Glycocalyx: What is it and what is it important?

Ulises Baltazar, MD, FACS, CLT
Assistant Professor of Vascular Surgery Weill-Cornell University
Director of the Veno-Lymphatic Service

Houston Methodist Hospital Sugar Land
Sugar Land, Texas

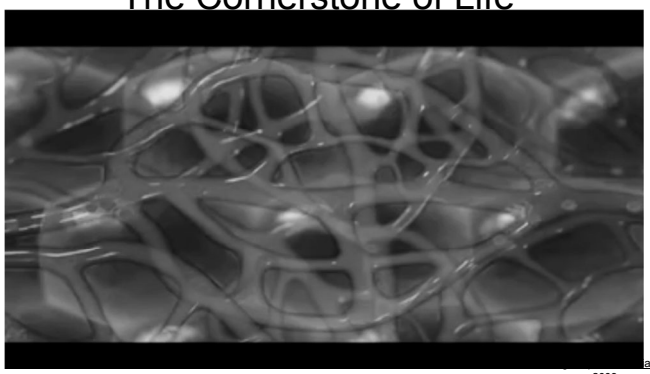
"When you are a hammer….
everything looks like a nail"

What is

The Cornerstone of Life?

Microcirculation

The Cornerstone of Life



a.com
2009

Microcirculation

- Capillary flow regulation (Getting the blood there)
- Hydrostatic - Oncotic equilibrium (Osmosis Starling principle)
- Filtration (Lymph formation revised Starling principle)

Microcirculation

- Capillary flow regulation

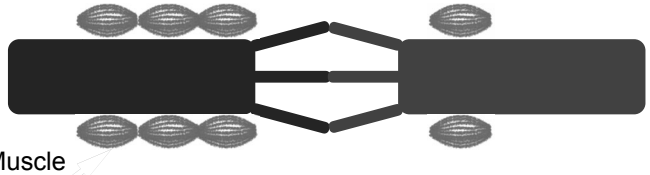
Microcirculation

- Capillary flow regulation
  - Arteriolar myogenic response
  - Venoarteriolar reflex
  - Pre-capillary arteriolar vasomotion

Wienersperger NF, Bouskela, E Microcirculation in insulin resistance and diabetes: more than and compilation" Diabetes Metab 2003:29, 6S77-6S87

Microcirculation

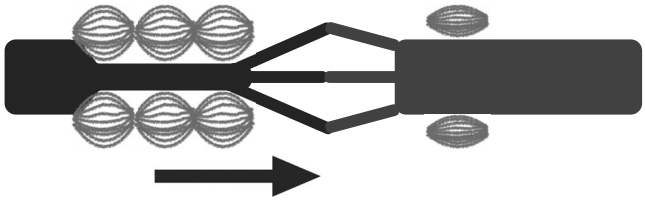
- Capillary flow regulation
  - Arteriolar myogenic response



Muscle Cells

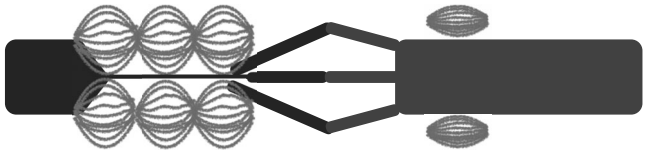
Microcirculation

- Capillary flow regulation
  - Arteriolar myogenic response



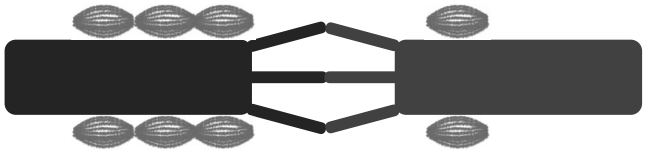
Microcirculation

- Capillary flow regulation
  - Arteriolar myogenic response



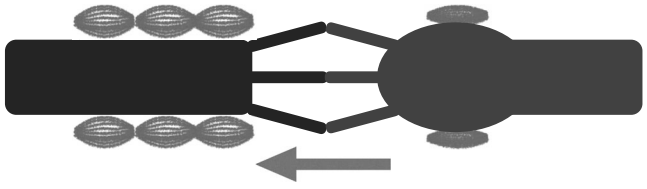
Microcirculation

- Capillary flow regulation
  - Venoarteriolar reflex



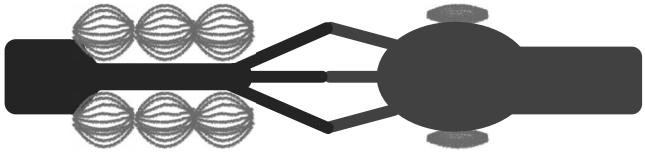
Microcirculation

- Capillary flow regulation
  - Venoarteriolar reflex



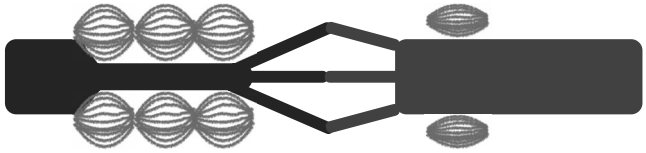
Microcirculation

- Capillary flow regulation
  - Venoarteriolar reflex



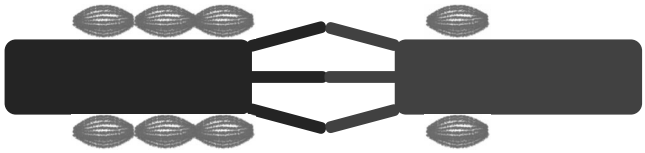
Microcirculation

- Capillary flow regulation
  - Venoarteriolar reflex



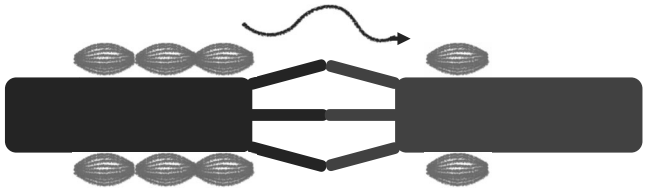
Microcirculation

- Capillary flow regulation
  - Pre-capillary arteriolar vasomotor



Microcirculation

- Capillary flow regulation
  - Pre-capillary arteriolar vasomotor



Microcirculation

- Arteriolar vasomotion
  - Nitric Oxide (NO)
  - Endothelium-Derived Hyperpolarizing Factor (EDHF)

Pohl U, De Wit C, "A unique role of NO in the control of blood flow": News Physiol Sci, 1999:19, 74-80,
Ozkor MA, Quyyumi AA: Endothelium,-Derived Hyperpolizing Factor and Vascular Function" Cardiology Research and Practice 2011, 1-12

Microcirculation

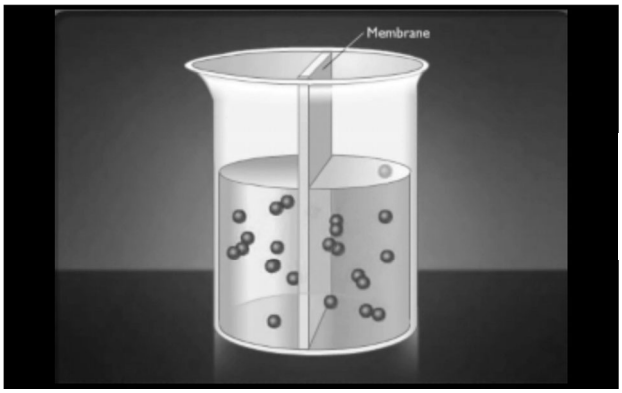
- Arteriolar vasomotion
  - Slow-wave
    - Arteriolar contraction oscillations of membrane potential
  - High amplitude
  - 1-10 Hz

Intlagietta M, "Vasomotion and flow motion: physiological mechanisms and clinical evidence" Vasc Med Rev, 1990 1, 101-112,
Bartlet II, Crane GJ, Neidl T, et al, "Electrophysiolocial basis of arteriolar vasomotion in vivo" J Vasc Res 2000, 37, 568-575

Microcirculation

- Hydrostatic - Oncotic equilibrium

Microcirculation



McGraw-Hill animations
Published June 4, 2017

Microcirculation

- Filtration

Microcirculation

Carl Friedrich Weilheim Ludwig
1816-1895

- Suggested that lymph was formed by plasma filtration through capillary walls

Microcirculation

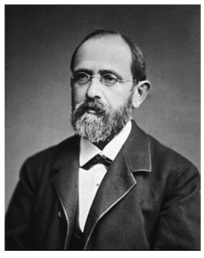
Julius Friedrich Cohnheim
1839-1884

- Expanded Ludwig's concept to vascular pressure and different capillary permeability throughout the body

Microcirculation

Rudolph Peter Heinrich Heidenhain
1834-1897

- 1854 published his secretion theory and his work on lymphagogues substances (crayfish extract)



Microcirculation

Ernest Henry Starling
1866-1927

- 1893 Intravenous injection of peptones

Microcirculation

Starling Principle

- 1896 Basic concept of tissue fluid absorption
- Incomplete

Microcirculation

ON THE ABSORPTION OF FLUIDS FROM THE CONNECTIVE TISSUE SPACES. By ERNEST H. STARLING. (Two Figures in Text.)

(*From the Physiological Laboratory, Guy's Hospital.*)

Until within the last few years, all workers, who investigated the question of absorption by the blood vessels, confined their experiments to cases in which some substance, not occurring normally in the blood, was introduced into some connective tissue space. That, under these conditions, absorption by the blood vessels does take place, was shown by Majendie, and confirmed in recent years by Ascher[1] as well as by Tubby and myself[2]. Although the ease, with which this interchange by a process of diffusion between blood and extravascular fluids takes place, must be of great importance for the normal metabolism of the tissues (as, *e.g.* the much discussed supply of CaO to the mammary gland-cells), yet such processes will not serve to explain the absorption by the blood vessels of fluids having the same tonicity and the same approximate constitution as the circulating plasma. The fluids contained in the tissue-spaces have the same tonicity and the same composition in salts as blood-plasma. We have to inquire first whether the blood vessels do absorb such isotonic fluids, and secondly the manner in which this absorption takes place.

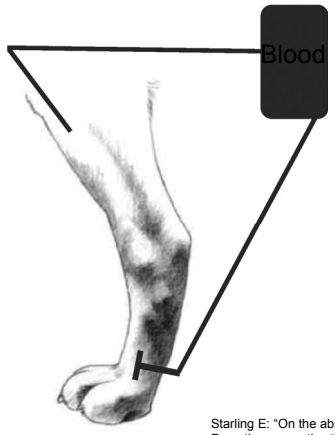
Evidence as to Absorption by Blood Vessels.

1. *Absorption from the serous cavities.*

A number of experiments have been made recently on the subject of the absorption of isotonic fluids (*e.g.* 1 ¾ salt solution or serum) from the serous cavities. Orlow[3] showed that isotonic fluids were absorbed from the peritoneal cavity with considerable rapidity without producing any corresponding lymph-flow from the thoracic duct, and concluded

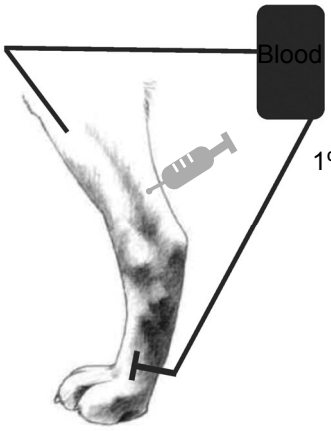
[1] *Zeitschrift f. Biologie*, 1893. 247. [3] This *Journal*, xvi. 140. 1894.
[2] *Pflüger's Archiv*, lix. 170. 1894.

Microcirculation

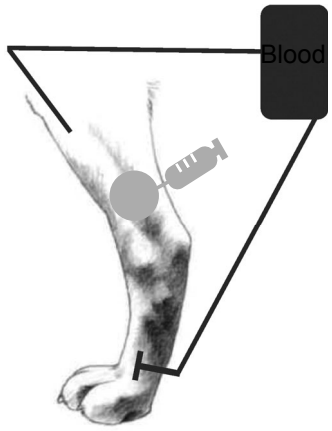


Starling E: "On the absorption of fluids From the connective tissue spaces",Journal of Physiology 1896:19; 312-326

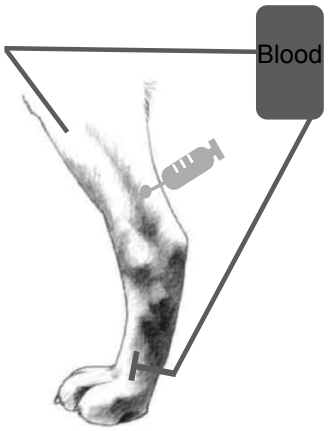
Microcirculation



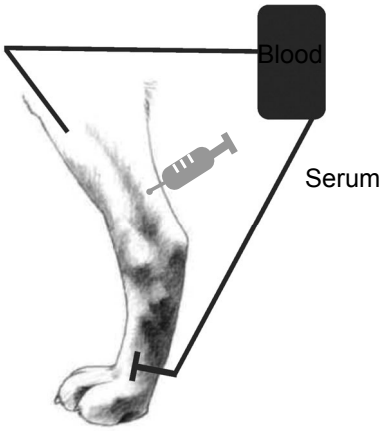
Microcirculation



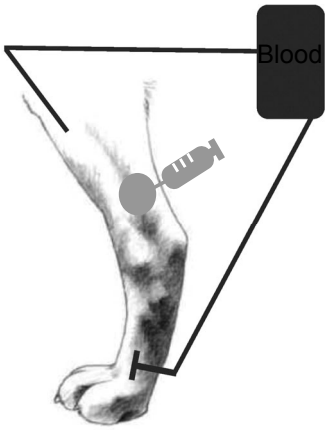
Microcirculation



Microcirculation



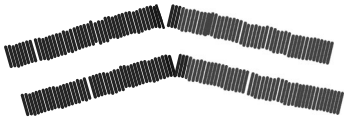
Microcirculation



Starling's Hypothesis

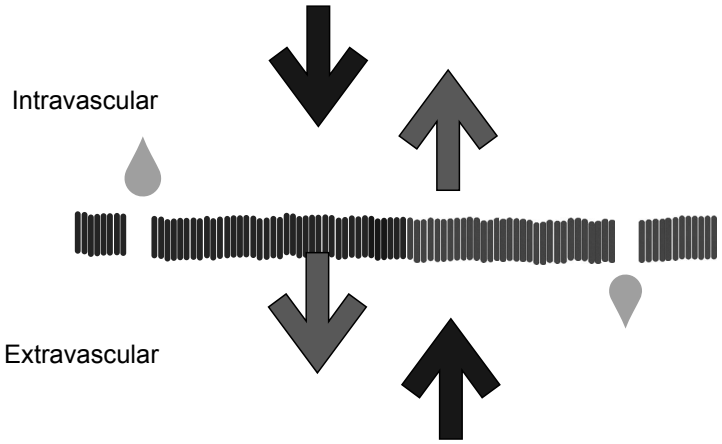
Capillary

Starling's Hypothesis



Starling's Hypothesis

Starling's Hypothesis



Microcirculation

Luigi Liciani
1840-1919

- 1911 thought Starling concepts were "too simple"

Microcirculation

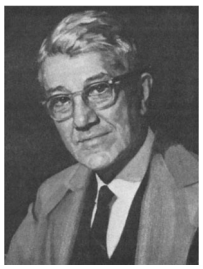
William D Haliburton
1860-1931

- 1904 disputed Starling's discoveries and he believed that filtration dominated over secretion

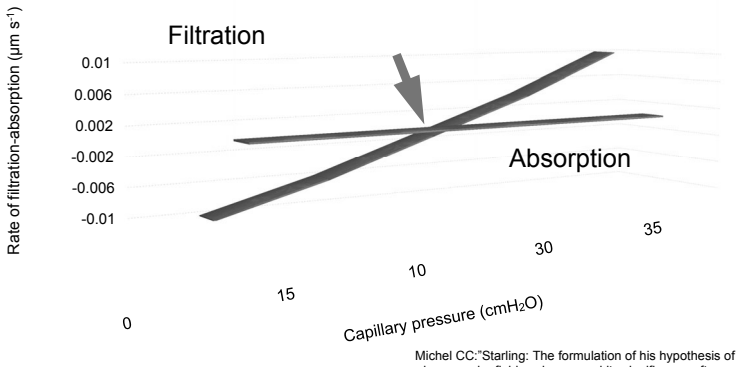
Microcirculation

Eugene Markley Landis
1901-1987

- 1927 first direct experimental evidence of Starling's principle
- He measured hydrostatic pressure and filtration in frog's mesentery

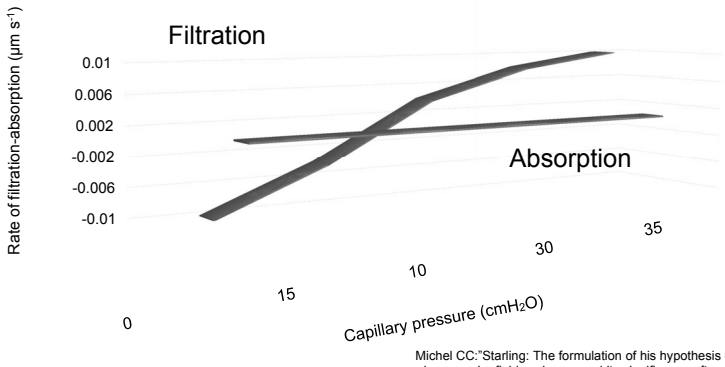


Microcirculation



Michel CC:"Starling: The formulation of his hypothesis of microvascular fluid exchange and its significance after 100 years"; Experimental Physiology 1997:82;1-30

Microcirculation



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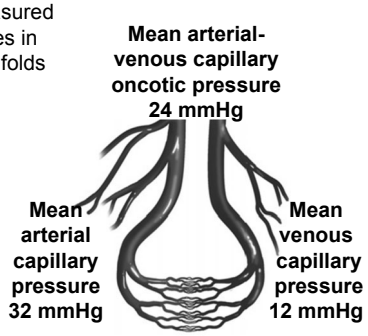
Microcirculation

- 1930 Landis measured capillary pressures in human fingernail folds at heart level



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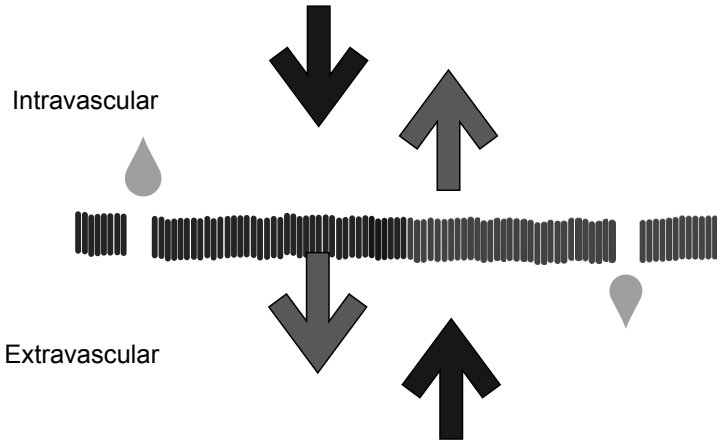
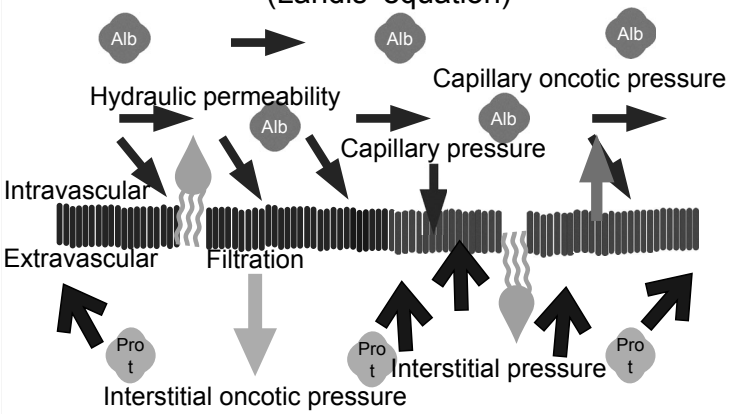
Starling's Hypothesis



Starling's Principle
(Landis' equation)



$$Jv/A = L_p\{(P_c - P_i) - (\pi_c - \pi_i)\}$$

Starling's Principle
(Landis' equation)

$$Jv/A = L_p\{(P_c - P_i) - (\bar{\pi}_c - \bar{\pi}_i)\}$$

Microcirculation

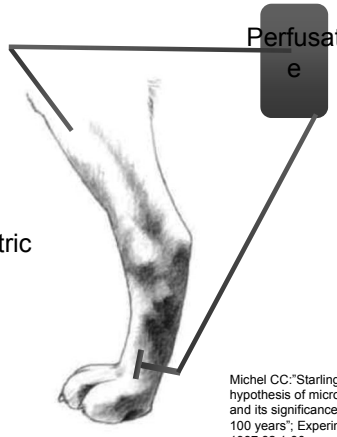
Armando Soto-Rivera
1920-2004

John Richard Pappenheimer
1915-2007

- 1948 coined the term isogravimetric state

Microcirculation

Constant limb weight=0 net fluid movement=isogravimetric state

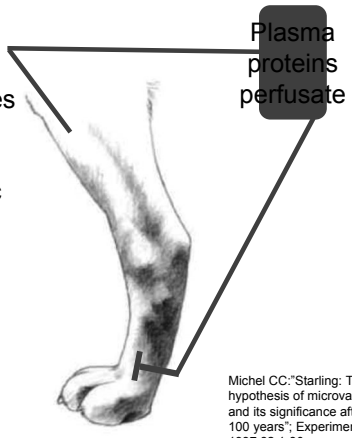


Michel CC:"Starling: The formulation of his hypothesis of microvascular fluid exchange and its significance after 100 years"; Experimental Physiology 1997:82;1-30

Microcirculation

Capillary pressure varies according the plasma oncotic pressure to achieve isogravimetric state

Rate of filtration-reabsorption=P_c-$P_{c(iso)}$



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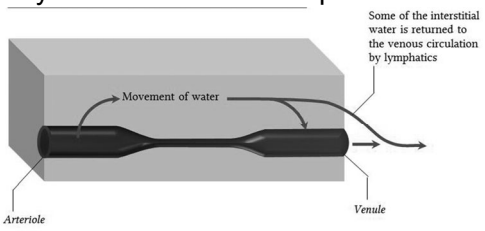
Microcirculation

Classic Starling Principle

- Landis, Pappenheimer and Soto-Rivera
- Krogh, Landis and Turner 1931
  - Hydrostatic and oncotic pressures



Microcirculation

Albert Jan Staverman
1912-1993

Microcirculation

1951 Staverman's Reflection Coefficient
University of van Leiden
Netherlands

NON-EQUILIBRIUM THERMODYNAMICS OF MEMBRANE PROCESSES

By A. J. Staverman
Plastics Research Institute T.N.O., Delft, Netherlands

Received 21st May, 1951; in final form 10th August, 1951

By applying the theory of non-equilibrium thermodynamics to membrane processes it is found that the action of a membrane in a system containing n components is completely characterized by $\frac{1}{2}n(n+1)$ thermodynamical constants.

In measurements of transference numbers, membrane potentials and electrokinetic constants, a set of phenomenological constants can be determined from which, if sufficient independent data are obtained, the thermodynamical constants may be computed.

The relations between phenomenological and thermodynamical constants are given. Also a number of relations between different phenomenological constants is given, which must hold independently of any model of the action of the membrane. Some of these relations, such as Nernst's equation for the diffusion potential and some relations between electrokinetic constants have been derived before by quasi-thermodynamical reasonings or from calculations on models. Others, such as the relation between mechanical and

Microcirculation

- Reflection coefficient

$$\sigma = \frac{\pi_{(obs)}}{\pi_{(theory)}}$$

Microcirculation

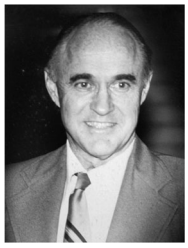
$$\mathbf{Jv/A = L_p\{(P_c - P_i) - (\pi_c - \pi_i)\}}$$

Microcirculation

$$Jv/A = L_p\{(P_c - P_i) - \sigma(\pi_c - \pi_i)\}$$

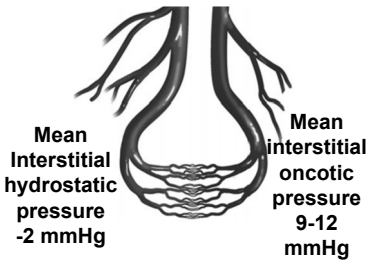
Microcirculation

Arthur Clifford Guyton
1919-2003

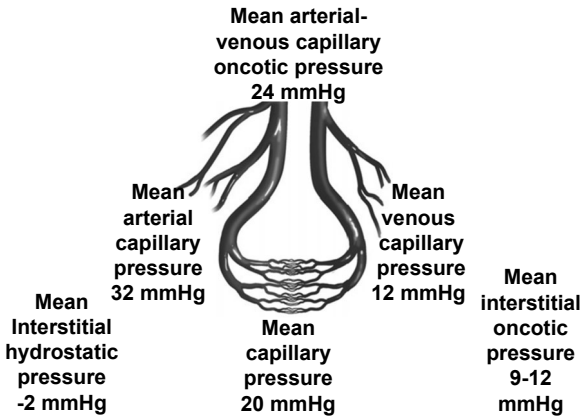


- 1963 Guyton measured the interstitial hydrostatic and oncotic pressures

Microcirculation



Microcirculation



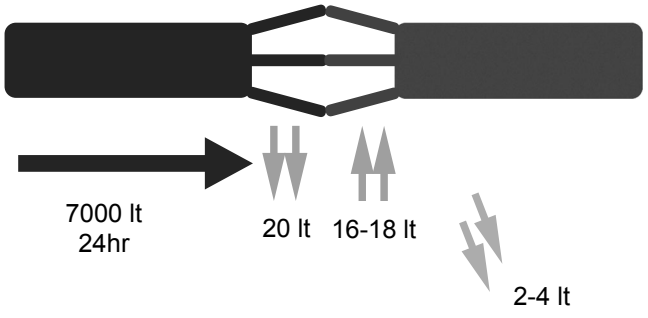
Microcirculation

Eugene Markley Landis
1901-1987

John Richard Pappenheimer
1915-2007

- 1963 quatinfied the values of filtration-absorption

Microcirculation



Microcirculation

Ernest Ruska
1906-1988

Max Knoll
1897-1969

- 1931 developed the electron microscope10 nm resolution
- 1944 2 nm resolution

Microcirculation

James Frederic Danielli
1911-1984

- 1940 First postulated the presence of the "fussy" endothelial layer

Microcirculation

John H Luft

- 1966 First demonstrated the Glycocalyx using cationic dye

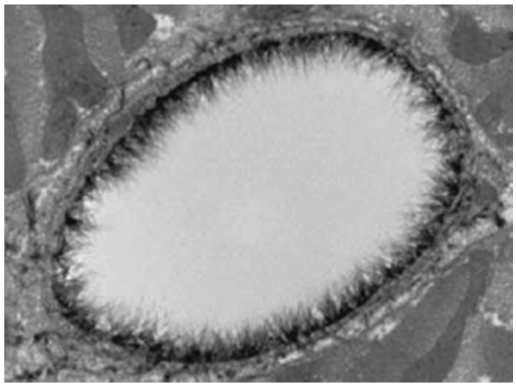
Microcirculation

John H Luft

- 1966 First demonstrated the Glycocalyx using cationic dye

Microcirculation

Glycocalyx



Reitsma S, Slaaf D, Vink H, et al:"The endothelial glycocalyx: composition, functions and visualization" Arch-Eur J Phisiol 2007

Microcirculation

Glycocalyx

- Hydrogel-like layer
- 500-2000 nm depending on the anatomy and size of the vessel
- Total surface 4000 - 7000 m^2 (0.98 - 1.7 acres)
- Negative net charge

Yang Y, Schmidt E: "The endothelial glycocalyx: Am important regulator of the pulmonary vascular barrier" Tissue Barriers 2013:1:1

Microcirculation

Glycocalyx

- Functions
  - Molecular sieve determine oncotic forces across the endothelium
  - Hydrodynamic exclusion layer preventing interaction between red cells and cell membranes
  - Modulating leukocyte attachment and rolling
  - Transducer of mechanical forces to the intracellular cytoskeleton

Weinbaum S, Zhang X, Han Y et al:"Mechanotransduction and flow across the endothelial glycocalyx" PANS 2003:100;13

Microcirculation

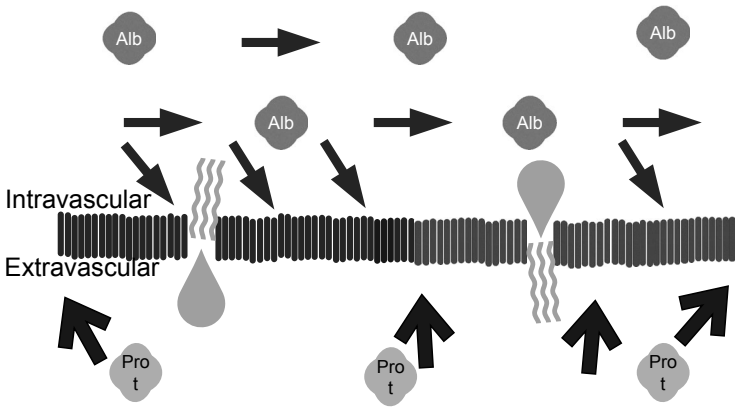
Glycocalyx

- Structure
  - Proteoglycans: Syndecans,Glypicans, Perlecan,Versicans, Decorins, Biglycans, Mimecans
  - Glycoproteins: Selectins, Integrins and Immunoglobulins
  - Glycosaminoglycans: Heparan Sulfate, Condroitin Sulfate, Dermatan Sulfate, Keratan Sulfate and Hyaluronan (hyaluronic acid)
  - Soluble components: Albumin, sialic acid, orosomucoid, etc

Reitsma S, Slaaf D, Vink H, et al:"The endothelial glycocalyx: composition, functions and visualization" Arch-Eur J Phisiol 2007

Microcirculation

Glycocalyx

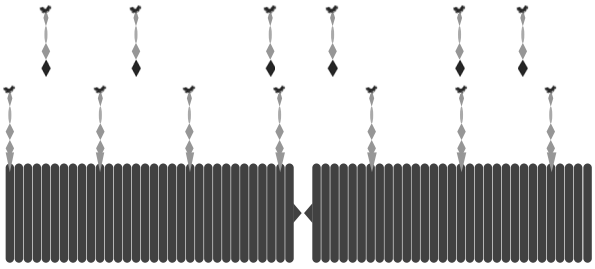


Microcirculation

Glycocalyx

Microcirculation

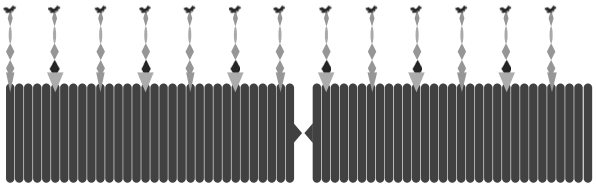
Glycocalyx



Proteoglycans: Syndecans (Specific domain)

Microcirculation

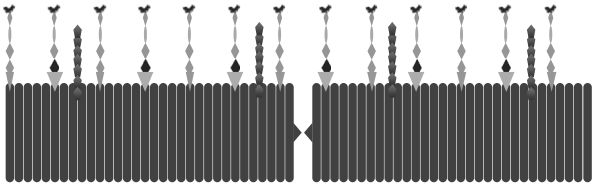
Glycocalyx



Proteoglycans: Glypicans(Glycosylphosphatidilinositol anchor)

Microcirculation

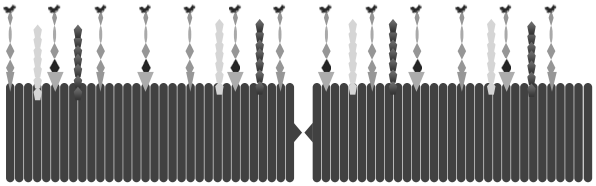
Glycocalyx



Proteoglycans: Perlecan (secreted)

Microcirculation

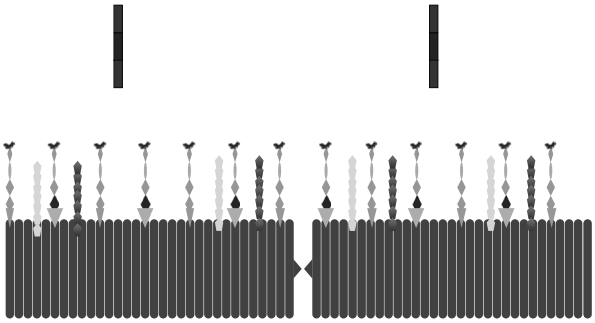
Glycocalyx



Proteoglycans: Versican (secreted)

Microcirculation

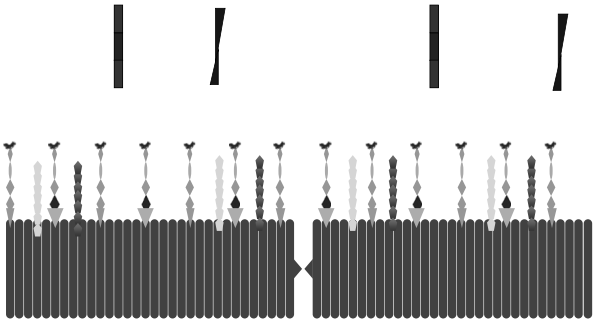
Glycocalyx



Glycoproteins: Selectins: P and E leukocyte endothelial interaction

Microcirculation

Glycocalyx



Glycoproteins: Integrins: heterodimeric molecules α or β platelet endothelial interaction

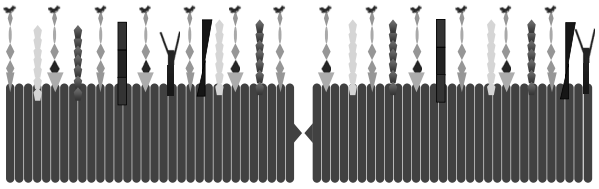
Microcirculation

Glycocalyx

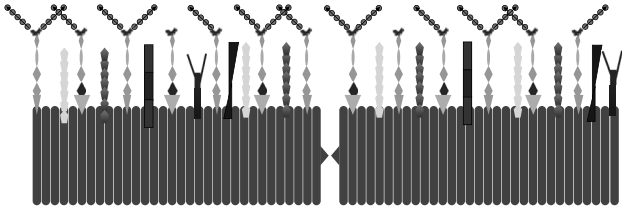
Glycoproteins: Immunoglobulin superfamily: ICAM-1 and 2, VCAM-1 and PCAM-1

Microcirculation

Glycocalyx

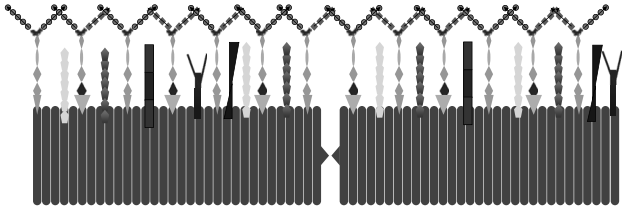


Microcirculation
Glycocalyx



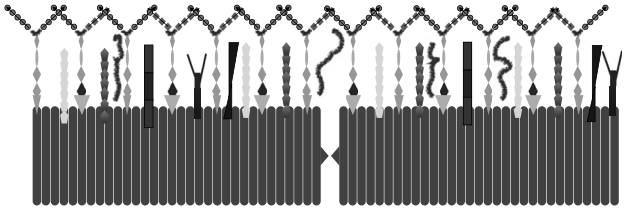
Glycosaminoglycans: Heparan Sulfate (50%-90%)

Microcirculation
Glycocalyx



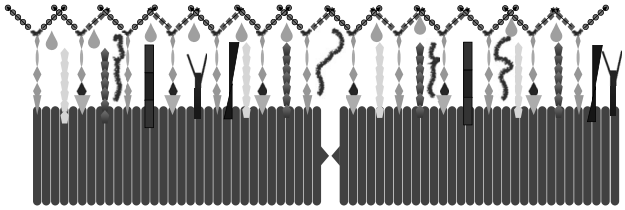
Glycosaminoglycans: Condroitin Sulfate

Microcirculation
Glycocalyx



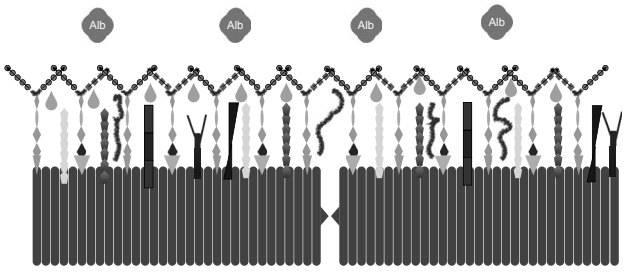
Glycosaminoglycans: Hyaluronan

Microcirculation
Glycocalyx

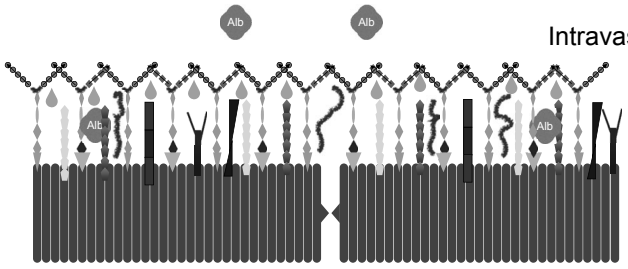


Hydrated

Microcirculation
Glycocalyx



Microcirculation
Glycocalyx



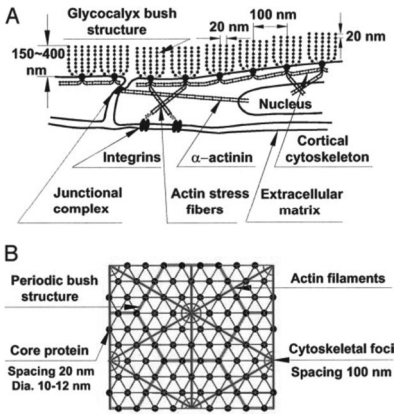
Intravascular

Extravascular

Endothelial Surface Layer (ESL)

Microcirculation

Glycocalyx



Weinbaum S, Zhang X, Han Y et al:"Mechanotransduction and flow across the endothelial glycocalyx" PANS 2003:100;13

Microcirculation

Charles C Michel

Roger H Adamson

J Rodney Levick

Sheldon Weinbaum

Microcirculation

Glycocalyx

- Functions
  - Hydrodynamic exclusion layer preventing interaction between red cells and cell membranes
  - Modulating leukocyte attachment and rolling
  - Transducer of mechanical forces to the intracellular cytoskeleton
  - Molecular sieve determine oncotic forces across the endothelium
  - "Reservoir"

Weinbaum S, Zhang X, Han Y et al:"Mechanotransduction and flow across the endothelial glycocalyx" PANS 2003:100;13

Microcirculation

Sheldon Weinbaum

2003 "Mechanotransduction and flow across the endothelial Glycocalyx"

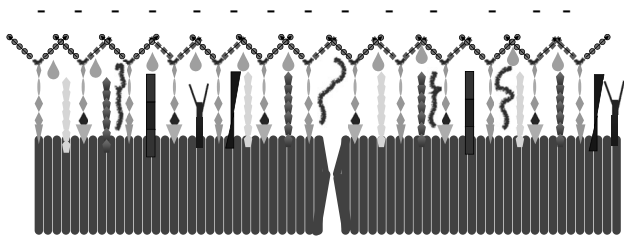
Microcirculation

Glycocalyx

Exclusion layer

Negative charge

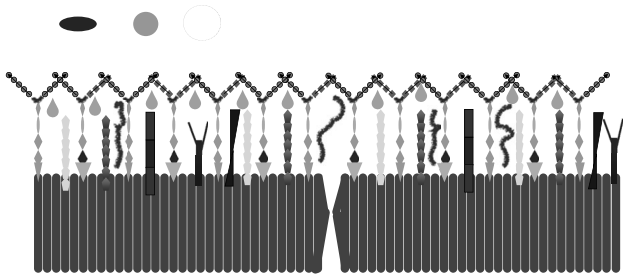
Intravascular



Extravascular

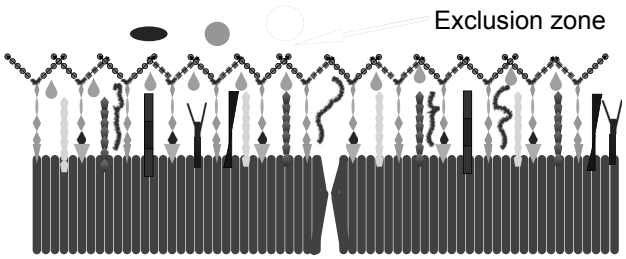
Microcirculation

Glycocalyx



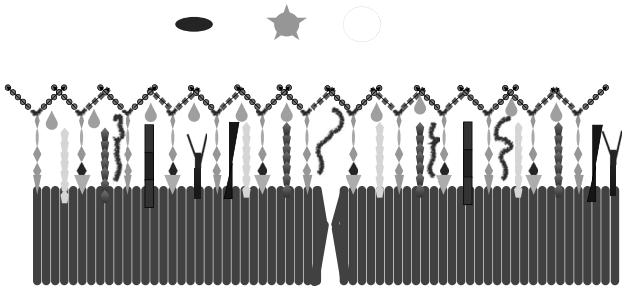
Microcirculation

Glycocalyx



Microcirculation

Glycocalyx



Microcirculation

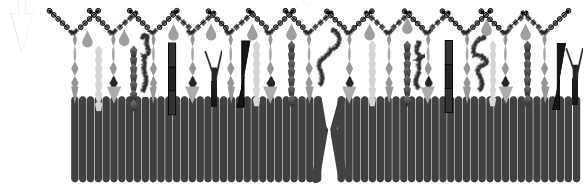
Glycocalyx

Mechano-transductor

Mechano receptors

NO

Intravascular



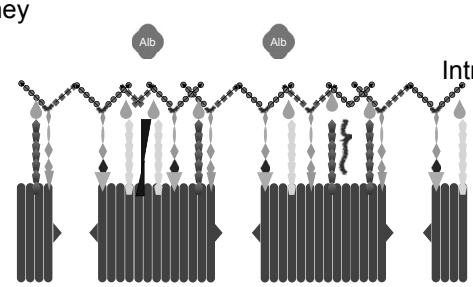
Extravascular

Microcirculation

Glycocalyx

Molecular sieve

Kidney



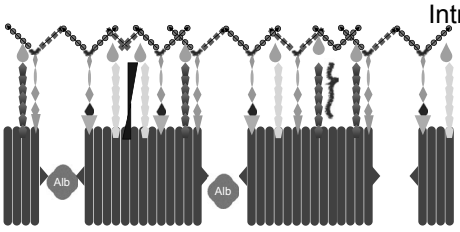
Intravascular

Extravascular

Microcirculation

Glycocalyx

Kidney



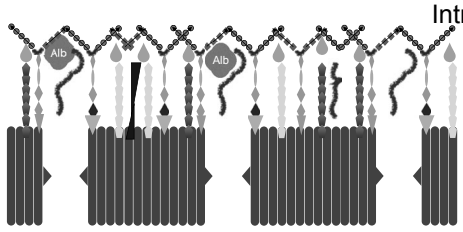
Intravascular

Extravascular

Microcirculation

Glycocalyx

Kidney



Intravascular

Extravascular

Microcirculation

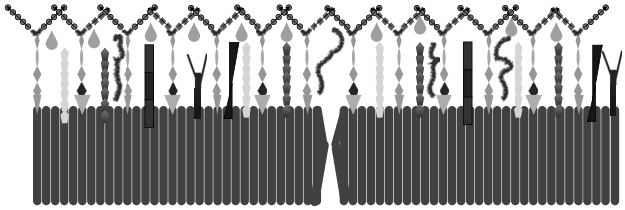
Roger H Adamson

2004 “Oncotic pressures opposing filtration across non-fenestrated rat micro vessels”
Despite having the same oncotic Pressure in the lumen and the Interstitium there was a 70% difference

Microcirculation

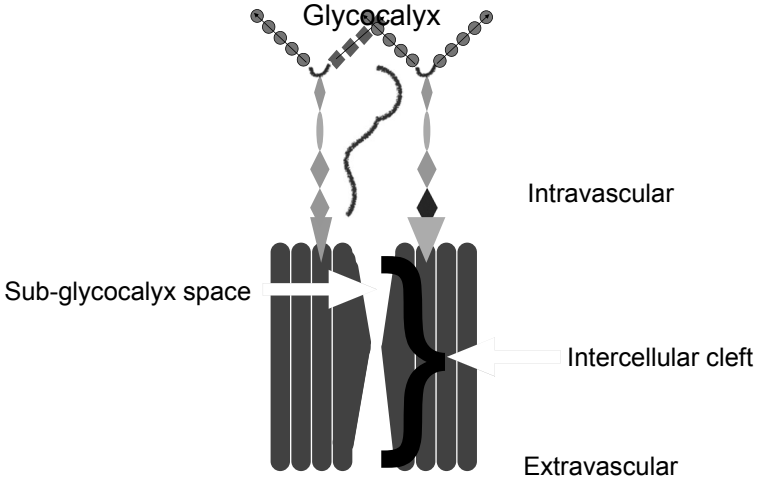
Glycocalyx

Intravascular

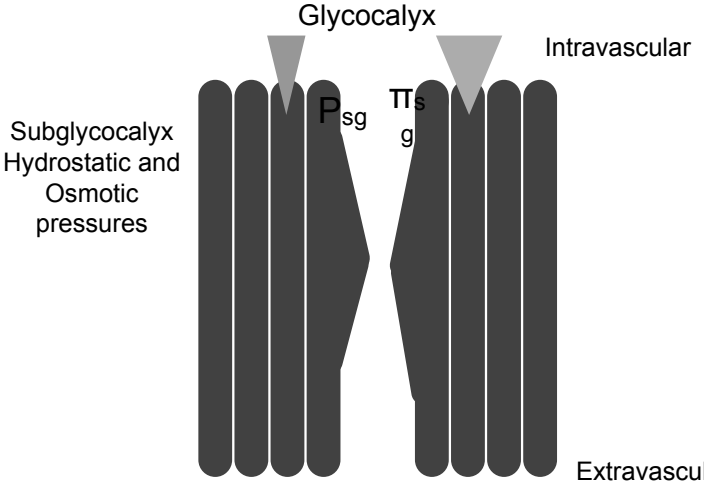


Extravascular

Microcirculation

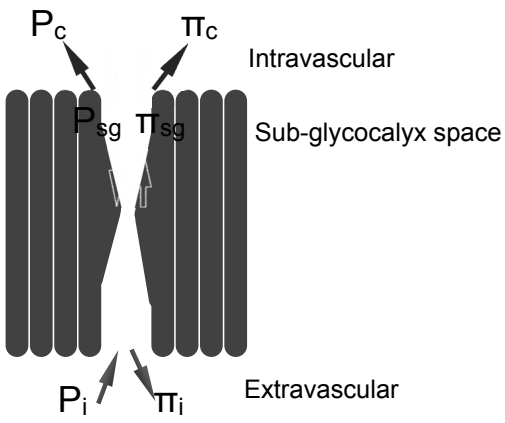


Microcirculation



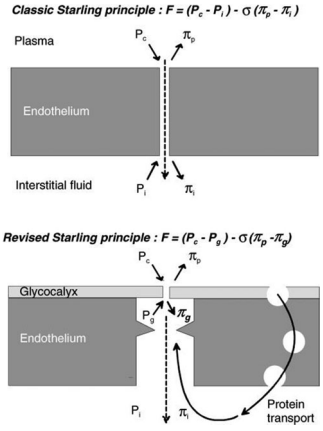
Microcirculation

Glycocalyx



Microcirculation

Glycocalyx



Levick JR, “Revision of the Starling Principle: new views of tissue fluid Balance” J Physiol 2004:15;557

Microcirculation

Glycocalyx

- "Hemodynamic states"
  - Steady state: Constant capillary hydrostatic pressures produce constant filtration throughout the capillary
  - Transient state: Sudden variation on capillary hydrostatic pressure favoring absorption for a short period of time until a "new" steady state is achieved and filtration resumes

Microcirculation

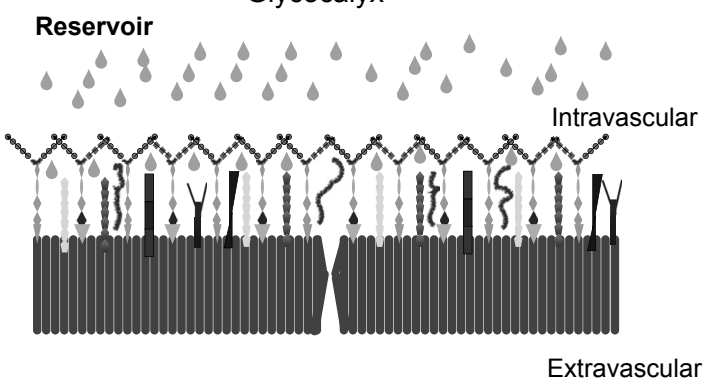
$$Jv/A = L_p\{(P_c - P_i) - \sigma(\pi_c - \pi_i)\}$$

Microcirculation

$$Jv/A = L_p\{(P_c - P_{sg}) - \sigma(\pi_c - \pi_{sg})\}$$

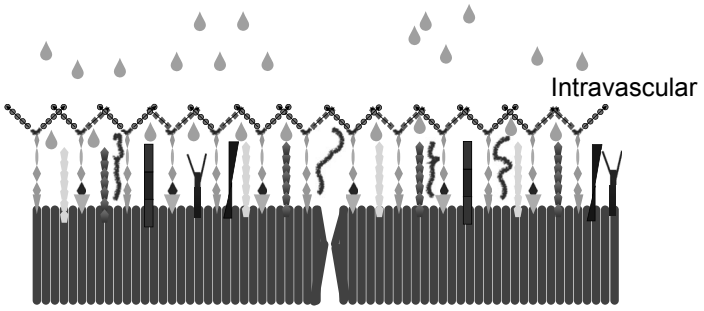
Microcirculation

Glycocalyx



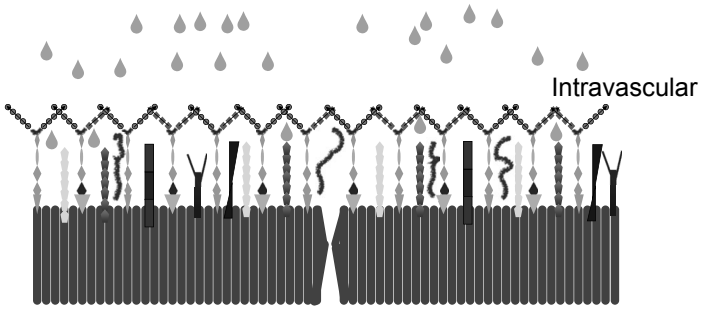
Microcirculation

Glycocalyx



Microcirculation

Glycocalyx



1-1.7 liters of fluid

Microcirculation

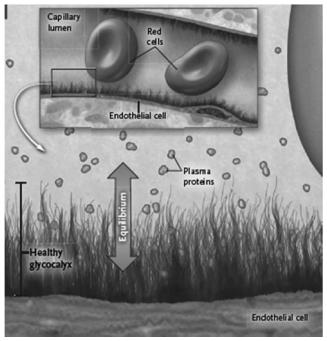
Glycocalyx

- Concepts that now "make sense"
  - Hypoalbuminemia is a marker of the severity of the disease, nevertheless treating it is a no clinical benefit
  - ARDS have low plasma and transferrin concentrations, treating this with albumin with or without diuretics has no benefit
  - Negative fluid balance rather that COP difference improves alveolar to arterial oxygen tension ratio in ARDS
  - In septic and non-septic patients fluid resuscitation with albumin improves cardiac output but not pulmonary edema

Woodcock TE, Woodcock TM. "Revised Starling equitation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy" BJA 2012:108;3

Microcirculation

Glycocalyx

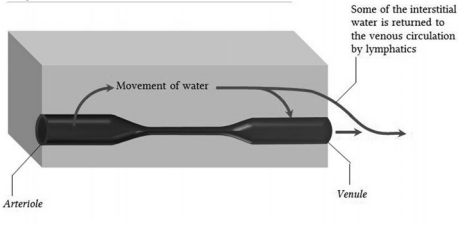


Mayburgh JA, Mythen MG: "Resuscitation Fluid" N Engl J Med 2013;369:13

Microcirculation

Classic Starling Principle

- Landis, Pappenheimer and Soto-Rivera
- Krogh, Landis and Turner 1931
  - Hydrostatic and oncotic pressures



www.derangedphysiology.com 2017

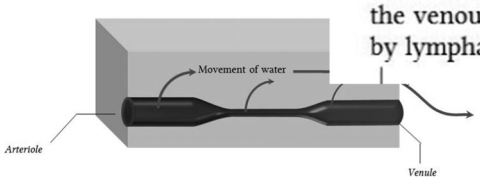
Microcirculation

Glycocalyx

Revised Starling Principle

- Adamson 2004, Weinbaum 2004, Levick and Michel 2010
  - Glycocalyx function

Most of the interstitial water is returned to the venous circulation by lymphatics



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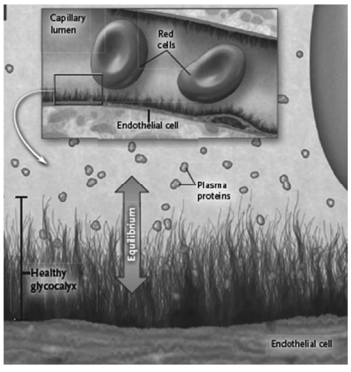
Microcirculation

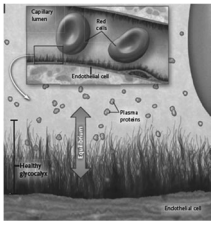
Glycocalyx

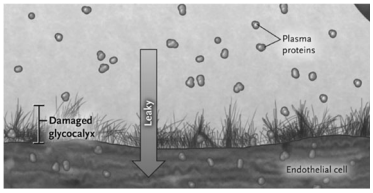


Mayburgh JA, Mythen MG: "Resuscitation Fluid" N Engl J Med 2013;369:13

Microcirculation

Glycocalyx



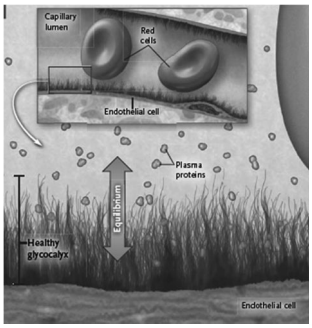


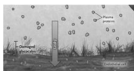
- Hyperglycemia
- Hypercholesterolemia
- Hypervolemia
- Ischemia-reperfussion
- Trauma
- Inflammation

Mayburgh JA, Mythen MG: "Resuscitation Fluid" N Engl J Med 2013;369:13

Microcirculation

Glycocalyx

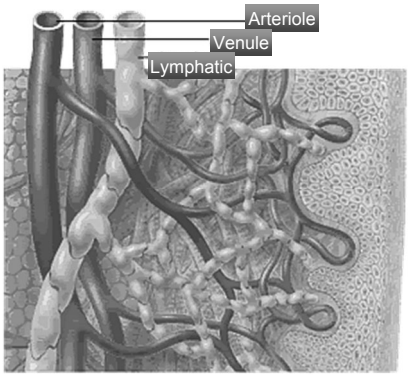




- Antioxidants
- N-acetyl-cysteine
- Albumin
- FFP
- Heparin
- Antithrombin III
- Sulodexide

Mayburgh JA, Mythen MG:
"Resuscitation Fluid"
N Engl J Med 2013;369:13

Microcirculation



If you want to go fast, go alone.

If you want to go far, go together.

-African Proverb-

Lymphedema treatment

TEAM EFFORT

Including the patient

THANK YOU