Wall permeability of isolated small arteries. Role of the endothelial surface layer
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chapter 1:

GENERAL INTRODUCTION
1.1 VASCULAR PERMEABILITY

1.1.1 Retrospection

The maintenance of interstitial fluid balance can be described as follows: „..... whereas capillary pressure determines transudation, the osmotic pressure of the proteids [proteins] of the serum determines absorption. Moreover, if we leave the frictional resistance of the capillary wall to the passage of fluid through it out of account, the osmotic attraction of the serum for the extravascular fluid will be proportional to the force expended in the production of this latter, so that, at any given time, there must be a balance between the hydrostatic pressure of the blood in the capillaries and the osmotic attraction of the blood for the surrounding fluids.” This passage from Ernest Henry Starling’s elaboration ‘on the absorption of fluids from the connective tissue spaces’ from 1896 (300) expresses the most fundamental knowledge about the driving forces that regulate exchange of fluid and solutes between the circulation and tissues. This knowledge led Starling (fig. 1.1) to postulate the famous hypothesis that was named after him and that describes the relation between transvascular fluid and solute transport on the one hand, and hydrostatic blood pressure and osmotic pressure on the other hand. The consequence of this relation was postulated to be that, since the hydrostatic pressure at the arterial end of the capillary bed is greater than at the venous end, fluid (and solutes) should be filtered from blood to tissues at the arterial end and reabsorbed at the venous end (210:300). Until today Starling’s hypothesis is the foundation of practically all research that has been performed to unravel the mysteries of transvascular transport and vascular permeability.
1.1.2 Fluid transport: theoretical

Direct evidence for Starling's hypothesis was for the first time provided in 1926 (169) by Eugene M. Landis (fig. 1.2), who developed a technique to puncture and perfuse single capillaries. Using this micro-injection technique, Landis was able to measure capillary pressure and by an ingenious method based on the movements of red blood cells (RBCs) inside these capillaries he was able to estimate fluid fluxes through the capillary walls (169-174;176;177). His findings led to the general formulation of Starling's hypothesis for transvascular fluid transport (210):

\[ J_v/A = L_p \left[ (P_c - P_i) - \sigma (\pi_c - \pi_i) \right] \]  

where:
- \( J_v \) = transvascular fluid flux \([m^3/s]\)
- \( A \) = exchange area of the vascular wall \([m^2]\)
- \( L_p \) = hydraulic conductivity of the vascular wall \([m^3/s/mmHg]\)
- \( P_c, P_i \) = capillary resp. interstitial hydrostatic pressure \([mmHg]\)
- \( \sigma \) = cosmotic reflection coefficient of the vascular wall [-]
- \( \pi_c, \pi_i \) = capillary resp. interstitial osmotic pressure \([mmHg]\).

1.1.3 Fluid transport: experimental

Transvascular fluid transport is directly driven by the Starling forces, hydrostatic and osmotic pressure gradients, and can thus be described by equation 1.1. The earliest studies of vascular permeability focussed on transvascular fluid transport, since it was the easiest to observe (69;76;170-172;175-177;229;230;298). There is a continuous turnover of fluid in the body, caused by the fact that fluid is filtered from blood to tissues at the arterial end of the circulation and reabsorbed at the venous end, as a consequence of the Starling forces. Excess fluid not taken up at the venous end is removed from the tissue by lymphatics (32;326). Thus fluid transport can be studied on individual blood vessels, for example by micro-injection techniques introduced by Landis (170-172;176;177), on whole organs, for example by (iso-)gravimetric measurements (355), or in the lymphatics, for example by studying fluid clearance rates from the circulation to the lymphatics (356). Landis' micro-injection technique using tracer-particles such as RBCs is the most frequently used method to study hydraulic conductivity of individually perfused (micro-) vessels and has provided a relatively easy tool to elucidate the physiological mechanisms responsible for fluid exchange and to intervene with these regulatory issues (357).
Figure 1.3: Schematic representation of hydraulic conductivity measurement technique.

Figure 1.4: Measurement of hydraulic conductivity \( L_p \) from the slope of the relation between fluid flux per surface area \( J_s / A \) vs. pressure gradient \( \Delta P \).
The principle of this technique is illustrated in figure 1.3. A certain microvessel is pressurized and perfused via a micropipet with a solution containing tracer particles (RBCs). At a certain moment the vessel is occluded with an occlusion-rod. As long as the hydrostatic pressure gradient over the vessel wall exceeds the osmotic pressure gradient, fluid will move from the vessel outward to the extravascular space (filtration). When the hydrostatic pressure gradient is lower than the osmotic pressure gradient fluid will move from the extravascular space into the vessel (absorption). Upon a fluid flux through the vessel wall, the RBCs will move inside the vessel.

The displacement of the RBCs over time is a measure for the fluid flux according to (171, as originally postulated by Landis):

$$J_v = \frac{\pi r^2 \cdot \Delta x / \Delta t}{A} = \frac{\Delta x \cdot r}{\Delta t \cdot 2x_0}$$

(1.2)

where:

- $\Delta x / \Delta t = \text{rate of RBC movement [m:s$^{-1}$]}
- $r = \text{vessel radius [m]}
- x_0 = \text{initial distance of marker RBC to side of occlusion [m]}

By measurement of $J_v / A$ at different perfusion pressures ($P_p$) and by plotting this quantity against $\Delta P$ (fig. 1.4), one can obtain the hydraulic conductivity of the vessel wall, $L_p$ (i.e. the vessel wall permeability to water), from the slope of the curve, and the effective osmotic pressure gradient, $\sigma \Delta \pi$, from the intercept at the $\Delta P$-axis, according to equation 1.1.

Until today, this technique is referred to as the (modified) Landis-method.

Since Landis’ pioneering work, micro-injection techniques have been extensively applied in vascular permeability studies in a wide variety of preparations. While measurements of transvascular fluid transport and hydraulic conductivity are usually performed by micro-injection of relatively large tracer-particles such as RBCs, measurements of transvascular solute transport and vascular permeability to the solute molecules generally depends on injection or infusion of dye tracers such as Evans Blue (358), radioactive-labeled tracer molecules such as radiolabeled proteins (359), or fluorescent-labeled molecules such as proteins or dextrans (360).
1.1.4 Solute transport: classical theory

To describe transvascular solute transport three processes can be taken into account: 1) convection or solvent drag, 2) diffusion and 3) active transport (by vesicles). Total solute flux \( J_s \) can be expressed as the sum of the fluxes due to each transport process (60;250;253):

\[
J_s = J_v (1-\sigma_s) C_{lum} + P_s A \cdot \Delta C \cdot \left( \frac{Pe}{e^{Pe}-1} \right) + \alpha Q_{ves} \cdot \Delta C
\]

\( \text{convection} \quad \text{diffusion} \quad \text{vesicular transport} \)

where:
- \( J_v \) = solute flux \([\text{mol} \cdot \text{s}^{-1}]\)
- \( \sigma_s \) = reflection coefficient of the vascular wall barrier to the solute [-]
- \( C_{lum} \) = luminal solute concentration \([\text{mol} \cdot \text{m}^{-3}]\)
- \( P_s \) = permeability of the vascular wall barrier to solute transport \([\text{m} \cdot \text{s}^{-1}]\)
- \( \Delta C \) = transvascular solute concentration difference \([\text{mol} \cdot \text{m}^{-3}]\)
- \( \alpha \) = partition of solute molecules between lumen and vesicular contents [-]
- \( Q_{ves} \) = vesicular volume flux \([\text{m}^3 \cdot \text{s}^{-1}]\)
- \( \frac{Pe}{e^{Pe}-1} \) = modified Pélet number [-], expressing the ratio between convective and diffusive solute transport (60)(and many others). When fluid and solute transport (partially) use the same pathway, convective and diffusive solute transport are coupled (60;337).

The correction factor using the Pélet number is introduced to correct the total vascular wall property ‘solute permeability \((P_s)\)’ for the contribution of convective solute transport (solvent drag), to retain the contribution of diffusive solute transport, as characterized by the molecular property ‘solute diffusion coefficient \((D)\)’.

The principle of diffusion of molecules had already been described in 1855 (81) by Adolf E. Fick (fig. 1.5), as what is now known as Fick’s law of diffusion:

\[
J_s / A = -D \cdot \Delta C / \Delta x
\]

\( D \) = diffusion coefficient \([\text{m}^2 \cdot \text{s}^{-1}]\)
\( \Delta C / \Delta x \) = concentration gradient \([\text{mol} \cdot \text{m}^{-3}]\).

It was however not until 1919 that the concept of diffusion was applied to transcapillary exchange (254) by S. August S. Krogh (fig. 1.6). According to Renkin, Krogh’s publication on
Figure 1.5: Adolph Eugen Fick (380).

Figure 1.6: S. August Krogh (reprinted by permission from the Nobel e-Museum (381)).

Figure 1.7: Principle of solute permeability measurement technique. Top images show rapid filling and wash-out with a fluorescent tracer of a vessel, cannulated with a 0-pipet. Bottom graph shows the total fluorescence signal over time, measured in the depicted window, from which the vessel wall permeability is calculated.

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capillary diffusion, together with Landis' verification of Starling's hypothesis, can be considered the starting point of the "modern history of transcapillary exchange" (254).

The contribution of vesicular transport to total solute transport is subject of an ongoing discussion (361). Probably this contribution is very low in comparison to convective and diffusive solute transport (250;310), therefore vesicular solute transport will not be discussed further in this survey.

1.1.5 Solute transport: classical experimental approach

A frequently used method in transvascular solute transport and solute permeability measurements is based on perfusion of individual (micro-)vessels with fluorescent-labeled molecules (362). Rapid inflow of tracers into the vessel can be obtained by perfusion of two branches of a Y-shaped segment, one branch with the normal perfusate and the other with the perfusate containing the tracer molecules (6;7;63;85;141;142;280). Another method to obtain rapid inflow of tracers has been developed by Huxley, who applied double-barreled θ-pipets to be able to perfuse microvessels with two different perfusate solutions (149-151;203). By measurement of the total fluorescence intensity in a window surrounding the vessel one is able to estimate the permeability of the vessel wall to the concerned fluorescent tracer molecules (6;7;63;85;141;142;149-151;203;280).

The principle of this technique is illustrated in figure 1.7. Upon the rapid filling of the vessel lumen with fluorescent perfusate a stepwise increase in fluorescence is observed. While the tracer molecules slowly permeate outward through the vessel wall a slow increase in fluorescence over time can be observed. After wash-out of the tracer a stepwise decrease in fluorescence is observed and, provided that no binding of tracer molecules occurs, eventually all tracer will be removed from the measurement window. Vessel wall permeability to the concerned solute (Pw) can thus be calculated from the time-dependent intensity-signal (6;7;63;85;141;142;149-151;203;280):

\[
P_w = \frac{1}{\Delta I} \cdot \frac{\partial I}{\partial t} \cdot \frac{r}{2}
\]

(1.5)

where:

\(\Delta I\) = stepwise increase in intensity upon filling of the vessel [a.u.]

\(\partial I / \partial t\) = slope of intensity increase over time [a.u. s⁻¹]

\(r\) = vessel radius [m].

A disadvantage of this method is that only measurements of total integrated fluorescence in a window containing the vessel and some extravascular space are made. Therefore, these
measurements do not provide any insight in the spatial distribution of the tracer molecules over time or the localization of possible permeability barriers to solute transport. Furthermore, these measurements are usually made within a time period of several seconds to minutes of dye perfusion. The slope ($\partial I/\partial t$) of the intensity-curve is assumed to be constant during this period. Obviously, this assumption is only valid as long as tracer accumulation within the measurement window does not reach saturation, which is likely to happen for longer tracer-perfusion periods.

1.1.6 Recent experimental studies of transvascular transport and vascular permeability

A wide variety of experimental approaches have been applied to study transvascular transport of fluid (363) or molecules such as proteins (364), dextrans (365), ions (158;159;246), oxygen (245;246) or lipoproteins (366). Considerable research has been devoted to the dependence of vascular permeability and transvascular transport on amongst others preparation (67;277), location in the circulation (24;208;213;290;305), flow patterns (367), protein composition (368), agonists (369), charge (370), temperature (100;103;177;220;246;264), pH (100), inflammatory stimuli (21;115;132;138;165;218;264;352) or other pathophysiological conditions (371).

All this research is generating an increasing body of evidence to elucidate the structural elements and control mechanisms responsible for the regulation of vascular wall permeability. One topic of special interest for this survey is the influence of charge of the structural vascular wall elements, especially of the endothelial surface layer (ESL), as well as charge of the solutes on vascular wall permeability and transvascular solute transport. Many authors have investigated transvascular or glomerular transport properties of charged molecules (372).

Most studies involving charge show diminished transport of anionic molecules in comparison to neutral molecules of similar size and conformation, whereas transport of cationic molecules is generally enhanced, but some contradictory results have also been reported (225;226;235). Although a diminished transport of anionic solutes is in agreement with the presence of fixed negative charges on the luminal endothelial surface, the exact dimension of the endothelial structures carrying these negative charges has not yet been determined satisfactorily. Imaging of the fixed negative charges after staining with cationic probes (20;228;293;294;322), shows that the charges are mainly located in membrane bound structures (glycocalyx) of the ESL such as glycoproteins and proteoglycans, but these techniques usually fail to obtain insight in the true in vivo dimensions of the charge carrying structures.

Another topic of special interest includes the influence of protein composition of the perfusate on vascular wall permeability and transvascular solute transport. Many investigators have studied
the effects of proteins, such as albumin and orosomucoid, on vascular wall permeability to water and solutes (373). These studies indicate that proteins lower vascular wall permeability. Hydraulic conductivity of vessels perfused with protein-free solutions is ~2.4-5 times higher compared to vessels perfused with albumin in frog capillaries (139;140;202;214;215) and ~1.4 times higher in rabbit carotid arteries (308). An albumin concentration of 0.001% (0.01 mg/ml) is generally sufficient to accomplish these effects, while higher concentrations show only small additional contributions in lowering hydraulic conductivity.

Furthermore, vascular wall permeability to solutes is ~1.5-2.6 times higher for frog capillaries perfused with protein-free solutions in comparison to vessels perfused with solutions containing 0.1-1% albumin (141;214). Higher albumin concentrations generally reduce solute permeability further, whereas the presence of full plasma is necessary to maintain vascular wall permeability to solutes at in vivo values (106;111;113;141;143).

The exact dimension and location of the structures interacting with proteins have not yet been determined satisfactorily. It has been suggested that the effect of proteins on vascular permeability are the result of interactions of these proteins with the endothelial glycocalyx (64;106;111;113;202;212;214;215;243).

Obviously, many more protocols for measurements of transvascular transport and vascular permeability have been reported, that all fall back on the basic principles formulated by Fick and Starling over 100 years ago. It would be beyond this survey to discuss all these experiments individually.

### 1.1.7 Recent theoretical models of transvascular transport and vascular permeability

Many authors have developed models to describe fluid and solute transport from the circulation to tissues (374). Long time it was the established paradigm that the endothelial cell layer forms the main permeability barrier and that exchange of fluid and solutes between the circulation and tissues takes place through pores of different sizes and vesicles (375). More recently it was postulated that endothelial cells, pores and vesicles are covered with a fibrous matrix that contributes to the permselective nature of the vessel wall (376). Theoretical models describing transvascular fluid and solute transport have accordingly been based on the vascular wall structures held responsible for either transport or barrier properties, whose structures are mainly known from morphological studies such as electron microscopy.

The above pore theory of capillary permeability failed to offer internal consistency in explaining results from a variety of experimental observations. In 1980, Curry and Michel introduced a fiber matrix model of capillary permeability (64) that did provide internal consistency in explaining a
diversity of experimental permeability measurements, such as capillary permeability to water, ions, small solutes or serum proteins. In this model, endothelial cells and pores through which fluid and solute transport takes place, are covered by a random array of fibers of constant radius, occupying only 5% of the volume in the porous region. This model will be discussed in more detail in chapter 3. In recent years the concept of transvascular transport has been altered in the sense that the fibrous matrix concept has been extended to what is described as the endothelial surface layer (ESL), also known as the glycocalyx. Several authors, including Michel (210) and Weinbaum and coworkers (134;135) have proposed that the Starling forces responsible for transvascular transport, namely hydrostatic pressure and osmotic pressure, act primarily across the ESL and not simply across the entire vascular wall. This theoretical modification of Starling's hypothesis is in agreement with the predominant role that the ESL appears to play in transvascular transport and vascular permeability (2;131;132;150;328;329), as will be discussed below. Recently, Stace and Damiano (299) developed an electrochemical model to describe transport of molecules, especially charged solutes, over the endothelial surface layer. This model predicts that negative charges within the ESL set up an electrical field that limits transport of anionic molecules from the vascular lumen into the ESL, resulting in partial exclusion of anionic molecules from the ESL and attenuated transport of these molecules over time. Both the exclusion factor and the accumulation rate are predicted to depend on the volume density of fixed charges within the ESL and on the valence of solute tracers. According to the model, cations present in blood (partially) counterbalance fixed negative charges in the ESL.

1.1.8 Vascular permeability in relation to vascular pathophysiology

Permeability of blood vessels forms an important parameter in the physiological regulation of exchange of water, proteins, nutrients, oxygen, hormones and other solutes between the circulation and tissues. An altered vascular permeability to water and solutes is one of the earliest detectable symptoms in several pathophysiological circumstances, including atherosclerosis, diabetes, shock or tumors (371). Consequently, considerable research has been devoted to the regulation of vascular wall permeability and its dependence on amongst others protein composition, agonists, temperature and pH (100;103;177;220;246;264;368;369).

Increased hydraulic conductivity of vessel walls can lead to increased accumulation of fluid (water) in the extravascular space, a well-known phenomenon called edema, observed in several pathophysiological circumstances. The occurrence of edema can also be the result of altered vascular permeability properties to plasma proteins such as albumin, causing increased
transvascular fluid transport by osmosis (152;164;234;301;302).

Increased permeability of large blood vessels to certain macromolecules leads to inclusion of amongst others albumin and low-density lipoproteins (LDL) into the subendothelial space, a well-known phenomenon in the process of atherogenesis (377). But also alterations in microvascular permeability, an important indicator of microcirculatory dysfunction, have been associated with several vascular pathologies such as atherosclerosis (52;179;191).

1.2 THE ENDOTHELIAL SURFACE LAYER (ESL)

1.2.1.1 The layer

The luminal surface of endothelial cells is covered by a gel-like layer, the endothelial surface layer (ESL) or glycocalyx (243). Part of the ESL is formed by a layer of membrane-bound macromolecules, such as proteoglycans, glycosaminoglycans, glycoproteins and glycolipids, that can be visualized by means of electron microscopy (EM). This layer was for the first time visualized by Luft in 1966 (197), using the cationic dye ruthenium red to stain the fixed structures within this layer. This staining is based on binding of this cationic dye to the fixed negative charges within the ESL. Since the development of this staining and visualization procedure several cationic probes have been used, such as ruthenium red (19;31;68;197;306;335), cationized ferritin (2;4;20;223;284;335) and alcian blue (102;294;306;324).

However, visualization of the ESL at its true in vivo dimension has been hampered by the fact that the ESL consists of not only membrane-bound macromolecules but for a major part of water and associated plasma proteins (243), which are usually removed upon dehydration during fixation for EM. The existence of a relatively thick ESL was postulated by Desjardins and Duling (75), to explain low levels of capillary hematocrit. More direct evidence from intravital microscopy was provided by Vink and Duling (328;329), demonstrating a gap between red blood cells (RBCs) and the endothelium in the order of 0.5 μm. Moreover, these authors demonstrated that molecular penetration rates in the ESL depend on size and charge of the molecules (329). The existence of an ESL in larger arterioles with barrier properties similar to those in capillaries was postulated from a 2.3-fold increase in permeability after treatment of the arterioles with pronase and heparinase (150), enzymes that are known to degrade the ESL (2;75). Model interpretation of these data resulted in an estimate of ESL thickness up to 7 μm (150). Regional variations in glycocalyx properties have been reported in literature (54;96;102), which might be reflected in regional variations in vascular properties such as permeability characteristics as well (24;208;213)
Recently, new procedures for visualization of the fixed structures within the ESL have been developed, which indicate that the dimensions of the ESL are indeed larger than revealed by the classical EM studies. Rostgaard and Qvortrup (276) reported a new perfusion-fixation technique using glutaraldehyde dissolved in an oxygen-carrying blood substitute, that revealed ‘bush-like filamentous sieve plugs’ covering the fenestrae of endothelial cells, consisting of 20-40 filaments with a length of 0.3-0.4 μm.

Van den Berg et al. (324) developed a new perfusion-fixation procedure using Alcian Blue, that enabled visualization of the non-collapsed ESL, leading to estimates of a glycocalyx thickness of 0.5-1 μm (see figure 1.8). However, many of its properties, especially in larger blood vessels, still remain to be elucidated. Only recently the functional properties of the ESL are becoming more extensively described. Biochemical research elucidates the receptor function of glycosaminoglycans within the ESL and binding patterns of proteins to heparan sulfates (79;271;292). Biodegradation of sialic acid, an important constituent of the ESL, by neuraminidase inhibits shear-induced NO production (129;240). Thus the ESL might play a role in the regulation of vascular tone. The role of the ESL in the control of vascular wall permeability has been addressed in experimental studies on microvessels (2;150;329) and in new theoretical transport models (134;135;299), as will be discussed below.

Figure 1.8: Electron microscopic recording of the glycocalyx in a rat left ventrical capillary, stained with Alcian Blue (courtesy of B. van den Berg, Dept. Medical Physics, University of Amsterdam).
1.2.2 The endothelial surface layer in relation to vascular permeability

Contact between blood and the luminal endothelial cell surface of the vascular system is mediated by the endothelial surface layer (ESL), an extended hydrated mesh of negatively charged proteoglycans (243). It is therefore likely that the ESL plays an important role in the regulation of the permselective barrier properties of the vascular wall and that it can specifically interact with plasma constituents such as proteins, whose interactions are essential for vascular function.

Many authors have investigated transvascular or glomerular transport properties of charged molecules (372). Most studies involving charge show diminished transport of anionic molecules in comparison to neutral molecules of similar size and conformation, whereas transport of cationic molecules is generally enhanced. Diminished transport of anionic solutes is likely to be due to the presence of fixed negative charges on the luminal endothelial surface (20;228;293;294;322), located in membrane bound structures of the ESL such as glycoproteins and proteoglycans. Furthermore, many studies have reported the influence of plasma or plasma proteins, such as albumin and orosomucoid, on vascular permeability to water and solutes (373).

These studies confirm the general principle that the presence of proteins lowers vascular wall permeability. It has been suggested that the effects of proteins on vascular permeability are the result of interactions of these proteins with the ESL (64;106;111;113;202;212;214;215;243). It has already been shown that the presence of plasma proteins influences the dimension to which the ESL extends into the vessel lumen (4).

Evidence for a predominant contribution of the ESL to vascular wall permeability was provided by some studies using proteolytic enzymes. Huxley and Williams demonstrated by means of treatment of the ESL with pronase and heparinase that the ESL may account for up to 60 % of the total vessel wall permeability barrier to macromolecular solute exchange (150). A similar contribution of the ESL to the hydraulic conductivity of the vessel wall was demonstrated by Adamson, using pronase to digest the ESL (2).

Direct evidence for the pronounced barrier properties of the ESL to solute transport was provided by Vink and Duling. By studying the distribution of different fluorescent-labeled solutes, plasma and blood cells within capillaries, these authors showed that an endothelial surface layer of 0.4-0.5 μm in thickness is capable of confining large macromolecules and cells to a core volume in the lumen of the capillaries (328). Permeation of smaller solutes into this layer was strongly dependent on size and charge of the solutes (figure 1.9) (329). An important factor in these ESL permeation characteristics is hyaluronan, a glycosaminoglycan of the ESL, as demonstrated by an increased permeation of certain anionic macromolecules after treatment of the ESL with hyaluronidase (131).
1.2.3 The endothelial surface layer in relation to vascular pathophysiology

Damage of the ESL is possibly the first onset to the development of pathophysiological conditions such as atherosclerosis. The pathogenesis of atherosclerosis is in the first stage accompanied by loss of proteoglycans from the ESL (96;102;200). One of the earliest detectable symptoms of atherosclerosis is an increased permeability of the vessel wall to plasma proteins such as albumin or lipoproteins such as LDL (221;222;272-275).

Damage to the ESL can occur by amongst others oxidative stress (328), for example as a result of oxidized LDL-cholesterol (57;58;327), by enzymes, such as heparinase, pronase, hyaluronidase and neuraminidase (2;75;95;150), by ischemia-reperfusion (31;68), or by inflammatory agents, such as tumor necrosis factor- (TNF-) (132). From permeability studies it is known that damage to or removal of the ESL results in a marked increase in vessel wall permeability (2;150).

Furthermore, it was demonstrated that oxidative stress-induced ESL damage results in an increased adhesiveness of the endothelium to platelets and leukocytes (58;327).

Also under 'normal' physiological circumstances there is a relation between regional variations in composition or dimension of the ESL and accumulation of amongst others lipoproteins in the arterial wall. For example, it was demonstrated that the carotid artery glyocalyx is twice as thick as the glyocalyx on the left and right descending coronary arteries, while those of the aorta and vena cava are of intermediate thickness (102). It was also reported that there are focal regions in permeability in the aorta and that the glyocalyx is thinner in regions of high permeability. In addition to its
thickness, studies of Görög and Born (95;96) revealed that the charge density of the glycocalyx may also influence vessel wall permeability. Enzyme treatment of charge bearing glycocalyx constituents increased uptake of low-density lipoproteins (LDL) into the rabbit carotid wall (95).

1.3 RATIONALE OF THE PRESENT STUDIES

1.3.1 Hypotheses

We hypothesized that the endothelial surface layer (ESL) forms the main barrier to transvascular solute transport. The thickness of the ESL may vary along the circulation, thus its dimension in conduit vessels or in resistance arteries might exceed that in capillaries [1]. The composition of the ESL may also vary along the circulation, but we hypothesized that its barrier properties are roughly similar to those in capillaries [2].

To study these hypotheses [1,2] we planned experiments to probe the permeability characteristics of the ESL in isolated arteries to anionic macromolecules of different sizes under several conditions. According to our hypotheses the ESL should form a profound barrier to transvascular transport of FITC-Aₘ in isolated small arteries of \(-150\text{–}200\ \mu\text{m}\). Accordingly, large FITC-A₁₄₈ (148 kD) should be excluded from the ESL and should thus be confined to a core volume inside the arteries. Smaller FITC-Aₘ should be able to penetrate the ESL in a size and charge dependent fashion (illustrated in figure 1.10). Furthermore we investigated whether the ESL in resistance arteries is sensible to oxidative stress such as induced by phototoxicity. We next tested the hypothesis that the fixed negative charges in the ESL play a predominant role in its

Figure 1.10: Schematic representation of the arterial wall consisting of amongst others smooth muscle cells (SMC), the internal elastic lamina (IEL), endothelial cells (EC), the latter covered on the luminal side with the endothelial surface layer (ESL), which can interact with protein present in the perfusate, such as albumin, and that forms a profound barrier to transport of anionic macromolecules, such as FITC-dextran. Transport of FITC-dextran through the ESL is thought to be dependent on their size. The lipophilic membrane tracer Dil, used to label the endothelial cells, is depicted as well.
barrier function to transport of anionic molecules [3]. We hypothesized that modulation of the effective charge density of the ESL by alterations in solvent ionic strength results in altered barrier properties of the ESL to transport of anionic solutes such as FITC-50 (50 kD). Furthermore, we tested the hypothesis that proteins such as albumin present in the perfusate of resistance arteries influence the arterial barrier properties to solute transport by interaction with the ESL [4].

**1.3.2 Outline of the thesis**

The development of a new experimental model, aimed to localize the permeability barrier to anionic macromolecules of different size and charge in isolated rat mesenteric resistance arteries, is described in chapter 2. In this chapter, we also aimed to determine the dependence of the barrier properties on size or charge of the tracers used. Finally, we investigated whether the permeability barrier that is regulating transport of these tracers, was sensitive to oxidative stress, such as phototoxic stress induced by light-dye-treatment.

In chapter 3 we developed a theoretical model to describe transvascular transport of the tracers used in resistance arteries, based on diffusive and convective solute transport equations. Calculated fluorescence kinetics from the model were compared to measured fluorescence kinetics from the isolated arteries (chapter 2) to estimate the physical parameters characterizing diffusive and convective solute transport, namely mobility coefficients, solute velocities and the dimensions of the arterial wall layers.

Chapter 4 describes experiments aimed to determine the influence of modulation of the effective charge density of the ESL on distribution and transport of anionic macromolecules (FITC-Δ50) in isolated resistance arteries. Modulation of ESL charge density was performed by modification of the ionic composition of vessel perfusion and superfusion solutions.

The influence of the presence of albumin in the perfusate on distribution and transport of anionic macromolecules (FITC-50) in isolated resistance arteries, is described in chapter 5.

In chapter 6 we analyzed the fluorescence characteristics during incorporation of the lipophilic tracer DiI in the endothelial cell membranes of the isolated arteries, as recorded during the permeability studies described in chapter 2, 4 and 5, and the dependence of these fluorescence characteristics on the experimental conditions such as the presence of different FITC-s, solvent ionic composition or the presence of albumin in the perfusate.

The results are evaluated in the general discussion in chapter 7 and the most important findings have been summarized in chapter 8.
1.4 REFERENCES


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