Wall permeability of isolated small arteries. Role of the endothelial surface layer
van Haaren, P.M.A.

Citation for published version (APA):
van Haaren, P. M. A. (2003). Wall permeability of isolated small arteries. Role of the endothelial surface layer
s.l.: s.n.

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s),
other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating
your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask
the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam,
The Netherlands. You will be contacted as soon as possible.
chapter 3:

MODELING MACROMOLECULAR TRANSPORT ACROSS THE ARTERIAL WALL; ESTIMATION OF PARAMETERS INFLUENCING DIFFUSION AND CONVECTION
3.1 ABSTRACT

This study describes a model for interpretation of macromolecular solute transport properties of the arterial wall. Solute transport was characterized by diffusion and convection. The arterial wall model consisted of two different layers: (1) the arterial wall including the endothelial cell layer (EC) was considered as one homogeneous layer; (2) the endothelial surface layer (ESL) that covers the luminal side of the endothelium. Diffusion, driven by concentration gradients and electrostatic forces, was characterized by the mobility coefficient $M$, dimension $m^2s^{-1}$. Convection was characterized by the effective solute velocity $v_e$, derived from the fluid flux and the reflection coefficient of the arterial wall to the solute. Solution of diffusive and convective transport equations for fluorescent solutes resulted in calculated fluorescence distributions over the arterial wall as developing over time. Kinetics of these fluorescence distributions were quantified by calculation of the position at which the fluorescence equals half-maximal value ($X_{50}$), which can be seen as a measure for the position of the dye front over time. Calculated $X_{50}$'s were compared with experimental $X_{50}$'s derived from fluorescence distributions of fluorescein-isothiocyanate (FITC)-labeled dextrans (FITC-As) of different sizes as measured in cannulated small arteries by means of confocal microscopy. We found that an ESL that may be as thick as 3-8 μm forms a profound barrier to solute transport. Mobility coefficients in the ESL were in the order of $10^{-6}$-$10^{-4}$ of the free diffusion coefficients. Reflection coefficients to convective solute transport were close to unity. The influence of the rest of the arterial wall on solute transport characteristics was negligible. In conclusion, we were able to predict FITC-A transport characteristics across the ESL and the arterial wall with a model that was based on diffusive and convective transport equations.

3.2 INTRODUCTION

It is the established paradigm that pores of different sizes and vesicles form the main pathway for solute transport across the endothelial cell layer (3;6;12;14-16;22). According to this theory endothelial cells, pores and vesicles are covered with a fibrous matrix contributing to the permselective nature of the vessel wall (3;6;12;22). Solute transport over this composed barrier can be described by three processes: diffusion, convection and vesicular transport.

In recent years the concept of solute transport has been evolved in the sense that the fibrous matrix concept has been extended to what is described as the endothelial surface layer (ESL)
or glycocalyx (4:8-10;13:17-20), a layer that extends up to 0.5-1 μm into the lumen of capillaries (2:13:18-20). By studying the distribution of different fluorescent-labeled solutes, plasma and blood cells within capillaries it has been shown that permeation of solutes into the ESL is strongly dependent on size and charge of the solutes (20). In an experimental study we investigated transport properties of fluorescein-isothiocyanate (FITC)-labeled dextrans (FITC-Δs) of different sizes in cannulated small arteries by means of confocal microscopy (chapter 2). We found that an ESL, with a thickness as large as 2-3 μm, is confining large anionic molecules (FITC-Δ148; 148 kD) to a core volume inside the arteries. Smaller FITC-Δs were able to slowly penetrate this layer, and the small FITC-Δ4 (4 kD) was able to accumulate in the arterial wall within 30 minutes.

The purpose of the present study was to interpret these experimental findings in terms of effective diffusion coefficients, denoted as mobility coefficients, effective solute velocities for convective transport and the dimensions of the different layers of which the arterial wall consists.

The model is based on diffusive and convective solute transport equations, resulting in calculated fluorescence distributions over the wall developing over time.

3.3 MATERIALS AND METHODS

3.3.1 Experiments

A full description of the experiments, in which the data that was used for verification of the model was collected, is given in chapter 2. Briefly, kinetics of arterial filling with FITC-dextrans (FITC-Δs) of different sizes and accumulation in the endothelium of the fluorescent membrane probe Dil (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) were recorded in isolated cannulated rat mesenteric small arteries (average internal diameter ~150 μm, as determined from Dil fluorescence profiles) by means of confocal laser scanning microscopy (CLSM). Rapid inflow of fluorescent tracers into the arteries was accomplished by using a double-barreled θ-pipet. Radial fluorescence profiles of all confocal recorded images were analyzed in a region spanning from 10 μm abluminally to 15 μm luminally of the endothelium. Average results (n=7 experiments per FITC-Δ used) of the dynamics of the fluorescence profiles within this 25 μm wide region around the endothelium are summarized in figure 3.1. The kinetics of these fluorescence profiles were quantified by calculation of the position at which the fluorescence equals half-maximal value (X0), which can be seen as a measure for the position of the dye front over time. Average results of the time dependence of X0 for the different FITC-Δs are shown in figure 3.2.
Figure 3.1: Dynamics of average normalized fluorescence in a region from 10 μm abluminally of the endothelium to 15 μm luminally of the endothelium for arteries perfused with FITC-Δ148 (A), FITC-Δ50 (B) or FITC-Δ4 (C). Fluorescence was normalized to luminal fluorescence in the center of the arteries. Position of the endothelium was determined from Dil fluorescence profiles and is depicted with the dotted line. Negative x-values indicate abluminal from the endothelium (arterial wall), positive x-values indicate luminal from the endothelium. Profiles are mean values ± SEM (n=7 experiments for each FITC-Δ). Reprinted from fig. 2.3.

Figure 3.2: Time dependence of the position at which the fluorescence profiles (see fig. 3.1) equal their half-maximal value, X_{50}.
3.3.2 Outline of parameter estimation procedure

Using diffusive and convective solute transport equations in a theoretical model of the arterial wall as depicted in figure 3.3, we first calculated solute concentration distributions for a wide set of parameter values. These parameters include the dimensions of the arterial wall layers, the mobility coefficient that determines diffusive transport and the solute velocity for convective transport. Next, the calculated concentration distributions were convolved with an optical 'line-spread function', reflecting the optical transfer function of the CLSM system, to obtain calculated fluorescence profiles. From these fluorescence profiles we calculated as a function of time the position ($X_{50}$) at which the fluorescence reaches 50% of the value at mid-luminal position. By fitting calculated $X_{50}$-values to the experimental $X_{50}$-values as a function of time, we estimated the physical parameters that describe diffusive and convective solute transport over the arterial wall. This procedure was performed for three cases: 1) diffusion-limited transport (no convection), 2) convection-limited transport (no diffusion), 3) combined, diffusive and convective transport.

3.3.3 Theoretical model.

Three barrier layers of the arterial wall are indicated in figure 3.3: the endothelial surface layer (ESL), the endothelial cell layer (EC), and the rest of the arterial wall, abluminally of the EC. In real arteries the latter consists of the internal elastic lamina, smooth muscle cells, adventitia and other adjacent tissue. The model considers two homogeneous layers: 1) the ESL, 2) the endothelium and the rest of the arterial wall (subscript w).

Concentration profiles. Considering diffusive and convective solute transport, the development of solute concentration distributions over time can be predicted, according to (5.11):

$$\frac{\partial C}{\partial t} (r, t) = M(r) \cdot \frac{\partial^2 C}{\partial r^2} (r, t) - v_s (r) \cdot \frac{\partial C}{\partial r} (r, t)$$  \hspace{1cm} (3.1)

where:

- $C$ = solute concentration [mol·m$^{-3}$]
- $r$ = radial position [m] ($r = 0$ represents the center of the lumen)
- $t$ = time [s]
- $M$ = mobility coefficient of the solute [m$^2$·s$^{-1}$]
- $v_s$ = $v_s (1 - \sigma)$, effective solute velocity for convective transport [m·s$^{-1}$]
- $v_f$ = velocity of fluid (water) moving through the arterial wall [m·s$^{-1}$]
- $\sigma$ = reflection coefficient of the arterial wall to the solute [-].
Figure 3.3: Schematic representation of an artery, used in the presented model. The artery consisted of an endothelial cell layer (EC) at radial position $R_{EC}$, which is covered on the luminal side with an endothelial surface layer (ESL; inner radial position $R_{ESL}$) and on the abluminal side by the rest of the arterial wall ($w$; outer radial position $R_w$). An enlargement of the arterial wall region studied in radial direction ($r$) is shown in the bottom, where the different layers and the properties determining solute transport inside these layers are indicated ($D$: diffusion coefficient; $M$: mobility coefficient; $O$: reflection coefficient; $d$: thickness). Also shown are the shapes of the Point Spread Function (PSF) of the confocal microscope in $x$- and $z$-direction.

PSF(z)
Both concentration gradients and electrostatic forces, due to the charge of the ESL and of the FITC-A, contribute to diffusive solute transport (17). Combining these effects, we used the so-called mobility coefficient $M \ [m^2 \cdot s^{-1}]$ rather than the conventional diffusion coefficient $D \ [m^2 \cdot s^{-1}]$ to describe diffusive transport processes, in order to prevent confusion.

Equation 3.1 was solved using routines written in Matlab (The Mathworks Inc., USA), using the following boundary conditions:

- Stepwise filling of only the lumen of the artery with FITC-A at $t = 0$ min:
  \[
  C(r, t) = 0 \quad \text{for} \ r \geq R_{ESL}, \ \text{for all} \ t < 0,
  \]
  \[
  C(r, 0) = C_{lum} \quad \text{for} \ r < R_{ESL},
  \]
  \[
  C(r, 0) = 0 \quad \text{for} \ r \geq R_{ESL}
  \]

where:

$C_{lum}$ = luminal FITC-A concentration as applied in the experiments (chapter 2)

$R_{ESL}$ = radial position of the luminal ESL border (see fig. 3.3).

- Solute concentration outside the artery was kept equal to zero, since continuous superfusion was applied in the experiments (chapter 2):
  \[
  C(r, t) = 0 \quad \text{for} \ r > R_w, \ \text{for all} \ t
  \]

where:

$R_w$ = radial position of the outer abluminal border of the arterial wall (see fig. 3.3)

Solving equation 3.1 with boundary conditions 3.2 and 3.3 resulted in calculated concentration distributions for a given set of parameters. These concentration distributions were transformed into fluorescence profiles using a line-spread function as described below. From such fluorescence profiles the $X_{50}$-values was calculated for a wide range of parameter values and compared with experimental $X_{50}$-values, resulting in estimations of the physical parameters.

### 3.3.4 Point- & line-spread functions.

To compare calculated concentration distributions with measured fluorescence intensity profiles, the diffraction due to the optical measurement system (CLSM) must be taken into account. This diffraction is generally characterized by the Point Spread Function (PSF). The shape of the PSF was estimated from CLSM recordings of fluorescent microspheres of ~0.175 μm in diameter and could be suitably described by Gauss-shaped curves in $x$- and $z$-direction:

\[
PSF(x) = \text{Const} \cdot \exp \left( -\frac{1}{2} \left( \frac{x - x_0}{\sigma_x} \right)^2 \right) ; \quad PSF(z) = \text{Const} \cdot \exp \left( -\frac{1}{2} \left( \frac{z - z_0}{\sigma_z} \right)^2 \right)
\]
where:

\(x_n\) = x-position of the observed fluorescent object

\(z_n\) = height of the plane of observation

\(\sigma_x\) = 'width' of the PSF in x-direction (\(\sigma_x = 1.3\) \(\mu m\))

\(\sigma_z\) = 'width' of the PSF in z-direction (\(\sigma_z = 5.5\) \(\mu m\); optical section thickness = 13 \(\mu m\)).

Since we were only interested in fluorescence profiles in the x-direction, assuming axial symmetry (y-direction), we estimated a line-spread function (LSF) as a function of x, which characterizes the diffraction in the plane of focus perpendicular to the arterial wall. We obtained this LSF by simulating a tube of 167 \(\mu m\) in diameter uniformly filled with dye in which fluorescence as function of x, y, and z would follow the measured PSF (eq. 3.4). This resulted in a simulated fluorescence profile as function of x for the plane \(z = z_n\). Based on the form of the PSF we postulated the shape of the LSF to be:

\[
\text{LSF}(x) = \text{Const} \cdot \exp\left(-\frac{1}{2}\left(\frac{x - x_n}{b_x}\right)^2\right)
\]  

(3.5)

where:

\(b_x\) = 'width' of the LSF in x-direction

\(c_x\) = 'steepness' of the LSF

Application of this LSF to relate the concentration distribution in the model tube to the calculated fluorescence profile in the plane \(z = z_n\) resulted in \(b_x \approx 2.1\) \(\mu m\) and \(c_x \approx 1.8\).

In figure 3.4 the predicted fluorescence profile of this model tube is compared to the measured fluorescence profile from a glass tube of 167 \(\mu m\) in diameter (chapter 2). The correspondence was considered to be satisfactory.

### 3.4 RESULTS: CHARACTERIZATION OF THE MODEL

#### 3.4.1 Lumped model (4 parameters: \(d_{\text{ESL}}, d_w, M, v\)).

In the first set of model simulations, denoted as the 'lumped model', both model layers were lumped to a homogeneous combination: mobility coefficients and solute velocities in the ESL and in the wall were chosen to be equal; \(M_{\text{ESL}} = M_w\) and \(v_{\text{ESL}} = v_{\text{wall}}\). The dimensions of interest are the thickness of the ESL (\(d_{\text{ESL}}\)) and the wall thickness (\(d_w\)). It should be stressed that both layers are separated by the endothelium. Independent experimental observations were available for the localization of the endothelium (see chapter 2, Dil measurements), allowing separate estimation of both layer thicknesses.
3.4.2 Concentration and fluorescence profiles.

Typical results of this model for a certain set of parameter values are depicted in figure 3.5. The left panels show the calculated concentration profiles after 2, 10, 20, and 30 min of dye perfusion, the right panels the corresponding fluorescence profiles. The position of the endothelium is taken as 0 µm, and is depicted with the vertical dotted lines. Thickness of the ESL is here taken as 5 µm. Typical profiles for the diffusion-limited case are depicted in the top panels. The concentration profiles (top left panel) are characterized by a decay, all starting from the boundary value \( C_{\text{lim}} \) at the boundary position \( R_{\text{ESL}} \). This stationary point disappears after convolution with the line-spread function (LSF) resulting in the fluorescence profiles shown in the top right panel. Typical profiles for the convection-limited case are shown in the middle panels. The concentration profiles (middle left panel) now decay from the boundary value but from a position advancing in time, away from the boundary position. This characteristic difference with the diffusion-limited case is attenuated after convolution with the LSF as demonstrated in the middle right panel. The case of combined diffusion and convection is depicted in the bottom panels.

3.4.3 Calculation of \( X_{50}(t) \) from fluorescence profiles.

The rates by which the fluorescence profiles in figure 3.5 develop over time as characterized by \( X_{50} \) are compared in figure 3.6. In the diffusion-limited case \( X_{50} \) has a square-root-like
Figure 3.5: Left panels: Examples of calculated FITC-Δ concentration profiles. Shown are the profiles after 2, 10, 20, and 30 min of dye perfusion, due to diffusive (top), convective (middle), and combined (bottom) solute transport. Concentration profiles are normalized to maximal luminal value. The position of the endothelium is taken as 0 μm, and is depicted with the vertical dotted lines. Parameters of these simulations are depicted in the panels. Right panels: Examples of calculated FITC-Δ fluorescence profiles. Fluorescence profiles resulted from convolution of the concentration profiles in the left panels with the line-spread function (LSF). Again, the profiles after 2, 10, 20, and 30 min of dye perfusion, due to diffusive (top), convective (middle), and combined (bottom) solute transport, are shown. Fluorescence profiles are also normalized to maximal luminal value.
Figure 3.6: Time dependence of the position at which the calculated fluorescence profiles (see fig. 3.5, right panels) equal their half-maximal value, $X_{so}$.

course over time, while for the convection-limited case $X_{so}$ shows a linear decrease over time, which is understandable since solute is transported by an already developed constant flow field. Combination of diffusion and convection results in a course for $X_{so}$ initially determined by diffusion, but eventually dominated by convection as time develops. Obviously, the exact fluorescence profile developments and displacement rates are dependent on parameters chosen.

### 3.4.4 Effects of parameters on $X_{so}(t)$.

Since the $X_{so}(t)$-curves start at $t = 0$ min approximately at the boundary position of the luminal ESL border, varying the parameter $d_{ESt}$ (thickness of the ESL) results in parallel vertical shifting of the $X_{so}(t)$-curves. The shape of these curves is almost independent of $d_{ESt}$. The influence of the parameter $d_w$ (thickness of the wall) on the $X_{so}(t)$-curves is shown in figure 3.7. For certain sets of parameter values the $X_{so}(t)$-curves show a deviation from the characteristic curves after long periods of time, especially when $d_w$ is small. This effect is more pronounced in the diffusion-limited case (upper panel) than in the convection-limited case (middle panel) or the combined case (lower panel). Note that $X_{so}$ for the diffusion-limited case (upper panel) is plotted against the square-root of time, to emphasize the deviation from the characteristic square-root like time course. In the diffusion-limited case the slope of the $X_{so}(\sqrt{t})$-curve is dependent on the mobility coefficient $M$: the higher $M$ is, the steeper the decay. In the convection-limited case the slope of the $X_{so}(t)$-curve is dependent on the solute velocity $v$: the higher $v$ is, the steeper the
Dependence of $X_{w,t}(t)$ on wall thickness $d_w$

(d_{sel} = 5 \mu m; M = 4 \times 10^{-14} m^2 s^{-1}; v_i = 0)

Dependence of $X_{w,t}(t)$ on wall thickness $d_w$

(d_{sel} = 3 \mu m; M = 0; v_i = 4 \times 10^{-4} ms^{-1})

Dependence of $X_{w,t}(t)$ on wall thickness $d_w$

(d_{sel} = 3 \mu m; M = 10^{-14} m^2 s^{-1}; v_i = 10^{-5} ms^{-1})

Figure 3.7: Dependence of the time course of $X_{w,t}$ on the model parameter $d_w$ (wall thickness). The top row panels show $X_{w,t}$'s in case of diffusive transport, the middle row in case of convective transport, and the bottom row when both diffusion and convection is taken into account. For diffusive transport $X_{w,t}$'s are plotted against $\sqrt{t}$ to emphasize the deviation from the characteristic square-root like time course. Chosen values for the other parameters are depicted in the panels.

Figure 3.8: Example of fitting $X_{w,t}$'s from the calculated fluorescence profiles (see fig. 3.5) to the $X_{w,t}$'s from the measured fluorescence profiles (see fig. 3.2) of individual arteries perfused with FITC-A4, FITC-A50 or FITC-A148. The top panel shows the calculated $X_{w,t}$ due to diffusive solute transport, the middle panel due to convective solute transport, and the bottom panel due to combined transport. The values of the parameters are given in the panels.
decay. In the case of combined diffusion and convection both M and \( \nu \) contribute to the rate of decay in \( X_{50} \). (For additional results on calculated \( X_{50}'s \) see appendix B.)

3.5 RESULTS: COMPARISON TO EXPERIMENTAL DATA

3.5.1 Estimation of the physical parameters.
We fitted the \( X_{50} \)'s from the calculated fluorescence profiles (see example in fig. 3.6) to the \( X_{50} \)'s from the measured fluorescence profiles (see averages in fig. 3.2), an example of which is shown in figure 3.8. The top panel shows the calculated \( X_{50} \) due to diffusive solute transport, the middle panel due to convective solute transport, and the bottom panel due to combined transport. The values of the parameters are also given in the panels.

Table 3.1 summarizes the results of these fitting procedures (n=7 experiments per FITC-\( \Delta \)). Results are mean ± SEM for \( d_{\text{est}} \) and \( d_w \). Results are geometric mean and 95% confidence interval for M and \( \nu \). The top panel in table 2 shows the results when only diffusive solute transport was taken into account, the middle panel when only convective transport was taken into account, and the bottom when both diffusive and convective transport was taken into account. Overall, we found an ESL thickness of about 8.5 \( \mu \)m. No differences in ESL thickness (\( d_{\text{est}} \)) were found between the different FITC-\( \Delta \)s used. For FITC-\( \Delta \)4 and FITC-\( \Delta \)50, ESL thickness found with convective transport was smaller than for diffusive or combined transport. Wall thickness (\( d_w \)) was estimated to be on average 4.2 \( \mu \)m, but this parameter had very limited predictive value (see sensitivity analysis). Mobility coefficients (M) were higher for FITC-\( \Delta \)4 and FITC-\( \Delta \)50 than for FITC-\( \Delta \)148. Calculated ratios of M / \( D_0 \) (see discussion) were also higher for the smaller two dextrans, indicating relatively less hindrance of the ESL and the arterial wall to diffusive transport of the smaller two dextrans. Overall the ratios M / \( D_0 \) were in the order of \( 10^{-6} \)-\( 10^{-4} \), indicating severe hindrance in especially the ESL to diffusive solute transport. Solute velocities (\( \nu \)) were also higher for the smaller two dextrans than for the large FITC-\( \Delta \)148. Overall solute velocities were in the order of \( 10^{-11} \)-\( 10^{-9} \) m·s\(^{-1} \), which would, when compared to an estimated fluid velocity of 3.8\( \times \)10\(^{-7} \) m·s\(^{-1} \), imply reflection coefficients close to 1. This may seem an overestimation, especially for the smaller two dextrans (see discussion). Calculated Péclét numbers (see discussion) tended to be higher at higher molecular weight, indicating a relatively larger contribution of convection to solute transport than diffusion, but this was not significant. We never found significantly different results when comparing FITC-\( \Delta \)4 and FITC-\( \Delta \)50.
Table 3.1: Summary of results 4 parameter continuous model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FITC-A48</th>
<th>FITC-A50</th>
<th>FITC-A148</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (R²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.09 ± 0.02</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>0.06 ± 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.03 ± 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Values are mean ± SEM. Geometric mean (95% confidence interval) * p<0.05 vs. FITC-A148; † p<0.05 vs. FITC-A50; ‡ vs. diffusion, ‡ vs. convolution.
Figure 3.9: Sensitivity analysis to the 4 parameters ($d_{w,0}$, $d_{w}$, $M$, and $v$) of the lumped model when fitting calculated $X_{i,0}$ to experimental $X_{i,j}$. The left column of panels shows the influence of variations in ESL thickness $d_{w,0}$ on $R'$ of the fit, the second column shows the influence of wall thickness $d_{w}$, the third column shows the influence of the mobility coefficient $M$, and the right column shows the influence of solute velocity $v$. The top row panels show the results in case of diffusive transport, the middle row in case of convective transport, and the bottom row when both diffusion and convection is taken into account. For $d_{w,0}$, $M$ and $v$, $R'$ was normalized according to $(R'-\text{min}(R'))/(\text{max}(R')-\text{min}(R'))$ over the range for $d_{w,0}$, $M$ or $v$, used, to prevent negative values for $R'$. For $d_{w}$ this was not done, since it would deceptively enhance the small differences in $R'$ over the range for $d_{w}$. 

WALL PERMEABILITY OF ISOLATED SMALL ARTERIES

Modeling macromolecular transport
3.5.2 Sensitivity analysis.

A sensitivity analysis to the 4 parameters of the lumped model is shown in figure 3.9. From left to right the panel-columns show the influence of variations in resp. ESL thickness \( d_{\text{ESL}} \), wall thickness \( d_w \), mobility coefficient \( M \) and solute velocity \( v_s \) on the average \( R^2 \) of the fits of \( X_{\text{fit}} \)'s. From top to bottom the panel-rows show the results in case of resp. diffusive, convective and combined transport. \( R^2 \)-values were normalized to maximal value per experiment. The optimum for ESL thickness \( d_{\text{ESL}} \) was around 7-9 \( \mu \text{m} \), independently of which FITC-\( \Delta \) was used. Wall thickness \( d_w \) had negligible influence on the quality of the model fits, except in case of diffusive and especially combined FITC-\( \Delta \)50 transport. Mobility coefficient was optimal but rather insensitive at low values for FITC-\( \Delta \)148, and reached a clear optimum around 1-2 \( \cdot 10^{-11} \text{ m}^2\text{s}^{-1} \) for FITC-\( \Delta \)4 and FITC-\( \Delta \)50. Solute velocity was optimal and also rather insensitive at low values for FITC-\( \Delta \)148, and reached an optimum around 2 \( \cdot 10^{-9} \text{ m} \text{s}^{-1} \) for FITC-\( \Delta \)4 and FITC-\( \Delta \)50.

3.5.3 Second model (5 parameters: \( d_{\text{ESL}}, M_{\text{ESL}}, v_{s\text{ESL}}, \text{fact}_w, \text{fact}_M \)).

Based on the results of the lumped model, we adjusted this model to study separate contributions of the ESL and the arterial wall. We expressed mobility coefficients and solute velocities in the wall as a fraction of their corresponding variables in the ESL: \( M_w = \text{frac}_w M_{\text{ESL}} \) and \( v_{s,w} = \text{frac}_{v_s} v_{s\text{ESL}} \). Wall thickness (\( d_w \)) was chosen equal to 10 \( \mu \text{m} \) in this model. This model was characterized by 5 parameters: \( d_{\text{ESL}}, M_{\text{ESL}}, v_{s\text{ESL}}, \text{fact}_w (= M_w / M_{\text{ESL}}), \) and \( \text{fact}_M (= v_{s,w} / v_{s\text{ESL}}) \). Similar to the lumped model, the influence of arterial wall properties on estimation of the physical parameters was negligible. Solute transport characteristics and corresponding fluorescence profiles were mainly determined by ESL properties. Thus variation of \( M_w \) and \( v_{s,w} \) in comparison to the values in the ESL had negligible influence on parameter estimation. Based on this model estimates of ESL thickness (\( d_{\text{ESL}} \), mobility coefficients (\( M_{\text{ESL}}, M_w \)) and solute velocities (\( v_{s\text{ESL}}, v_{s,w} \)) were not significantly different from the values mentioned in table 3.1.

3.6 DISCUSSION

We developed a model to predict macromolecular solute transport properties of the arterial wall. Solute transport was described by convection and diffusion. The arterial wall model considered two homogeneous layers: 1) the endothelial surface layer (ESL), 2) the endothelium and the rest of the arterial wall. Diffusion, driven by concentration gradients and electrostatic forces, was
characterized by the mobility coefficient \( M \) [m\(^2\) s\(^{-1}\)]. Convection was characterized by the effective solute velocity \( \nu \) [m s\(^{-1}\)], derived from the reflection coefficient of the arterial wall layers to the concerned solute and the fluid velocity \( \nu_f \). We found that an ESL (layer 1) that might be as thick as 3-8 \( \mu \)m forms the main barrier to solute transport. Mobility coefficients in the ESL were in the order of \( 10^{-6} \text{ to } 10^{-4} \) of the free diffusion coefficients. Reflection coefficients to convective solute transport were close to unity. The influence of the rest of the arterial wall (layer 2) on solute transport characteristics was negligible.

### 3.6.1 Comparison to literature

Many authors have developed models to describe (macro) molecular transport from the circulation to tissues (3;4;6;8-11;16;17;22). Long time it was the established paradigm that the endothelial cell layer forms the main permeability barrier allowing solute transport through pores and vesicles of different sizes (3;6;12;14-16;22). According to this theory endothelial cells, pores and vesicles are covered with a fibrous matrix that contributes to the permselective nature of the vessel wall (3;6;12;22). Only recently this concept of solute transport has been extended with the incorporation of the endothelial surface layer (4;8-10;17).

Hu and coworkers (8;9) postulated that for the application of the classical Starling forces to model transvascular fluid and solute transport, it is not simply the hydrostatic and osmotic pressure gradients across the entire vascular wall that count, but that these forces exert their primary effect across the endothelial surface layer. Assuming an ESL thickness of 0.15 \( \mu \)m and reflection coefficients to albumin or Ficoll70 of 0.94, these authors estimated diffusion coefficients in the ESL that were \( 10^{3} \text{ to } 10^{4} \)-fold smaller than free diffusion coefficients.

Stace and Damiano (4;17) developed an electrochemical model to predict transport properties of (anionic) molecules through the capillary ESL. This model predicts partial exclusion of anionic tracers from the ESL and attenuated transport of these tracers into the ESL over time, dependent on their size and charge. These authors assumed a constant ESL dimension of 0.5 \( \mu \)m, based on intravital microscopic recordings of the capillary ESL made by Vink and Duling (19;20). Assuming a severely negatively charged ESL these authors were able to predict the observed prolonged transport times for FITC-\( \Delta \)s smaller than 70 kD, while larger FITC-\( \Delta \)s remained excluded from the ESL.

Curry and Michel (3) assumed a fibrous matrix covering endothelial cell, pores and vesicles that consisted of fibers with a radius \( (r_f) \) of \( \sim 0.6 \) nm, occupying only 5% of the volume in the porous region (i.e. volume fraction \( (\varepsilon) \) occupied by aqueous solution equals 0.95). This would result in effective diffusion coefficients for molecules with radius \( a \) [nm] according to:
\[ D = D_0 \cdot \exp \left[ - \frac{1 - \varepsilon}{r_f^2} (a + r_f) \right] \approx D_0 \cdot 0.80 \cdot (0.69)^a \] (3.6)

where \( D_0 \) is the free diffusion coefficient. For the FITC-\( \Delta \)s used in our studies this would result in ratios of effective diffusion coefficient to free diffusion coefficients of respectively:

\[
\begin{align*}
\frac{D_{\Delta^+}}{D_{0,\Delta^+}} & = 0.48 & (a_{\Delta^+} = 1.4 \text{ nm}) \\
\frac{D_{\Delta^{50}}}{D_{0,\Delta^{50}}} & = 0.12 & (a_{\Delta^{50}} = 5.0 \text{ nm}) \\
\frac{D_{\Delta^{148}}}{D_{0,\Delta^{148}}} & = 0.04 & (a_{\Delta^{148}} = 8.2 \text{ nm})
\end{align*}
\]

Radii of the FITC-\( \Delta \)-molecules were extrapolated from the data provided by Granath and Kvist (7). Since most authors have been interested in the dimensions of the structures responsible for solute transport, namely pores, vesicles and more recently the fibers, most of the described models observe solute distributions and transvascular solute transport on a sub-microscopic scale in the order of Ångströms or nanometers. We described solute distributions on a more macroscopic scale in the order of micrometers, to be able to compare the calculated fluorescence kinetics with the measured kinetics of FITC-\( \Delta \)s in isolated arteries (chapter 2).

We found an ESL thickness of approximately 8.5 \( \mu \text{m} \). This might seem a rather unrealistic high value. In our experimental study (chapter 2) in which the data was collected that was used for verification of the model and estimation of the physical parameters, we estimated an ESL thickness of 2-3 \( \mu \text{m} \). This was done by comparison of the fluorescence profile of FITC-\( \Delta \)s inside a small glass tube, which profile was shifted over a distance \( X \), with the fluorescence profile of FITC-\( \Delta^{148} \) inside a cannulated small artery, after 30 min of dye perfusion.

In the present study the thickness of the ESL was estimated from comparison of calculated \( X_{50}(t) \)-values of fluorescence profiles that resulted from the model calculations, with \( X_{50}(t) \)-values from the measured fluorescence profiles of the different FITC-\( \Delta \)s in cannulated arteries.

ESL thickness was here mainly determined by the initial \( X_{50} \)-values. Thus we found an ESL thickness of approximately 8.5 \( \mu \text{m} \), independent of which FITC-\( \Delta \) we observed.

If we assume that FITC-\( \Delta^{50} \) is able to penetrate the ESL, but does not accumulate in the arterial wall within 30 min of dye perfusion, and that FITC-\( \Delta^{148} \) does not penetrate the ESL, as has been demonstrated in our experimental study (chapter 2), we can also take the change in \( X_{50} \) for FITC-\( \Delta^{50} \) during 30 min as a measure for ESL thickness. Indeed \( X_{50} \) did not change significantly during 30 min for FITC-\( \Delta^{148} \) (from 8.6 \( \pm \) 0.6 \( \mu \text{m} \) after 2 min to 7.4 \( \pm \) 0.6 \( \mu \text{m} \) after 30 min), but for FITC-\( \Delta^{50} \), \( X_{50} \) decreased significantly from 7.1 \( \pm \) 1.6 \( \mu \text{m} \) after 2 min to 3.7 \( \pm \) 0.9 \( \mu \text{m} \) after 30 min, indicating an ESL thickness of 3.2 \( \pm \) 1.1 \( \mu \text{m} \). This value appears in better agreement...
with the 2-3 μm found in our previous study.

Evidence for a relatively thick ESL was provided from intravital microscopic recordings in cremaster capillaries by Vink and Duling (19;20), which revealed an ESL of ~0.5 μm in thickness. Furthermore, these authors estimated that diffusion coefficients in the ESL can be ~5·10⁶-fold smaller than free diffusion coefficients, accounting for the long ESL transport times observed for certain molecules, such as FITC-Δs and albumin (20). The predominant contribution of the ESL to vascular permeability has also been demonstrated by a study using ESL-degrading enzymes on coronary arterioles (10), in which an ESL thickness was estimated that might be as large as 7 μm. We also expressed the estimated mobility coefficients (see table 1) for the different FITC-Δs as a fraction of free diffusion coefficients, to indicate to what extent diffusive solute transport is hindered in the arterial wall layers. Free diffusion coefficients of the FITC-Δs were extrapolated from the data provided by Granath and Kvist (7), resulting in

\[ D_{0,Δ4} \approx 1.35·10^{-10} \text{ m}^2\text{s}^{-1} \]
\[ D_{0,Δ5} \approx 4.35·10^{-11} \text{ m}^2\text{s}^{-1} \]
\[ D_{0,Δ148} \approx 2.60·10^{-11} \text{ m}^2\text{s}^{-1}. \]

Thus, we found mobility coefficients in the ESL that were ~5·10⁵-fold smaller than free diffusion coefficients for the large FITC-Δ148, and ~10⁴-fold smaller than free diffusion coefficients for the medium-sized FITC-Δ50 and the small FITC-Δ4. Our estimated \( \frac{M_{\text{ESL}}}{D_0} \)-ratios are much smaller than the ratios predicted by Curry and Michel (3), slightly smaller than those estimated by Hu and coworkers (9), but slightly larger than those estimated by Vink and Duling (20). Diffusion coefficients in extravascular mesenteric tissue have been reported to be ~3-30-fold smaller than free diffusion coefficients (1;5;6;9), thus still remarkably higher than those in the ESL. Consequently, the ESL exerts a much higher hindrance on (diffusive) solute transport than the rest of the arterial wall or extravascular tissue and is therefore likely to form the main barrier to solute transport.

### 3.6.2 Criticism of the methods

Comparison of the lumped model to the experimental data resulted in an estimation for the wall thickness (\(d_w\)) of 4.2 μm. It is likely that the real wall thickness is somewhat larger (~10 μm) for the arteries used in the experiments, ranging from 105-191 μm in diameter. Wall thickness has been reported to be ~6 μm for coronary arterioles from 13-101 μm in diameter (10). Nevertheless, wall thickness (\(d_w\)) had very limited predictive value and had negligible influence on the quality of the fits of model \( X_{\text{mod}} \)'s to experimental \( X_{\text{exp}} \)'s. Similar to the lumped model, the influence of
arterial wall properties \((M, v_w)\) on estimation of the physical parameters in the second model was negligible. Solute transport characteristics were mainly determined by ESL properties. Reflection coefficients for the different FITC-\(A\)s used, as estimated from:

\[
\sigma_s = 1 - v_s \cdot v_{fl}
\]  

(see eq. 3.1)

were close to unity, since \(v_s << v_{fl}\).

Fluid velocity \((v_{fl})\) could be estimated according to \((8;9;16;22)\):

\[
v_{fl} = J_v \cdot A = L_p \left( \Delta P - \sigma \Delta \pi \right)
\]  

(3.7)

where:

\(J_v\) = transvascular fluid flux \([m^3.s^{-1}]\)

\(A\) = exchange area of the vascular wall \([m^2]\)

\(L_p\) = hydraulic conductivity of the vascular wall \([m^3.s^{-1}.mmHg^{-1}]\)

\(\Delta P\) = hydrostatic pressure gradient \([mmHg]\)

\(\sigma\) = osmotic reflection coefficient of the vascular wall \([-]\)

\(\Delta \pi\) = osmotic pressure difference \([mmHg]\)

Using equation 3.7 the fluid velocity in our experimental study was estimated to be \(0.38 \, \mu m\cdot s^{-1}\).

This value was based on assumptions about the value for \(L_p\) of mesenteric vessels \((6.7 \times 10^{-9} \, m^3.s^{-1}.mmHg^{-1}; (14-16))\) and on estimated values for \(\Delta P\) (60 mmHg) and \(\sigma \Delta \pi\) (3 mmHg) from the experimental conditions (chapter 2). Lower estimates for \(v_s\), e.g. when \(L_p\) for the vessels in our experiments would be lower, would result in reflection coefficients \(\sigma_s < 1\), which appears to be more realistic, especially for the smaller two FITC-\(A\)s.

By comparison of the estimated mobility coefficients and solute velocities we can evaluate the contributions of diffusive and convective transport to total solute transport. The ratio of these contributions is expressed by the modified Péclét number and can be calculated using \((16;21)\):

\[
Pe = \frac{J_v \cdot (1 - \sigma_s)}{P_s \cdot A} = \frac{v_{fl} \cdot (1 - \sigma_s)}{v_s \cdot (d_{ESL} + d_w)}
\]  

(3.8)

where:

\(P_s\) = vascular wall permeability to the solute \([m^3.s^{-1}]\)

\(d_{tot}\) = total arterial wall thickness \([m]\).
Thus we found Pe-values in the order of 1-10 (see table 3.1), which tended to be higher at higher molecular weight of the FITC-Ás (P=NS), indicating a relatively larger contribution of convection to total solute transport. Nevertheless, the Pe-values were never significantly different from unity, indicating that the contributions of diffusion and convection were comparable.

3.6.3 Implications of the study

An endothelial surface layer of several micrometers in thickness with barrier properties as described in the present study, will have major influence on transvascular fluid and solute transport. The ESL might thus play a predominant role in the regulation of physiological processes such as ligand-receptor-interactions, transvascular exchange of oxygen, nutrients or proteins, and protection of the endothelium against atherogenic stimuli. Damage to the ESL by for example oxidative stress (2;18;19) can thus be the first onset of pathophysiological conditions such as atherosclerosis (13).

3.6.4 Conclusion

In conclusion, we were able to predict FITC-Á transport characteristics across the ESL and the arterial wall with a model that was based on diffusive and convective transport equations. The main barrier to FITC-Á transport was formed by the ESL, while the rest of the arterial wall exerted a negligible influence on solute transport.
3.7 REFERENCES