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Speaker: Ioana Grigoras

The physiology and pathology of glycocalyx

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Endothelial glycocalix

Glycocalix – Bennett 1963

extracellular polysaccharide coating on cells

INTRAVASCULAR VOLUME

3 compartments:

Voluminous intravascular compartment

cellular volume (erithrocytes)

playing an important role in

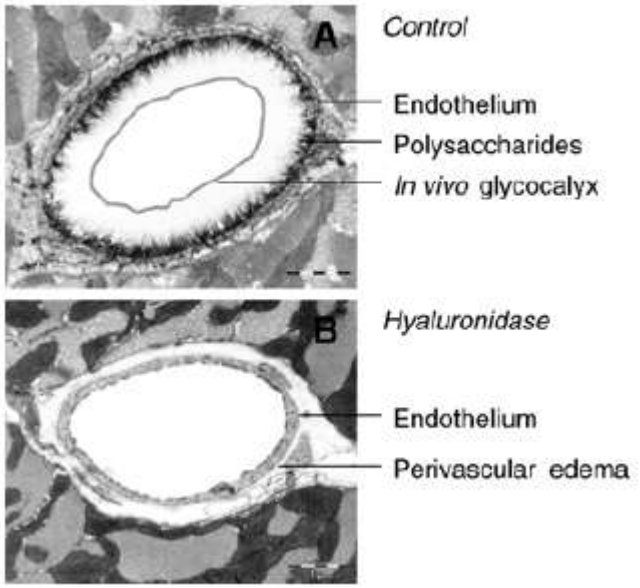
plasma volume

vascular wall homeostasis

glycocalix volume

blood flow regulation

tissue exchanges



TCM Vol. 17, No. 3, 2007

The first line barrier to regulate cell and macromolecule trasport

Endothelial glycocalix - structure

Glycoproteins
Proteoglycans
Glycosaminoglycans

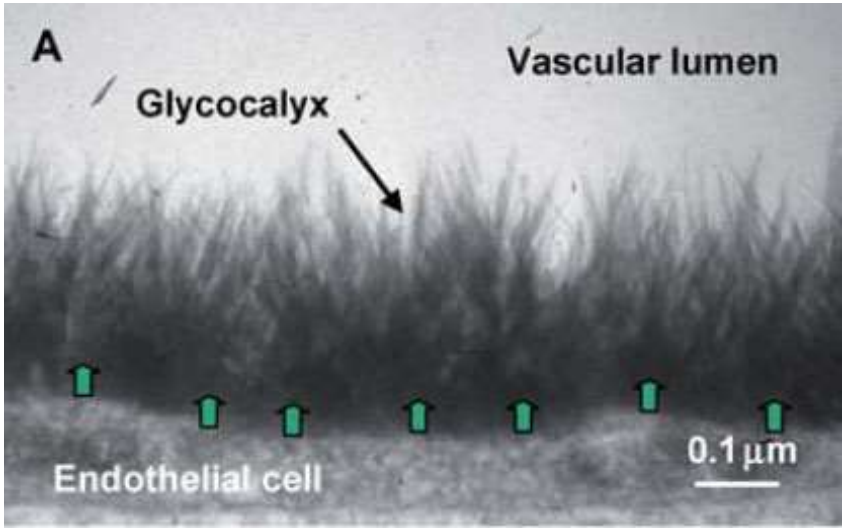
Plasma proteins
albumin
fibrinogen

+

Enzimes
lipoprotein lipase
superoxid dismutase

Growth factors
vascular endothelial growth factor

Glycocalix + endothelial cells + Water = endothelial glycocalix layer (EGL)



Becker, Cardiovascular Research, 2010,87:300

Physiology of glycocalyx

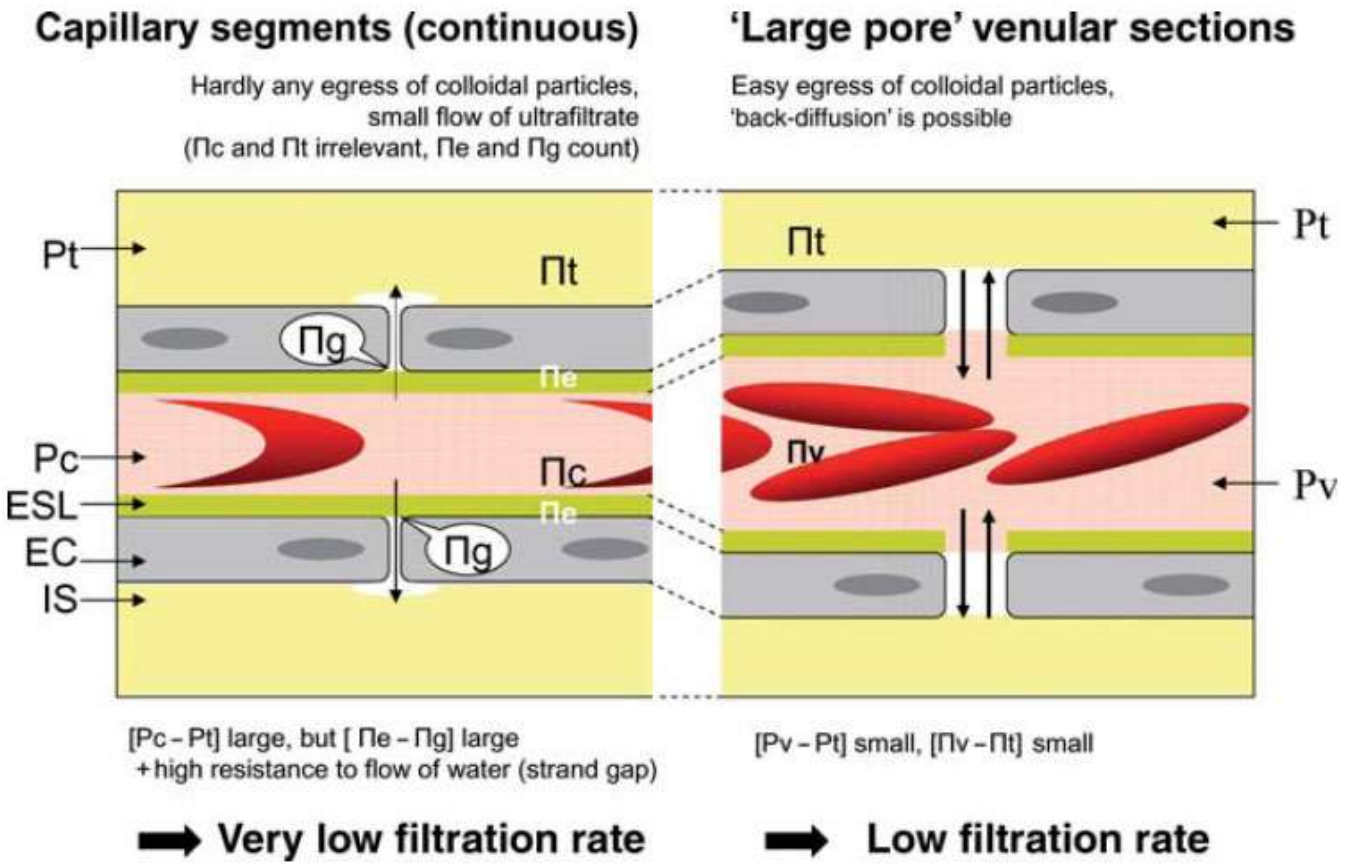
Regulation of vascular permeability in peripheral vessels

Mediation of shear stress

Attenuation of leucocyte and platelets adhesion

Becker, Cardiovascular Research, 2010

Physiology of glycocalyx



Becker, Cardiovascular Research, 2010

Cardiovascular Research (2010) **87**, 320–330
doi:10.1093/cvr/cvq146

SPOTLIGHT REVIEW

Shear stress and the endothelial transport barrier

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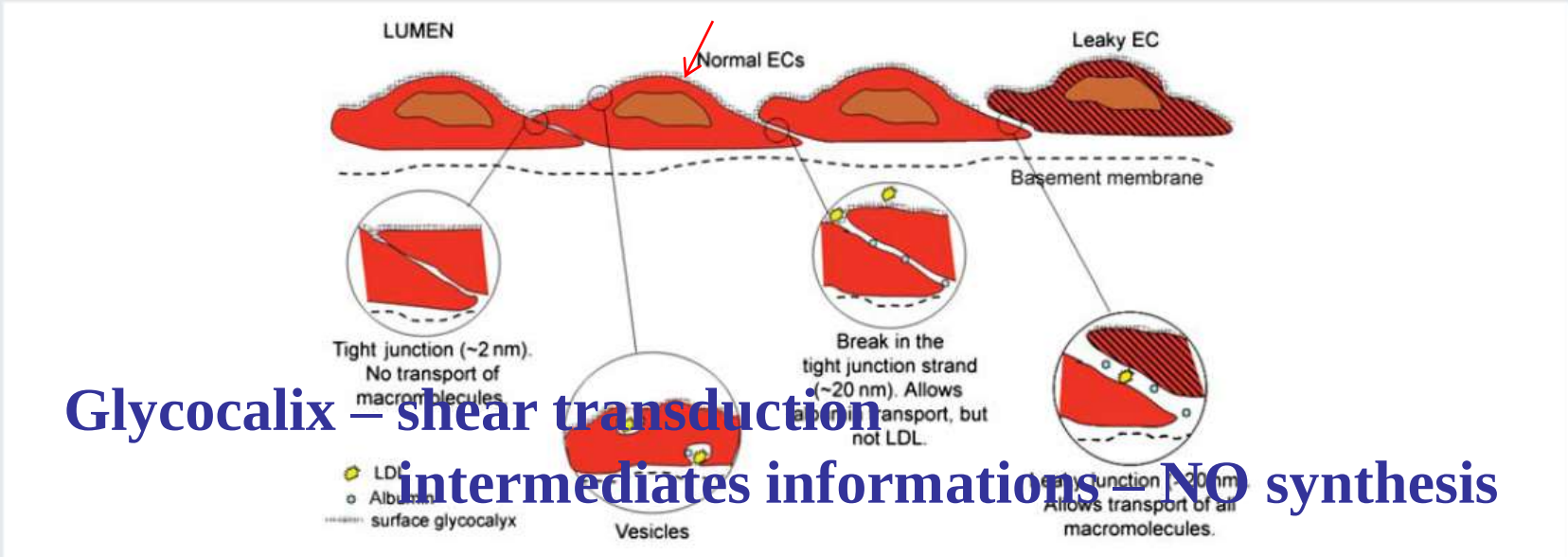


Glycocalix – shear transduction

intermediates informations – NO synthesis

Figure 1 Transport pathways across the endothelium. The major transport pathways are: the tight junctions, breaks in the tight junctions, vesicles, and leaky junctions. The surface glycocalyx covers the entrance to all but the leaky junctions (figure courtesy of Limary Cancel).

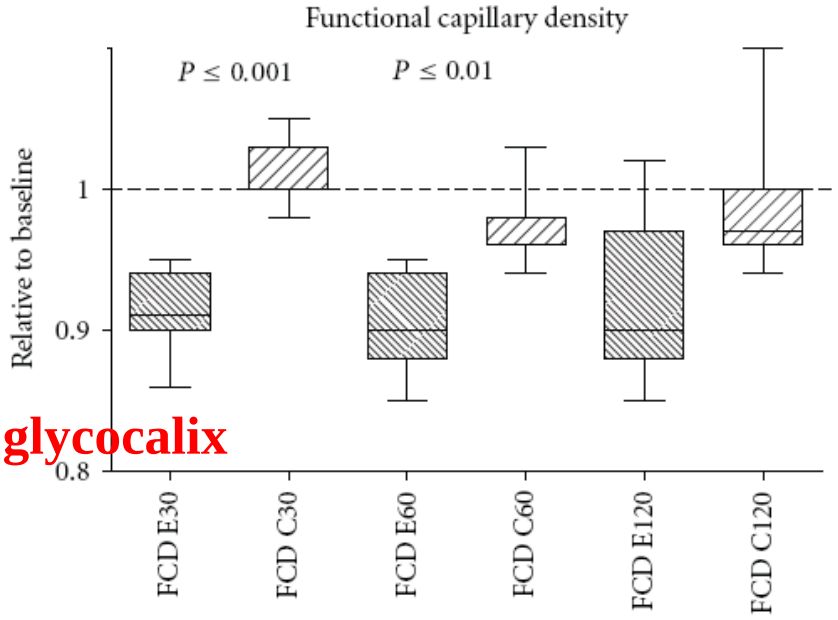
Gycocalix – role in adapting vascular beds to metabolic demand

Research Article

Impact of Enzymatic Degradation of the Endothelial Glycocalyx on Vascular Permeability in an Awake Hamster Model

S. A. Landsverk,[1,2] A. G. Tsai,[2] P. Cabrales,[2] and M. Intaglietta[2]

[1] Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093, USA
[2] Department of Anesthesiology, Oslo University Hospital, 0424 Oslo, Norway



Effects of enzimatic degradation of glycocalix

Reduced functional capillary density at 30 and 60 min vs control

FIGURE 3: Effect of enzymatic degradation of the glycocalyx on functional capillary density (FCD). Data shown as values relative to baseline. Significant changes were found, between the enzyme group (E30) and control group (C30) after 60 minutes (E60, C60). After 120 minutes (E120, C120) there was a non significant trend ($P = 0.07$).

Critical Care Research and Practice
Volume 2012, Article ID 842545, 8 pages

Endothelial glycocalix

Prevents cell adhesion

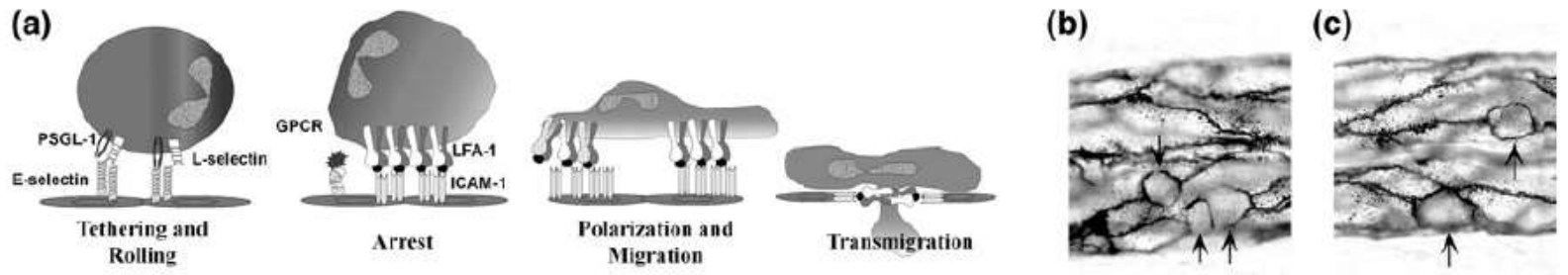


FIGURE 3. (a) The multistep process of leukocyte recruitment requires interaction of membrane-bound macromolecules near the surface of the leukocyte and the endothelium.[67] The adhesion molecules extend only tens of nm from the cell surface. The common cartoon of this cascade does not take into account the presence of a thick endothelial surface layer which limits access to the cell surface receptors. (b) Upper and (c) lower sides of same vessel in frog mesentery show that leukocytes (arrow) preferentially localize to endothelial cell borders as revealed by silver staining[27] (a) Courtesy of Professor Scott I. Simon, and (b) and (c) used with permission from the American Physiological Society.

Annals of Biomedical Engineering, Vol. 40, No. 4, April 2012 (© 2011) pp. 828–839

Pathology of glycocalyx

Glycocalix injury
shedding, fragmantation

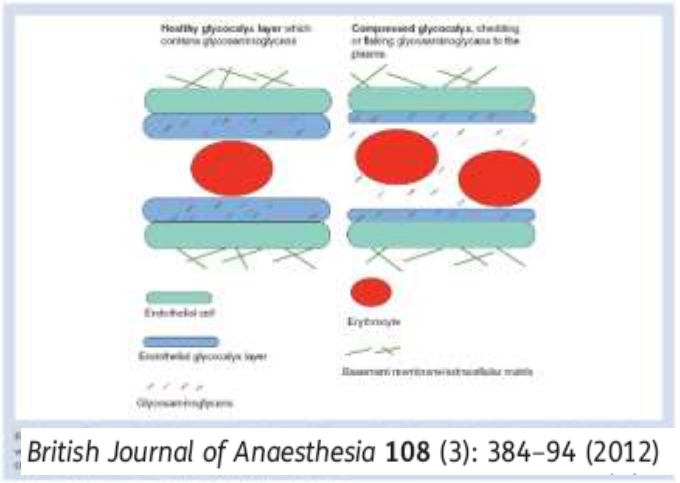
Types:

Compaction of EGL and ↑ heparan, hyaluronic acid, condroitin in plasma

- Partial (shedding)
  - Selective cleavage of sulfate side chains (heparinase) or of receptor-bound chains (hialuronidase)
- Total distruction of glycocalyx

Consequences:

- Capillary leak syndrome
- Oedema formation
- Accelerated inflammation
- Platelet hyperaggregation
- Hypercoagulation
- Loss of vascular tone response



British Journal of Anaesthesia **108** (3): 384–94 (2012)

Becker, Cardiovascular Research, 2010

Pathology of glycocalyx

Reperfusion injury

Microvascular dysfunction

Enhanced adhesion of platelets and leukocytes

Activation of coagulation

Detachment of swollen endothelial cells

Oxidative stress of endothelial cells

Increased vascular permeability

Degradation of glycocalyx

Becker, Cardiovascular Research, 2010

Pathology of glycocalyx

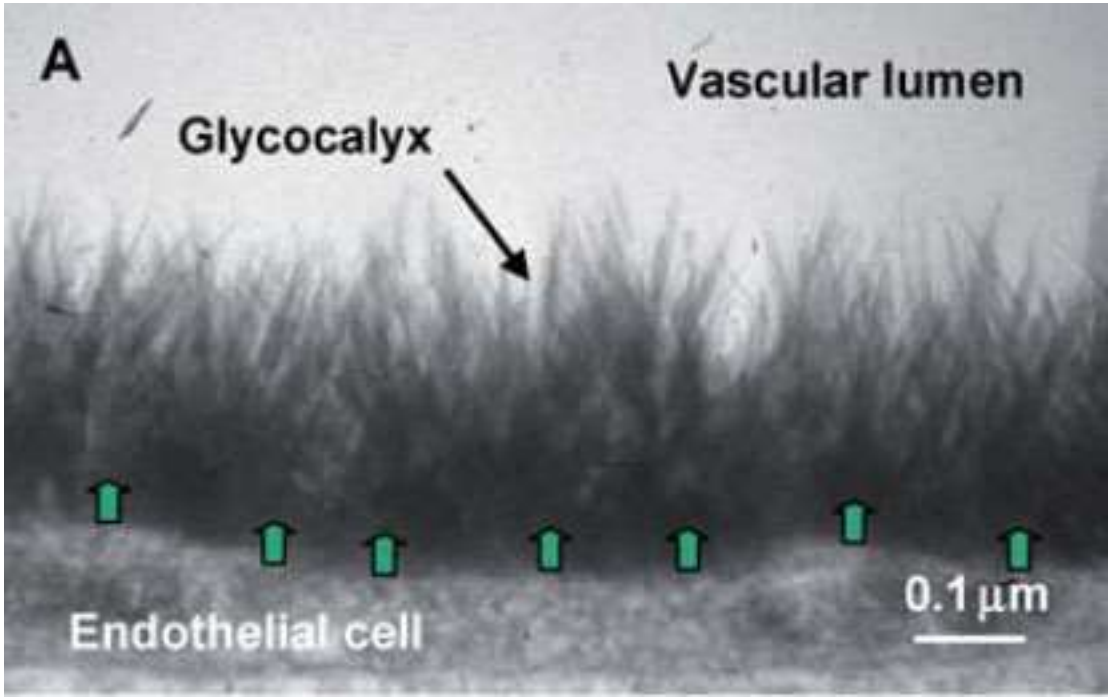
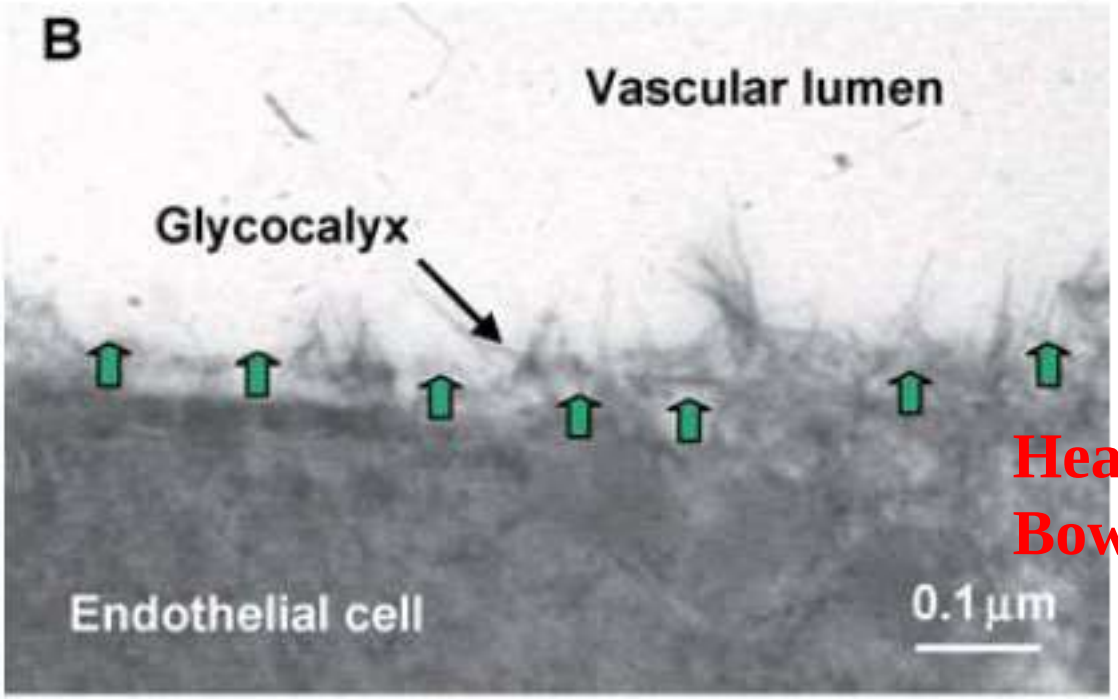


Figure 4 The glycocalyx is shown for guinea pig hearts **under normal perfusion** . The green arrows represent the estimated maximal extension (10 nm) of the bonds between membrane molecules for firm endothelial adhesion of leucocytes and platelets. Adhesion molecules in question are the ICAMs, VCAMs, PECAM, integrins, etc.

Becker, Cardiovascular Research, 2010

Pathology of glycocalyx



Heart 20min
Bowel 60 min

Figure 4 The glycocalyx is shown for guinea pig hearts **after I/R** . The green arrows represent the estimated maximal extension (10 nm) of the bonds between membrane molecules for firm endothelial adhesion of leucocytes and platelets. Adhesion molecules in question are the ICAMs, VCAMs, PECAM, integrins, etc.

Becker, Cardiovascular Research, 2010

Pathology of glycocalyx

Inflammation and trauma

Surgical trauma - systemic inflammatory response

Vascular endothelium inflammation

- **Swelling of endothelial cells**
- **Proinflammatory and procoagulant fenotype**
- **Damage of cell membrane (loss of content)**
- **Necrosis**
- **Degradation of glycocalyx by inflammatory mediators**

Glycocalyx constituents detected in plasma of patients with septic shock – related to mortality

Becker, Cardiovascular Research, 2010

Pathology of glycocalyx

Hypervolemia

- Acute normovolemic hemodilution in major surgery – 60% of infused volume in interstitial space within minutes (HAES or albumin)
- Hypervolemia – release of atrial natriuretic peptide by heart (wall stress) alteration (shedding) of glycocalyx

Becker, Cardiovascular Research, 2010

Table 1 Factors affecting the endothelial glycocalyx—known injurious mechanisms and potential protective agents

Degradation	Protection
Ischaemia/reperfusion; hypoxia/reoxygenation; inflammatory cytokines proteases; atrial natriuretic peptide	Sevoflurane; hydrocortisone; antithrombin

Anesthesiology 2007; 107:776–84

Hydrocortisone Preserves the Vascular Barrier by Protecting the Endothelial Glycocalyx

Daniel Chappell, M.D., Matthias Jacob, M.D.,* Klaus Hofmann-Kiefer, M.D.,* Dirk Bruegger, M.D.,* Markus Rehm, M.D.,† Peter Conzen, M.D.,‡ Ulrich Welsch, M.D., Ph.D.,§ Bernhard F. Becker, M.D., Ph.D.‖*

Hidrocortison 20 min 20 min warm ischemia and reperfusion

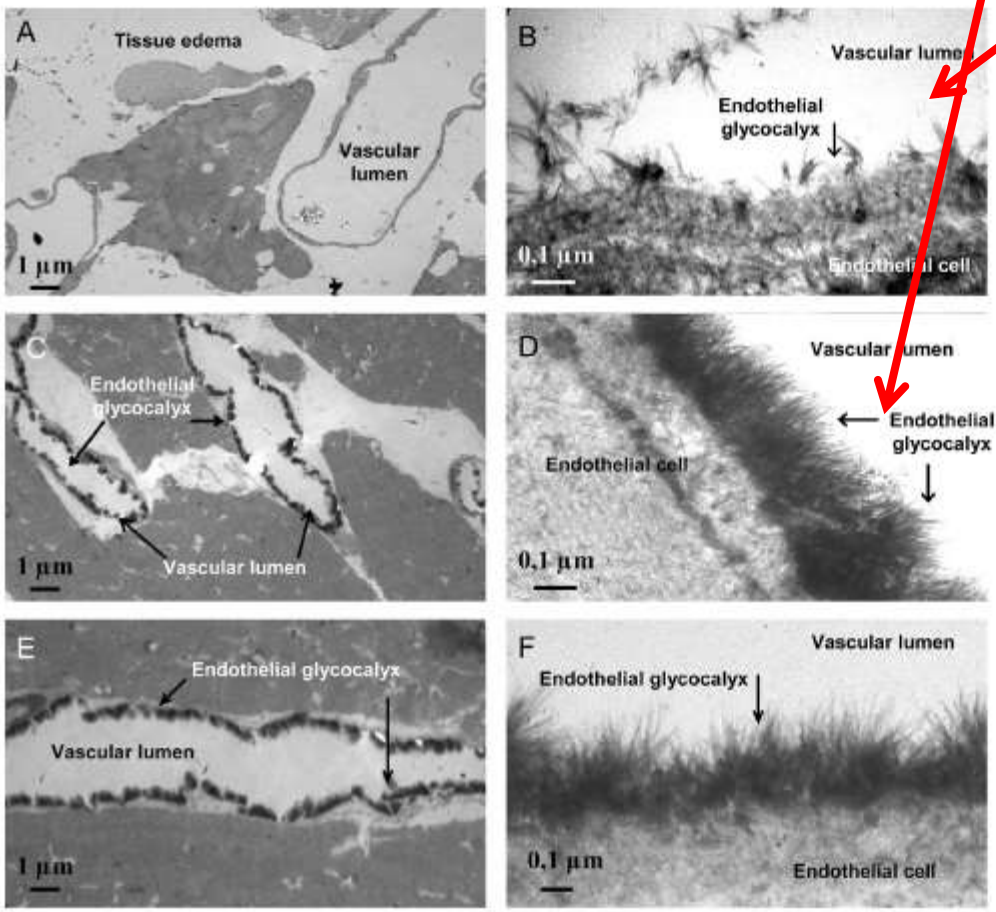
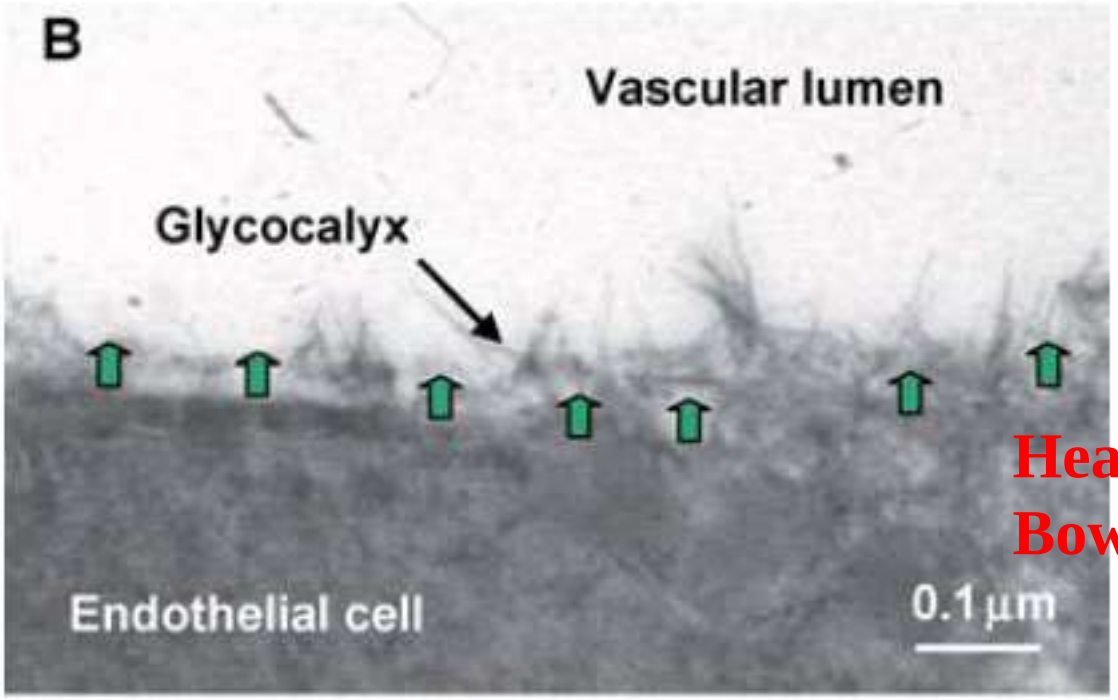


Fig. 5. Electron microscopic views of the hearts stained to reveal the glycocalyx. (*A* and *B*) Control experiment after 20 min of warm ischemia and reperfusion. The endothelial glycocalyx is nearly completely degraded, and a significant formation of edema is visualized. *A* is an overview, and *B* is a close-up view of the degraded glycocalyx. (*C* and *D*) After treatment with hydrocortisone and 20 min of warm ischemia and reperfusion. Endothelial glycocalyx is mostly intact, and less edema formation can be seen. *C* is an overview, and *D* is a close-up view of the intact glycocalyx. (*E* and *F*) Heart perfused for 35 min without ischemia. *E* is an overview, and *F* is a close-up view of the intact glycocalyx.

Pathology of glycocalyx



Heart 20min
Bowel 60 min

Figure 4 The glycocalyx is shown for guinea pig hearts **after I/R** . The green arrows represent the estimated maximal extension (10 nm) of the bonds between membrane molecules for firm endothelial adhesion of leucocytes and platelets. Adhesion molecules in question are the ICAMs, VCAMs, PECAM, integrins, etc.

Becker, Cardiovascular Research, 2010

Pathology of glycocalyx

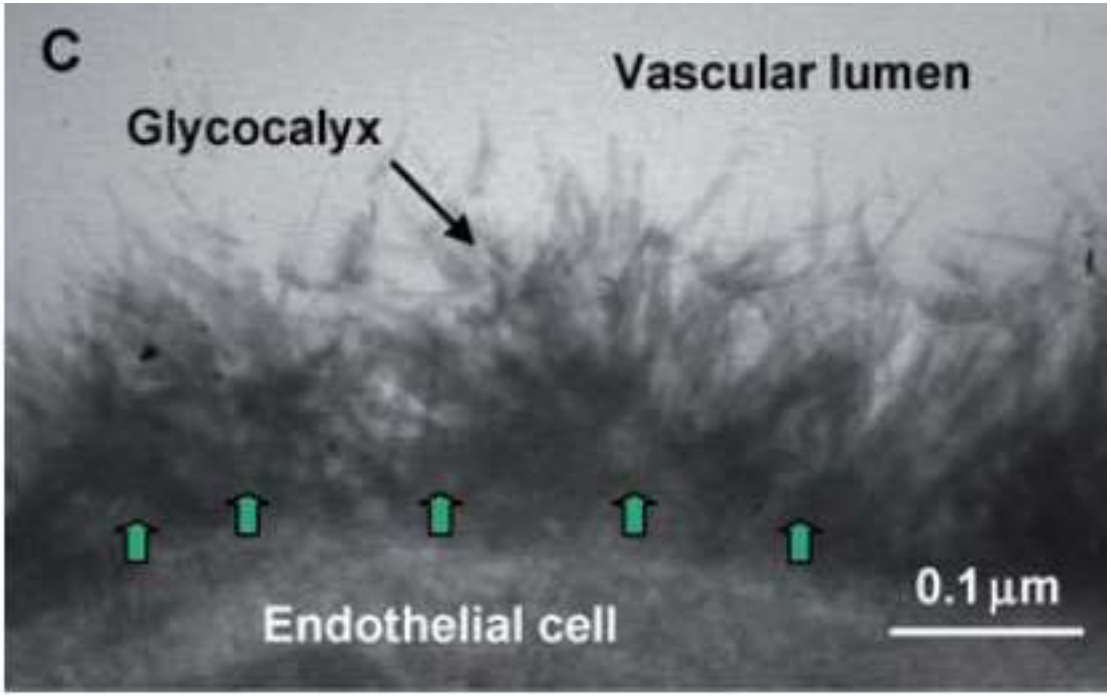


Figure 4 The glycocalyx is shown for guinea pig hearts **after I/R with protection by antithrombin** . The green arrows represent the estimated maximal extension (10 nm) of the bonds between membrane molecules for firm endothelial adhesion of leucocytes and platelets. Adhesion molecules in question are the ICAMs, VCAMs, PECAM, integrins, etc.

Becker, Cardiovascular Research, 2010

Physiology of glycocalyx

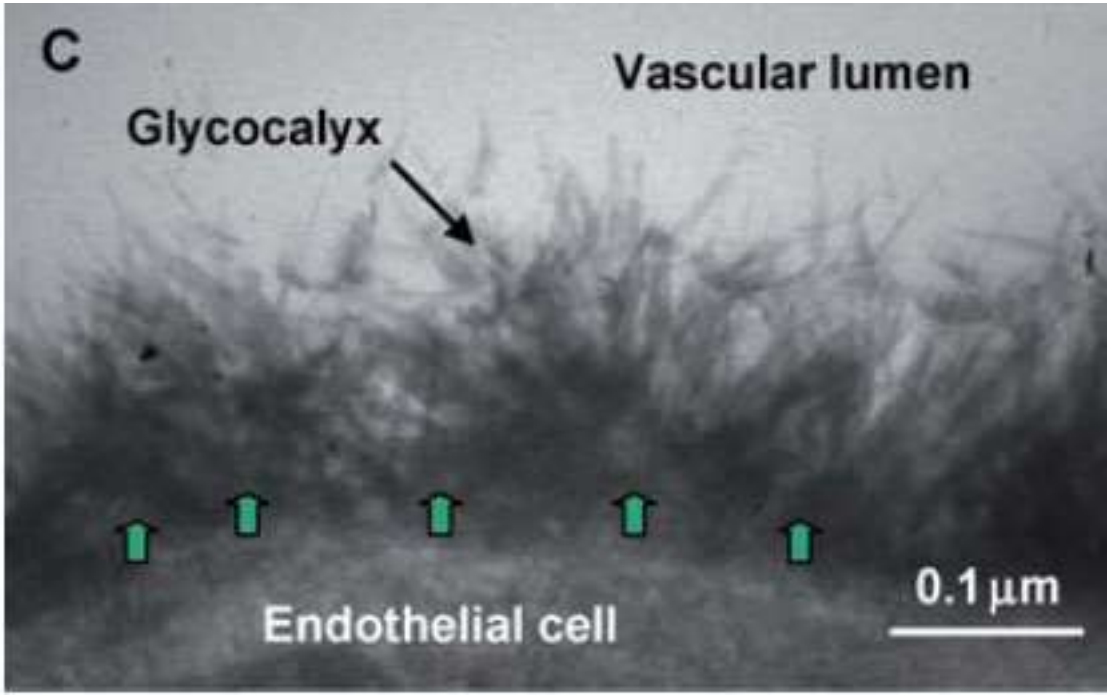


Figure 4 The glycocalyx is shown for guinea pig hearts **after I/R with protection by antithrombin** . The green arrows represent the estimated maximal extension (10 nm) of the bonds between membrane molecules for firm endothelial adhesion of leucocytes and platelets. Adhesion molecules in question are the ICAMs, VCAMs, PECAM, integrins, etc.

Becker, Cardiovascular Research, 2010

Q J Med 2008; **101**:513–518
doi:10.1093/qjmed/hcn024 Advance Access published on 4 March 2008

Review

QJM

Hypothesis: arterial glycocalyx dysfunction is the first step in the atherothrombotic process

M.I.M. NOBLE[1], A.J. DRAKE-HOLLAND[2] and H. VINK[3]

From the [1]Department of Medicine and Therapeutics, University of Aberdeen Medical School, [2]School of Pharmacy and Life Sciences, The Robert Gordon University, Aberdeen, UK, and [3]Department of Physiology, Maastricht University, The Netherlands

F1000Reports BIOLOGY

Published: 02 October 2012

Glycocalix and Na regulation

An emerging concept of vascular salt sensitivity

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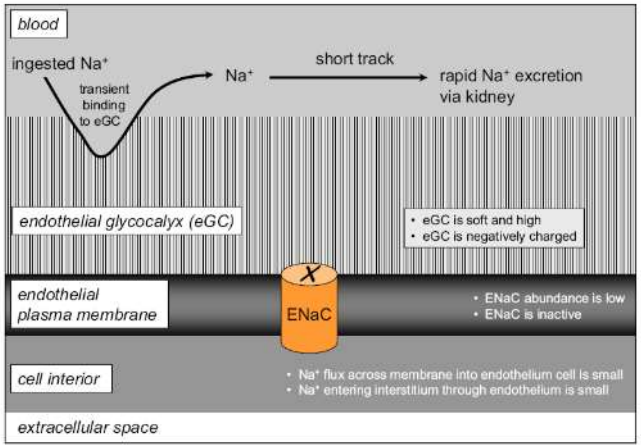
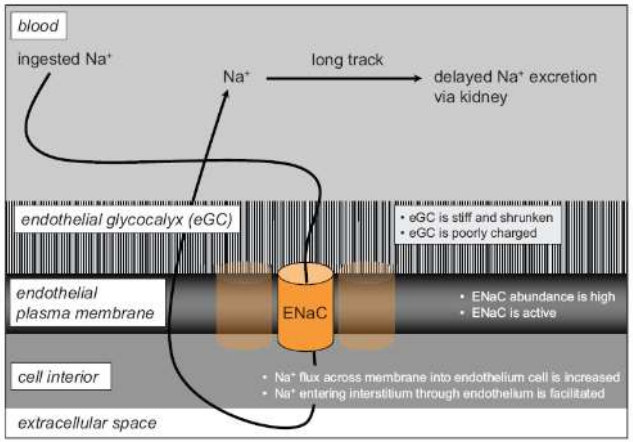
F1000 Biology Reports 2012, 4:20 (doi:10.3410/B4-20)

Figure 1. Model explaining *low* vascular sodium sensitivity



This state of vascular function is associated with low daily sodium intake, and/or low aldosterone and/or favorable genetics. Abbreviations: ENaC, epithelial sodium channel.

Figure 2. Model explaining *high* vascular sodium sensitivity



This state of vascular function is associated with high daily sodium intake, and/or high aldosterone and/or unfavorable genetics. Abbreviations: ENaC, epithelial sodium channel.

Rationale of fluid therapy

REVIEW ARTICLE

Anesthesiology 2010; 113:470–81

David S. Warner, M.D., Editor

Volume Kinetics for Infusion Fluids

Robert G. Hahn, M.D., Ph.D.*

VOLUME kinetics is an adaptation of pharmacokinetic theory that makes it possible to analyze and simulate the distribution and elimination of infusion fluids.

By the use of volume kinetics we can study the disposition of different infusion fluids in terms of parameter values or, by simulation, compare the rates of infusion required to reach a predetermined plasma volume expansion. Volume kinetics has also made it possible to quantify changes in the distribution and elimination of fluids that result from stress, hypovolemia, anesthesia, and surgery.

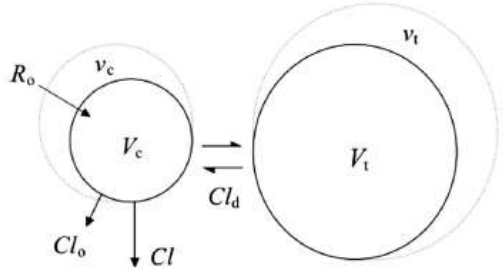


Fig. 2. The two-volume kinetic model. Fluid is infused at the rate R_o into the body fluid space V_c, which is then expanded to v_c. Fluid exchanges with V_t and becomes eliminated *via* a dilution-dependent mechanism, Cl. All sources of baseline fluid losses are accounted for by Cl_o. When v_c approaches V_c, the fractional increase in volume approaches zero. When this occurs, the total elimination clearance approaches Cl_o. Cl_d = distribution clearance.

Robert G. Hahn

REVIEW ARTICLE

Anesthesiology 2010; 113:470–81

David S. Warner, M.D., Editor

Volume Kinetics for Infusion Fluids

Robert G. Hahn, M.D., Ph.D.*

25ml/kg acetated Ringer solution over 30 min

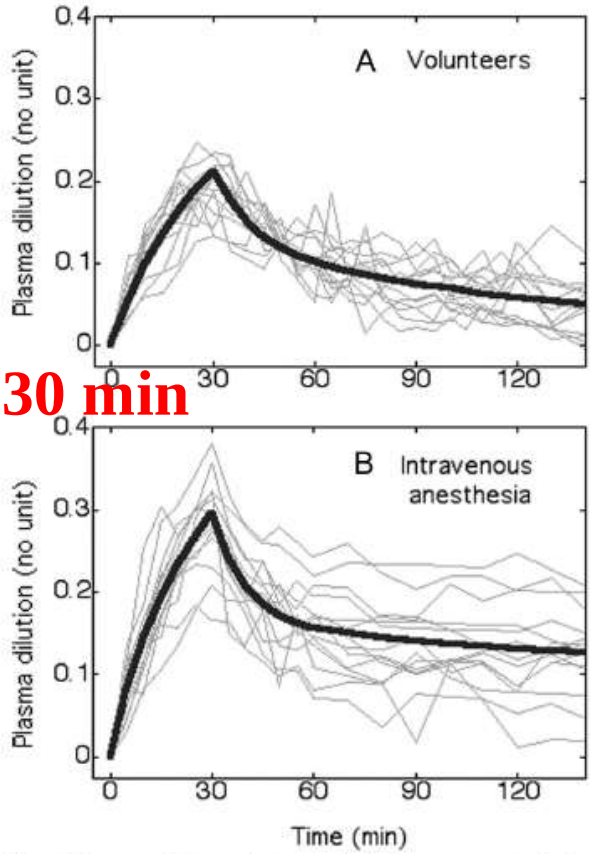


Fig. 2. Plasma dilution during and after intravenous infusion of 25 ml/kg acetated Ringer's solution over 30 min in 14 representative normovolemic volunteers (*A*, selected from Refs.[11,44]) and in 14 patients undergoing thyroid surgery with intravenous anesthesia (*B*, based on Ref.[17]). *Thin lines* = individual experiments. *Dark lines* = optimal curve fit for kinetic analysis based on all experiments.

higher volume effect in anesthetized vs healthy volunteers

REVIEW ARTICLE

Anesthesiology 2010; 113:470–81

David S. Warner, M.D., Editor

Volume Kinetics for Infusion Fluids

Robert G. Hahn, M.D., Ph.D.*

Low Elimination Clearance during Surgery

The elimination clearance (*Cl*) for isotonic crystalloid fluid varies greatly depending on whether a patient is conscious or anesthetized. Other factors such as hydration, stress, and trauma also seem to play a role.

Table 2. Elimination Clearance of Acetated Ringer's Solution under Various Physiologic Circumstances in Adults

	Clearance (ml/min)	References
Healthy volunteers	60–110	3,8,10,11
Pre-eclampsia	125	19
Normal pregnancy	36	19
Thyroid surgery	10	17
Laparoscopic cholecystectomy	7	16
Open abdominal surgery*	21	21

* Patients received lactated Ringer's solution.

REVIEW ARTICLE

Anesthesiology 2010; 113:470–81

David S. Warner, M.D., Editor

Volume Kinetics for Infusion Fluids

Robert G. Hahn, M.D., Ph.D.*

The volume effect of
the same amount of cristalloids

over 30min in healthy volunteers
vs
over 60 min in anesthetized patients

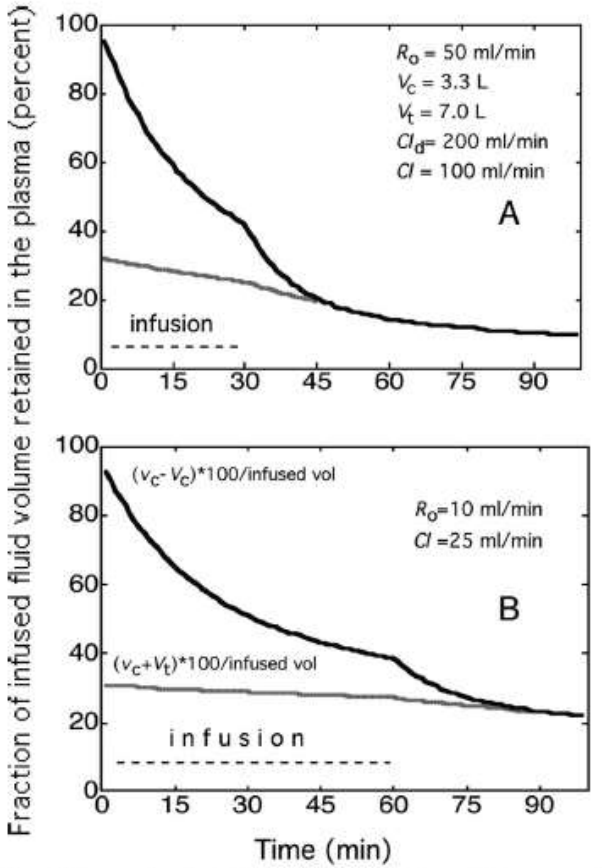


Fig. 6. Computer simulation of the percentage of the amount of infused Ringer's solution that still remains in the plasma, calculated as $(v_c - V_c) \cdot 100$/infused volume, based on typical kinetic data for a brisk 30-min infusion in volunteers (*A*) and a much slower infusion during 60 min in perioperative patients (*B*). The *light lines* show what the fraction would have been if distribution from the plasma to the interstitial fluid space was immediate. Cl = clearance; Cl_d = distribution clearance; R_o = rate of infusion; V_c and V_t = size of central and peripheral fluid spaces, respectively, which are termed v_c and v_t when expanded.

REVIEW ARTICLE

Anesthesiology 2010; 113:470–81

David S. Warner, M.D., Editor

Volume Kinetics for Infusion Fluids

Robert G. Hahn, M.D., Ph.D.*

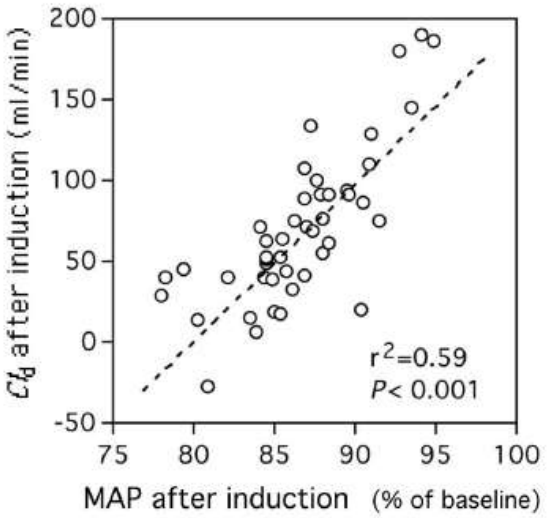


Fig. 9. The mean arterial pressure (MAP) after induction of either general anesthesia with propofol or epidural anesthesia with ropivacaine *versus* the distribution clearance (Cl_d) for lactated Ringer's solution measured after the induction. A lowered MAP retards distribution of the fluid from the plasma to the interstitial fluid space so much as to finally become arrested when Cl_d = 0. Based on data from Ref. 31.

esthesia than in the conscious state. However, it is most apparent during the onset of spinal, epidural, and general anesthesia. Then, the distribution of fluid from the plasma to the interstitium might even be arrested. The effect is dependent on the decrease of the arterial pressure and boosts the plasma volume expansion in response to infused fluid.

Fluid therapy in major surgery

Interstitial space

- anatomical - functional ECV
- nonanatomical – nonfunctional ECV (third space)

The third space doesn’t exist

Fluid therapy in major surgery

The endothelial glycocalix

The gateway to the interstitial space

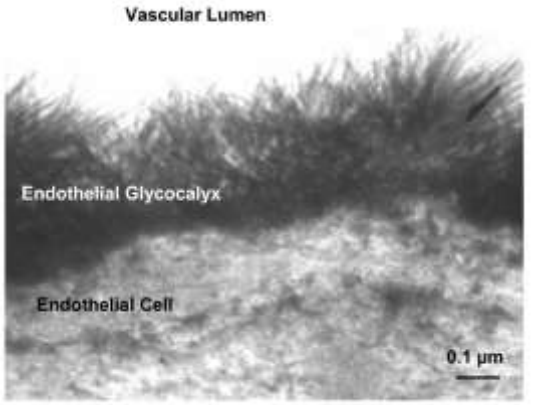


Fig. 6. Electron microscopic view of the endothelial glycocalyx.

The vascular permeability – **THE DOUBLE BARRIER CONCEPT**

- endothelial cell line
- glycocalix

- fluid shift depends on hydrostatic and oncotic pressure between blood and the space beneath glycocalix inside the anatomical lumen of the vessel

Chappell, Anesthesilogy, 2008, 109:723-40

British Journal of Anaesthesia **109** (1): 69–79 (2012)

Fluid therapy in major surgery

Types of fluid shift

- type I – physiologic shift
  - all the time
  - shift of colloid-free water and electrolytes
  - from intravascular to interstitial space
  - the vascular barrier is intact
  - in pathologic amount, if large volume of isotonic cristalloids are given
- type II – pathologic shift
  - shift of colloid-rich (proteins) fluids
  - altered vascular barrier
  - inconstant
  - depends on type, extent and duration of surgery
  - endothelial damage – endothelial glycocalix alteration
  - 2 iatrogenic causes
    - surgery: mechanical stress, endotoxin exposure, ischemia-reperfusion, inflammation
    - anesthesia: acute hypervolemia

Perioperative fluid shift: trigger or effect of liberal fluid handling ?

Chappell, Anesthesilogy, 2008, 109:723-40

Fluid therapy in major surgery

Diminution of endothelial glycocalix

Consequences

- platelet aggregation
- leukocyte adhesion
- increase in endothelial permeability
- tissue oedema

Diminution of endothelial glycocalix

Causes

- ischemia-reperfusion
- proteases
- TNFα
- atrial natriuretic peptide

} **surgery**

acute hypervolemia - **anesthesia**

Chappell, Anesthesiology, 2008, 109:723-40

Fluid therapy in major surgery

Minimizing type I shifting

- ***cristalloids***
  - for replacement of urine losses and insensible perspiration
- ***colloids***
  - for replacement blood loss

Minimizing type II shifting

- prophylaxis - protection of endothelium surface layer
  - ***surgery*** - atraumatic surgical technique
    - minimally invazive surgery
    - gentle handling
    - mechanical sutures
  - ***anesthesia***
    - neuraxial block - continuous epidural analgesia over 48-72h
    - avoiding hypervolemia
- treatment - ???

Chappell, Anesthesilogy, 2008, 109:723-40

British Journal of Anaesthesia **108** (3): 384–94 (2012)
Advance Access publication 29 January 2012 · doi:10.1093/bja/aer515

Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy

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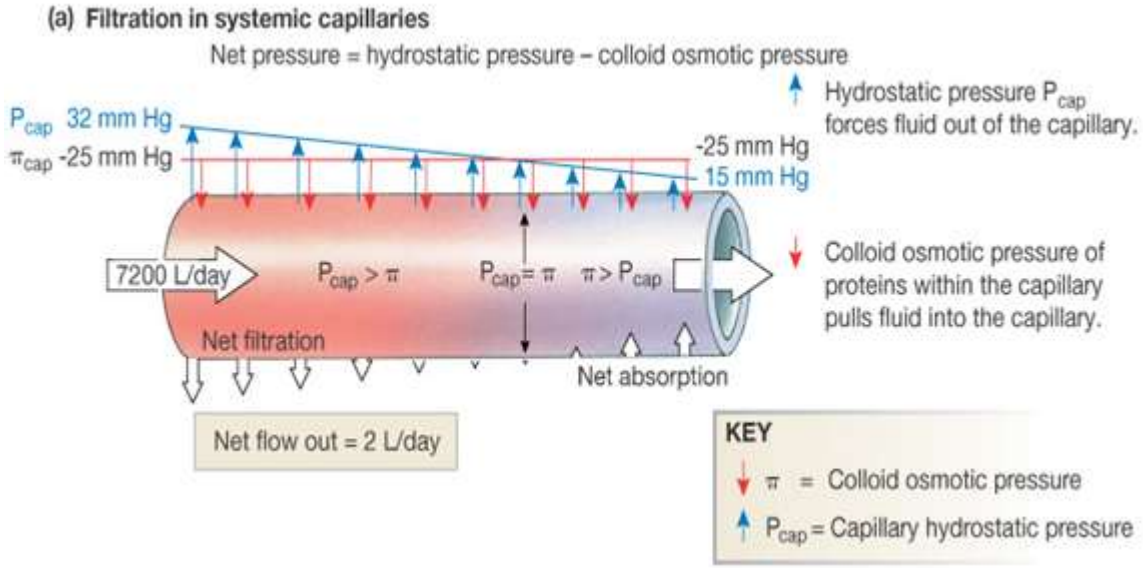
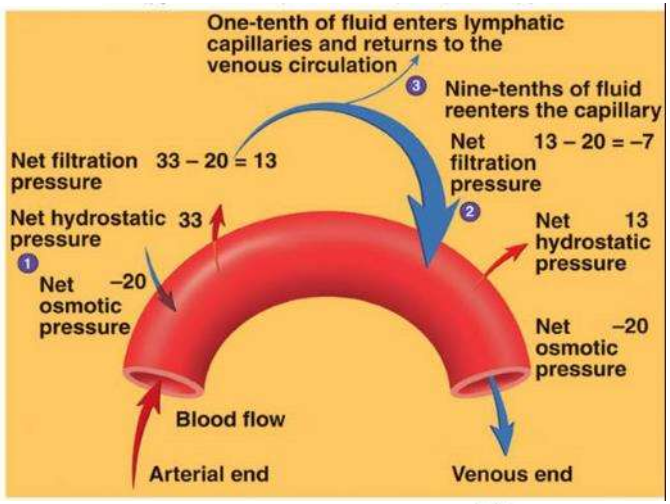
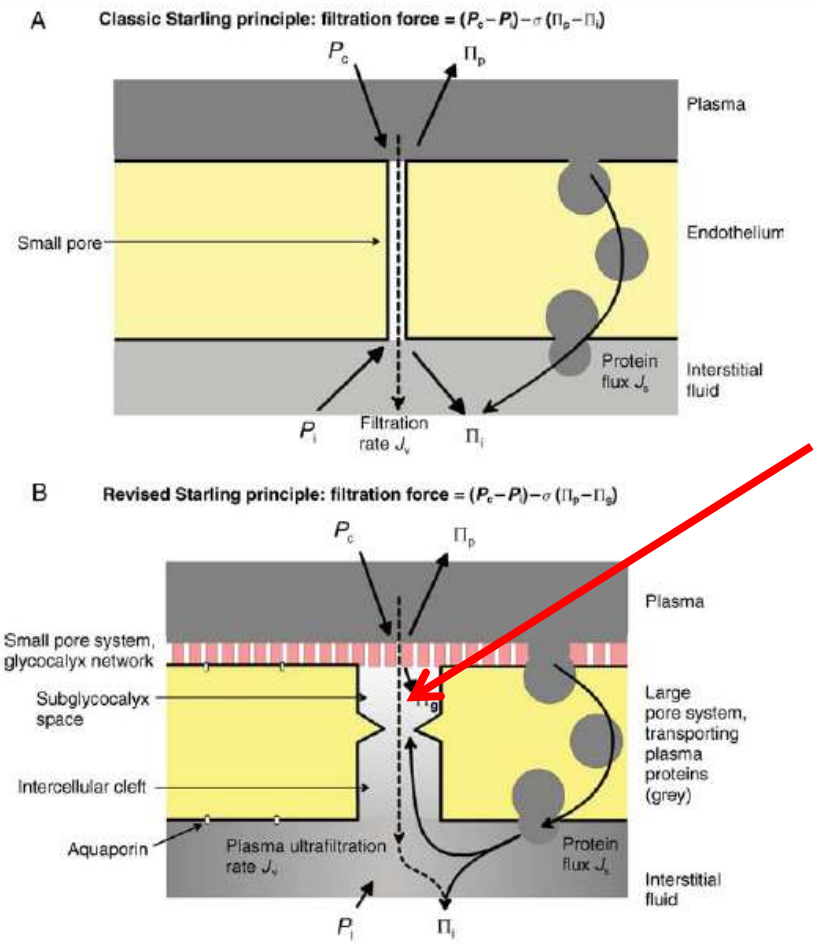
[2] The Australian School of Advanced Medicine, Macquarie University, NSW 2109, Australia

Capillary Starling equilibrium





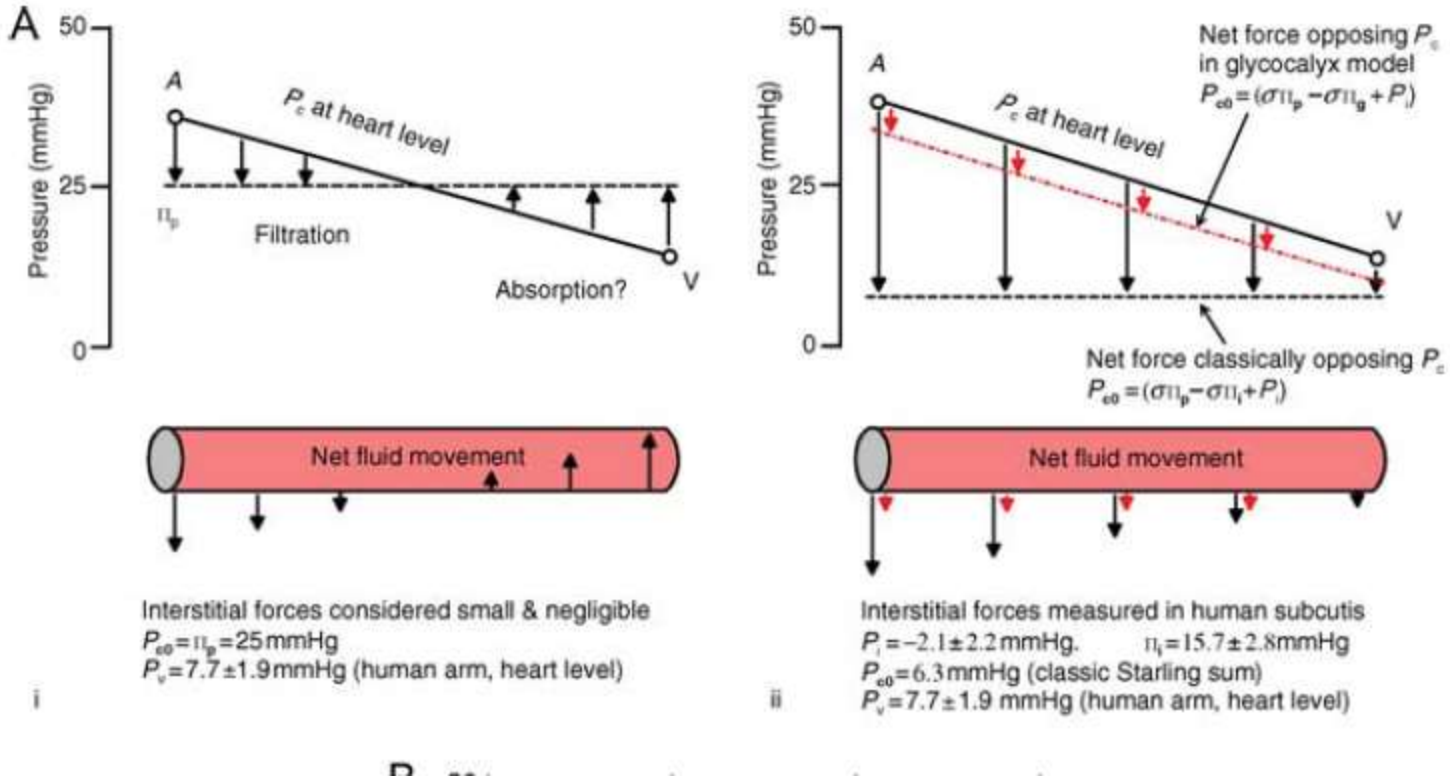


Subglycocalix space
protein free

Figure 1 Comparison of traditional and revised views of the endothelial semipermeable membrane and the forces acting on it. (*A*) Traditional view of continuous endothelium as a semipermeable membrane. (*B*) The glycocalyx–cleft model identifies glycocalyx as a semipermeable layer. Its underside is subjected to the COP of fluid high inside the intercellular cleft rather than ISF, with important functional consequences.[80,82] Symbols defined in main text. Grey shade denotes concentration of plasma protein.

Classical Starling equation

Revised Starling equation



Cardiovascular Research (2010) **87**, 198–210

At very low capillary pressure – transient water influx

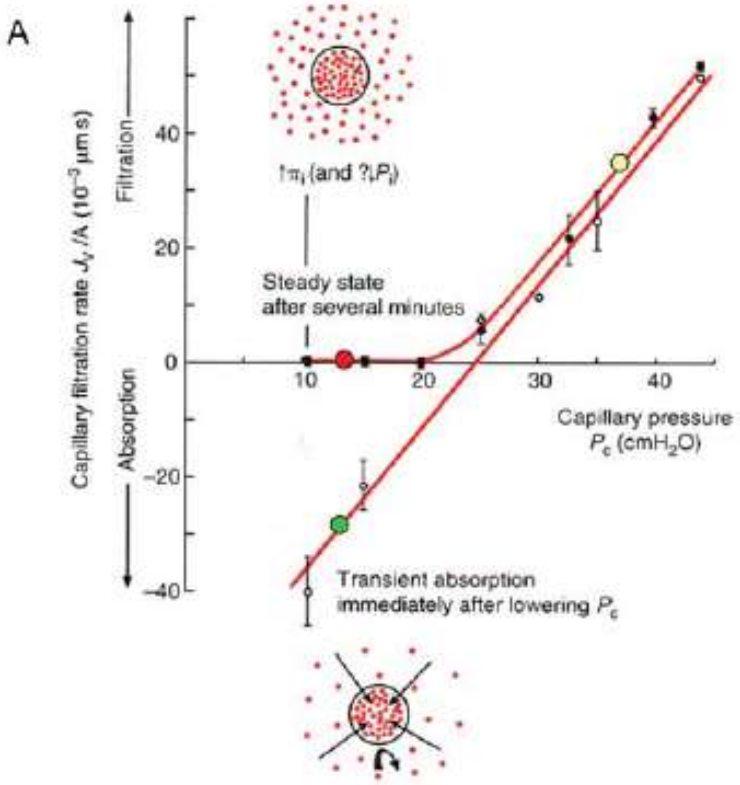


Table 1 Comparison of the original and revised paradigms for prescribing fluid therapy

Original Starling principle	Revised Starling equation and glycocalyx model
Intravascular volume consists of plasma and cellular elements	Intravascular volume consists of glycocalyx volume, plasma volume, and red cell distribution volume
Capillaries separate plasma with high protein concentration from ISF with low protein concentration	Sinusoidal tissues (marrow, spleen, and liver) have discontinuous capillaries and their ISF is essentially part of the plasma volume Open fenestrated capillaries produce the renal glomerular filtrate Diaphragm fenestrated capillaries in specialized tissues can absorb ISF to plasma Continuous capillaries exhibit 'no absorption' The EGL is semi-permeable to anionic proteins and their concentration in the intercellular clefts below the glycocalyx is very low
The important Starling forces are the transendothelial pressure difference and the plasma–interstitial COP difference	The important Starling forces are the transendothelial pressure difference and the plasma–subglycocalyx COP difference. ISF COP is not a direct determinant of J_v
Fluid is filtered from the arterial end of capillaries and absorbed from the venous end. Small proportion returns to the circulation as lymph	J_v is much less than predicted by Starling's principle, and the major route for return to the circulation is as lymph
Raising plasma COP enhances absorption and shifts fluid from ISF to plasma	Raising plasma COP reduces J_v but does not cause absorption
At subnormal capillary pressure, net absorption increases plasma volume	At subnormal capillary pressure, J_v approaches zero. Auto transfusion is acute, transient, and limited to about 500 ml
At supranormal capillary pressure, net filtration increases ISF volume	At supranormal capillary pressure, when the COP difference is maximal, J_v is proportional to transendothelial pressure difference
Infused colloid solution is distributed through the plasma volume, and infused ISS through the extracellular volume	Infused colloid solution is initially distributed through the plasma volume, and infused ISS through the intravascular volume At supranormal capillary pressure, infusion of colloid solution preserves plasma COP, raises capillary pressure, and increases J_v At supranormal capillary pressure, infusion of ISS also raises capillary pressure, but it lowers COP and so increases J_v more than the same colloid solution volume At subnormal capillary pressure, infusion of colloid solution increases plasma volume and infusion of ISS increases intravascular volume, but J_v remains close to zero in both cases

Fluid therapy in major surgery

Cristalloids versus colloids

Cristalloids and colloids

Liberal ? Standard ? Restrictive ?

The right amount

Fluid therapy in major surgery

We must use
the right kind of fluid
in appropiates amounts
at the right time
to reduce collateral damage.