

An HPLC system (Waters, Milford, MA) was used to determine size and concentration of Ficoll and dextran in perfusate and plasma. Size-exclusion chromatography was achieved using an Ultrahydrogel-500 column (Waters). The mobile phase was driven by a pump (Waters 1525) and fluorescence was detected with a fluorescence detector (Waters 2475) with an excitation wavelength set at 492 nm and an emission wavelength of 518 nm. The samples were loaded to the system using an autosampler (Waters 717 plus), and the system was controlled by Breeze Software 3.3 (Waters). The column was calibrated using Ficoll and protein standards described in a previous paper [13]. θ for FITC-Ficoll and FITC-Dextran were determined as the polysaccharide concentration ratio between filtrate and perfusate during the period of measurement (5 min).

Two-pore analysis

A two-pore model [4, 5, 10, 12, 14], was used to analyze the θ data for Ficoll and dextran as a function of Stokes-Einstein radius (a_e = 13-80Å). A nonlinear least-squares regression analysis was used to obtain the best curve fit, using scaling multipliers, as described at some length previously [4]. The major parameters of the two-pore model are: 1) the small pore radius (r_s), 2) the large pore radius (r_L), 3) the unrestricted pore area over unit diffusion path-length ($A_0/\Delta X$), and 4) the fraction of the ultrafiltration (UF)-coefficient accounted for by the large pores (α_L).

Log-normal distributed + shunt model

A log-normal distributed + shunt model was also employed to analyze the θ data for Ficoll and dextran as a function of a_e, as described at some length in Appendix I.

Statistical Analysis

Values are presented as means ± SE. Differences among groups were tested using non-parametric analysis of variance with the Kruskal-Wallis test and post-hoc tested using the Mann Whitney U-test. Bonferroni corrections for multiple comparisons were made. A Pearson chi-square test was used for testing the "goodness of fit" for the data fitted to the two-pore model and the log-normal distributed model, respectively, for all three membranes investigated. Significance levels were set at *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. All statistical calculations were made using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL).

Results

Experimental setup and parameters

Experimental setup and parameter characteristics of these experiments are shown in Fig 1 and Table 1, respectively. For calculating the dialyzer filtration coefficient the moderate upconcentration of the perfusate colloid (estimated with RISA) towards the distal end of the fibers (~2 mmHg for the HCO dialyzer and ~1 mmHg for the HF-Revaclear® dialyzer) and the phenomenon of concentration polarization of the colloid at the membrane surfaces (see below) were not accounted for.

Dialyzer size-selectivity to Ficoll compared to that of the GFB

Figure 2a demonstrates the sieving coefficient (θ) *vs.* Stokes-Einstein radius (a_e) curves for Ficoll together with θ for RISA and Myo, for the HF-Revaclear® (hatched line) and the HCO filter (solid line), respectively, compared to those obtained for the rat GFB from previous studies in our laboratory (dotted line) [12, 15]. According to the two-pore model, size-selectivity in terms of small pore radius increased in the order HF-Revaclear®<rat glomerulus<HCO (Table 2a). However, size-selectivity in terms of permeability to high molecular weight Ficoll and to albumin increased in the order: rat glomerulus<HF-Revaclear®<HCO. Indeed, the HCO showed the highest cut-off, but at the expense of an increased permeability to high molecular weight solutes (Table 3 and 2a). The HCO filter showed nearly an order of magnitude higher flux through the large pore system (Jv_L/Jv) than did the HF-Revaclear® filter, which, in turn, showed a 60-fold higher Jv_L/Jv than the rat glomerulus (Table 3). Note that θ for albumin (a_e ~36Å) will fall on the large pore curves (θ for Ficoll for a_e >50Å) when retropolated to the ordinate, which was particularly evident for the two synthetic filters investigated [5]. According to the log-normal distributed (+ shunt) model, both the HCO and the HF-Revaclear® showed a more heterogeneous pore size distribution than did the rat GFB (Table 2b).

Dialyzer size-selectivity to dextran compared to that of the GFB

Similar to the GFB both synthetic filters investigated showed a lower selectivity (higher θ) to dextran than to Ficoll. In turn, there was a higher θ for Ficoll than that for the two proteins investigated at equivalent a_e. Fig 2b and Table 3b thus demonstrate sieving data for the two filters investigated using dextran in comparison to our previous measurements with dextran for the rat GFB [16]. The small pore radius was similar among the HCO filter and the GFB, 62.1±1.14 and 64.2±1.02Å, respectively, but much larger than for the HF-Revaclear® dialyzer (46.7±1.5Å). The fractional large pore flow (Jv_L/GFR) increased in the order: rat glomerulus< HF-Revaclear®<HCO. Analyzed according to the log-normal distributed (+ shunt) model the HF-Revaclear® filter and the HCO filters showed markedly higher pore size heterogeneity than did the rat GFB (Table 3b).

Individualized dialyzer permeability with respect to molecular species (proteins, Ficoll and dextran)

Fig 3a and 3b and Table 4 demonstrate each membrane separately; Fig 3a the HF-Revaclear® membrane and Fig 3b the HCO membrane, demonstrating that for each membrane, permeability with respect to the species of the molecular test probe increased in the order of: proteins<Ficoll<dextran. For HF-Revaclear® there was thus a significant difference among θ for Myo, θ for $Ficoll_{20Å}$ and θ for $Dextran_{20Å}$ (Table 4a). For HCO the same separation was seen, i.e. θ for myoglobin<θ for $Ficoll_{20Å}$<θ for $Dextran_{20Å}$. The same pattern was also noted for albumin, as shown in Table 4b, for both the HF-Revaclear® and the HCO membrane.

Two pore modeling

The best curve fits of θ *vs.* a_e for Ficoll and dextran according to the two-pore model were obtained using the parameters listed in Table 2a and 3a. The small pore radii were generally larger in the HCO filter than in the HF-Revaclear® filter, regardless of test probe, and the fractional fluid flow through the large pores (Jv_L/Jv) for dextran was markedly higher than for Ficoll for both the dialyzers. The latter parameter was in turn much higher than that for the rat GFB. Thus, artificial dialyzers with high

cut-off properties leak much more macromolecules (albumin) than the rat GFB. With respect to pore surface area, the HCO filter showed a much higher $A_0/\Delta X$ than the HF-Revaclear® (twice the value), the latter having, fortuitously, similar $A_0/\Delta X$ as one gram of rat kidney. Also, the UF coefficient (L_pS) of both the HF-Revaclear® and the HCO dialyzer Revaclear® was of similar order of magnitude as that for one gram of kidney 0.054±0.0023 mL/min/mmHg and 0.109±0.004 mL/min/mmHg, respectively, *vs.* ~0.08-0.13 mL/min/mmHg per gram rat kidney. $A_0/\Delta X$ was here obtained from sieving data by the curve fitting procedure, but can also be calculated from L_pS (UF-coefficient), since the "unrestricted pore area for diffusion" (A_0) and "the membrane thickness" (ΔX), as well as the (small) pore radius (r_s) are all determinants of both L_pS and PS (solute diffusion capacity or mass transfer coefficient; see eq. 8 in [10]). For the HF-Revaclear® dialyzer and the rat GFB there was an excellent agreement between $A_0/\Delta X$ obtained by curve fitting of Ficoll sieving data and $A_0/\Delta X$ calculated from the independently determined L_pS value.

Log-normal distributed+ shunt model and Peclet number analysis

A log-normal distributed + shunt model was also used to analyze the Ficoll and dextran θ *vs.* a_e data from the experiments (Appendix I). Results are listed in Table 2b and 3b. The average pore radius (u) was found to be much lower in the distributed model, the lowest radius being found for the HF-Revaclear® for both Ficoll and dextran. The distribution parameter of the pore radius (s) was markedly higher in the two artificial membranes compared to that for the rat GFB for both Ficoll and dextran. Furthermore, the distributed + shunt model tended to markedly overestimate $A_0/\Delta X$. A Peclet number analysis was carried out using the distributed model for the different membranes investigated. Figure 4a and 4b show that the Peclet number *vs.* a_e were closely similar among the three different membranes for both Ficoll (Fig 4a) and dextran (Fig 4b).

Comparison of "goodness of fit" for the two-pore model vs. the log-normal distributed+ shunt model

The "goodness of fit" of data to the two membrane models employed was found to be generally better for the two-pore model compared with the log-normal distributed +shunt model, with an exception for the behavior of dextran in the rat renal glomerulus (Table 5).

Discussion

The essential result of the present study is that the two synthetic dialyzing membranes investigated, exhibiting widely differing sieving properties, generally showed a lower selectivity (higher θ:s) to large size polysaccharides and to albumin and a higher degree of heteroporosity than the rat glomerular filtration barrier. In terms of the two-pore theory of membrane permeability [10], both artificial membranes investigated thus exhibited a markedly increased number of large pores (radius ~120Å) than the GFB. However, for molecules of radii <50Å the small pore radius was either lower (HF-Revaclear®) or higher (HCO) than that for the rat GFB. Furthermore, according to the log-normal distributed + shunt model a significantly higher degree of pore size variation was found for the synthetic membranes than for the GFB. The HCO filter investigated showed sieving coefficients to solutes of the size of small proteins (myoglobin) similar to those of the GFB, but at the expense of a markedly elevated permeability to high MW solutes. As for the rat GFB, the permeability of the synthetic membranes was dependent upon the molecular species investigated, the permeability increasing in the order: proteins<Ficoll<dextran. Also, similar to the GFB, there was a rather good consistency between $A_0/\Delta X$ assessed from Ficoll sieving data analyzed according to the two-pore model and that independently obtained from the ultrafiltration coefficient (LpS). In that respect, the two synthetic membranes investigated and the GFB in many respects behaved as "simple" membranes, as opposed to most continuous capillary walls [10, 17, 18].

At present it is well established that dextran, due to its random-coil nature, is hyperpermeable across the GFB [1-3, 19, 20]. Recent studies, both *in vivo* and *in vitro*, strongly indicate that this in some respects also applies to Ficoll, a highly cross-linked copolymer of sucrose and epichlorohydrine, namely when the molecules approach the size of the size-limiting structures ("pores") of the restricting

barrier [3, 5, 6, 12]. This has been demonstrated for Ficoll molecules of radius 25-45 Å ($Ficoll_{25Å-45Å}$) across the GFB (exhibiting a small pore radius (r_s) of ~37.5Å when measured for proteins, but a r_s of ~45-47Å when measured for Ficoll) and was found also for the present synthetic dialyzers with similar molecular cut-off characteristics as the GFB [6]. For the present uncharged artificial membranes $Ficoll_{36Å}$ was thus much more permeable than albumin, the latter passing the GFB mainly through rare large pores according to the "two-pore" theory. For $Ficoll_{50Å-75Å}$, also permeating the GFB (and the present synthetic membranes) through large pores, such Ficoll hyperpermeability was not evident, however. Thus, despite the hyperpermeability of $Ficoll_{36Å}$ (compared to albumin), θ for albumin could be accurately predicted from the Ficoll large pore curve by retropolating the large pore θ *vs.* a_e curve (for $Ficoll_{50Å-75Å}$) to a_e = 36Å. This indicates that $Ficoll_{50Å-75Å}$ and albumin actually share the same (large) pores for their passage, and that large Ficoll molecules (if not exceeding 80Å in radius) do not show anomalous membrane permeability.

The hyperpermeability of dextran, and to some extent of Ficoll, has been discussed at some length in previously [3, 14, 16, 17]. Current mathematical models describing restricted solute transport through porous or fibrous barriers assume that the probe molecules behave as rigid spheres and not as flexible macromolecules. Whereas globular proteins seem to behave in a way not very different from hydrated hard spheres, polydisperse dextran, and to some extent Ficoll, show *in vitro* SE-radii that are larger for any molecular weight than for proteins. It thus seems that polysaccharides show an increased molecular "extension", paralleling the increased permeability across the rat GFB [3, 16]. Furthermore, it seems that the size of flexible macromolecules, such as most polysaccharides, is not as constant as that of rigid macromolecules [21-23]. For the glomerular filter the "pores" are short and straight, allowing a high permeability for molecules with high flexibility or asymmetry. Obviously, the critical size separation layer in the two artificial membranes investigated must be thin, on the order of a μm, to allow for the hyperpermeability of dextran. This is in opposition to the behavior of the blood-tissue-barriers in most continuous capillary beds, showing complex, paracellular, winding exchange pathways for solute transport [10, 17]. Paradoxically, it thus seems that the artificial membranes

investigated and the GFB behave more like "simple membranes" than, e.g., most peripheral microvascular walls [18].

Due to the apparent hyperpermeability of dextran across the membranes investigated, not only was the average membrane small pore radius (r_s) overestimated, but also the value of $A_0/\Delta X$ determined from dextran sieving data. Furthermore, due the falsely high values of r_s determined with dextran, the $A_0/\Delta X$ value calculated from L_pS was underestimated (due to the fact that the $A_0/\Delta X$ estimate is reciprocal to the pore radius squared; cf. eq 8 in [10]). Thus, in the dextran experiments there was a large discrepancy between $A_0/\Delta X$ obtained from sieving data and that calculated from L_pS. Another contributing factor causing an inflated $A_0/\Delta X$ is the phenomenon of concentration polarization, which was particularly evident in the HCO filter (see below). Thus, concentration polarization will tend to markedly increase the diffusion gradient across the membrane, and hence, $A_0/\Delta X$, while reducing the L_pS. Also, the log-normal distributed (+ shunt) model is known to markedly overestimate $A_0/\Delta X$ [4, 24]. Conceivably, this is due to the distributed nature of the pore model, which will overestimate L_pS due to a tendency of the model to "inflate" fluid flows through the larger pores of the pore distribution. This is owing to the fact that the fluid flow is dependent on r_s^4 (and not r_s^2). Despite this obvious shortcoming of the log-normal distributed (+ shunt) model it was still deemed accurate enough for evaluating the pore size distribution of the membranes investigated.

To be able to make realistic comparisons of sieving properties among different sieving membranes, the relationships between the solutes' convective clearance, and their diffusive clearance (the latter determined by $A_0/\Delta X$) in terms of Peclet (Pe) numbers, should be of similar order of magnitude for the membranes investigated. Indeed, a Pe-number analysis demonstrated closely similar Pe-values *vs.* a_e for all three membranes compared. Thus, the filters investigated were at reasonably similar conditions with respect to the relative rates of filtration/diffusion and therefore sieving data could be directly compared among the different membranes.

In the short-term experiments of the present study the dialyzer priming period was just 5-8 min. During this period there is no reason to believe that there was any marked clogging (fouling) of the membranes except with Myo. Myo was found to markedly bind to the HCO membrane (15% binding, as retropolated from the 5 and 30 min distribution spaces) and then continuously adsorb to both the HCO and the HF-Revaclear® membranes, at a similar rate for both. Despite of this adsorption, θ for myoglobin was high and more or less identical between the HCO membrane and the GFB. Although a very small fraction of albumin was bound to the membrane, there was no continuous adsorption of albumin over time affecting its permeability. Furthermore, as discussed above, it seems that θ for albumin (a_e=36Å) could be predicted from back-extrapolating the large-pore Ficoll sieving curve (to 36Å) for both the synthetic filters investigated. This again indicates that the impact of albumin binding to the membrane was quite small. The high level of adsorption of Myo to the two synthetic membranes is probably, at least partly, due to the absence of "cold" (unlabeled) myoglobin in the perfusate (plasma + 0.9% saline). This is in contrast to the presence of a high fraction of "cold" albumin in the diluted plasma perfusate used, markedly reducing the ability of radiolabeled albumin to bind to the membrane.

The present synthetic membranes were investigated during conditions mimicking clinical hemofiltration using a perfusate containing diluted plasma. Because of that, there may have been concentration polarization of solutes in the membranes, including a sharp rise in colloid osmotic pressure in close proximity to the membrane surface, most notably for the HCO membrane. This is obvious from the L_pS values given by the manufacturer, which were sevenfold higher for the HCO membrane and threefold higher for the HF-Revaclear® membrane, when assessed for protein-free perfusate (saline). Overall, the upconcentration of the perfusate at the venous side of the filter was approximately (only) 20% and 10% for the HCO and the HF-Revaclear®, respectively, as measured using RISA. Hence, the colloid osmotic pressure rise at the "venous" end was calculated to be 1-2.5

mmHg. In the absence of concentration polarization this change in colloid osmotic pressure is not enough to cause filtration equilibrium early in the fiber (reducing the effective fiber filtering surface area). In the presence of concentration hyperpolarization, however, filtration equilibrium is indeed possible and highly likely, even in proximal parts of the filter. The consequence of a reduced filtering surface area would be that L_pS and $A_0/\Delta X$ are about equally reduced with little impact on the Pe number. In fiber sections with a high filtration rate, however, the presence of concentration polarization will markedly reduce the effective transmembrane pressure and also increase the solute diffusion gradient. This may be the major reason why a discrepancy was seen between L_pS (underestimated) and $A_0/\Delta X$ (overestimated) for Ficoll in the HF-Revaclear® membrane. Finally, however, it should be pointed out that, despite the fact that concentration polarization most likely occurred in these filters, this phenomenon is regularly occurring in clinical hemofiltration.

The remarkably low sieving coefficient for albumin and those for large Ficoll molecules (50-80Å in radius) in the GFB, reflects the fact that the relative number of large pores (shunt pathways) in the GFB can be calculated to be on the order of only 1 per 10^7 of the total population of pores (cf. eq 27 in Ref [10]). Hence, the most important finding of the present study is that the relative number of large pores was found to be much lower for the GFB than for the synthetic dialyzers investigated. According to the heteroporous + shunt model it was further evident that the two synthetic membranes investigated were much more heteroporous than the GFB. Thus, the normal GFB is apparently a rather homoporous membrane with only a very low fraction of large pores, whereas the synthetic membranes investigated showed a high degree of heteroporosity, with a relatively greater abundance of shunt-like pores. In that sense, the synthetic membranes apparently mimic the nephrotic GFB [25]. The clinical implication of these properties is that leakage of albumin to the dialysate has to be accounted for when the HCO filters are used for e.g. the removal of small proteins, such as light chains, from the circulation, at least during long treatment times. Hence, a major challenge to producers of artificial dialyzing membranes is to produce membranes with a high degree of homoporosity with only very few shunt pathways present.

In conclusion, the present study aimed at investigating the size-selective properties of two synthetic dialyzing membranes and at comparing them with those of the rat GFB using sieving techniques and sieving probes previously extensively employed in our laboratory. Similar to the GFB, the two artificial dialyzers investigated showed a bimodal (small pore/large pore) selectivity. The sieving coefficients for solutes <50Å in radius increased in the order: HF-Revaclear®<rat glomerulus<HCO. Although the HCO filter showed the highest cut-off, suitable for removal of small proteins from the circulation, this occurred at the expense of a markedly increased permeability to albumin and Ficoll molecules >50Å in radius. Furthermore, the two artificial membranes showed a greater heteroporosity than the GFB. Similar to the GFB, however, the two synthetic membranes investigated showed a membrane permeability which was dependent on the molecular species probed, increasing in the order: proteins<Ficoll<dextran. In that respect, the two synthetic membranes investigated showed striking similarities with the rat GFB, and all three membranes differed in many ways functionally from the walls of systemic microvessels [18].

Appendix I

Log-normal distributed + shunt model

A heteroporous model [4] with a log-normal distributed population of pores in parallel with a non-selective shunt was used to describe the experimental data in addition to using the two-pore model (TPM) [10, 14]. The theoretical fractional clearance values (θ_{model}) were fitted to the experimental data using the Levenberg-Marquardt non-linear least-squares algorithm to calculate values of parameters u (average pore radius), s (distribution parameter), $A_0/\Delta X$ (total pore area over diffusion path length), and f_L (fractional fluid flow through shunts) that minimizes the sum of squares using the well-known MINPACK library [26]. A log-transform was used to compensate for the large (five orders of magnitude) range of the experimental data. The theoretical θ data values (θ_{model}) were calculated from the non-linear global convection/diffusion equation [10] with a non-selective shunt (f_L = 1-f_D) according to

$$\theta_{model} = f_D \frac{1-\sigma}{1-\sigma e^{-Pe}} + f_L \quad (1)$$

where the Peclet number (Pe) is defined by

$$Pe = f_D \frac{GFR(1-\sigma)}{PS} \quad (2)$$

where PS is the permeability-surface area product and σ is the reflection coefficient. In an n-pore model such as the TPM the pore radii r_i are assumed to be *discretely* distributed according to their respective weights $\alpha_i = L_{p,i}/L_p$ so that the total reflection coefficient for a n-pore barrier is given by

$$\sigma_n(a_e) = \sum_{i=1}^{n} \frac{L_{p,i}}{L_p} \sigma_h(a_e, r_i) \quad (3)$$

where the hydrodynamic estimation for the reflection coefficient is given by

$$\sigma_h(a_e, r_i) = 1 - \frac{(1-\lambda)^2(2-(1-\lambda)^2)(1-\frac{\lambda}{s})}{1-\frac{\lambda}{s}+\frac{2\lambda^2}{s}} \quad (4)$$

and $\lambda = a_e/r$ is the solute radius (a_e) to pore radius ratio [10]. In the distributed model used in this paper it is assumed that the pore radii are *continuously* distributed according to the ordinary log-normal distribution

$$g(r) = \frac{1}{\sqrt{2\pi}\, r \ln s} e^{-\frac{1}{2}\left(\frac{\ln r - \ln u}{\ln s}\right)^2} \quad (5)$$

where u is the mean pore radius and s is the distribution spread. According to Poiseuille's law the hydraulic conductivity through pores in the distribution of a fixed radius R is given by

$$L_{p,R} = \frac{\pi R^4}{8\eta\Delta x} g(R) \quad (6)$$

where η is the viscosity of water at 37ºC and Δx is the thickness of the barrier. Hence, the hydraulic conductivity for the entire barrier is

$$L_p = \int_0^\infty \frac{r^4\pi}{8\eta\Delta x}\, g(r)\, \mathrm{dr} \quad (7)$$

Analogous to the discrete case the reflection coefficient for the distributed model is thus

$$\sigma = \int_0^\infty \frac{L_{p,R}}{L_p} \sigma_h(a_e, r)\, dr = \frac{\int_0^\infty r^4 g(r)\sigma_h(r)dr}{\int_0^\infty r^4 g(r)dr} \quad (8)$$

Using a similar argument as for the solute reflection, the capillary diffusion capacity for the distributed model can be calculated from

$$PS = \frac{\int_0^\infty r^2 PS_h(r) g(r) dr}{\int_0^\infty r^2 g(r) dr} \quad (9)$$

where the homoporous permeability-surface area product is given by

$$PS_h = D_s \frac{A_0}{\Delta x} \frac{(1-\lambda)^{9/2}}{1 - 0.3956\lambda + 1.0616\lambda^2} \quad (10)$$

and D_s is the free diffusion coefficient, A the apparent (effective) pore area and A_0 the total cross-sectional pore area [10]. All improper integrals were evaluated numerically using a subroutine package for automatic integration [25].

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Table 1 - Experimental parameters

	HF-Revaclear®	HCO
Filtration rate, mL/min	0.85 ± 0.04	1.50 ± 0.06
Mid capillary pressure, mmHg	22.6 ± 0.23	20.8 ± 0.14
Net filtration pressure, mmHg	15.6 ± 0.23	13.8 ± 0.14
Filtration coefficient, $mL \times min^{-1} \times mmHg^{-1}$	0.054 ± 0.002	0.109 ± 0.004

Values are given as means ± SE.

GFR = glomerular filtration rate

Table 2a - Two pore parameters, Ficoll

	Rat glomerulus – Ficoll [15]	HF-Revaclear® – Ficoll n=6	HCO – Ficoll n=7
Small-pore radius (r_s), Å	45.7 ± 0.2	43.5 ± 0.33**	50.4 ± 0.23**
Large-pore radius (r_L), Å	138.1 ± 3.76	110.1 ± 6.79	166.28 ± 10.75
$\alpha_L \times 10^3$	0.041 ± 0.008	1.51 ± 0.88**	8.58 ± 0.93**
$J_{vL}/GFR \times 10^3$	0.12 ± 0.026	1.91 ± 0.34**	12.9 ± 1.47**
$A_0/\Delta X$, cm/g $\times 10^{-5}$ †	3.76 ± 0.59	2.96 ± 0.41*	8.36 ± 0.43**
Calculated $A_0/\Delta X$, cm/g $\times 10^{-5}$ (from L_pS) †	2.69 ± 0.38 (3.74 ± 0.52) ††	2.06 ± 0.05	3.12 ± 0.13
Filtration coefficient, mL×min^{-1}×mmHg^{-1}×g^{-1} †	0.08 ± 0.01	0.054 ± 0.002*	0.109 ± 0.004*

Values are given as mean ± SE. Symbols are given in the text.

r_s = small pore radius; r_L = large pore radius; α_L = fractional ultrafiltration coefficient accounted for by large pores; Jv_L/GFR = fractional fluid flow through large pores; GFR = glomerular filtration rate; $A_0/\Delta X$ effective pore area over unit diffusion path-length. Statistical differences between GFB and filter devices are as follows: *p<0.05, **p<0.01 and ***p<0.001. † refers to g kidney for the rat glomerulus. †† if calculated for a filtration coefficient of 0.11 ml x min^{-1} x mmHg^{-1} per gram kidney [27].

Table 2b – Log-normal distributed + shunt model, Ficoll

	Rat Glomerulus – Ficoll	HF-Revaclear® – Ficoll n=6	HCO – Ficoll n=7
Pore radius (u), Å	35.2 ± 0.82	25.3 ± 1.94**	36.2 ± 1.79
Distribution parameter (s)	1.16 ± 0.01	1.28 ± 0.03*	1.22 ± 0.02*
$A_0/\Delta X$, cm/g x 10^{-5}	23.9 ± 6.28	32.9 ± 6.41	31.5 ± 3.93
Shunt x 10^3 (Jv_{shunt}/GFR or Jv_{shunt}/Jv)	0.035 ± 0.00	0.7 ± 0.02**	8.0 ±1.7**

u = pore radius (Å); s =distribution spread of the pore radius; $A_0/\Delta X$ = effective pore area over unit diffusion path-length.

Statistical differences between rat glomerulus and synthetic membranes: *p<0.05, **p<0.01 and ***p<0.001.

Table 3a - Two pore parameters, dextran

	Rat glomerulus – dextran [16]	HF-Revaclear® – dextran n=6	HCO – dextran n=7
Small-pore radius (r_s), Å	64.2 ± 1.02	46.7 ± 1.50**	62.1 ± 1.14
Large-pore radius (r_L), Å	119.0 ± 3.0	121.7 ± 12.35	146.8 ± 7.55**
$\alpha_L \times 10^3$	1.15 ± 0.38	8.63 ± 1.41**	56.4 ± 15.1**
$J_{vL}/GFR \times 10^3$	3.44 ± 1.13¤	10.9 ± 1.68*	80.8 ± 21.8**
$A_0/\Delta X$, cm/g $\times 10^{-5}$ †	4.58 ± 0.99	4.05 ± 0.35	8.31 ± 0.81*
Calculated $A_0/\Delta X$, cm/g $\times 10^{-5}$(from L_pS) †	1.44 ± 0.34 (1.98 ± 0.47) ††	1.66 ± 0.25	2.08 ± 0.16
Filtration coefficient mL×min^{-1}×mmHg^{-1}×g^{-1} †	0.08 ± 0.02	0.054 ± 0.002	0.109 ± 0.004

Values are given as mean ± SE. Symbols are given in the text.

¤ = calculated value from Asgeirsson et al. 2007 [16]

r_s = small pore radius; r_L = large pore radius; α_L = fractional ultrafiltration coefficient accounted for by large pores; Jv_L/GFR = fractional fluid flow through large pores; GFR = glomerular filtration rate; $A_0/\Delta X$ effective pore area over unit diffusion path-length. Statistical differences between GFB and filter devices are as follows: *p<0.05, **p<0.01 and ***p<0.001. † refers to g kidney for the rat glomerulus. †† if calculated for a filtration coefficient of 0.11 ml x min^{-1} x mmHg^{-1} per gram kidney [27].

Table 3b – Log-normal distributed + shunt model, dextran

	Rat Glomerulus – Dextran	HF-Revaclear® – Dextran n=6	HCO – Dextran n=7
Pore radius (u), Å	53.3 ± 1.55	28.3 ± 1.42**	43.7 ± 2.24*
Distribution parameter (s)	1.16 ± 0.008	1.28 ± 0.020**	1.26 ± 0.007**
$A_0/\Delta X$, cm/g x 10^{-5}	6.26 ± 1.20	26.39 ± 4.29**	21.23 ± 3.38*
Shunt x 10^3 (Jv_{shunt}/GFR or Jv_{shunt}/Jv)	0.4 ± 0.1	2.8 ± 0.9*	31.0 ± 8.9**

u = pore radius (Å); s =distribution spread of the pore radius; $A_0/\Delta X$ = effective pore area over unit diffusion path-length.

Statistical differences between rat glomerulus and filter devices are as follows: *p<0.05, **p<0.01 and ***p<0.001.

Table 4a – Sieving coefficients to myoglobin, Ficoll$_{20Å}$ and dextran$_{20Å}$

	Rat Glomerulus	HF-Revaclear®	HCO
θ Myoglobin	$0.73 \pm 0.05^{\Delta}$	0.29 ± 0.011 (0.31 ± 0.012) †	0.73 ± 0.035
θ Ficoll 20 Å	$0.82 \pm 0.003^{\circ}$	0.78 ± 0.007	0.90 ± 0.001
θ dextran 20 Å	$0.99 \pm 0.003^{\blacklozenge}$	0.87 ± 0.03	0.94 ± 0.002

Table 4b – Sieving coefficients to albumin, Ficoll$_{36Å}$ and dextran$_{36Å}$

	Rat glomerulus	HF-Revaclear®	HCO
θ Albumin	$4.71 \times 10^{-4} \pm 1.56 \times 10^{-5}$ °	$2.60 \times 10^{-3} \pm 9.32 \times 10^{-5}$	0.014 ± 0.002
θ Ficoll$_{36Å}$	$0.06 \pm 0.001^{\circ}$	0.023 ± 0.002	0.15 ± 0.003
θ dextran$_{36Å}$	$0.40 \pm 0.01^{\blacklozenge}$	0.072 ± 0.02	0.43 ± 0.03

Values are given as means ± SE.

† = corrected for binding (trapping)

$^{\Delta}$ = data from Lund et. al 2003 [12]

$^{\circ}$ = data from Axelsson et. al 2009 [28]

$^{\blacklozenge}$ = data from Asgeirsson et. al 2007 [16]

Table 5. Goodness of fit. χ^2 tests for two-pore fit *vs.* log-normal distributed plus shunt model fit

	Two-pore model χ^2	Distributed model χ^2
Glomerulus - Ficoll	0.58 ± 0.07	1.65 ± 0.19**
HF-Revaclear® - Ficoll	0.18 ± 0.04	0.35 ± 0.05*
HCO - Ficoll	0.11 ± 0.01	0.15 ± 0.01**
Glomerulus - dextran	1.13 ± 0.30	0.55 ± 0.37
HF-Revaclear® - dextran	0.15 ± 0.03	0.28 ± 0.06
HCO - dextran	0.14 ± 0.02	0.22 ± 0.04

Statistical differences between two-pore model and distributed model are as follows: *p<0.05, **p<0.01 and ***p<0.001.

Figure legends

Figure 1

Schematic representation of the perfusion system used for testing the HCO and the HF- Revaclear® filters.

Figure 2a

Sieving coefficients (θ) *vs.* SE-radius (a_e) for FITC-Ficoll, for the HCO filter (solid line and upper curve) and the HF-Revaclear® dialyzer (hatched line). For comparison, data from sieving experiments in Ref. [28] are included (dotted line). θ for albumin for the HF-Revaclear® (filled square), for the HCO filter (filled circle), and for the rat GFB (reproduced from Ref. [15]; filled diamond) are also included. θ for Myo for HF- Revaclear® (black pentagon), for HCO (open star), and for the rat GFB (Ref. [12]; open pentagon) are also indicated.

Figure 2b

Sieving coefficients *vs.* a_e for FITC-dextran for the HCO filter (finely hatched line and upper curve) and for the HF-Revaclear® filter (broken-hatched line and lower curve), and for the rat GFB (dotted line; reproduced from Ref. [28]). Symbols for albumin: filled diamond (rat GFB), filled square (HF-Revaclear®) and filled circle (HCO), and for Myo are the same as in Fig. 2.

Figure 3a

θ *vs.* a_e-curves for FITC-dextran (hatched-broken line and upper curve) and FITC-Ficoll (hatched line and medium curve) for one and the same filter, namely HF-Revaclear®. The sieving curve for FITC-Ficoll (dotted line) for the rat GFB (reproduced from Ref. [28]) is also indicated.

Figure 3b

Sieving curves for the HCO filter using either FITC-dextran (finely hatched line and upper curve) or Ficoll (solid line and medium curve). The Ficoll sieving curve for the rat GFB (reproduced from Ref. [28]) is also shown for comparison (dotted line).

Figure 4a

Figure shows the Peclet numbers of the distributed model vs. molecular radius (a_e) for experiments using Ficoll. Rat Glomerulus is represented by the dotted line, HF-Revaclear® by the dashed line and HCO by the solid line.

Figure 4b

Figure shows the Peclet numbers of the distributed model vs. molecular radius (a_e) for experiments using dextran. Rat Glomerulus is represented by the short dotted line, HF-Revaclear® by the dash dotted line and HCO by the short dashed line.