Pathophysiology of kidneys – part I

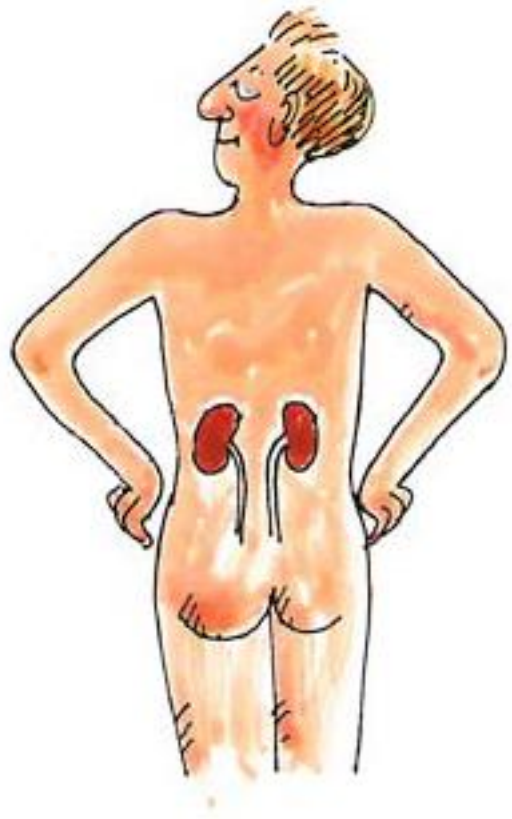
Glomerular hemodynamics and GFR

Methods for measurement of GFR

Glomerular filtration membrane and its pathologic changes

Proteinuria

Glomerular diseases



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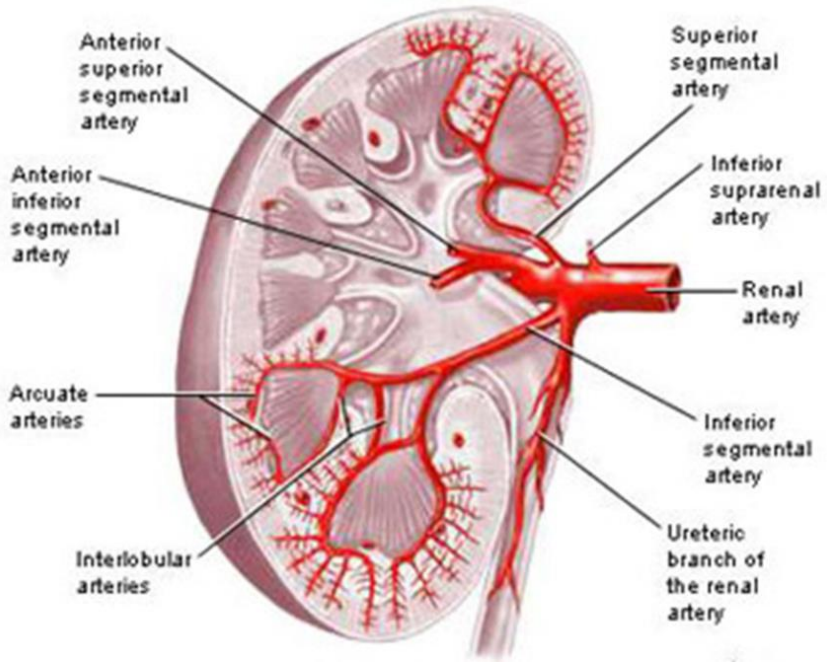
Blood supply & functions of the kidney

- blood flow through kidney ~1200 ml/min, this represents ~20-25% cardiac output
  - cortical flow >>> medullar flow
- arterio-venose difference in oxygen saturation of hemoglobin is very low
  - given nearly 100% saturation of Hb with O2 in arterial blood, high Hb saturation in venous blood proves that great perfusion serves primarily to regulation purposes and not to nutrition !!!
    - heart ~35%, brain ~50%, kidney ~90%
- blood suply to kidney
- a. renalis → aa. interlobares → aa. arcuates → aa. interlobulares → afferent arteriols → glomerular capillaries → efferent arteriols
  - → peritubular capillary network (cortical nephrons)
  - → vasa recta (juxtamedullar nephrons)
- regulation of
  - extracellular volume
  - tonicity and osmolarity
  - acid-base balance
  - nitrogen metabolism
  - calcium and phosphate homeostasis
  - hematocrit



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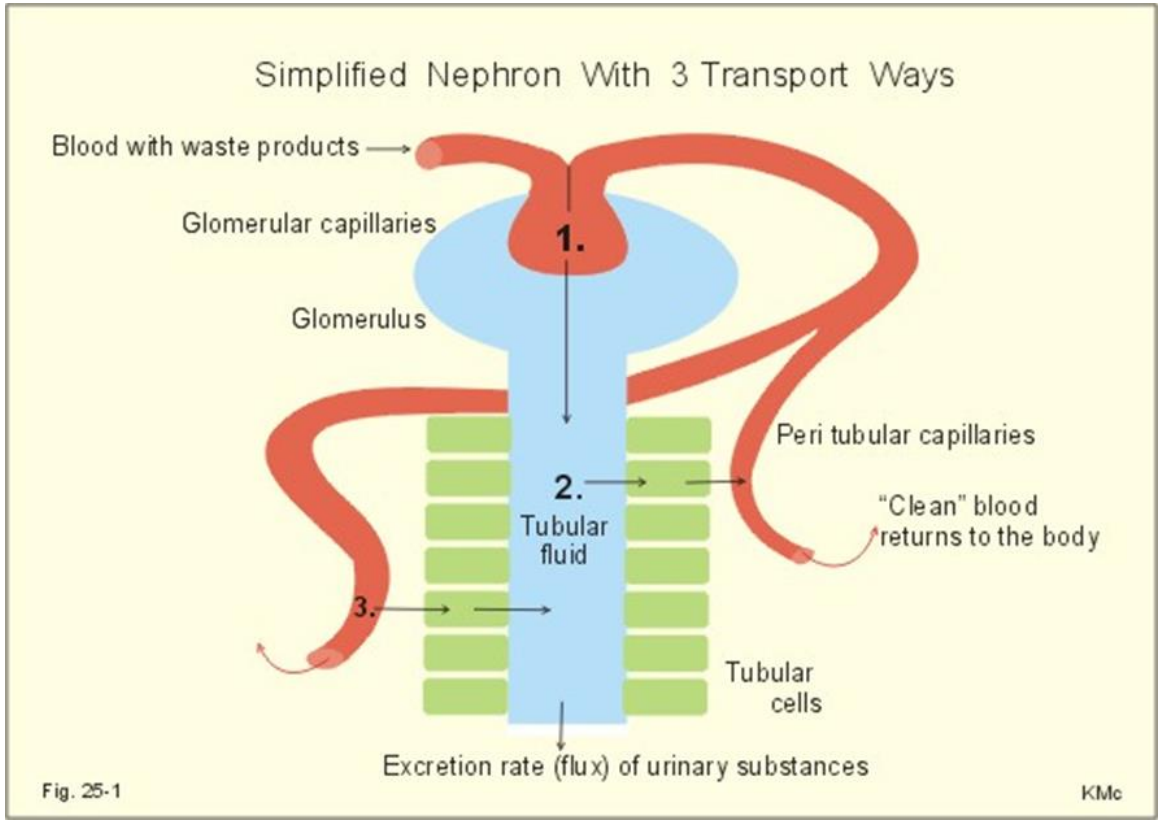
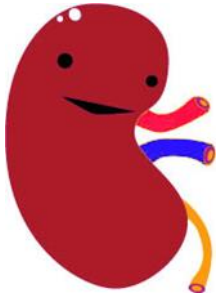
Nephron – transport processes



- glomerular filtration
  - driven by hydrostatic and osmotic pressure gradients (Starling forces)
  - limited by size of filtered compounds (<65kDa) and other criteria
- tubular reabsorption
  - typical symports
    - e.g. Na/Glc, Na/AAs, …
  - saturable capacity (transport maximum, Tm)
    - renal thresholds (e.g. Glc)
- tubular secretion
  - active (ATP)
  - secondary active



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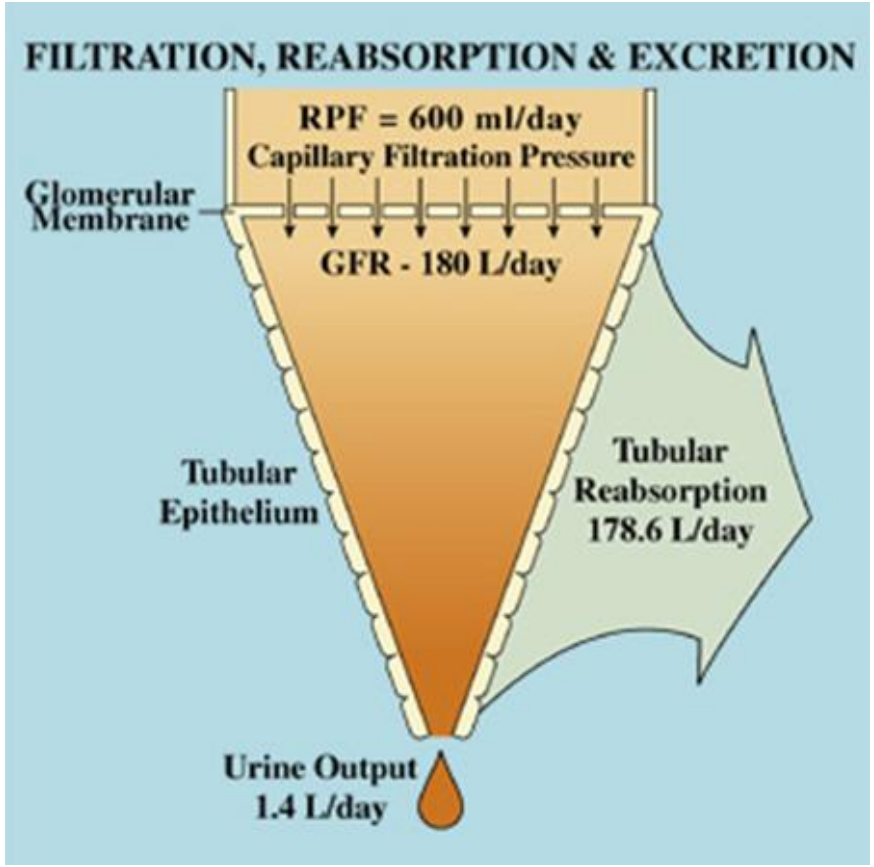
Requirements for a normal control of homeostatic parameters by kidney



- sufficient kidney perfusion
  - autoregulation vs. systemic effects
- sufficient volume of glomerular filtrate
  - ultrafiltration of the plasma is the first step in formation of urine
    - ultrafiltrate is free of cells and proteins, concentration of low molecular weight substances is equal to plasma
  - GFR is a crucial parameter of kidney function
    - volume of glom. filtrate per min
  - low range of normal interval ~100 ml/min/1.73m^2
  - natural age-related decline (>40 yrs) 0.4 - 1.2 mL/min per year
- normal function of tubular epithelia
  - tubular reabsorption of ~99% of glom. filtrate
- normal function of peritubular capillaries
  - in both cortical and juxtamedullar nephrons

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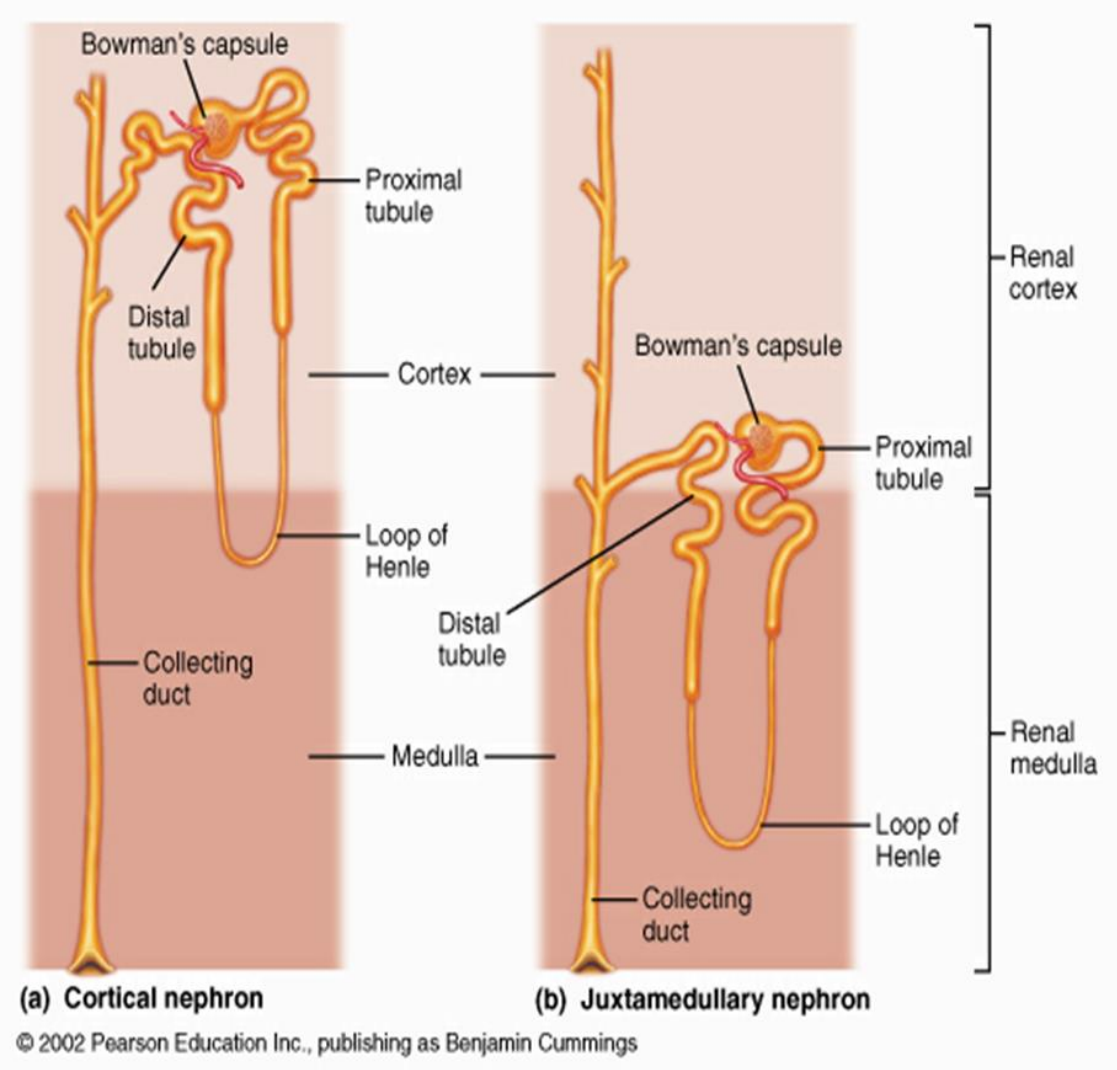
Two types of nephrons



- cortical nephrons (~80%)
  - outer 2/3 of cortex
  - shorter tubules and loops of Henle (LH)
  - participates in reabsorption of solutes, not in urine concentration
    - loops of peritubular capillaries
  - important for autoregulation
    - tubulo-glomerular feedback
- juxtamedullar nephrons
  - inner 1/3 of cortex
  - longer LH radiating deeply into the osmotically concentrated medulla
  - important for production of concentrated urine
    - capillaries - vasa recta (from efferent arteriole) together with LH form „counter-current“ concentration system
- various diseases can affect variably these two populations and thus have deferent effects on renal processes

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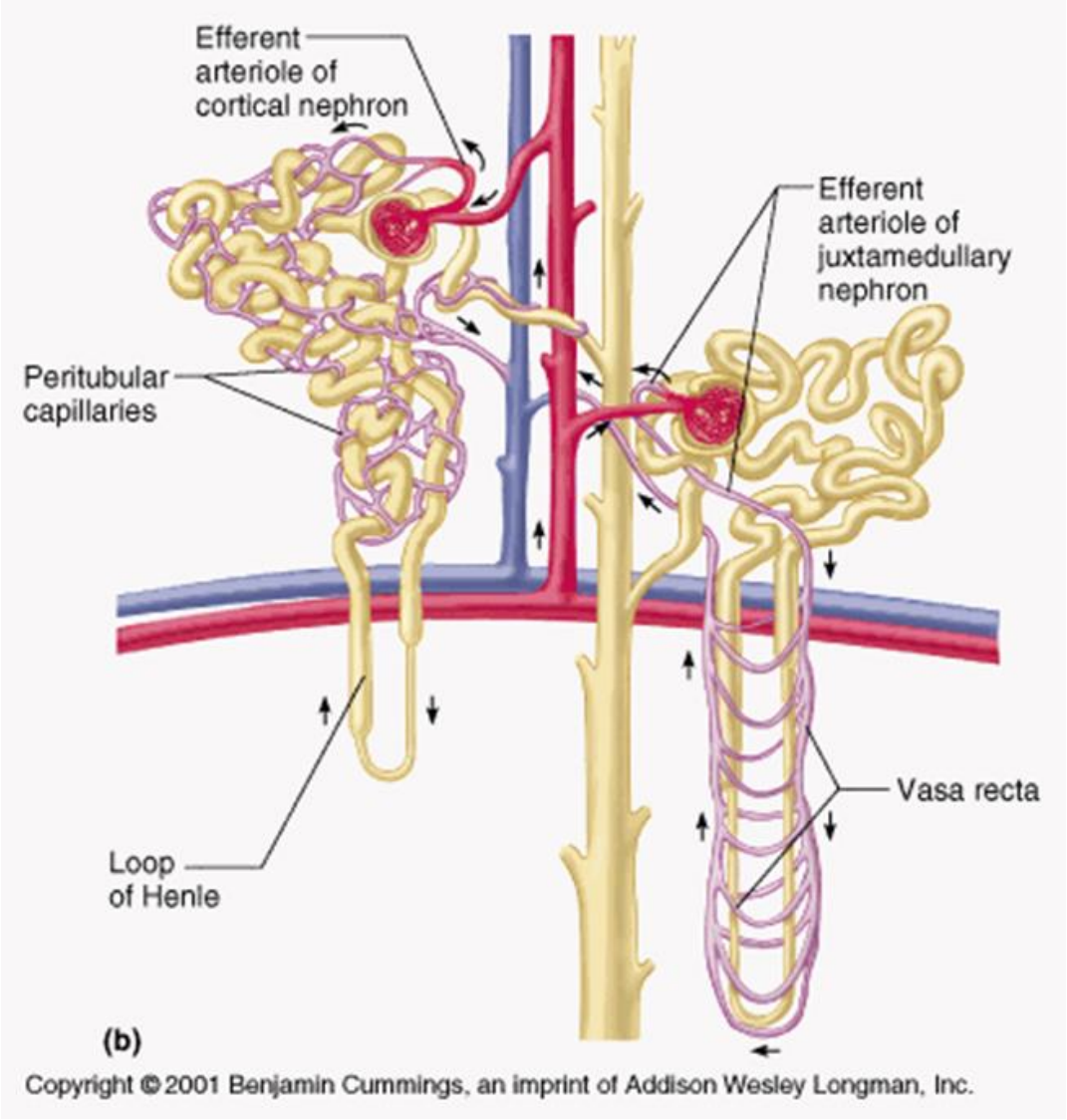
Variable length of LH and capillaries → different function



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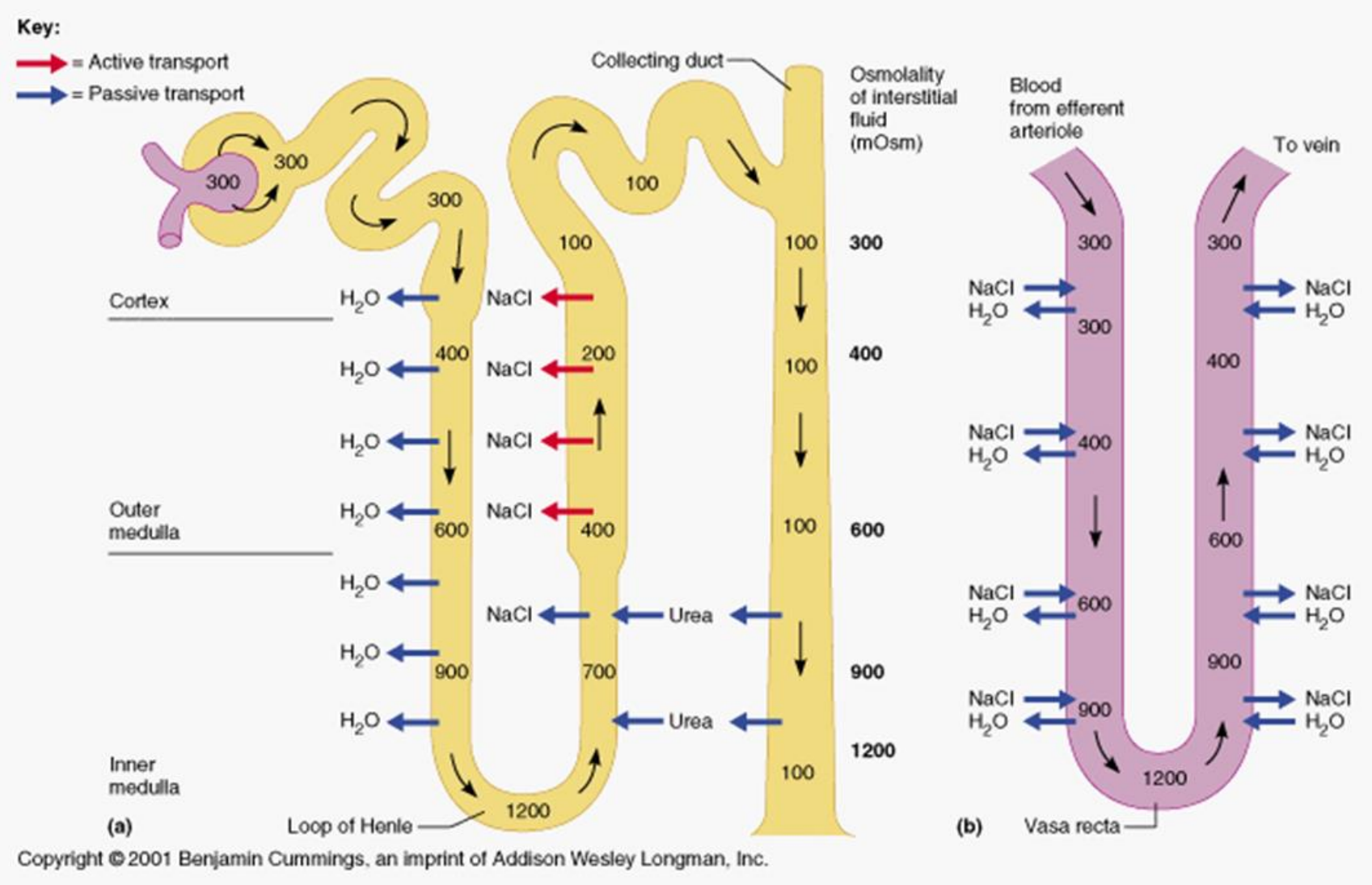
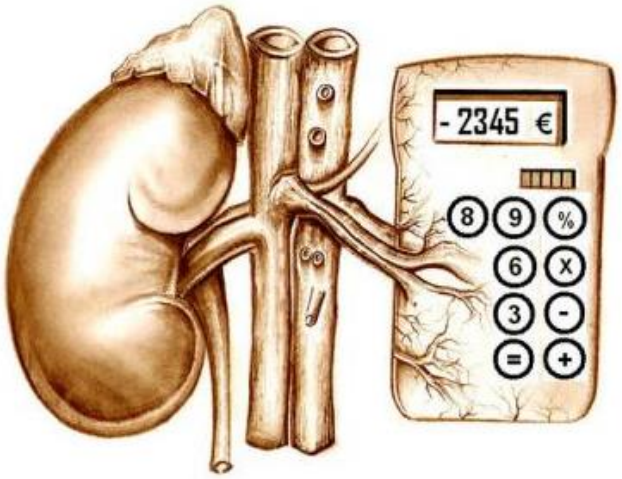
Counter-current system in medulla





GLOMERULAR FILTRATION RATE (GFR)

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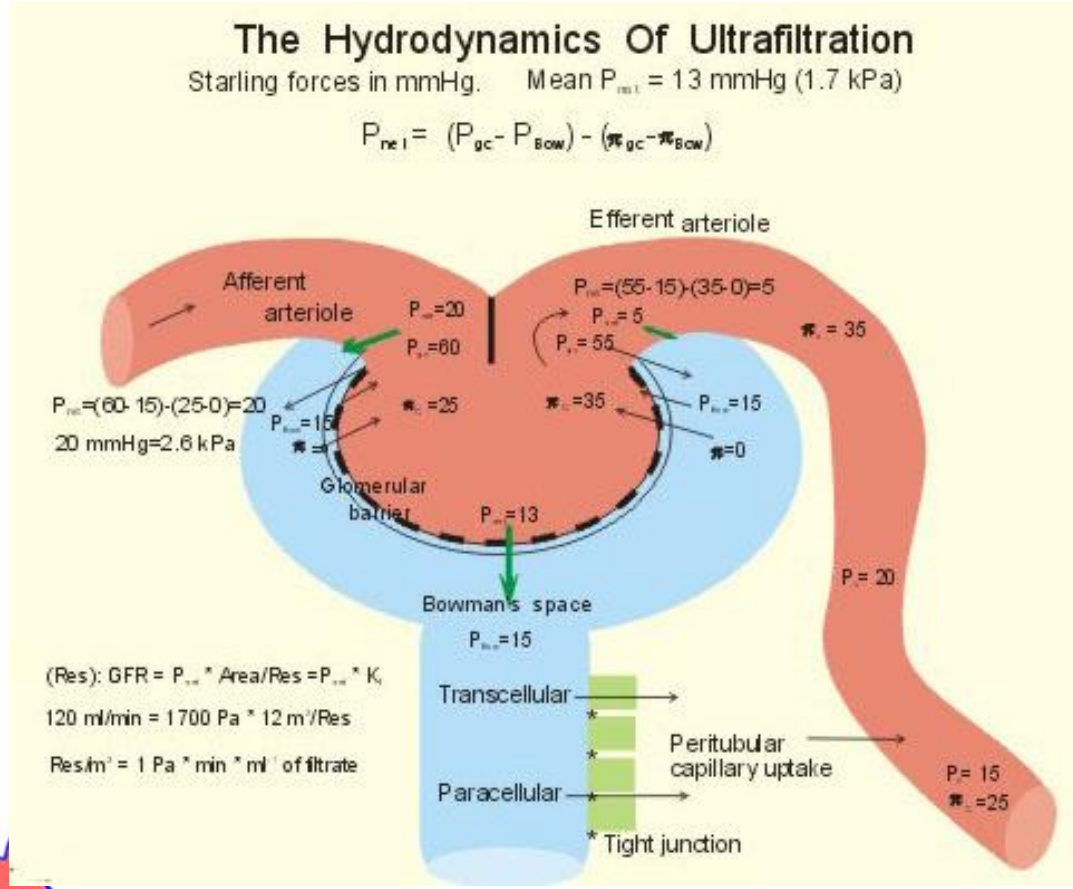
Determinants of GFR

- rate of ultrafiltration of plasma to Bowman capsule is determined:
  - **GFR = A × K × P_f**
- depends on:
  - A = a total area available for filtration (~100m^2)
    - number of glomeruli (approx. 500,000 – 1,000,000 per kidney)
      - changes with loss of functional glomeruli
    - effect of mesangial cells
      - capable of contraction (a thus ↓ A)
  - K = permeability of filtration membrane
    - changed by diseases affecting structure of glom. filtr. membrane (see further)
  - P_f = effective ultrafiltration pressure
    - Starling forces (see further)



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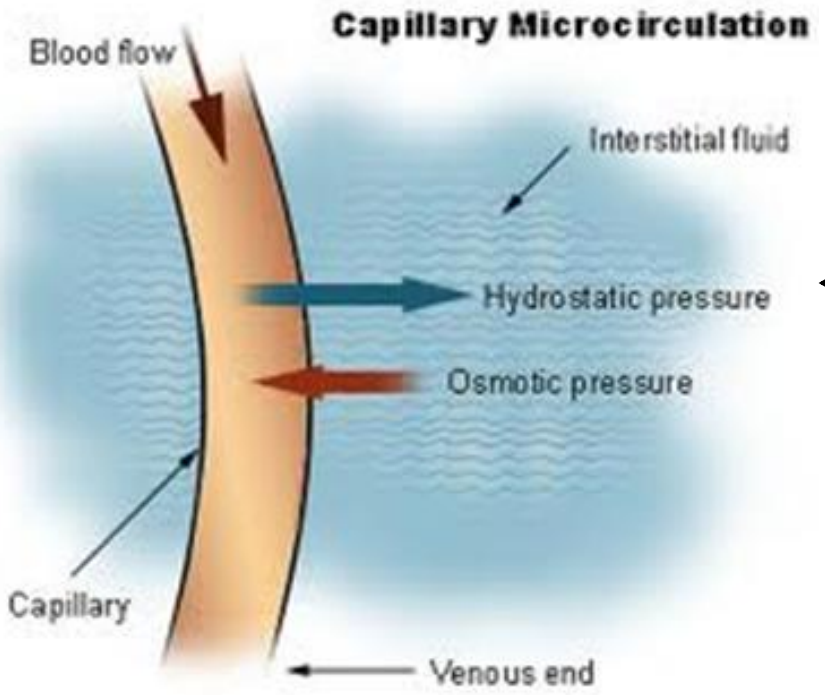
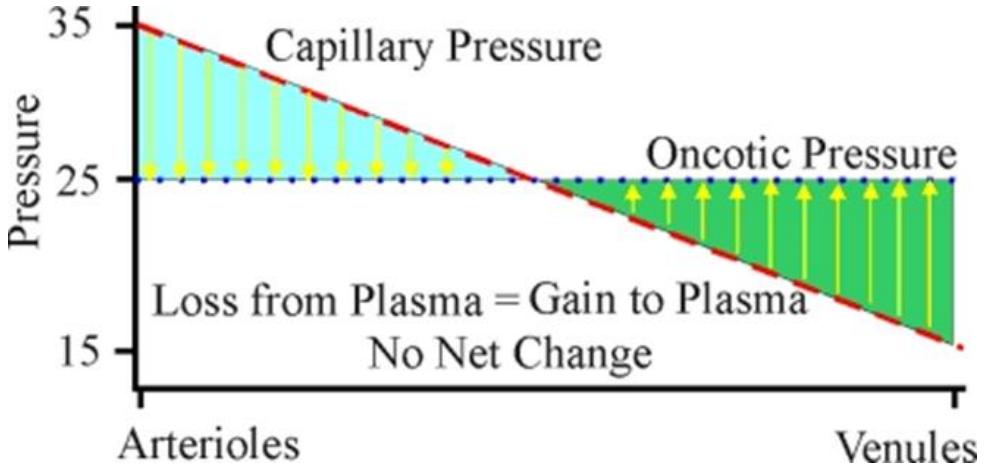
Microcirculation – Starling forces



situation elsewhere in microcirculation apart from glomerulus



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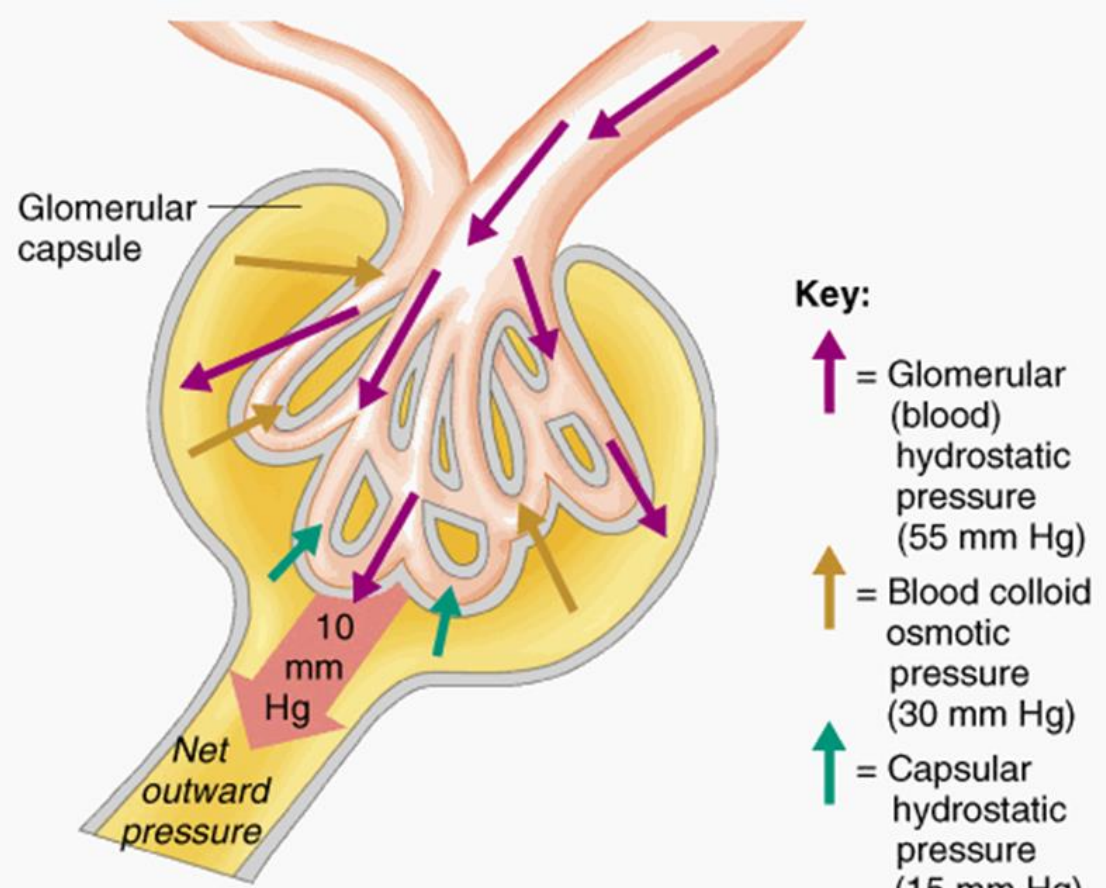
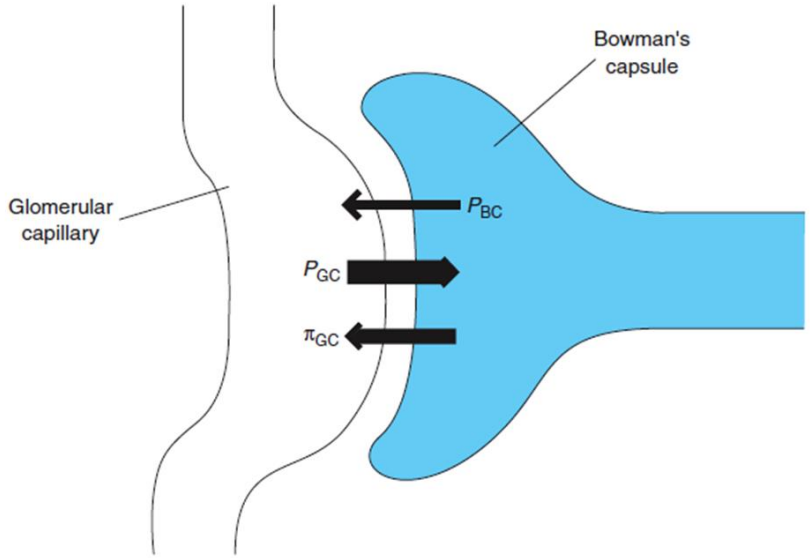
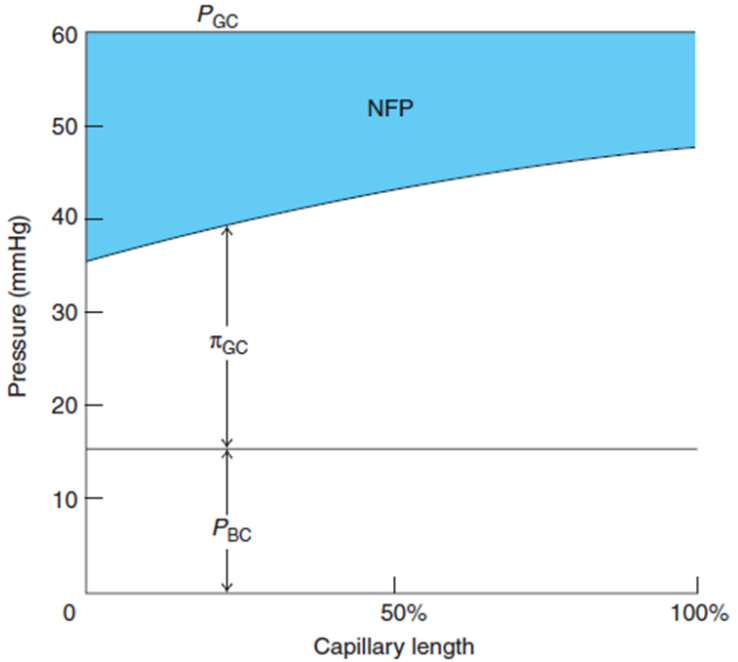
Glomerular filtration pressure



Glomerular capillaries – Starling forces





- net filtration pressure (P_f) in the renal corpuscle equals glomerular-capillary hydraulic pressure (P_{GC}) minus Bowman's capsule hydraulic pressure (P_{BC}) minus glomerular capillary oncotic pressure (π_{GC})
- contrary to common capillaries **hydrost. pressure in the whole length of glom. capillary decreases minimally** (due to autoregulation), therefore filtration is about 100-times higher compared to other capillaries
- hydrostatic pressures
  - P_{GC} is high and constant ~45 - 55mmHg
  - P_{BC} ~10 - 15mmHg

  } **net filtration pressure P_f ~35 mm Hg**
- osmotic pressures
  - π_{GC}~25 - 30mm Hg
  - thanks to large filtration π_{GC} increases along the capillary up to ~35 mm Hg at which point pressures reach equilibrium

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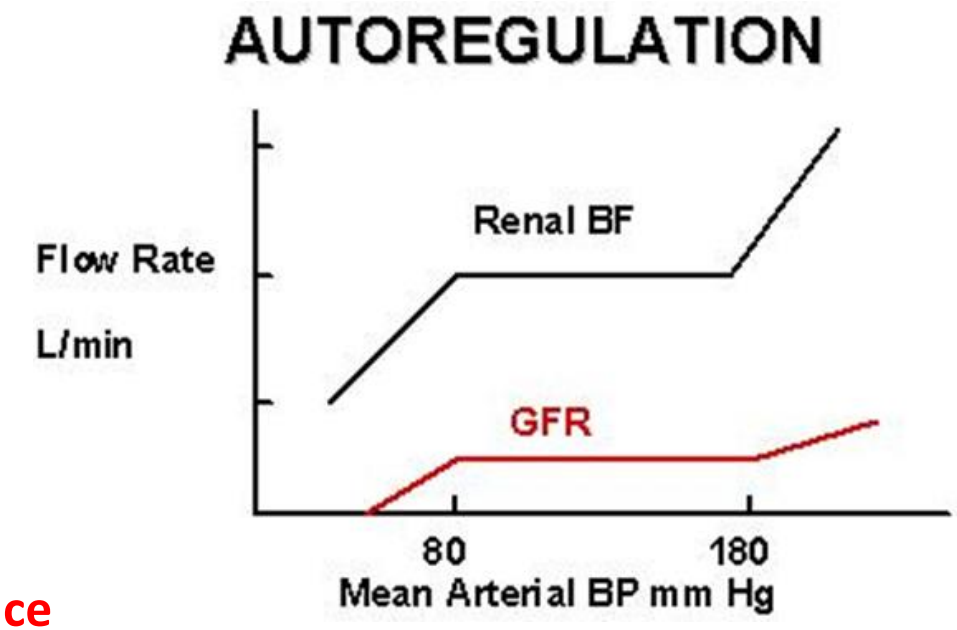
Renal blood flow (RBF) and GFR

- RBF in a healthy man (and GFR too) are thanks to the autoregulation quite stable
  - all plasma volume circulate through kidney in approx. 20 min
  - systemic pressure typically fluctuates
  - however, RBF stays rather constant in the range of 80 – 180 mmHg due to the autoregulation
  - only after significant drop of syst. BP RBF falls
    - → risk of ischemia, tubular necrosis
- RBF vs. renal plasma flow (RPF)
  - RBF ~ 20-25% of CO (cortex >>> medulla)
    - i.e. ~1000 - 1200 ml/min
    - rather high considering the weight of kidneys (~350 g)
  - RPF (hematocrit 0.45) ~600 - 700 ml/min
- glom. filtration
  - GFR ~20 - 25% RPF → **GFR ~ 120 – 140 ml/min**
  - ratio GFR/RPF = filtration fraction (~ 120/600 = ~ 0.2)
  - daily filtered ~ 180 l, but 99% reabsorption → 1.5–1.8 l of urine/day
- GFR and RPF can be assessed by various methods based on **clearance**
  - RPF (RBF) - PAH
  - GFR – creatinin, inulin (experimental) etc.



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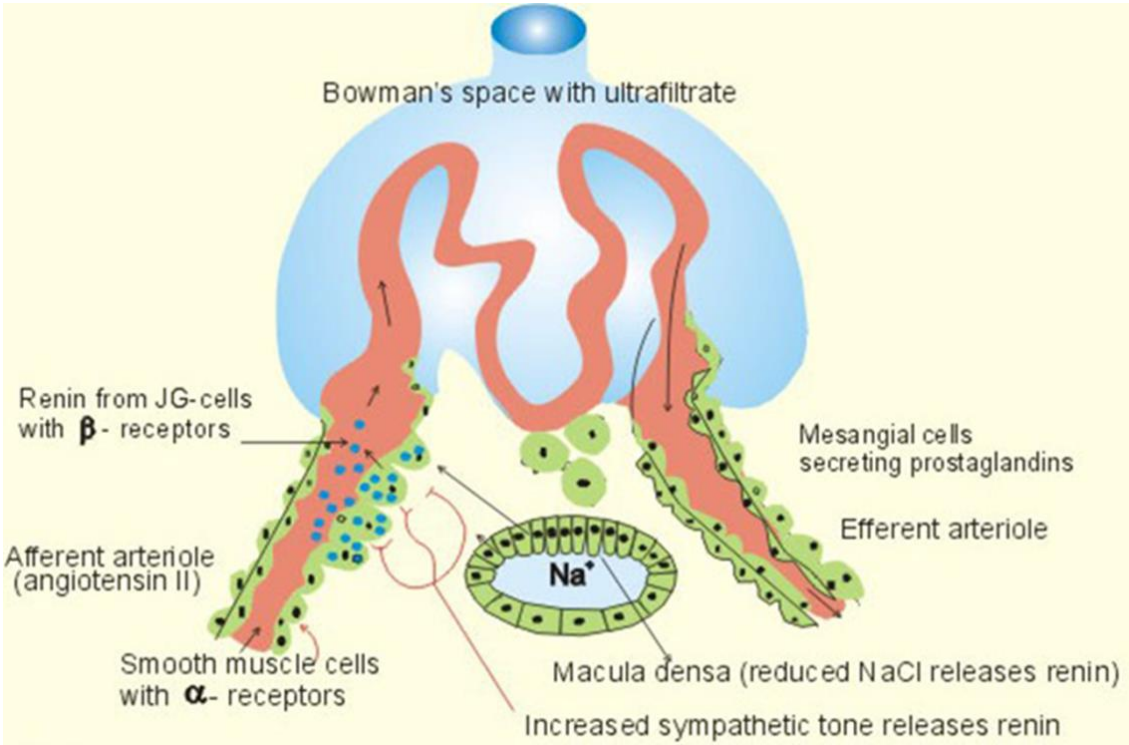
Regulation of RBF

- **autoregulation** of RBF
  - (1) **myogenic reflex**
    - SMC of the aff. and eff. arterioles detect wall tension and modify their resistance
  - (2) **tubuloglomerular negative feedback**
    - juxtaglomerular apparatus – macula densa - changes of NaCl – renin release – local RAS (dose-dependent effect)
- other **paracrine factors**
  - prostaglandins, adenosine and NO
- **sympathetic** nervous system
  - NE from adrenergic nerve endings and circulating E from adrenal medulla mediate constriction of afferent and efferent arterioles (α1-receptors)
    - drop of RBF and GFR
  - NE stimulates release of renin from granular JG-cells (via β1-receptors) and thus activation of systemic RAAS
  - NE ↑Na^+-reabs.in prox. tubule
- systemic RAAS



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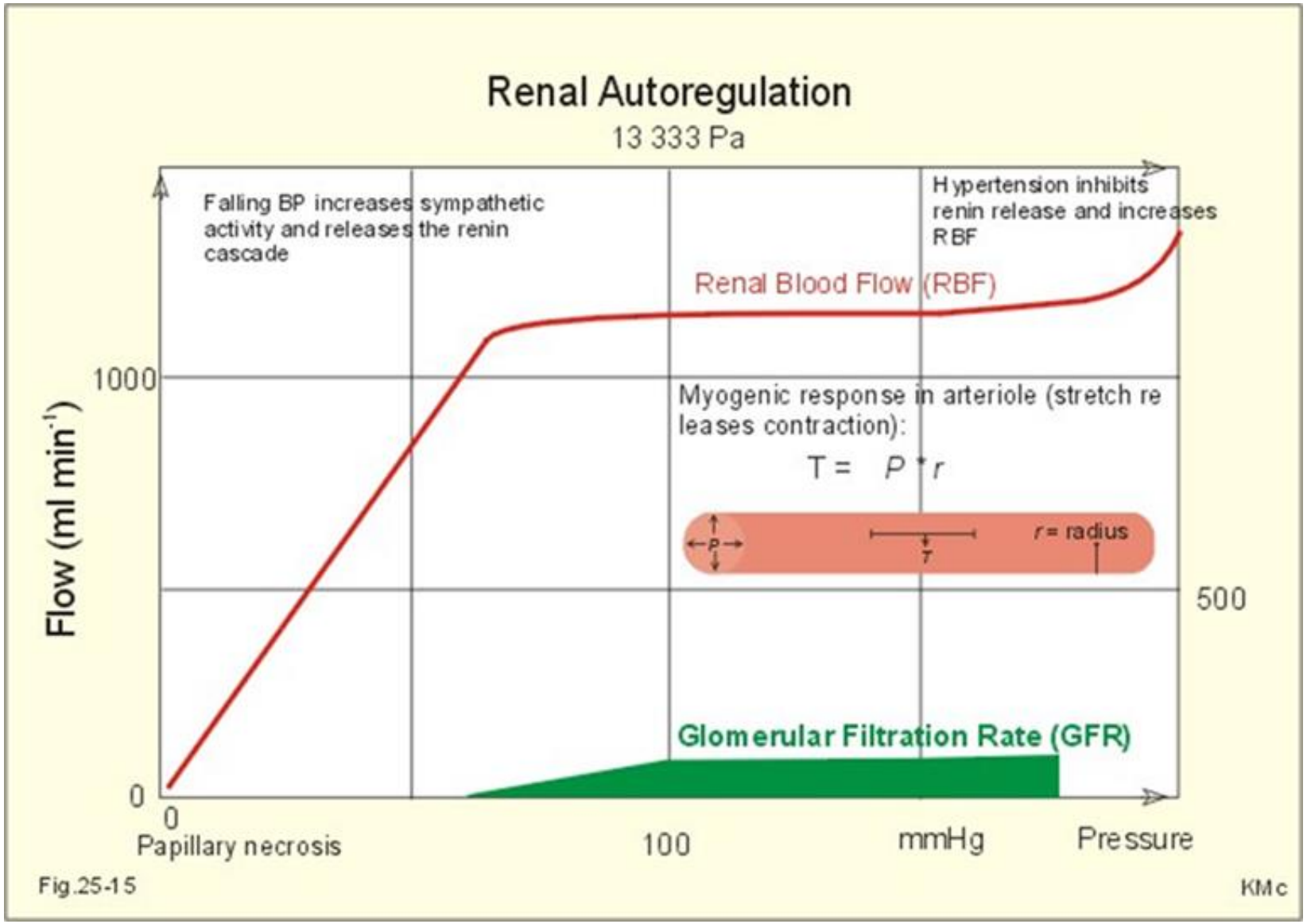
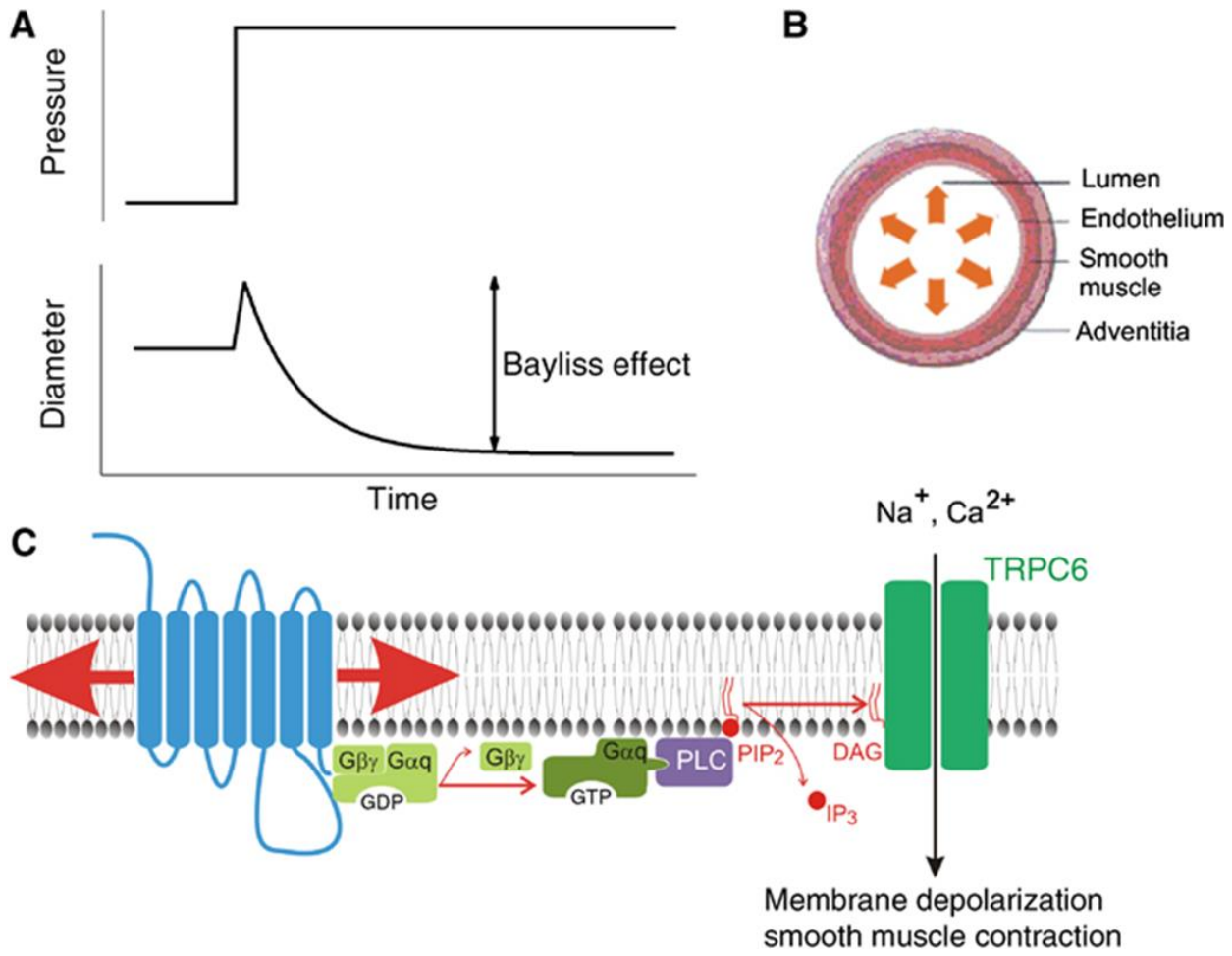
Autoregulation of RBF vs. systemic interest



(1) Myogenic regulation (Bayliss effect)



(**A**) Increasing pressure causes vasoconstriction.(**B**) The BE is mediated by the smooth muscle layer, independent of the inner layer of endothelial cells. (**C**) Proposed mechanism for stretch-induced activation of stretch-activated receptors in vascular smooth muscle membranes.

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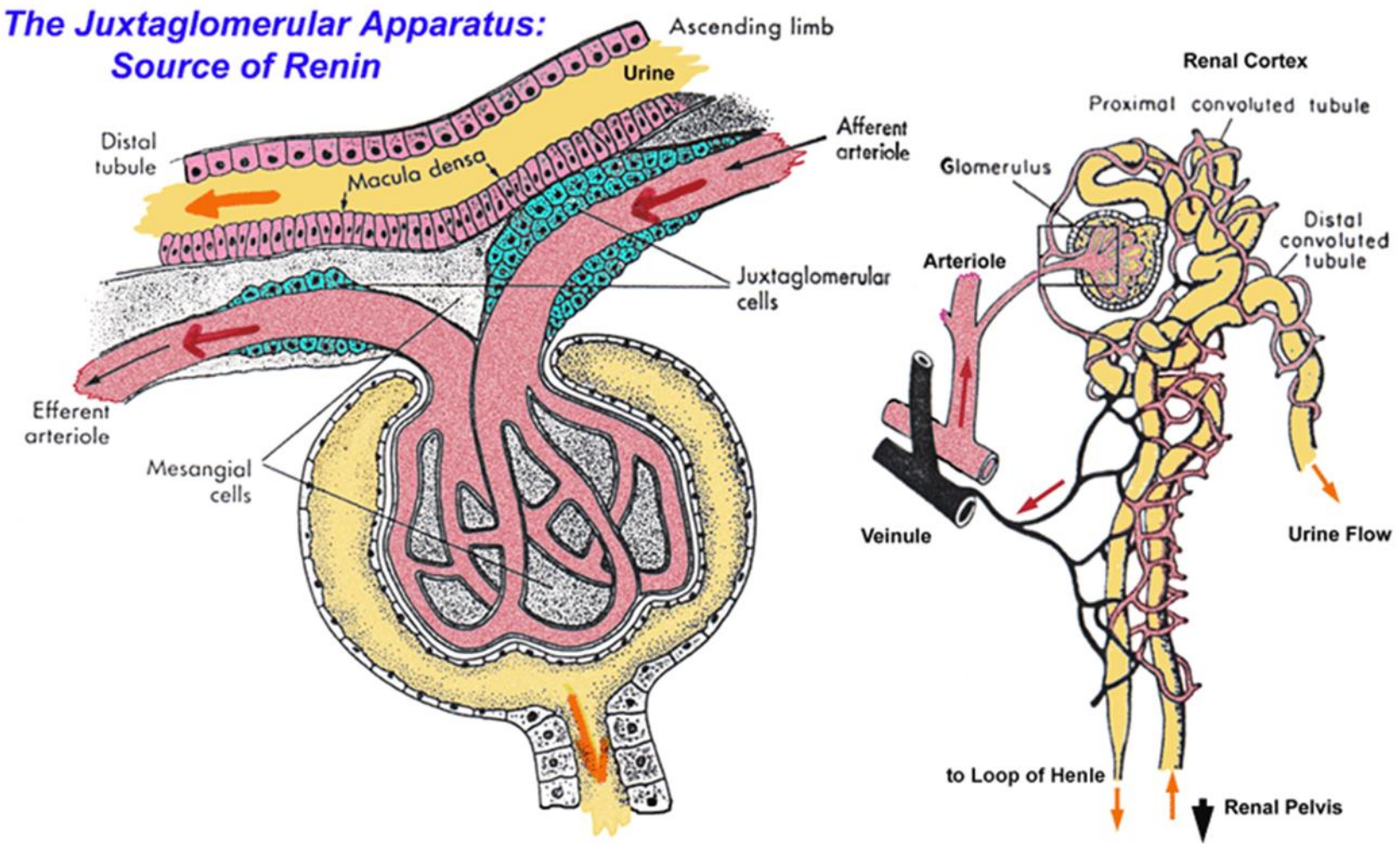
(2) Tubulo-glomerular feedback



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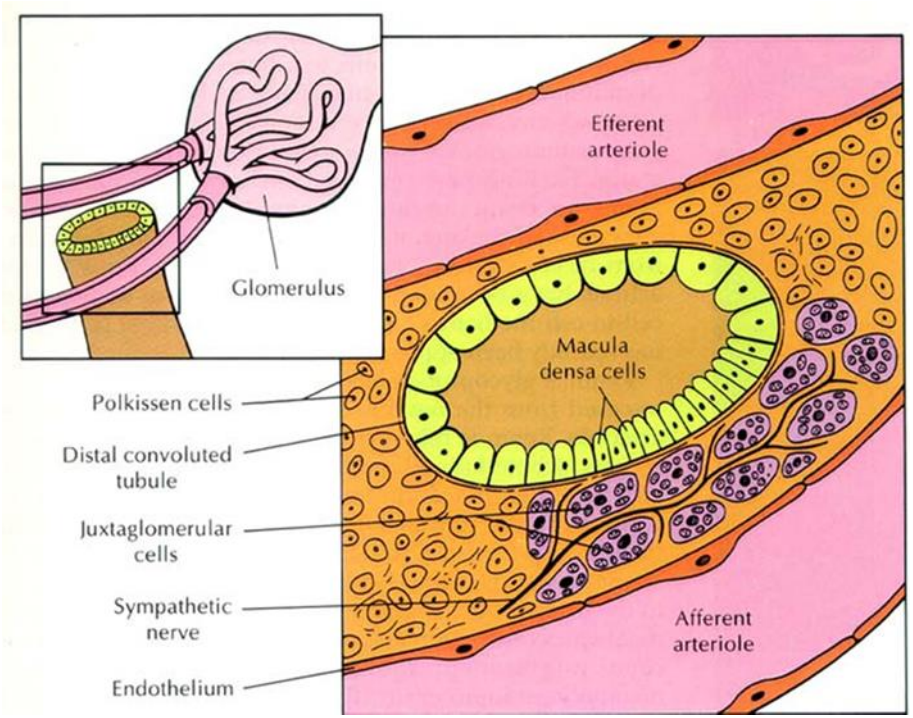
Juxtaglomerular apparatus (JGA)

- tubular and vascular component
  - (1) tubular component
    - specialised parts of distal tubule near afferent and efferent arterioles (**macula densa**)
    - cells of macula densa are sensitive to NaCl and control production of renin in **granular cells** of JGA
  - (2) vascular component
    - **afferent and efferent arterioles**
    - extra-glomerular **mesangium**
- JGA granular cells are specialized SMC producing and storing **renin**
  - cells of macula densa do not have a basal membrane to ensure tight contacts with granular cells
- both vascular and tubular components are **innervated by SNS**
  - renal nerve stimulation increases renin secretion by NE-induced stimulation of beta-adrenergic receptors



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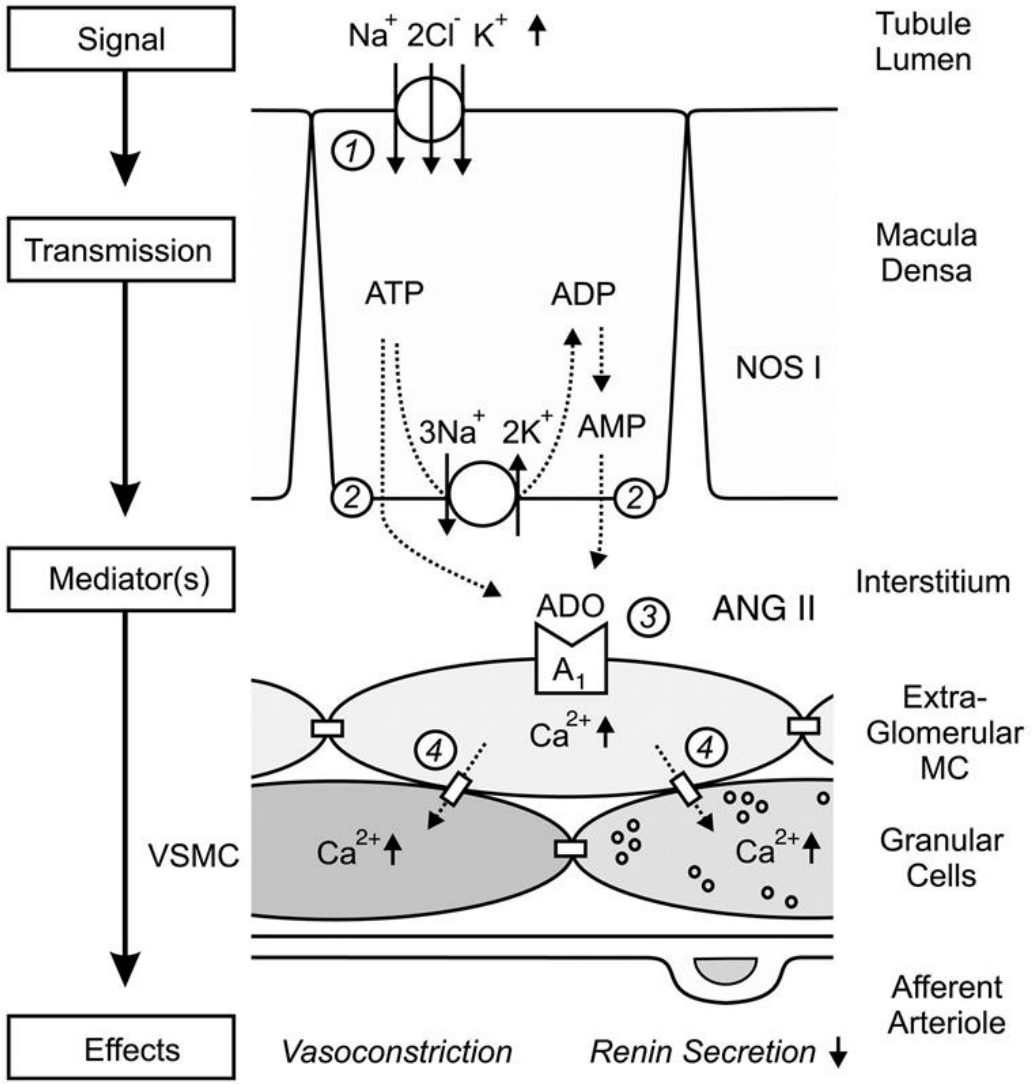
Detail mechanisms of TGF



Vallon V Physiology 2003;18:169-174

- macula densa cells (at the junction of ascending limb of loop of Henle and distal convoluted tubules)
  - presence of Na-K-2Cl symporter
    - when ↑ NaCl content at macula densa cells →- ↑ NaCl uptake → swelling of macula densa cells → release of ATP
      - stimulation of purinergic P2 receptors on mesangial cells and afferent arteriole smooth muscles
      - alternatively ATP may be metabolized to adenosine, which also causes vasoconstriction here
        - adenosine normally causes vasodilation in other tissues !!!
- effect of increased NaCl content
  - contraction of mesangial cells and contraction of glom. arterioles
    - reduction in effective filtration area
    - decreases GFR and RBF
  - NaCl content at macula densa also ↓ renin release
- effect of decreased NaCl content
  - opposite

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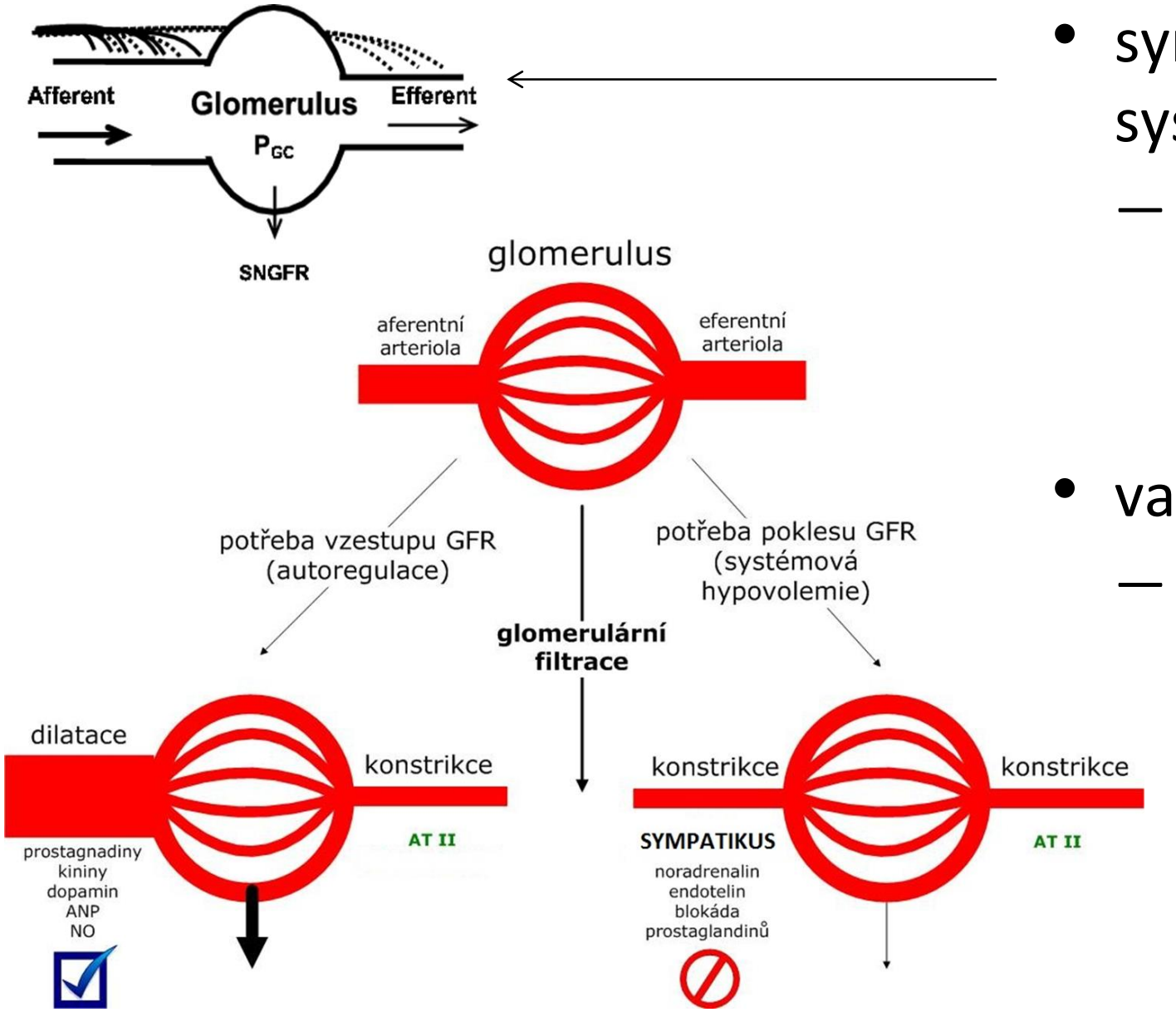
Other regulators of glom. hemodynamics



- sympathetic nervous system
  - sympathetic innervation of glom. arterioles
    - 3-time denser in afferent arteriole
- vasoactive peptides
  - receptors for vasodilators mainly in AA
    - their pharmacological blockade (e.g. by COX1 inhibitors) can decrease GFR without BP change

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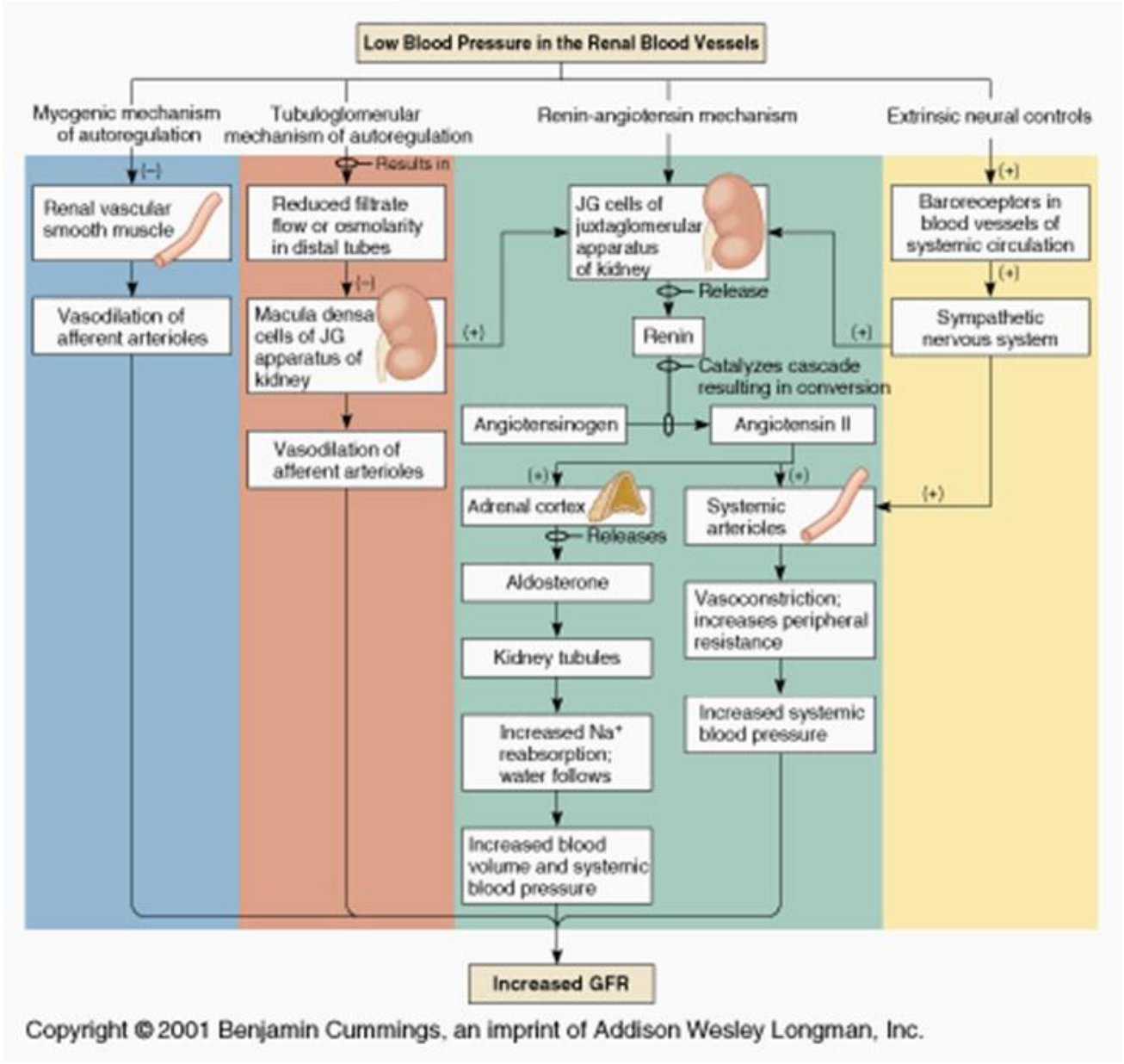
Summary of regulatory mechanisms



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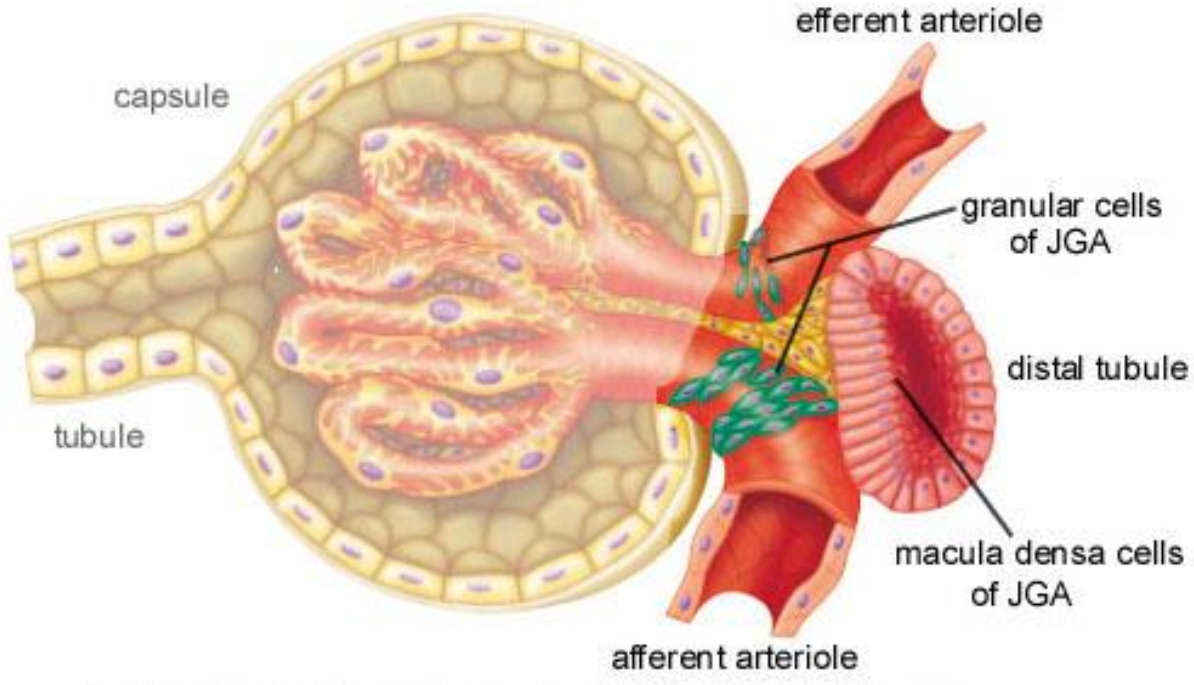
Three major mechanisms governing renin release

- (1) signals at the individual nephron
  - decreased NaCl load at the macula densa
  - decreased afferent arteriolar pressure (probably mediated by a cellular stretch mechanism)
- (2) signals involving the entire kidney
  - beta1-adrenergic receptor stimulation at the juxtaglomerular cells
  - at the same time, negative-feedback inhibition by AT II at the JG cells
  - other hormonal factors
- (3) local effectors
  - prostaglandins E2 and I2
  - nitric oxide
  - adenosine
  - dopamine
  - arginine vasopressin



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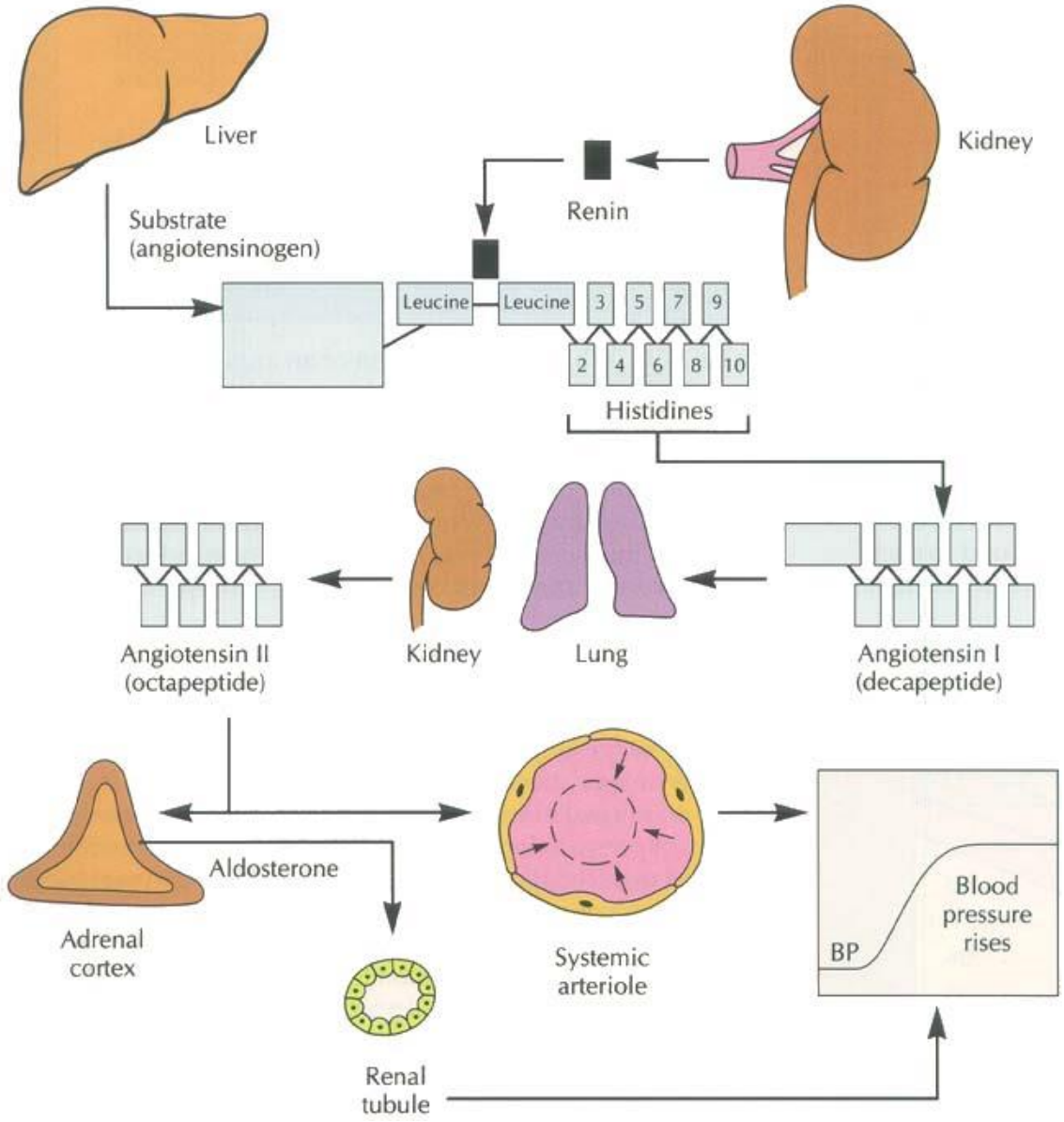
Systemic RAAS



- prorenin is converted to active renin by a trypsin-like activating enzyme
- renin enzymatically cleaves AGT to form the decapeptide AT I
  - this step can be blocked by renin inhibitors
- AT I is hydrolyzed to the octapeptide AT II by angiotensin- converting enzyme (ACE)
  - this step is blocked by ACE inhibitors
- AT II acts at a specific receptor
  - this interaction can be blocked by a variety of peptide or non-peptide AT II antagonists

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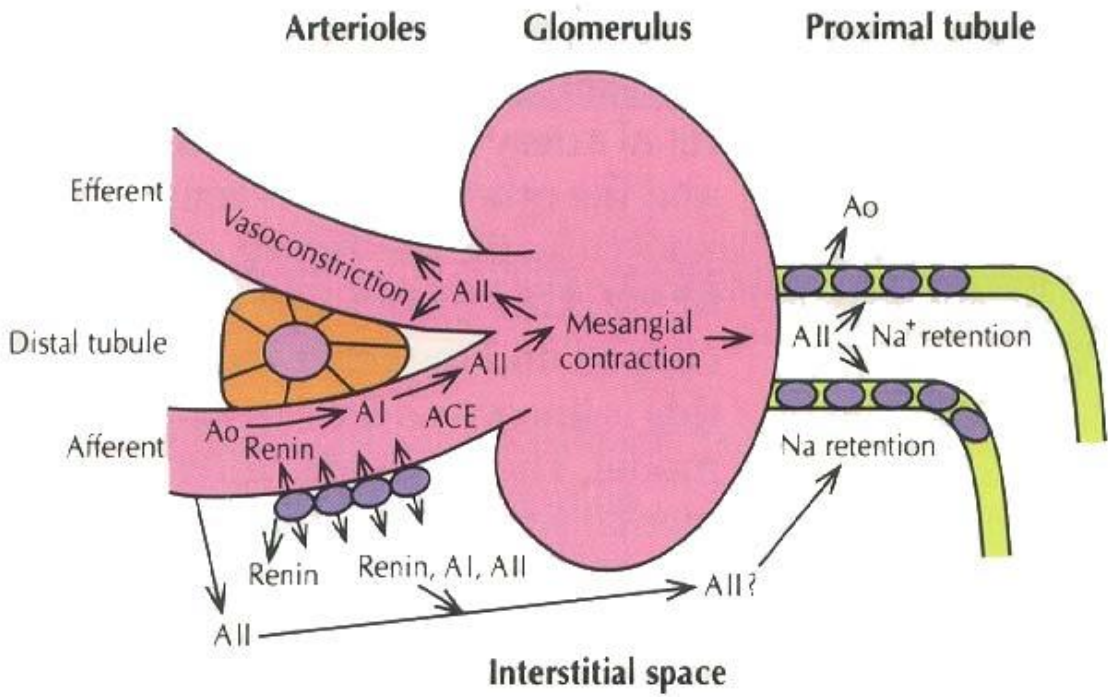
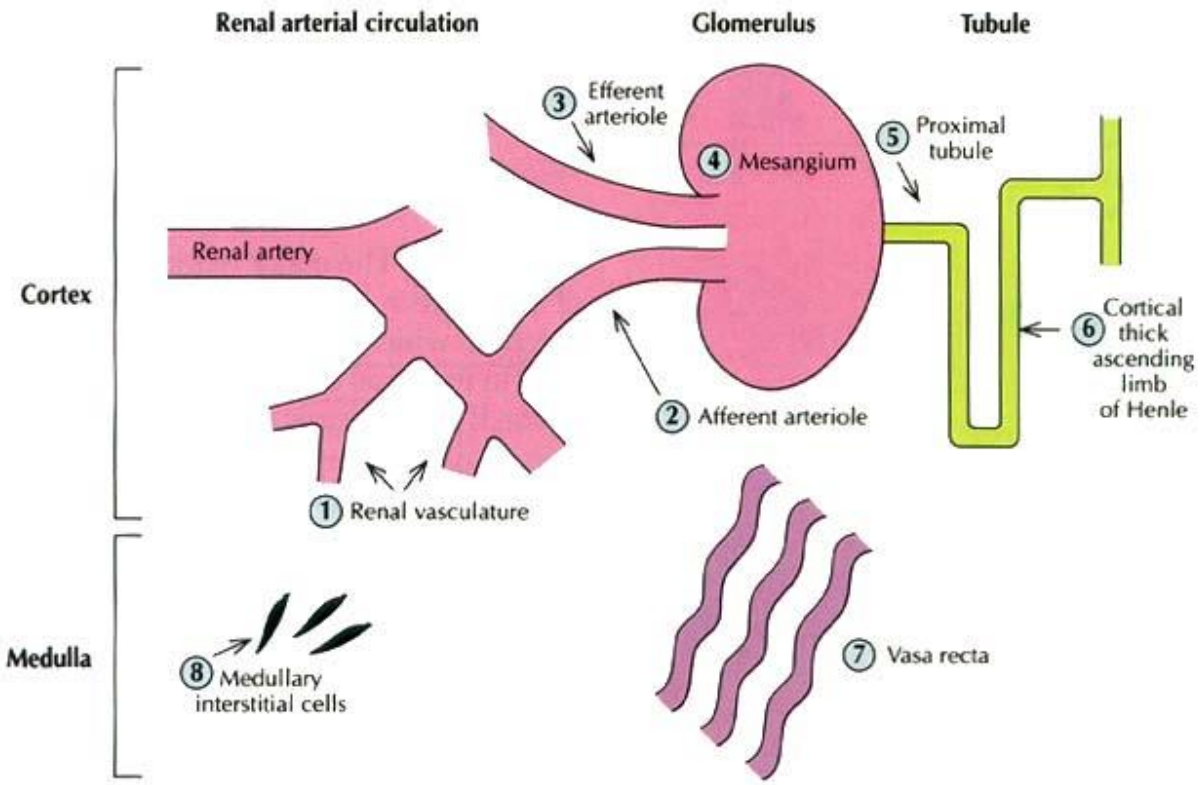
Paracrine effects of AT II in the kidney

- AGT either circulates to the kidney from the site of production in the liver or is synthesized locally in proximal tubular cells in the kidney
- renin is synthesized and released from the JG cells into the afferent arteriolar lumen or into the renal interstitium
- AT I is generated in the afferent arteriole and is converted to AT II by ACE and acts on efferent arteriole
- AT II can also be filtered at the glomerulus and may subsequently act at the proximal tubular cells to increase sodium reabsorption
- in the renal interstitium renin can cleave AGT to produce angiotensin peptides
  - these peptides may act at vascular and tubular structures



The renal tissue localization of AT II receptors and their physiologic action



- vasoconstriction occurs when AT II acts at receptors in the arcuate and interlobular arteries **[1]**, the afferent **[2]** and efferent **[3]** arterioles and the medullary vasa recta **[7]**
  - AT II preferentially constricts the efferent arteriole, thereby increasing glomerular filtration pressure
- AT II also acts on mesangial cell receptors [4] to produce cellular contraction and reduce glomerular filtration
- AT II receptors also are localized to the proximal tubule **[5]** and the cortical thick ascending LH cells **[6]** which cause sodium reabsorption
- AT II receptors are expressed also elsewhere in kidney - medullary interstitial cells **[8]** - but the physiologic significance of these receptors is still unknown
- in summary, AT II has three major effects all of which result in sodium retention
  - 1) arteriolar vasoconstriction
  - 2) renal sodium retention
  - 3) increased aldosterone biosynthesis
- these effects work together to maintain arterial blood pressure as well as blood volume
- AT II also stimulates the sympathetic nervous system, particularly the thirst center in the hypothalamus

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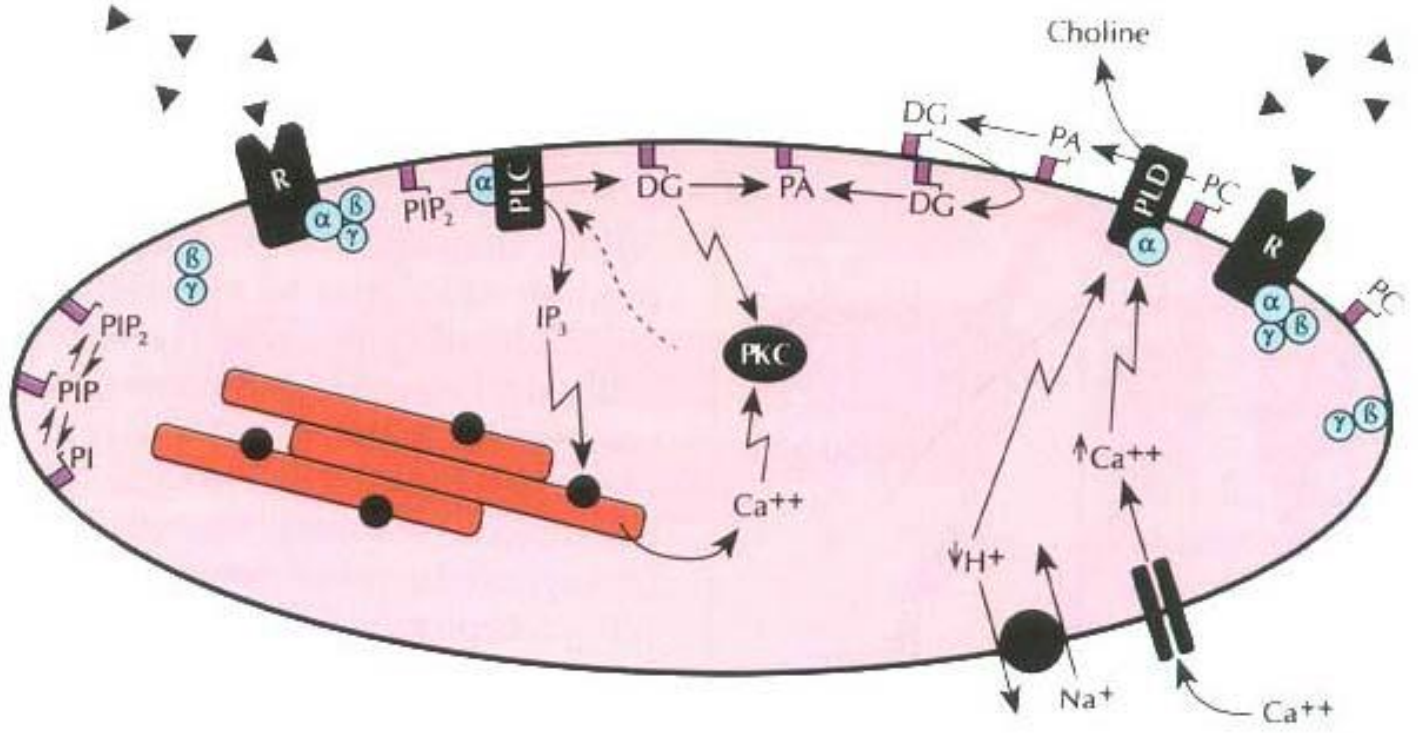
Cellular action of AT II



- when ATII activates AT1 receptors in vascular cells the peptide initiates a biphasic signalling response
  - the initial phase comprises PLC-mediated break-down of the inositol polyphospholipids to generate IP3 and DAG as well as to mobilize intracellular calcium
  - the second phase is characterized by a sustained accumulation of DAG, activation of PKC, hydrolysis of phosphatidylcholine-mediated by PLD, and intracellular alkalinization

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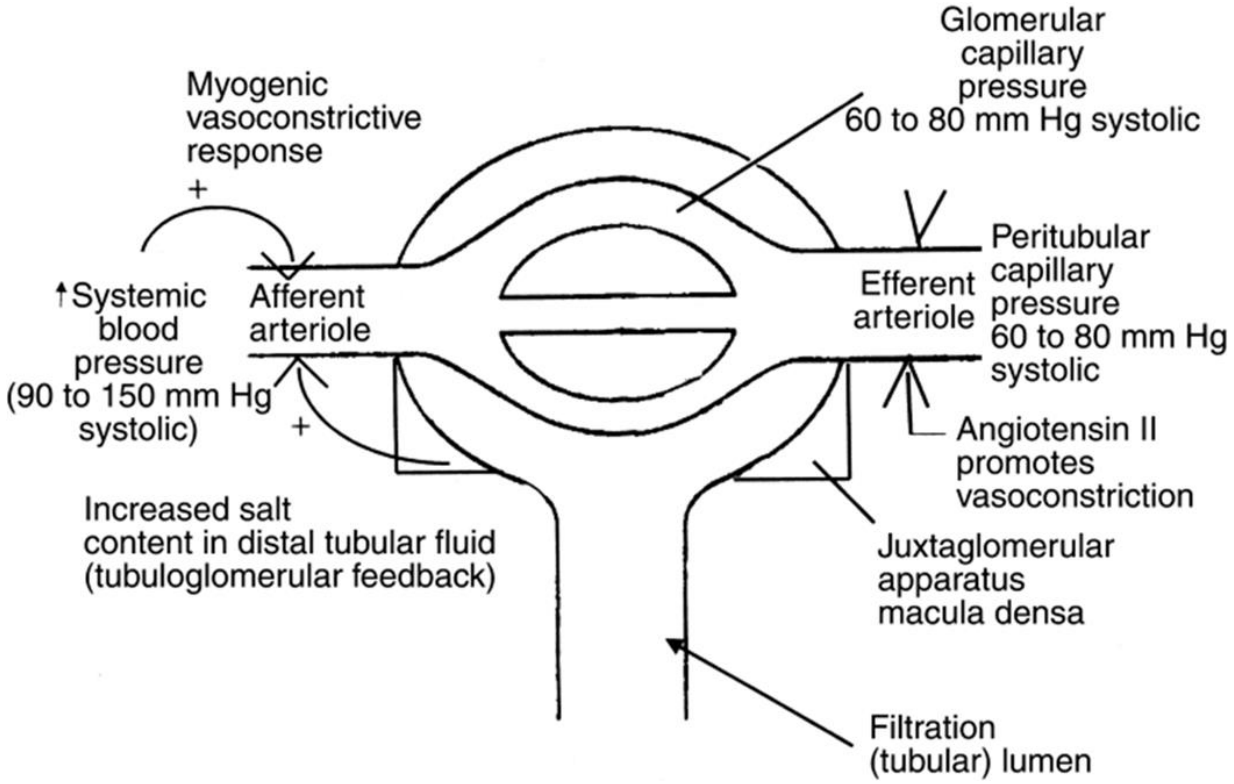
RBF is regulated in conflicting manner



- during light to moderate decrease of systemic pressure by autoregulation
  - the aim is to maintain renal perfusion, GFR a homeostasis
- during significant decrease (circulation emergency) perfusion of kidney drops in “systemic interest”
  - **pre-renal azotemia**
  - eventually with morphological consequences (**acute tubular necrosis**)

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Measurement of GFR

- GFR is a main parameter characterizing kidney function
  - — however the volume of glomerular filtrate produced per time unit is **not directly measurable**
- it can be assessed precisely enough by
  - — (1) determining **clearance** of certain substances fulfilling certain criteria (see further)
    - endogenous substances – creatitinin, urea
    - exogenous
      - — unlabeled tracer – inulin,
      - — radio-contrast - iohexol
      - — radioactive isotope – [^{51}Cr] EDTA, [^{125}I] iothalamate, [^{99}Tcm] DTPA
  - — (2) **estimation** of GFR based on plasma levels of endogenous substances by **formula**
    - creatinin - Cockroft-Gault, MDRD, CKD-EPI, …
    - other endogenous markers (freely filtred and completely degraded by tubular cells)
      - — β2-microglobulin, cystatin C

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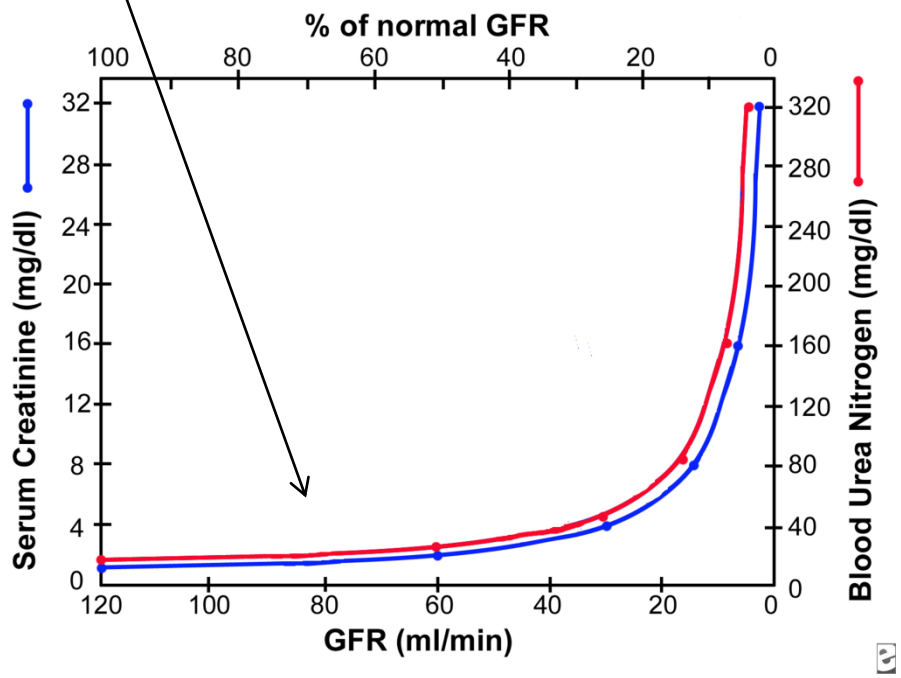
Clearance

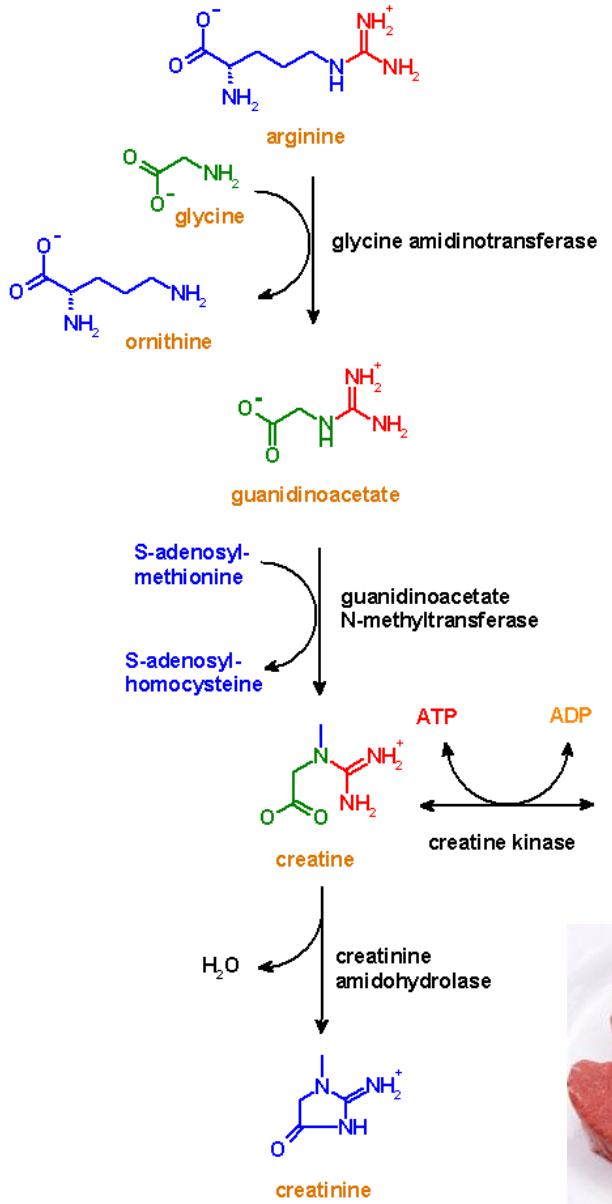
- substances must fulfill following criteria
  - LMW, freely filtered to urine, unbound to plasma carriers
  - not undergoing further degradation
  - no tub. reabsorption nor secretion
  - concentration in plasma and analogic volume of glom. filtrate stable
  - detection method is simple, cheap and standardized
- concentration in urine is proportional to changes of GFR:
  - **$[P] \times GF = V \times [U]$**
- clearance of substance X = volume of plasma that is cleared of substance X per unit time
  - units: volume/time
  - timed urine collection is necessary
    - ideally 24 hrs, often shorter

hyperbolic relationship is clinically important

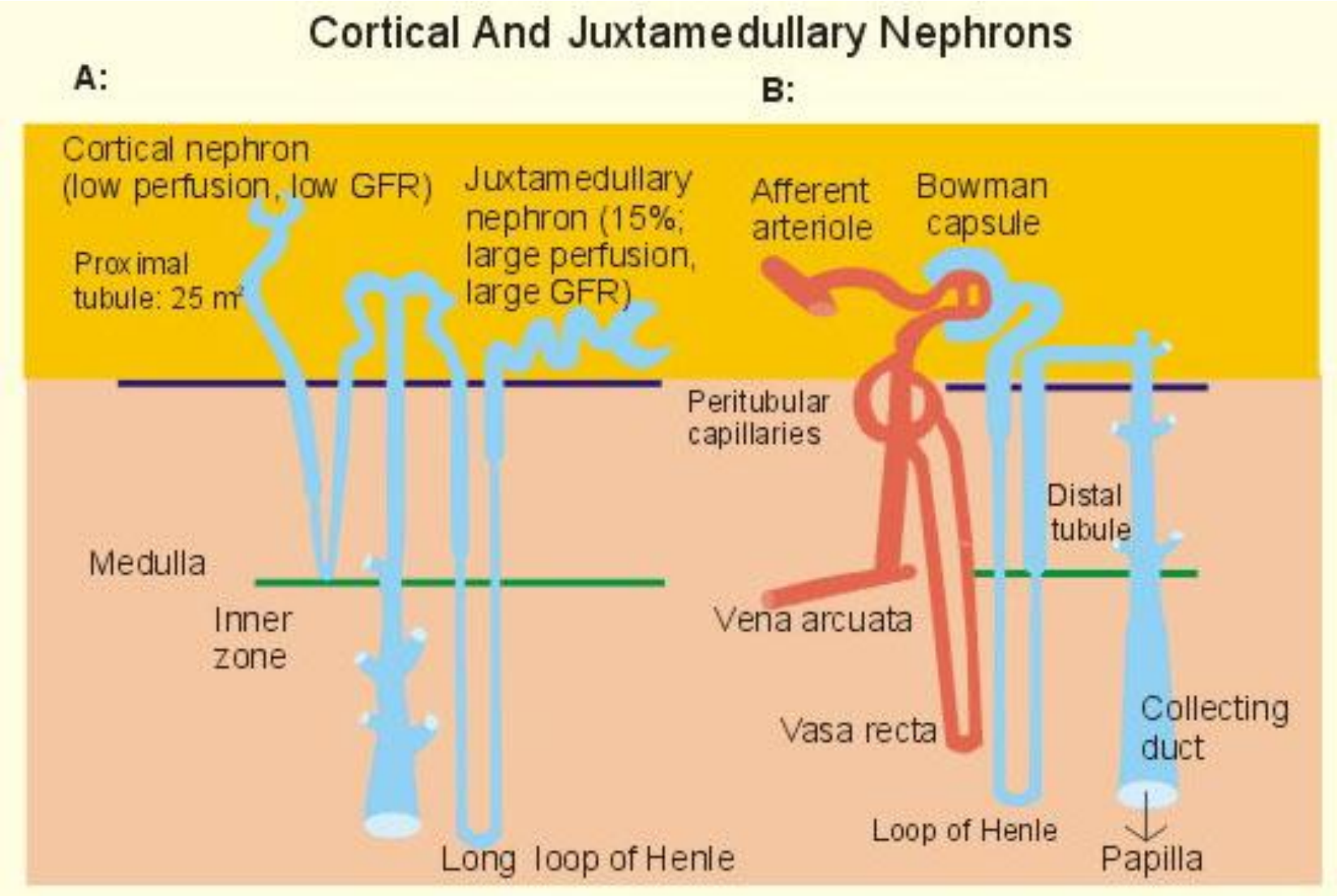


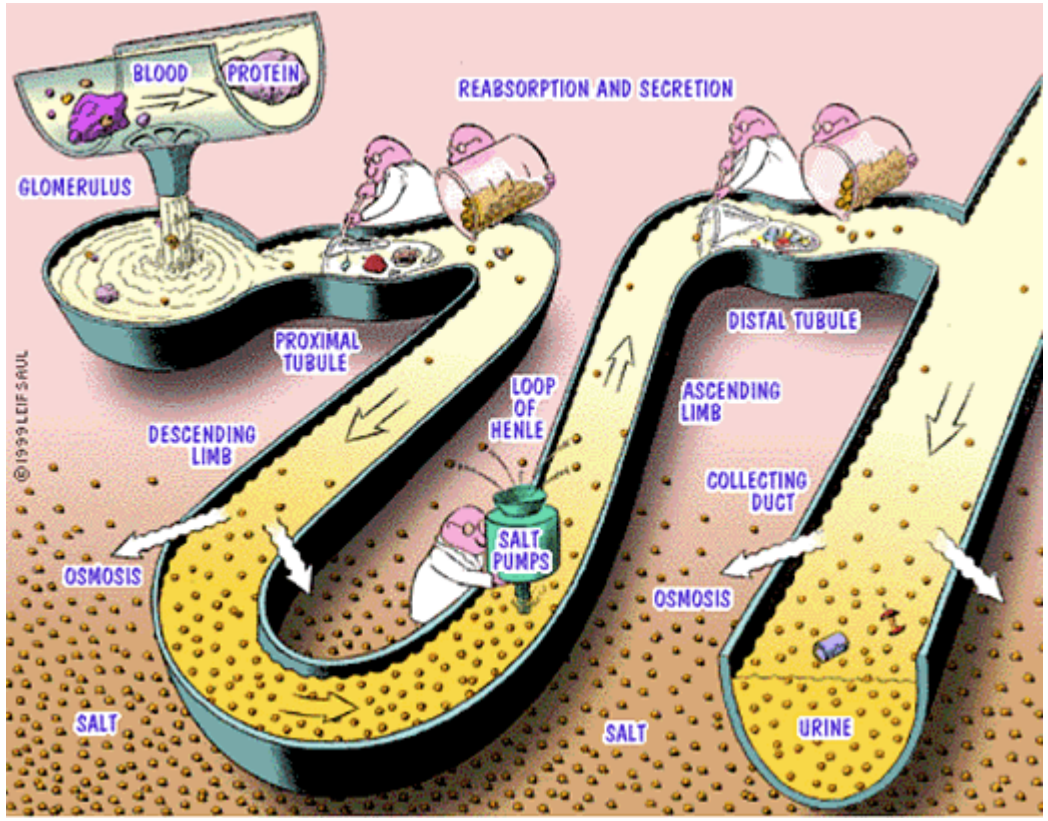
Creatinin

- produced in muscles from creatin
- in kidneys 90% filtered, z 10% secreted to urine by tubules
  - the contribution of tubular secretion rises with ↓ filtration (↓ number of functional nephrons)
  - i.e. the lower the GFR the less precise assessment of GFR by Ccr, but still the best endogenous marker of GFR
- there are possible technical problems with timed urine collection
  - suboptimal cooperation of patient
- concentration of S-creatinin directly related to muscle mass (therefore depends on age and gender)
  - plasma creatinin = 35 – 100 µmol/l, production 1.2mg/min
  - usually corrected for body surface area (1.73m^2), but still there are discrepancies due to body composition
    - 25-yrs old athlete vs. 60-yrs old obese man with the same weight and body surface
- intra-individual fluctuation not more than 10 - 15%
  - concentration rises after the strenuous physical exercise and after intake of exogenous creatinin (meat)
    - especially fried



Heterogenous GFR in individual nephrones





TUBULAR REABSORPTION AND SECRETION

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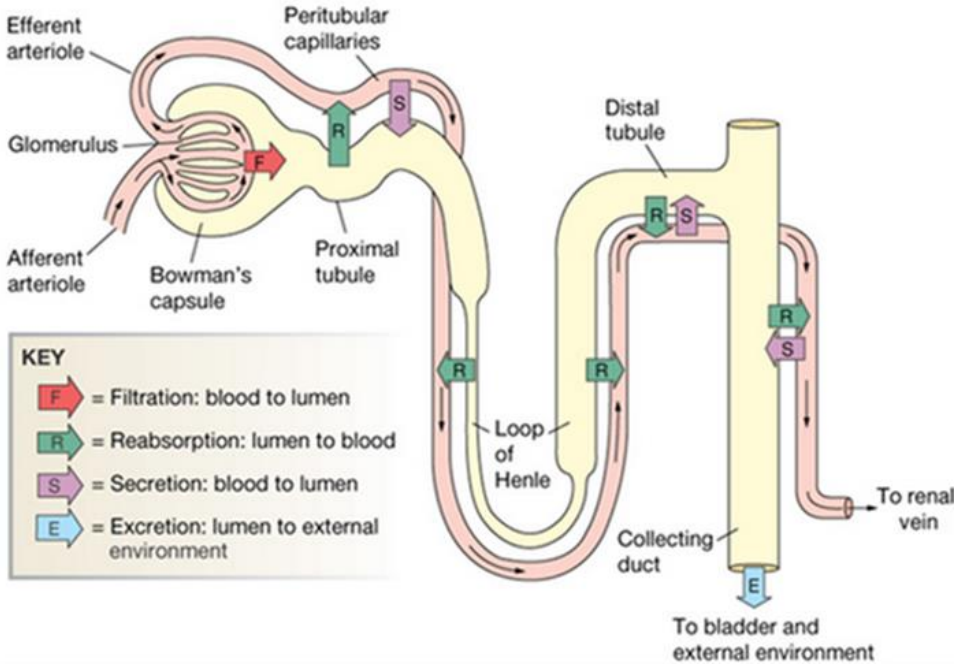
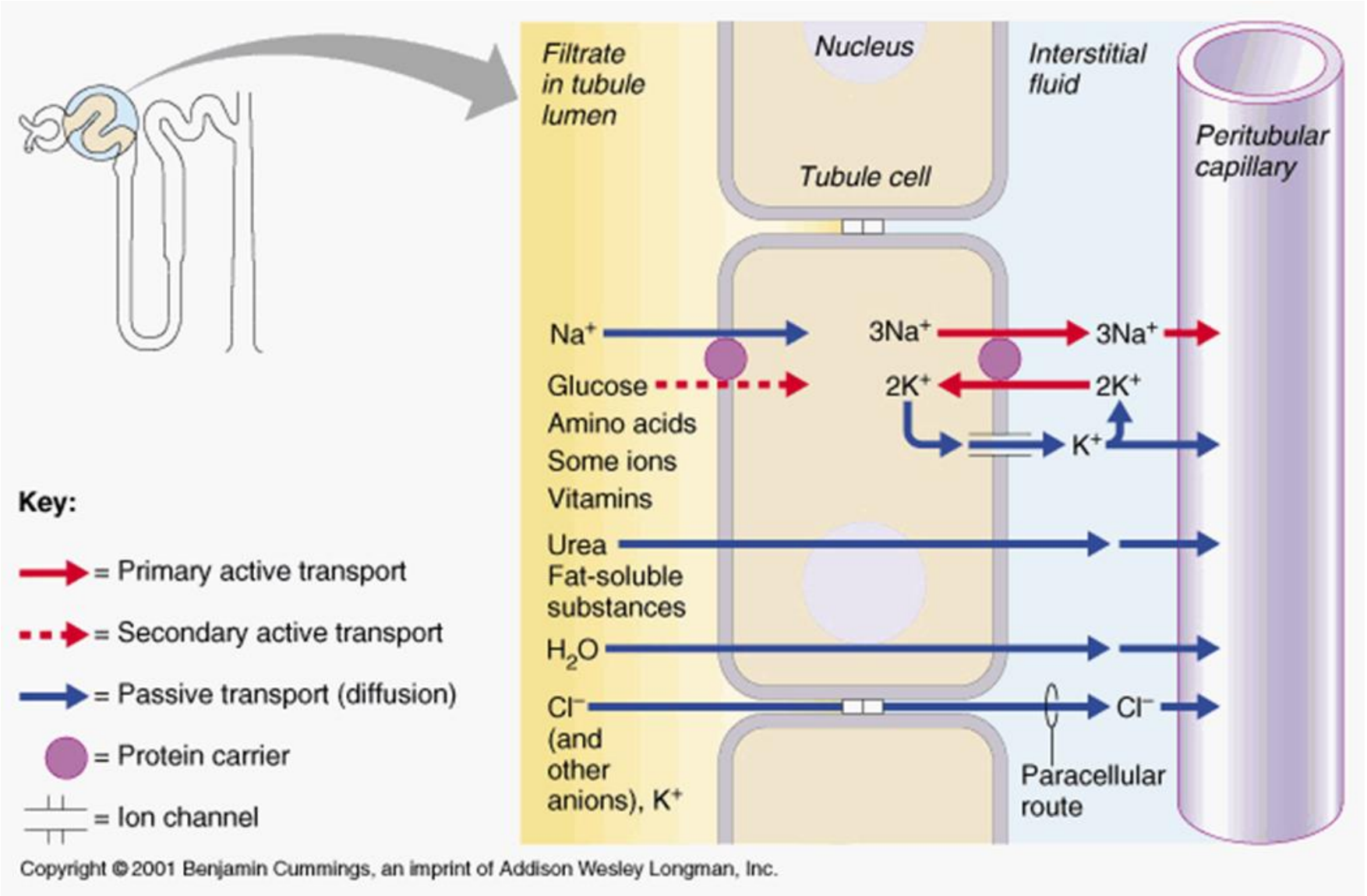
Tubular reabsorption and secretion

- complex processes (active and passive)
  - — epithelial cells of kidney tubules (and their hormonal control)
    - reabsorption means transport from apical to basolateral side
    - secretion is a transport from basolateral to apical side
- different parts of tubules have different function - specialization of tub. segments
  - — nearly 90% complete reabsorption in prox. tubule by various forms of transport
    - ~75% Na^+ (and HCO_3^-), Cl^- and H_2O
    - ~90% K^+
      - — secretion of K+ in collecting tubules, total amount depends on Na+ concentration in particular segment (aldosterone)
  - — distal tubule and collecting duct are under the influence of ADH & aldosterone



Types of transport



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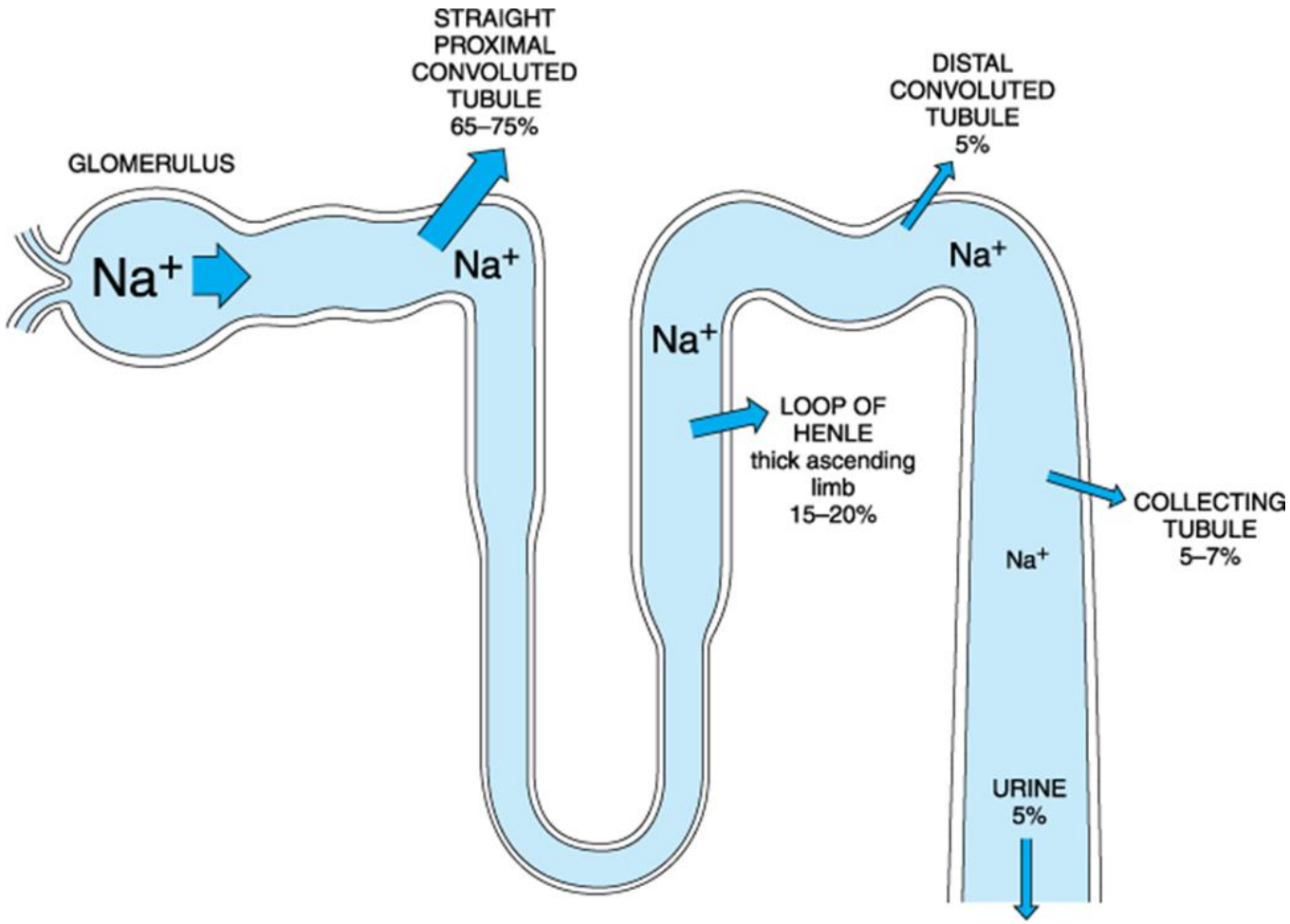
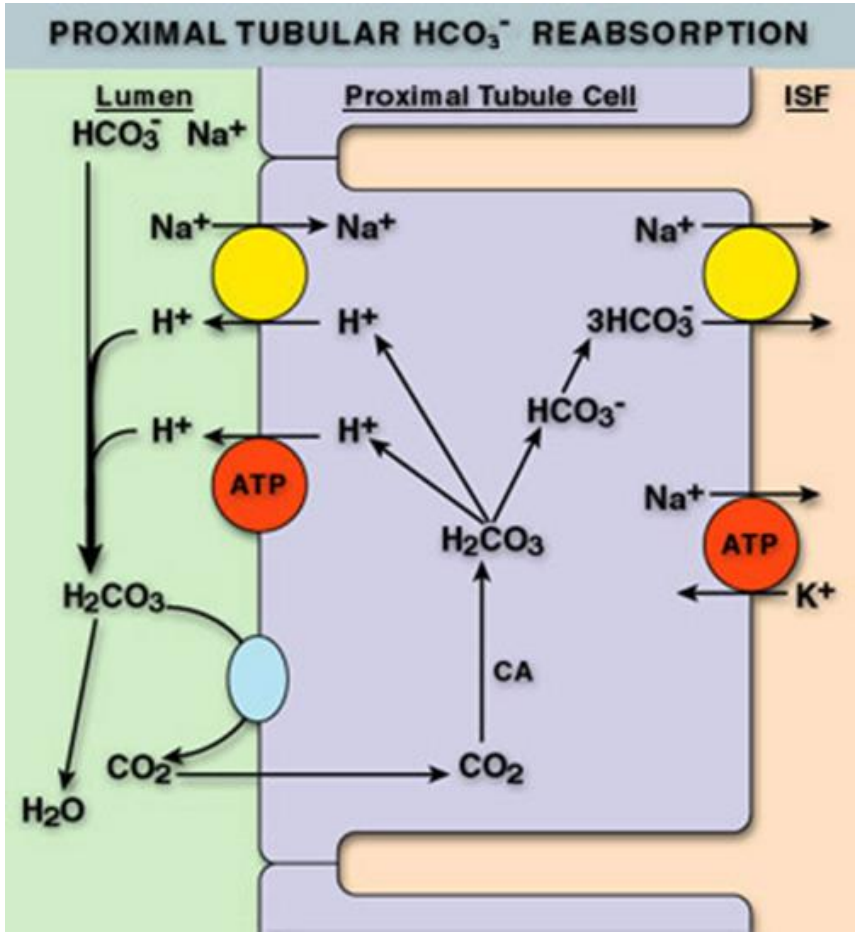
Reabsorption of Na^+ (and HCO_3^-)





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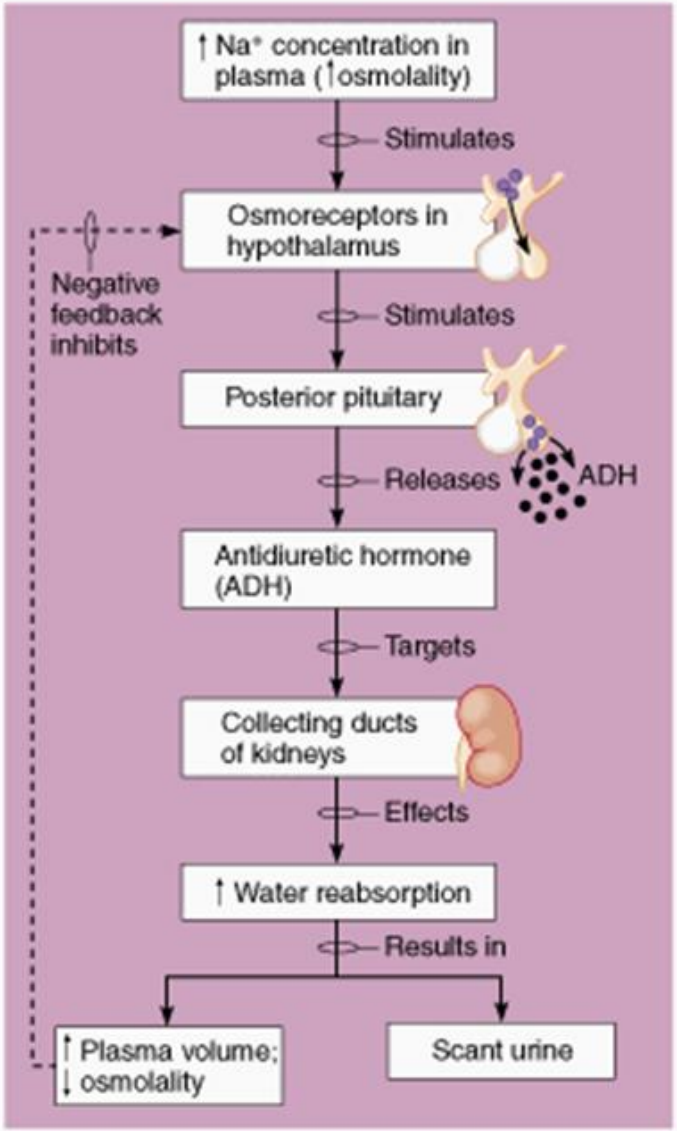
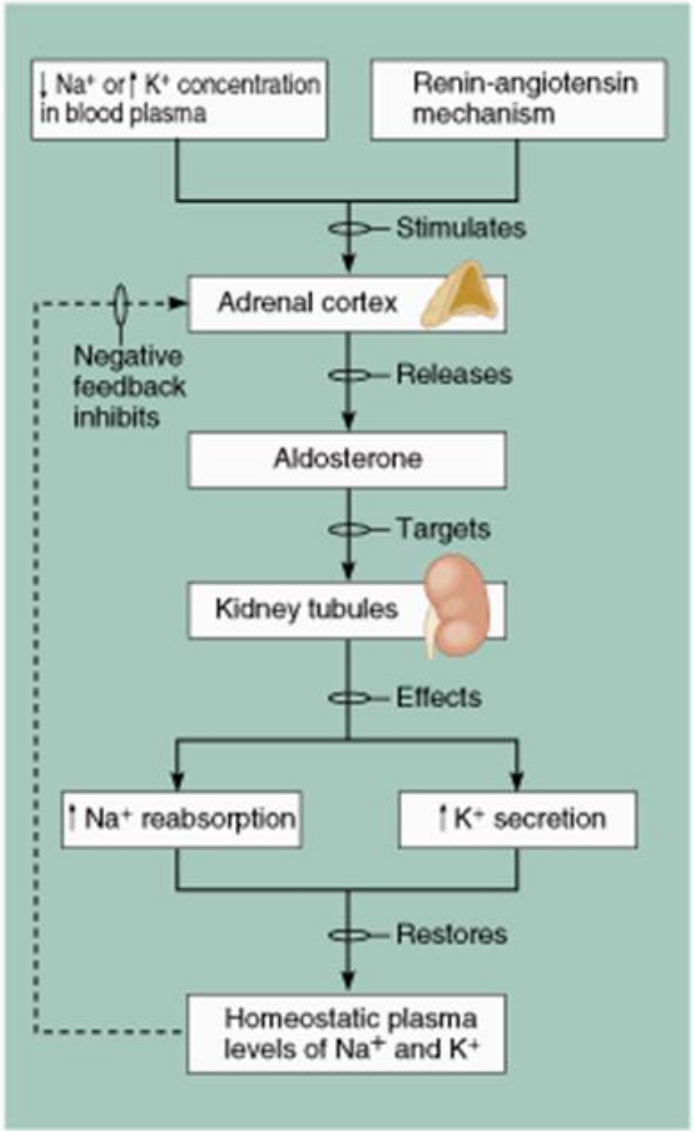
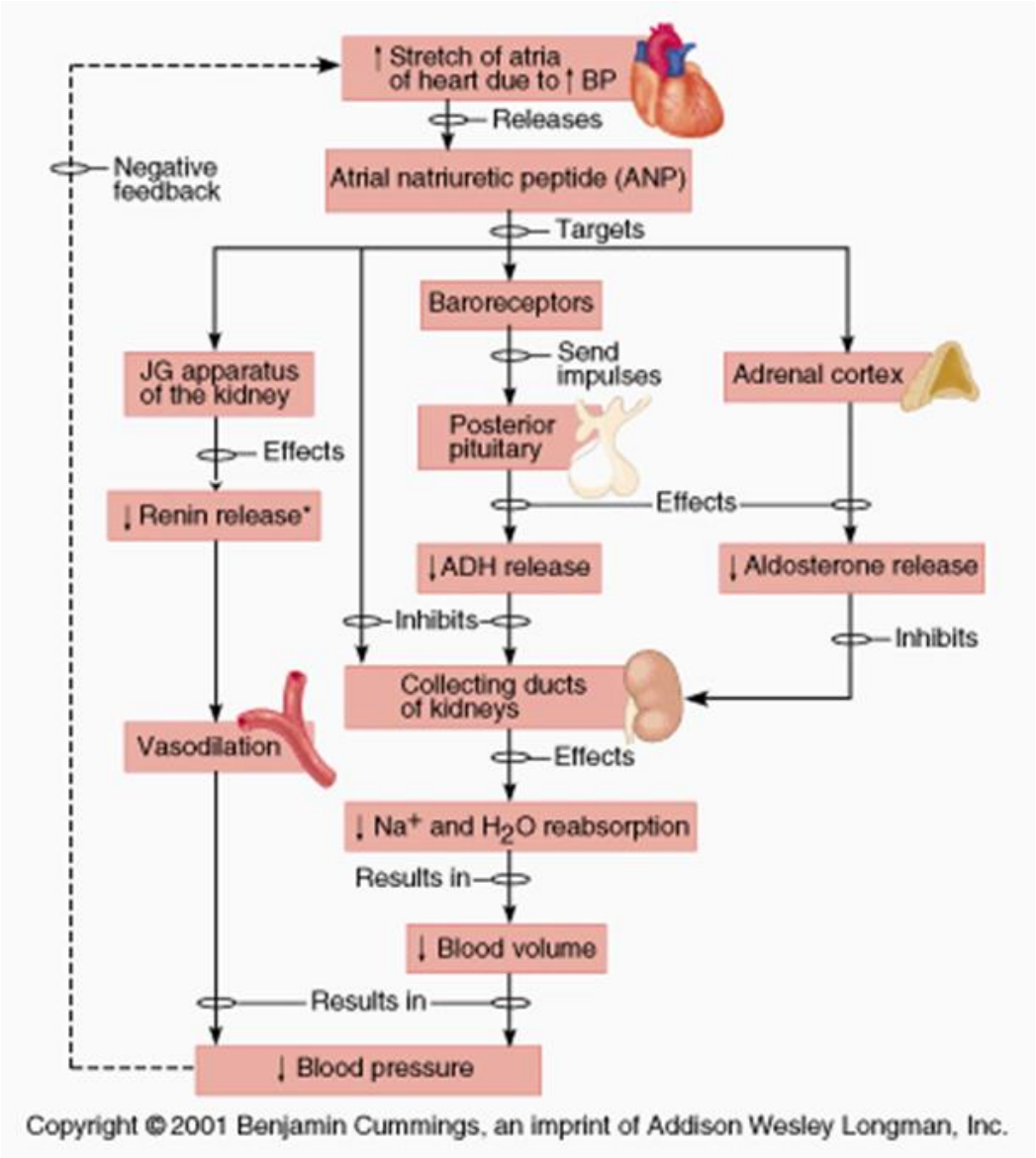
Hormones affecting renal function





MUNI MED

Hormones affecting renal function



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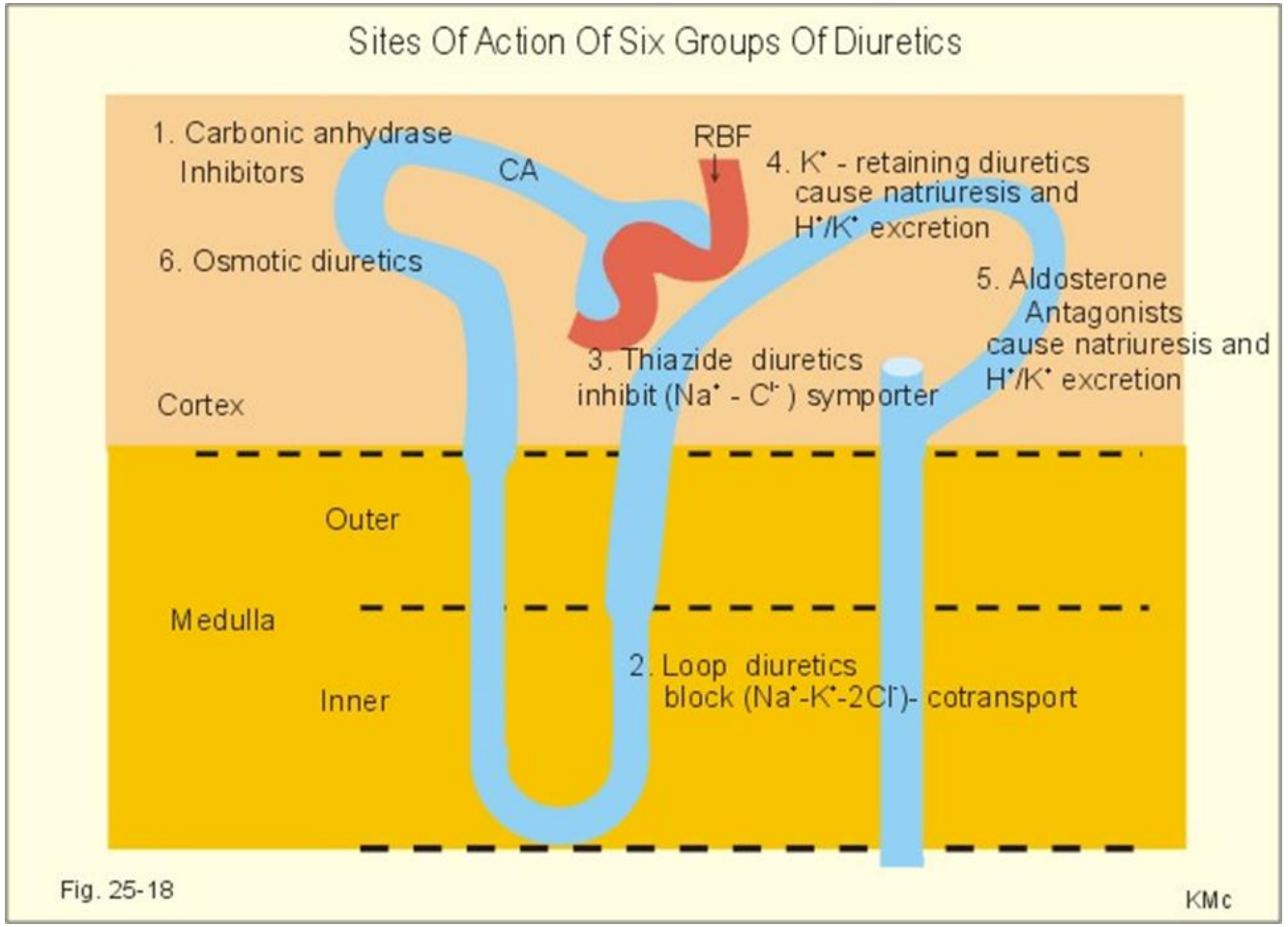
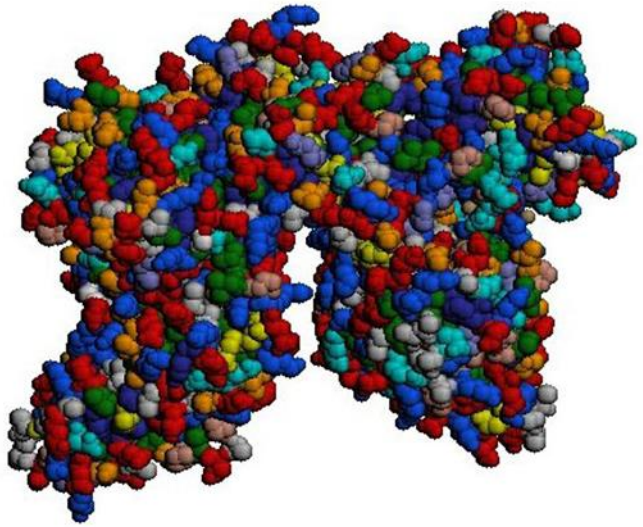
Diuretics

diuretics increase volume of urine (more exactly a proportion of glomerular filtrate excreted as urine)

- types
  - inhibitors of carboanhydrase
    - ↑ osmotic pressure of filtrate
  - loop diuretics
    - inhibit NaCl transport in ascendent part of LH
  - thiazide diuretics
    - inhibit NaCl reabsorption in the 1st segment of DCT
  - osmotic diuretics
  - kalium sparing diuretics
  - combined





FILTRATION MEMBRANE

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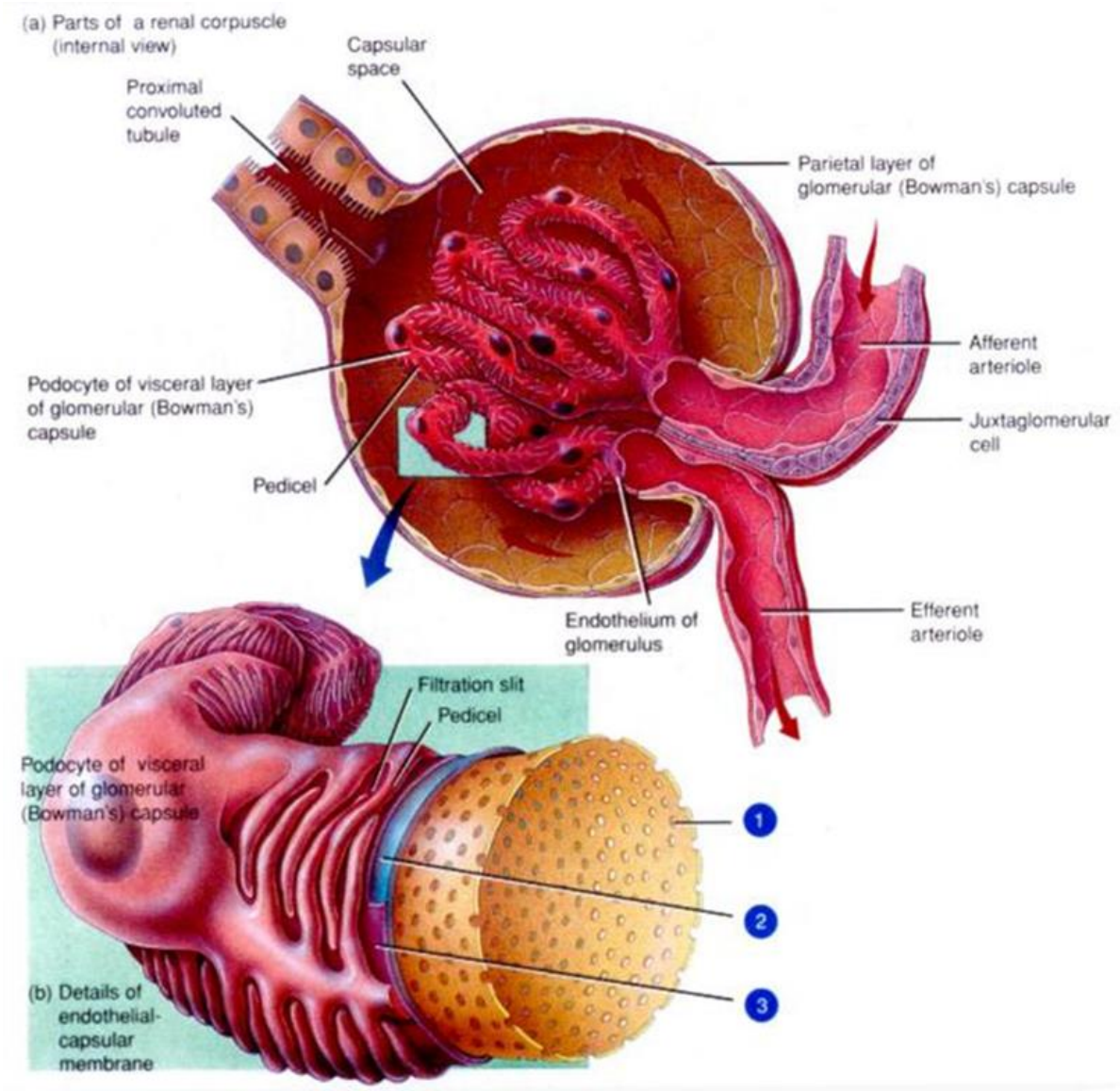
Properties of filtration membrane



- size-selectivity, i.e. limit of mol. weight of filtered substances
  - <7kDa freely
  - 7-70kDa concentration dependent
  - >70kDa not filtered
- charge-selectivity
  - negative charge (also for proteins <70kDa)

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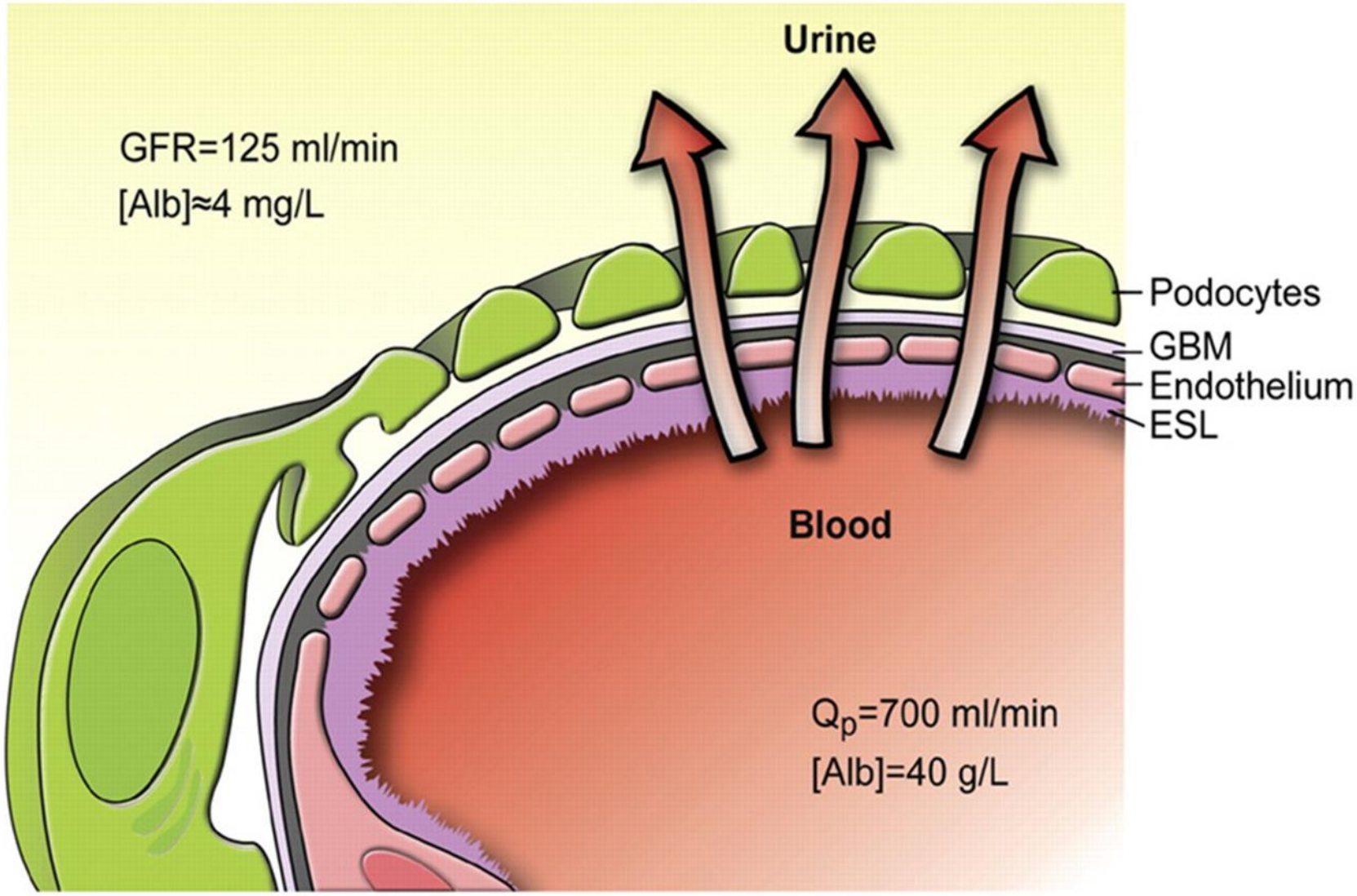
Glomerular filtration barrier



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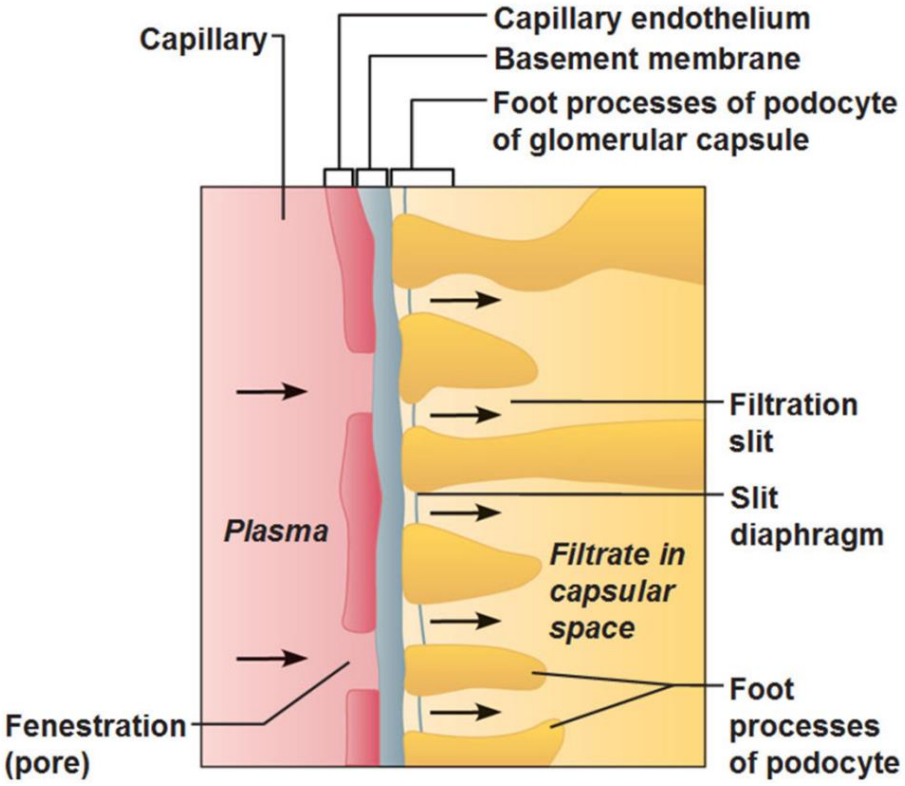
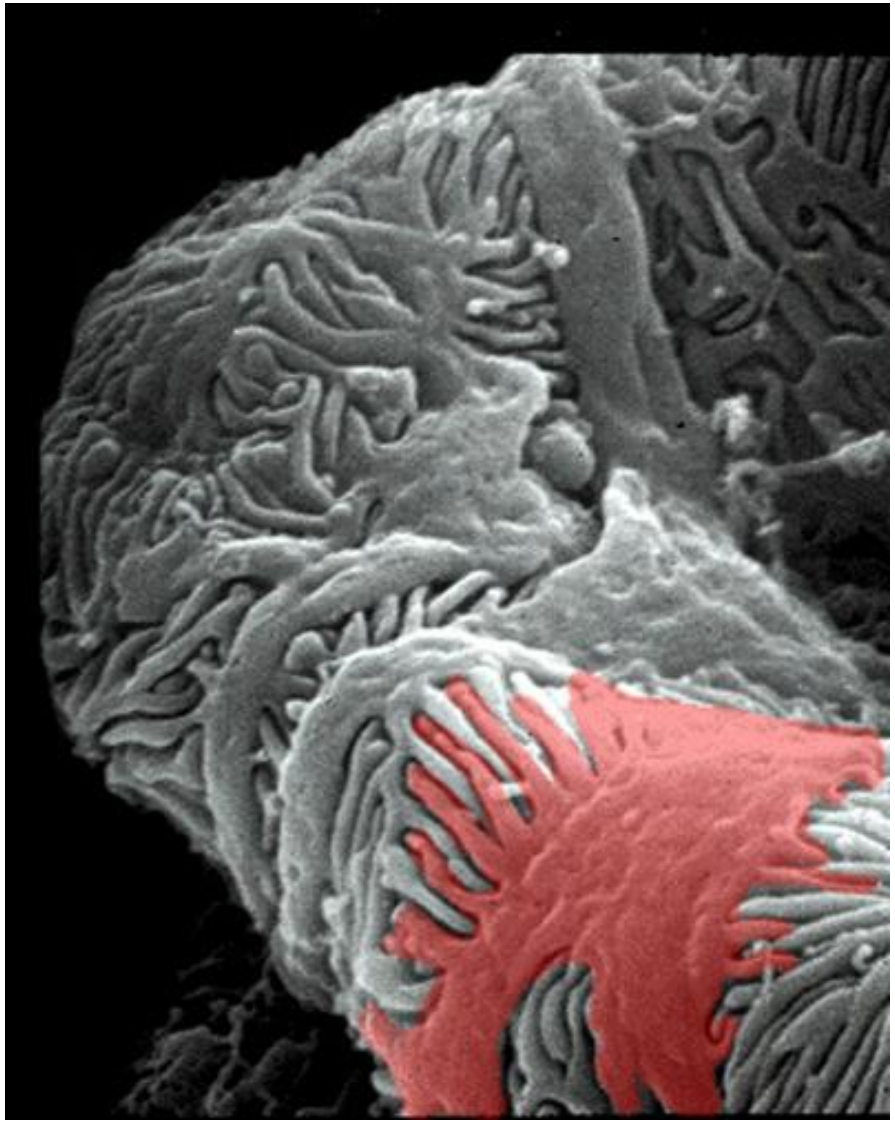
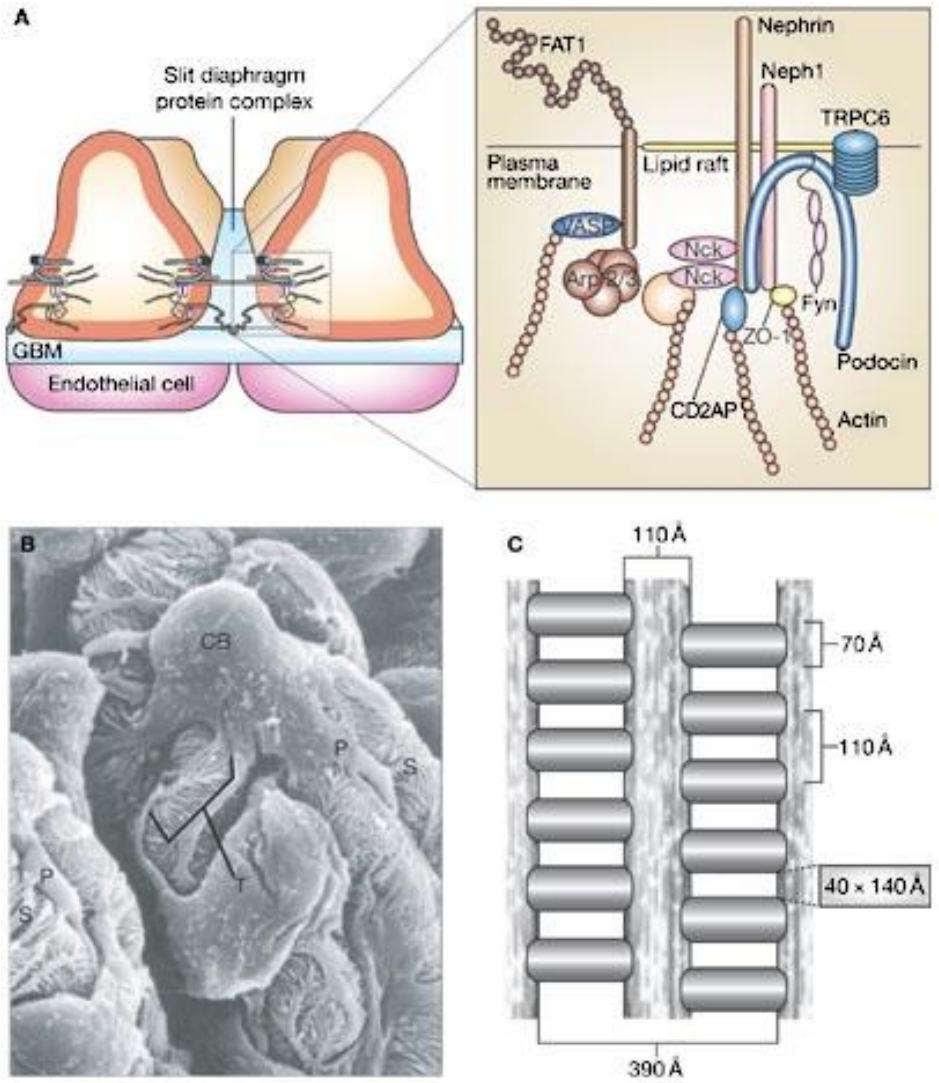
Structure of glom. filtration membrane

- (1) endothelium
  - **fenestrae** – filter ~50-100nm ∅
    - separation of blood cells
- (2) glom. basal membrane (GBM)
  - network of glycoproteins (collagen type IV, laminin, entactin, agrin, …) and mucopolysacharides ~300nm thick with summary **negative charge**
    - size-selective separation of majority of plasma proteins >70kDa (~ 4nm ∅)
      - hemoglobin (~ 40kDa) yes
      - myoglobin (~ 17kDa) yes
      - β2-mikroglobilin (12kDa) yes, but reabsorbed
      - paraproteins (<70kDa) yes
    - neg. charge – heparansulphate, hyaluronic and sialic acid
      - albumin (~ 67kDa) mostly no/in limited extent yes
- (3) visceral epithelium of Bowmann capsule = **podocytes**
  - primary, secondary and tertiary foot processes (pedicles)
  - slit diaphragm (cell-cell junction)
    - important contribution to size (as well as charge) separation of proteins
- (4) mesangium
  - indirectly affect filtration of proteins – mesangial cells contract and ↑ filtration pressure



Podocytes – slit diaphragm





Johnstone DB and Holzman LB (2006) Clinical impact of research on the podocyte slit diaphragm. *Nat Clin Pract Neprol* **2:** 271–282 doi:10.1038/ncpneph0180

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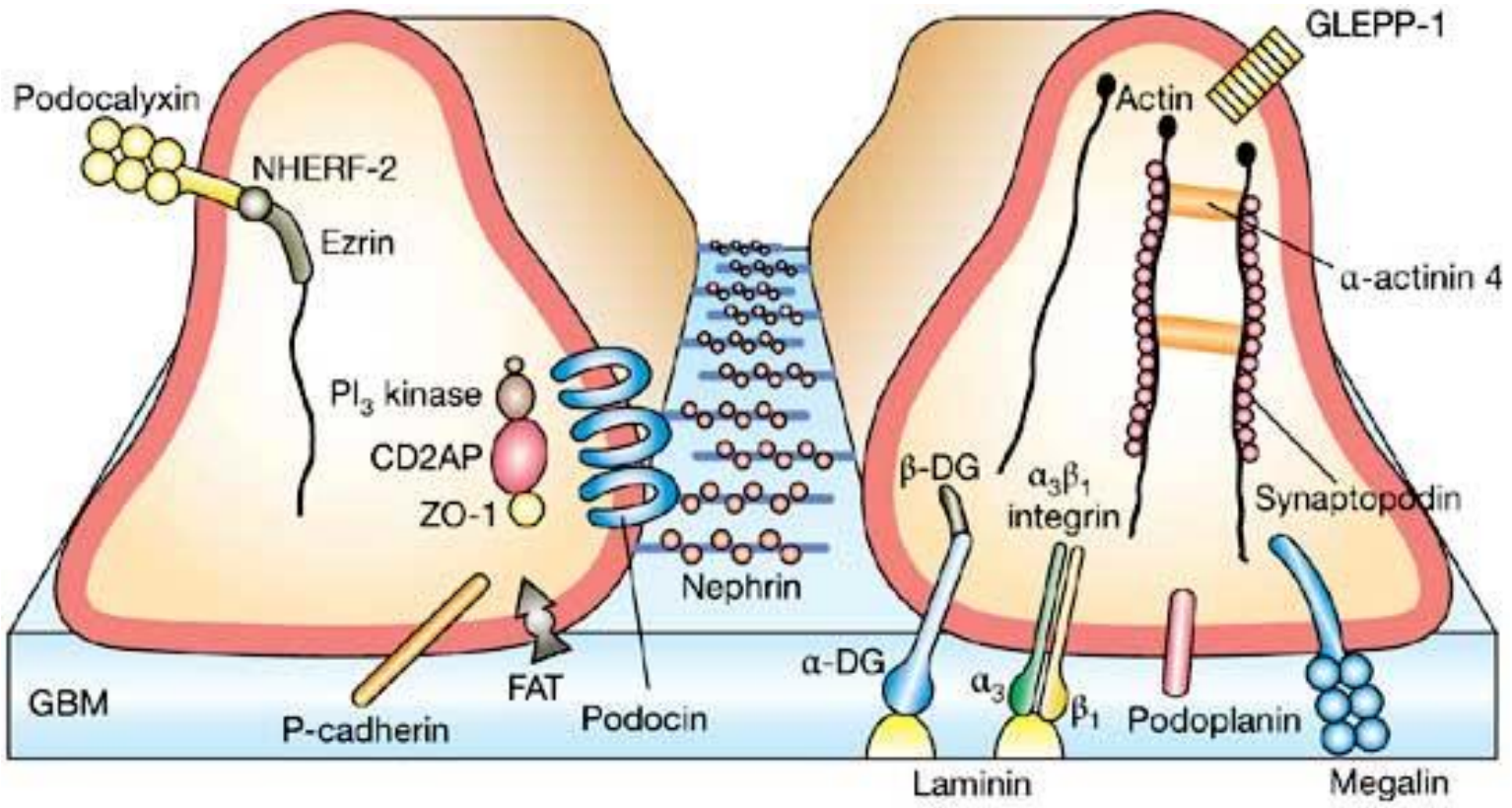
Proteins of slit diaphragm



- (1) basal domain – anchoring to GBM
  - integrins, DG = dystroglycan
- (2) cytoskeleton - shape
  - actin, myosin, synaptopodin, actinin
- (3) junction domain – slit diaphragm
  - nephrin, Neph1, podocin, CD2AP = CD2-associated protein, ZO-1 = zona occludens-1 protein, densin, FAT = mammalian homolog of Drosophila fat protocadherin
- (4) apical domain – neg. charge
  - podocalyxin, podoplanin, podoendin, GLEPP-1 = glomerular epithelial protein-1, other proteins and receptors (NHERF-2 = Na+/H+ exchanger regulatory factor-2, …)

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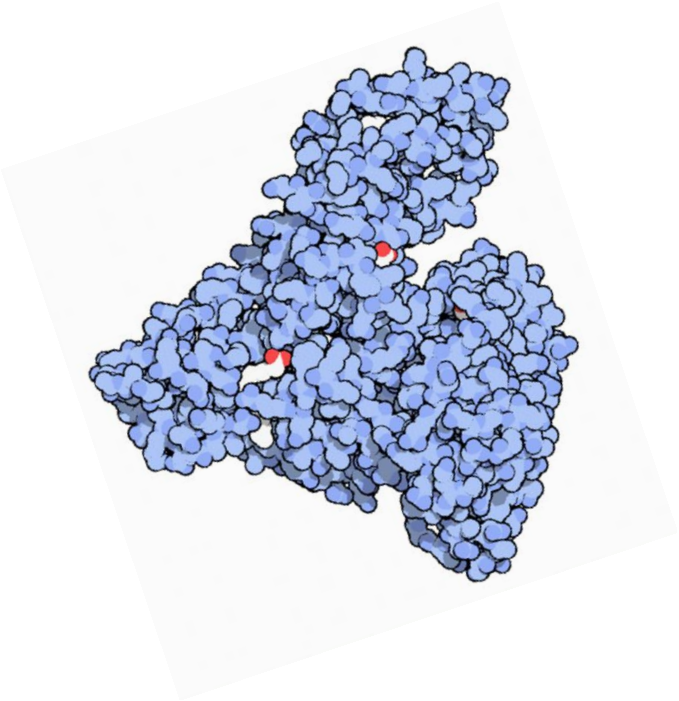
Proteins in urine (1) - normal

- physiological presence of proteins in urine
  - proteins produced by tubular cells
    - Tamm-Horsfall protein (= uromodulin)
      - glycoprotein produced by cells of asc. arm of LH
      - unknown function (immunomodulation, protection against crystala or infection?)
      - main component of hyaline casts
    - uropontin
    - IgA immunoglobulin
    - nephrocalcin
  - filtered but reabsorbed and degraded in tubule
    - **albumin** (see further details)
    - α2- a β2-microglobulins, enzymes, apoproteins, peptide hormones, …
- sensitivity of routine dg. methods ensures, that these proteins and albumin fragments are not detected
- only clinically significant proteinuria (>0.5 g protein) gives positive results

MUNI MED

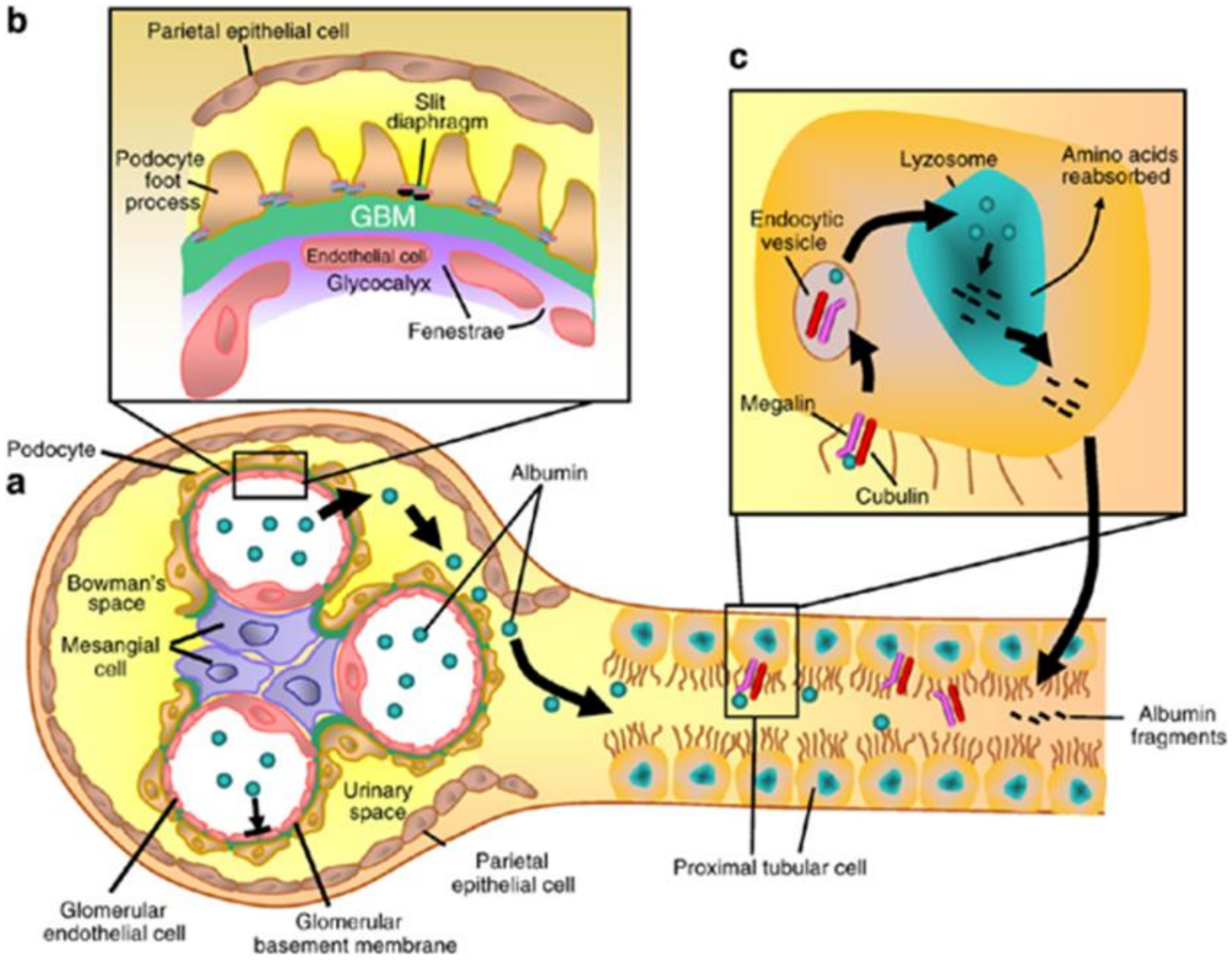
Human serum albumin (HSA) paradox

- HSA ~65kDa
- the molecule contains ~ 185 charged residues (Asp, Glu, Lys)
  - their surface distribution and overall charge is variabile due to multiple functions of albumin:
    - transport (FFA, bilirubin, Ca, Mg, hormones, drugs, vitamins, …)
    - buffer / AB balance
    - enzyme activity (antioxidant, esterase)
    - oncotic pressure
    - AA pool
- handling of albumin by kidneys
  - (1) limited filtration
    - electrostatic repulsion of albumin was not always exp. proved
    - this concept was dominantly base on the absence of albumin in urine
  - (2) tubular reabsorbtion
    - endocytosis = degradation ($\rightarrow$ AA and small fragments)
  - (3) tubular degradation



MUNI MED

Normal renal handling of albumin



(**a**) Albumin (represented by green spheres) normally remains within the capillaries of the glomerular tuft, and does not escape into the urinary (Bowman's) space. (**b**) Fenestrae within specialized endothelial cells are covered by a negatively charged glycocalyx. Podocytes attach to the outermost aspect of the GBM by foot processes, between which are proteins comprising the size barrier slit diaphragm. (**c**) The albumin that is physiologically filtered at the level of glomerulus into the urinary space is taken up by the megalin/cubulin receptor lining the brush border of proximal tubular cells. Albumin is internalized by vesicles, and upon lysozyme action, the resultant fragments are either reabsorbed or secreted back into the tubular lumen as albumin fragments

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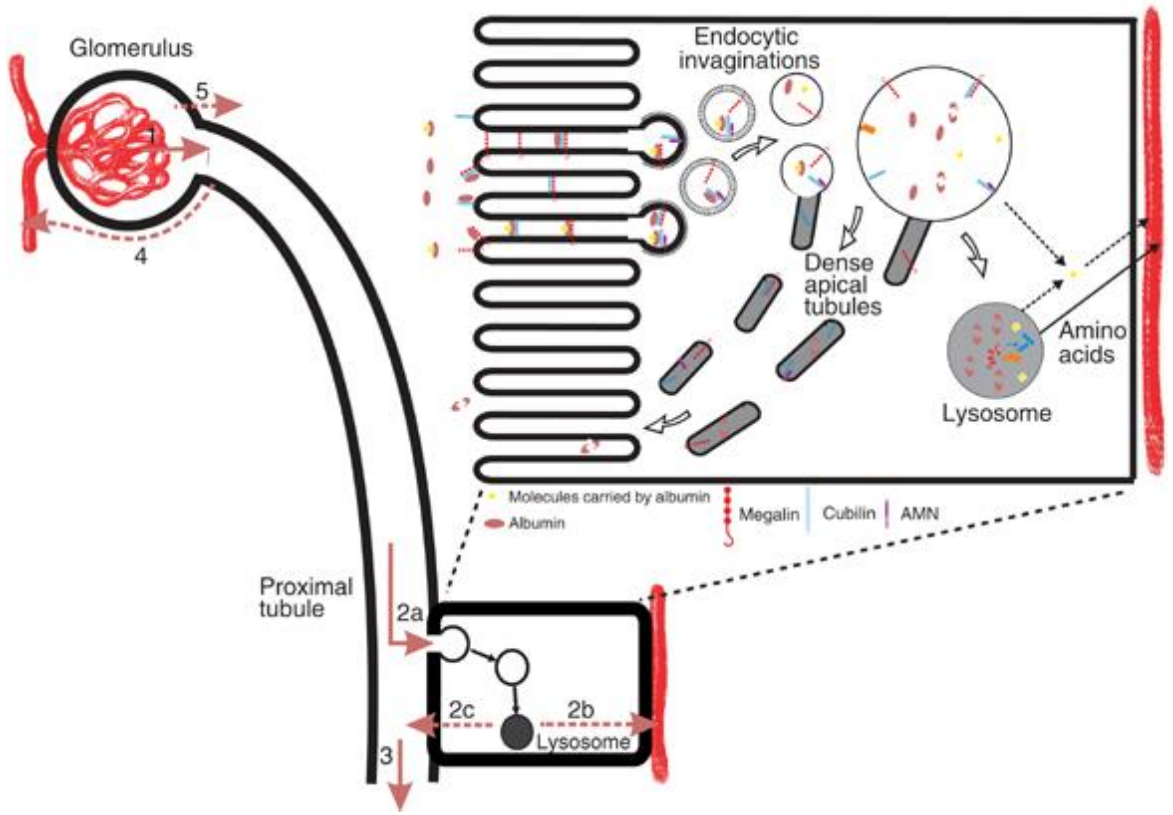
Mechanism of proximal tubular re-uptake of albumin

- receptor-mediated endocytosis
  - — high capacity/low affinity
    - the same mechanism is used elsewhere (e.g. absorption of complex of vit. B12/intrinsic factor in ileum)
  - — endocytic complex
    - megalin/cubilin – binding of albumin
      - — Imerslund-Graesbeck disease (mutation in cubilin gene) - **proteinuria**
      - — Fanconi syndrome (mutation in megalin genu) - **proteinuria**
    - NHE3 – necessary for acidification of endosome/lysosome
      - — NHE3 KO animals - **proteinuria**
    - ClC5 – interaction with cytoskeleton
      - — Dent's disease (mutation in ClC5 gene) - **proteinuria**
    - H-ATPase – necessary for acidification of endosome/lysosome



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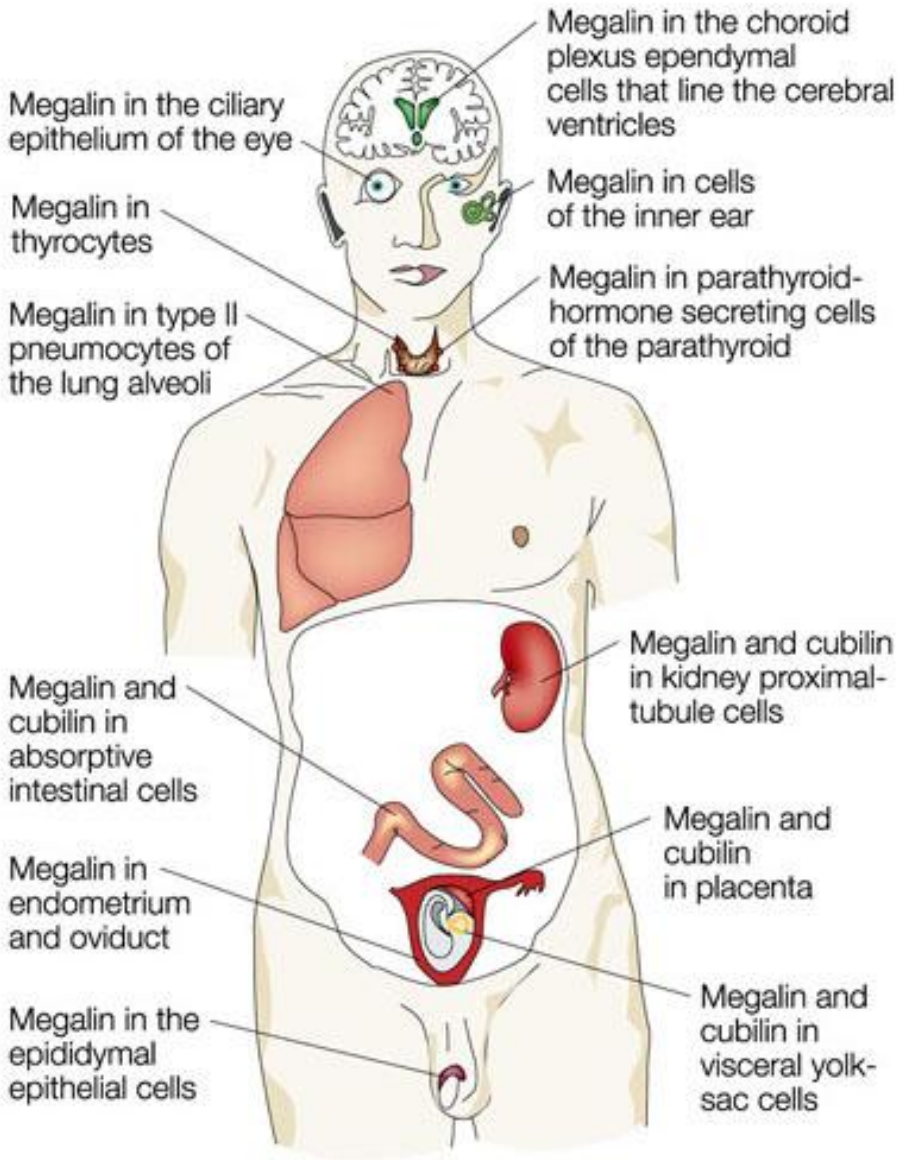
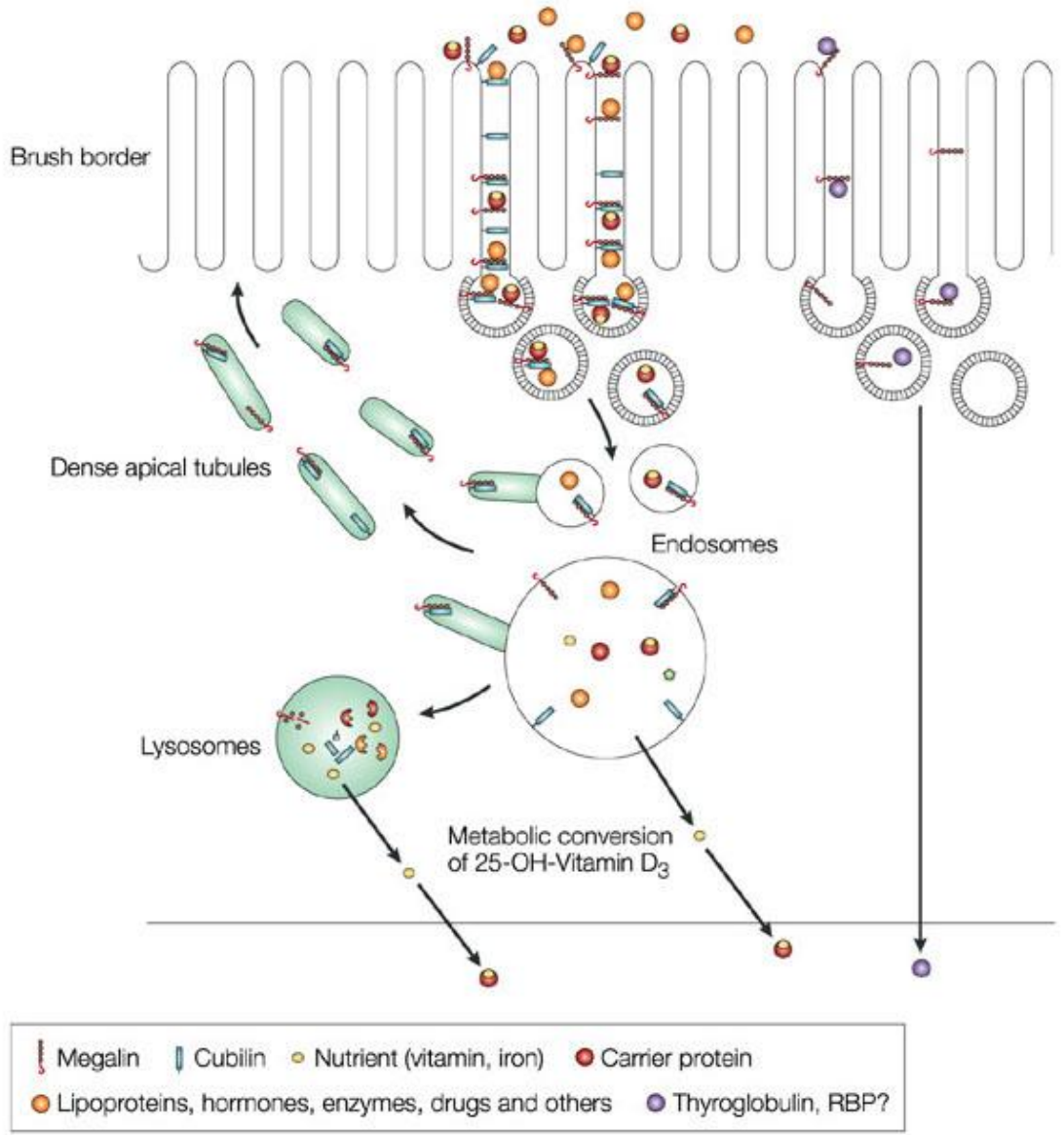
Megalin/cubilin





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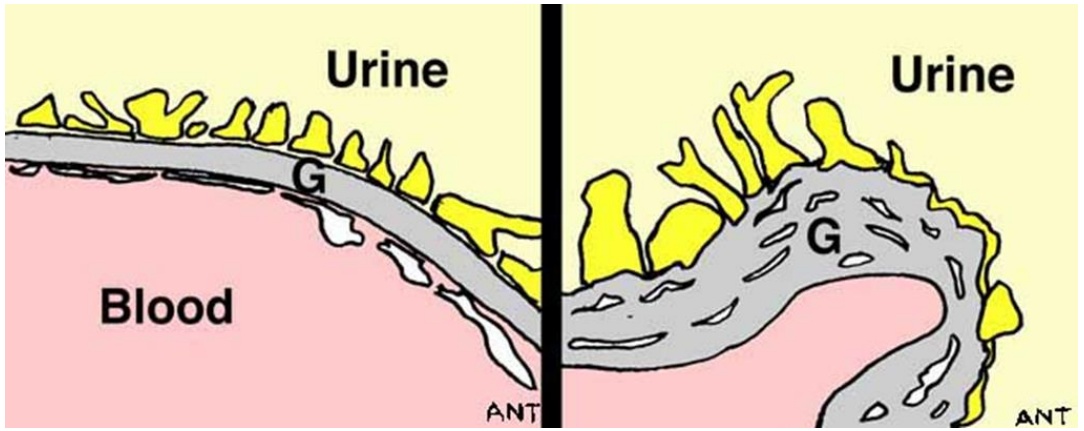
Proteins in urine (2) - proteinuria

- **(a) functional proteinuria**
  - appears occasionally as a result of altered glomerular hemodynamics (↑ hydrostat. pressure in capillaries), glomerular membrane intact
    - orthostatic, exercise, fever, …
    - non-selective proteinuria
- **(b) glomerular proteinuria** (often >1g/day)
  - pre-renal – pathological increase of "small" proteins capable of passing to glomerular filtrate
    - e.g. hemolysis (α-β-dimers globin), rhabdomyolysis (myoglobin), paraproteins (leight chains of Ig κ and λ (so called Bence-Jones protein)
  - selective - intact lamina densa (loss of glycocalyx from the surface of endothelia and podocytes)
    - albuminuria, larger proteins retained
  - non-selective – gross structural damage incl. lamina densa and podocytes
- **(c) tubular proteinuria** (often <1g/den)
  - decreased reabsorption of small plasma proteins (mainly albumin and β2-microglobulin in urine)
    - congenital (Dent's disease (ClC5), Imerslund-Graesbeck disease (cubilin), Fanconi syndrome (megalin) etc.)
    - acquired (e.g. hypertension)

Pathogenesis of glomerular proteinuria

- congenital
  - — disorders of GBM
    - e.g. Alport syndrome (mutation in collagen gene)
  - — disorders of podocytes (slit diaphragm)
    - mutations in genes for nephrin, podocin, …
- acquired – affect any part of glomerulus
  - — immune mechanisms - typically **glomerulonephritis**
    - circulating or in situ immune complexes (90 %)
      - — antigen: bacteria (β-hemolyt. Strepto-, Staphylo-, Pneumococci), parasites, viruses, endotoxin, cell organelles (in SLE), drugs, …
      - — it depends on the size whether immune complexes remain in circulation (→ vasculitis), will be scavenged by phagocytizing cells or become deposited (owing to large perfusion and fenestrated endothelia) in kidneys
    - antibodies against GBM, anti-neutrophil or against glomerular cells (10 %)
  - — non-immune – ischemia, hyperfiltration, toxins, infection, …
    - e.g. diabetes, hypertension, amyloidosis, HIV, ...



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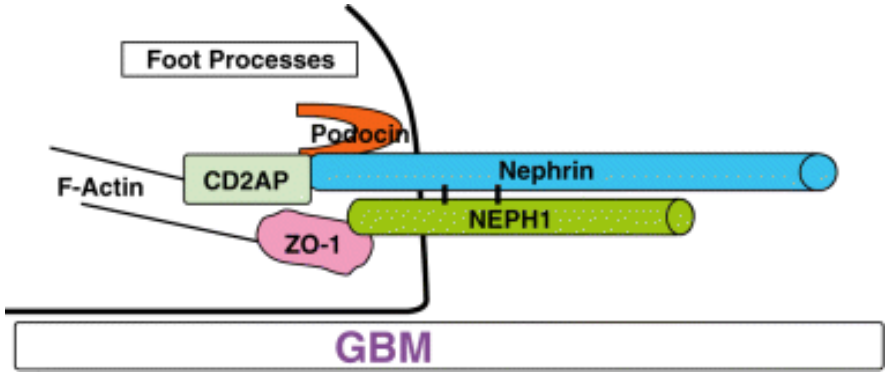
Importance of podocyte slit diaphragm

- (1) study of familiar forms of nephrotic syndrome led to the identification of majority proteins of slit diaphragm of podocytes
  - nephrin (Finnish-type congenital nephrotic syndrome, NPHS1)
    - congenital. defect of development of pedicles and slit diaphragm
    - massive and potentially lethal proteinuria starting during the fetal period
    - parenteral nutrition and peritoneal dialysis necessary till the transplantation
      - in comparison Alport syndrome (collage IV) leads to only moderate proteinuria
      - deletion of heparansulphate in mouse models do not lead to any proteinuria
  - podocin (familiar steroid-resistant nephrotic syndrome, NPHS2)
    - early postnatal proteinuria
  - other syndromes with significant proteinuria (CD2AP, NEPH1, FAT, TRPC6, …)



- (2) it is possible to induce nephrotic syndrome experimentally by polyclonal antibodies against slit diaphragm or by monoclonal ab. against nephrin, podocin, …
- (3) clinical importance
  - glomerulopathies are dominant cause of proteinuria
    - current classification of glomerulopathies based histopathologic appearance (= non-specific)
  - future (?} molecular-biologic classification
    - diagnostics, prognosis, treatment (steroids y/n), benefit of transplantation (family member/donor), …

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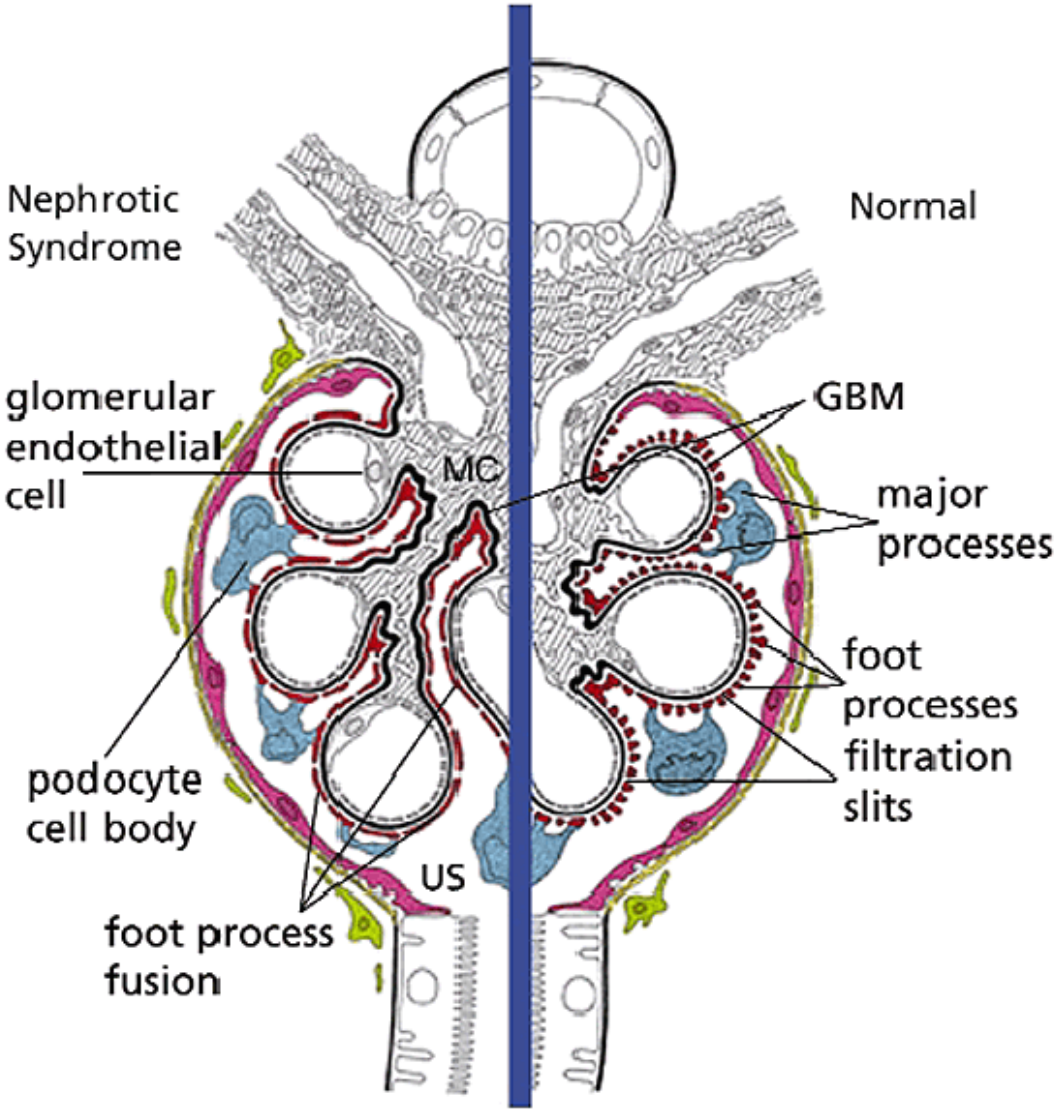
Podocytes - foot-process effacement



- = “smoothening” of podocytes - universal sign of damage to podocytes
- correlate with degree of proteinuria (“chicken or egg”?)
- variable etiology of podocyte damage
  - ROS (→ DNA damage, apoptosis, peroxidation of lipids)
  - AT II (→ apoptosis, hypertrophy, ↑ TGF-b, ↓ nephrin)
  - MMPs (→ ↓ GBM, ↓ nephrin-Neph complex)
  - mechanical stress (→ apoptosis, hypertrophy)
  - growth factors (→ ↑ MMPs, GBM, …)
  - hyperglycemia (→ ↓ neg. charged apical protein)
- **loss of podocytes ⇒ proteinuria ⇒ glomerulosclerosis**
  - synechia between naked GBM and parietal epithelium of Bowmann capsule → sclerotisation (FSGS)
  - **podocytes do not regenerate**

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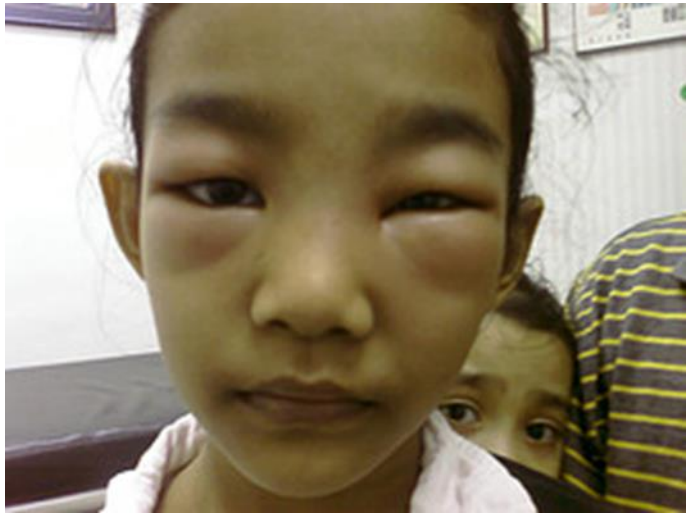
Consequences of glomerulopathies resp. proteinuria

- extra-renal
  - — hemodynamics
    - ↓ oncotic pressure (edema)
  - — composition of ECF
  - — dyslipidemia
  - — loss of substances bound to proteins
    - hypovitaminoses
  - — nutrition
- intra-renal
  - — albumin in small concentration necessary survival factor for tubular cells
  - — however, larger quantities of proteins in tubules lead to inflammation and interstitial sclerotisation
    - perpetuation of renal damage!!!



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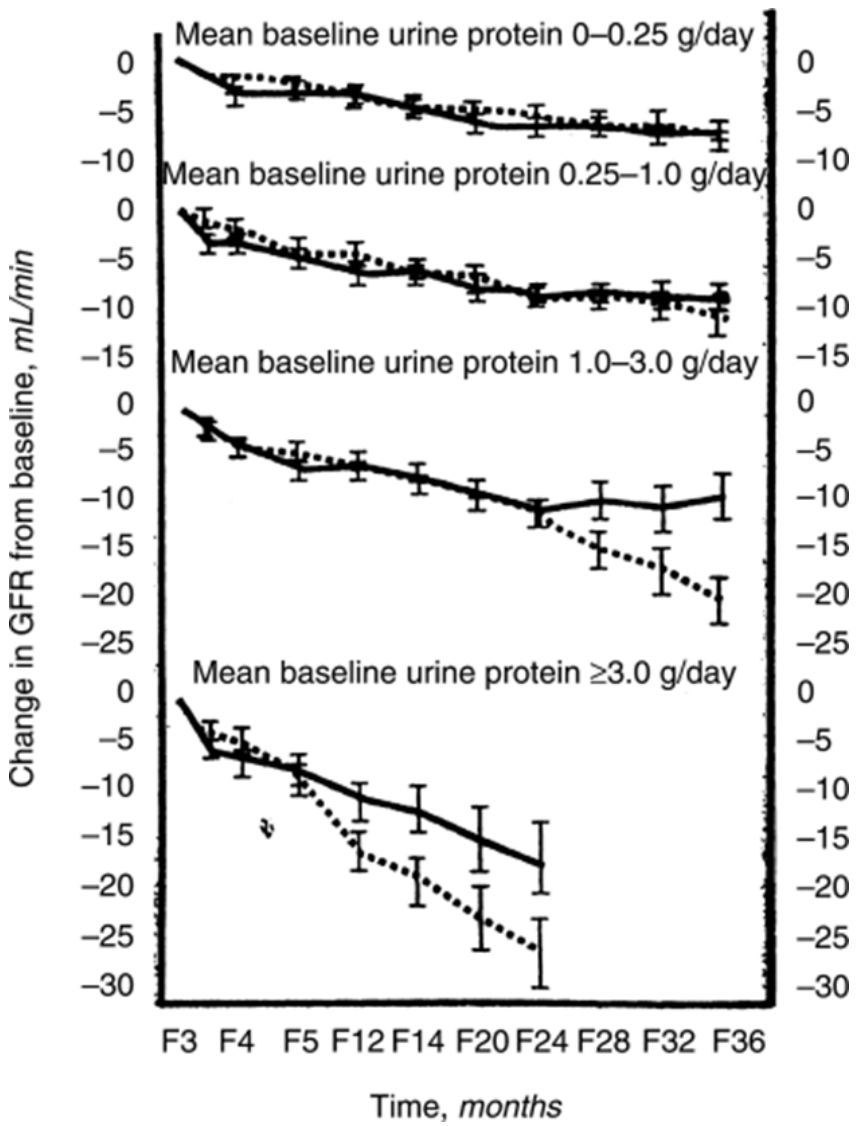
Mean decline in GFR depends on proteinuria



- mean decline in GFR (mL/min) over a 36-month period in groups with four different mean baseline 24-hour urine protein levels in non-diabetic patients with chronic renal failure in the MDRD study
  - — compared in each of these four groups are the
    - normal blood pressure group (dashed line; 140/90 mm Hg; 102-107 mm Hg MAP)
    - intensive control group (solid line; 125/75 mm Hg; 92 mm Hg MAP)

Progressive renal and cardiovascular disease: Optimal treatment strategies
Matthew R Weir

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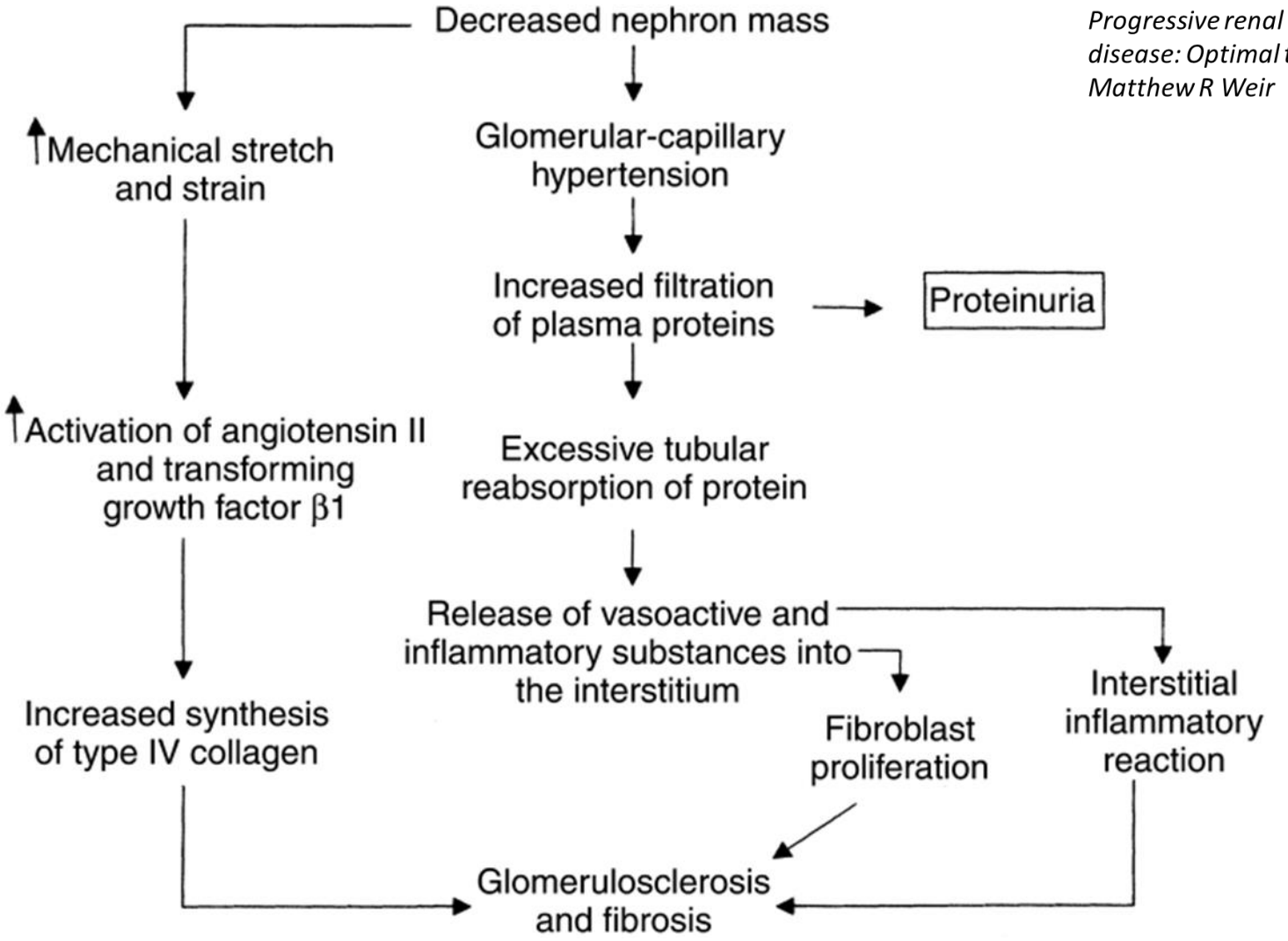
Proteinuria results in the development of glomerulo-sclerosis and fibrosis



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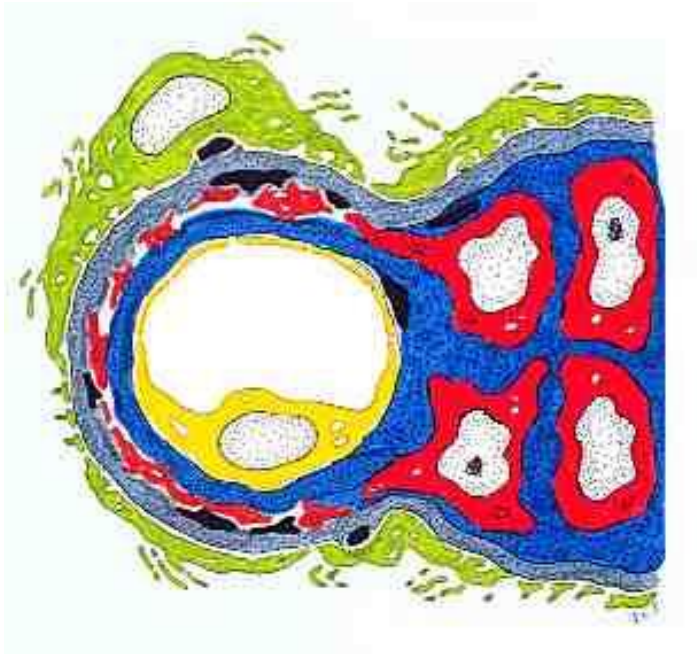

GLOMERULAR DISEASES (GLOMERULOPATHIES)

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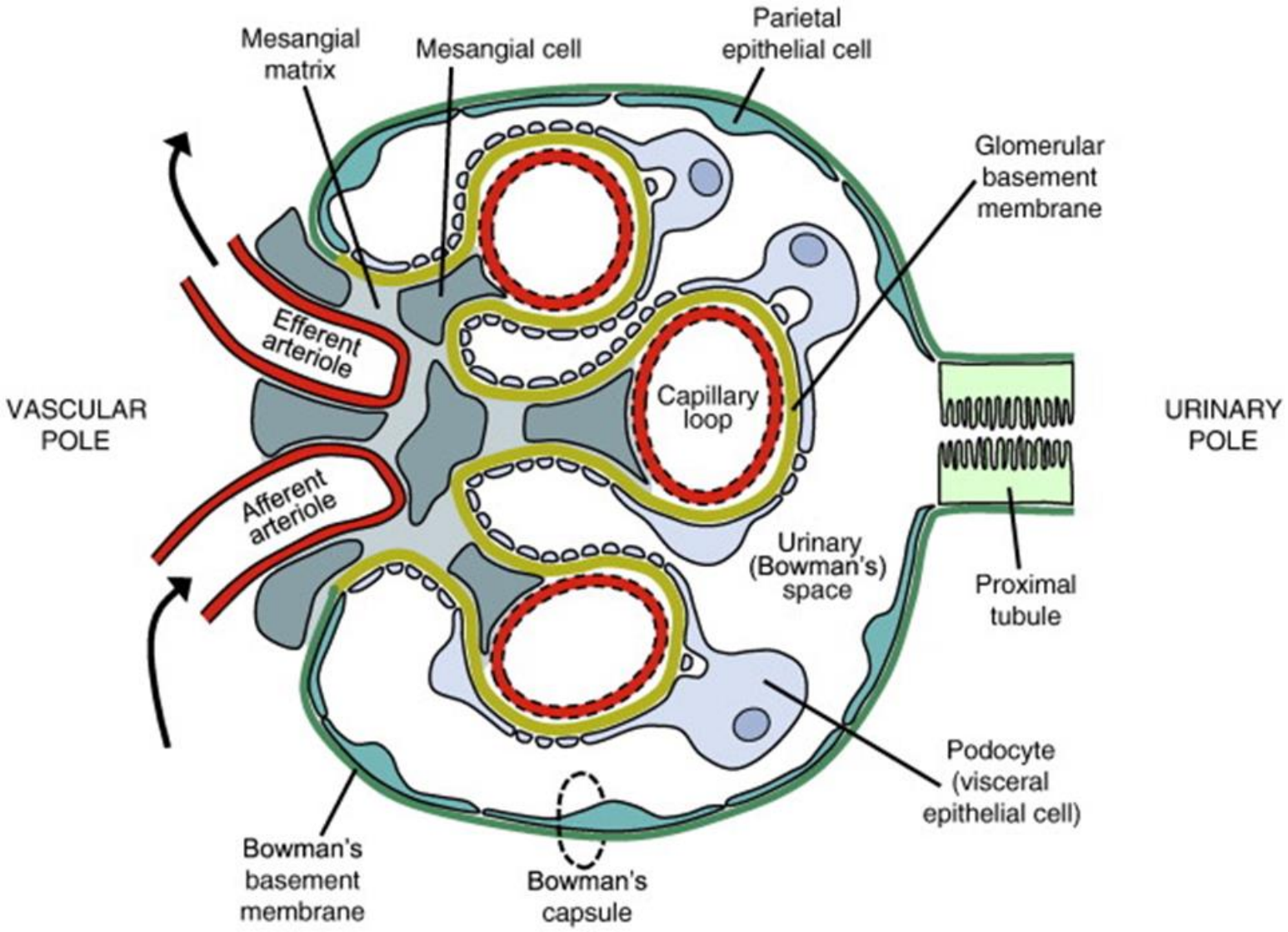
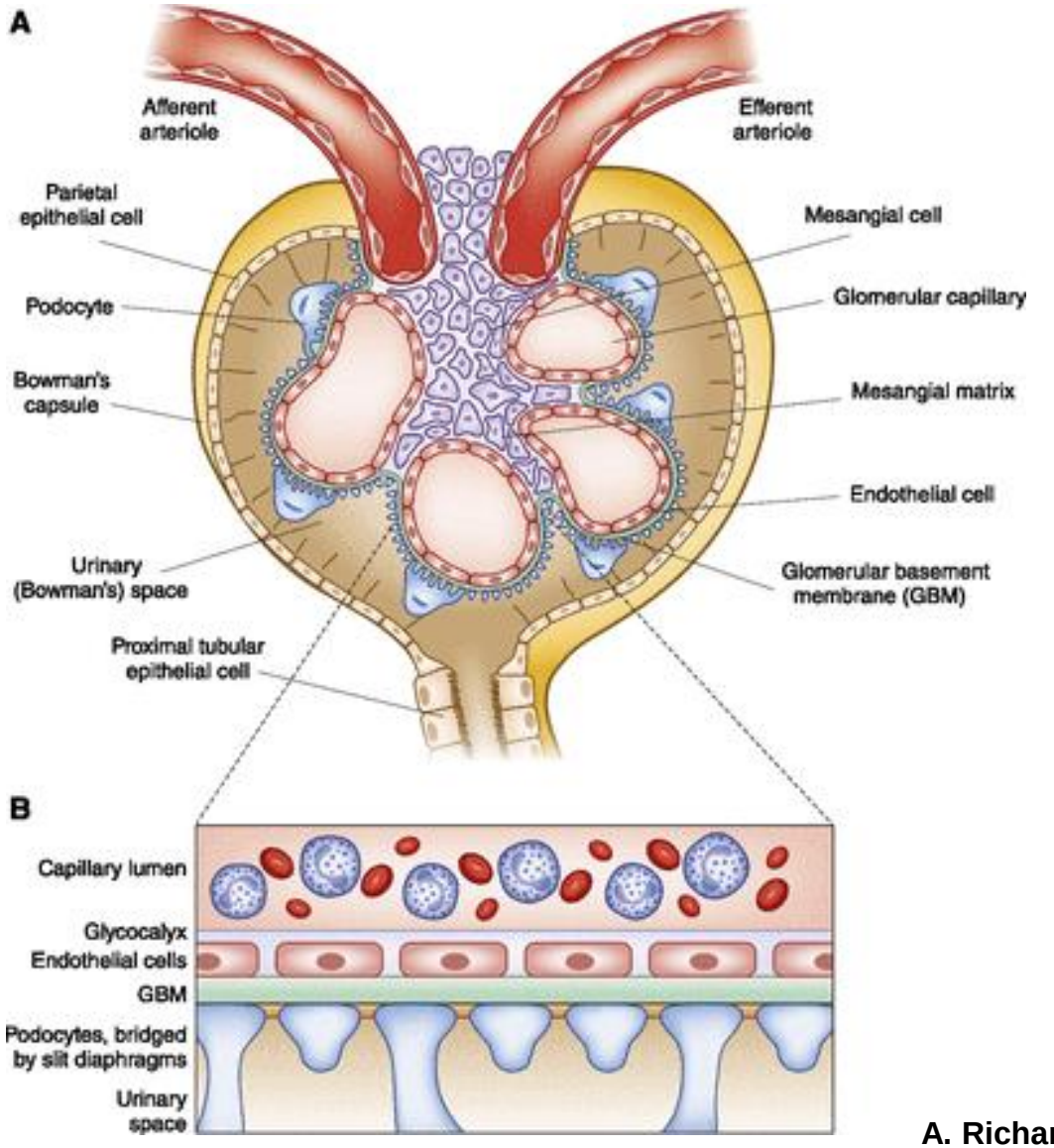
Glomerulus - cells



The players: cells Involved in glomerular disease



- Basic structure of the glomerulus and the glomerular filtration barrier. (A) Each glomerulus is composed of an afferent arteriole, which supplies the glomerular capillaries, and an efferent arteriole, into which they drain. Mesangial cells and mesangial matrix provide structural support for the glomerular capillaries, lined by specialized fenestrated endothelium, and then the glomerular basement membrane. On the urinary side of the glomerular basement membrane are podocytes, with foot processes that wrap around the glomerular capillaries. The urinary space is lined by a cup-like layer of parietal epithelial cells which adhere to the basement membrane of Bowman's capsule. (B) The glomerular filtration barrier is a specialized molecular sieve, with properties that aid filtration of small solutes from the blood to the urine, while limiting the passage of macromolecules such as albumin.

A. Richard Kitching, and Holly L. Hutton CJASN 2016;11:1664-1674

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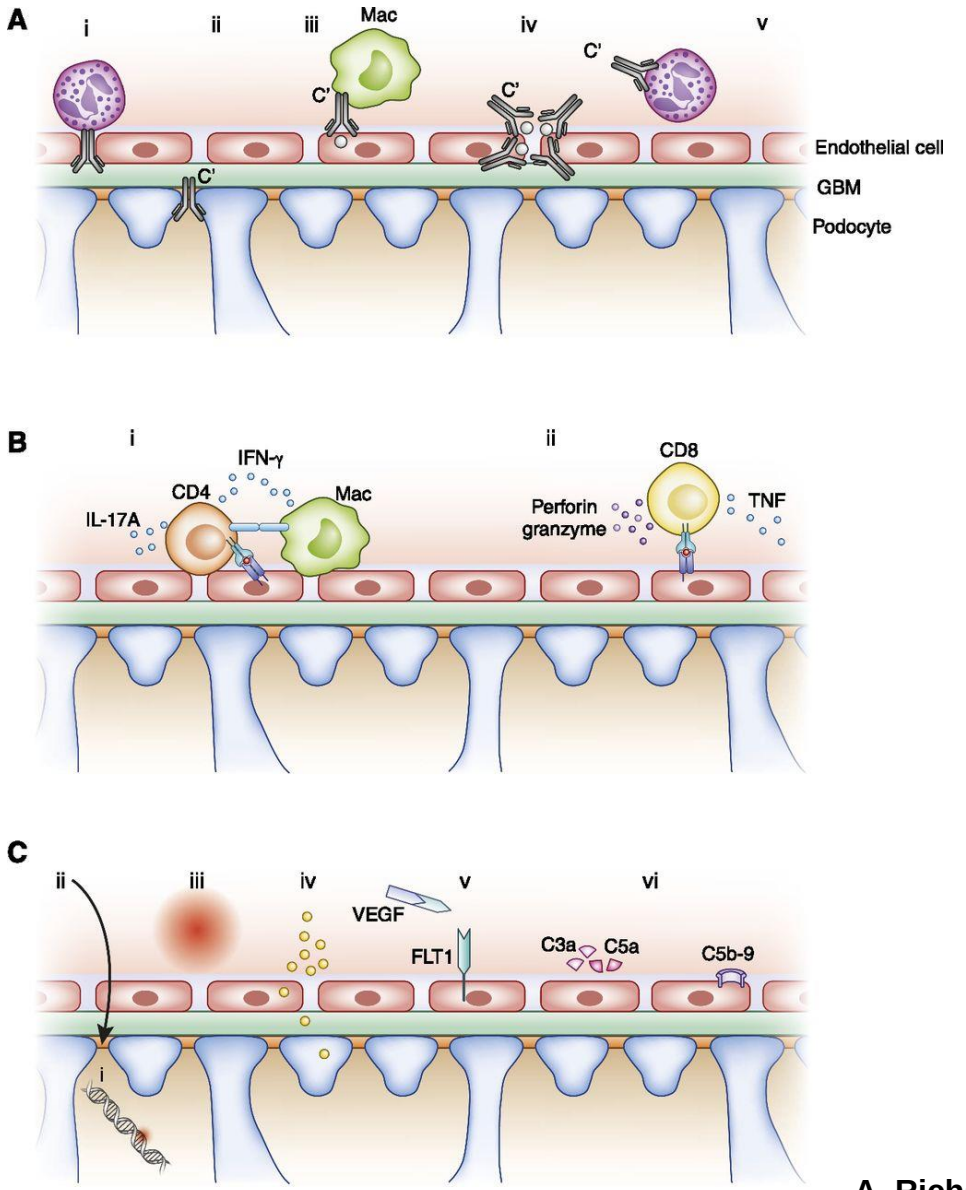
Simplified representation of mechanisms of glomerular injury



- (A) Antibody-mediated glomerular injury. From left to right, (i) neutrophils (shown) and macrophages induce injury after anti-α3(IV)NC1 autoantibodies bind to the GBM in anti-GBM GN; (ii) in membranous glomerulopathy autoantibodies against PLA2R1 (and other antigens) on podocytes are deposited subepithelially, with the involvement of complement; (iii) antibodies can bind to antigens lodged in the glomerulus (grey dots) with recruitment of macrophages (shown) and neutrophils, and the activation of complement; (iv) circulating immune complexes can be deposited in glomeruli, activate complement, and recruit leukocytes; (v) ANCA, (with complement) activates neutrophils and enables their recruitment to the glomerulus. Not shown, but important, is IgA deposition in mesangial areas.
- (B) Cell-mediated immune mechanisms. (i) Effector CD4+ cells (often Th1 or Th17 type) recognize antigens that can be intrinsic to or planted in the glomeruli. This occurs via their T cell receptor recognizing MHC class II peptide complexes (several cell types could possibly be involved in this process). Activated T cells produce cytokines (IL-17A and IFN-γ as examples) that have direct effects on intrinsic kidney cells and activate, together with costimulatory molecules (e.g., CD154/CD40), innate leukocytes such as macrophages. Not shown are interactions between intrinsic renal cells and T cells that include costimulation and cytokines. (ii) CD8+ cells can recognize antigenic peptides with MHC class I on intrinsic cells and secrete cytokines or induce cell death.
- (C) Metabolic, vascular, and other mechanisms of injury. Podocyte and foot process injury and dysfunction occurs due to (i) genetic abnormalities of slit diaphragm proteins and (ii) in minimal change disease and FSGS due to circulating permeability factors. Metabolic factors such as (iii) systemic and intraglomerular hypertension and (iv) hyperglycemia and its consequences are common, and affect both the cells and the structural components of the glomerulus. Both glomerular endothelial cell and podocyte injury are important consequences of preeclampsia, involved a number of mediators including soluble fms-like tyrosine kinase-1. C3 glomerulopathy, as well as some types of atypical hemolytic uremic syndrome (vi), can be induced by autoantibodies to, or genetic abnormalities in, complement regulatory proteins, resulting in complement activation. α3(IV)NC1, the non-collagenous domain of the α3 chain of type IV collagen;
- Abbreviations: FLT1, fms-like tyrosine kinase-1; GBM, glomerular basement membrane; Mac, macrophage; M-type PLA2R1, phospholipase A2 receptor 1; Th, T helper; VEGF, vascular endothelial growth factor.

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Classification of glomerular diseases

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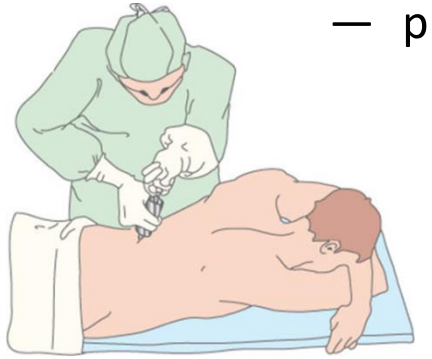
Classification of glomerular diseases

- the exact cause of the majority of glomerular disease unknown, therefore etiologic classification does not exist
- **clinically**
  - primary (affect only kidneys)
  - secondary (renal manifestation of systemic disease)
    - autoimmune (SLE, IgA nephropathy, m. Henoch-Schonlein, Goodpasteur syndrome etc.)
    - vascular (vasculitis)
    - metabolic (diabetes, amyloidosis)
    - tumors (multiple myeloma)
    - genetic
- but even this is ambiguous (immunopathologic process responsible for development of vast majority glomerulonephritis is predominantly systemic)
- according to **time course**
  - acute
  - chronic



- unless exact etiology is known GN are classified based on clinical-laboratory-histologic criteria
  - **kidney biopsy**
    - → light, immunofluorescent, electron microscopy
- **histologic** classification of GN dominantly focuses on the number of affected glomeruli and predominantly affected cell type
  - focal (only some glomeruli affected)
  - diffuse (mote than 80% of glomeruli)
  - segmental (only certain structures)
  - global (all cell types affected)
- and also degree of **cellularity**
  - non-proliferative
  - proliferative

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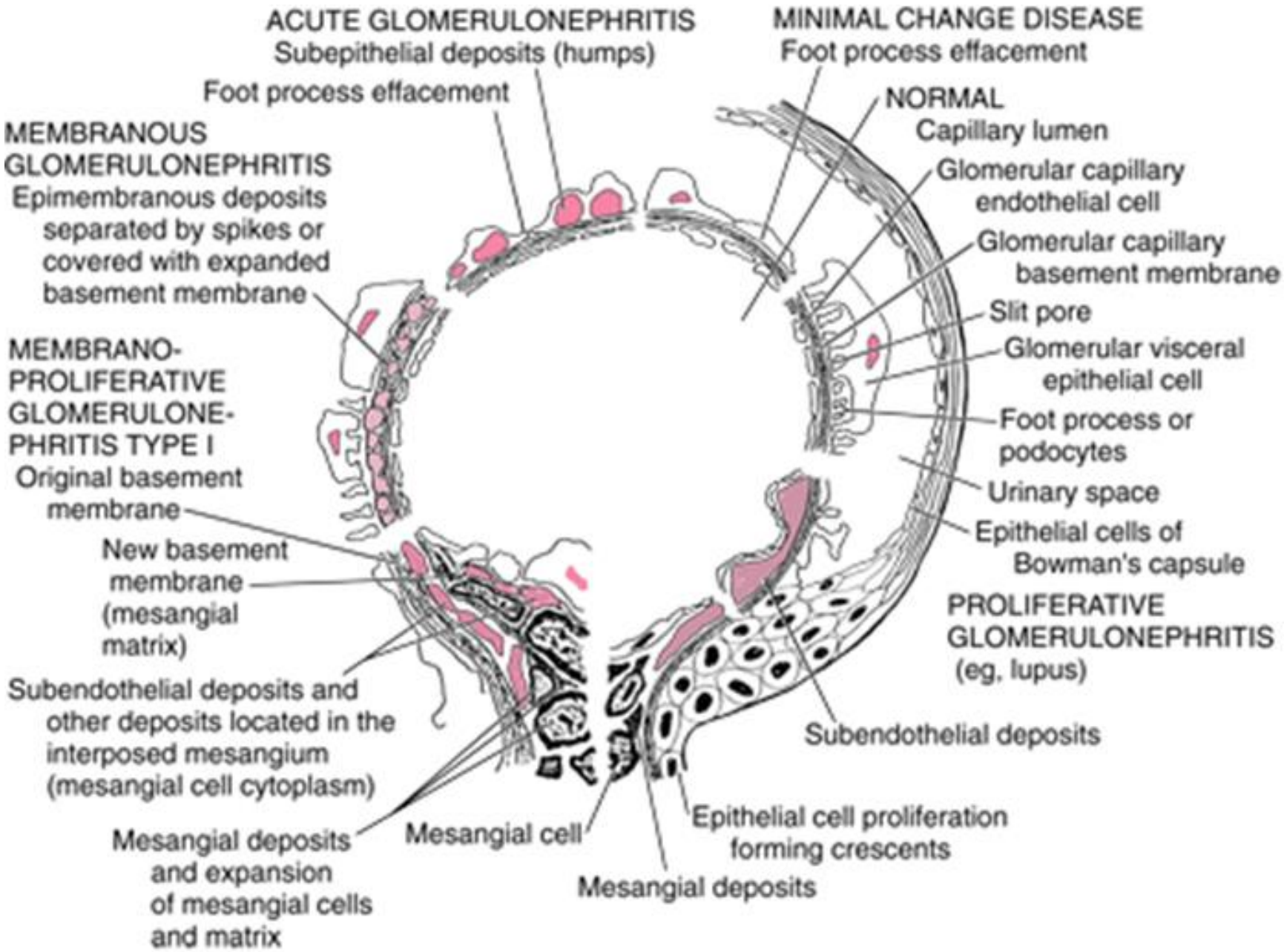
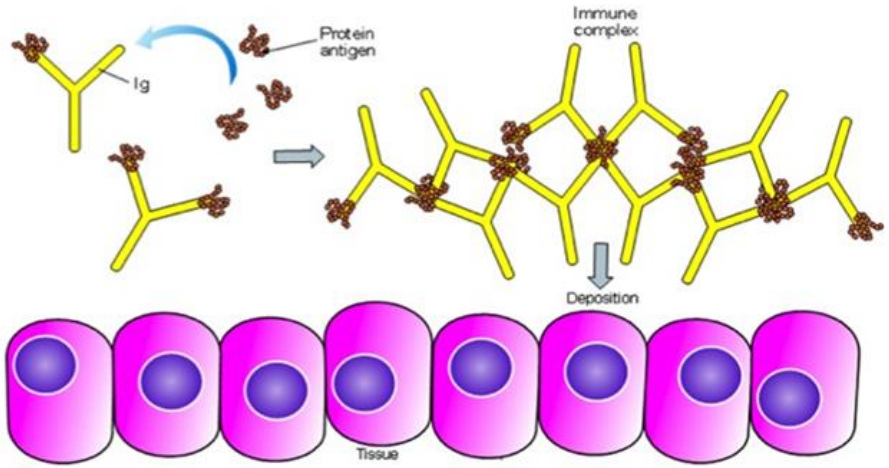
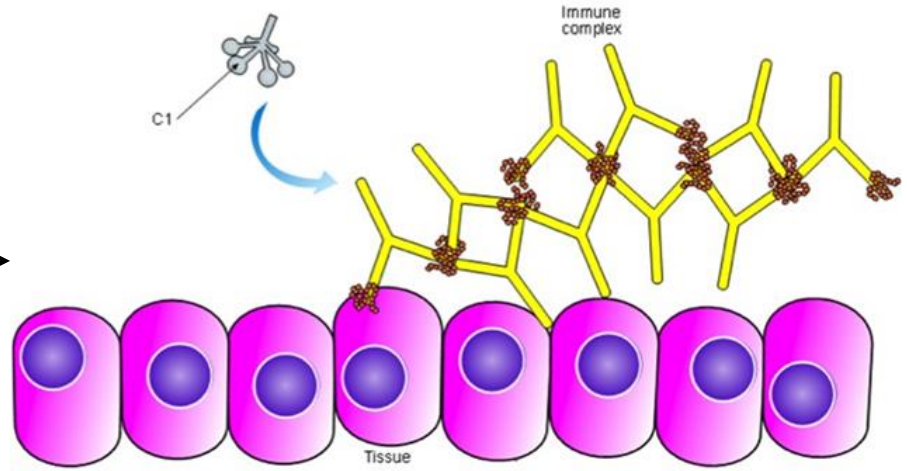
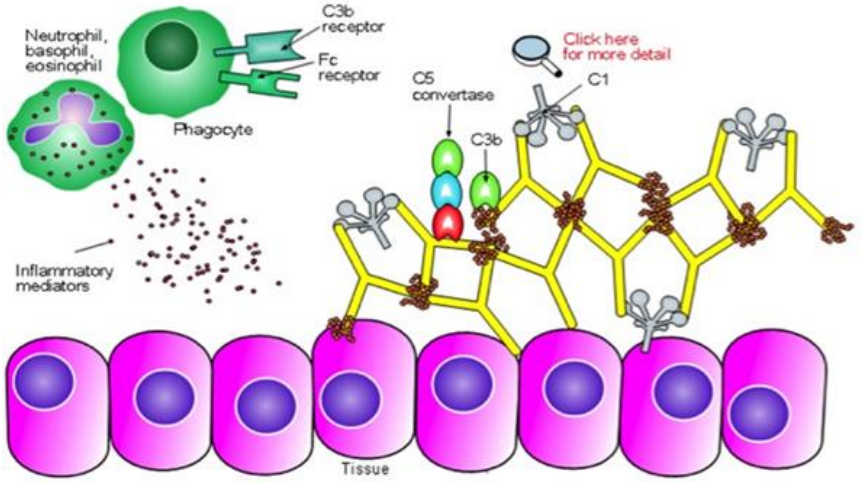

ACUTE GLOMERULONEPHRITIS
Subepithelial deposits (humps)
Foot process effacement
MINIMAL CHANGE DISEASE
Foot process effacement
NORMAL
Capillary lumen
Glomerular capillary endothelial cell
Glomerular capillary basement membrane
Slit pore
Glomerular visceral epithelial cell
Foot process or podocytes
Urinary space
Epithelial cells of Bowman's capsule
MEMBRANOUS GLOMERULONEPHRITIS
Epimembranous deposits separated by spikes or covered with expanded basement membrane
MEMBRANO-PROLIFERATIVE GLOMERULONE-PHRITIS TYPE I
Original basement membrane
New basement membrane (mesangial matrix)
Subendothelial deposits and other deposits located in the interposed mesangium (mesangial cell cytoplasm)
Mesangial deposits and expansion of mesangial cells and matrix
Mesangial cell
Mesangial deposits
PROLIFERATIVE GLOMERULONEPHRITIS
(eg, lupus)
Subendothelial deposits
Epithelial cell proliferation forming crescents

General pathogenesis of acute GN





- formation of **immune complexes**
  - in circulation or in situ
- **complement activation**
  - formation of opsonins, chemotaxis, glomerular **cell lysis**
- **inflammatory infiltration** by neutrophils and macrophages
  - **protease degradation** of cells and ECM
- healing
  - growth factors – **cell proliferation**
  - connective tissue (mesangium) – **fibrosis and sclerosis**



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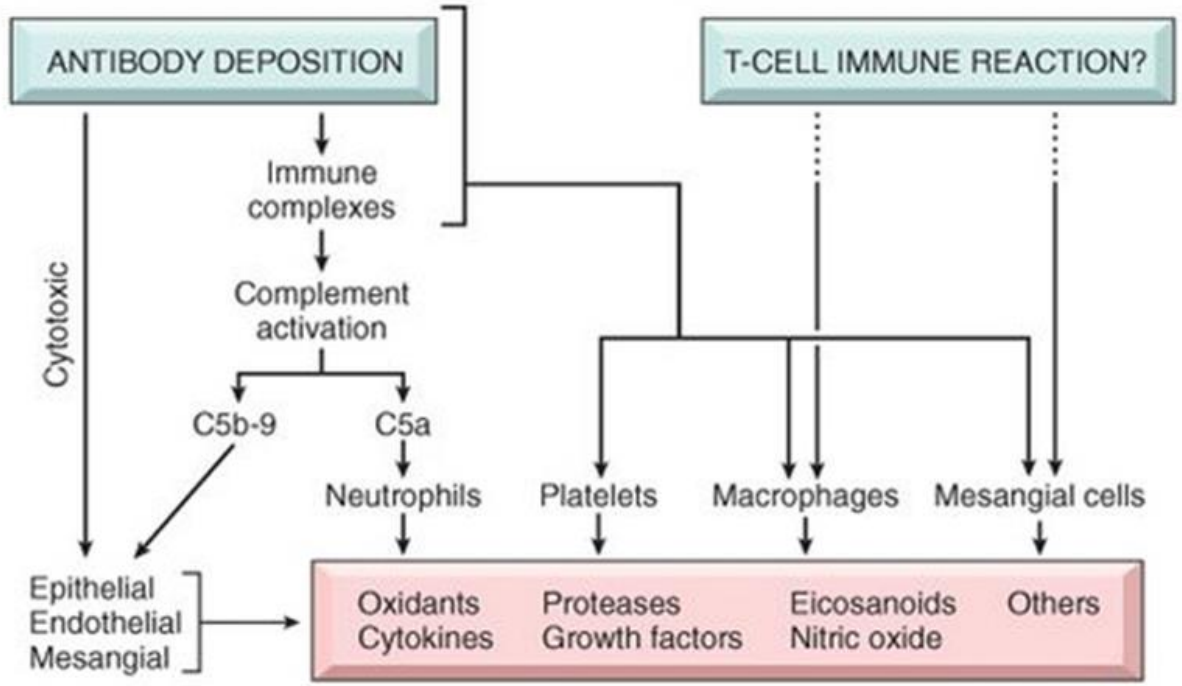
General pathogenesis of GN

- glomerulus has only limited spectrum of reactions to damage
  - — inflammation
    - proliferation of glomerular cells
    - infiltration by other cells
    - proteolytic destruction of filtration membrane
      - — **proteinuria**
  - — deposition of ECM
    - fibrosis
    - scarring
    - sclerosis
      - — **decrease of GFR**
  - — histologic appearance depends on which particular part of glomerulus is affected
    - proliferative GN – mesangium
    - membranous GN – GBM thickening
    - sclerotising GN – capillaries and synechia



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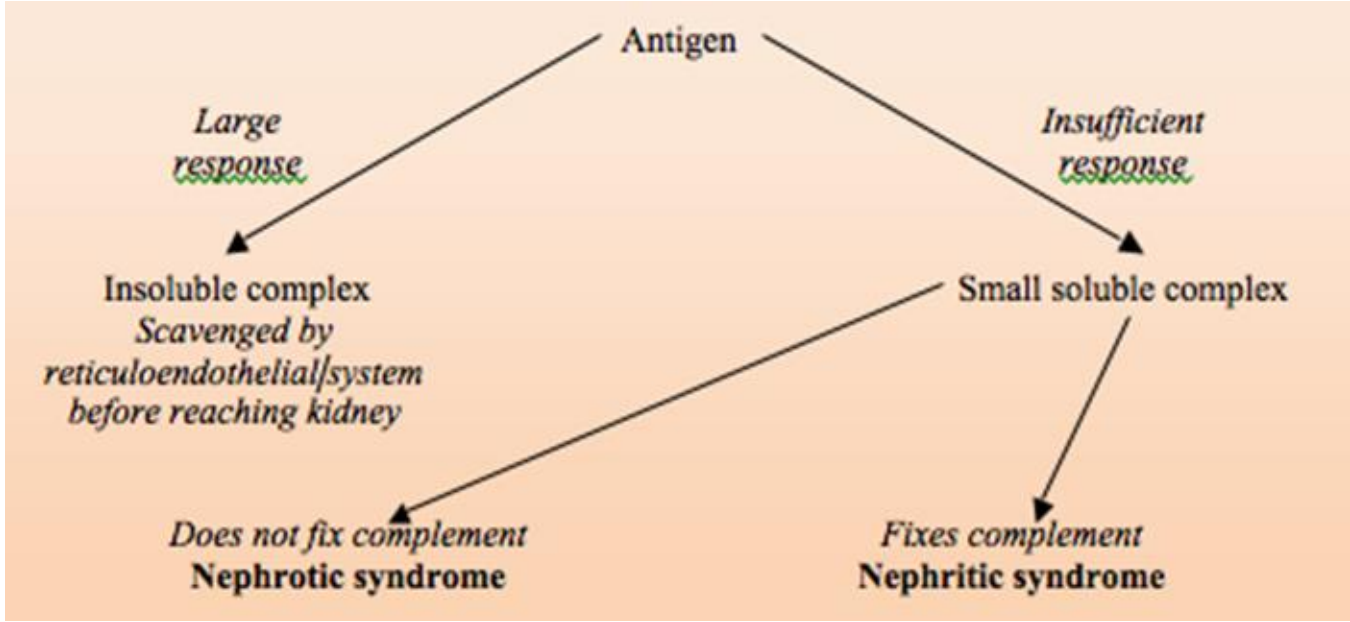
Clinical picture: Nephritic vs. nephrotic syndrome



- Both syndromes are caused by the formation of soluble complexes of antigens after an insufficient clearing from the immune system
  - **nephrotic syndrome** – no activation of complement
    - soluble antigen/antibody complexes are deposited within the slit pores (between opposing podocyte foot processes) or within the mesangial artery
  - **nephritic syndrome** - activation of complement
    - soluble antigen/antibody complexes are deposited in sub-endothelial space

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Nephrotic vs. nephritic syndrome

- nephritic syndrome
  - gross damage to filtration barrier (involving endothelium and GBM) = **oliguria**
    - activation of complement
  - inflammation causes blockade of filtration and **hypertension** due to hypervolemia
  - leak of red blood cells = glomerular **hematuria**
    - diff. dg. post-renal!
    - macroscopic (visible by naked eye)
    - microscopic (urine analysis)
  - **mild proteinuria** in comparison with nephrotic syndrome
    - only as much protein as in the given volume of blood leaking into urine

- nephrotic syndrome
  - damage to filtration barrier (involving mainly podocytes)
    - no activation of complement
  - no blockade to filtration therefore no hypertension
  - **large proteinuria** dominates
    - typically >3g/day
  - as a result of hypoalbuminamia / hyporoteinemia
    - oncotic pressure decrease → **edema**
    - infections
    - hypocalcemia, hypovitaminosis, …
  - protein loss becomes compensated by liver, the non-selectivity of this globally increased protein synthesis explains
    - **dyslipidemia**
      - ↑ lipoproteins, ↓ plasma LPL
    - hypercoagulation (risk of thrombosis)

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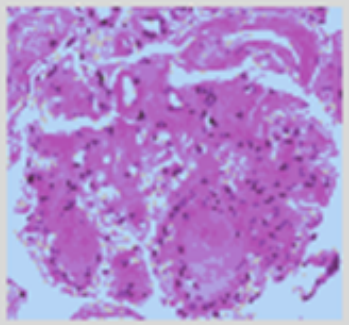
The spectrum of glomerular diseases



Nephrotic

Mechanism

- Injury to podocytes
- Changed architecture:
  - Scarring
  - Deposition of matrix or other elements

SLE

IgA nephropathy

Minimal change nephropathy

FSGS

Membranous nephropathy

Diabetic nephropathy

Amyloidosis

MCGN

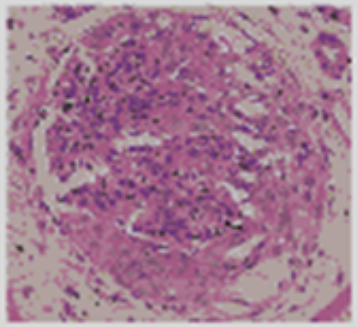
Post-streptococcal glomerulonephritis

Small vessel vasculitis

Anti-GBM disease

Haematuria

Proteinuria

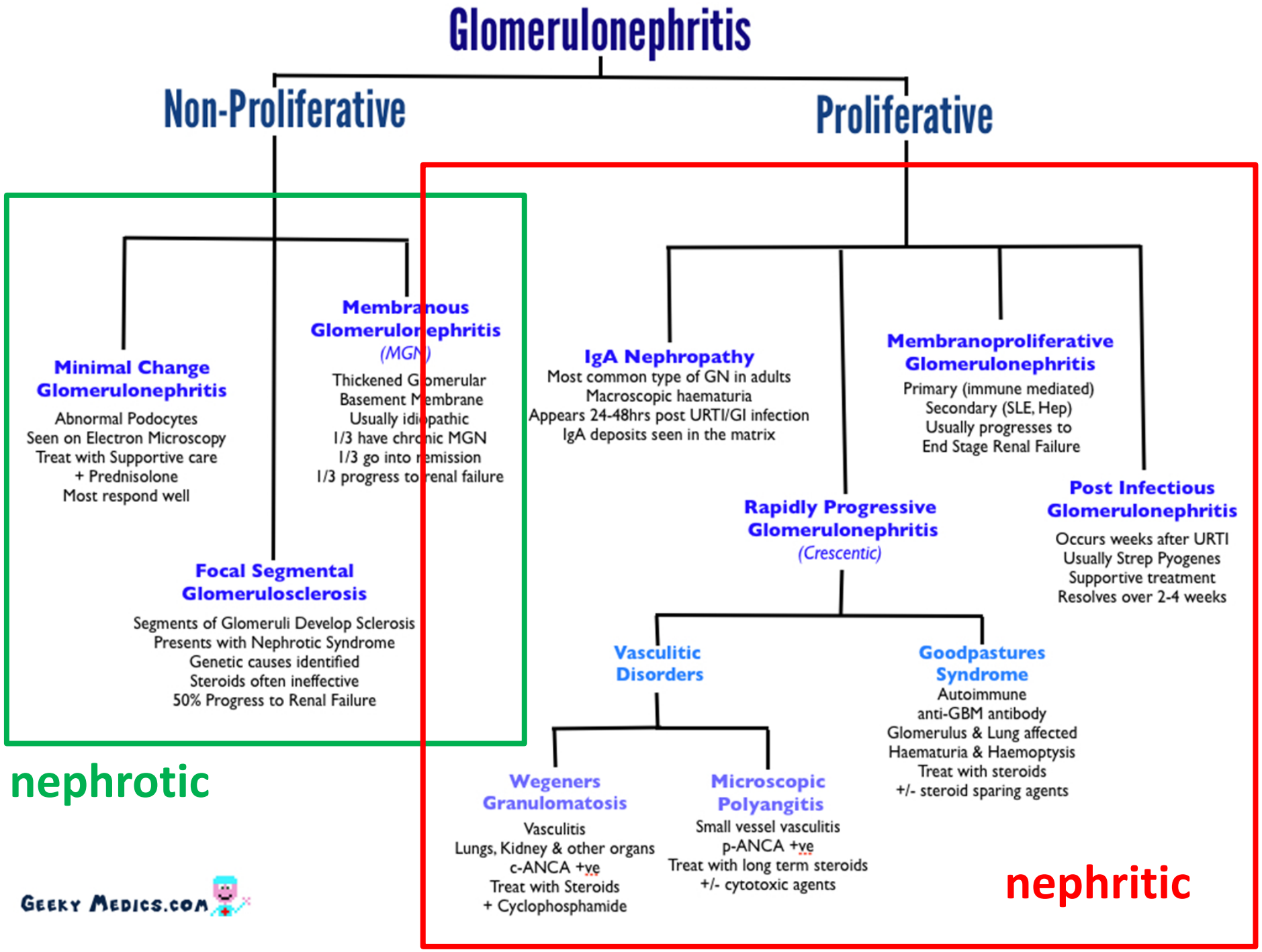


Nephritic

Mechanism

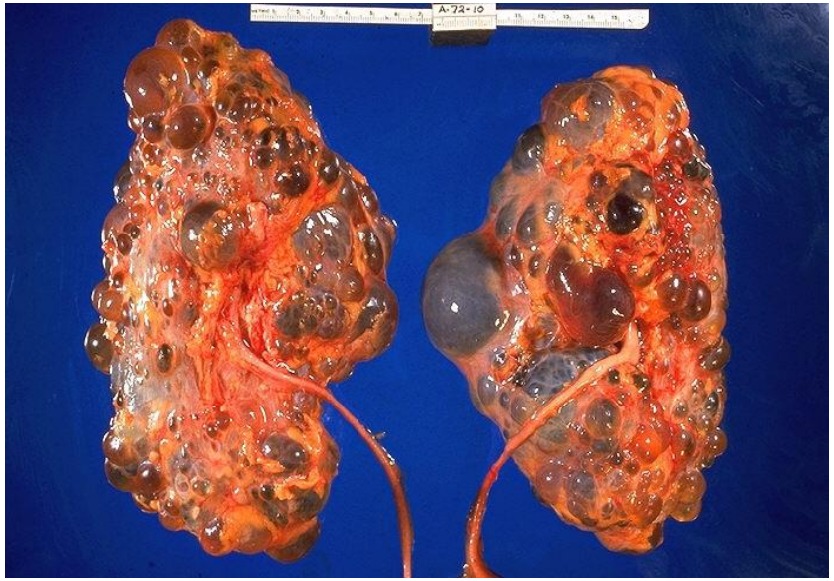
- Inflammation
- Reactive cell proliferation
- Breaks in GBM
- Crescent formation

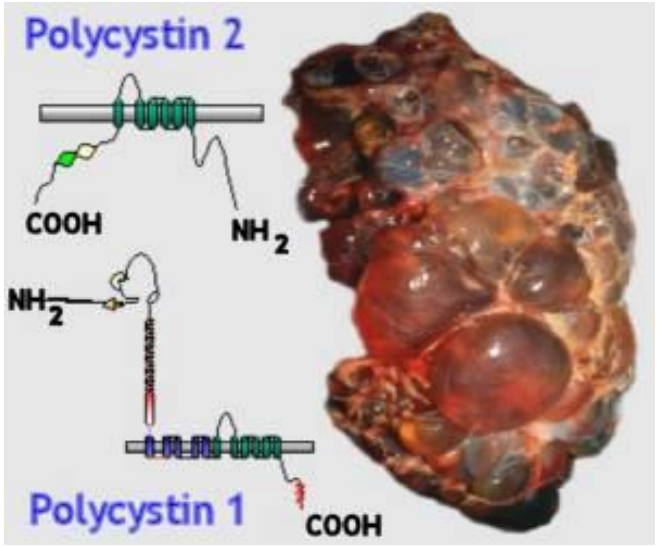
www.edren.org

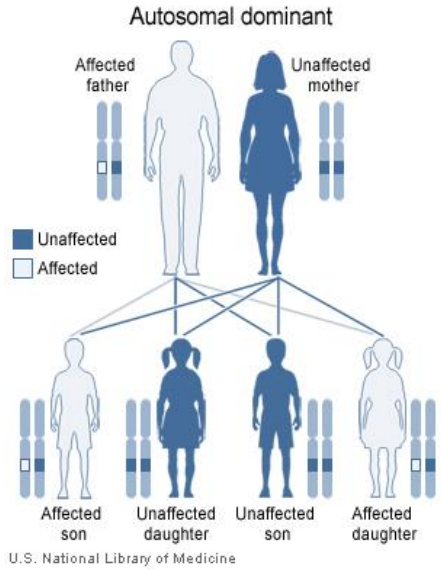


Rare kidney diseases

- polycystická choroba ledvin (PKD)
  - autosomálně dominantní (ADPKD)
    - mutace v genu PKD1 (ch. 16)v 85%, v 15% případů PKD2
    - progresivně se rozvíjející mnohočetné cysty oboustranně v ledvinách
    - hypertenze
    - renální insuficience
    - ~50% případů ESRD
  - recesivní forma vzácná, závažnější







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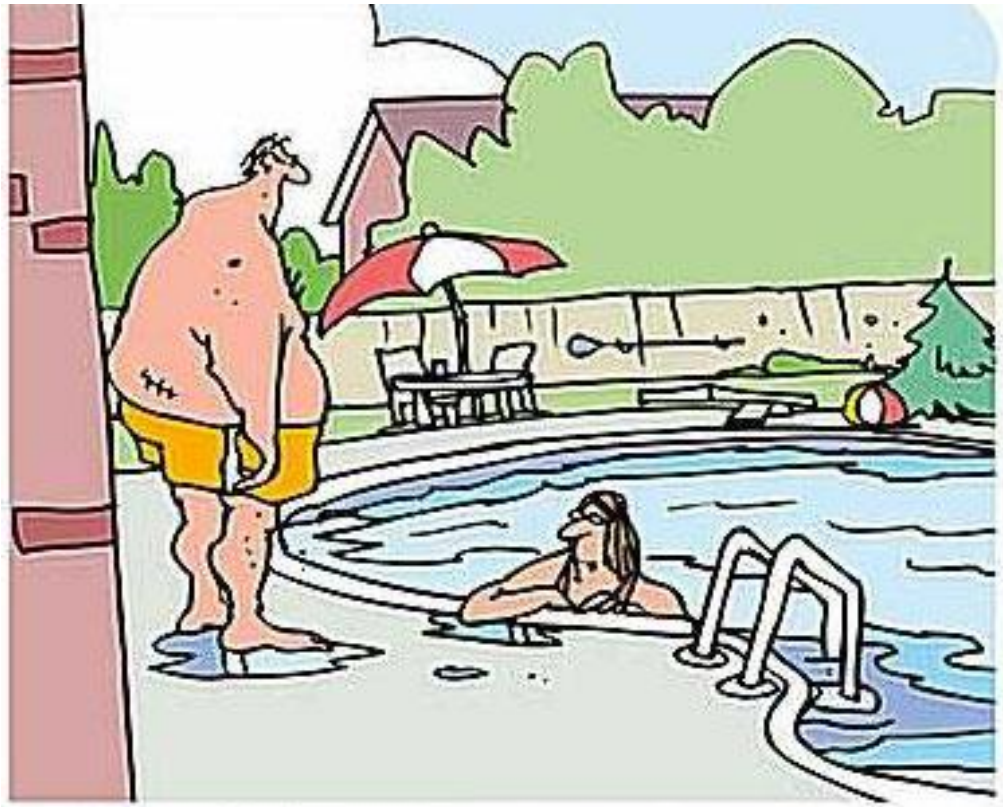

"The kidney shape was a cool idea.
Reminds us what you sold to pay for it."

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