

Endothelial glycocalyx and coronary vascular permeability: the fringe benefit

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Abstract Current concepts of vascular permeability are largely still based on the Starling principle of 1896. Starling's contribution to understanding vascular fluid homeostasis comes from realising that the transport of fluid to and from the interstitial space of peripheral tissues follows the balance between opposing oncotic and hydrostatic pressures. It is presumed that in peripheral tissues fluid is readily filtered from blood to tissues at the arterial/arteriolar side of the circulation and largely reabsorbed at the venular/venous aspect, excess fluid being removed from the tissue by the lymphatic system. This balance is determined particularly by the properties of the vascular barrier. Recent studies have shown that the endothelial glycocalyx, located with a thickness of at least 200 nm on the luminal side of healthy vasculature, plays a vital role in vascular permeability by constituting the vascular barrier together with the endothelial cells themselves. While water and electrolytes can freely pass through the glycocalyx, plasma proteins, especially albumin, interact strongly. Binding and intercalating plasma constituents with the structural elements of the glycocalyx creates the so-called endothelial surface layer. This is the actual interface between flowing blood and the endothelial cell membrane *in vivo*. The oncotic pressure difference pertinent to fluid homeostasis is not built up between the intravascular and the interstitial tissue spaces, but within a small protein-free zone beneath

the glycocalyx surface layer. This explains why perturbation of the glycocalyx leads to a breakdown of both fluid and protein handling in the coronary vascular bed. Preventing damage to the glycocalyx seems to be a promising goal in cardioprotection in many clinical scenarios, including acute ischaemia, hypoxia and inflammation, and chronic vascular disease as in atherosclerosis, diabetes and hypertension.

Keywords Albumin · Antithrombin · Endothelium · Glycocalyx · Heparanase · Hydraulic conductance · Mast cell · Permeability · Tissue factor · Vascular barrier

Basics

About 60% of the body mass of adults in good cardiopulmonary health consists of water, which is distributed to two-thirds intracellularly and to one-third extracellularly. Most of the latter is allocated in the interstitial space (80%), while 20% contribute as the plasma volume to cardiac preload. Intra- and extracellular spaces are separated by the cell membranes. This lipid double layer is not resistant against hydrostatic pressure, but is largely impermeable for electrolytes and proteins. In contrast, the vascular barrier, in total, permits hydrostatic pressure gradients to be established, but does not retain electrolytes, though it is supposedly largely impermeable for proteins. Thus, water distributes passively over the compartments, following the distribution of osmotically and oncologically active substances.

This review was aimed to provide an integrative update on current physiological knowledge of the properties of the vascular barrier, focusing on the coronary system and the role played by the endothelial glycocalyx. Other

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physiological functions of the glycocalyx such as the mediation of shear stress from flowing blood to the endothelial cells, the regulation of inflammatory interactions and the prevention of firm adhesion of blood platelets and leucocytes to the intact vessel wall will only be mentioned in passing. The readers are referred to recent reviews and papers covering these aspects [5, 14, 16, 26, 40, 54, 63, 79, 80, 85, 93, 102, 114].

Though the endothelial glycocalyx represents the primary, endothelially generated structure at the vessel wall/blood interface, *in vivo* we are dealing with the endothelial surface layer, formed when plasma constituents interact with the molecules expressed at the cell membrane [86]. The two concepts are often not differentiated in the following text, but there are graded differences between them in regard to hydraulic resistance and permeability.

Background

In 1896, the British physiologist Ernest Starling [100] introduced his famous concept of vascular fluid homeostasis, in which he schematically opposed hydrostatically driven fluid losses towards the tissues against reabsorption on the basis of an inwardly directed oncotic pressure gradient. This model is still favoured in most text books of physiology today and can be mathematically described by the equation

$$F/A = C_H \times [(P_{HV} - P_{HI}) - \sigma \times (\pi_{OV} - \pi_{OI})],$$

where F/A is filtration rate per area, C_H hydraulic conductivity, $P_{HV} - P_{HI} =$ hydrostatic pressure (P_H) gradient between the vascular lumen (V) and the interstitial space (I), σ reflection coefficient for the respective colloids at the vascular barrier and $\pi_{OV} - \pi_{OI}$ is oncotic pressure (π_O)

gradient between the vascular (V) lumen and the interstitial space (I).

Starling [100] suggested a high filtration–high resorption scenario even for non-fenestrated microvessels where fluid loss out of the vasculature on the arteriolar side caused by the higher blood pressure in comparison to the oncotic pressure is countered on the venular side by reabsorption due to the overbearing effect of intravascular oncotic pressure (Fig. 1). If these forces are in near balance when integrated along the microvascular length, the lymphatic system should be able to cope with any resulting, relatively low excess of fluid left in the interstitial space. The introduction of the term “sigma”—the reflection coefficient of colloids at the vascular barrier—within the equation results from an uncertainty within the concept concerning possible differences in behaviour of distinct colloidal molecular species and variability of permeability between different organs. In the special case of the blood–brain barrier, σ can be set as 1. However, also in all other vascular beds, one must expect σ to become totally unnecessary under steady-state conditions, once the actual net difference in oncotic pressure ($\Delta\pi_O$) is known.

Most clinical and experimental observations detailing tissue oedema have been based on this historical principle. It was also, apparently successfully, adopted for describing myocardial fluid homeostasis [69]. This appears to be inadequate in the face of several recent physiological findings, but theoretical physiological considerations clearly challenge the traditional view. For instance, Levick [62] first noticed the “low lymph flow paradox”, i.e., the phenomenon that the vascular barrier is still competent even when the interstitial oncotic pressure equals that of the vascular lumen. In rat mesenteric microvessels, the effective inwardly directed oncotic force opposing the hydrostatic gradient was still 70% of the intravascular

Fig. 1 The classical principle of Ernest Starling for fluid homeostasis in peripheral microvascular beds with continuous endothelium: a high filtration–high resorption concept relying on very low permeability of plasma proteins and low interstitial protein concentration

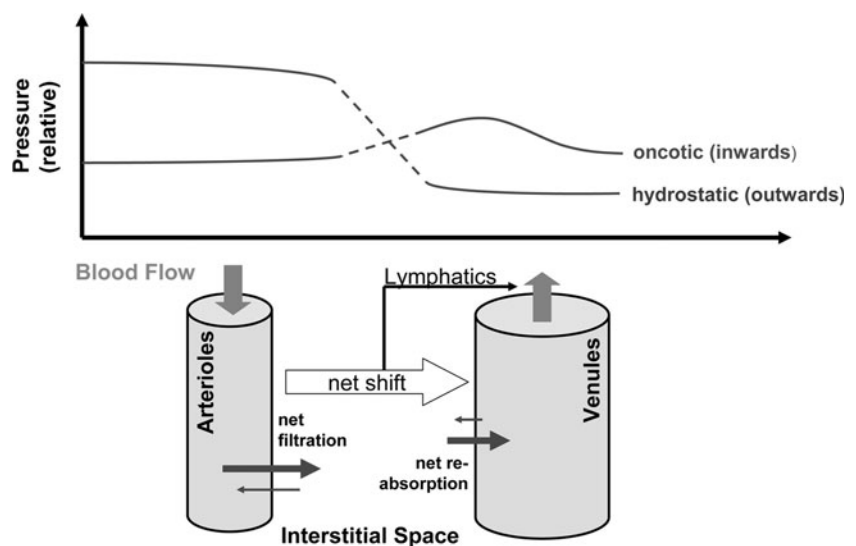
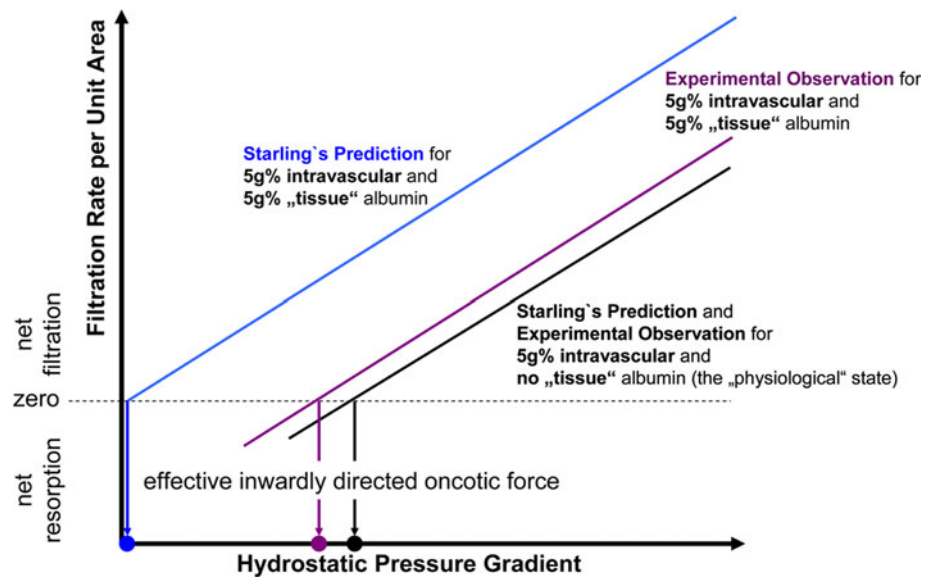


Fig. 2 The effective oncotic gradient across the vessel wall is not determined by the concentration gradient of colloid between intravascular and interstitial compartment (schematic summary of results of studies on isolated microvessels; for detailed description see [2])



colloid osmotic pressure even if the non-fenestrated microvessels examined in this model were surrounded by fluid containing colloid at the same concentration [2]. The Starling hypothesis would have predicted the absence of any inwardly directed force in this situation [100], hydrostatically driven outflow of fluid merely being mechanically hindered by physical properties of the vessel wall. As illustrated schematically in Fig. 2, the steepness of the graphs represents the hydraulic conductivity, i.e., the filtration rate per unit area per unit pressure gradient, which is the higher the steeper the line [2]. The intersection with the abscissa represents the effective inwardly directed oncotic force, which should lie at the point of origin if the oncotic pressure gradient across the vessel wall is nullified. Any resorptive force towards the vascular lumen should shift the curve rightwards. Obviously, a non-fenestrated isolated microvessel does not follow Starling's suggestions concerning a significant role of the interstitial oncotic pressure for vascular barrier competence. Experimental research using the isolated perfused heart, a whole organ model providing a complete vascular bed with large vessels, arterioles, capillaries and venules, plus the naturally surrounding tissue, confirmed these findings. Consistently, the interstitial and intravascular concentrations of any colloid applied into the coronaries came to within about 90–95% of each other inside of 20 min of onset of infusion [17, 48, 91]. Moreover, immunohistochemistry showed an even distribution of albumin throughout the whole interstitial compartment. The vascular barrier, however, was functionally intact in these isolated organs [47].

A further, clinically important contradiction to Starling's concept was revealed in the perfused whole organ, namely the "COP paradox" [48]. It is obviously not primarily the intravascular colloid osmotic pressure (COP)

which determines the power limiting transvascular fluid loss. Rather, the pivotal issue might be the *type* of colloid active at the endothelial surface. Similar to the isolated microvessel model, the coronary system of isolated guinea pig hearts was pressurised with artificial perfusate at different levels. Using a Langendorff setup, hearts were perfused with Krebs-Henseleit buffer alone, buffer diluted with isotonic saline, or buffer containing one of two different kinds of colloid at various concentrations [48]. The resulting transvascular filtration rates gave rise to transudate fluid appearing on the epicardial surface, comparable to the lymph flow in vivo, and were related to the respective perfusion pressures (Fig. 3). Just as in the isolated vessel model (Fig. 2) [2], a strictly linear dependency was observed. Adding a colloid to the artificial perfusate significantly lowered pressure-dependent transvascular filtration. The natural protein human albumin, however, appeared to be much more effective in sealing the vasculature than the artificial alternative hydroxyethyl starch, frequently used in clinically relevant bleeding to maintain the plasma COP [48]. The sealing effect of albumin was practically independent of the intracoronary COP it provided, down to a value only one quarter of the physiological level. The special ability of albumin to prevent coronary vascular extravasation of fluid has been noted repeatedly before [42–44]. If such behaviour were to be considered mathematically, then the reflection coefficient σ of the Starling formula would need to become greater than unity for low concentrations of albumin.

Neither the "low lymph flow paradox" [62] nor the "COP paradox" [48] is really compatible with the traditional concept of Ernest Starling [100]. Furthermore, it surprises that physiologists, including Starling, have ignored the fact that lymphatic fluid is potentially able to coagulate. This ability,

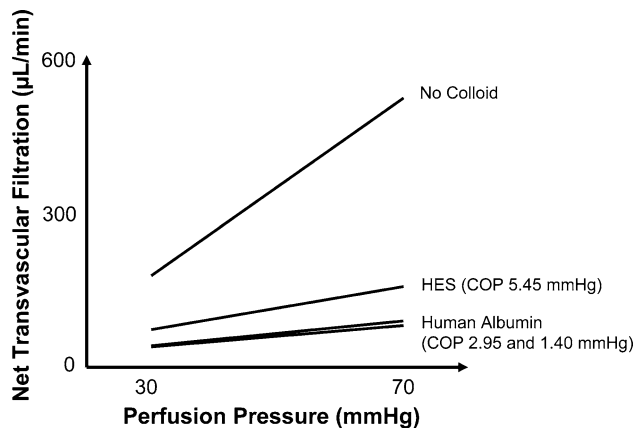


Fig. 3 The pressure dependency of net fluid filtration in the intact coronary system of guinea pig hearts perfused with Krebs-Henseleit buffer containing no colloid or 2 g% hydroxyethyl starch (HES) or human albumin (1.7 or 0.85 g%). *Lines* represent mean values of five hearts per condition, with the two albumin concentrations being practically equipotent. *COP* colloid osmotic pressure; data taken from [47, 48]

recognised already in 1653 (see [61]), implies that the extravascular fluid must contain significant amounts of coagulation factors derived from plasma and also all kinds of other plasmatic proteins. Indeed, the interstitial space of human heart tissue is rich in albumin, seen to be coating all cardiomyocyte cell surfaces (Fig. 4) [52].

Introducing the endothelial glycocalyx

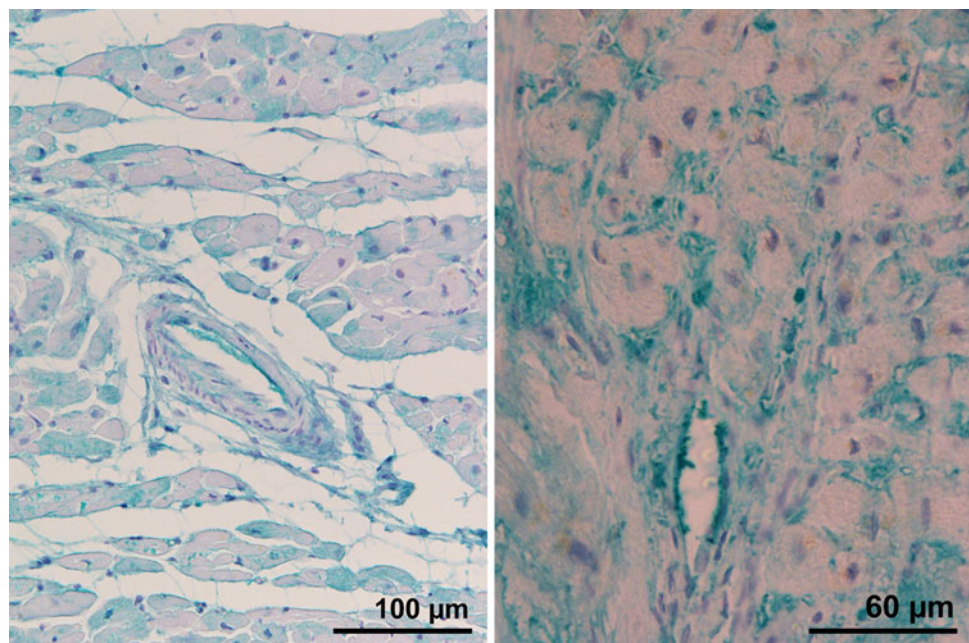
The endothelial glycocalyx is an intravascular fringe of astounding functional significance. It participates in

numerous physiological processes, foremost in regulating vascular permeability, preventing firm adhesion of leucocytes and blood platelets to the vessel wall, transmission of shear stress, and in modulation of inflammatory and haemostatic processes [5, 16, 85, 86, 93, 102, 114]. Pathophysiological sequelae of glycocalyx failure or perturbation include generation of tissue oedema, systemic inflammatory response syndrome, diabetic angiopathy and, possibly, atherogenesis. Situations in which damage to the glycocalyx has been reported include ischaemia/reperfusion, hypoxia, sepsis, volume overload, diabetes and atherosclerosis [74, 76, 88, 108, 112]. As already stated, this review shall focus on the role played in fluid homeostasis and colloid permeability in the heart.

Hydraulic conductance

First suspicions that there was something on the surface of the vessel wall helping plasma proteins to attenuate the development of oedema were published in 1940, but without any supportive optical evidence [29]. On the basis of chemical analysis, showing high content of sugars, this lining was soon termed the “endothelial glycocalyx”; the physiological properties and true dimensions, however, remained unclear for a long time [67]. There were first substantial considerations about a significant contribution of the endothelial glycocalyx to vascular barrier competence in the early 1980s [24]. Furthermore, the ability of plasma albumin to significantly lower hydraulic conductivity in conjunction with the endothelial glycocalyx was established [24, 42]. However, despite the formal

Fig. 4 Albumin (*green stain*) in samples of human heart tissue. *Left panel* immunohistology of a healthy human heart; *right panel* explanted human heart with dilated cardiomyopathy; for details see [52]. Albumin coats all extravascular cell surfaces and fills the interstitial matrix



attribution of a role as a “second barrier” [91], in addition to that posed by the endothelial cell bodies themselves, the exact contribution of the endothelial glycocalyx to vascular barrier competence remained unclear until very recently. Meanwhile, improved electron microscopical fixation techniques [106], in particular one based on stabilisation of the negatively charged structure (see below) by lanthanum ions during fixation reaction with glutaraldehyde [111], revealed that the first descriptions had gravely underestimated its actual dimension [67, 112]. It could be demonstrated repeatedly to be at least thicker than 200 nm in situ, both in the coronary system and the human umbilical vein (Fig. 5) [14, 15, 17, 19, 21, 47, 48, 54, 55, 91].

In its strictest sense, the endothelial glycocalyx consists mainly of membrane-bound glycoproteins and proteoglycans, chiefly syndecans and glypicans, carrying negatively charged heparan sulphate (about 70%) and chondroitin and dermatan sulphate side chains (30%), accounting for much of the biophysical properties [5, 85, 86, 114]. In addition, the glycocalyx harbours the outer aspects of membrane receptors, channels and adhesion molecules (within the closest 10–20 nm). The non-sulphated, receptor-attached, long-chain molecule hyaluronan is also often considered to be an essential part of the surface lining (Fig. 6) [86, 103]. The endothelial glycocalyx by itself, however, is relatively functionless in a model vasculature perfused with a pure crystalloid buffer since it poses no great resistance against passage of water, electrolytes and small uncharged osmolytes [47, 48, 63, 91].

The situation is dramatically different *in vivo* when natural proteins are present at the endothelial surface. Albumin, for instance, has been shown to be tightly attached to the endothelial surface, even after a protein-free washout procedure lasting several minutes [47, 54]. Obviously, many proteins, peptides (including cytokines and chemokines) and even lipids are bound to and intercalated in the endothelial glycocalyx, together constituting the so-called “endothelial surface layer” (ESL) [114]. This seems to possess a functional thickness of at least 1 μm [85], but general consensus is that the width of the glycocalyx proper and that of the ESL varies in different regions of the vascular system. Large vessels seem to support a wider ESL than microvessels. A dynamic equilibrium *in vivo* between the proteins of the flowing blood and those bound to or within this structure has been described before (Fig. 6) [85, 86].

The question exactly how this layer might be able to generate an inwardly directed oncotic gradient even when interstitial and intravascular protein concentrations are comparable was answered in 2004 using isolated rat mesenteric microvessels as model [2]. Adamson et al. [2] suggested a small space of much less than 100 nm width situated directly beneath the endothelial surface layer to

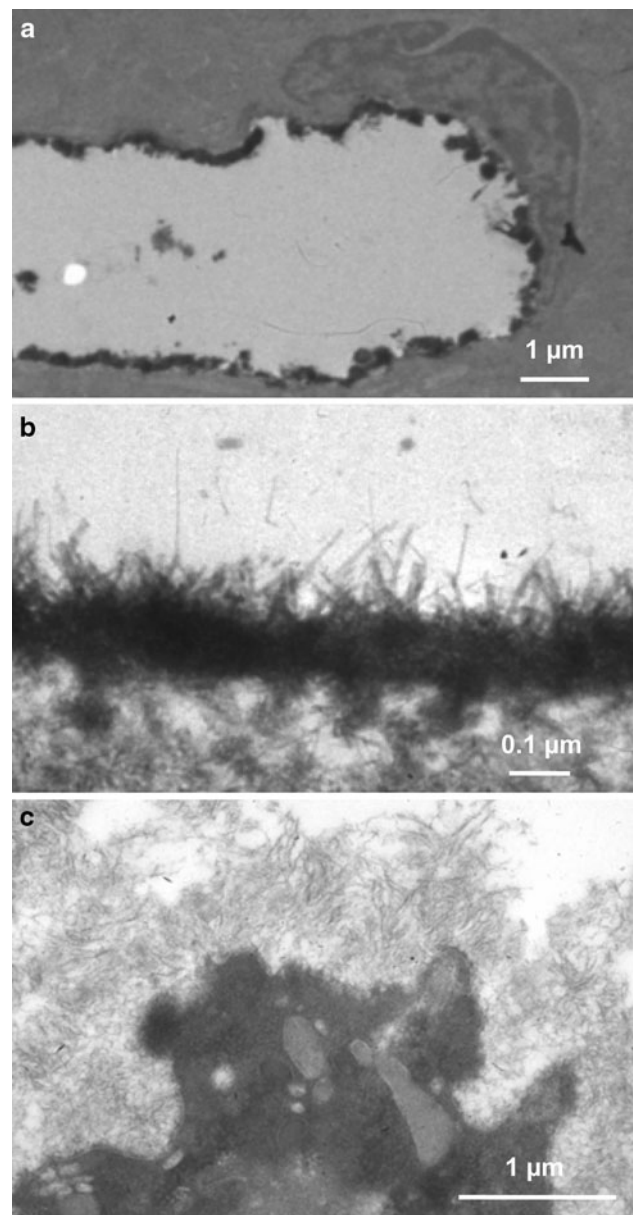


Fig. 5 Transmission electron microscopy of the endothelial glycocalyx fixed with glutaraldehyde in the presence of lanthanum nitrate. **a, b** Coronary capillary of guinea pig heart, in total and at high magnification, respectively, perfused *ex vivo* after isolation (taken from Chappell et al. [15]); **c** human umbilical vein, perfused *ex vivo* within minutes of birth (for details, see Chappell et al. [21])

remain practically protein-free when plasma is forced outwards hydrostatically as proteins are excluded or retained within the structure. Because the fluid, nonetheless, passing through will be extremely low in protein, an inwardly directed oncotic gradient will be generated between this small space and the protein-loaded endothelial surface layer (Fig. 7) [47]. This force limits the net outflow of ultrafiltrate towards the interstitial space. A further limitation of hydraulic conductivity occurs on the basis of

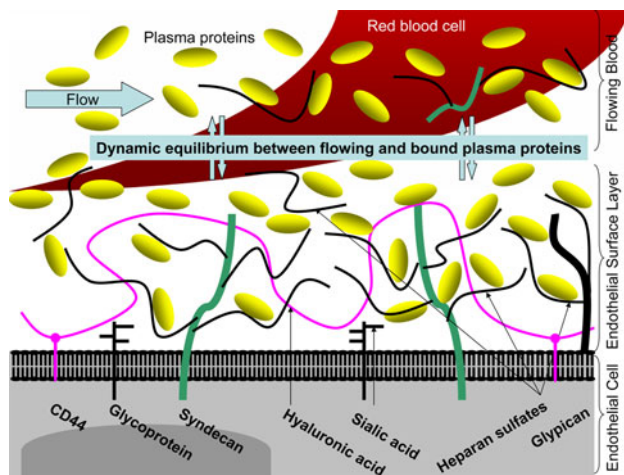


Fig. 6 Schematic arrangement of the chief constituents of the endothelial glycocalyx and the established endothelial surface layer. Glycoproteins include intercellular and vascular adhesion molecules, selectins and integrins. These all extend at most 10–20 nm from the lipid double layer and are, consequently, normally sequestered beneath a healthy surface layer [5]. Shed components of the glycocalyx are to be found in normal plasma [11, 88]. *CD44* membrane receptor binding hyaluronan (adapted from Pries et al. [86] and Tarbell and Prahakis [103])

the endothelial anatomy in continuous arteriolar and capillary vessel segments. As described in detail by Adamson et al. [2] as well as by Curry and Adamson [26] in a recent review, fluid can only pass into the interstitial space in these vessel segments via small infrequent breaks within the junction strands of narrow clefts between the endothelial cells. This normally small flow of ultrafiltrate is important, because it prevents back-diffusion of colloid from the interstitial space (see below) into the sub-glycocalyx zone, a process which would obliterate the oncotic gradient developed across the luminal glycocalyx.

As formulated by Levick and Michel [63], the revised Starling equation for continuous endothelia should read as

$$F/A = C_H \times [(P_{HV} - P_{HI}) - \sigma \times (\pi_{OV} - \pi_{OG})],$$

where F/A is filtration rate per area, C_H hydraulic conductivity, $P_{HV} - P_{HI}$ hydrostatic pressure (P_H) gradient between the vascular lumen (V) and the interstitial space (I), σ reflection coefficient for the respective colloids at the vascular barrier and $\pi_{OV} - \pi_{OG}$ is oncotic pressure (π_O) gradient between the vascular (V) lumen and the underside of the glycocalyx (g).

The coronary venules, in contrast, appear to be more porous than arterioles and capillaries. Qualitative observations in isolated heart preparations have shown that highly charged positive ionic species such as lanthanum ions, which should be retained by the strongly negatively charged glycocalyx, and also plasma proteins such as albumin pass into the interstitial space within minutes after

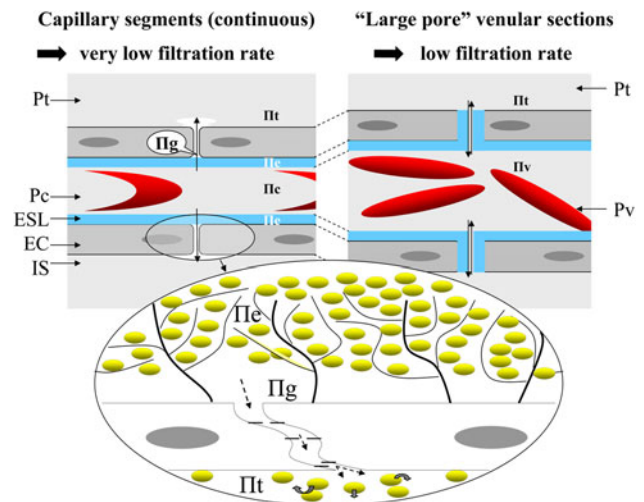


Fig. 7 The modern low filtration–(low) no resorption concept of fluid homeostasis in the coronary system. In the capillary segments there is a relatively high hydrostatic pressure gradient, but this is opposed by a large oncotic pressure gradient established between the endothelial surface layer and the underside of the glycocalyx. In addition, there is a high resistance to flow through the narrow interendothelial clefts. In the venular sections the hydrostatic pressure gradient is small and owing to the easy egress of colloids, there is hardly any oncotic pressure gradient. For details, see [47]. The enlargement of the interendothelial cleft illustrates the ability of the endothelial glycocalyx to establish an oncotic gradient and the maintenance of the protein-poor zone under the glycocalyx by the low flow of ultrafiltrate through the narrow cleft. This convection prevents interstitial proteins from diffusing up to the luminal side of the endothelial cells. P hydrostatic pressure, Π oncotic pressure, c capillary lumen, e endothelial surface layer (ESL), g underside of the glycocalyx, t tissue, IS interstitial space, v venular lumen

onset of intracoronary infusion, but only around venules [47]. This relatively facile passage of colloids explains why lymphatic fluid of the heart and the so-called “transudate” appearing on the epicardial surface of perfused heart preparations contain colloid in such high concentrations in the steady state ($\sim 90\%$ of the arterial value) [91]. As a consequence, there is practically no oncotic gradient hindering outflow of plasma in the venular vessel segments, or favouring resorption of interstitial fluid. On the other hand, there is only a very small hydrostatic pressure gradient across the vascular wall in this coronary section, limiting fluid extravasation. Accordingly, the actual amount of colloid passing into the interstitial space of the myocardium via passive transport should be quite small in a healthy coronary bed. Colloid that does extravasate will do so largely via diffusion.

The present concept describing fluid homeostasis in the intact cardiac microvascular bed is schematically outlined in Fig. 7 [47]. In the arteriolar and capillary segments we have a low-flow situation of protein-poor plasma filtrate; in the venular section we have a low-flow exchange of protein-rich plasma with little or no direct resorption from the

interstitium, but with ready exchange (via diffusion) of all dissolved constituents of the intra- and extravascular fluids. Allowing such exchange answers the age-old problem of how large molecules and essential nutrients such as low-density lipoprotein particles gain access to the parenchymal cells of the heart under physiological conditions. It, of course, also explains why cardiac lymphatic fluid is potentially able to coagulate.

Though neither σ , the colloid reflection coefficient, nor π_{og} , the oncotic pressure at the underside of the endothelial glycocalyx, of the revised Starling formula will generally be known, consequences of damaging or degrading the endothelial surface layer (see below) are readily apparent: Loss of the ability to form a transglyceal oncotic gradient will heighten filtration forces in arteriolar and capillary segments. Figure 7 also allows one to appreciate the “double barrier concept”, exemplified in experiments with histamine by Rehm et al. [91]. Histamine applied to isolated heart preparations perfused at constant flow with a purely crystalloid solution (Krebs-Henseleit buffer) did not elevate net filtration of coronary perfusate unless the glycocalyx had been enzymatically degraded beforehand. Conversely, using heparinase to cleave off the heparan sulphates causes collapse of the glycocalyx [22], but does not elevate net filtration under constant flow conditions unless histamine is applied to widen the endothelial clefts [91].

Vascular permeability of colloidal and electrically charged substances

With respect to short-term transport of substances across the vascular wall, one must distinguish between changes in vascular permeability and changes in hydraulic conductance. Alteration of the latter will influence solvent drag (provided permeability is not zero); alteration alone of the former will affect both diffusional transport and solvent drag. Transport via solvent drag is discussed in detail in recent reviews [46, 102].

Permeability of plasma proteins and of xenogenic colloids in the arterial and capillary sections of the coronary bed is governed by the glycocalyx according to selectivity based on size and charge. Especially, charge has been implicated to play a major role [64]. Because of the preponderance of heparan and chondroitin sulphate side chains, negatively charged molecules are excluded from entry, while positively charged species will be bound and retained. This has been shown for charged dextrans [7, 95, 110]. We have observed that infused antithrombin III, a molecule with negative charge, lines the luminal vessel wall, but does not enter the interstitial space of the heart unless the glycocalyx is removed [19]. Albumin is a special case; though the molecule carries a bulk negative charge at

physiological pH, there are also positively charged groups along the protein chain. This amphoteric nature allows albumin to interact particularly well with the glycocalyx. Consequences are increased hydraulic resistance [43, 44, 68, 91, 112], stabilisation against degradation [54], and facilitated transmission of shear stress as evidenced by enhancement of flow-mediated dilatation of coronary vessels [55].

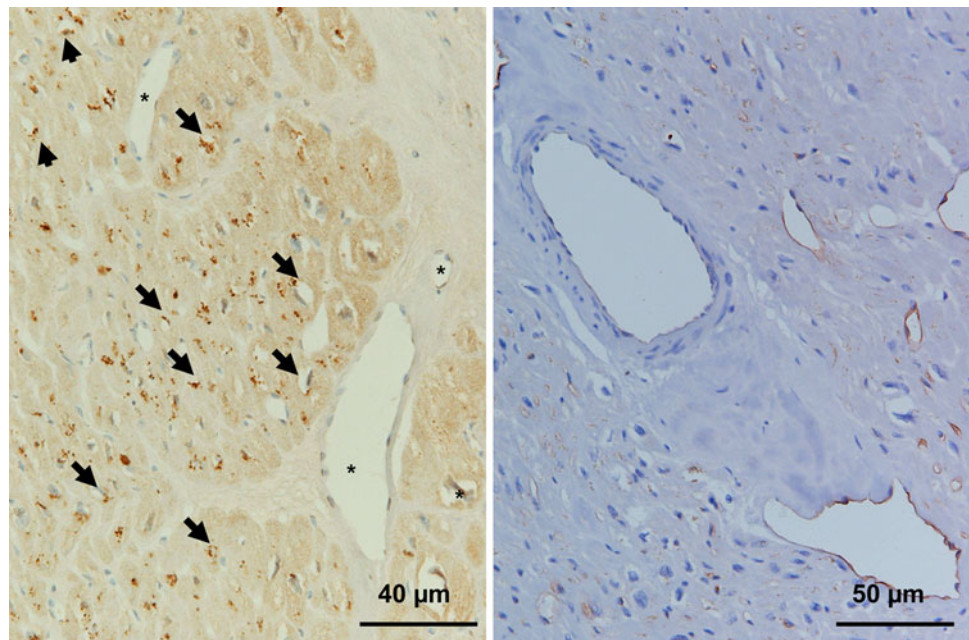
Even in the relatively permeable venular region (large-pore endothelial zone), the glycocalyx extends into the pore and exerts a limiting action on protein and colloid extravasation [112]. Equilibration of infused colloids (albumin and hydroxyethyl starches) with the interstitial space requires at least 20 min in isolated heart preparations [91] and is, thus, far from being instantaneous. Similarly, neither lanthanum ions nor antithrombin pass into the perivenular interstitial space readily as long as the glycocalyx is intact [19].

Testing the “low-filtration, venular egress” concept

An immediate consequence of plasma proteins being able to leave, albeit slowly, the coronary intravascular space via large venular pores and of bulk extravasation not being offset by high resorptive flow of interstitial fluid is that cardiac lymphatic fluid will also contain plasma proteins in appreciable concentrations. This is the case [4, 61]. While this also satisfies the observation concerning coagulability of lymphatic fluid, there is an evident problem: myocardial tissue is rich in tissue factor, the strongest initiator of the extrinsic pathway of coagulation [38, 101]. What then prevents coagulation from starting in the interstitial spaces of the heart under physiological conditions? Clearly, this would be a devastating scenario.

With this in mind, we have recently examined human heart tissue with respect to the distribution, in relation to the vasculature, of coagulation-initiating (tissue factor) and coagulation-inhibiting factors (thrombomodulin and activated protein C) [52]. Significantly, tissue factor was never found in the vicinity of venules or small veins of the heart. Instead, it was present subendothelially in arterioles and capillaries (Fig. 8). This distribution corresponds with that of pericytes in the vascular system, cells which have recently been shown to express tissue factor exclusively in the vessel wall under physiological conditions [56]. The considerable intra-organ heterogeneity of distribution observed supports the opinion that tissue factor forms a haemostatic envelope preferably around high-pressure segments of the vasculature to limit bleeding also in the face of rupture or trauma [34]. Thrombomodulin, in contrast, was present on all endothelial surfaces of the coronary system, indiscriminate of the vessel type or magnitude, and particularly high concentrations were

Fig. 8 Immunohistology showing the distribution of tissue factor (*left panel* brown granular deposits) and thrombomodulin (*right panel* brown luminal lining) in explanted human heart tissue. Arrows in the *left panel* indicate some of the capillaries; *asterisks* show some veins and venules. For details, see [52]



localised in the interendothelial clefts [52]. Thus, any thrombin being carried towards the interstitial space would be expected to initiate the strongly anticoagulatory protein C system [36]. Interestingly, there was little activated protein C anywhere in the heart tissue. In those instances in which it was detected, there were discrete amounts in areas where tissue factor had typically been found, i.e., near capillaries and arterioles [52].

Altogether, the inhomogeneous distribution of tissue factor, with its noteworthy absence around venules, and the strategic localisation of thrombomodulin at endothelial junctions are in full accordance with the proposed easy access of plasma constituents into the myocardial interstitial space because there is a minimised risk of generating fibrin. The result is also gratifying in the sense of Karl Popper (1902–1994). This great philosopher of science maintained that theories should be validated by asking questions and designing experiments potentially likely to disprove them. Fittingly, evidence for the important role of the endothelial glycocalyx in regulating coronary permeability comes from experiments designed to damage this sensitive structure.

Deterioration and shedding of the endothelial glycocalyx

Pathophysiological situations clearly leading to damage of the glycocalyx are ischaemia/reperfusion, hypoxia, volume loading and heart surgery and will be addressed in the following. Deterioration due to sepsis and chronic alteration as in atherosclerosis and diabetes mellitus are likely

but have not been expressly studied in the heart. There are several recent publications and reviews dealing with these aspects [5, 16, 70, 74, 76, 78, 81, 93, 105, 108].

Ischaemia/reperfusion and hypoxia

The hypoxia-induced increase in coronary permeability was, surprisingly for early investigators, not visualised in ultrastructural examinations of the endothelium [113]. However, these exact and detailed electron microscopical studies did not conserve the endothelial glycocalyx during fixation, so that it escaped notice.

Degradation of the endothelial glycocalyx due to ischaemia/reperfusion and hypoxia has been demonstrated in various experimental models. Rat intravital microscopy revealed that intestinal ischaemia/reperfusion led to a significant reduction of glycocalyx thickness due to shedding of glycosaminoglycan chains [73]. Intravital microscopy of the mouse cremaster muscle showed a rapid deterioration of the endothelial glycocalyx after reperfusion [82, 95]. Postischemic destruction of the endothelial glycocalyx could also be demonstrated in isolated heart models [3, 6, 10, 14, 15, 17, 19, 28, 48, 54, 91, 106]. Indicators for evaluation of disruption of the endothelial glycocalyx included rate of formation of coronary transudate (a direct measure of net fluid filtration), colloid permeability, shedding and washout of major components of the glycocalyx (syndecan, heparan sulphate and hyaluronan) and direct visualisation of the glycocalyx by electron microscopy [3, 12, 14, 17, 21, 54]. Hypoxia also disrupted the endothelial glycocalyx of isolated rat hearts [112].

Recently, damage of the endothelial glycocalyx was indirectly demonstrated in patients undergoing global or regional ischaemia [88]. The level of two components of the endothelial glycocalyx, syndecan and heparan sulphate, increased multifold in arterial blood of patients undergoing major vascular and heart surgery. Surprisingly, quantitatively identical increases in shed constituents were detected in plasma of patients undergoing coronary artery bypass, irrespective of whether the operation was performed with or with cardiac arrest (on- vs. off-pump mode, see below) [11].

Much remains unclear about the mechanisms involved in ischaemia/reperfusion injury. Possible mediators of this type of injury include free radicals [13, 23, 32, 95], complement activation [23], TNF- α expression [59, 118] and mast-cell degranulation [39, 57, 92]. The latter may be induced by elevated levels of endogenous adenosine, via adenosine A3 receptors [81], as recently discussed in a review of van Teeffelen et al. [107]. Leucocyte activation and adhesion have also been reported to contribute to reperfusion injury [13, 23, 41, 60, 87]. Chappell et al. [14] have recently demonstrated that intracoronary adhesion of human polymorphonuclear leucocytes in the coronary system, as in postischemic reperfusion, is only possible if the glycocalyx has been largely shed (see also [5, 54]).

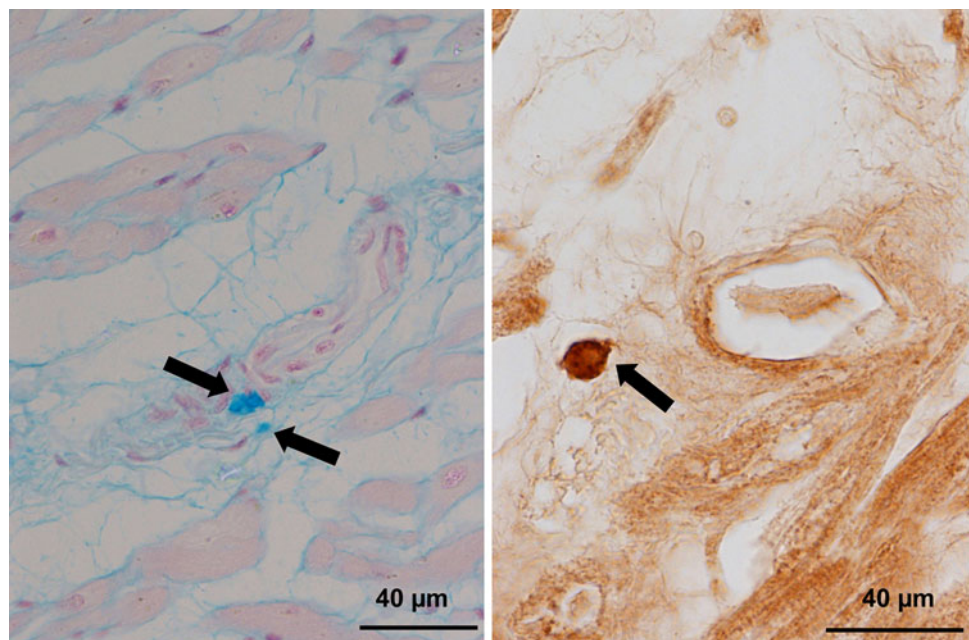
Postischemic or TNF- α -induced mast-cell degranulation liberates cytokines such as TNF- α [39, 92, 117], demonstrated to induce shedding of the glycocalyx [15], and proteases such as tryptase and cathepsin B [3], which could cleave syndecans and hyaluronan from the endothelial membrane. Of particular note is our hitherto unpublished finding that the resident mast cells are the sole store of the

enzyme heparanase in the human myocardium. This is shown in Fig. 9. Liberation of heparanase would be required to explain postischemic and postinflammatory shedding and washout of heparan sulphates from the heart as observed ex vivo [3, 10, 12, 14, 15, 17, 19, 54]. Heparanase acts just like heparinase, employed by us and others to selectively degrade the glycocalyx [10, 17, 22, 31, 48, 91]. Interestingly, heparinase has been applied to human patients undergoing coronary artery bypass grafting as an alternative means of abrogating heparin anticoagulation [99]. This clinical study was terminated ahead of time because of an inferior safety profile to protamine, hardly surprising in view of the strong degradative potential of heparinase versus glycocalyx [22].

Hypervolemia

Atrial natriuretic peptide (ANP), a small peptide released by the heart upon hypervolemia due to atrial stretch, acts acutely to reduce plasma volume by renal excretion of salt and water, vasodilatation and increasing vascular permeability [25]. While several studies demonstrated that ANP increased vascular permeability [45, 96, 104], and Curry et al. [27] have since been able to show that the permeabilising effect is mediated via endothelial ANP guanylyl cyclase type-A receptors, the exact mechanism remained undefined. In an isolated perfused heart model, we have been able to identify a general feature, namely that an increase in endothelial cyclic GMP always leads to an increase in coronary leak, irrespective of whether the agent is bradykinin, acetylcholine, nitroprusside or ANP [20].

Fig. 9 Mast cells and heparanase in human heart tissue. *Left panel* Alcian blue stain shows mast cells (*arrows*) in an explanted human heart with the typical perivascular localisation (for details, see Gilles et al. [39]). *Right panel* immunohistological detection shows heparanase (*intense brown stain*) exclusively in the granules of a cell in the same perivascular localisation as mast cells (rabbit anti-human heparanase antibody A00078, Genscript Corp., dilution 1:100 in formalin-fixed and paraffin embedded ventricular myocardium; for further details, see [52])



In this context, our group investigated a possible connection between the ability of ANP to acutely shift volume from the intra- to the extravascular space and the integrity of the endothelial glycocalyx [10]. Low-dose infusion of ANP (10^{-9} M) in isolated guinea pig hearts resulted in an increase in fluid leak and an accelerated extravasation of colloid. Moreover, ANP caused rapid shedding of syndecan core protein and histologically detectable degradation of the coronary glycocalyx [10]. Thus, the ANP-induced increase in vascular permeability described *in vivo* might be related to changes in the integrity of the endothelial glycocalyx.

The results of this investigation are in accordance with the findings of previous double-label blood volume measurements in humans [89, 90]. As a substitute during acute bleeding, iso-oncotic colloids had volume effects of more than 90%, provided normovolaemia was carefully maintained [90]. In contrast, volume loading of normovolaemic patients with the same iso-oncotic colloid led to volume effects of only approximately 40% [89]. Consequently, volume effects of colloids seem to depend on the “context”, i.e., the volume and hydration state of the patient [18, 50, 51]. The most likely explanation for this phenomenon is that volume loading resulted in an increased level of plasma ANP, thereby degrading the endothelial glycocalyx. Furthermore, a greater decrease in large vessel haematocrit (measured by centrifugation of arterial blood samples) in relation to whole body haematocrit (derived by double-label measurements of erythrocyte and plasma volume) was found during volume loading [89]. This observation leads one to suspect that a considerable decrease in the volume of the endothelial surface layer occurred during volume loading, leading to a larger distribution space for red blood cells. Again, ANP-induced alteration of the endothelial surface layer might be the underlying cause.

The double-tracer dilution technique based on indocyanin green (as label for the total plasma space) and fluorescein-labelled red blood cells (RBC) yielded a whole body volume of the endothelial surface layer in man amounting to about 730 ml, i.e., approximately 25% of the total plasma volume [89]. This volume was diminished to 250–350 ml upon volume loading [89]. Double-tracer dilution using fluorescent dextran and RBC has been applied by others to assess the glycocalyx volume in studies on goats and mice [7, 95, 110]. However, some of the results were physiologically rather unlikely and have been strongly criticised by Curry and Michel [71] for methodological reasons, but also on principle. The methodological shortcomings of the animal studies, for example redistribution of plasma space tracer into other compartments or inhomogeneous molecular size, were excluded in the measurements on humans [49, 53]. The principal

criticism centred on whether a large molecular weight tracer is at all able to distribute fully into a space filled by the branched chains of glycocalyx molecules. In other words, the tracer dilution values must fall short of the mark. The validity of this argument is surely given, but the extensive calculations performed to establish the magnitude of the under-representation of endothelial surface layer volume far overrate the error. Curry and Michel assume the glycocalyx to exist as a rigid, highly ordered geometrical structure. However, this is not the case *in vivo*, where fluctuations in blood flow and pressure and passage of cells, especially that of leucocytes, constantly deform and rearrange the glycocalyx fringe lining the endothelial cells [70, 75, 109].

Cardiac bypass surgery and reperfusion after acute myocardial infarction

Mechanical manipulation of the heart is another adequate stimulus for the release of ANP, and robust handling inadvertently takes place both in pump-assisted and off-pump bypass surgeries. Indeed, systemic shedding of the glycocalyx of equal magnitude was noted by us in patients irrespective of whether they were subjected to the on- or off-pump procedure, i.e., irrespective of whether the heart had experienced ischaemia/reperfusion or not [11]. At present it is not known whether shedding of the coronary glycocalyx itself occurs in bypass patients, but there is no reason to believe that the coronary system is exempt from the systemic vascular phenomenon.

As a point of interest, ANP was applied pharmacologically to patients during reperfusion by percutaneous coronary intervention after acute myocardial infarction [72]. The so-called J-WIND study was unable to show any benefit 6–12 months after ANP treatment versus control although the treatment had seemingly produced a 15% decrease in infarct size. Also in the AMISTAD II trial, in which adenosine was applied during reperfusion, there was no benefit for the patients at 6-month follow-up despite early pronounced reduction in infarct size [94]. It might be a coincidence, but both ANP and adenosine have the potential to deteriorate the coronary glycocalyx, thereby enhancing oedema as well as adhesion of leucocytes and, thus, secondary myocardial damage [87, 116].

Pathophysiological implications of damage to the glycocalyx

As outlined above, volume overload can induce impressive fluid and protein shifting towards the interstitium by deteriorating the vascular barrier [18, 51]. Consequently, and in accordance with clinical studies, volume loading in

normovolaemic patients should most likely be abolished in favour of demand-related fluid regimens [8, 65, 77]. Using fluid in adequate amounts seems to have the power to improve patient outcome by minimising perioperative fluid shifting.

Interstitial oedema is a common problem in the critically ill and in the surgical patients and appears to be related to mortality [66, 74]. Therefore, protecting vascular barrier competence is an important therapeutical issue. Until today, it remains unclear what the best strategy might be in practise to combine a sufficient cardiac preload with limiting oedema, especially in patients already suffering from capillary leakage. The introduced endothelial surface layer concept might provide some new aspects in this standoff. Therapeutic strategies targeting the endothelial glycocalyx are in view, but clinically unproven at this stage [5].

In the heart, oedema leads to a loss of myocardial compliance [30, 33]. As depicted in Fig. 10, this increase in wall stiffness is equivalent to a shift-to-the-left and steepening of the passive filling pressure curve of the ventricles. Accordingly, the ventricular pressure–volume loop for given pre- and afterload and inotropy will also be shifted to the left, amounting to a loss of stroke volume. To maintain cardiac output, preload enhancement, afterload reduction, inotropic stimulation and heart rate increase are required, either singly or in combination, to compensate. Thus, prevention of myocardial oedema must be awarded high priority in cardioprotection.

Improved magnetic resonance imaging techniques are allowing the detection of myocardial oedema early after cardiac infarction and coronary microembolisation in vivo [9, 58]. It is tempting to relate this formation of oedema to damage of the glycocalyx by ischaemia and secondary inflammatory responses. Unfortunately, to date, it is (still)

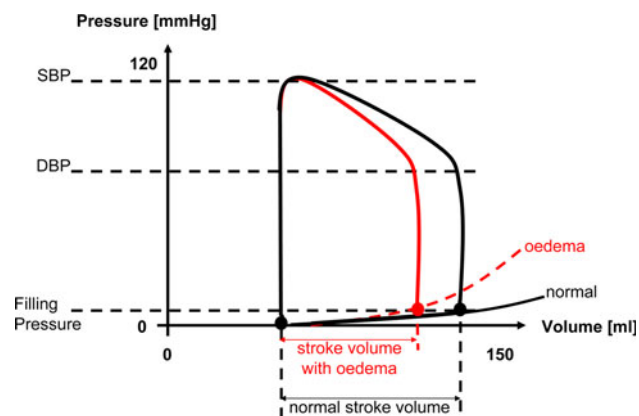


Fig. 10 Pressure–volume loop of the left ventricle (schematic drawing), illustrating the consequence of tissue oedema. The passive filling pressure curve becomes steeper, shifting the loop to the left and, thus, reducing stroke volume at given filling and aortic pressures and unaltered inotropic state

impossible to simultaneously and repetitively assess the state of the coronary endothelial glycocalyx by any means of non-invasive imaging. Concerning functional consequences, it is of interest that sites of myocardial oedema matched areas of contractile dysfunction [9].

Jacob et al. [54] have examined experimentally the ability of albumin, given as supplement to histidine–tryptophan–ketoglutarate (HTK) cardioplegic solution, to prevent postischemic damage in a glycocalyx-directed manner in a transplantation setting. Guinea pig hearts were arrested in situ by perfusion with either cold unaltered HTK solution or HTK solution containing 1% human albumin and stored for 4 h at 4°C. The hearts were reperfused with Krebs-Henseleit buffer containing 4% albumin at 37°C. Pertinently, albumin supplementation lessened shedding of syndecan and heparan sulphate, reduced myocardial oedema and postischemic adhesion of granulocytes in the coronary system, and improved pump function, especially that of the intrinsically weaker right ventricle.

Apart from albumin, other experimentally promising procedures aimed at preserving the coronary glycocalyx from damage under various conditions include cardiac (pre)treatment with hydrocortisone and antithrombin [5, 15, 17, 19]. Since full regeneration of the glycocalyx seems to require 5–7 days in vivo [84], prevention is certainly better than cure. However, partial recovery may occur more rapidly because the endothelial caveolae are filled with glycocalyx which could be externalised on short notice [21]. The restitution of fluid homeostasis of septic patients within just days in the advent of successful anti-infectious therapy, a familiar clinical observation, suggests pathways of quick repair of the glycocalyx. Indeed, nature shows that extremely rapid turnover is possible. Trypanosomes avoid immunological destruction by weaving a dense coat of surface glycoproteins of constantly changing composition to ward off attack by human antibodies [37].

Caveats and conclusions

1. Per definition, one may distinguish between the endothelial glycocalyx proper, and the layer formed when plasma constituents bind to and intercalate in the structure. Although there are basic differences between the endothelial glycocalyx and the endothelial surface layer, it is the latter we are concerned with in vivo.
2. Serious differences exist between endothelial cells in situ and cultured endothelial cells. This pertains, for one, to the dimensions of the glycocalyx (200–2,000 nm in situ, only 20–40 nm in culture [21, 55, 83]). For another, when studying permeability, responses of cultured endothelial layers and endothelial cells in situ differ so much that they cannot really

be compared. Curry and Adamson [26] have listed a great number of discrepant results and are adamant in the claim that cultured cells are at best a model for endothelium in a chronically inflamed state.

3. Gender may influence coronary permeability responses [46]. However, hard data for the human heart is still needed. Furthermore, the fundamental mechanisms regarding the role of the endothelial glycocalyx can hardly be different for males and females.
4. The main contradictions of the historical principle of Ernest Starling with the modern Endothelial Surface Layer Concept for the coronary system are:
 - The endothelial glycocalyx is the basal structure which creates, together with plasma proteins, the physiological oncotic gradient at the vascular barrier.
 - The interstitial protein concentration is of little consequence with respect to arteriolar and capillary fluid filtration under physiological circumstances.
 - Bulk colloid exchange between the vascular and the interstitial spaces is substantial, but not indiscriminate, and occurs mainly in the venular segments. Tissue factor is sequestered away from extravasating coagulation factors in intact heart tissue.
 - The endothelial cell bodies act as a second barrier, the width of interendothelial clefts and pores determining hydraulic conductivity, especially if the endothelial glycocalyx is altered.
5. The endothelial glycocalyx is important for regulating vascular permeability and fluid exchange in many organs besides the heart [1, 35, 97, 98, 115]. However, details are often still lacking, and the systemic importance is just beginning to emerge.
6. These findings are interesting from a physiological standpoint as they challenge old knowledge. More importantly, however, they are crucial for the treating physician. It is time to forcefully sound possibilities to protect the endothelial surface layer in critically ill patients [5].

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