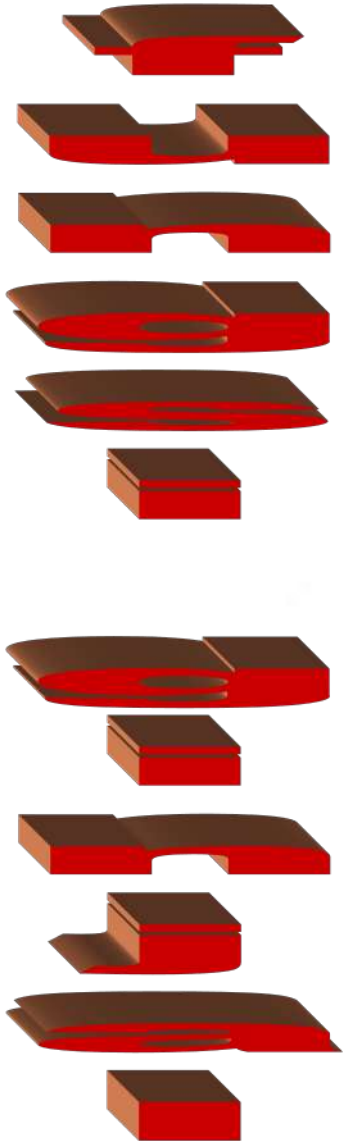
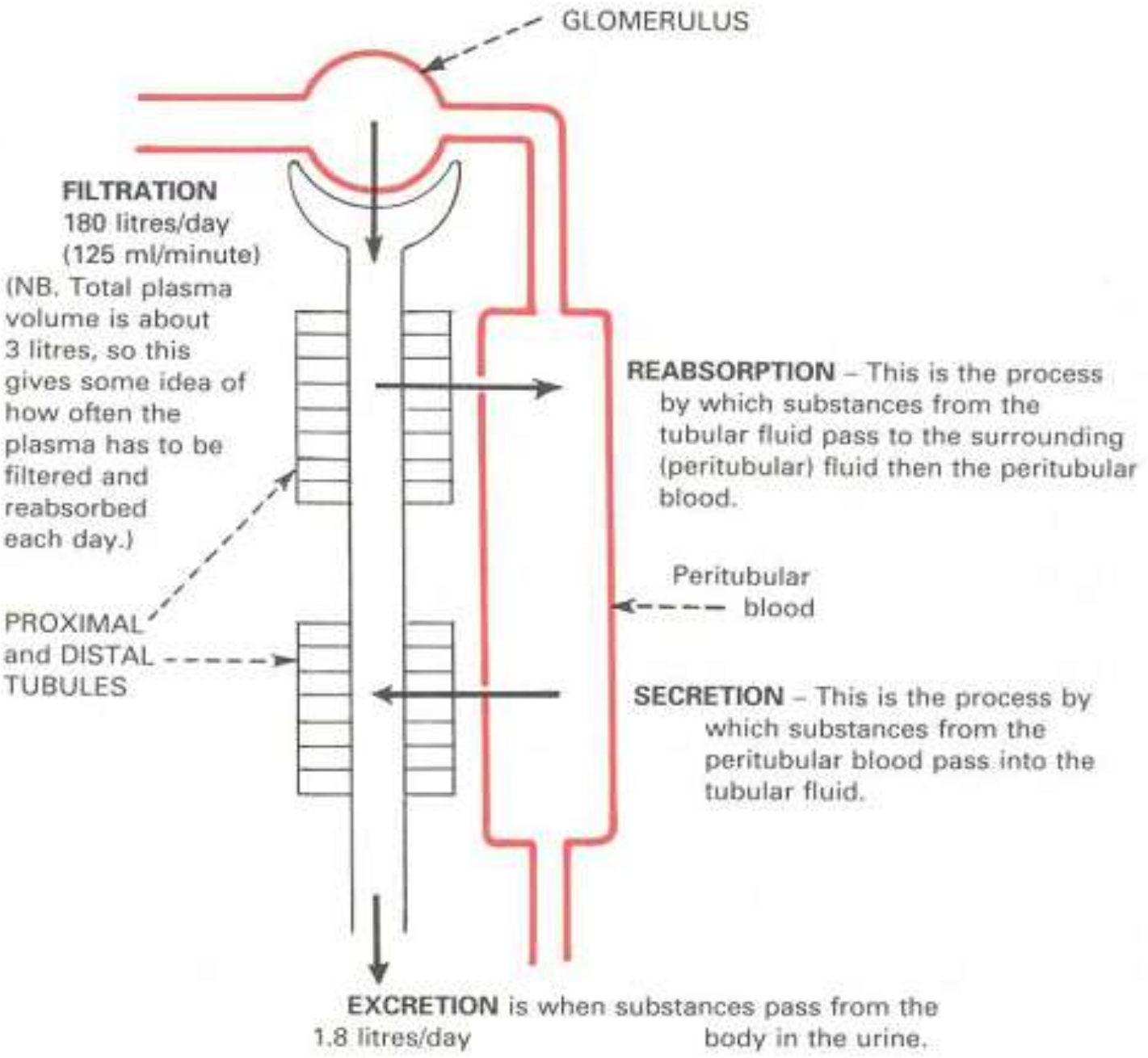
functional to pathological perspective with sellular, molecular aspects

Nur Arfian, dr, Ph.D



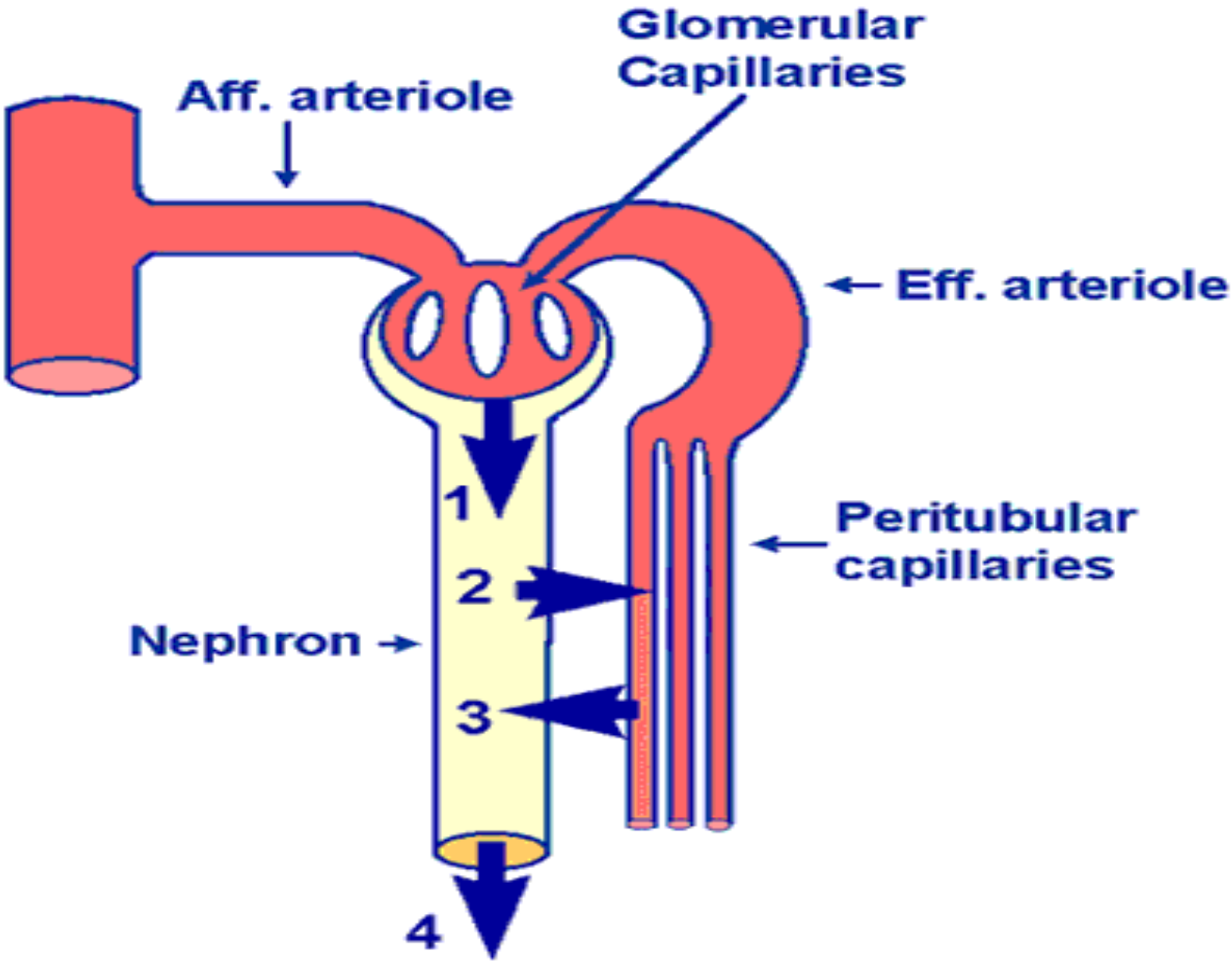

GLOMERULUS
FILTRATION
180 litres/day
(125 ml/minute)
(NB. Total plasma volume is about 3 litres, so this gives some idea of how often the plasma has to be filtered and reabsorbed each day.)
REABSORPTION – This is the process by which substances from the tubular fluid pass to the surrounding (peritubular) fluid then the peritubular blood.
Peritubular blood
PROXIMAL and DISTAL TUBULES
SECRETION – This is the process by which substances from the peritubular blood pass into the tubular fluid.
EXCRETION is when substances pass from the body in the urine.
1.8 litres/day

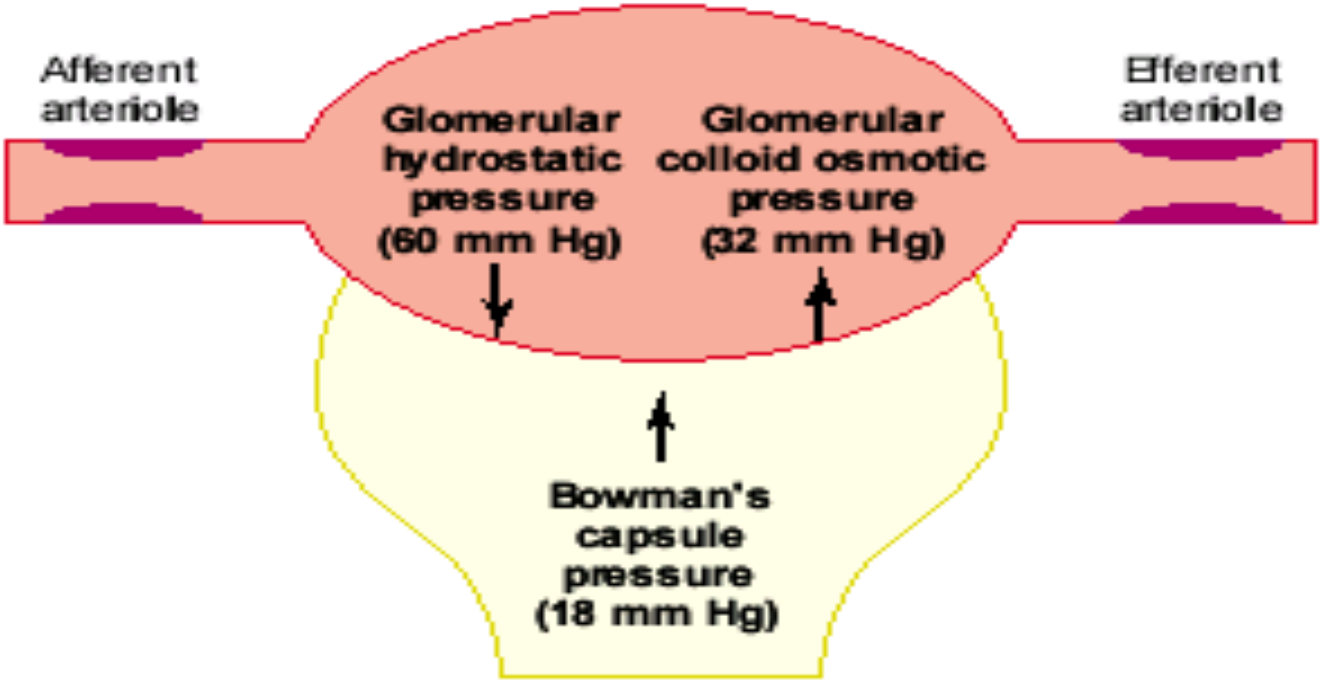
General Characteristics of Normal Urine

Characteristic	Normal Range
pH	4.5–8 (average: 6.0)
Specific gravity	1.003–1.030
Osmotic concentration (osmolarity)	855–1335 mOsm/L
Water content	93–97%
Volume	700–2000 mL/day
Color	Clear yellow
Odor	Varies with composition
Bacterial content	None (sterile)

Filtrasi

- Air, produk sampah, garam, glukosa dan substansi lain yang telah terfiltrasi disebut filtrat glomerulus
- Filtrat glomerulus terdiri dari air, garam (primarily Na+ and K+), glucose, & produk sisa yg disebut urea
- Urea dibentuk tubuh untuk mengeluarkan senyawa toksik amonia
- Rata-rata total filtrasi glomerulus disebut (Glomerular Filtration **rate** or **GFR**)
- Kira-kira 125 ml air dan senyawa terlarut difiltrasi keluar darah tiap menit


Glomerular
Capillaries
Aff. arteriole
Eff. arteriole
1
2
Peritubular
capillaries
Nephron
3
4

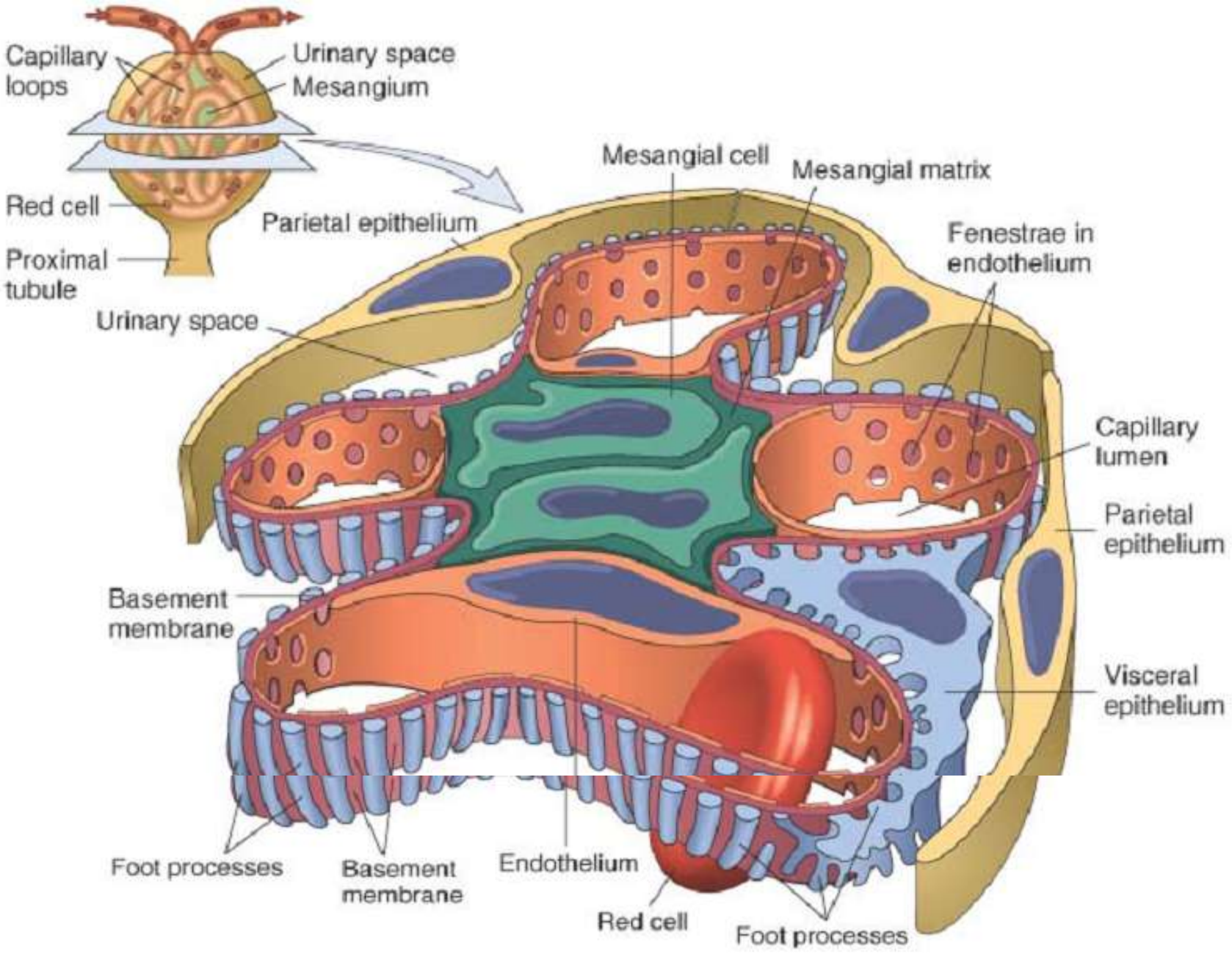


Net filtration pressure (10 mm Hg)	=	Glomerular hydrostatic pressure (60 mm Hg)	–	Bowman's capsule pressure (18 mm Hg)	–	Glomerular oncotic pressure (32 mm Hg)

Glomerular Filtration rate (GFR)

GFR : Kf X Net Filtration volume

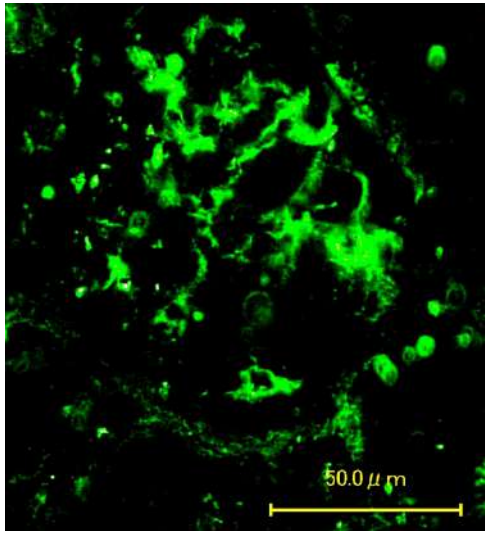
GFR : 125 mL / menit


Capillary loops
Urinary space
Mesangium
Red cell
Proximal tubule
Parietal epithelium
Mesangial cell
Mesangial matrix
Fenestrae in endothelium
Urinary space
Capillary lumen
Parietal epithelium
Basement membrane
Visceral epithelium
Foot processes
Basement membrane
Endothelium
Red cell
Foot processes

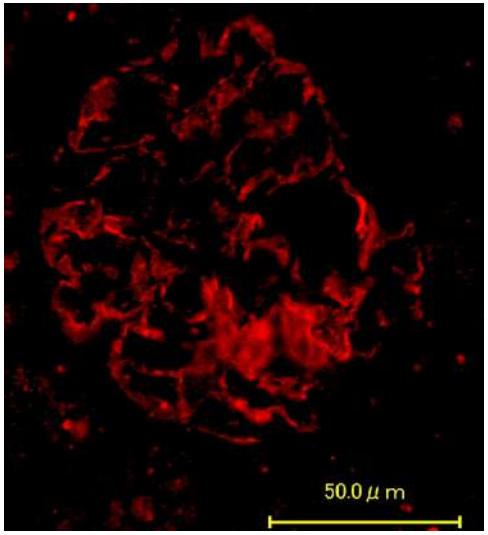
Filtration barrier

- Endothelium of glomerulus
  - Fenestrated endothelial cells
  - Endothelial surface layer (Glycocalyx)
- Podocyte
  - Visceral Epithelial cell
  - Markers: podocine, podocalyxin
  - Slit diaphragm: nephrin,
- Basement membrane
  - Proteoglycan
  - Negative charge

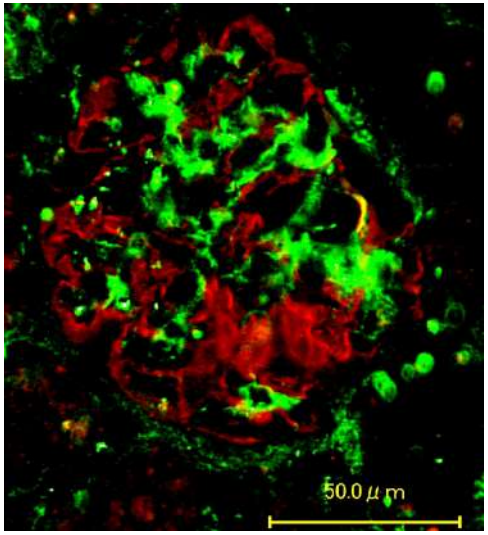
Von-Willebrand/EC



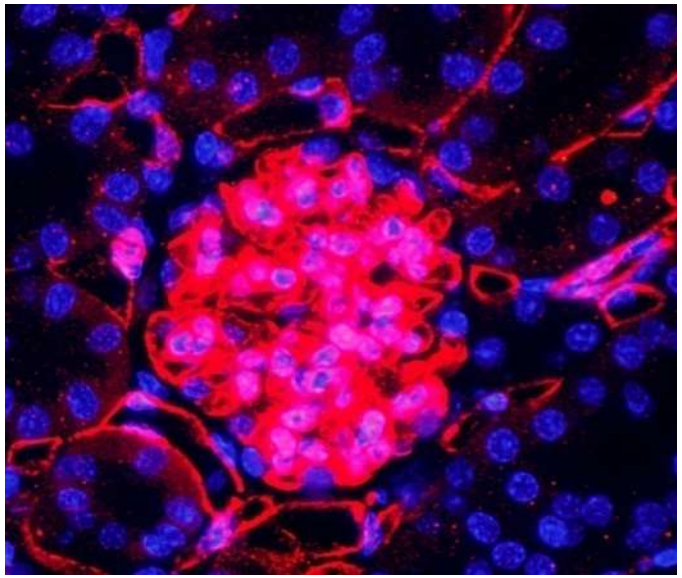
Nephrin

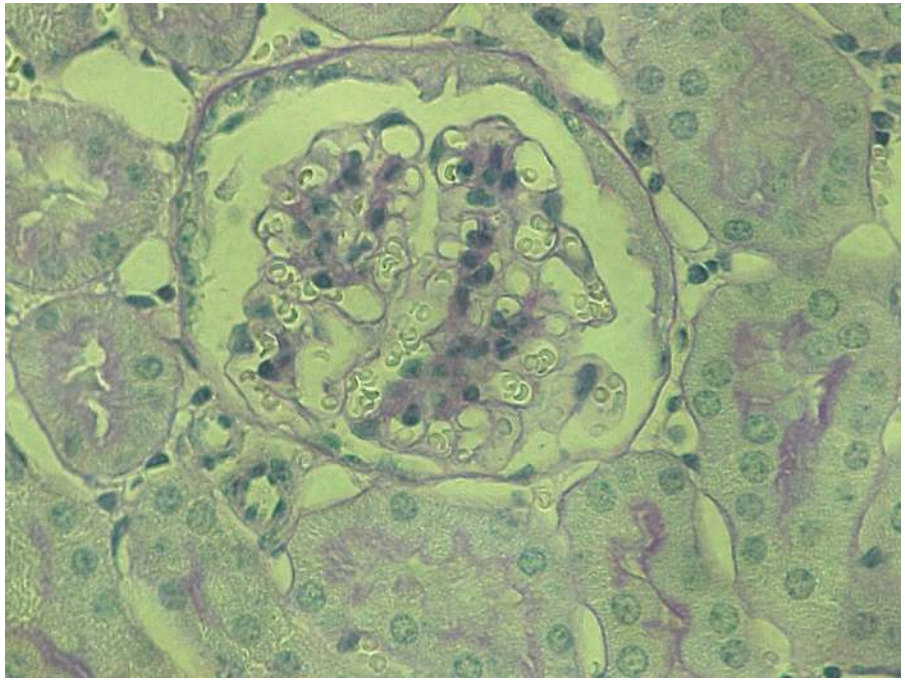


Overlay



Endothelial cell / CD31





Arfian, un-published data

Endothelial surface layer (ESL) atau glycocalyx

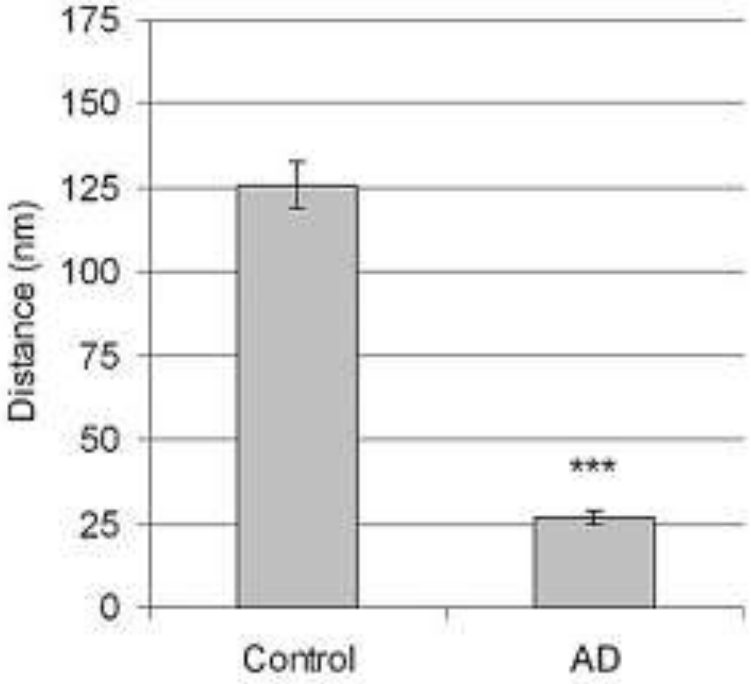


Figure 4. Mean ± SEM for the ESL in controls (four animals, n = 88 glomeruli) and AD mice (three animals, n = 79 glomeruli). *** $P < 0.001$.

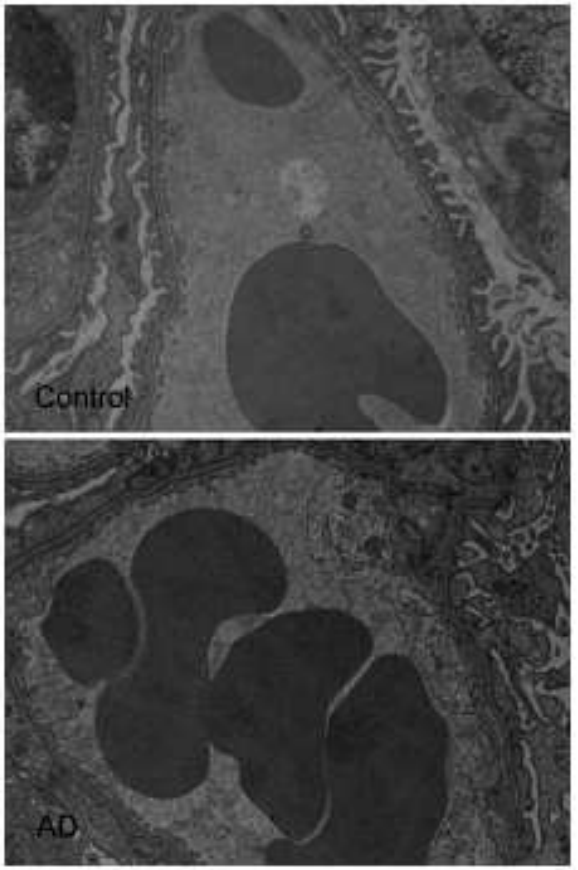
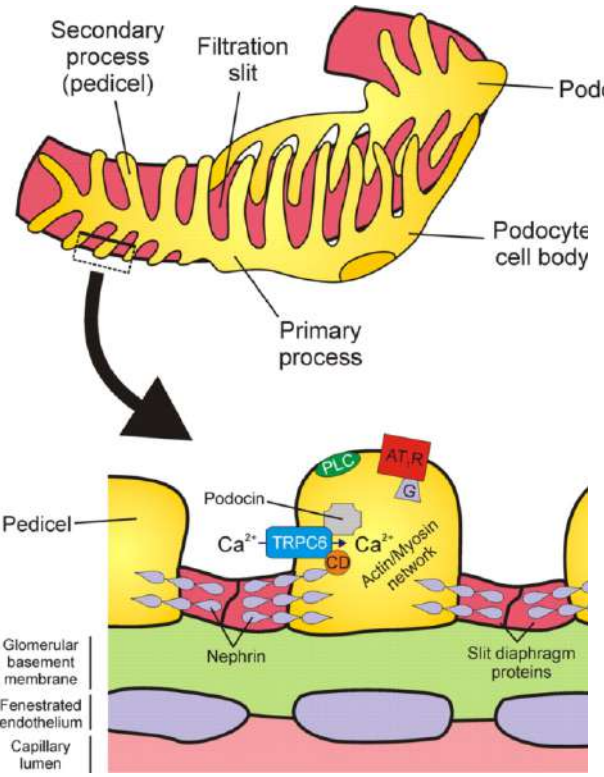
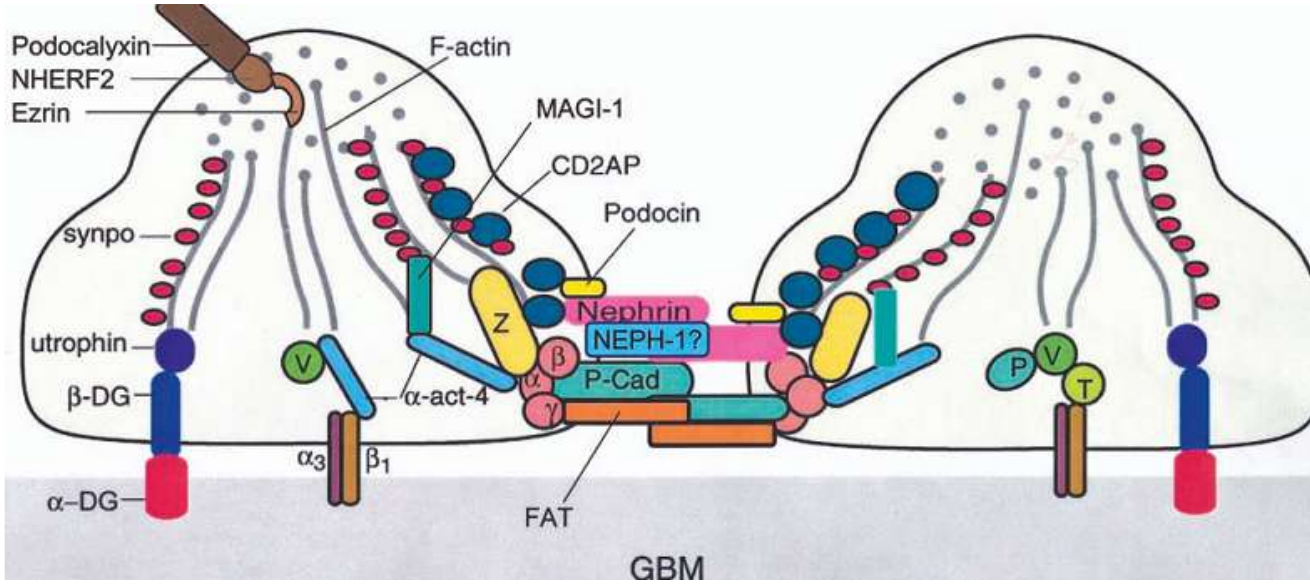


Figure 5. Electron micrographs of glomerular capillaries showing Intralipid droplets and the podocyte foot-process flattening in AD mice.

J Am Soc Nephrol 20: 114–122, 2009

The importance of podocyte

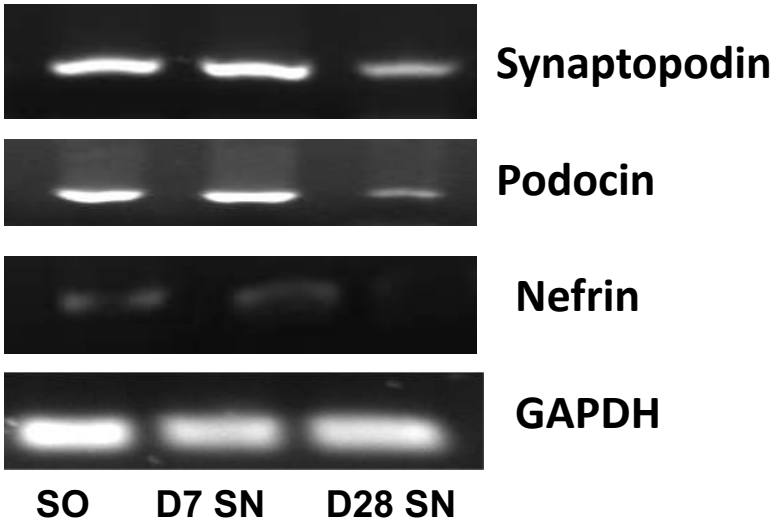


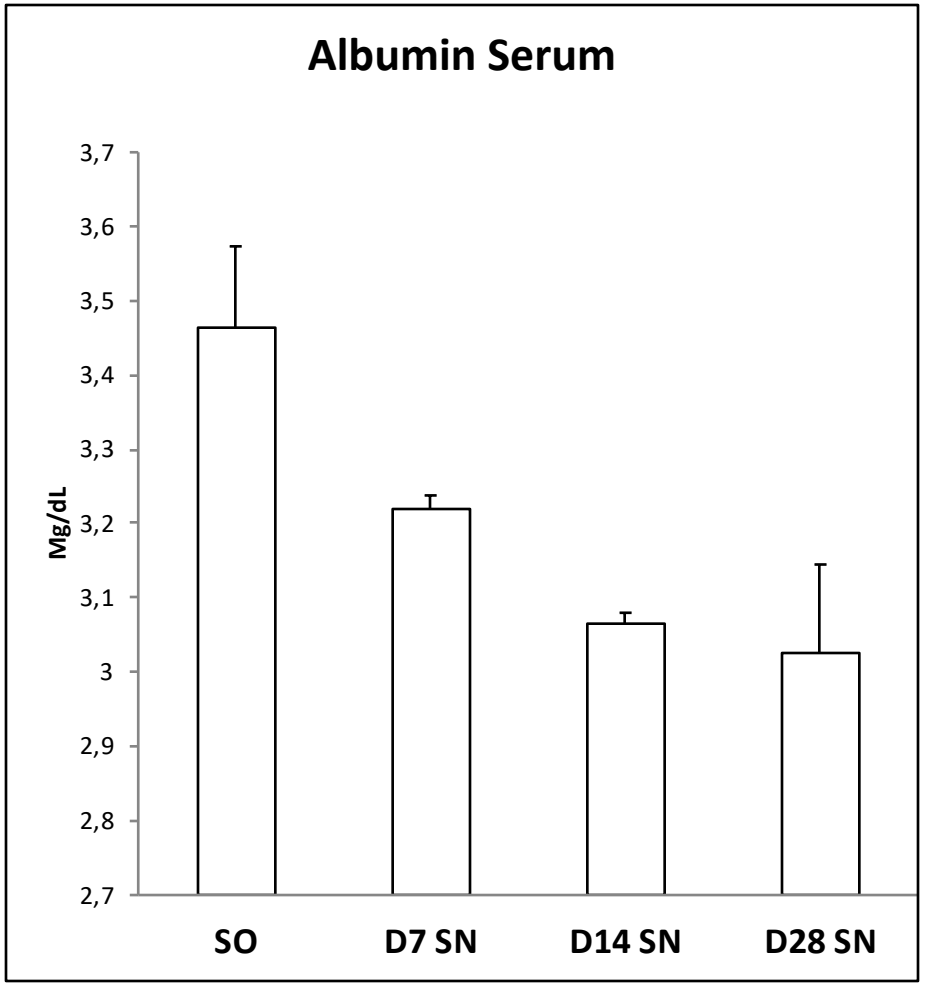


Podocytes are injured in many forms of human and experimental glomerular disease, including minimal change disease, FSGS, membranous glomerulopathy, diabetes mellitus, and lupus nephritis (8,27). Independent of the underlying disease, the early events are either characterized by alterations in the molecular composition of the SD without visible changes in morphology or, more obviously, by a reorganization of the FP structure with fusion of filtration slits and apical displacement of the SD (8,37,43).

Melihat ekspresi marker podocyte

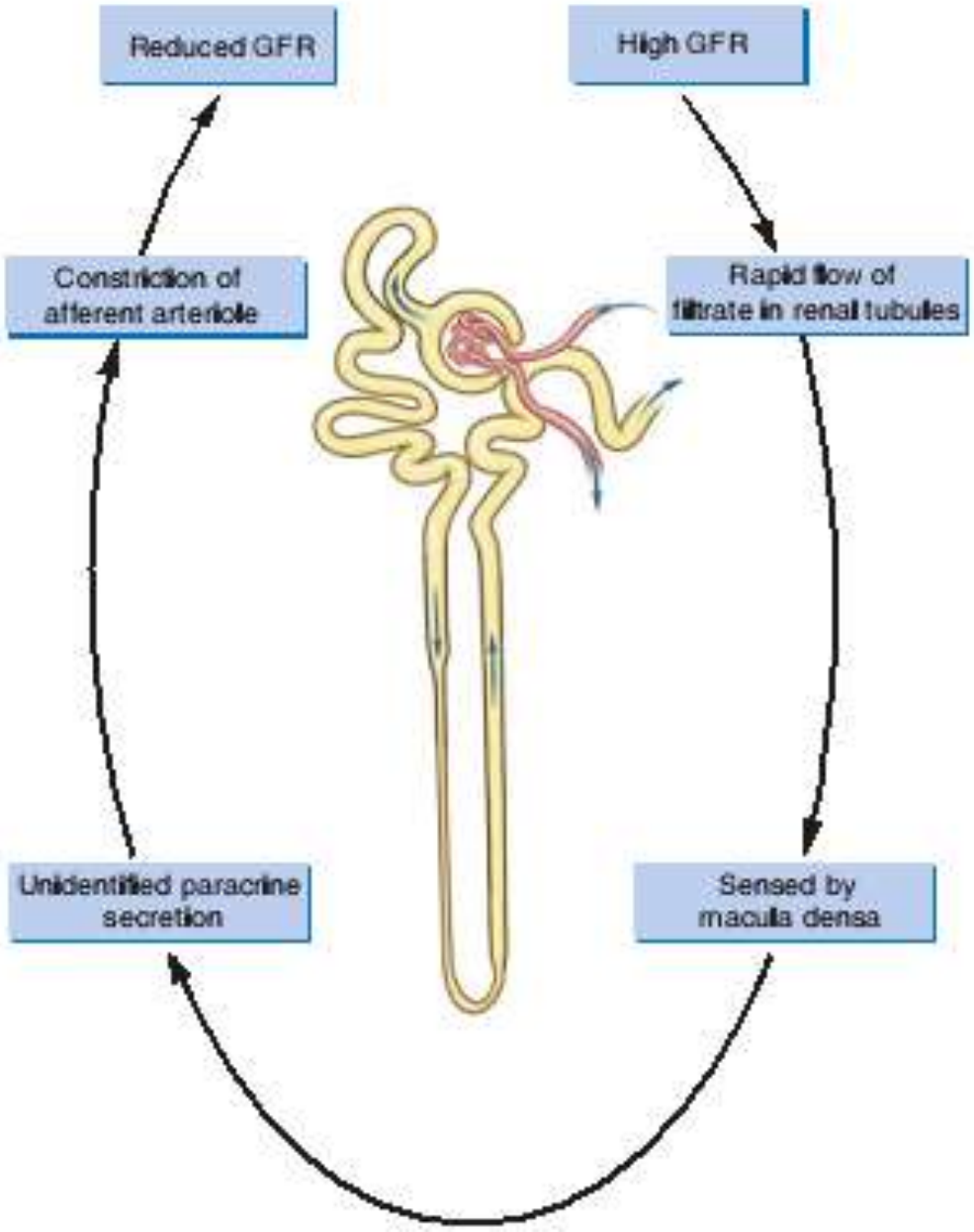
SN: Subtotal Nephrectomy
(Mencit Gagal Ginjal)





Autoregulasi ginjal

- Kemampuan ginjal dalam mengatur aliran darah dan GFR
- Mekanisme Miogenic : kontraksi otot polos krn peregangan; ktika tek.darah naik maka art.aferen konstraksi & tjd penurunan filtrasi
- Tubuloglomerular feedback



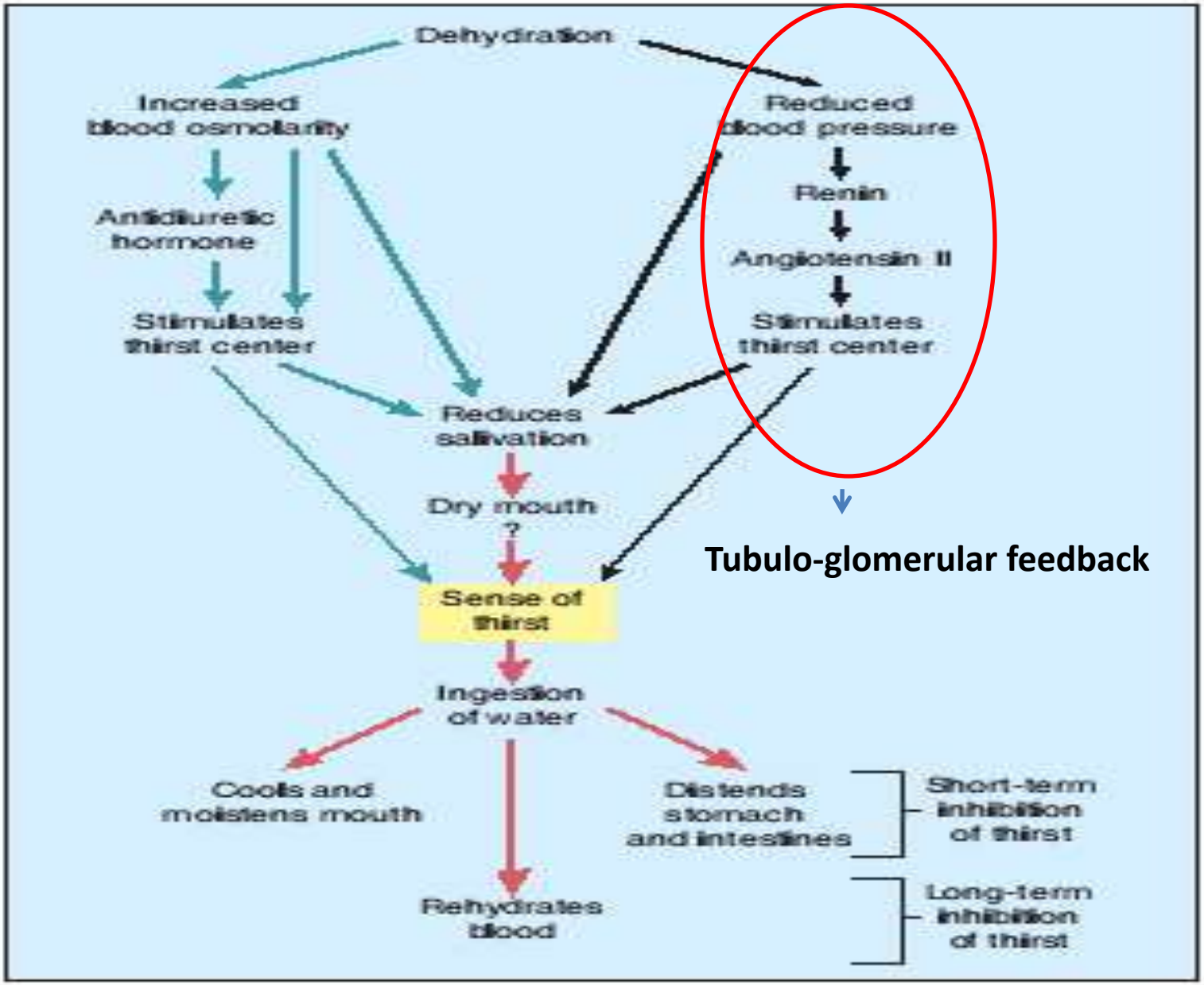


Figure 24.3 Dehydration, Thirst, and Rehydration.

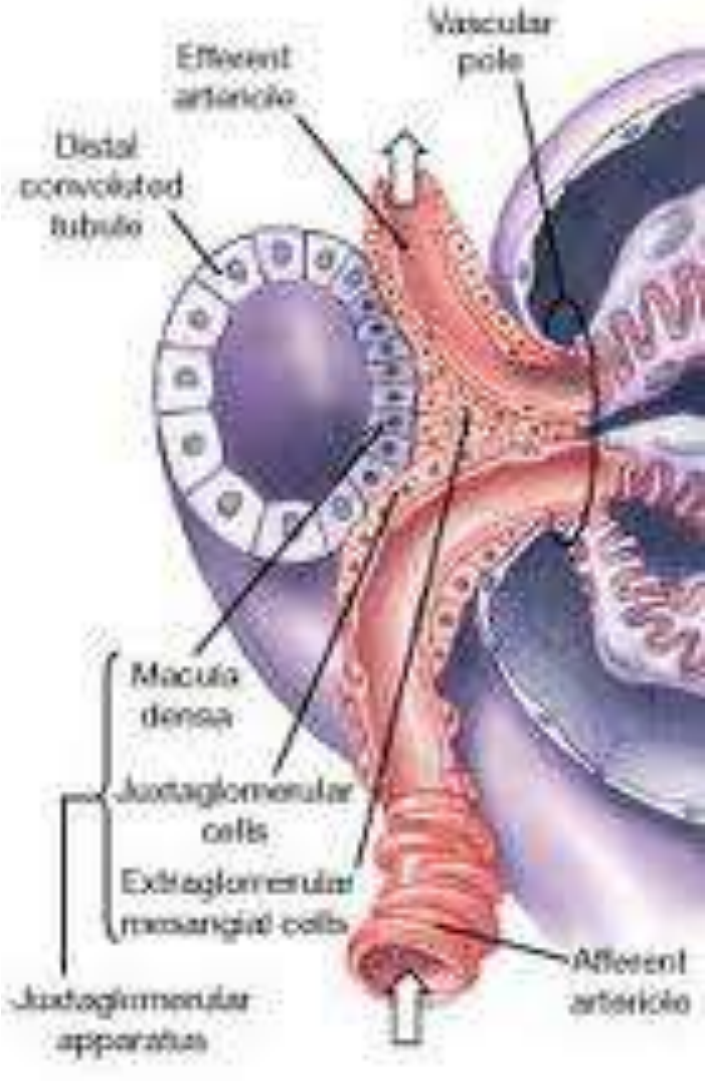
Juxta-glomerular apparatus dan Tubuloglomerular Feedback

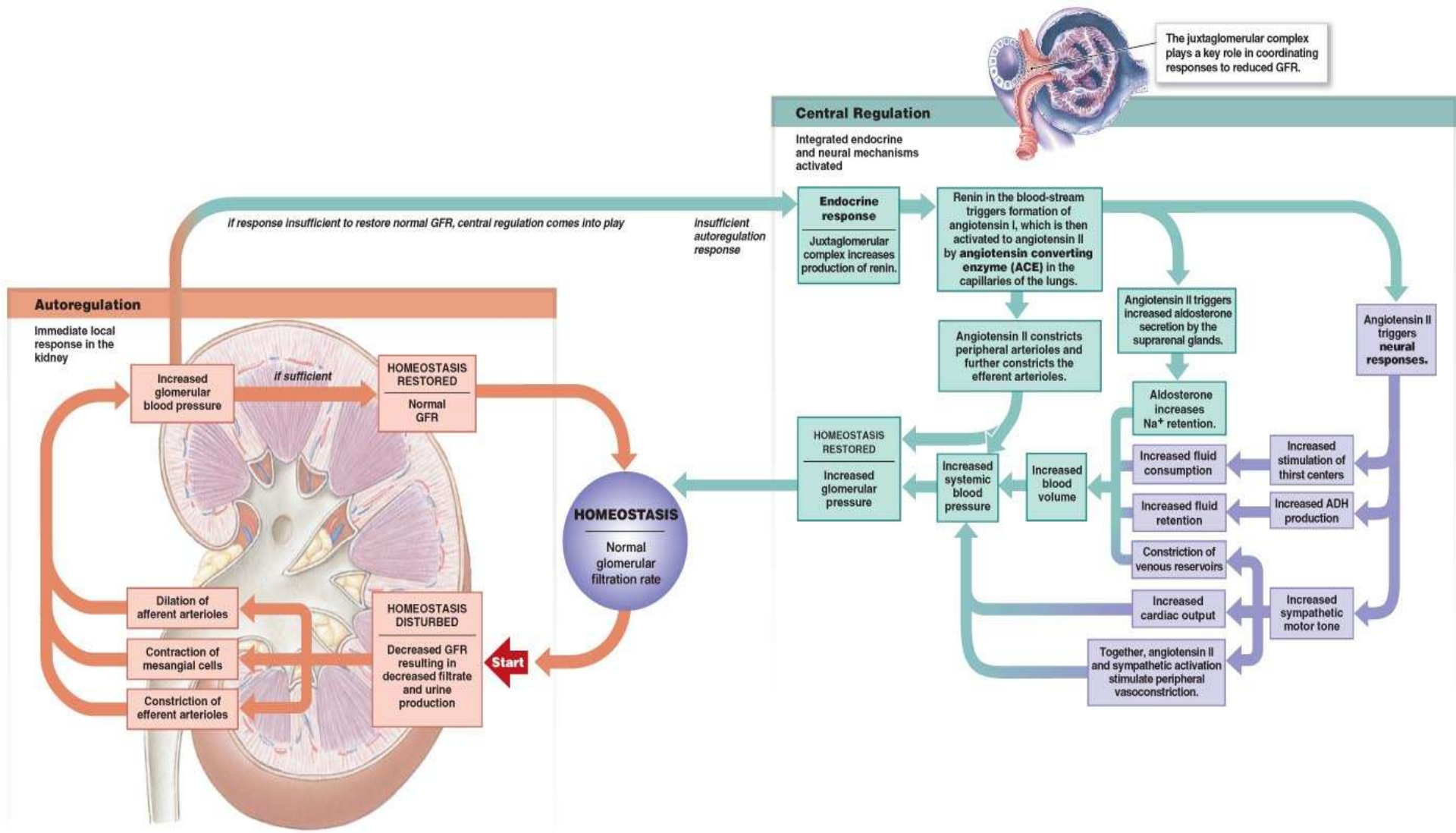
- The three components of the JGA are the following:

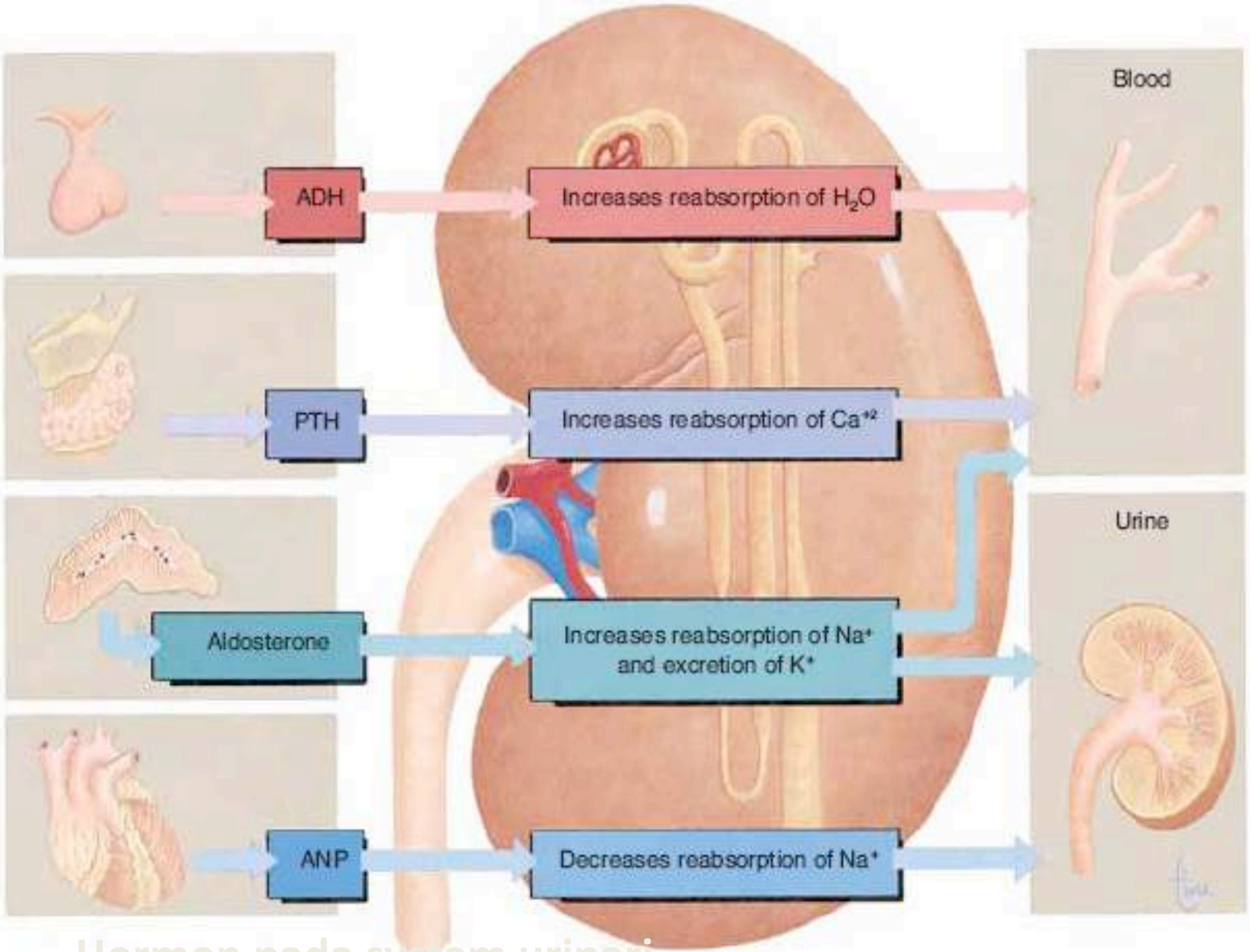
  (1) the juxtaglomerular cells of the afferent arteriole : renin production (stimuli: low blood flow, decreased NaCl delivery). Sebagai Effector

  (2) the macula densa, "sensory arm" of the renin-angiotensin-aldosterone axis in that these are the cells which sense decreased Na Cl delivery which determines downstream function. They are also involved in the mechanism of tubuloglomerular feedback.

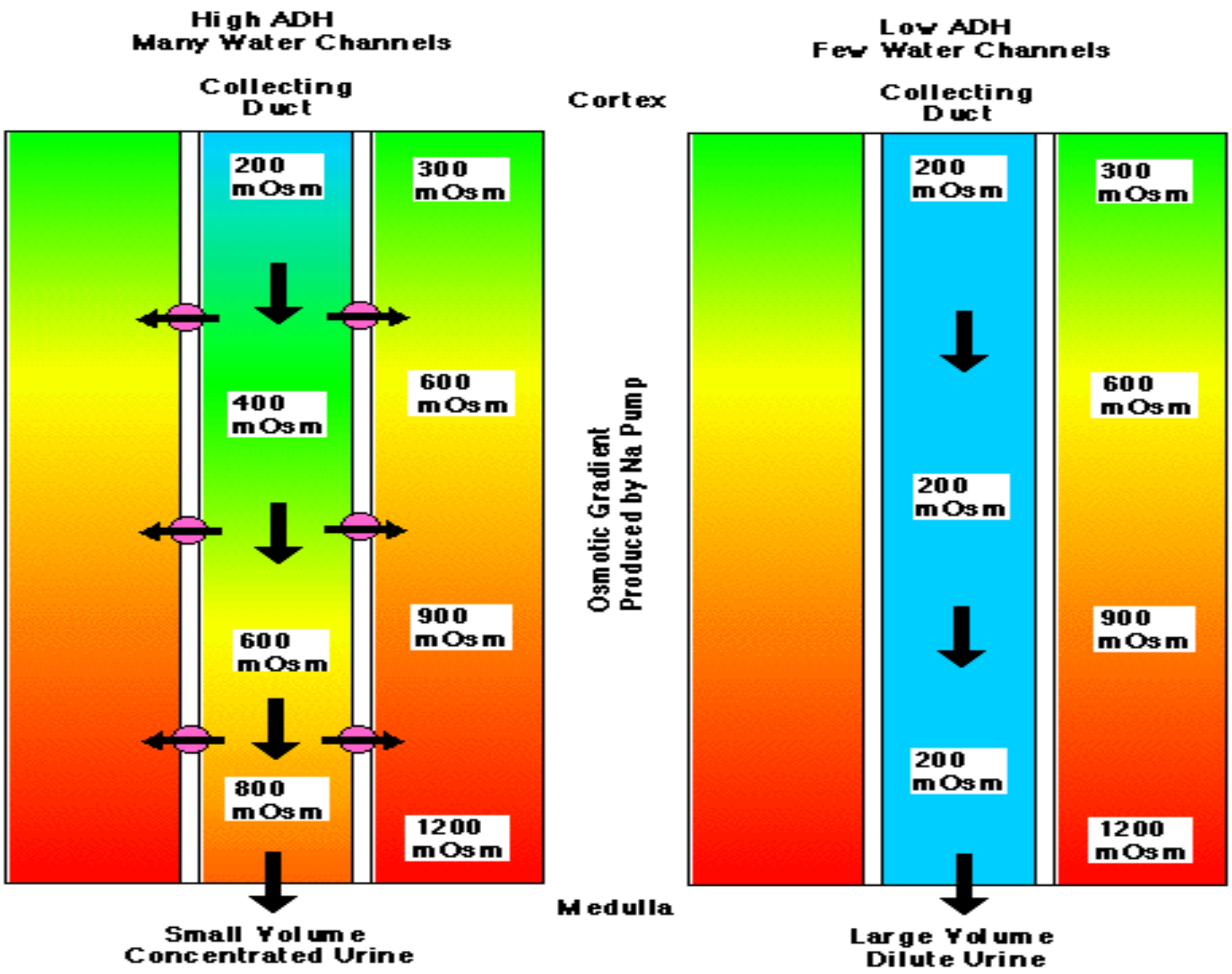
  (3) mesangial cells, which form connections via actin and microtubules which allow for selective vasoconstriction/vasodilation of the renal afferent and efferent arterioles with mesangial cell contraction.


Vascular pole
Efferent arteriole
Distal convoluted tubule
Macula densa
Juxtaglomerular cells
Extraglomerular mesangial cells
Juxtaglomerular apparatus
Afferent arteriole

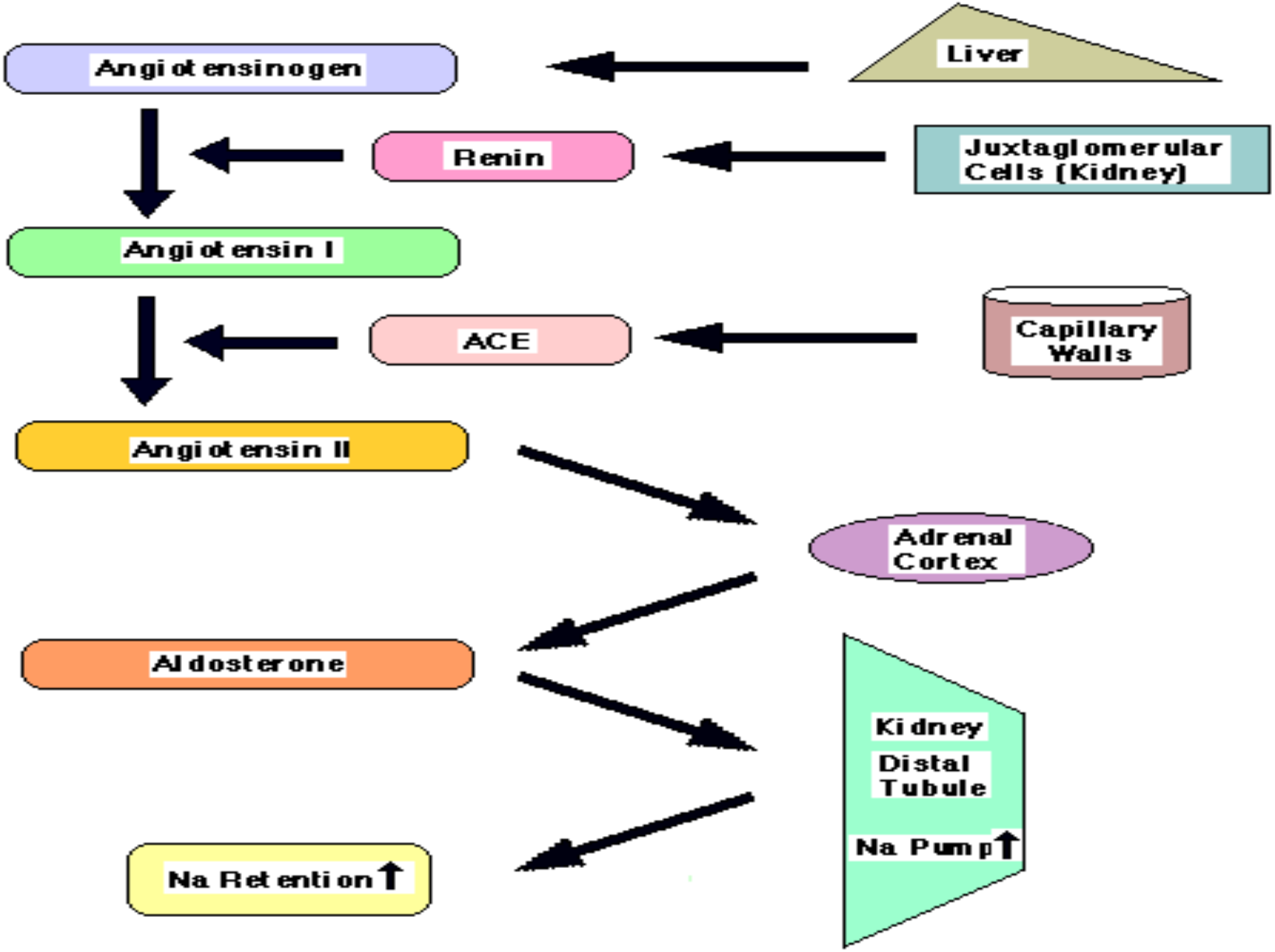




Hormon pada system urinari



ADH menyebabkan penurunan volume urine
Dan peningkatan konsentrasi urine


Angiotensinogen
Liver
Renin
Juxtaglomerular Cells (Kidney)
Angiotensin I
ACE
Capillary Walls
Angiotensin II
Adrenal Cortex
Aldosterone
Kidney
Distal Tubule
Na Pump
Na Retention

Blockade of the Renin-Angiotensin and Endothelin Systems on Progressive Renal Injury

Zemin Cao, Mark E. Cooper, Leonard L. Wu, Alison J. Cox, Karin Jandeleit-Dahm, Darren J. Kelly, Richard E. Gilbert

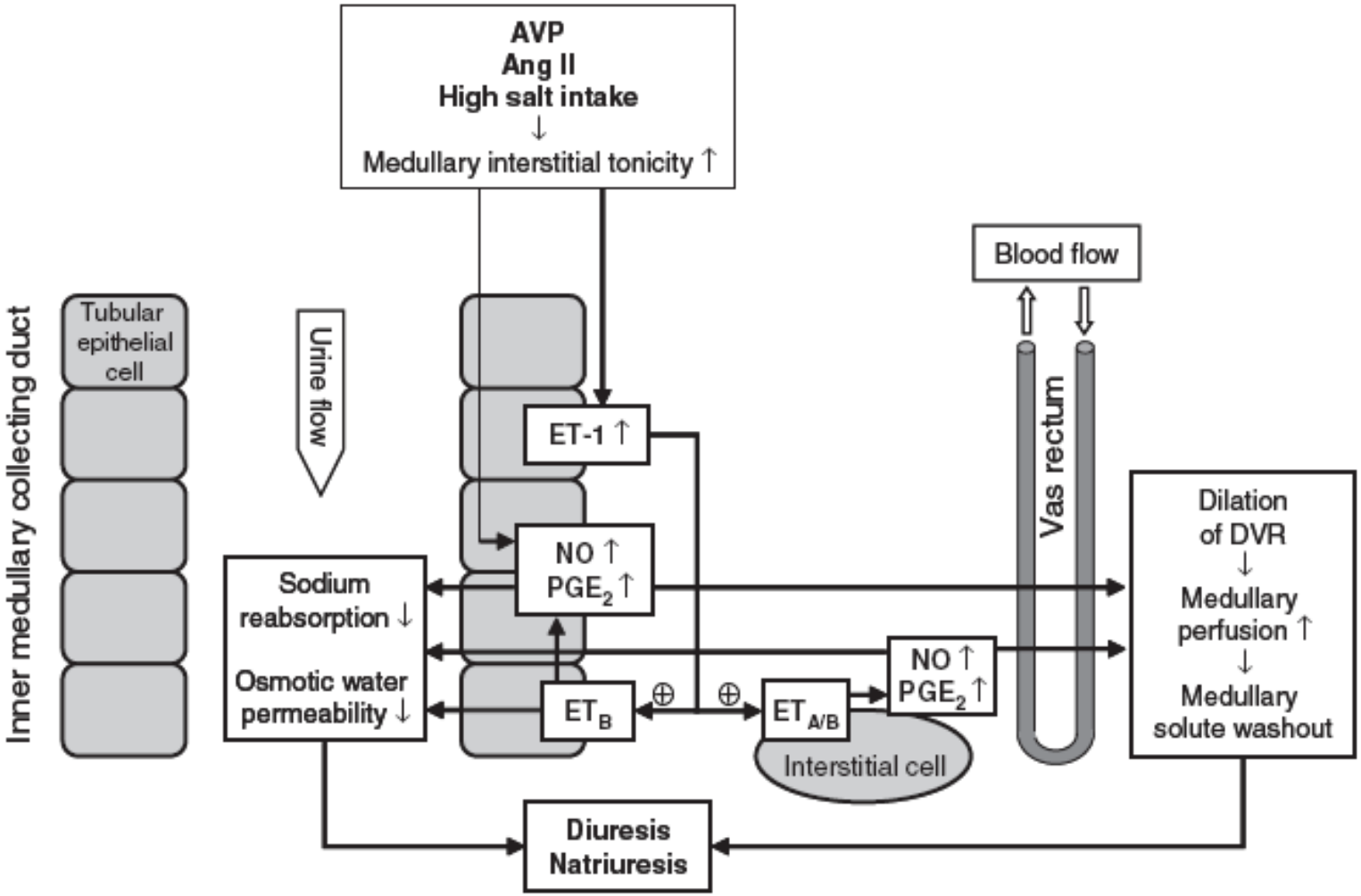
***Abstract*—**The renin-angiotensin system (RAS) and endothelin system may both play a role in the pathogenesis of progressive renal injury. The aims of the present study were 3-fold: first, to explore the possible benefits of dual blockade of the RAS with an ACE inhibitor and an angiotensin type 1(AT1) receptor antagonist; second, to examine the relative efficacy of endothelin A receptor antagonism (ETA-RA) compared with combined endothelin A/B receptor antagonism (ETA/B-RA); and third, to assess whether interruption of both RAS and endothelin system had any advantages over single-system blockade. Subtotally nephrectomized rats were studied as a model of progressive renal injury and randomly assigned to one of the following treatments for 12 weeks: perindopril (ACE inhibitor), irbesartan (AT1 receptor antagonist), BMS193884 (ETA-RA), bosentan (ETA/B-RA), and a combination of irbesartan with either perindopril or BMS193884. Treatment with irbesartan or perindopril was associated with an improved glomerular filtration rate and reductions in blood pressure, urinary protein excretion, glomerulosclerosis, and tubular injury in association with reduced gene expression of transforming growth factor-β_1 and matrix protein type IV collagen. The combination of irbesartan with perindopril was associated with further reductions in blood pressure and urinary protein excretion. No beneficial effects of either BMS193884 or bosentan were noted. Furthermore, the addition of BMS193884 to irbesartan did not confer any additional benefits. These findings suggest that the RAS but not the endothelin system is a major mediator of progressive renal injury after renal mass reduction and that the combination of an AT1 receptor antagonist with an ACE inhibitor may have advantages over the single agent of RAS blocker treatment. **(*Hypertension.* 2000;36:561-568.)**

Key Words: kidney failure ■ angiotensin II ■ endothelin ■ transforming growth factors

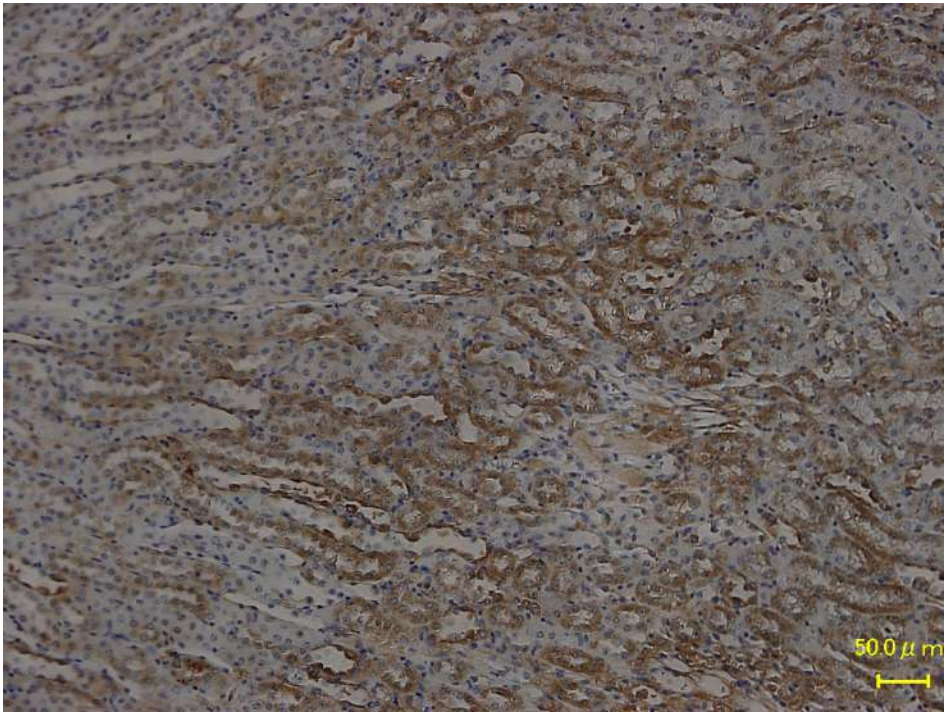
Apakah ada kebalikannya

- Aktifasi sistem renin-angiotensin menyebabkan hipertensi pada kasus sensitif garam (salf sensitive hypertension)
- Belajar dari ETBR recued rat: hypertensi karena sensitif garam.
- Renin-Angiotensin VS Endothelin-ETBR pada sistem kolektif ginjal.

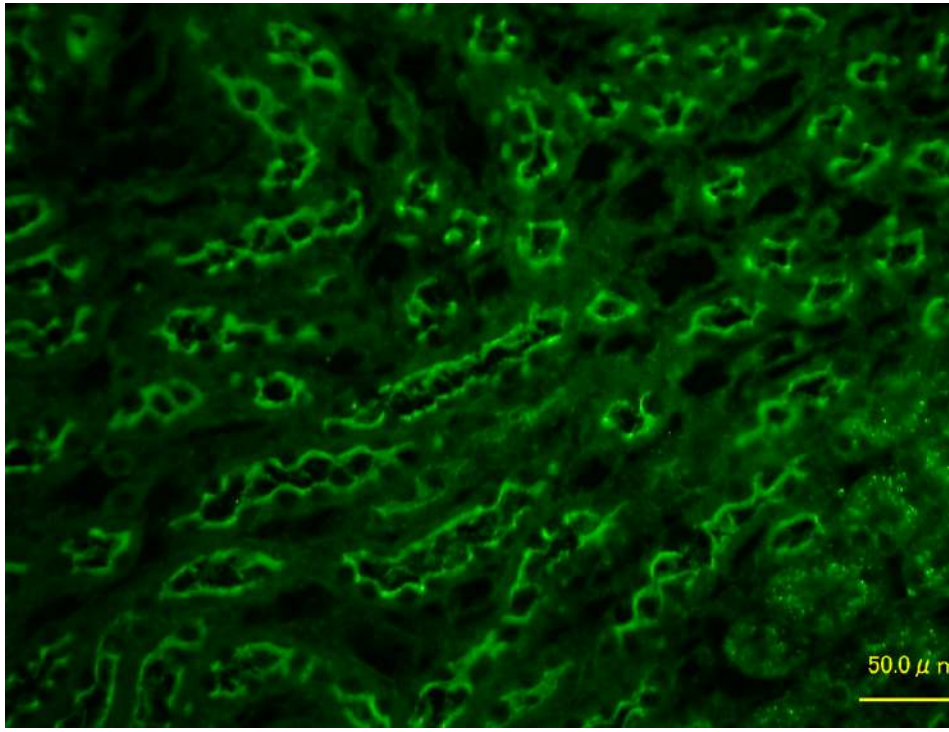
AVP-RAA vs Endothelin-ETBR in collecting duct



Endothelin-1 pada medula ginjal



ETBR pada medula ginjal-duktus kolektifus



Arfian, unpublished data

Proteinuria awal cedera from glomerulus to tubulus

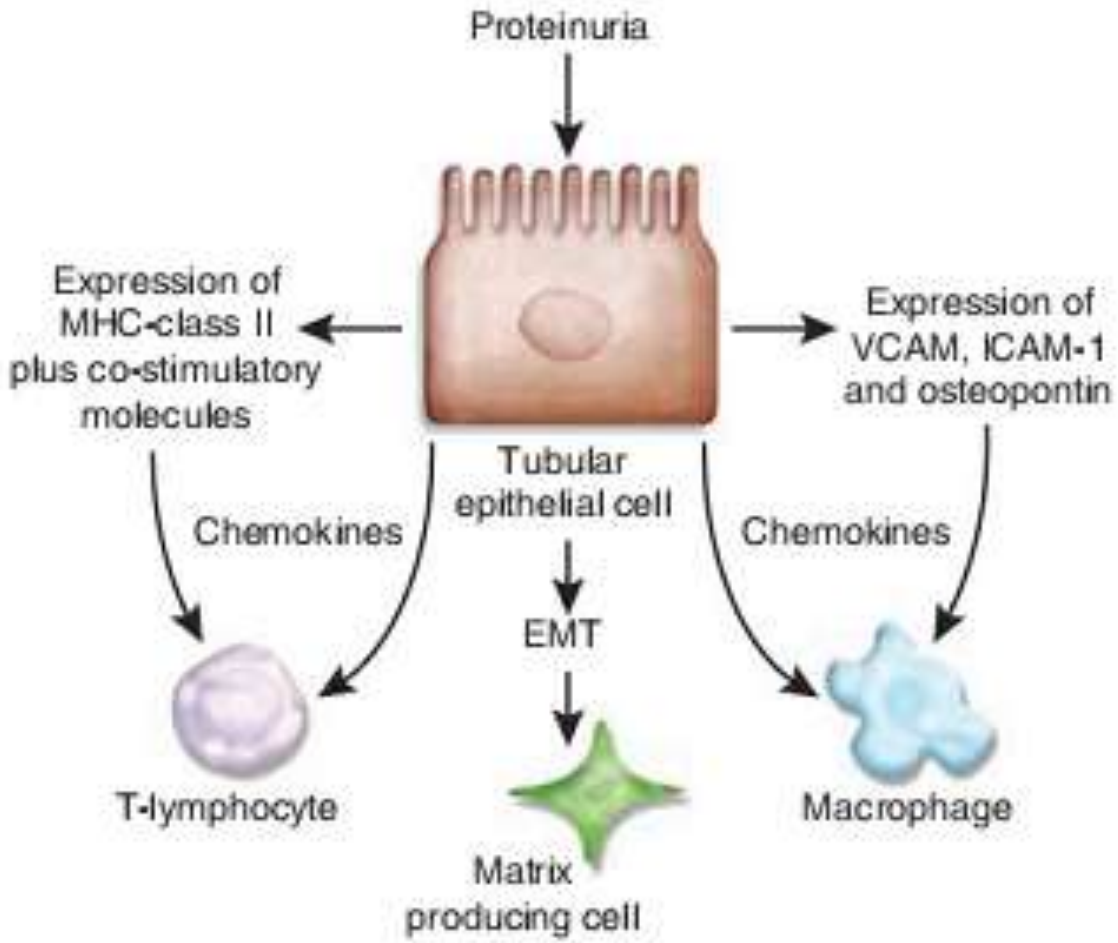
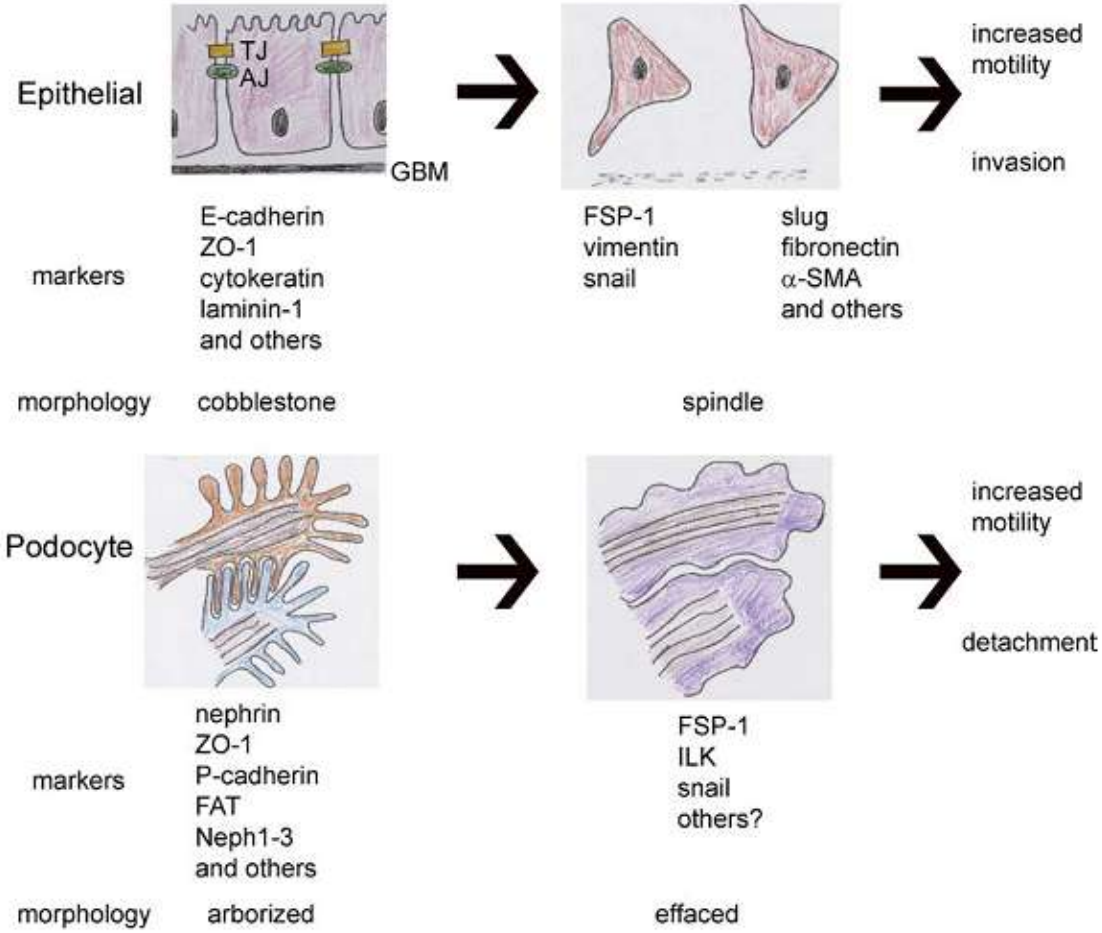


Figure 1 | Effects of proteinuria on tubular epithelial cells.

Podocyte injury and reduced podocyte density have been documented in patients with diabetic kidney disease and are among the strongest predictors of its progression.[4] When a critical proportion of the total podocyte population is lost, the remaining cells are unable to compensate and glomerulosclerosis develops.[5] Although the



Fungsi nefron

- **Tubulus proximal : absorbsi**
- **Ansa henle**
  - **Descenden : sangat permeabel thd air**
  - **Ascenden : sangat permeabel thd Natrium**
- **Tubulus distal : secresi; absorbsi**
- **Ductus kolectivus : secresi**

Reabsorption,

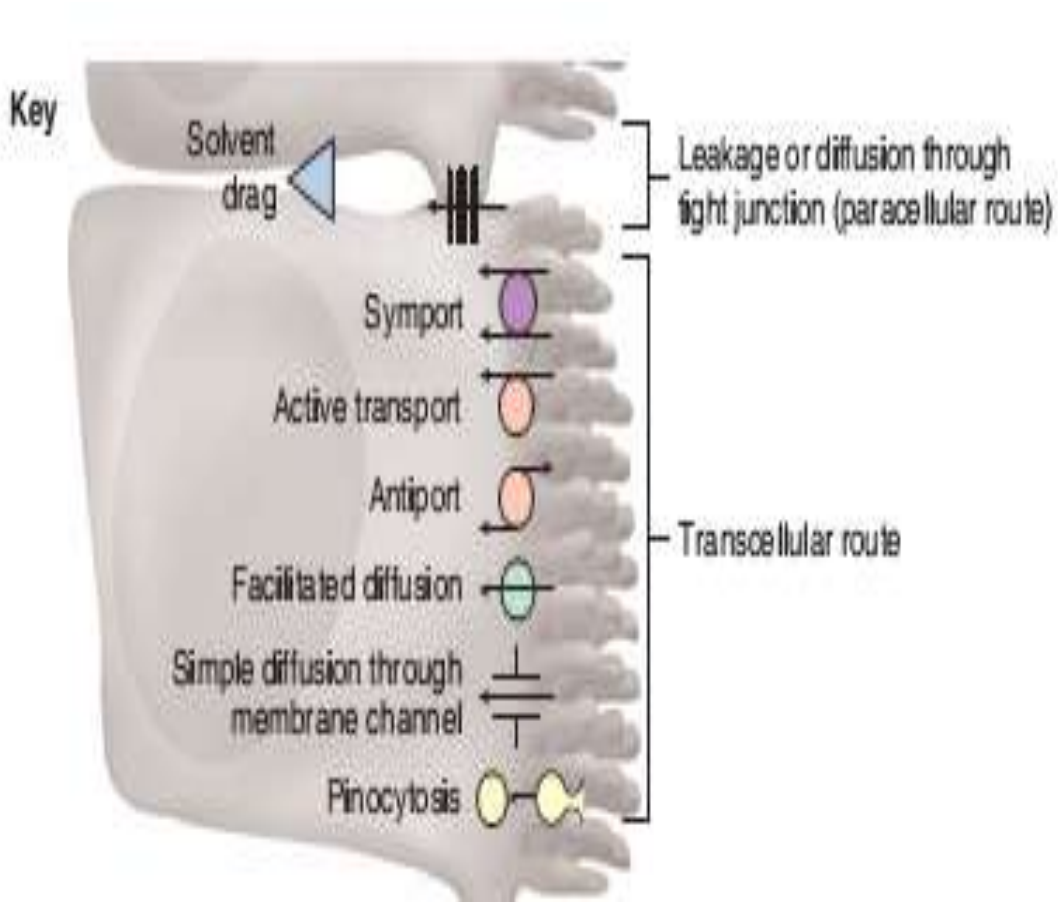
- Gerakan substansi keluar tubulus renal kembali ke kapiler darah disekitar tubulus (**peritubular capillaries**).
- Substansi yang diabsorbsi yang adalah air, glukosa, nutrisi lain, natrium
- Dimulai di tubulus tubulus convulated proksimal, dan berlanjut loop of Henle, tubulus convulatio distal, dan tubulus kolektivus

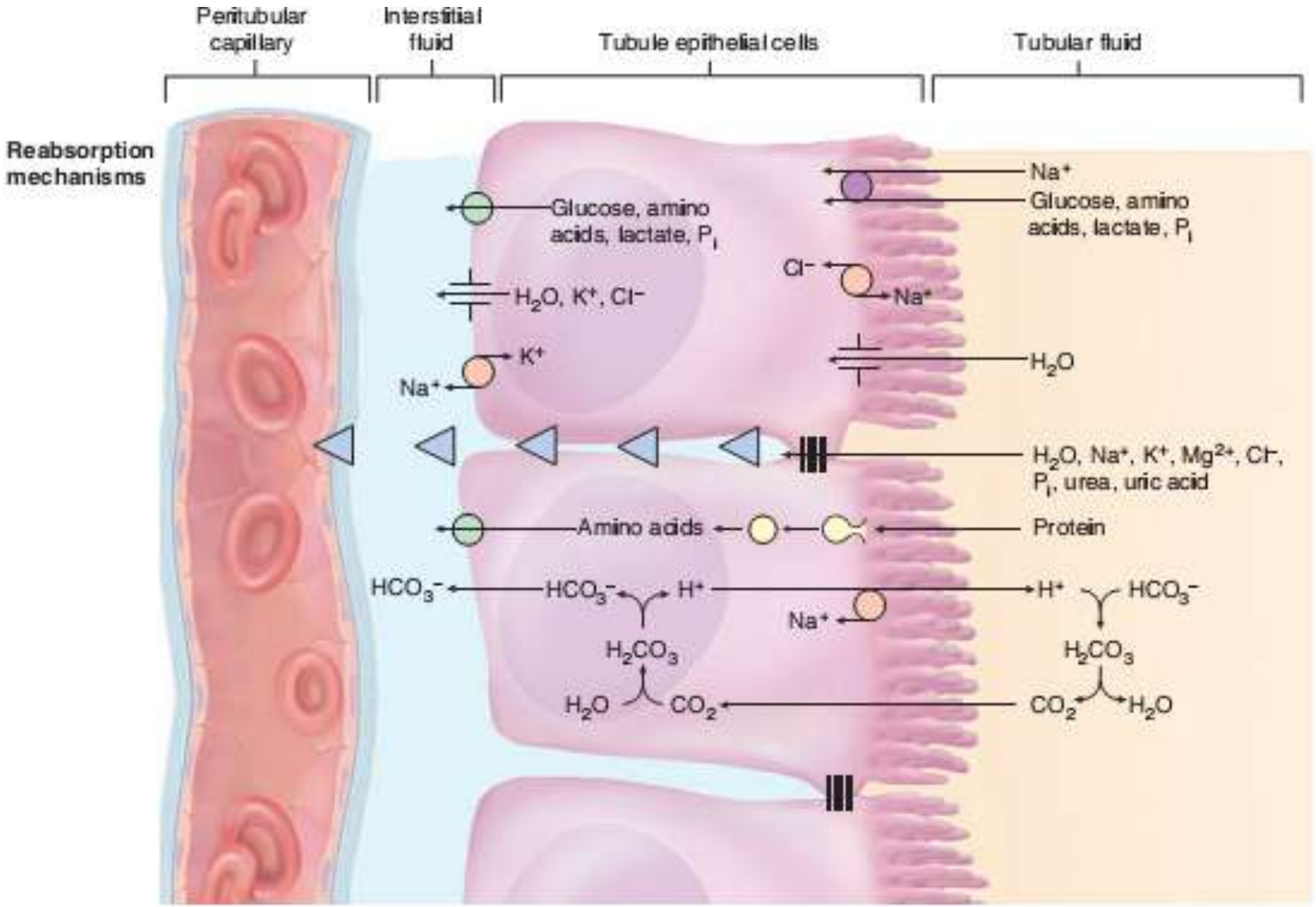
Tubular reabsorption

- Active tubular reabsorption - diffusion by ATP-dependent carrier; transport of glucose, amino acids, and vitamins
  - there is cotransportation of molecules
  - molecules being carried depends upon another molecule's (e.g. Na) transport across basolateral membrane
- Passive tubular reabsorption - diffusion, facilitated diffusion, and osmosis; substance moves along electrochemical gradients without the use of energy
  - sodium movement establishes osmotic gradient
  - water moves by osmosis into peritubular capillaries
  - causes obligatory water reabsorption

Route of absorbstion

- the **transcellular route,** which substances pass through the cytoplasm and out the base of the epithelial cells
- **paracellular route,** in which substances pass between the epithelial cells

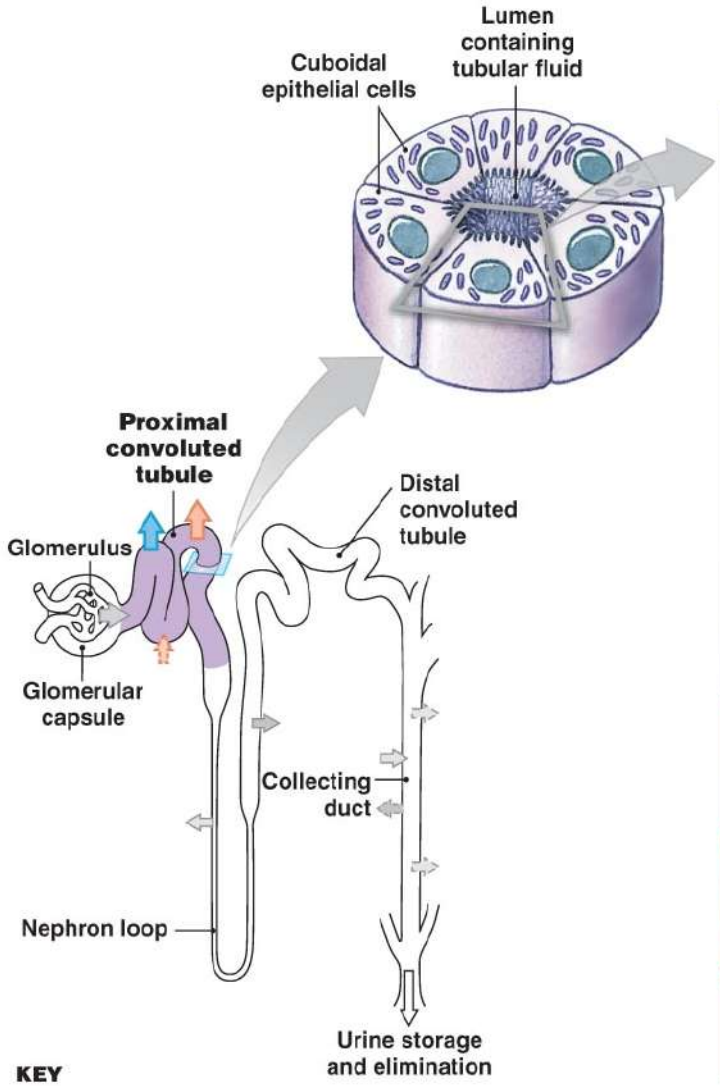


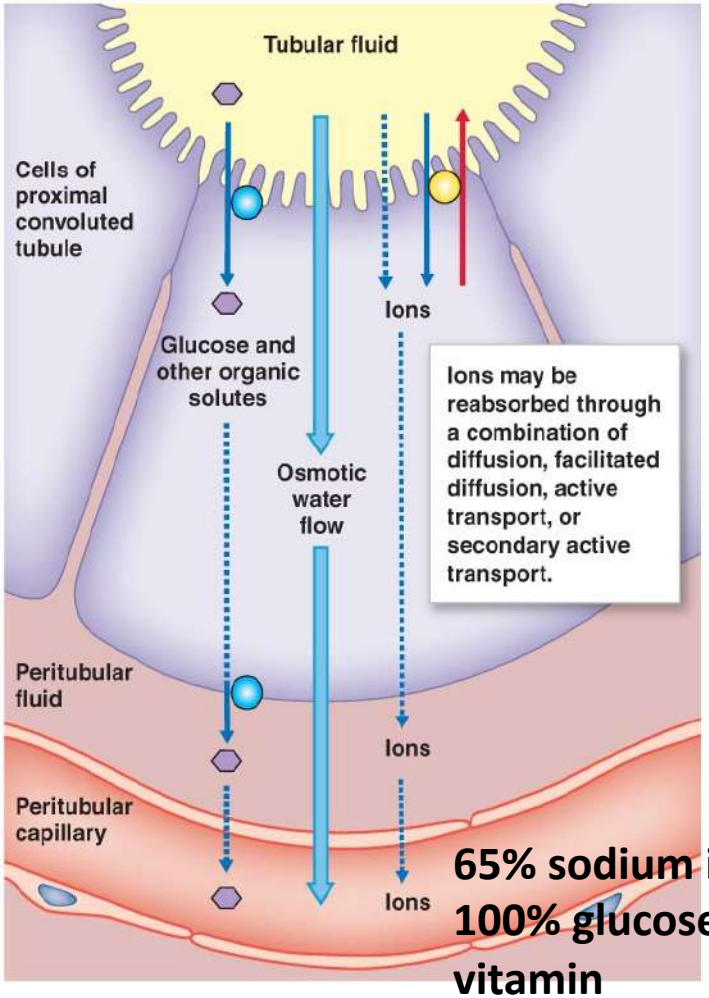


23.14 Mechanisms of Reabsorption in the Proximal Convoluted Tubule.

proximal convoluted tubule (PCT)

- Reabsorbs about 65% of the glomerular filtrate
- Removes some substances from the blood and secretes them into the tubule
- great length and prominent microvilli, which increase its absorptive surface area
- Mitochondria: 6% of resting ATP

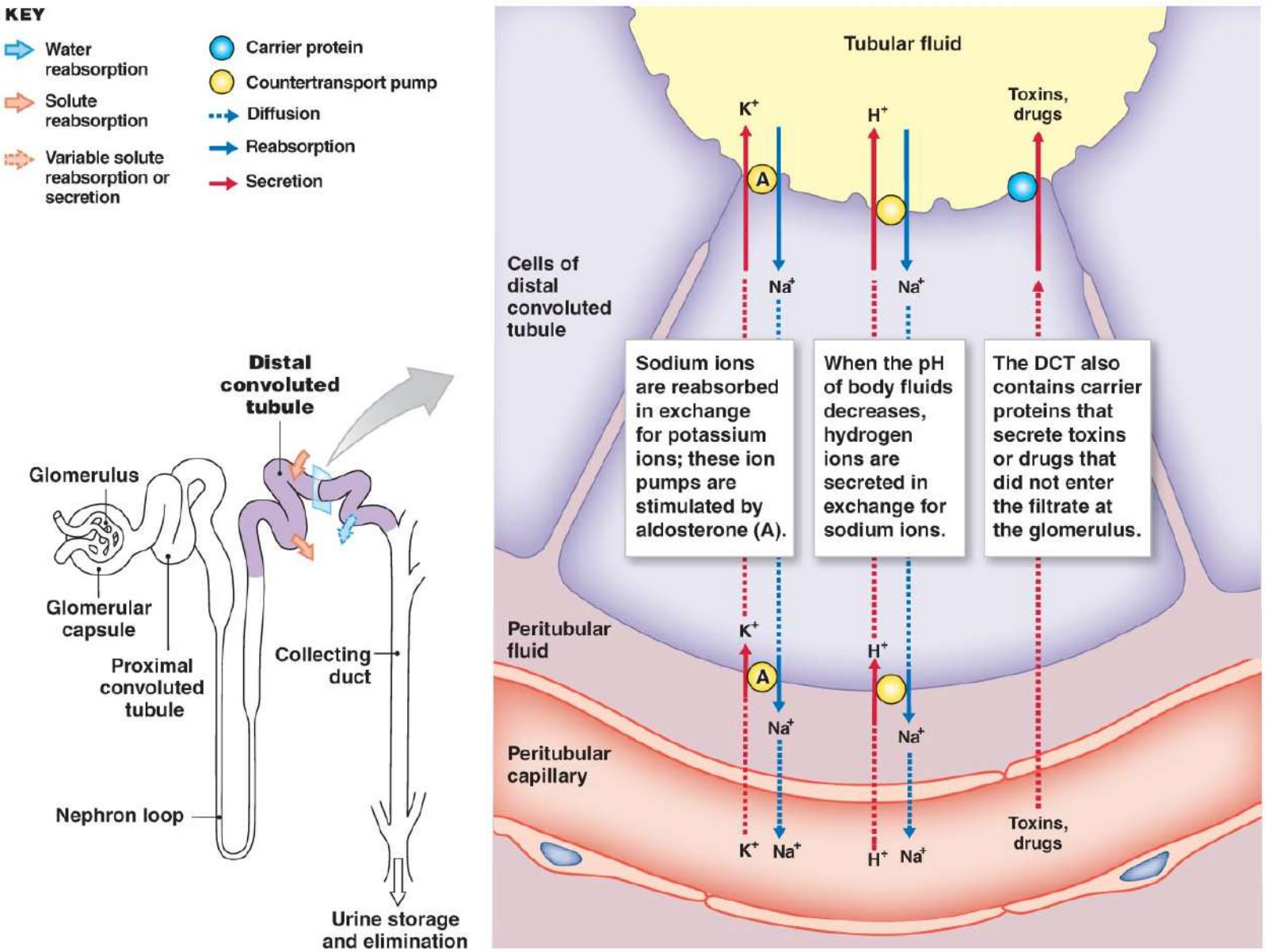




65% sodium ions and water
100% glucose, lactate, amino acids, vitamin
90% bicarbonate ions
50% chloride ions
55% potassium ions
calcium, phosphate, and magnesium ion (hormonally controlled)

Sodium / natrium

- creates an osmotic and electrical gradient that drives the reabsorption of water and the other solutes.
- transcellular and paracellular routes
- Conc: 140 mEq/L in the fluid entering the PCT and only 12 mEq/L in the cytoplasm of the



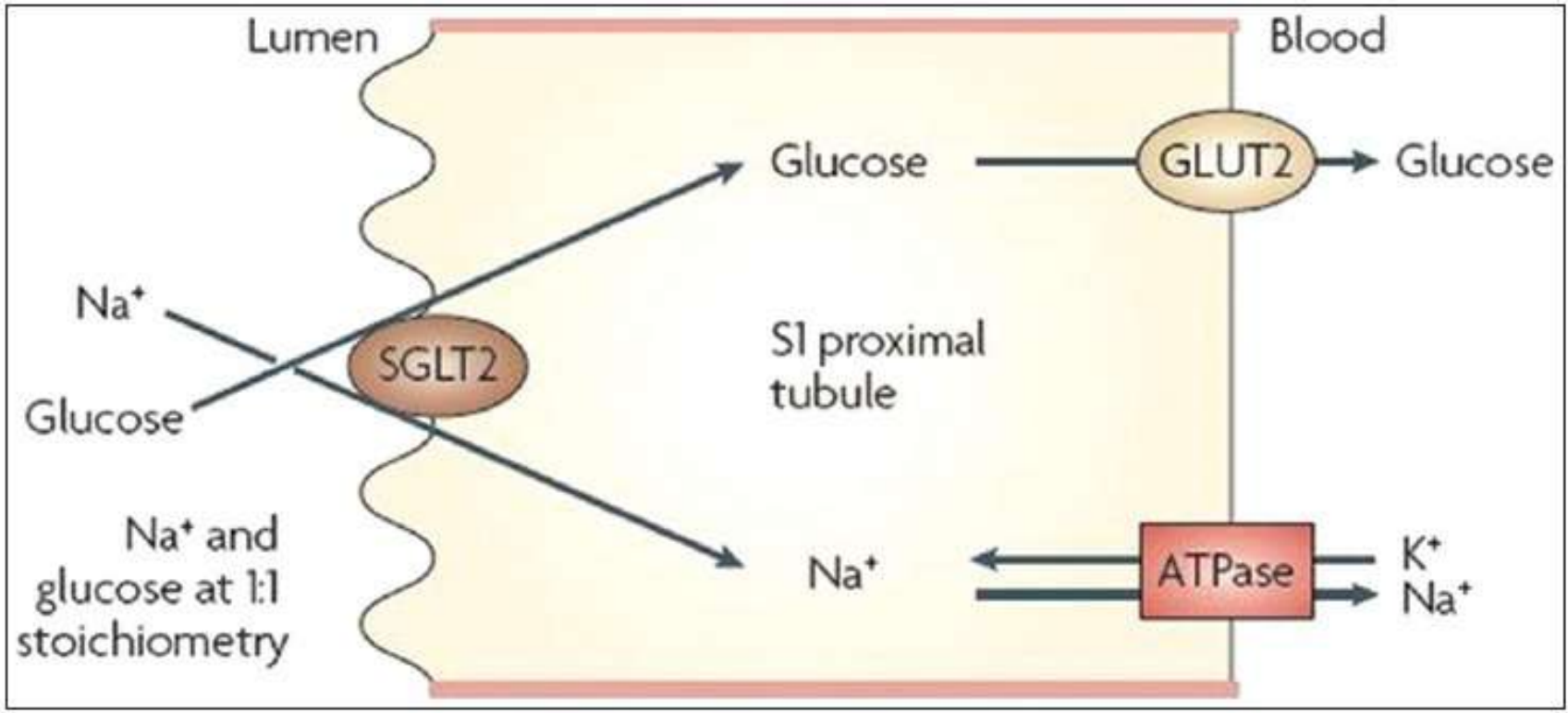
glucose

- Reabsorption of glucose occurs mainly in the proximal tubule and is mediated by 2 different transport proteins, SGLT1 and SGLT2.
- SGLT1, which are found in the straight section of the proximal tubule (S3), are responsible for approximately 10% of glucose reabsorption.
- The other 90% of filtered glucose is reabsorbed through by SGLT2, which are located in the convoluted section on the proximal tubule (S1)

	SGLT1	SGLT2
Site	Intestine, kidney	Kidney
Renal location	Late proximal straight tubule (S3 segment)	Early proximal convoluted tubule (S1 segment)
Sugar specificity	Glucose or galactose	Glucose
Glucose affinity	High (K_m=0.4 mM)	Low (K_m=2 mM)
Glucose transport capacity	Low	High
Renal glucose reabsorption (%)	~10%	~90%
Role	Dietary absorption of glucose and galactose, renal glucose reabsorption. Mutation in the gene for SGLT1 results in carbohydrate malabsorption and severe diarrhea	Renal glucose reabsorption. Inhibition of SGLT2 as a rational target of therapy for T2DM is based on pathology of familial renal glycosuria

SGLT: Sodium–glucose co-transporter, T2DM: Type 2 diabetes mellitus, Data from Ref.[5,10]

- The GLUT2 is present at many different sites including red blood cells, brain, and other tissues and is therefore not an appropriate site for pharmacological intervention.
- In contrast, **SGLT2** is specific to the **proximal tubule**, so that pharmacological inhibition will affect glucose reabsorption in the kidney but not in other tissues


Lumen
Blood
Glucose
GLUT2
Glucose
Na+
SGLT2
Glucose
S1 proximal tubule
Na+ and glucose at 1:1 stoichiometry
Na+
ATPase
K+
Na+

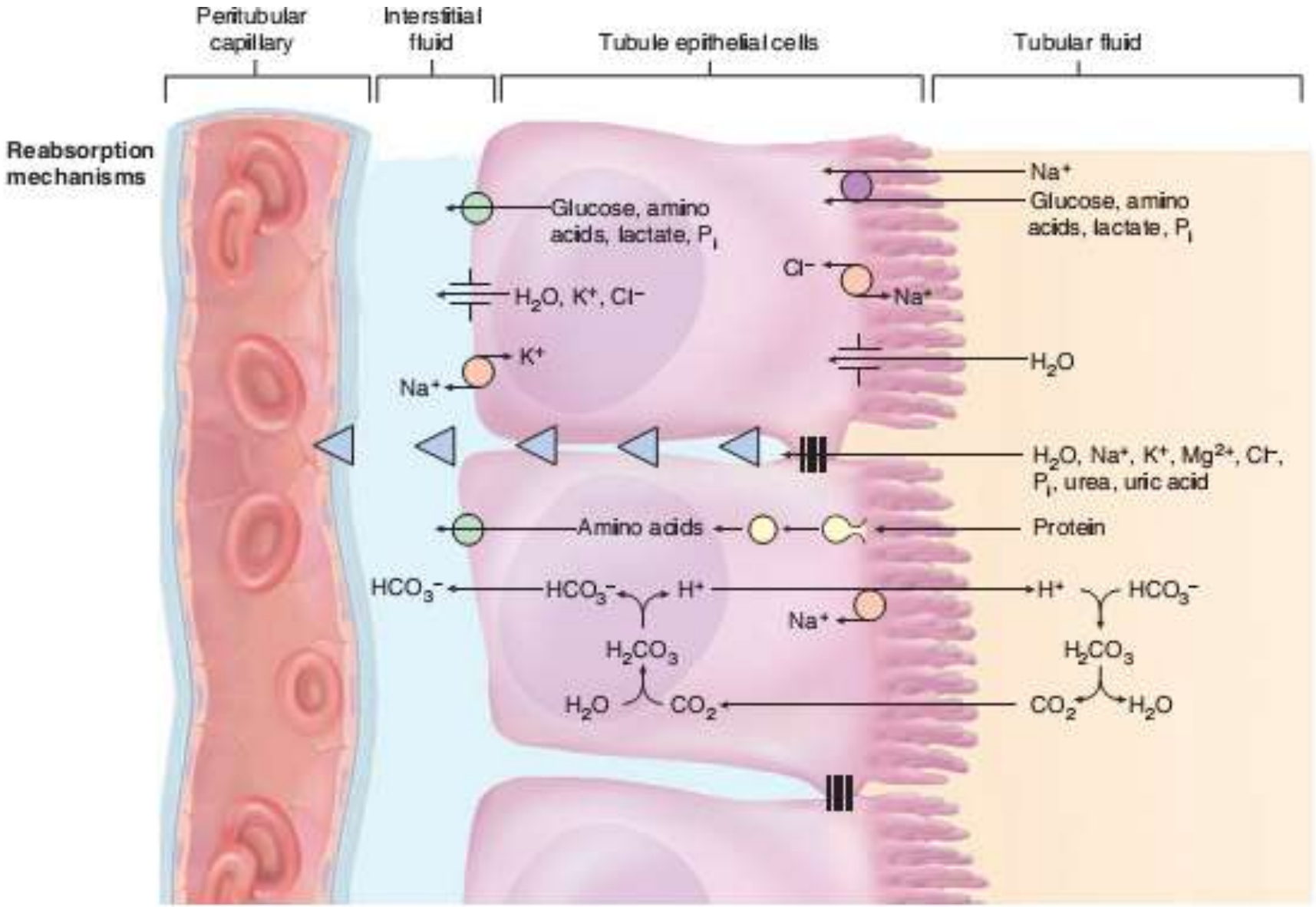
- Glucose is cotransported with Na by carriers called **sodium-glucose transport proteins (SGLTs).**
- Potassium, magnesium, and phosphate (Pi) ions diffuse through the paracellular route with water
- Urea diffuses through the tubule epithelium with water. The nephron as a whole reabsorbs 40% to 60% of the urea in the tubular fluid

The maximum rate of reabsorption

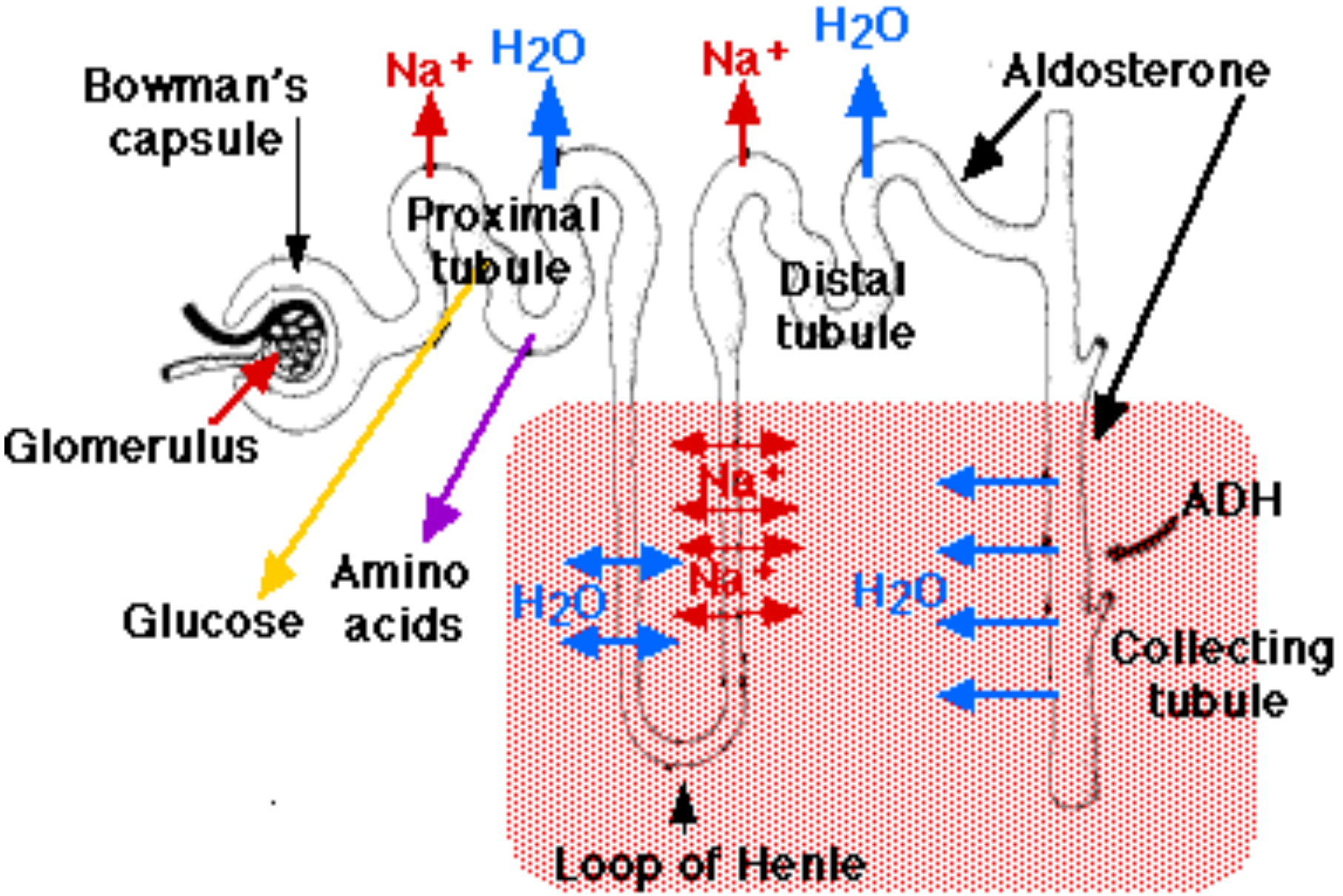
- is the *transport maximum (Tm), which is reached when the transporters* are saturated.
- Glucose: Tm 320 mg/min, normally: 125 mg/min
- Glucosuria

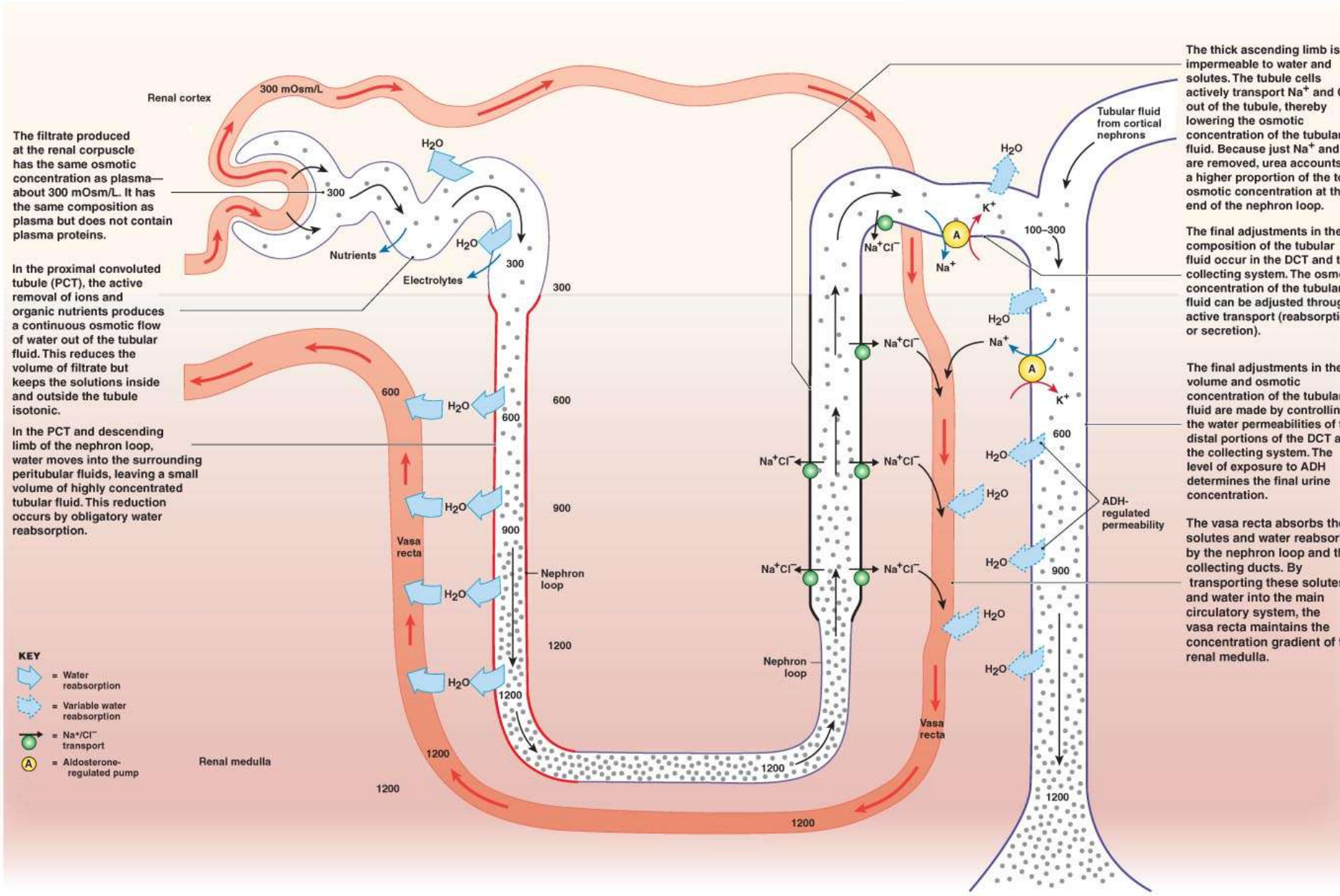
- At this threshold, the system becomes saturated and the maximal resabsorption rate, the glucose transport maximum (Tm_G), is reached.
- No more glucose can be absorbed, and the kidneys begin excreting it in the urine, the beginning of glycosuria.
- The Tm_G varies among individuals but it has an average value of approximately 375 mg/min for healthy subjects.
- Although 11 mmol/L (198 mg/dL) represents the theoretical threshold glucose concentration, the actual concentration varies due to nephron heterogeneity, resulting in slight differences in actual glucose reabsorption levels and Tm_G values between individual tubules.

- **> 178 liters per day** – air direabsorbsi kembali melalui **passive reabsorbstion**
- **glucose** (blood sugar) seluruhnya diabsorbsi melalui transport aktif
- **Sodium ions (Na+)** & ion lain diabsorbsi partial melalui **transport aktif**



23.14 Mechanisms of Reabsorption in the Proximal Convoluted Tubule.


Bowman's capsule
Na+
H2O
Na+
H2O
Aldosterone
Proximal tubule
Distal tubule
Glomerulus
Glucose
Amino acids
Na+
Na+
H2O
H2O
ADH
Collecting tubule
Loop of Henle



Chloride

- negative chloride ions tend to follow the positive sodium ions by electrical attraction,
- water reabsorption raises the Cl concentration in the tubular fluid, thereby creating a gradient favorable to Cl reabsorption, especially in the second half of the
- tubule.
- In the transcellular route, Cl is apically absorbed
- by various antiports that exchange Cl for other anions.
- K-Cl symport transports the chloride ions out the basolateral cell surfaces.

Bicarbonate

- tubule cells generate bicarbonate and hydrogen ions internally by the reaction of CO2 and water.
- The hydrogen ions are pumped into the tubular fluid by the Na-H antiport mentioned earlier, and neutralize the HCO3 in the tubule.
- The bicarbonate ions are pumped out the base of the cell and enter the blood.
- Thus one HCO3 disappears from the tubule fluid as one new HCO3
- appears in the blood, and the net effect is the same as if an HCO3 ion had actually crossed the epithelium from tubular fluid to blood.

Secretion

- Substansi bergerak ke tubulus kolektivus distal dari kapiler
- Meliputi : Hydrogen ions (H+), potassium ions (K+), ammonia (NH3), and certain drugs
- **Melalui : active transport** mechanism or as a result of **diffusion**

Secretion

- **Waste removal. Urea, uric acid, bile acids, ammonia,** catecholamines, and a little creatinine are secreted
- Tubular secretion of uric acid compensates for its reabsorption earlier in the PCT and accounts for all of the uric acid in the urine.
- Tubular secretion also clears the blood of pollutants, morphine, penicillin, aspirin, and other drugs.

Collecting duct

- the osmolarity of the extracellular fluid is four times as high deep in the medulla as it is in the cortex,
- the medullary portion of the CD is more permeable to water than to NaCl.
- Therefore, as urine passes down the CD through the increasingly salty medulla, water leaves the tubule by osmosis, most NaCl and other wastes remain behind, and the urine becomes more and more concentrated

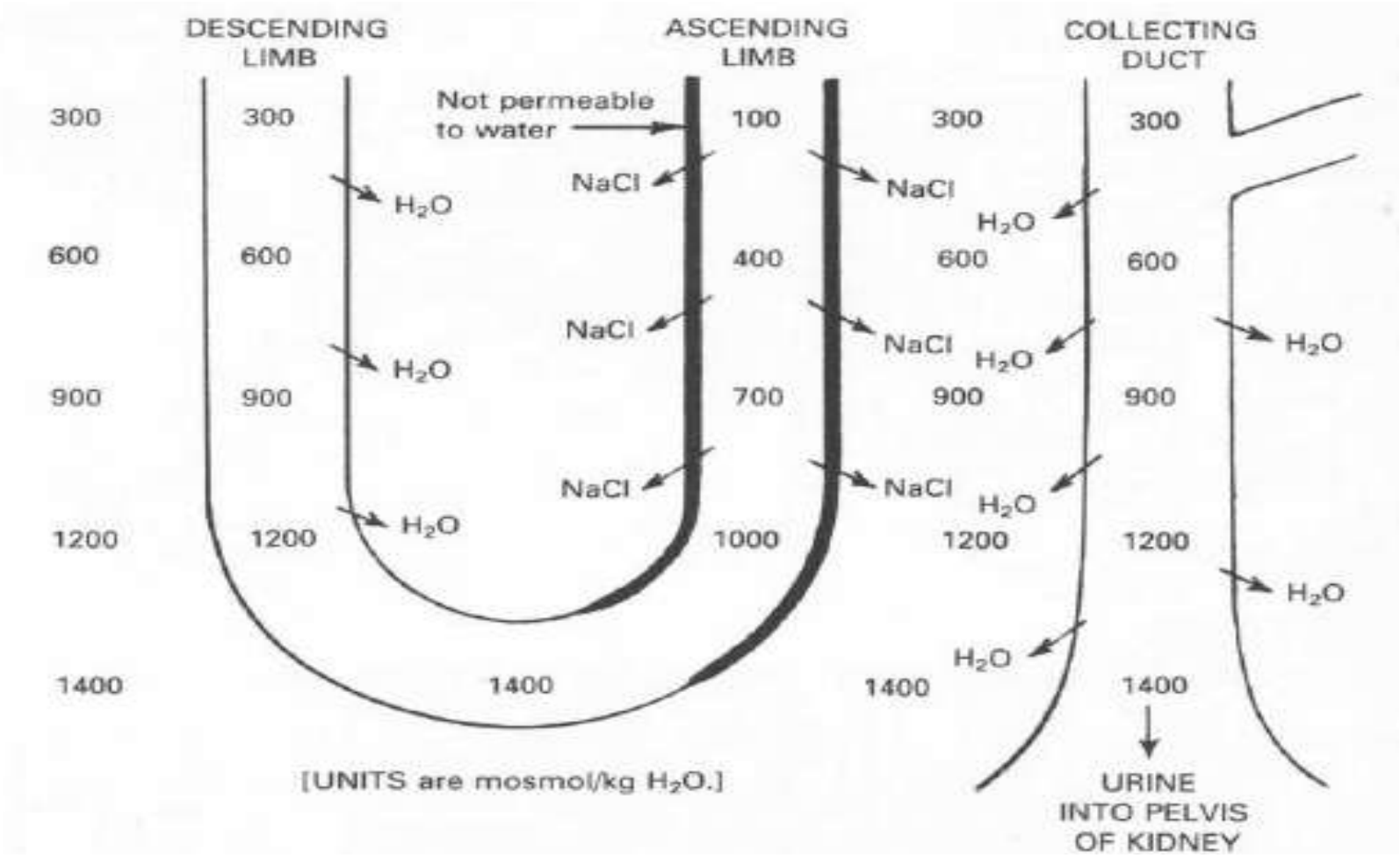
- The **principal cells are the more** abundant; they have receptors for ADH, Aldosteron and are involved chiefly in salt and water balance.
- The **intercalated cells are fewer in number**
  - **high density** of mitochondria, reabsorb K, secrete H into the tubule lumen, and are involved mainly in acid-base balance

Principal cells are the main Na+ reabsorbing **cells** and the site of action of aldosterone, K+-sparing diuretics, and spironolactone. Type A and B **intercalated cells** make up the second **cell** type in the **collecting duct** epithelium. Type A **intercalated cells** mediate acid secretion and bicarbonate reabsorption.

Countercurrent multiplier

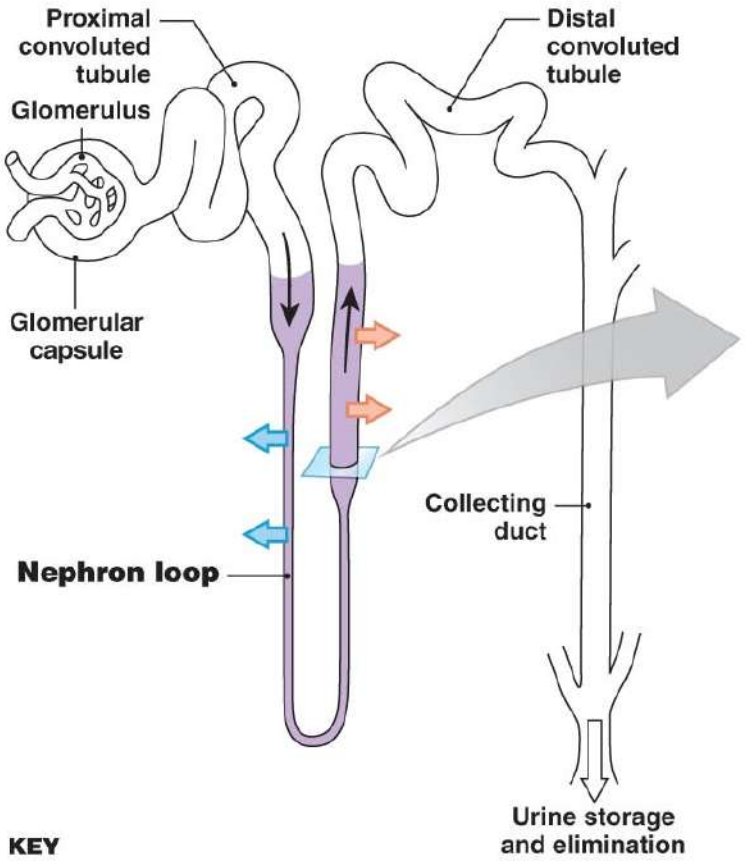
- By Wirz (1951) and Hargitay and Kuhn (1951)
- A small difference in osmotic pressure (the single effect) is multiplied by countercurrent flow in adjacent channels of the limbs of Henle's loop to produce a large axial difference in osmotic pressure between the renal cortex and the tip of the renal papilla; that is, the multiplier generates a hypertonic renal medulla

Loop of Henle

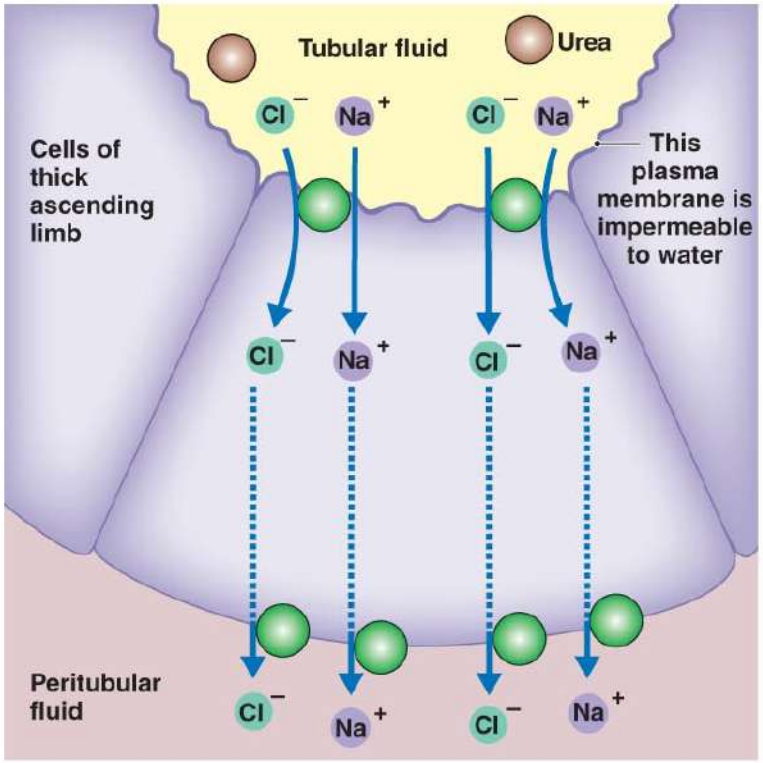


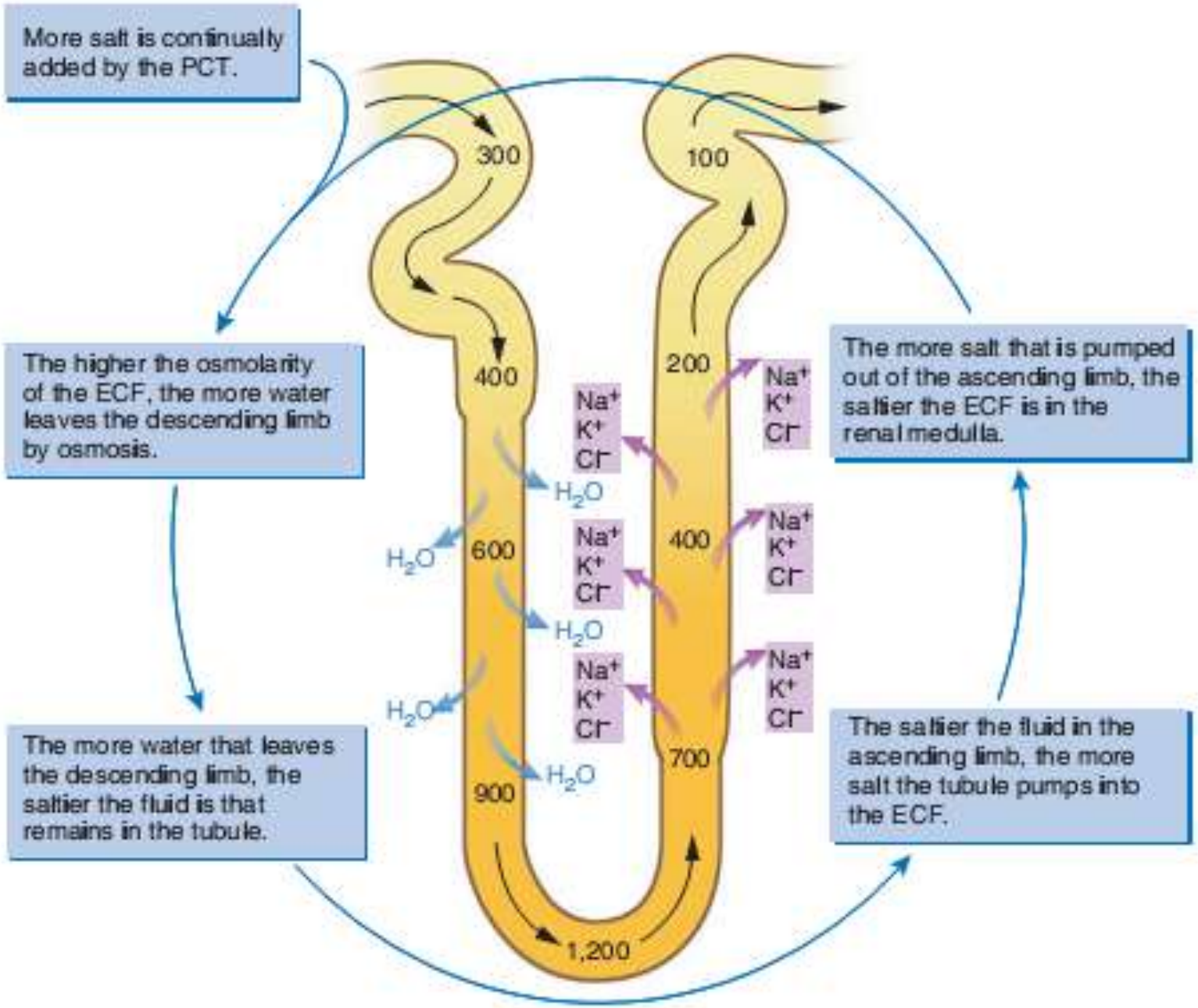
Absorbsi air pada loop henle descenden dan NaCl pada bag.ascenden menyebabkan osmolaritas naik di medulla ginjal

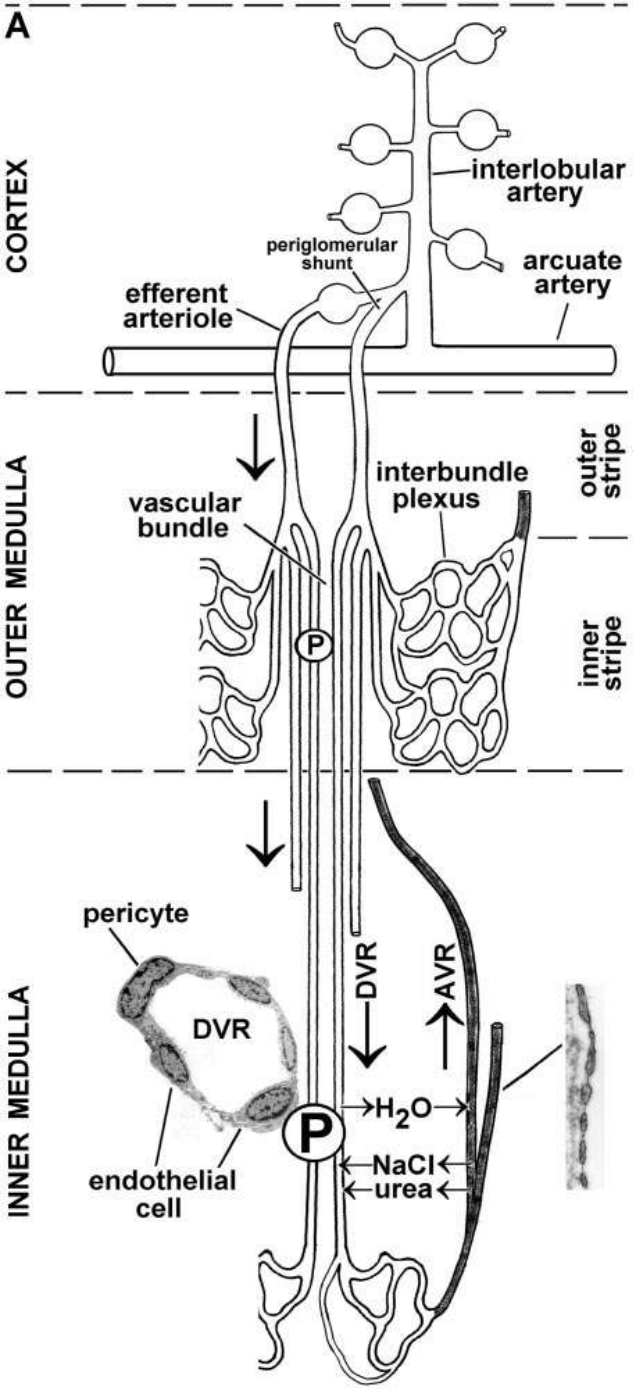
The close proximity of the thin descending and the thick ascending limbs of the nephron loop, which enables the exchange called countercurrent multiplication

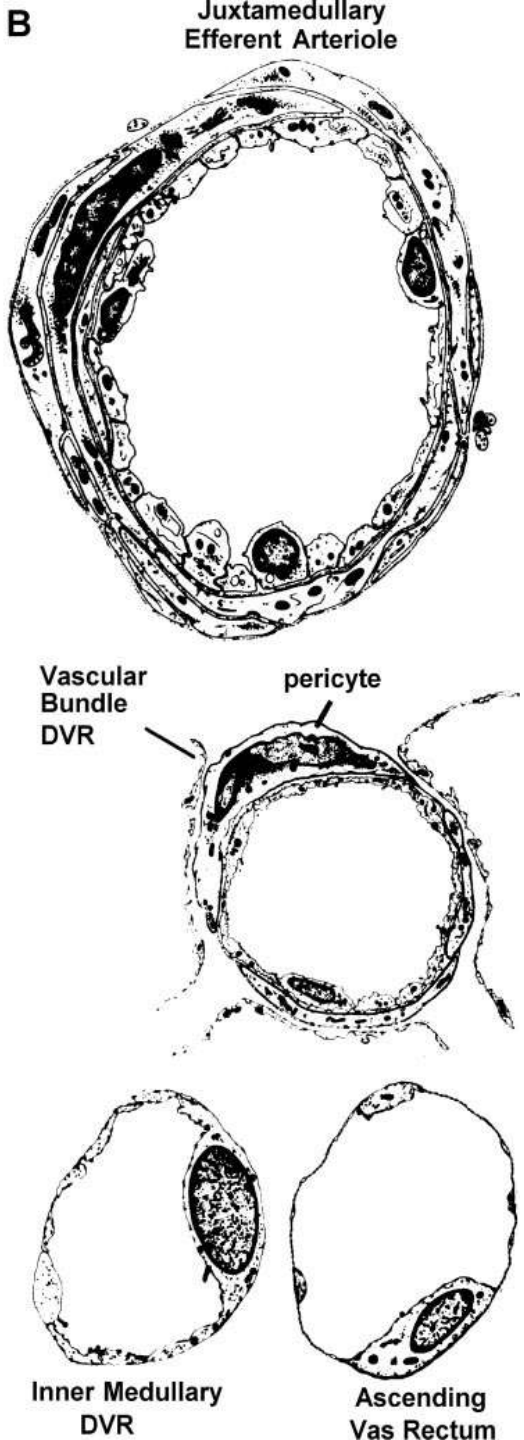


The transport activities performed by cells of the thick ascending limb




More salt is continually added by the PCT.
The higher the osmolarity of the ECF, the more water leaves the descending limb by osmosis.
The more water that leaves the descending limb, the saltier the fluid is that remains in the tubule.
The saltier the fluid in the ascending limb, the more salt the tubule pumps into the ECF.
The more salt that is pumped out of the ascending limb, the saltier the ECF is in the renal medulla.
300
400
600
900
1,200
100
200
400
700
Na+ K+ Cl−
H2O





Countercurrent exchanger
Role of vasa recta
Preserve medullary hypertonicity

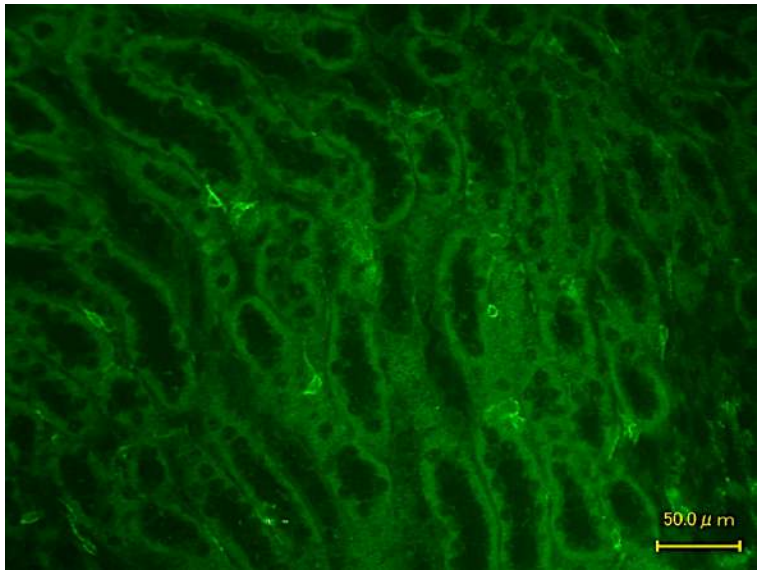
Deep VR: continous EC
With pericyte. Aquaporin transfer H20 from DVR to AVR in the inner Medula just before tip of medula

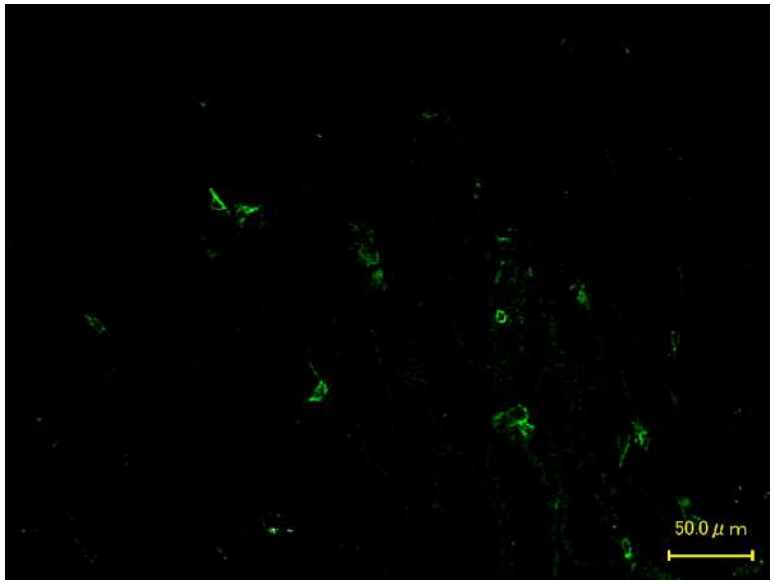
Ascending VR: fenestrated EC.
Trapped NaCl & urea in medula.
Urea Carrier in AVR

Am J Physiol Regul Integr Comp Physiol 284: R1153–R1175, 2003;
10.1152/ajpregu.00657.2002

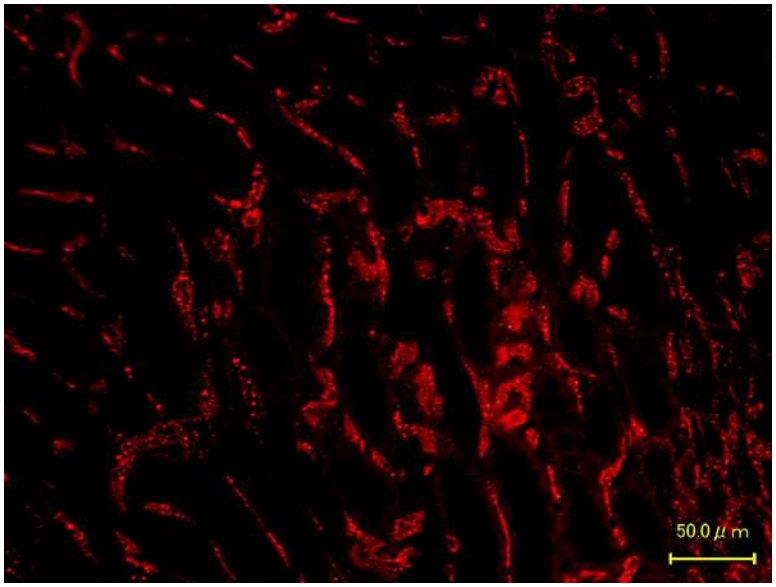
VASA RECTA

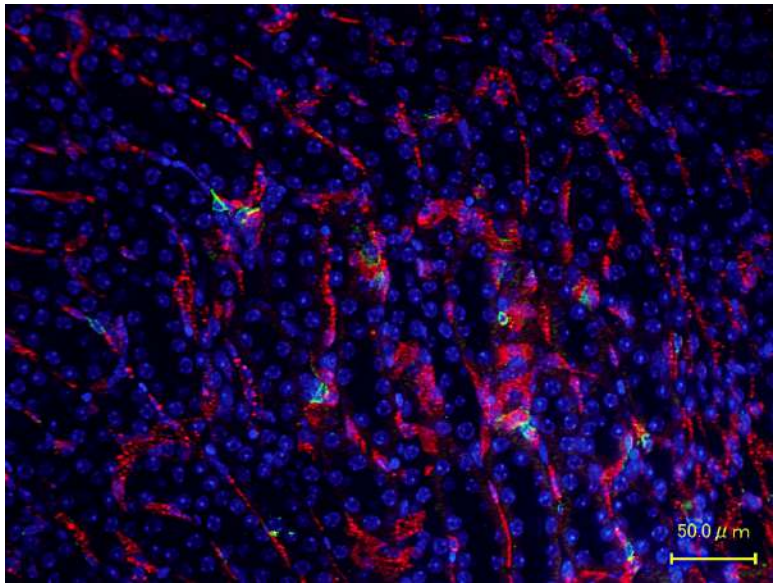
NG2 / pericyte





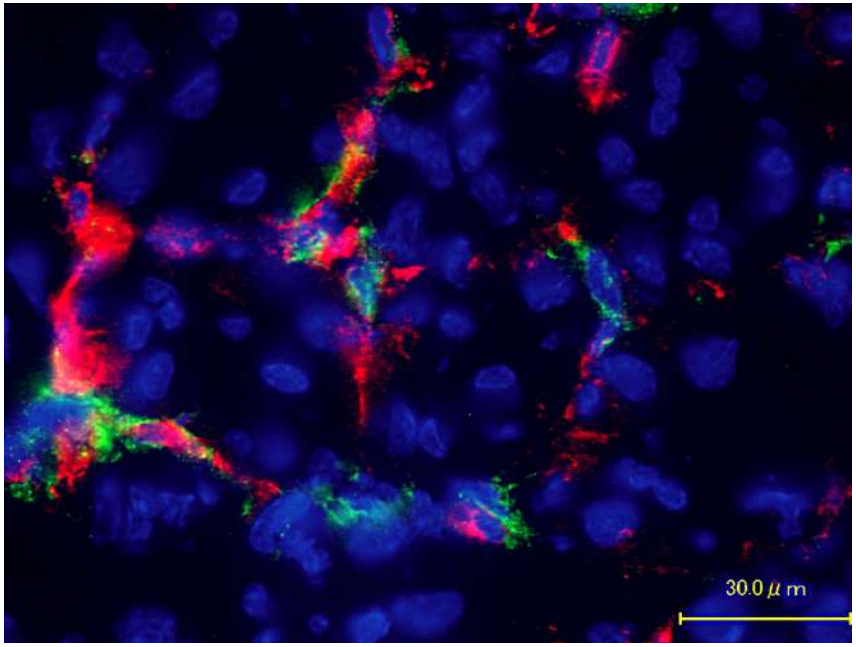
CD31 / EC



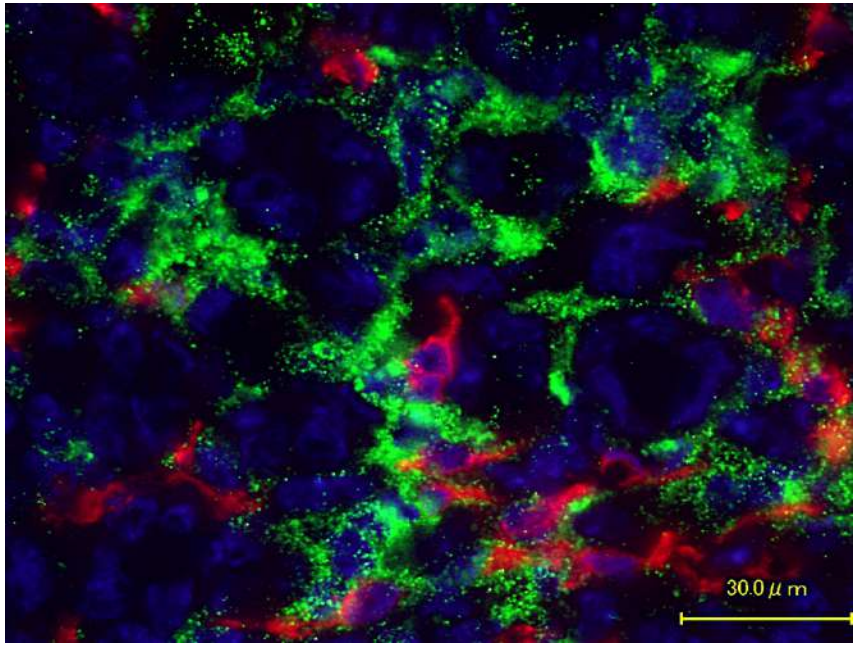


Pericyte detachment, early event to fibrosis and capillary loss

EC / Pericyte / normal kidney



EC / Pericyte / fibrosis kidney



Fungsi ginjal dlam regulasi pH darah

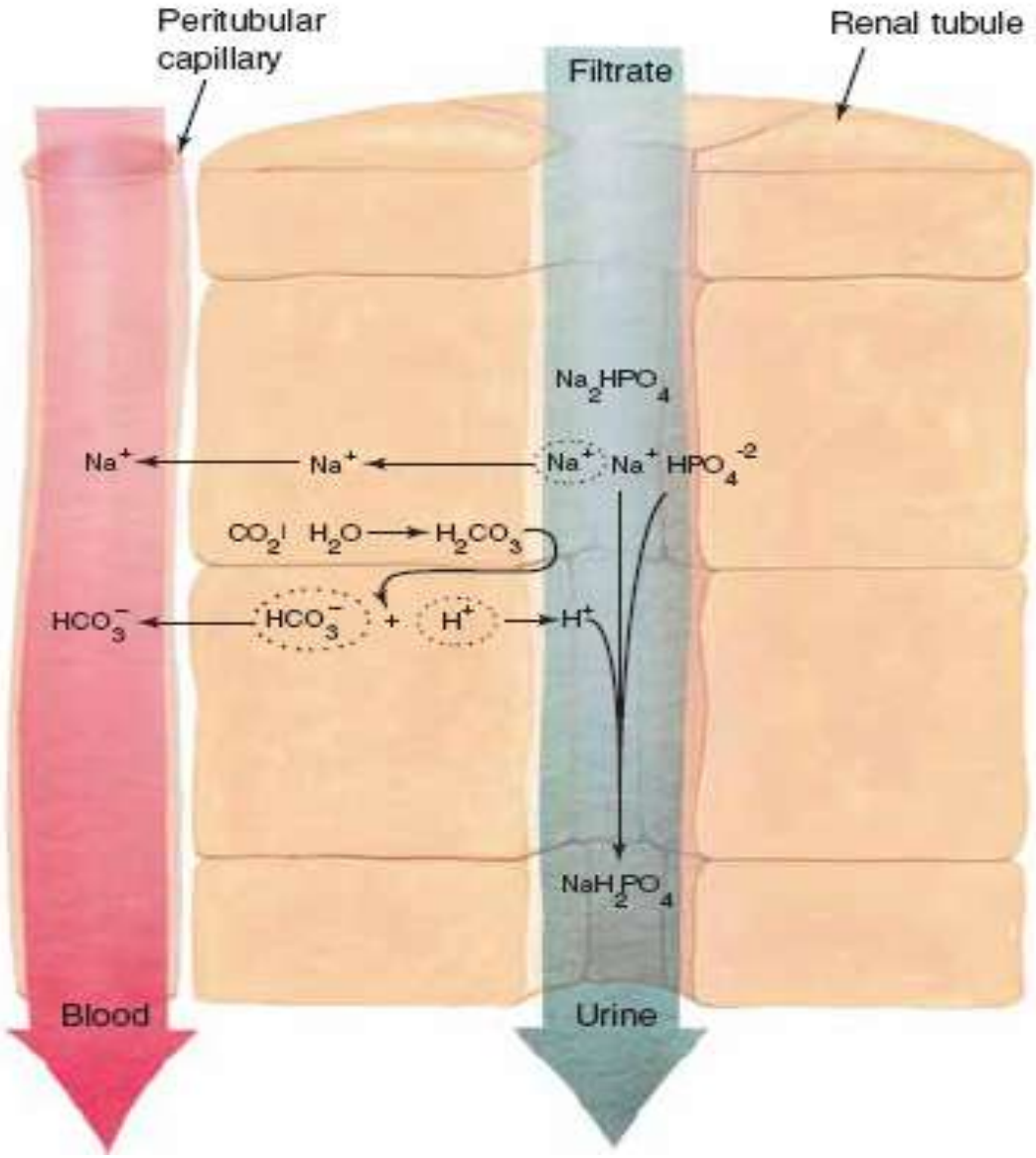


Figure 19–4. The phosphate buffer system. The reactions are shown in a kidney tubule. See text for description.
QUESTION: From where does the kidney tubule cell get a hydrogen ion to excrete?

Exresi H pada tubulus renalis : System Buffer

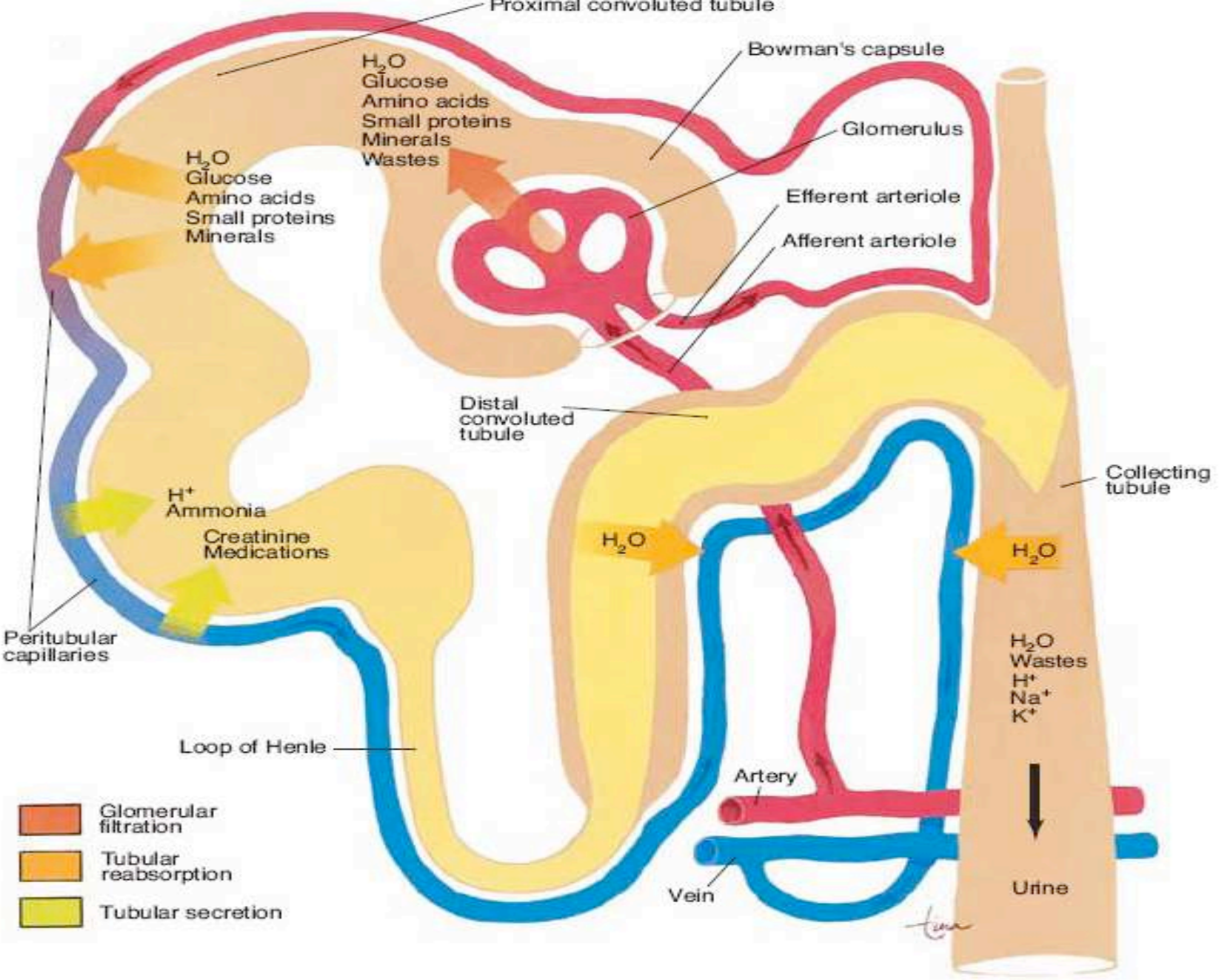
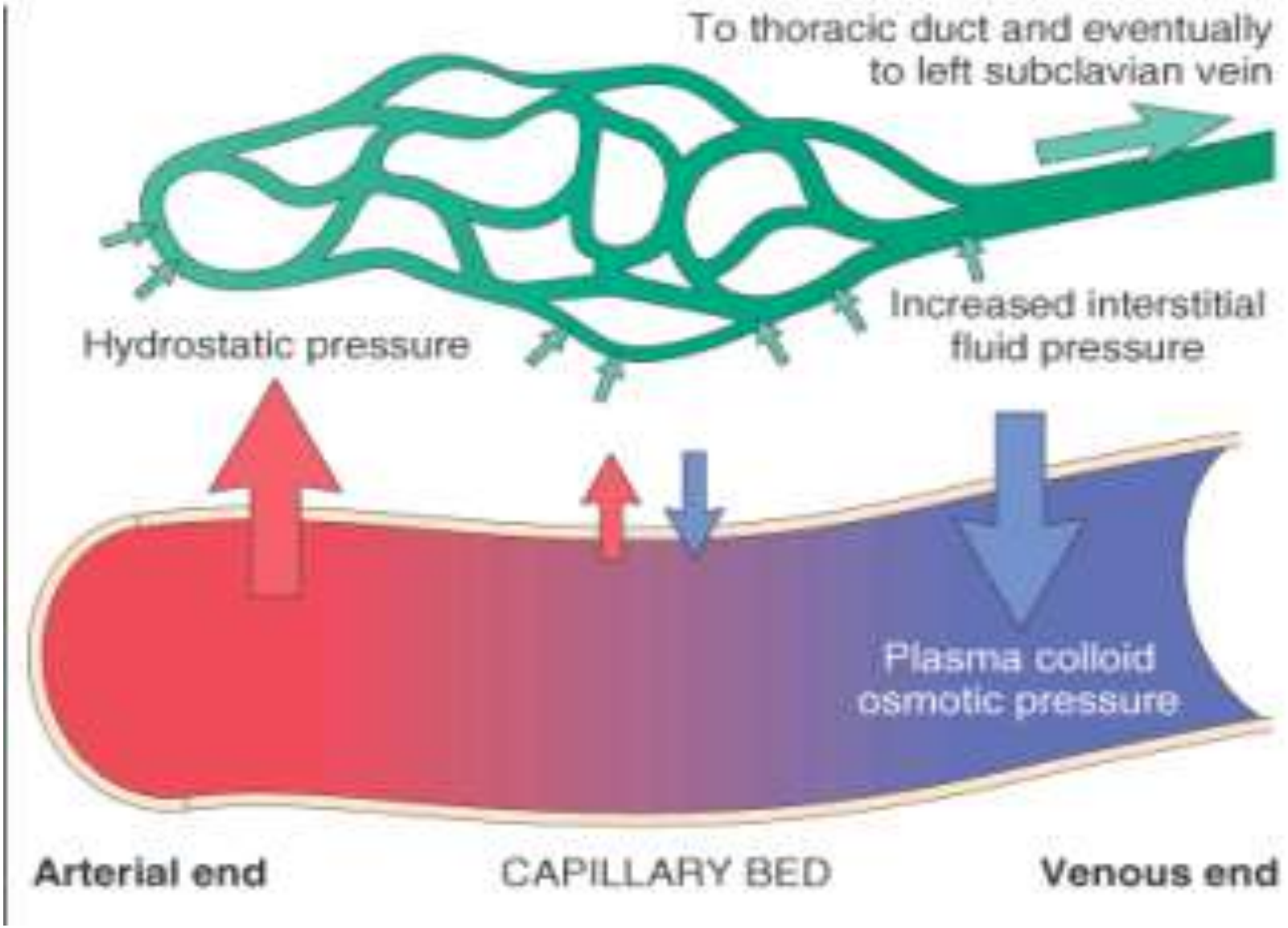


Figure 18–4. Schematic representation of glomerular filtration, tubular reabsorption, and tubular secretion. The renal tubule has been uncoiled, and the peritubular capillaries

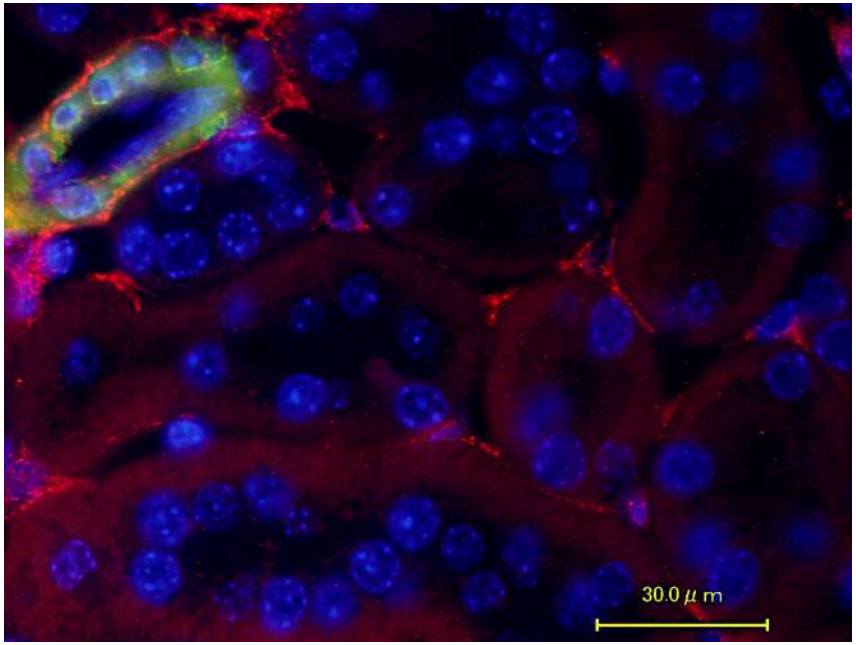
Faktor-faktor filtrasi



Gangguan pada faktor-faktor tersebut menimbulkan penumpukan cairan (edem)

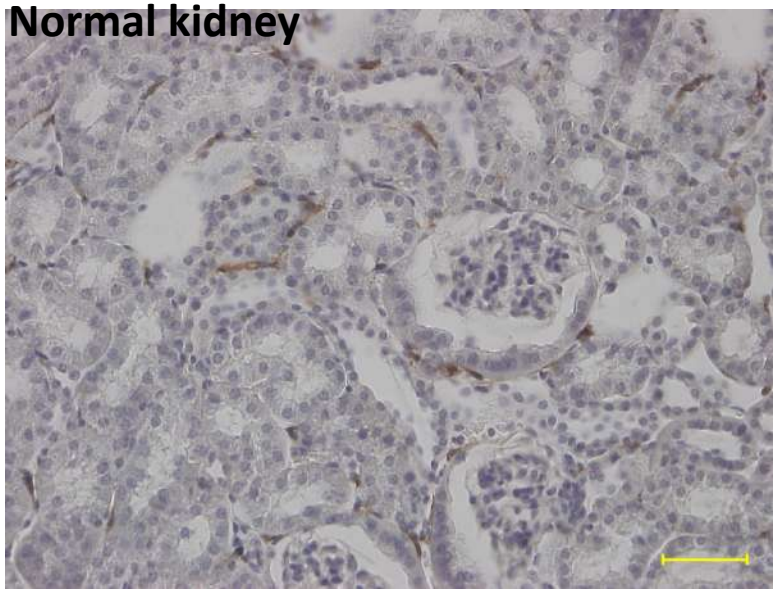
Cells of the kidney

Interstitial cells –fibroblast
Positive in smooth muscle cells in vessel
Same origin
Erithropoetin producing cells
PDGFRβ

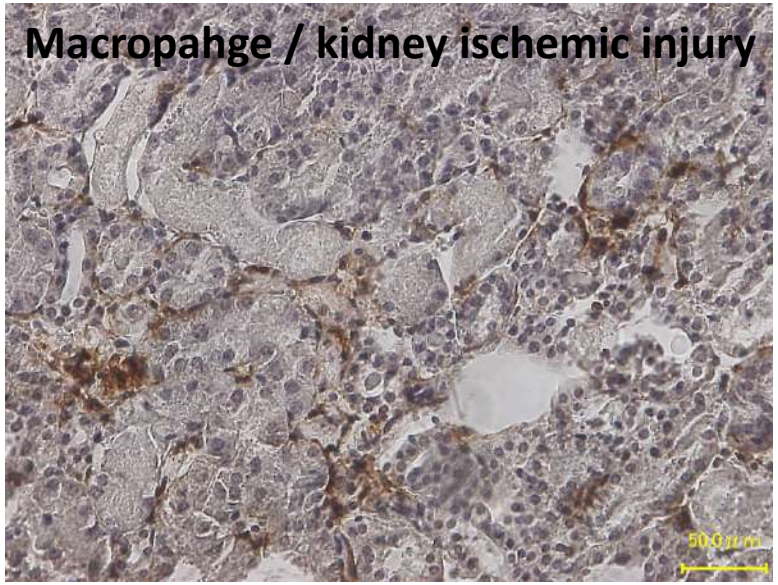


Dendritic cells
IHC of F4/80 (Macrophage & dendritic cells)

Normal kidney



Macropahge / kidney ischemic injury

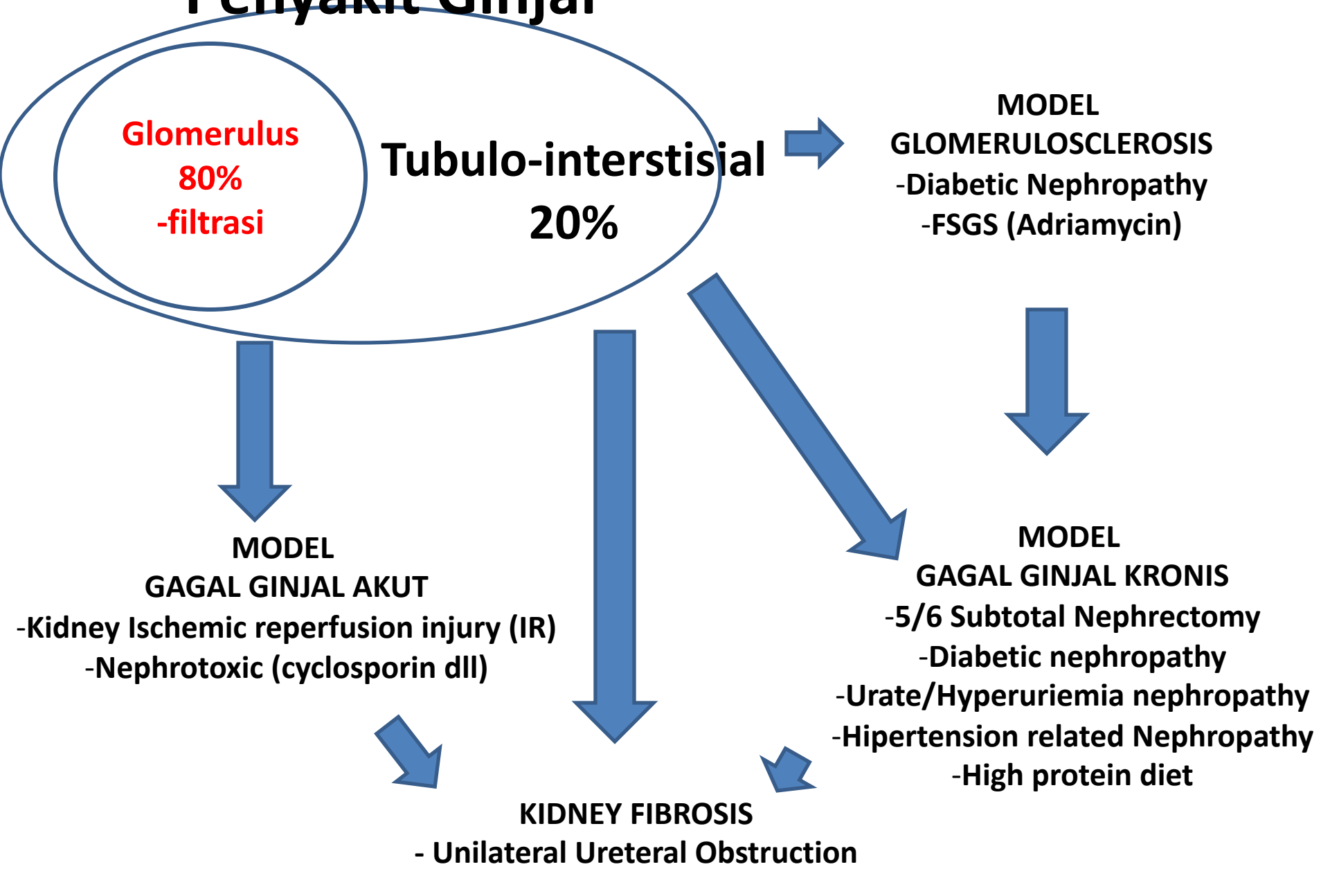


Gangguan ginjal

- 80% gangguan ginjal terdapat pada glomerulus
  - Gangguan filtrasi: proteinuria, albuminuria, penurunan GFR
  - Glomerulosclerosis, Syndrome alport
  - Podocytopathy, gangguan membrane basal, Endothelium glomerulus-glycocalyx

- 20% terdapat pada tubulo-interstisial
  - 2 faktor: iskemik-reperfusion dan nefrotoksik
  - Cedera tubulus: penipisan brush-border, apoptosis sel epithel tubulus, penumpukan cash intraluminal (Tom-horsfall body).
  - Fibrosis ginjal: hypoxia, fibrosis, ekspansi myofibroblast

Penyakit Ginjal



Model-model pada gangguan tubulo-interstisial

Gangguan filtrasi

- Glomerulosclerosis
  - Adriamycin nephropathy
- Diabetic nephropathy

Gangguan tubulointerstisial

- Model ischemic-reperfusion injury : acute kidney injury model
  - Bilateral Renal pedicles clamping
- Renal artery clamping
  - Blood pressure
- Nephrotoxic agent: cyclosporin, COX inhibitor
- Fibrosis : Unilateral ureteral ligation
- Gabungan
  - Renal ablation (5/6 sub-total nephrectomy)

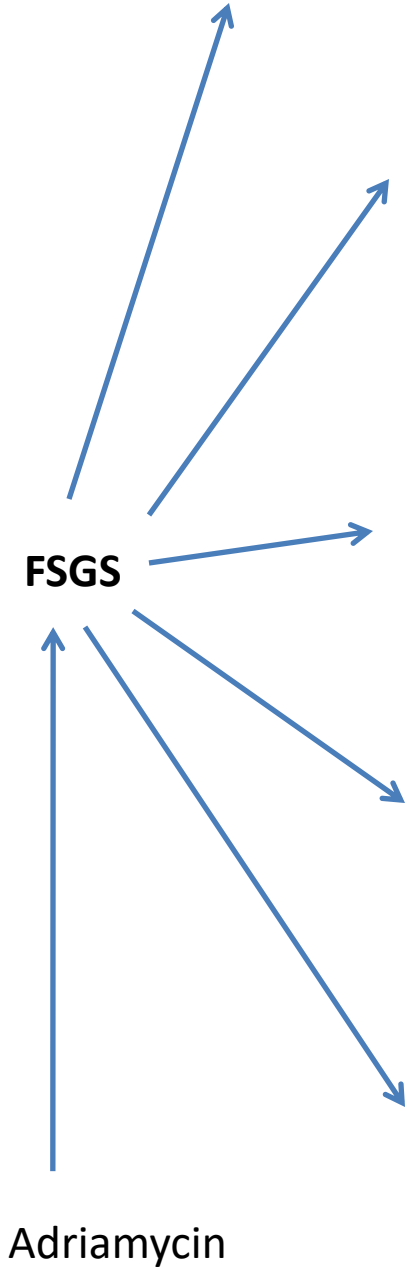
Gangguan filtrasi

- Diabetic nephropathy is the leading cause of end-stage renal disease and is clinically charac-terized by proteinuria and progressive renal insufficiency.
- Increase in mesangial matrix as the main lesion of diabetic glomerulopathy.
  - correlates closely with both proteinuria and deterioration of renal function
  - Effect of proteinuria in tubules
- Glomerular basement membrane (GBM) thickening (Kimmelstiel and Wilson)

Focal Segmental Glomerulosclerosis

Histopathology : Glomerulosclerosis

Tubular atrophy and Intertisial fibrosis



Endothelial Dysfunction / Vascular Toxycity due to Doxorubicin

- EC morphology (SEM)
- EC reactivity test by aorta/renal artery?

Podocyte damage :

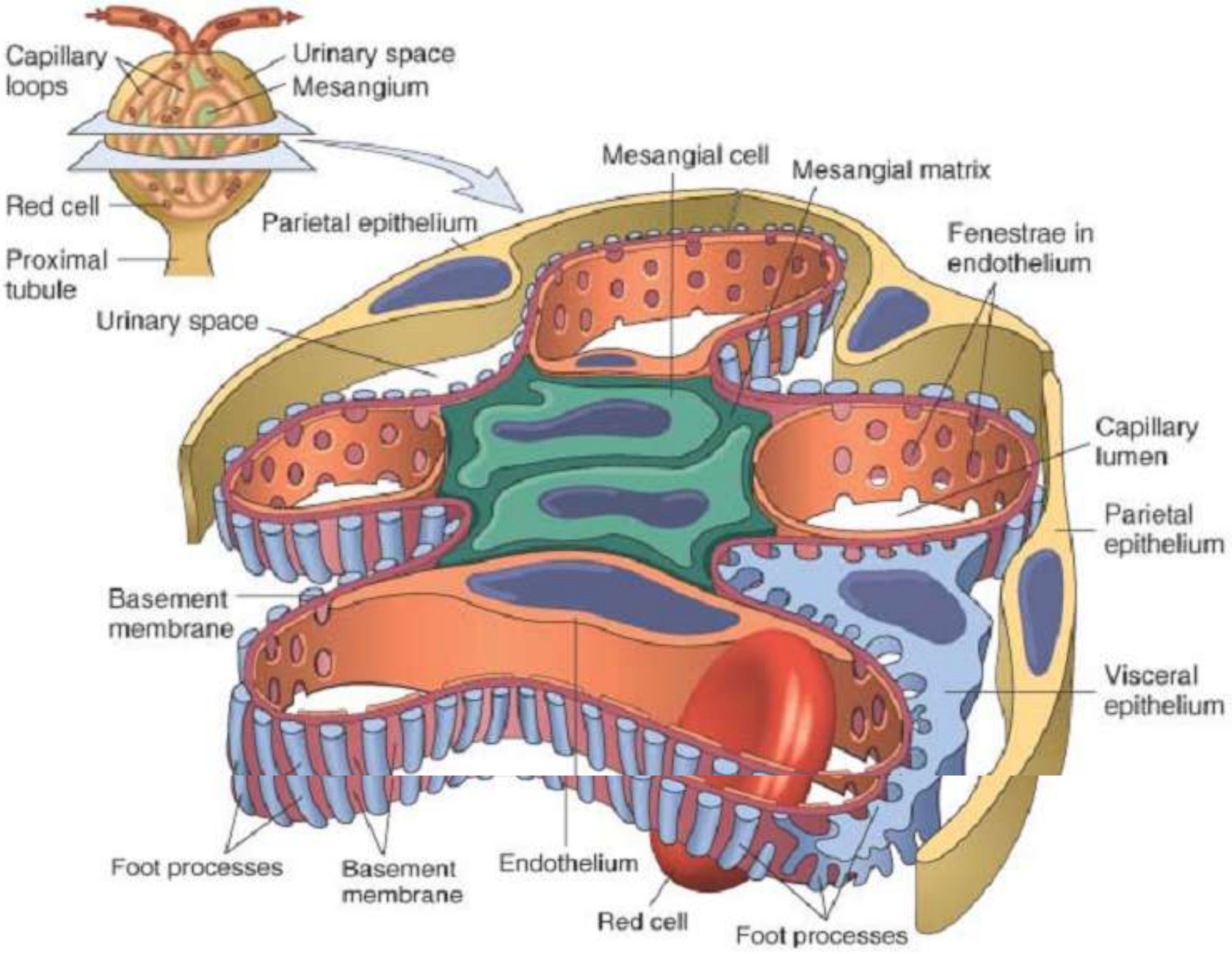
- Slit diaphragm protein expression (Immunofluorescence)
- Podocyte spesific marker (Immunoflurosence)
- podocyte morphology (SEM)
- in vitro : podocyte is treated with Adriamycin with Silencing ET-

Glomerular Basement membrane :

- Collagen type IV
- Heparan sulphat PG
- Proteoglycan synthesis analizes? GAG elongation?
- Enzymes that produces Heparan Sulphat

Tubular atrophy and interstitial fibrosis

- matrix deposition
- Proteoglycan composition and expression in matrix


Capillary loops
Urinary space
Mesangium
Red cell
Proximal tubule
Parietal epithelium
Mesangial cell
Mesangial matrix
Fenestrae in endothelium
Urinary space
Capillary lumen
Parietal epithelium
Basement membrane
Visceral epithelium
Foot processes
Basement membrane
Endothelium
Red cell
Foot processes

Podocyte

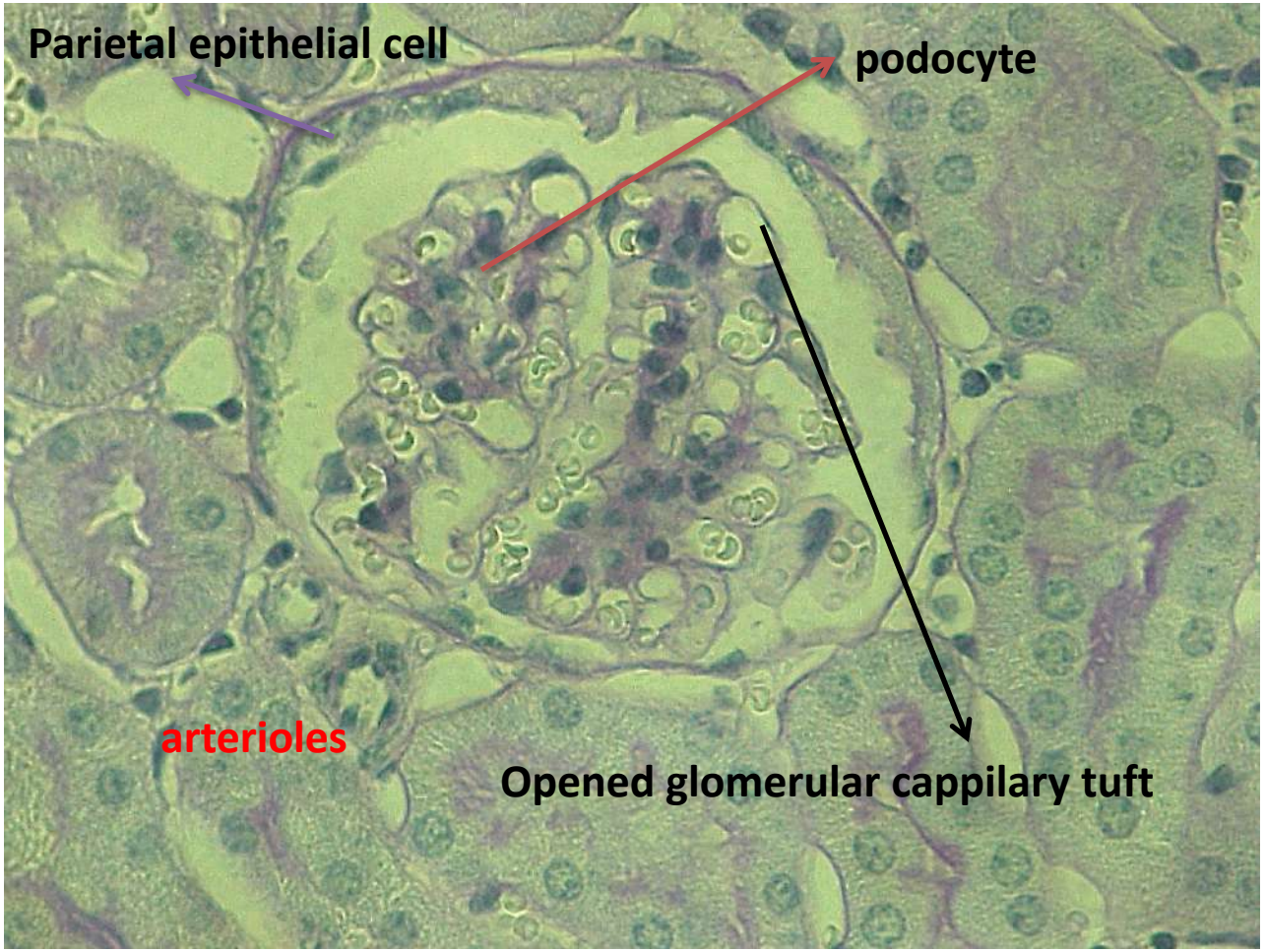
- the integrity of the slit diaphragm is one of the principal determinants of the permselective properties of the glomerular filtration bar-rier, and knowledge of its molecular architecture will help elucidate the role that the slit diaphragm plays in proteinuria.
- Podocytopenia may exacerbate the development of pro-teinuria because a denuded GBM can come into contact with Bowman’s capsule and promote synechiae formation, an initial step in the development of glomerulosclerosis

Glomerulosclerosis index, from PAS staining

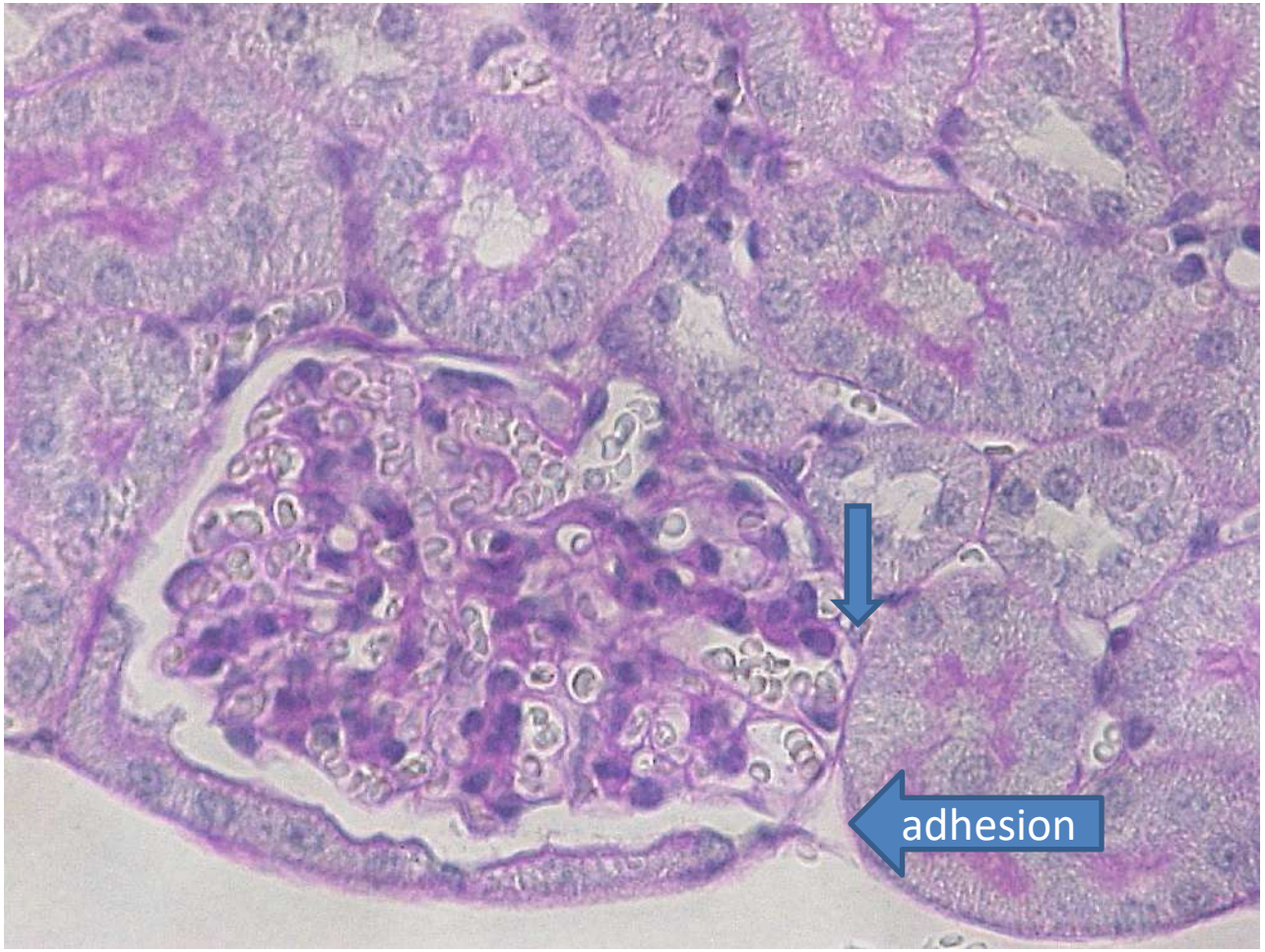
- Based on PAS staining
- Histopahology examination of kidney (glomerulus)
  - Adhesion of glomerular tuft and bowman capsule
  - Mesangial expansion and matrix accumulation
  - Glomerular tuft obstruction
  - cellular expansion in bowman space

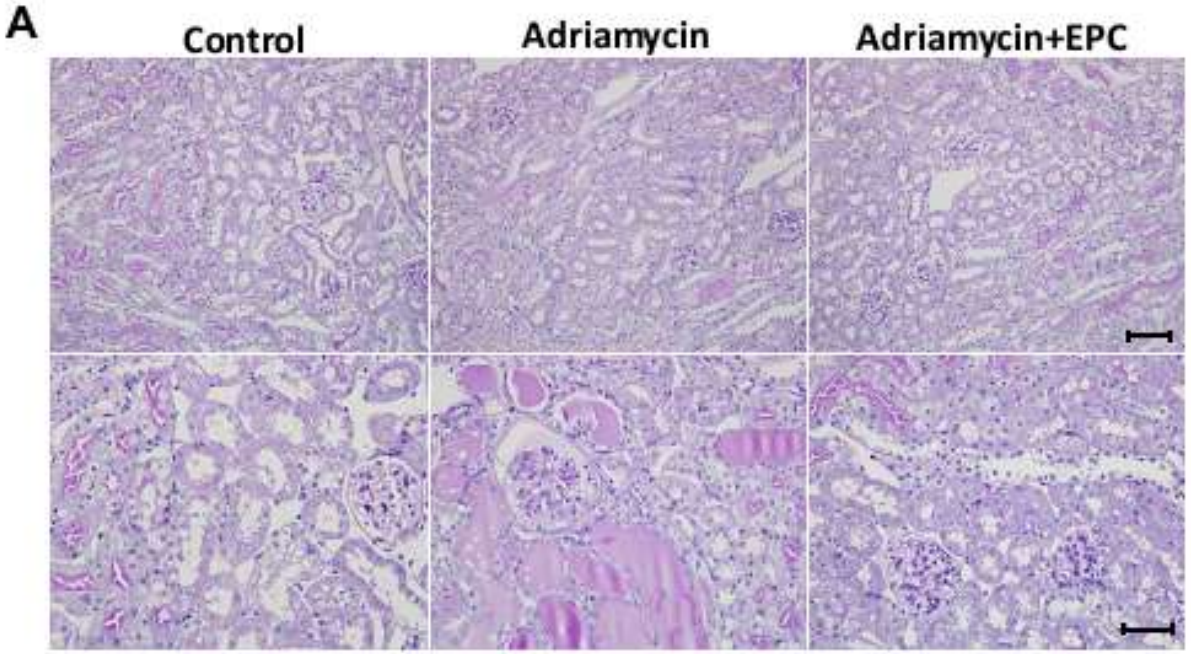
- 0, normal;
- 1,slight glomerular damage, the mesangial matrix and/or hyalinosis with focal adhesion, involving ,25% of the glomerulus;
- 2, sclerosis of 25% to 50%;
- 3, sclerosis of 50% to 75%; and
- 4, sclerosis of .75% of the glomerulus

Normal glomerulus



1,slight glomerular damage, the mesangial matrix and/or hyalinosis with focal adhesion, involving ,25% of the glomerulus;





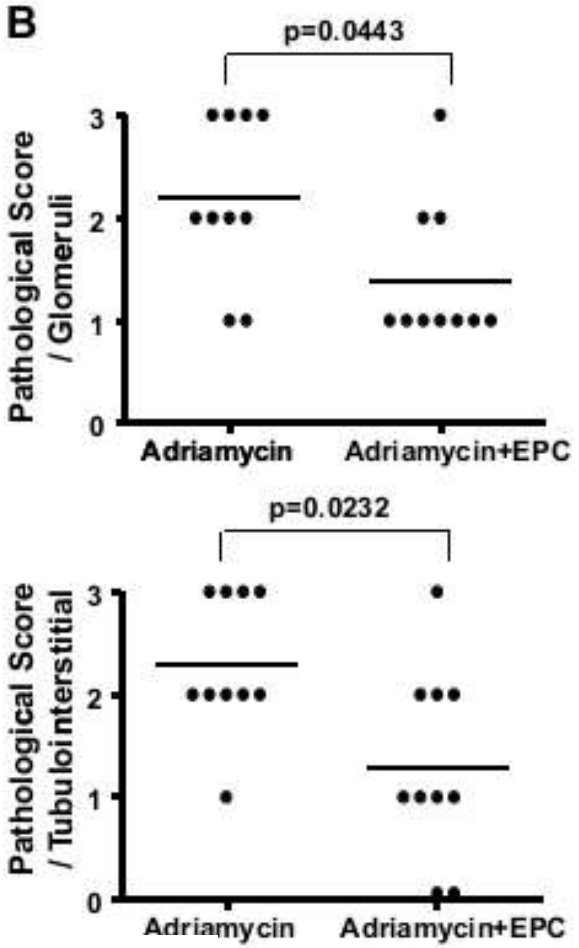
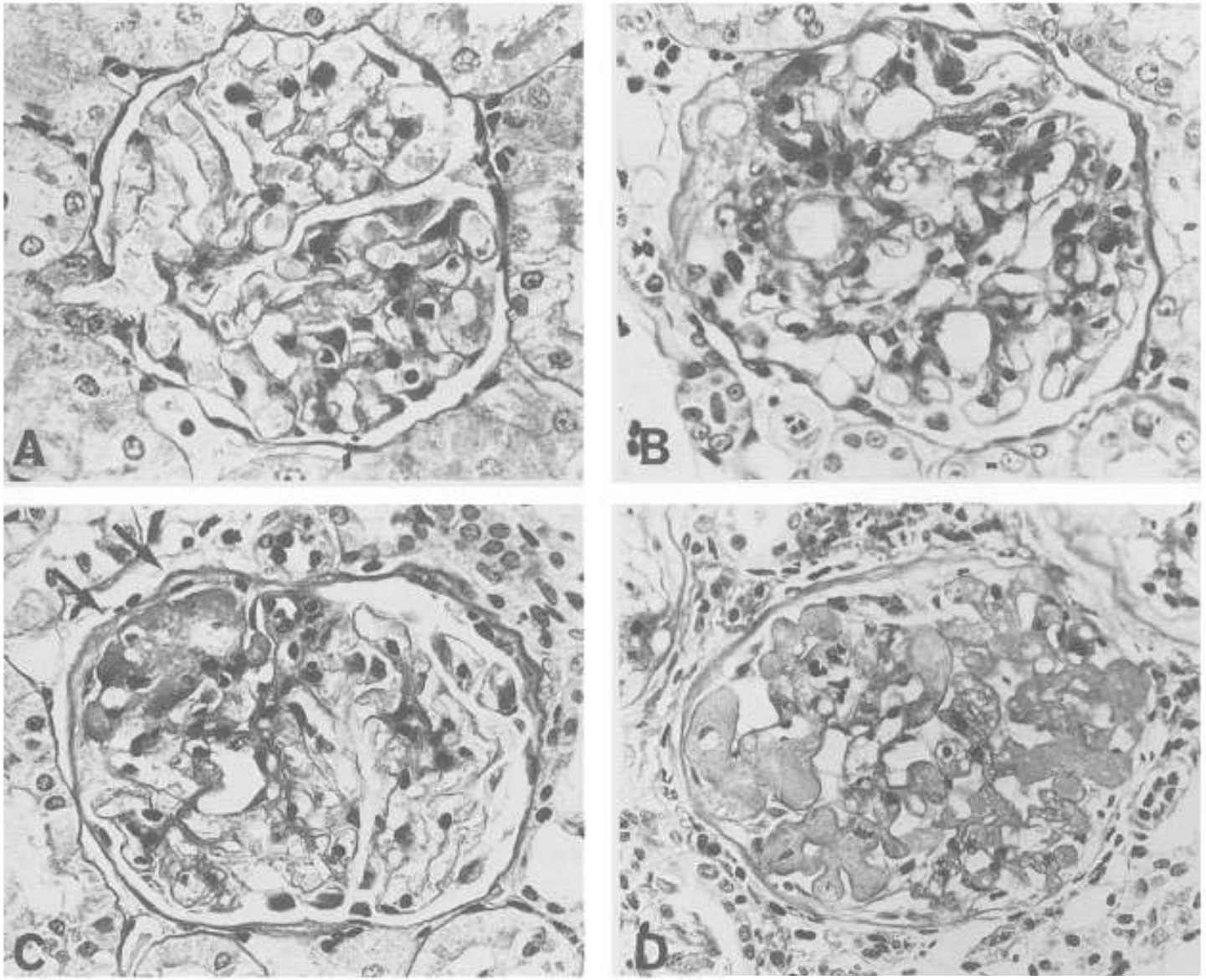


Figure 2. Representative periodic acid-schiff-stained kidney sections and pathological scores. **A:** Normal control (**left**); adriamycin-treated (**middle**); adriamycin followed by adoptive transfer of EPCs (**right**). **Upper panel**, bar = 100 μm; **lower panel**, Scale bar = 50 μm. See text for detailed description. **B:** Semiquantitative scores of glomerular and tubulointerstitial injury. All kidneys were harvested on day 21 postadriamycin.

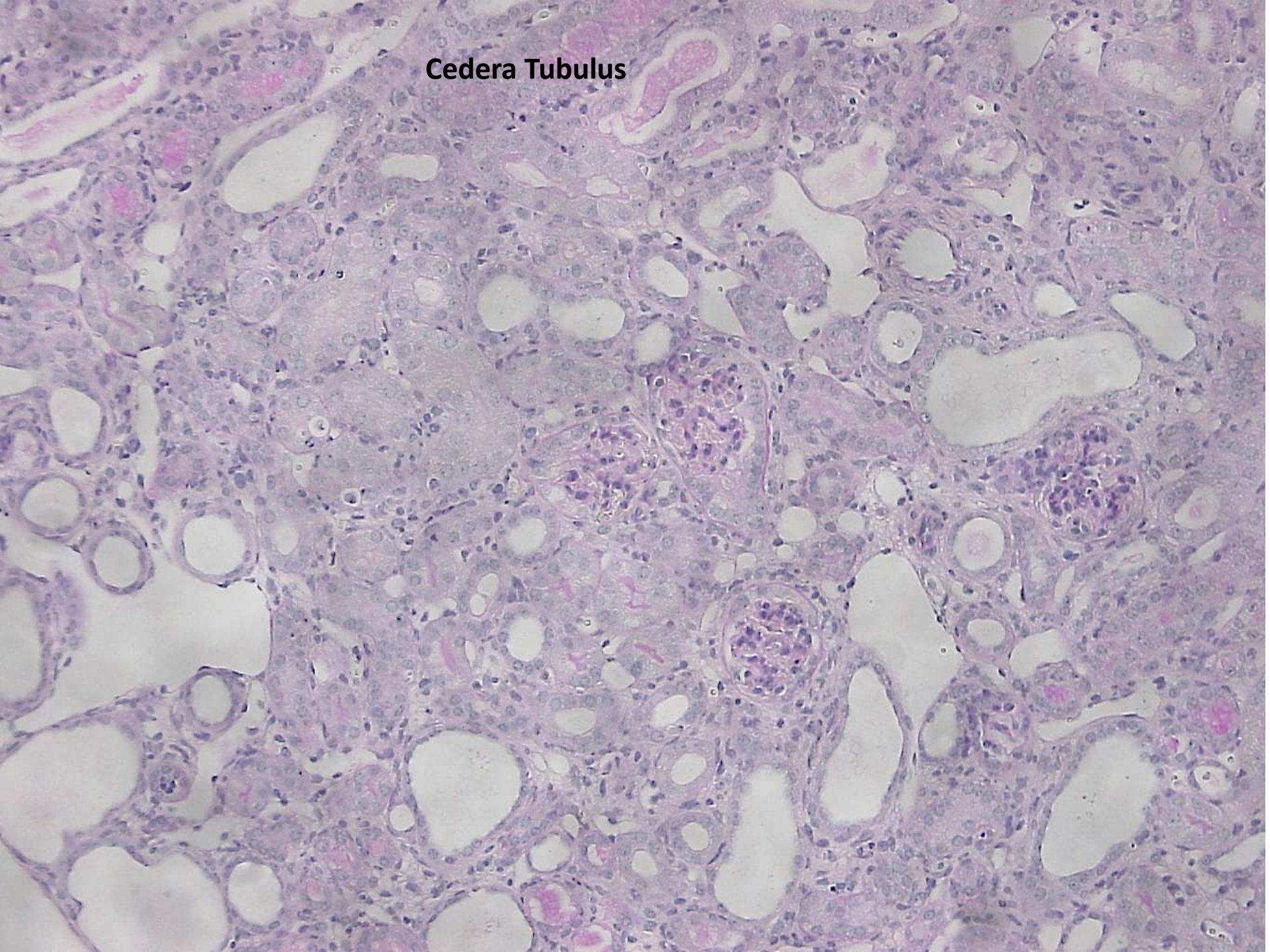
Adryamicin nephropathy



Kidney International, Vol. 29 (1986), pp. 502–510

Tubulo-intertitial injury

- Twenty fields of tubulointerstitial area in the
- cortex were observed and graded as following:
- 0, normal;
- 1, the area of interstitial inflammation and fibrosis, tubular atrophy, and dilation
- with cast formation involving ,25% of the field;
- 2, lesion area between 25% and 50% of the field; and
- 3, lesions involving .50% of the field.
- *(Hypertension* 2000;36;561-568)


Cedera Tubulus

Kidney Fibrosis

- Kidney fibrosis is regarded as the final common pathway for most forms of progressive renal disease, and involves glomerular sclerosis and/or interstitial fibrosis.

- Severity of interstitial lesions in many forms determined the deterioration of renal function of renal disease, both in animal models and in patients (Youhua Liu, JASN; 2008).

- Tubulointerstitial fibrosis is the best indicator of the progression of renal dysfunction (Risdom, 1968. Nath KA, 1992).

Models progressivitas

- leading within 24 h to reduced renal blood flow and glomerular filtration rate
- followed within several days by hydronephrosis, interstitial inflammatory infiltration (macrophages), and tubular cell death attributable to apoptosis and necrosis
- progression to a severely hydronephrotic kidney with marked loss of renal parenchyma takes place over 1–2 weeks, with more severe fibrosis in the neonate than the adult

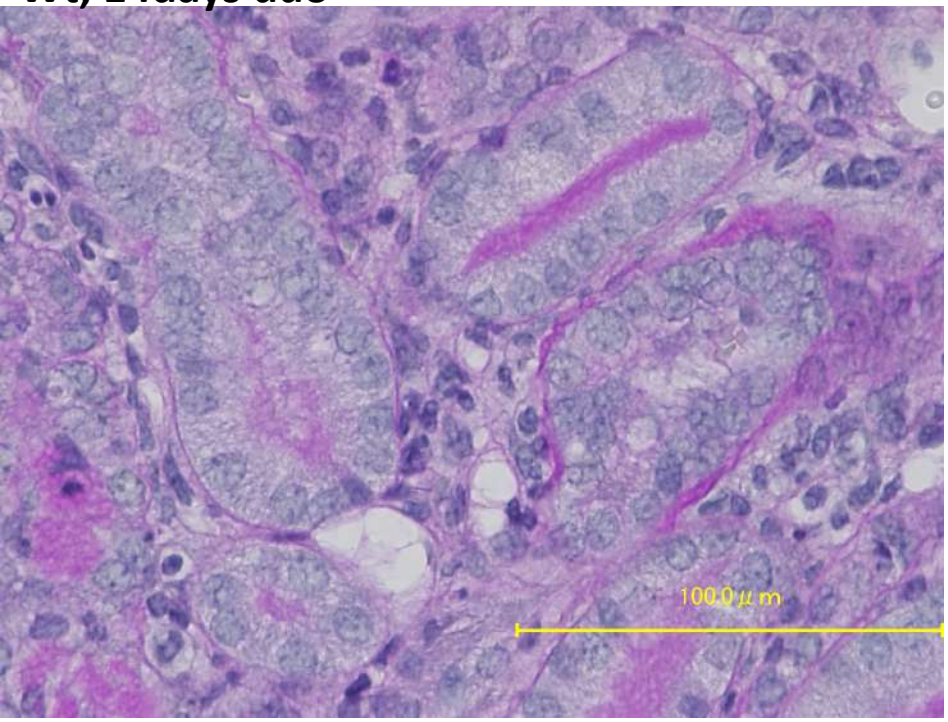
Alpha SMA, myofibroblast

- interstitial fibrosis is a remarkably monotonous process characterized by *de novo* activation of -smooth muscle actin (SMA)–positive myofibroblasts, the principal effector cells that are responsible for the excess deposition of interstitial ECM under pathologic conditions
- **The key cellular mediator of fibrosis is the myofibroblast, which when activated serves as the primary collagen-producing cell.**
- **Myofibroblasts are generated from a variety of sources including resident mesenchymal cells, epithelial and endothelial cells in processes termed epithelial/endothelial-mesenchymal (EMT/EndMT) transition, as well as from circulating fibroblast-like cells called fibrocytes that are derived from bone-marrow stem cells (***J Pathol 2008;* ***214: 199–210)***

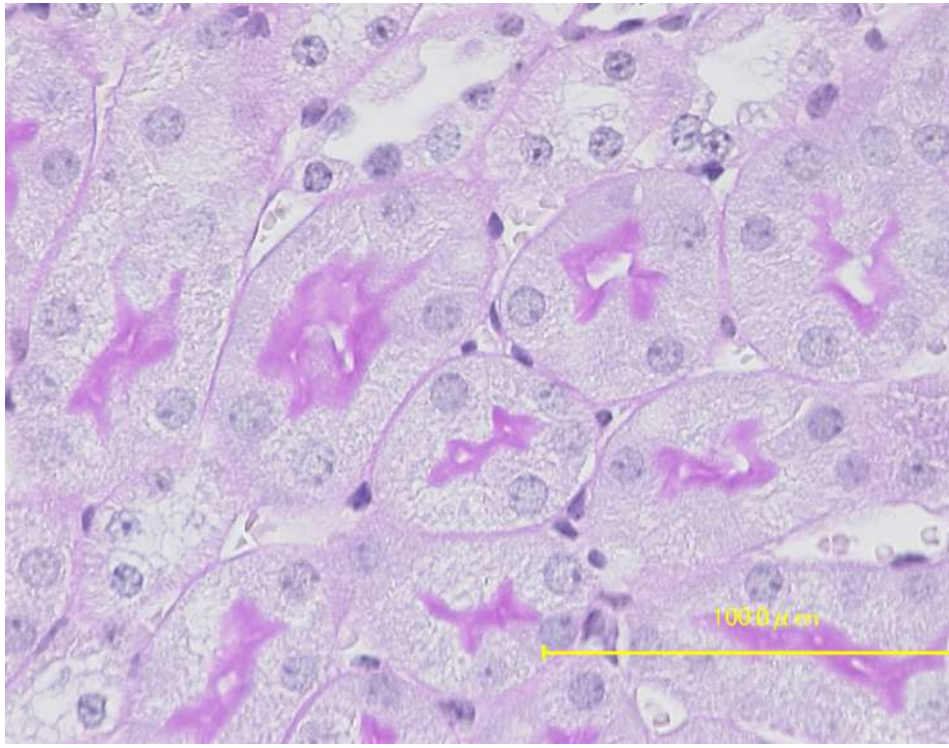
Tubular atrophy

Atrophic tubules were identified by their irregular, thickened, and sometimes duplicated basement membranes. The number of atrophic tubules per field at 400 magnification was counted, and 12 fields per kidney were analyzed (***The American Journal of Pathology, Vol. 171, No. 3, September 2007)***

Wt, 14days uuo



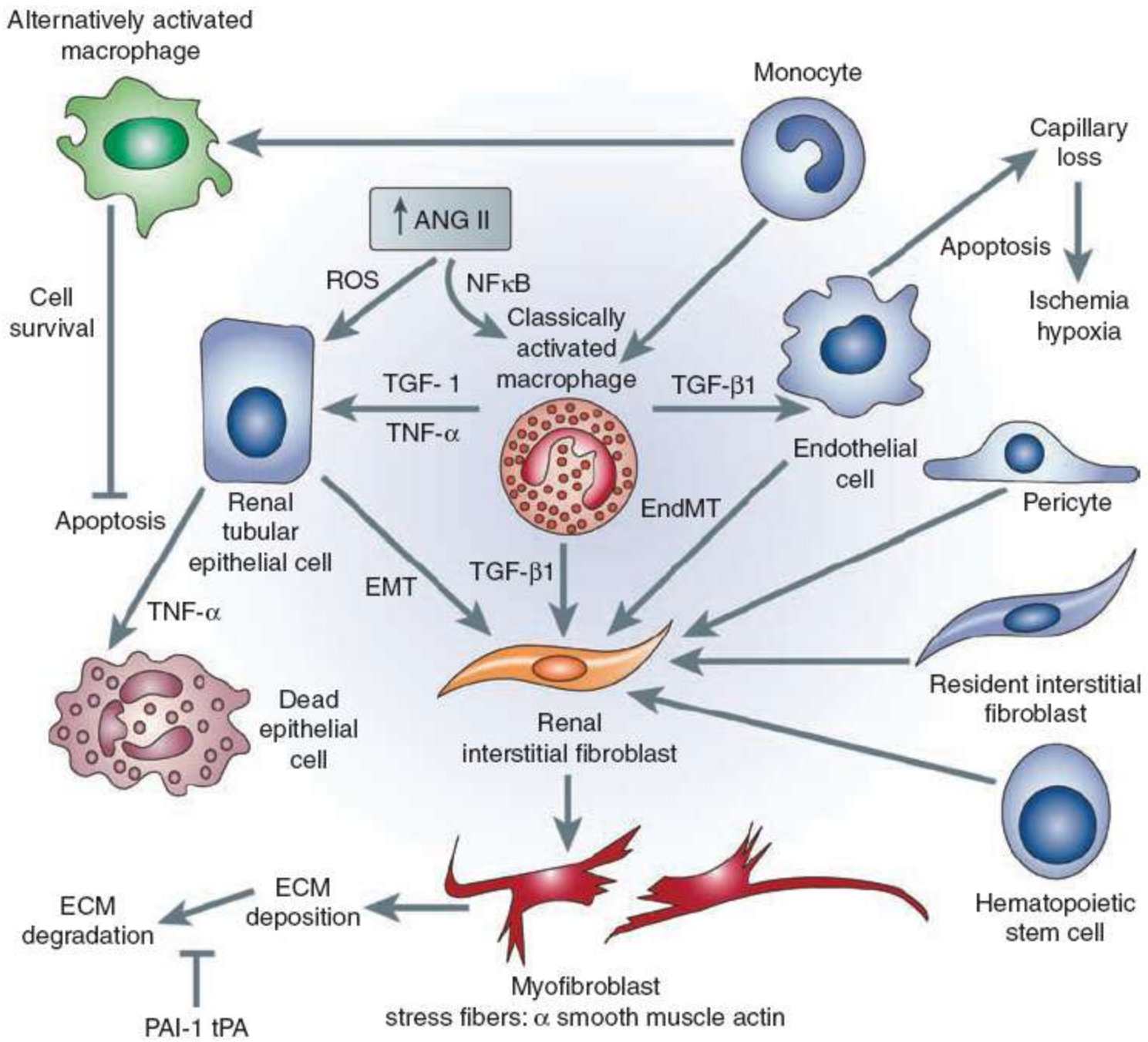
Normal tubule, non obstructed kidney



EMT

- Tubular EMT is proposed as an orchestrated, highly regulated process that consists of four key steps:
  - (*1) loss of epithelial cell adhesion;*
  - *(2) de novo –smooth* muscle actin expression and actin reorganization;
  - (*3) disruption* of tubular basement membrane; and
  - (*4) enhanced cell* migration and invasion

(J Am Soc Nephrol 15: 1–12, 2004)



Kidney International (2009) **75**, 1145–1152

Role of tgf beta signaling

Targeted disruption of TGF-β1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction

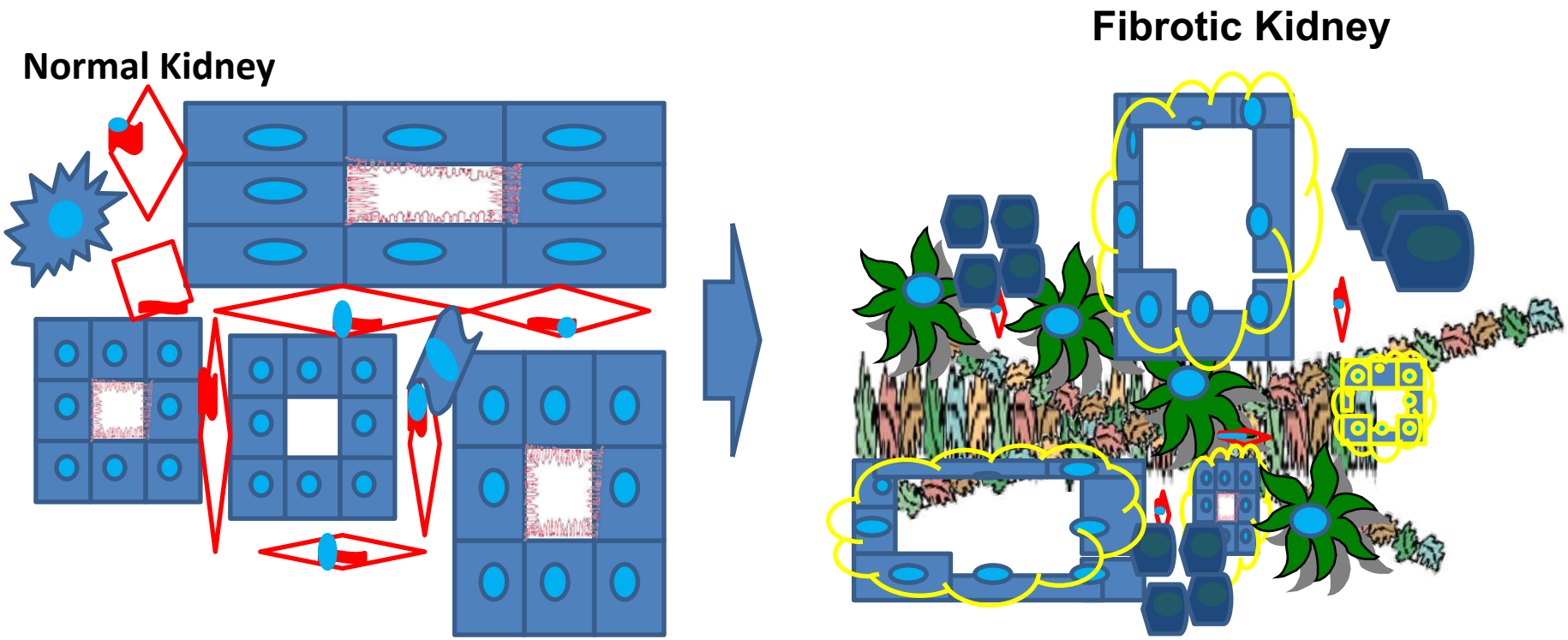
Misako Sato,[1] Yasuteru Muragaki,[1] Shizuya Saika,[1] Anita B. Roberts,[2] and Akira Ooshima[1]

J. Clin. Invest. **112**:1486–1494 (2003). doi:10.1172/JCI200319270.

Smad3 deficiency attenuates renal fibrosis, inflammation, and apoptosis after unilateral ureteral obstruction

Kidney International, Vol. 66 (2004), pp. 597–604

Normal Kidney vs Fibrotic Kidney



myofibroblast

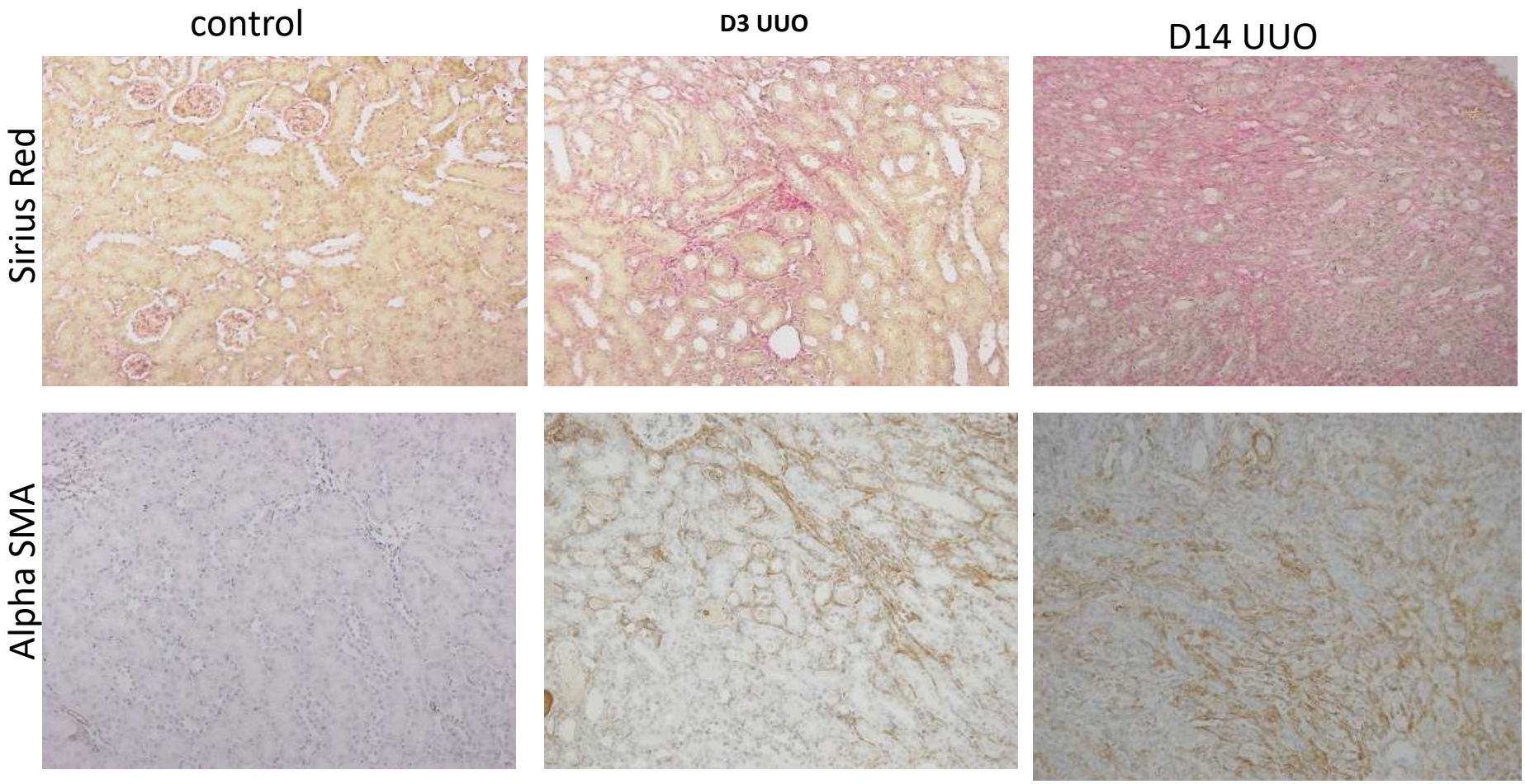
Matrix deposition

Macrophage infiltration

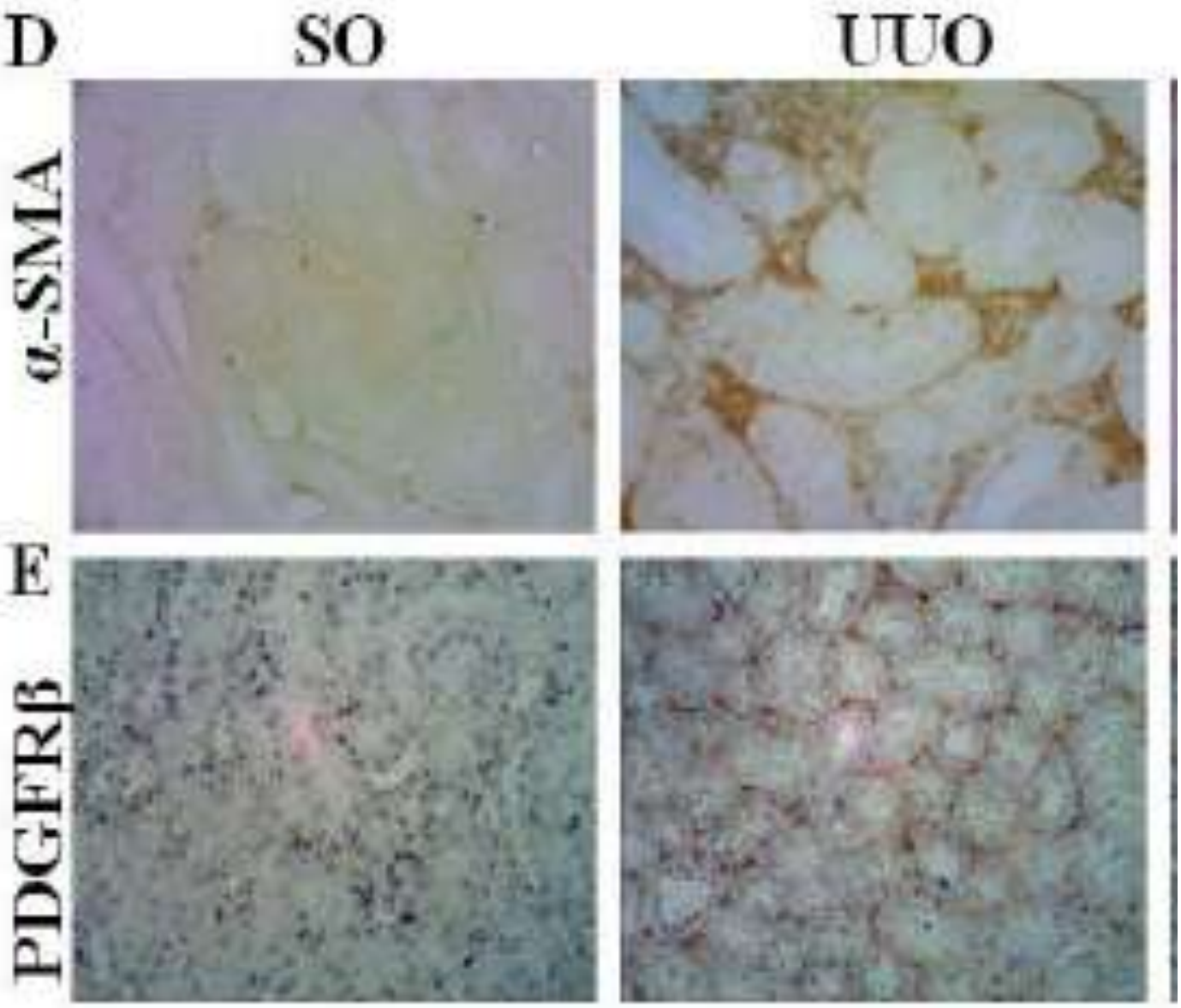
Peritubular capillary loss

Robert L. Chevalier; 2009
Won Kim, et all; 2006

Fibrosis dan myofibroblast



Arfian, unpublished data


D
SO
UUO
α-SMA
E
PDGFRβ

RBF
Dopler Imaging

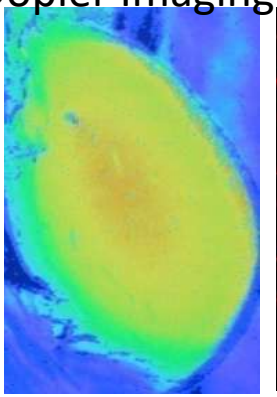
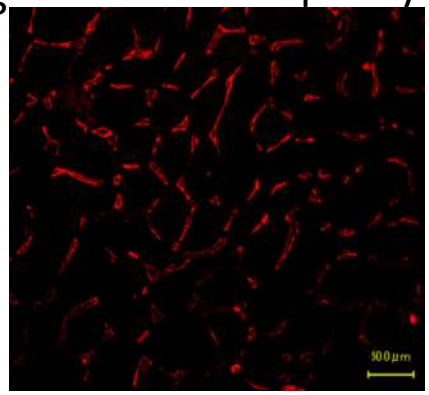
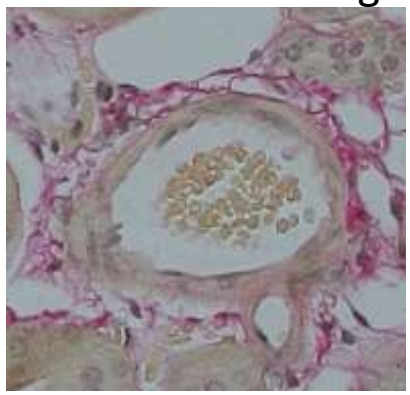
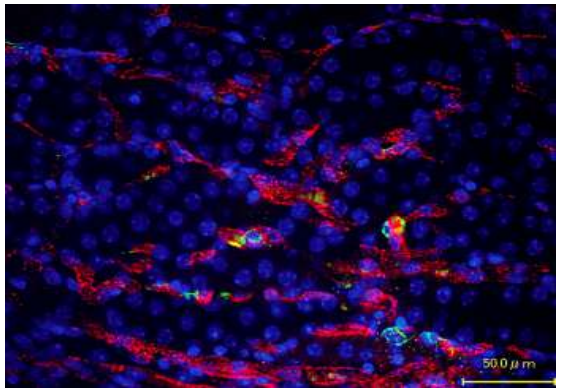
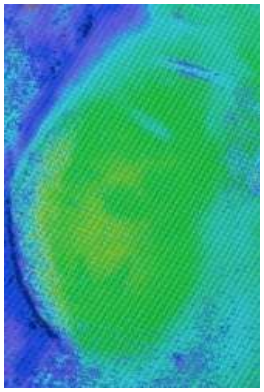
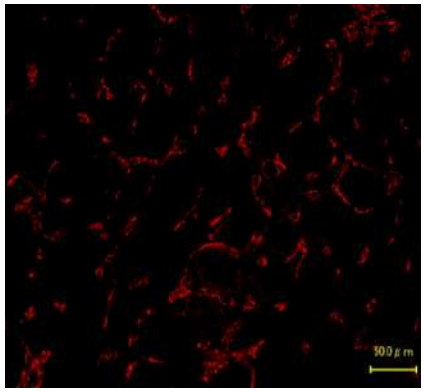
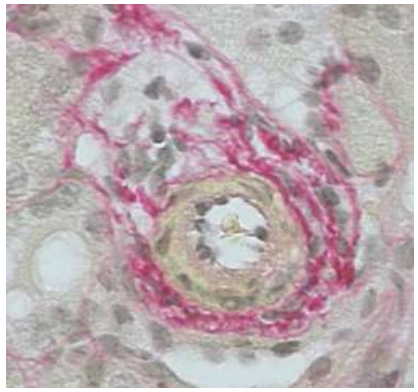
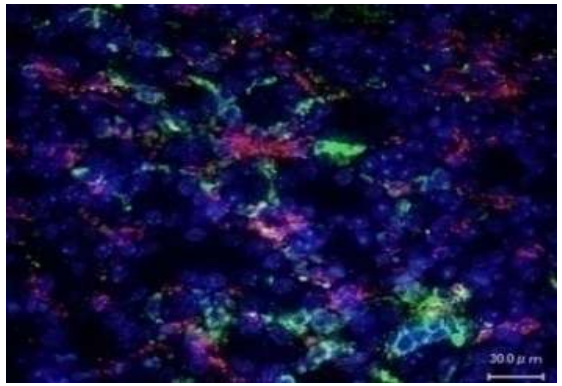
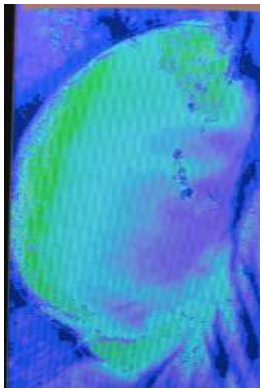
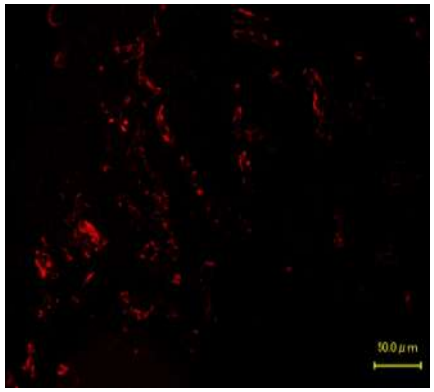
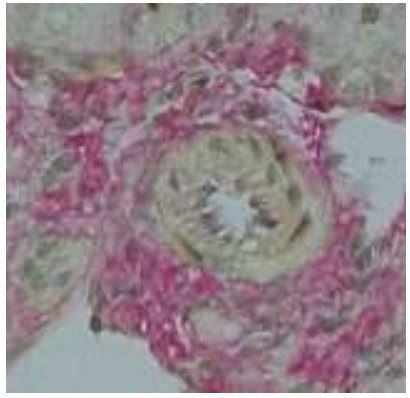
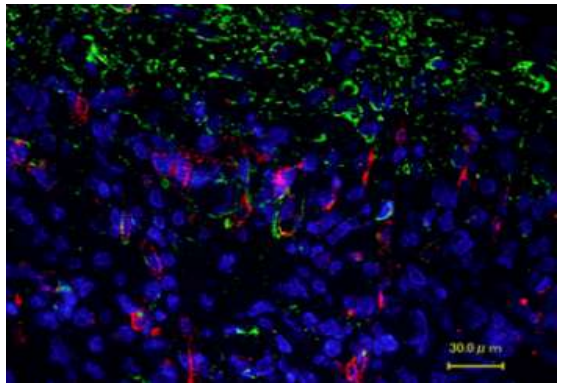
Peritubular capillary

Vessel remodeling

CONTROL

D3 UUO

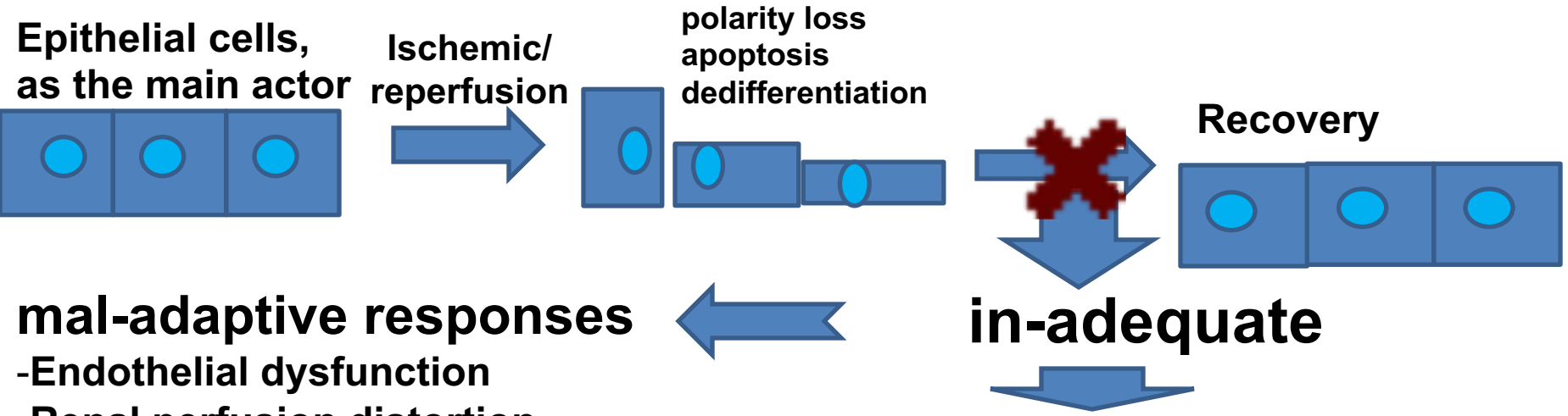
D14 UUO

Arfian, unpublished data

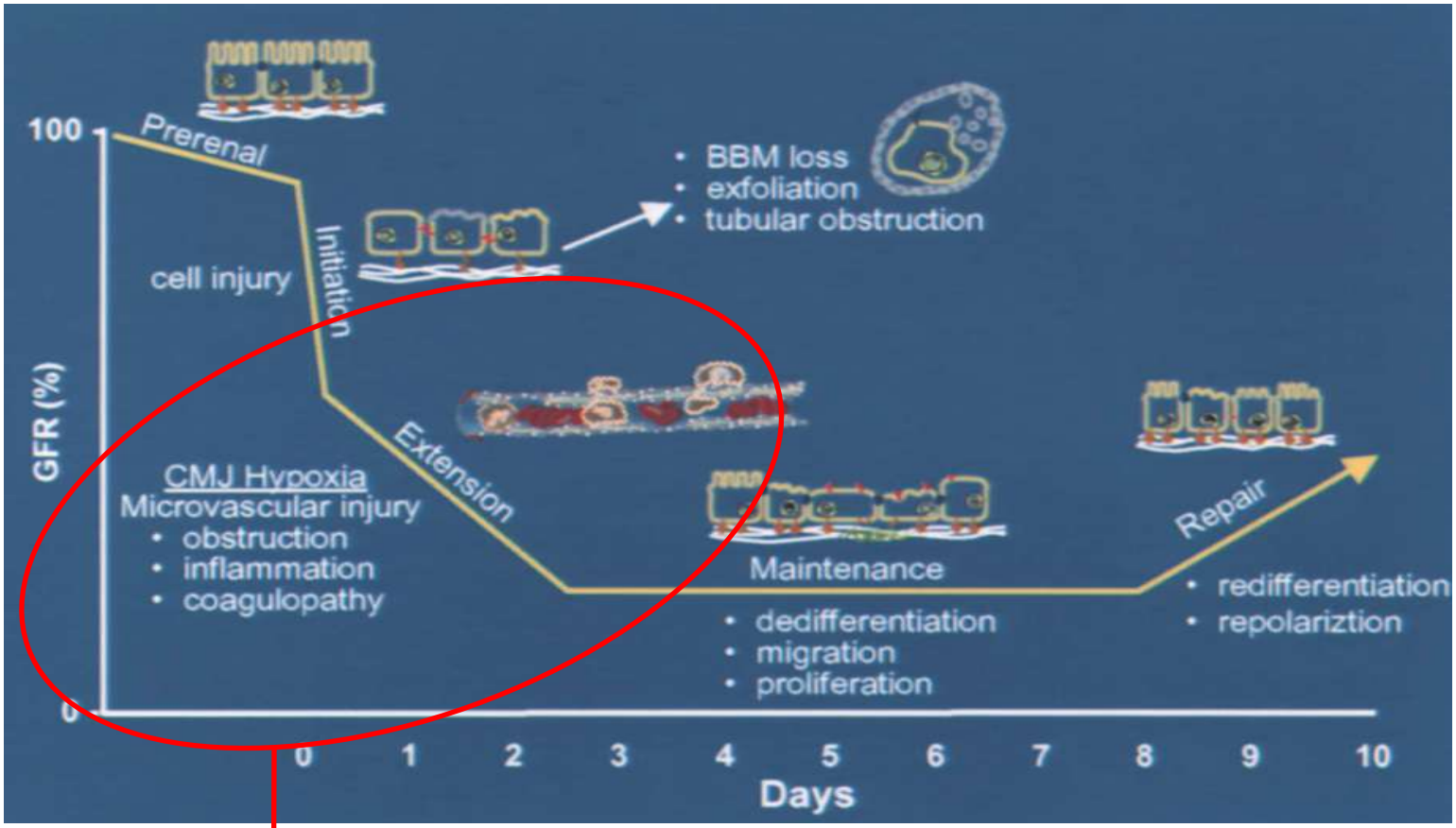
Kidney Ischemia/Reperfusion Injury (IRI)

- Ischemia/reperfusion injury contributes to the mortality and morbidity in a wide range of pathologies.
- Kidney IRI is the major cause of Acute Kidney Injury (AKI).



Extension phase of AKI

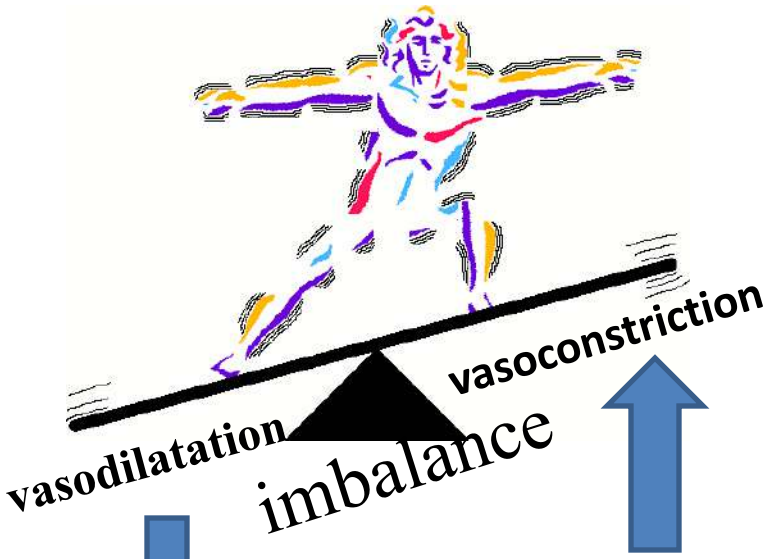
Endothelial injury induces mal-adaptive responses



continued hypoxia
inflammation responses
renal perfusion alteration

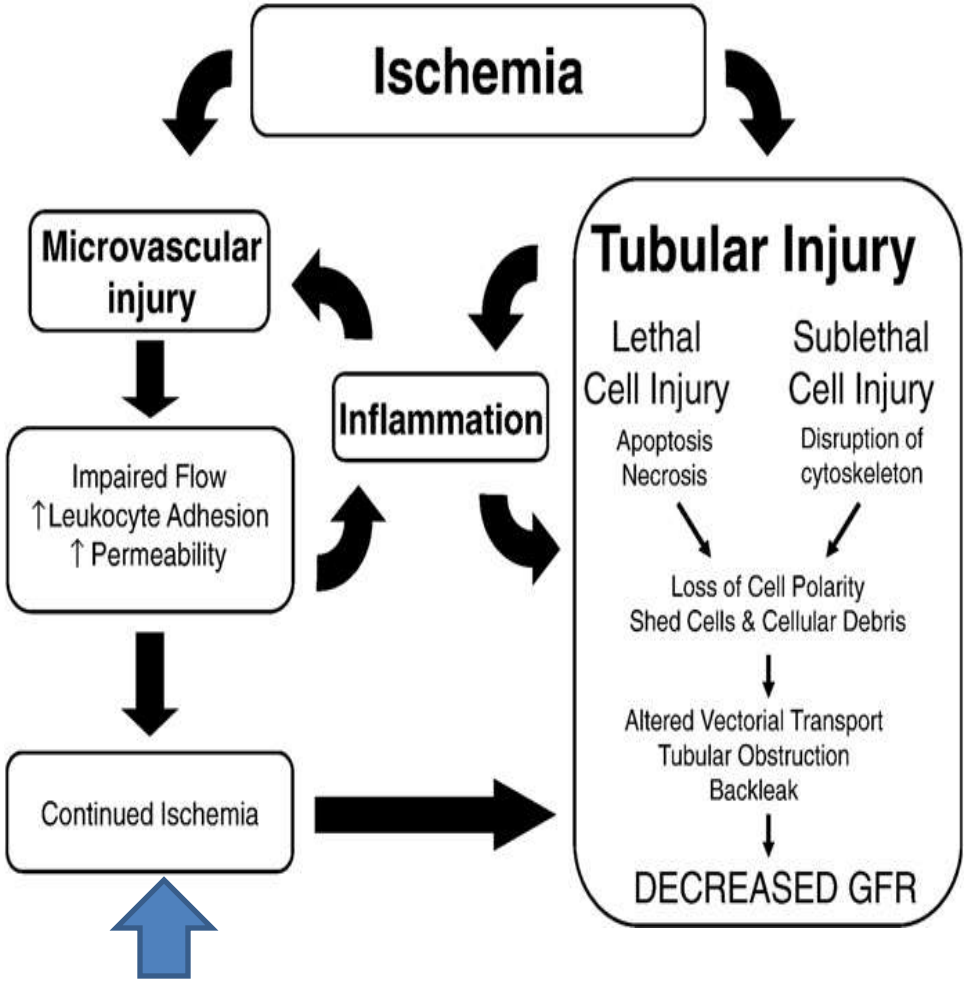
extend tubular injury

Endothelial dysfunction



Nitric Oxide
Bradykinin

Endothelin (ET-1)
Angiotensin II
Thromboxane A2
Adenosin
Sympathetic stimulation



Renal perfusion disturbance

Endothelin-1 (ET-1), a long lasting vasoconstrictor induces kidney fibrosis

Endothelin-1 Transgenic Mice Develop Glomerulosclerosis, Interstitial Fibrosis, and Renal Cysts but Not Hypertension

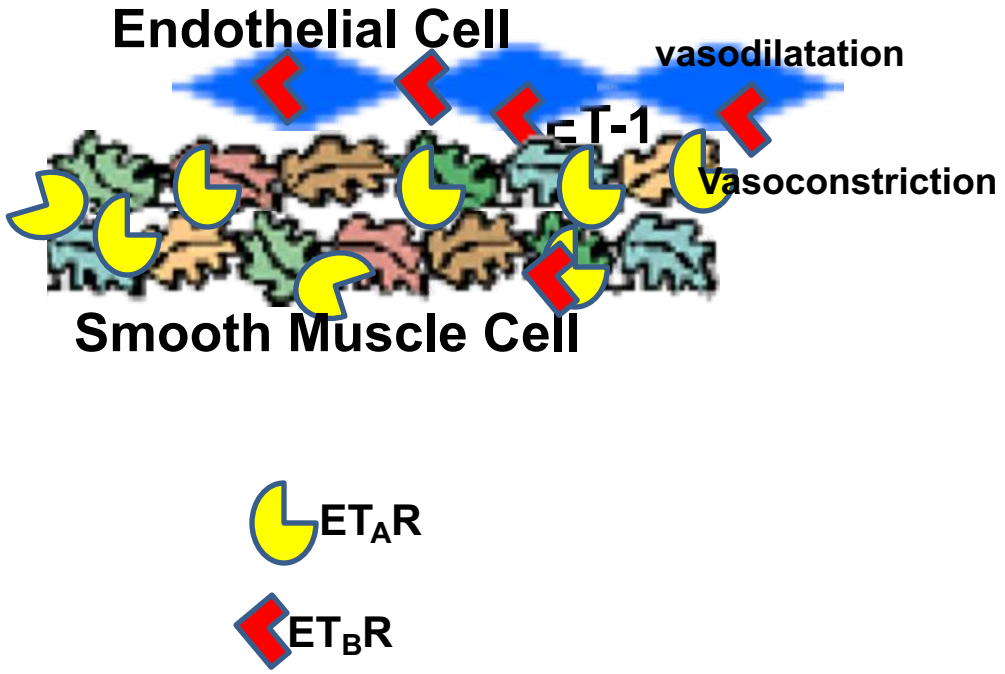
Hocher et al. J. Clin. Invest.
Volume 99, Number 6, March 1997, 1380–1389

ET-1 deletion from endothelial cells protects the kidney during the extension phase of ischemia/reperfusion injury

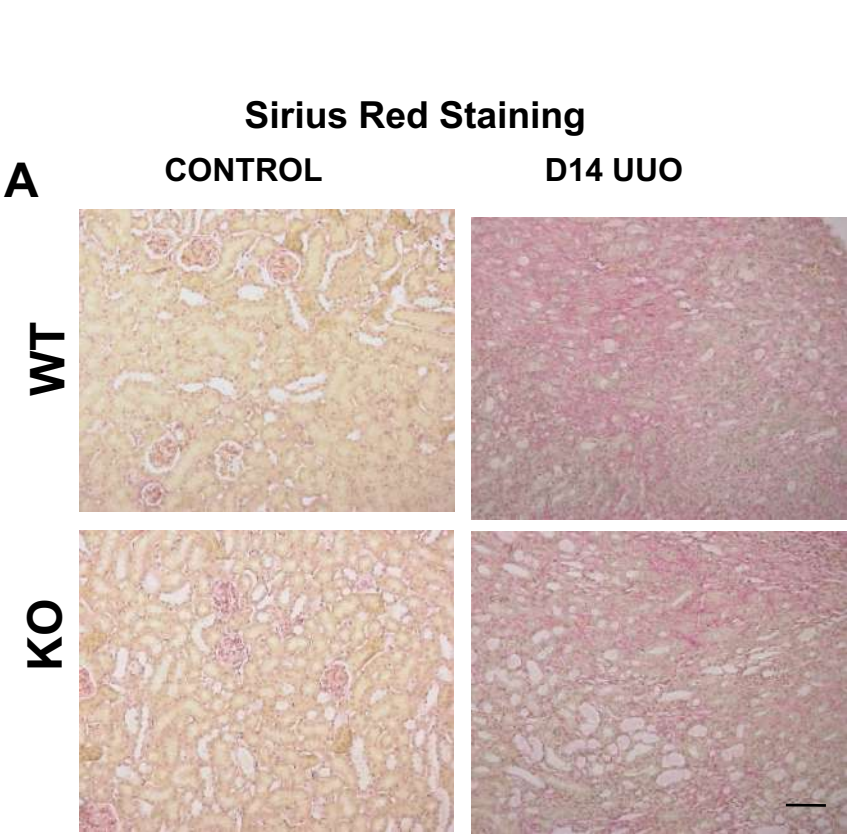
Biochemical and Biophysical Research Communications 425 (2012) 443–449

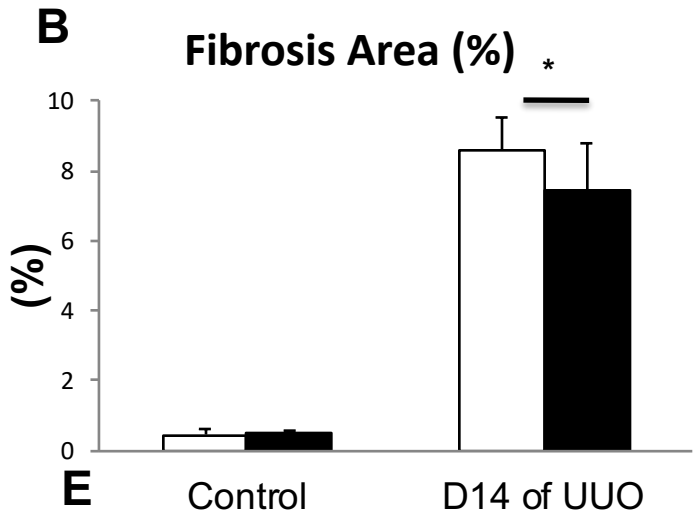
Endothelial Cell–Derived Endothelin-1 Promotes Cardiac Fibrosis in Diabetic Hearts Through Stimulation of Endothelial-to-Mesenchymal Transition

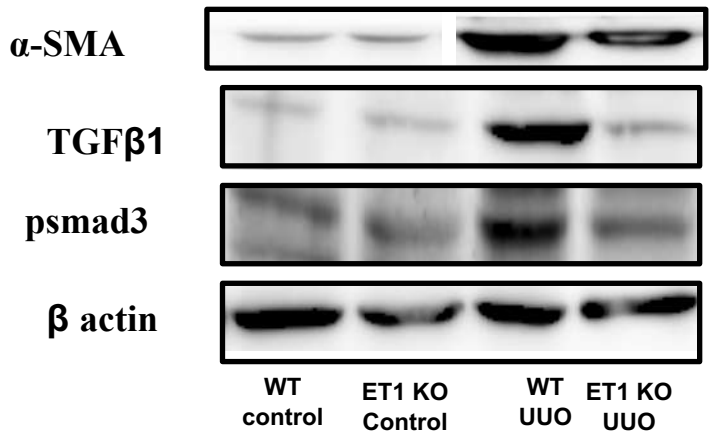
(*Circulation*. 2010;121:2407-2418.)



Endothelin-1 Knock-out from Endothelial Cells reduces kidney fibrosis through reducing TGFβ signaling



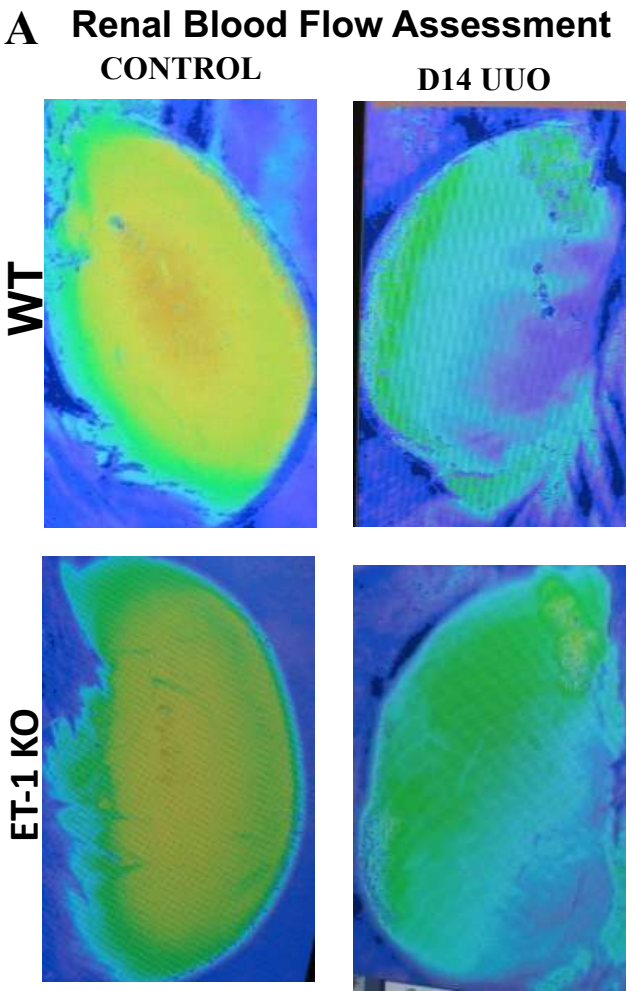


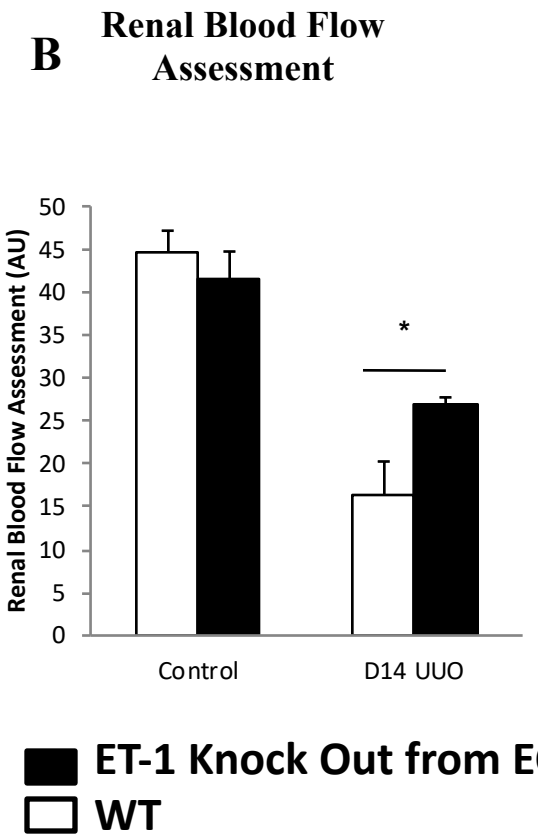


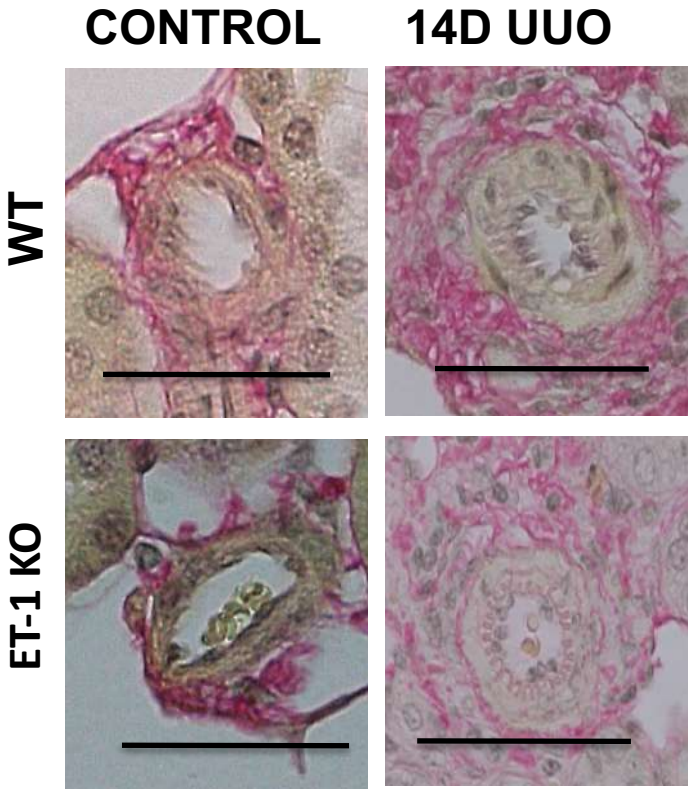
Nur Arfian, Noriaki Emoto, Un-published data

ET-1 deletion attenuated renal ischemia and vascular remodelling after kidney fibrosis

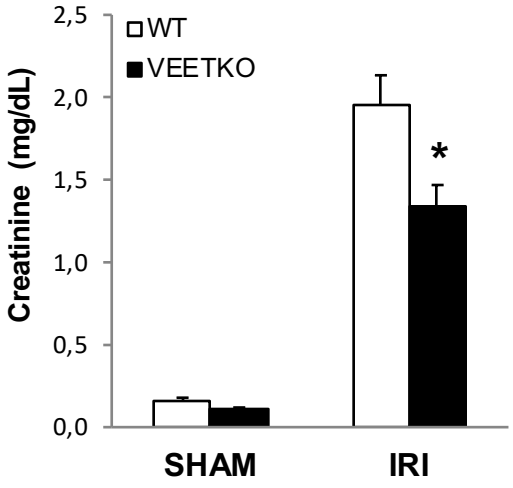
UUO: Unilateral Ureteral Obstruction, model to induce kidney fibrosis





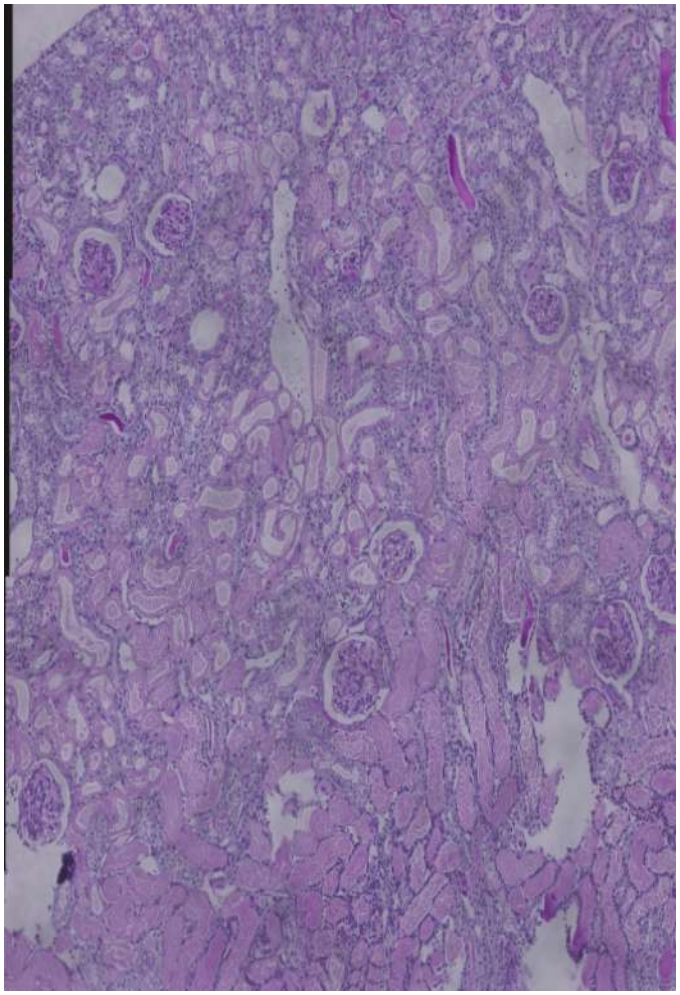


ET-1 deletion from EC attenuated kidney injury and cortical-spreading tubular injury

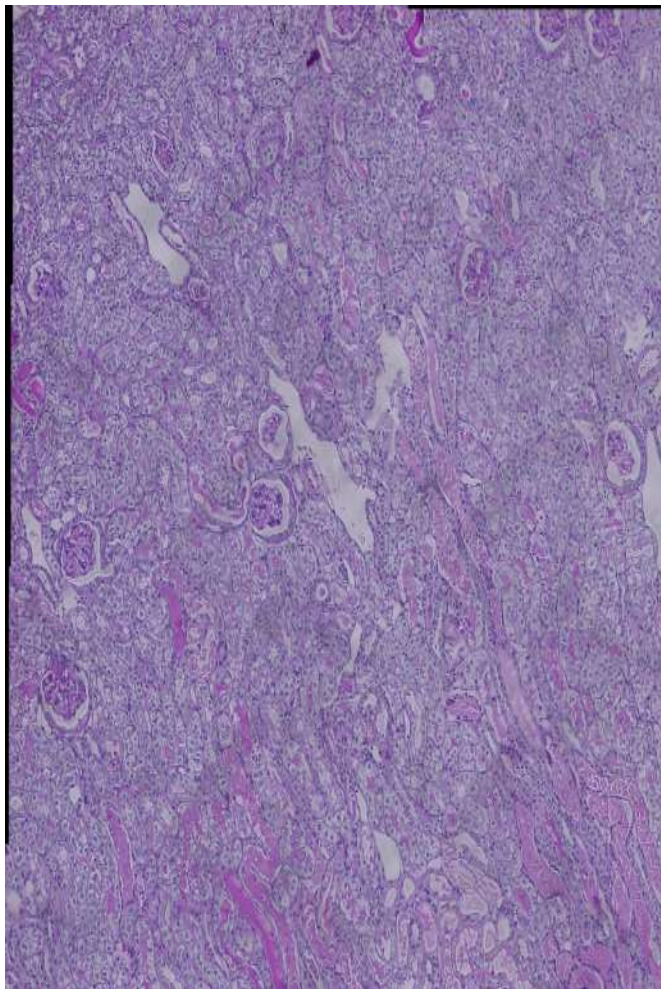


*P<0.05 vs WT IRI

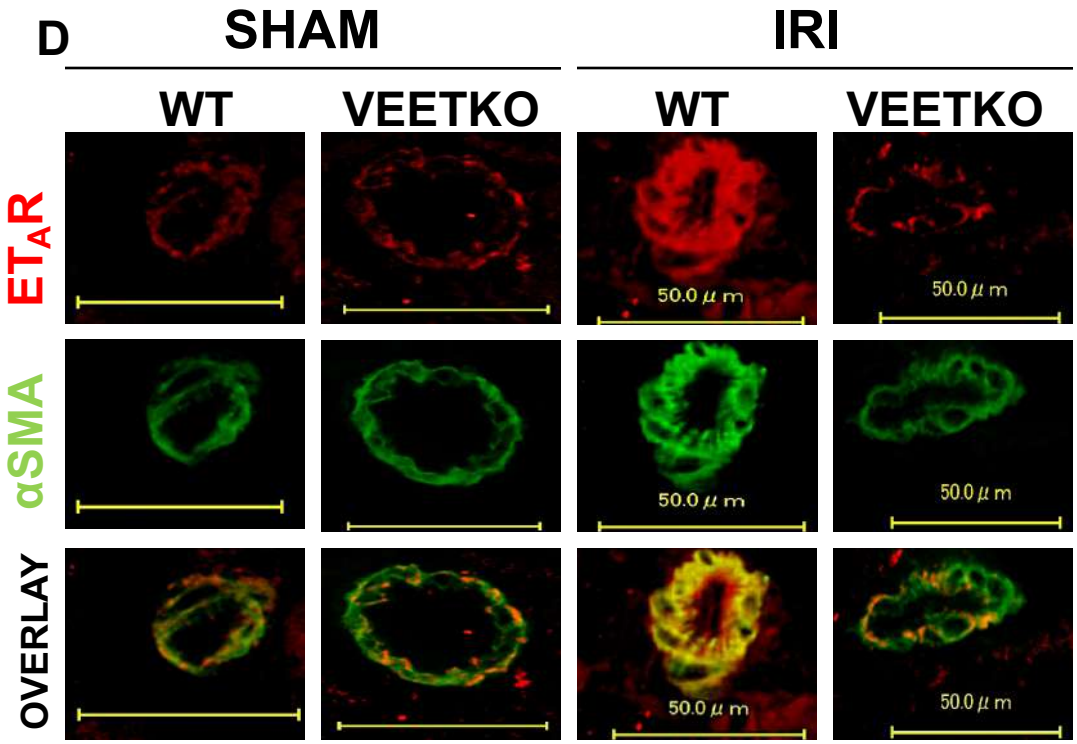
WT IRI

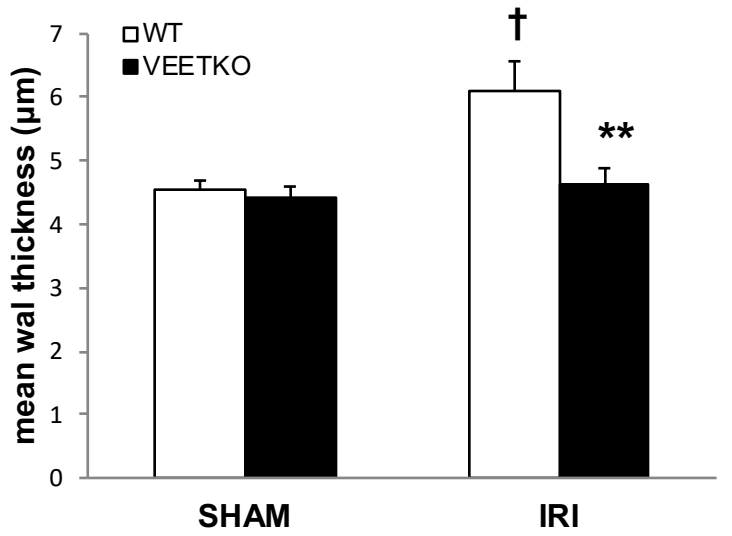


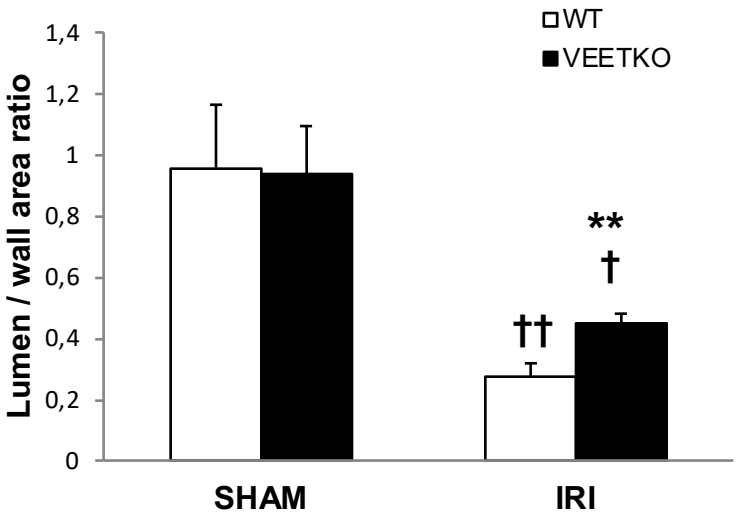
VEETKO IRI



ET-1 deletion from EC reduced vessel wall thickening after IRI

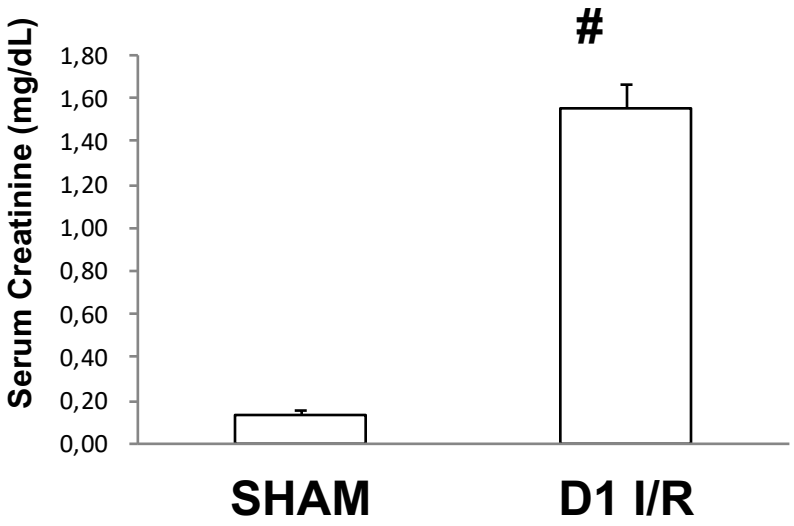




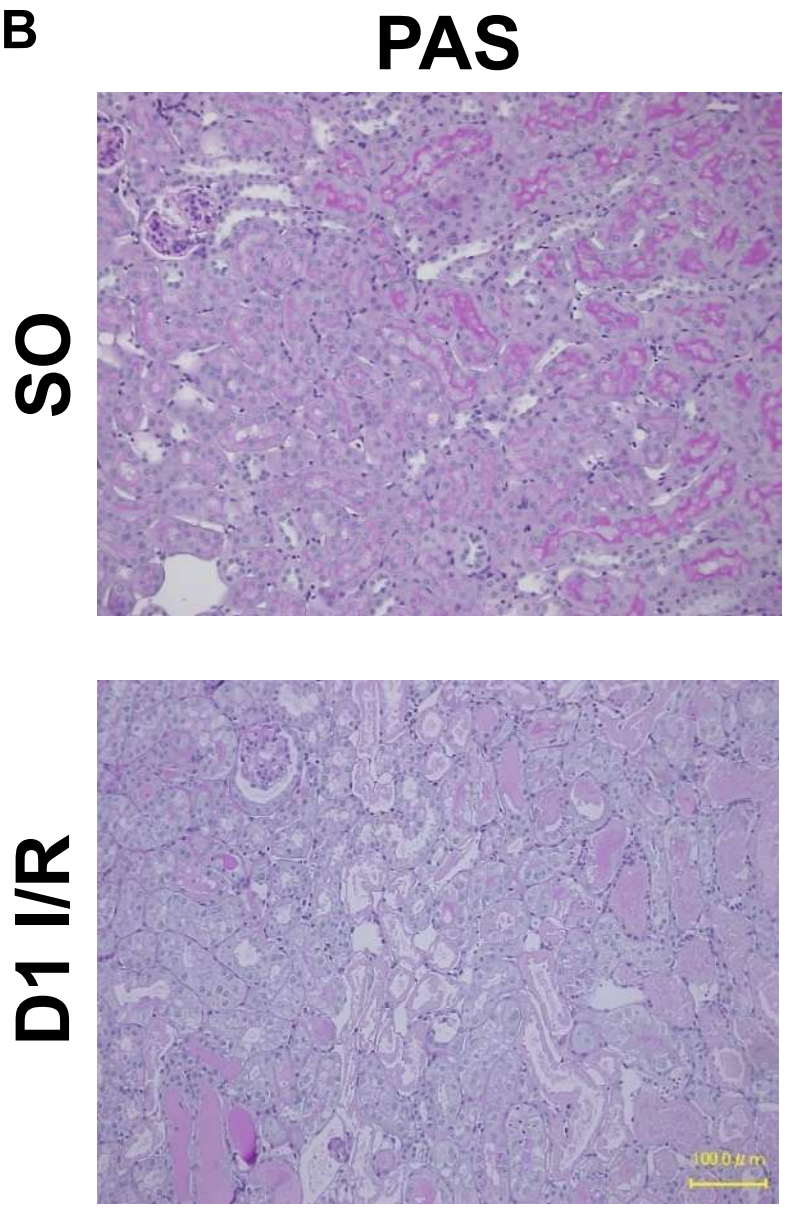


Wall thickness and lumen/wall area ratio quantification in renal small arteries based on αSMA staining.
†P<0.05 & ††P<0.01 vs WT Sham. *P<0.05 & **P<0.01 vs WT IRI.
Bar=50μm

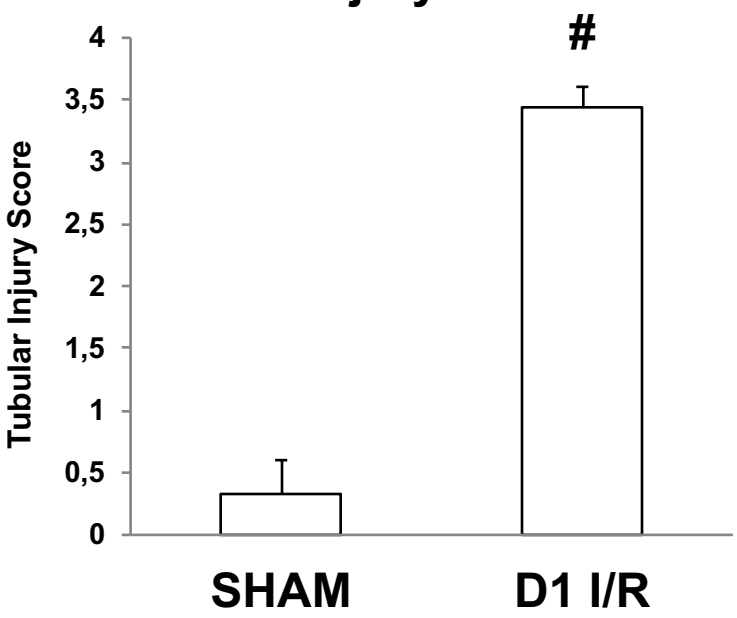
A


Serum creatinine (mg/dL)
Serum Creatinine (mg/dL)
1,80
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
#
SHAM
D1 I/R

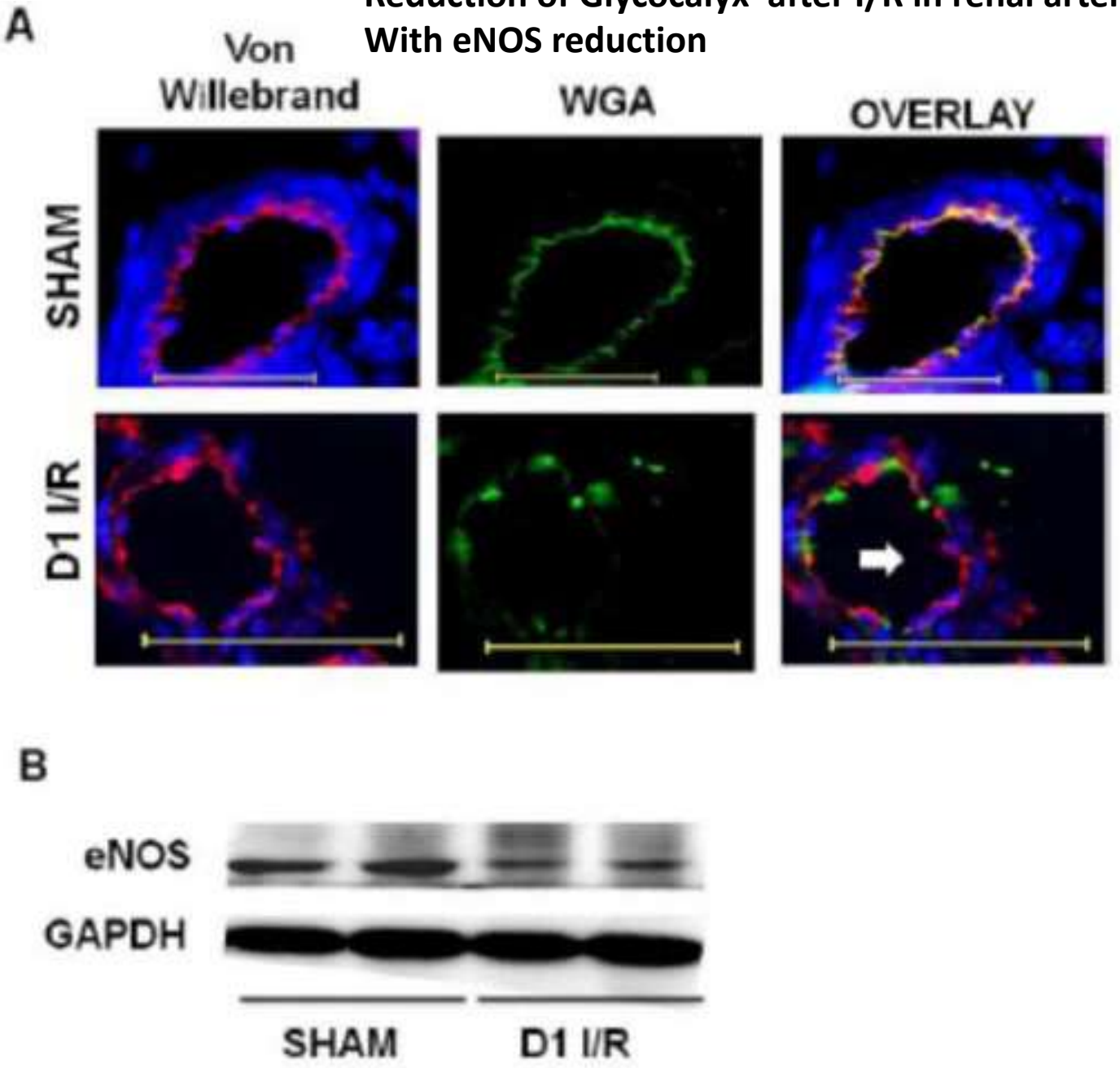
B


PAS
SO
D1 I/R

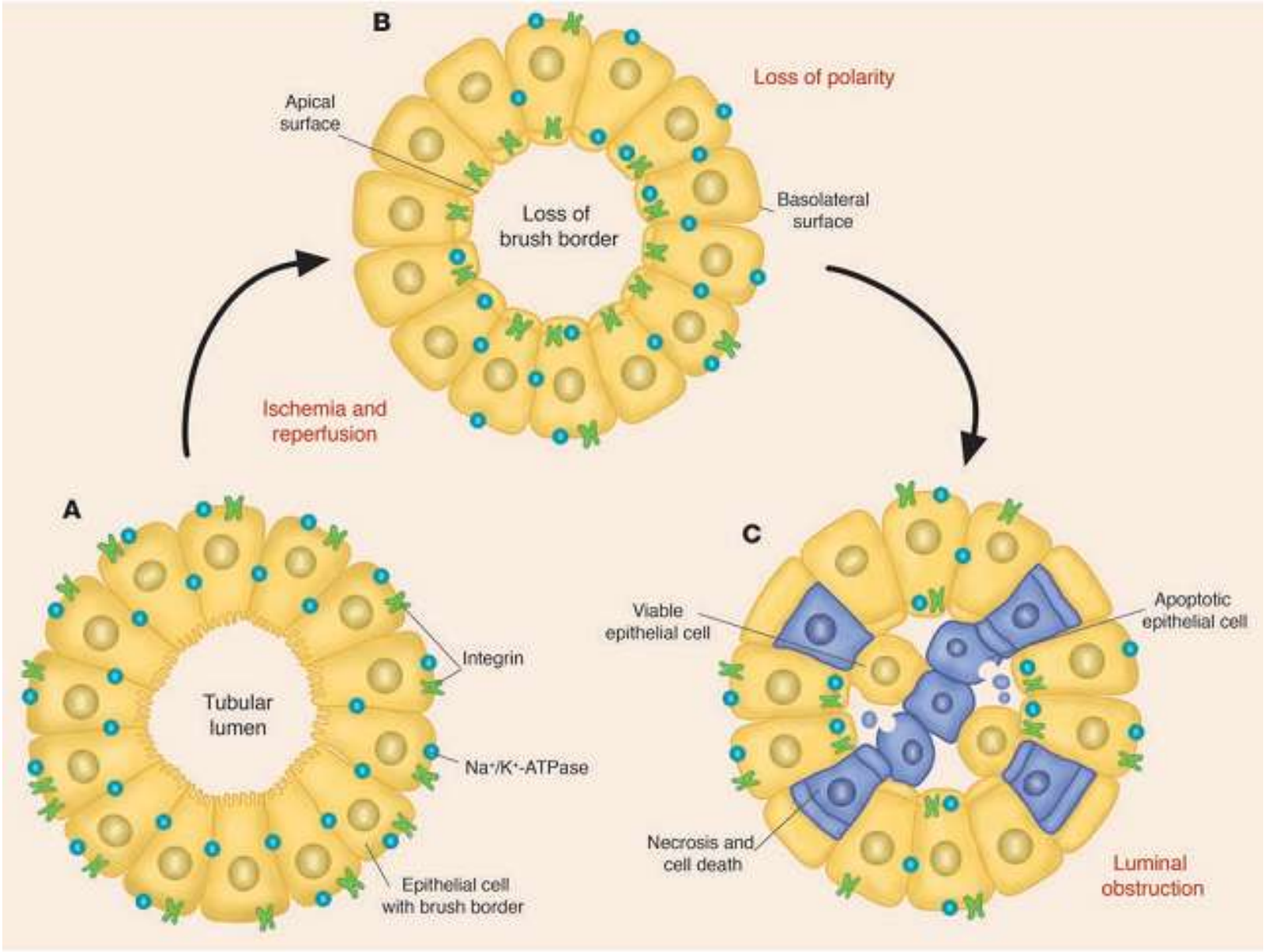
C


Tubular Injury score
Tubular Injury Score
4
3,5
3
2,5
2
1,5
1
0,5
0
#
SHAM
D1 I/R

Reduction of Glycocalyx after I/R in renal arteries associates With eNOS reduction

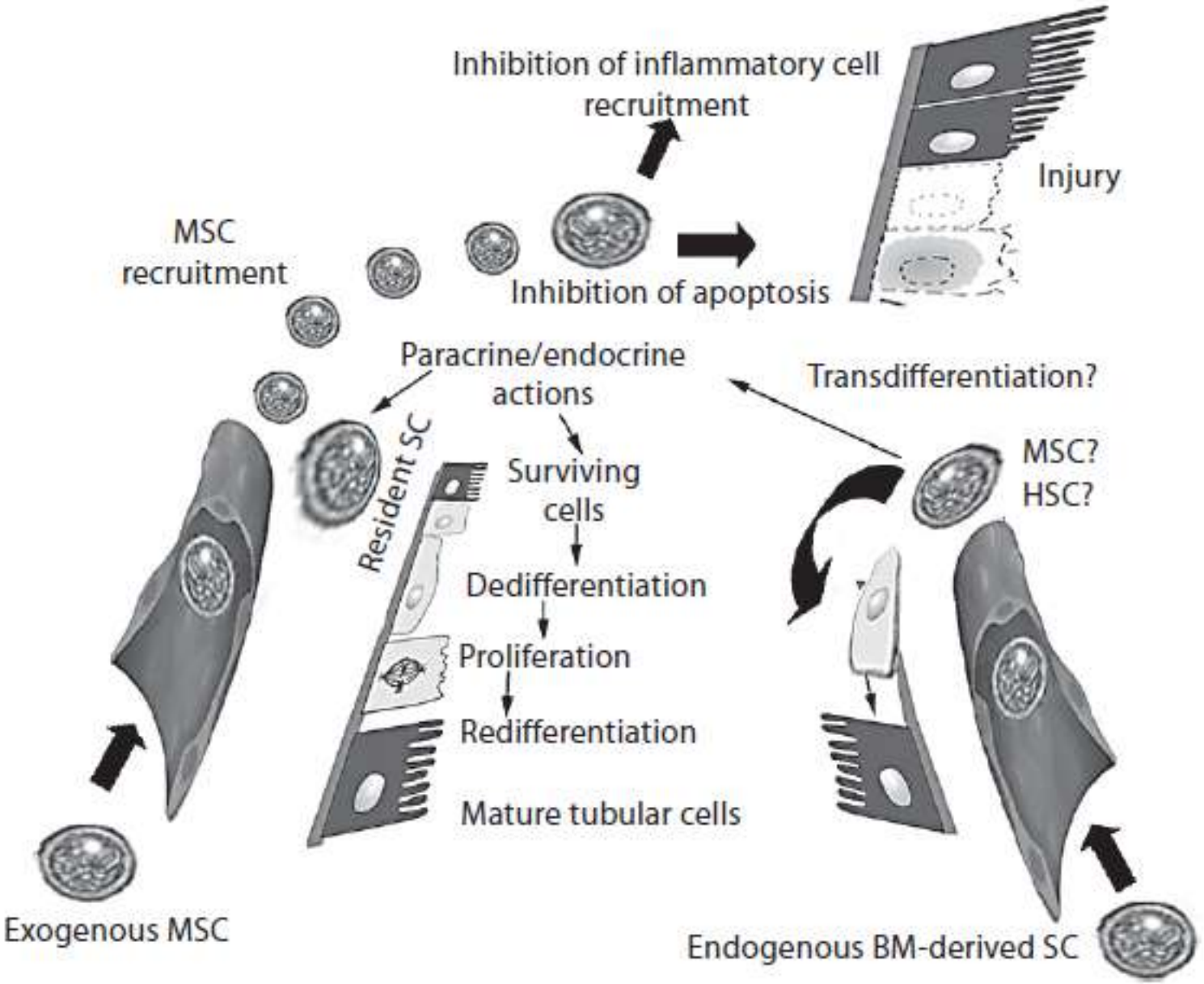


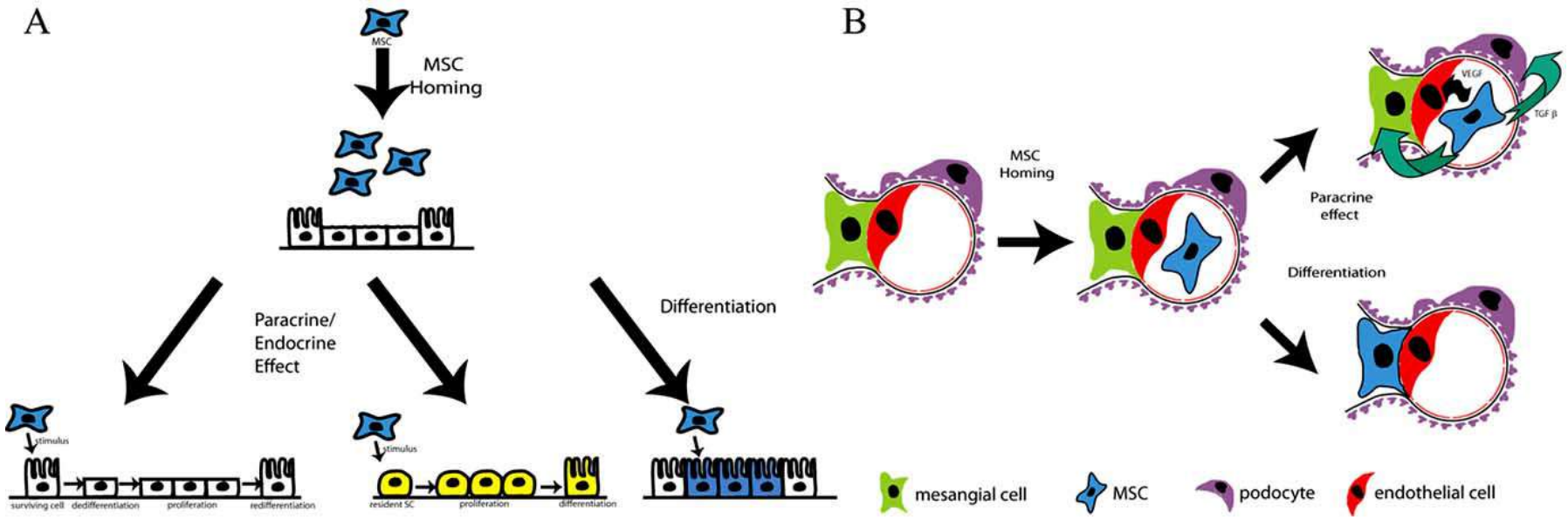
Arfian, et al, 2016


B
Loss of polarity
Apical surface
Loss of brush border
Basolateral surface
Ischemia and reperfusion
A
Integrin
Tubular lumen
Na+/K+-ATPase
Epithelial cell with brush border
C
Viable epithelial cell
Apoptotic epithelial cell
Necrosis and cell death
Luminal obstruction

- Characterisation of gene expression profi les of such cells and elucidation of corresponding signalling molecules will be important to clarify whether neonephrogenesis can theoretically be re-established in mammal adult life.
- Newly created transgenic mice with cell lineage tracing can be then used to assess the extent to which theoretical knowledge gained from cell biology and gene expression studies can be implemented in vivo

Therapy: Cell Reprogramming (Stem cells)





While the injected MSC had an immediate positive effect, they negatively contributed to the long term regeneration of the glomerular tissue and induced progression into glomerulosclerosis.

Kunter *et al. the injected MSC in a long tem analysis of the renal* tissue were found to undergo maldifferentiation in adipocytes.

Breakthrough treatment

REGULAR ARTICLE

Human mesenchymal stem cell-conditioned medium improves cardiac function following myocardial infarction

Stem Cell Research (2011) 6, 206–214

Pre-conditioned mesenchymal stem cells ameliorate renal ischemic injury in rats by augmented survival and engraftment

Masoud *et al. Journal of Translational Medicine* 2012, **10**:243

Mesenchymal stem cell-conditioned medium accelerates regeneration of human renal proximal tubule epithelial cells after gentamicin toxicity

Experimental and Toxicologic Pathology 65 (2013) 595–600

Renal Progenitor Cells

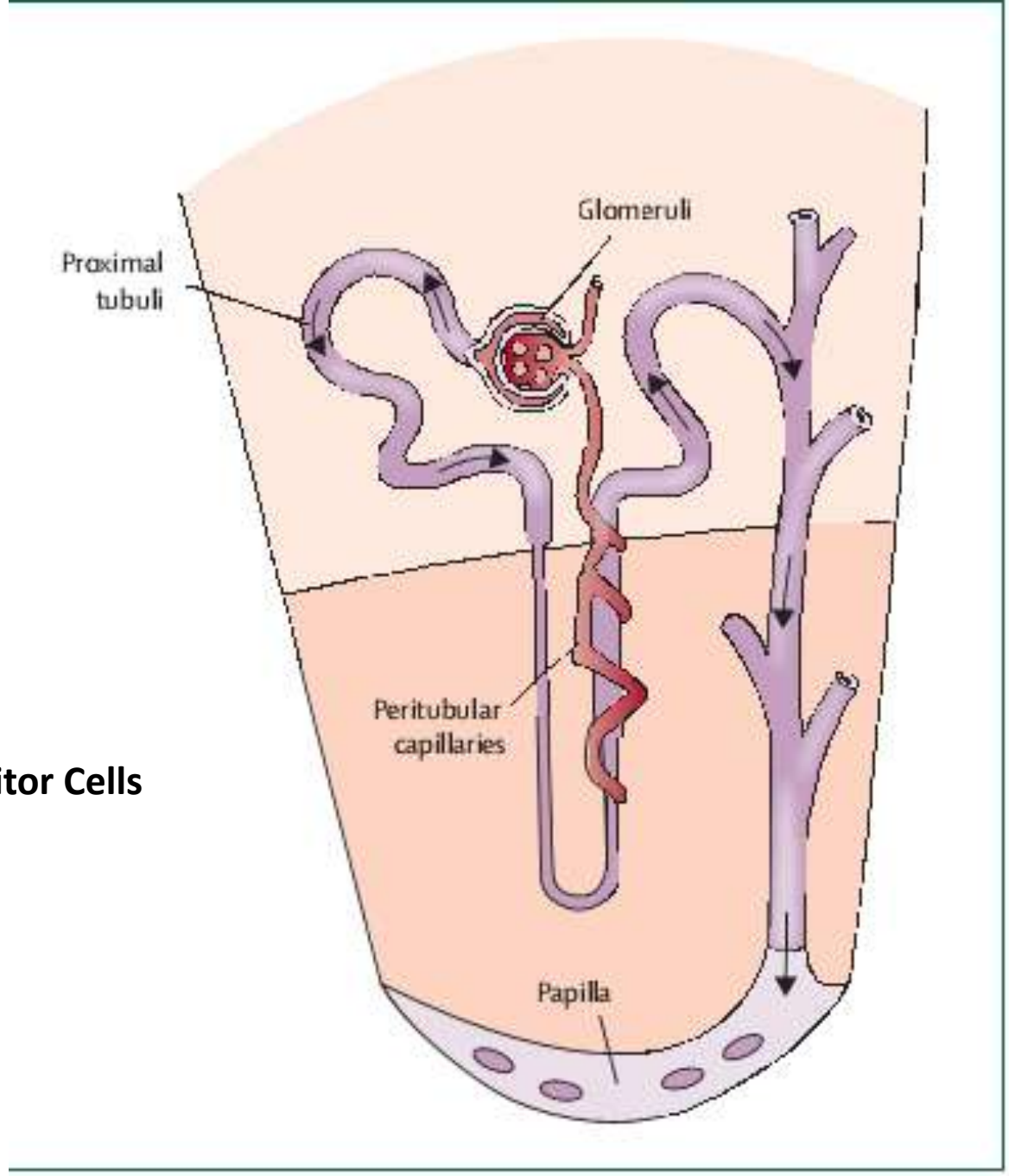
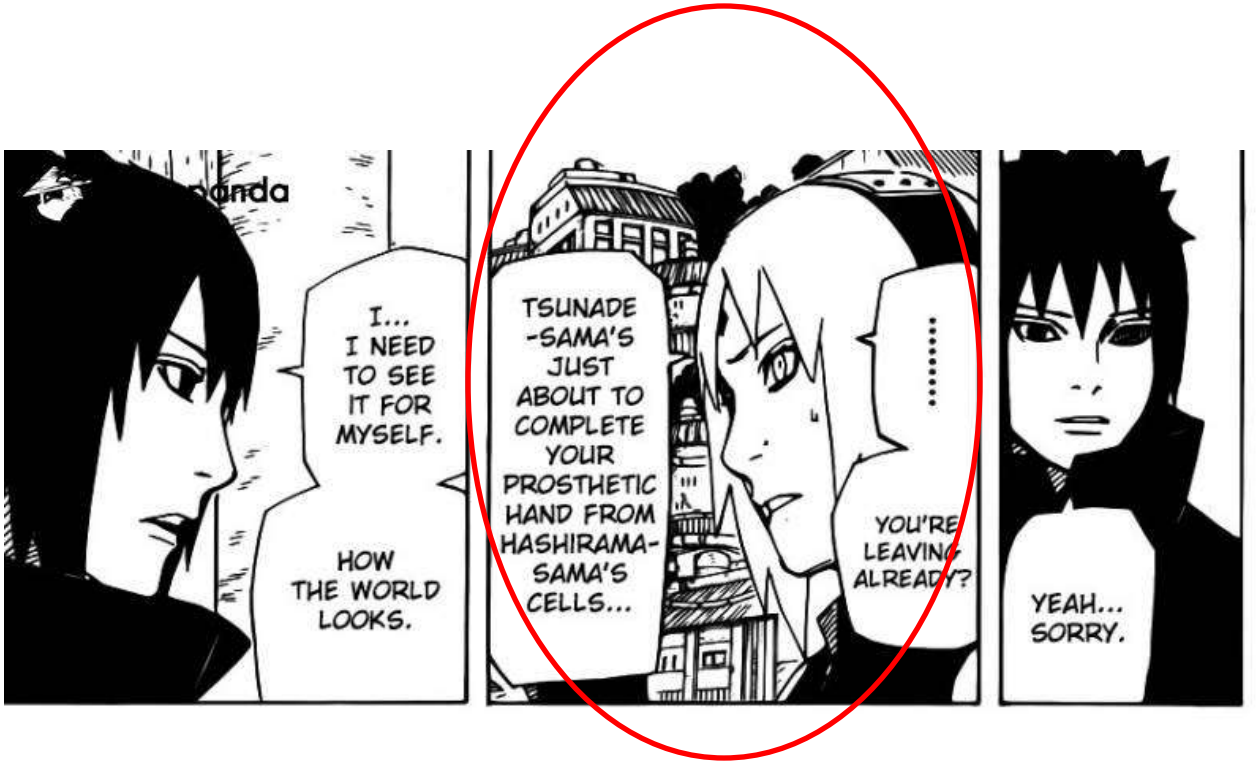
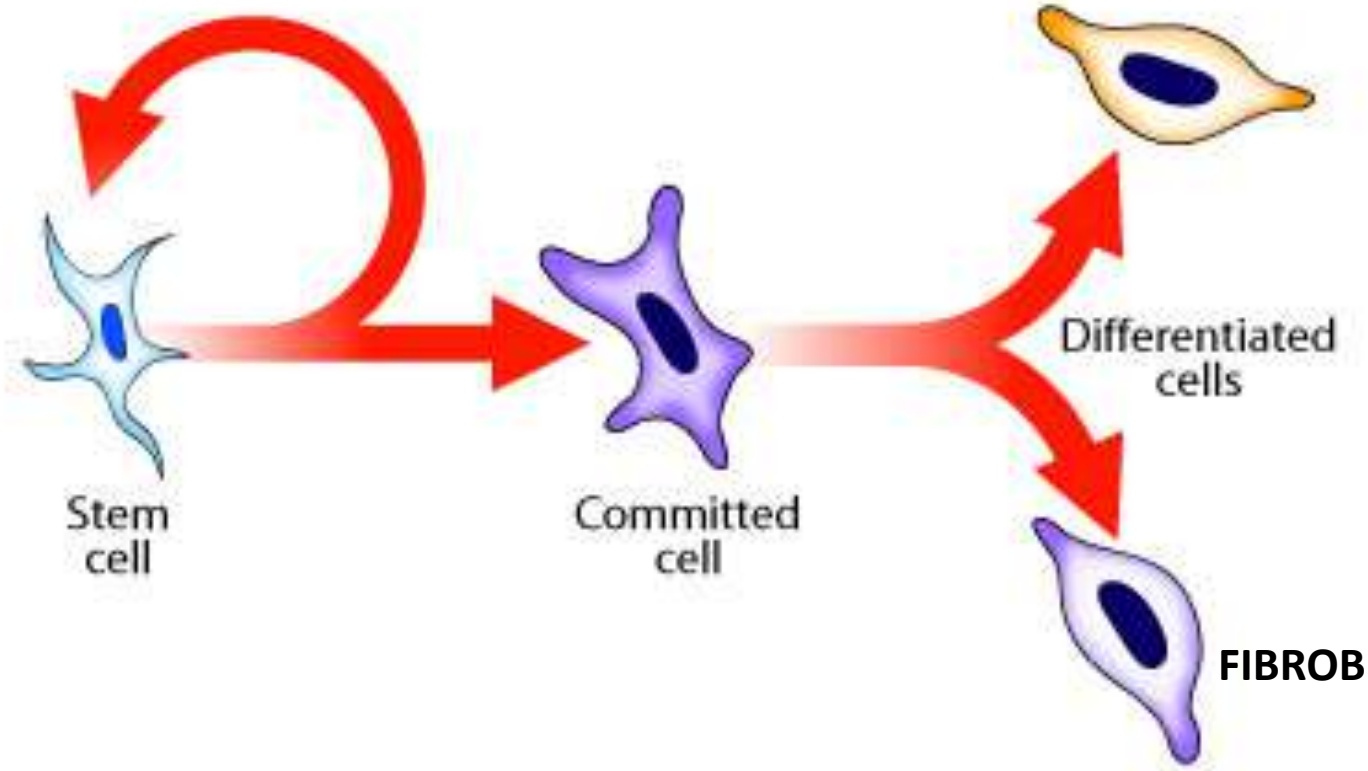


Figure 1: Potential niches for renal progenitor or stem cells in postnatal kidney

Naruto vs sasuke

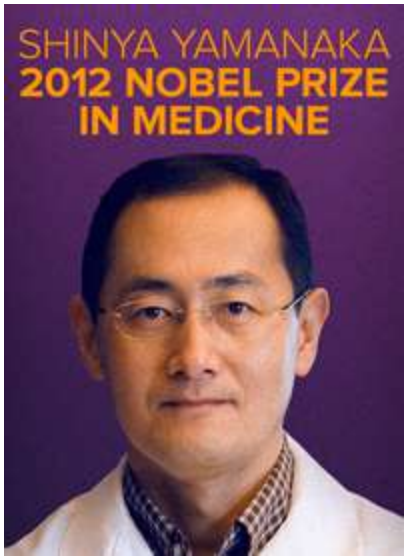


BISAKAH DIBALIK?

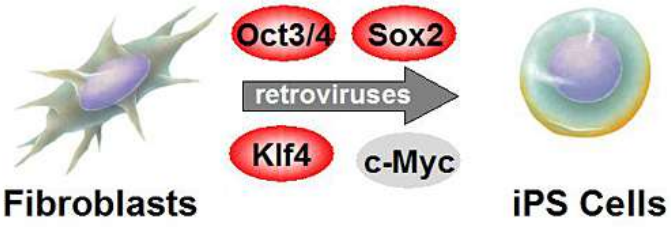


FIBROBLAST

Illustration by Cell Imaging Core of the Center for Reproductive Sciences.



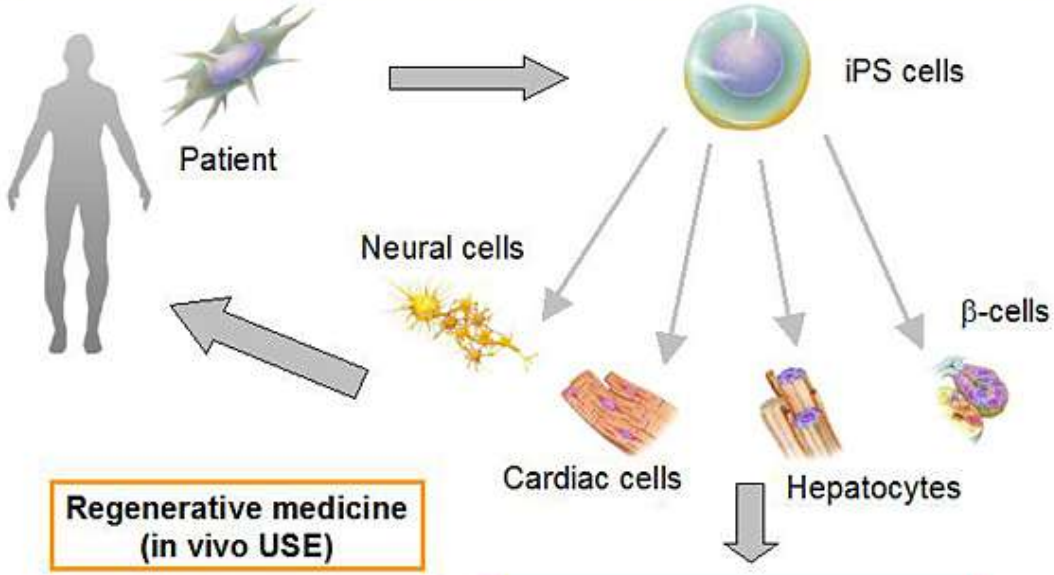
Induced Pluripotent Stem (iPS) Cells



Mouse iPS cells reported in 2006

Human iPS cells reported in 2007

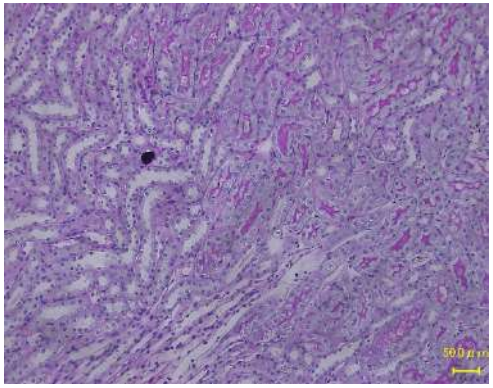
Applications of iPS Cells



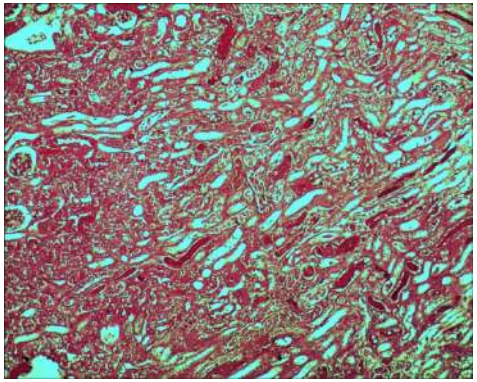
MSC Conditioned Medium ameliorated kidney tubular injury

PAS (magnification 100x)

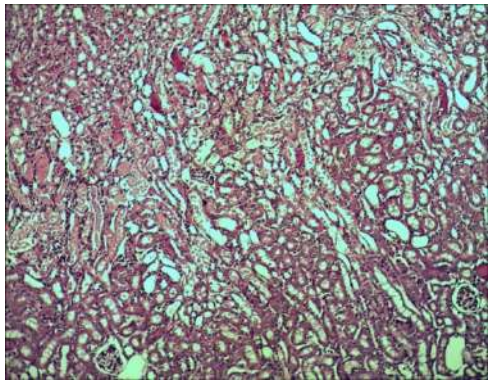
SO



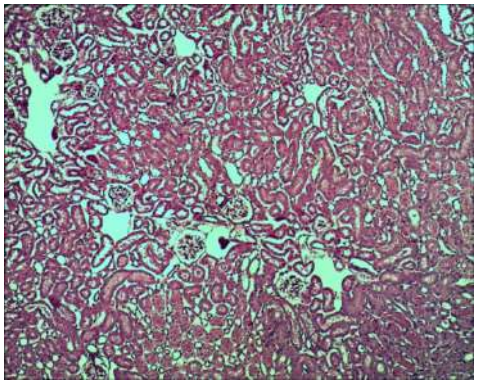
IR

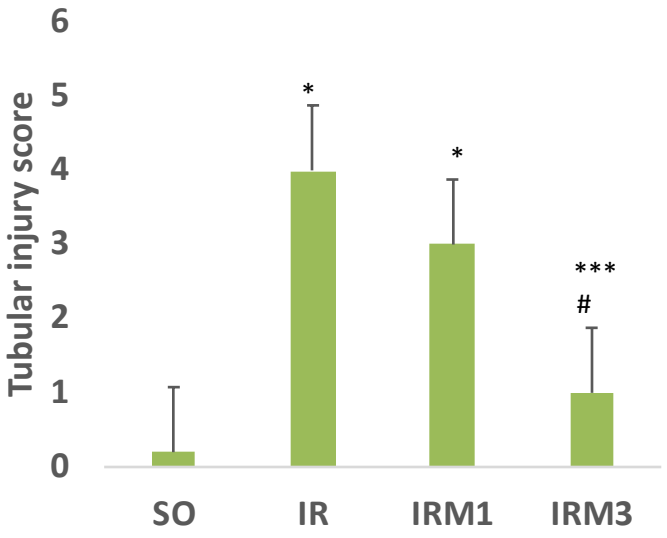


IRM1



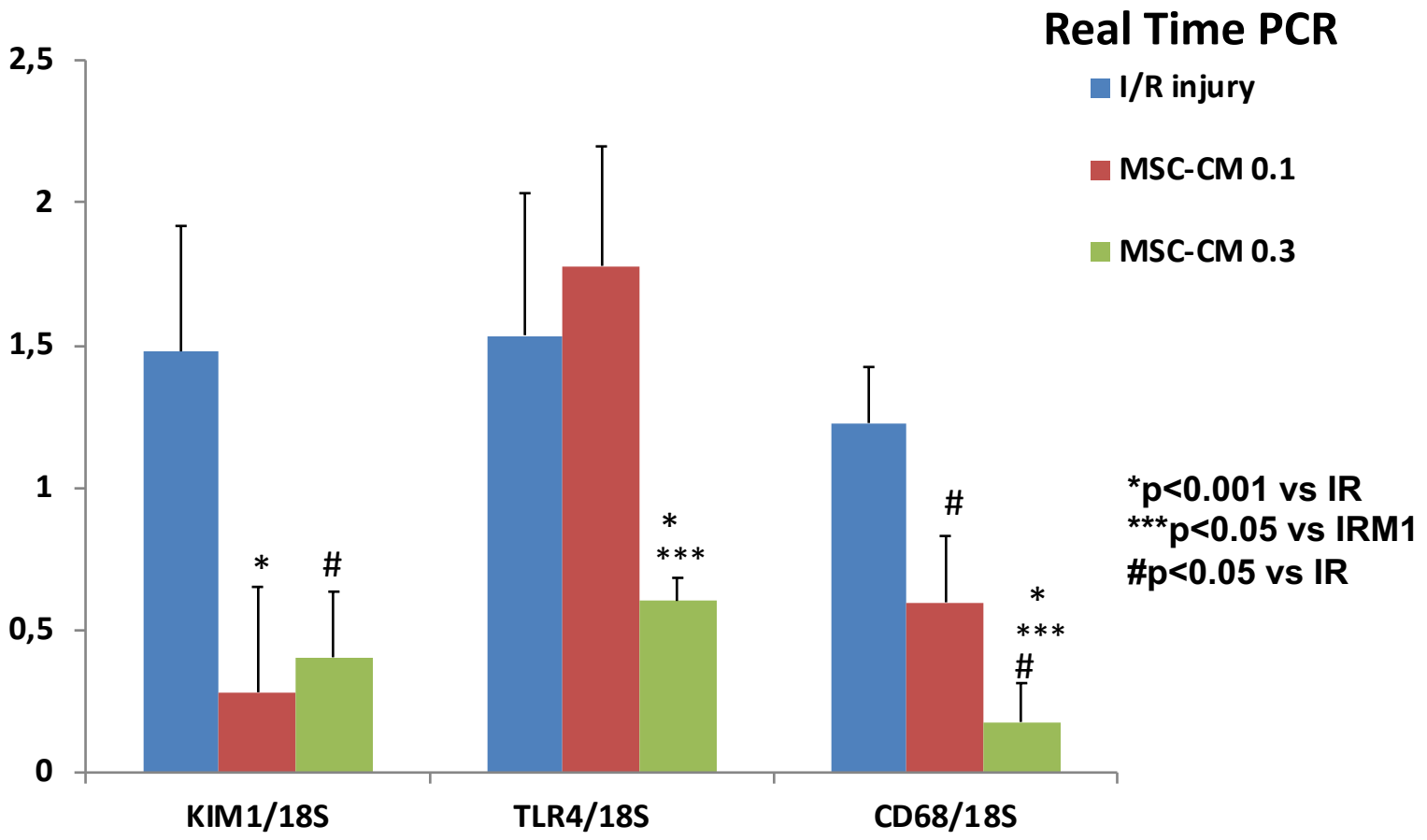
IRM3





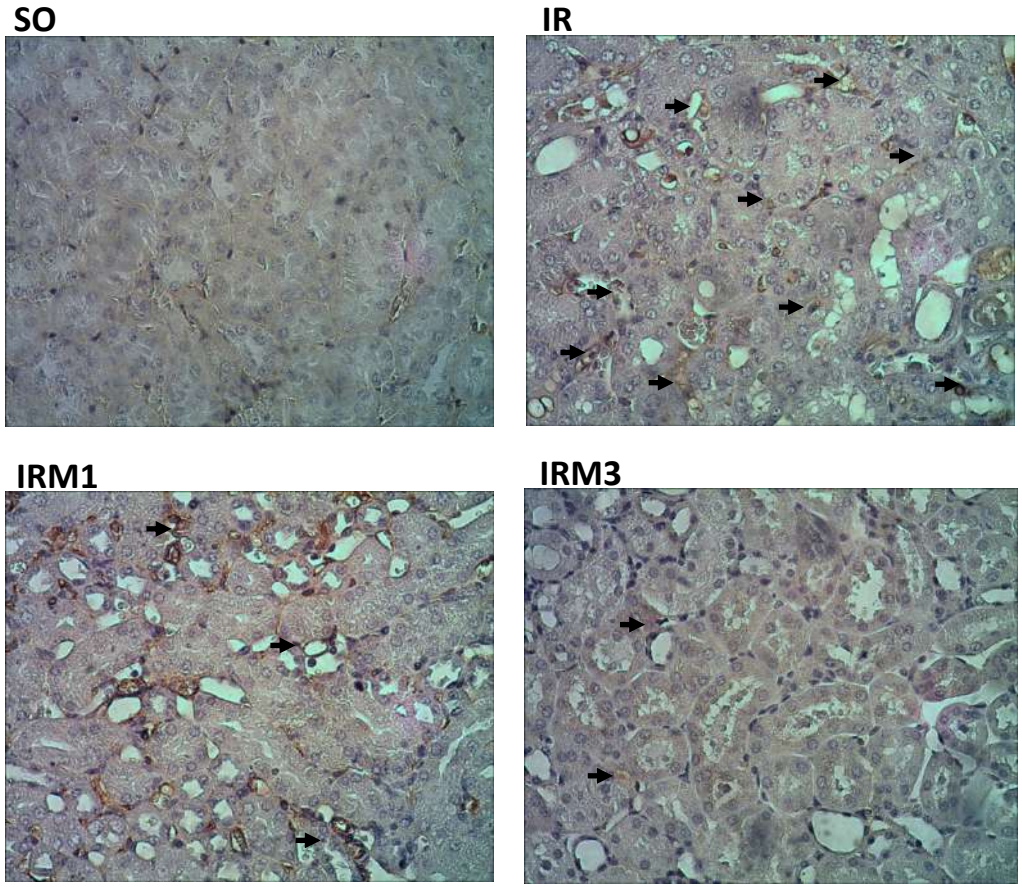
*p<0.001 vs SO
***p<0.05 vs IRM1
#p<0.05 vs IR

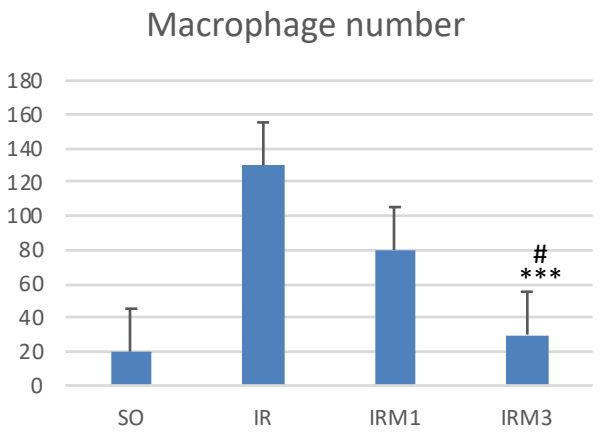
MSC Conditioned Medium ameliorated inflammation in kidney



MSC Conditioned Medium reduced inflammatory cells on kidney tubular injury

Immunostaining CD68
(magnification 400x)





***p<0.05 vs IRM1
#p<0.05 vs IR

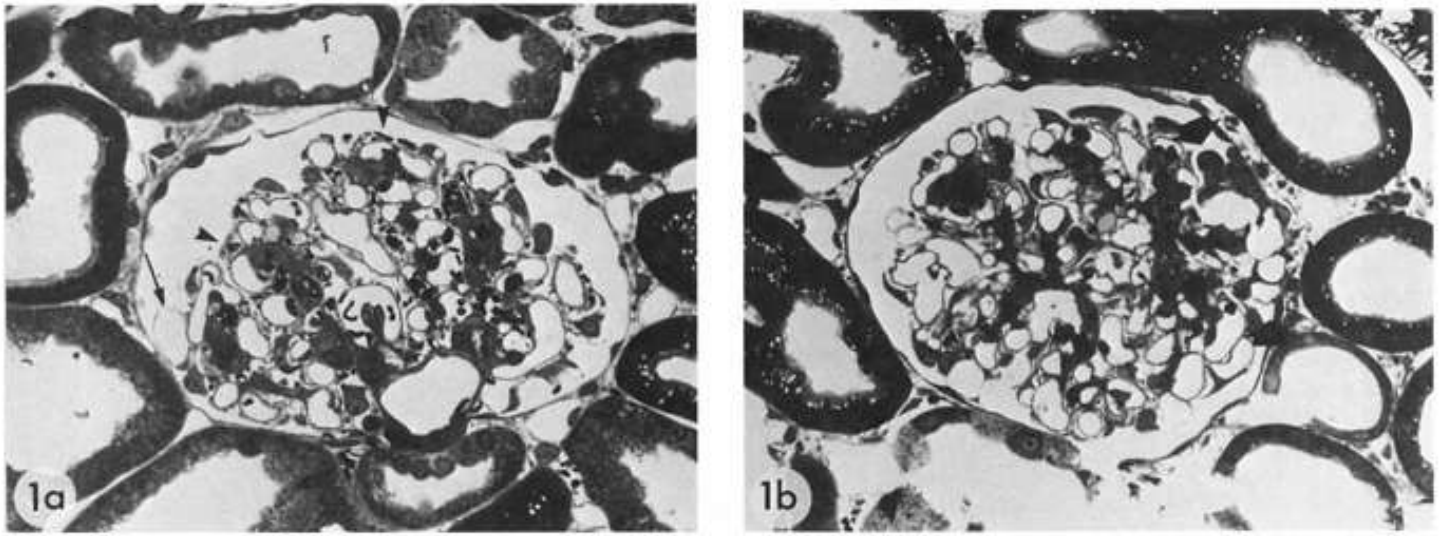


Fig. 1. *Light micrographs of glomeruli from experimental animals 14 days after surgery (group 3).* **A** *Glomerulus with dense epithelial reabsorption droplets* (arrowheads) *and cytoplasmic blebs* (arrow). Note also an expansion of the mesangium. **B** *Glomerulus with small adhesion between Bowman's capsule and segment of glomerular tuft* (arrows) (Toluidine blue, ×300).

Blockade of the Renin-Angiotensin and Endothelin Systems on Progressive Renal Injury

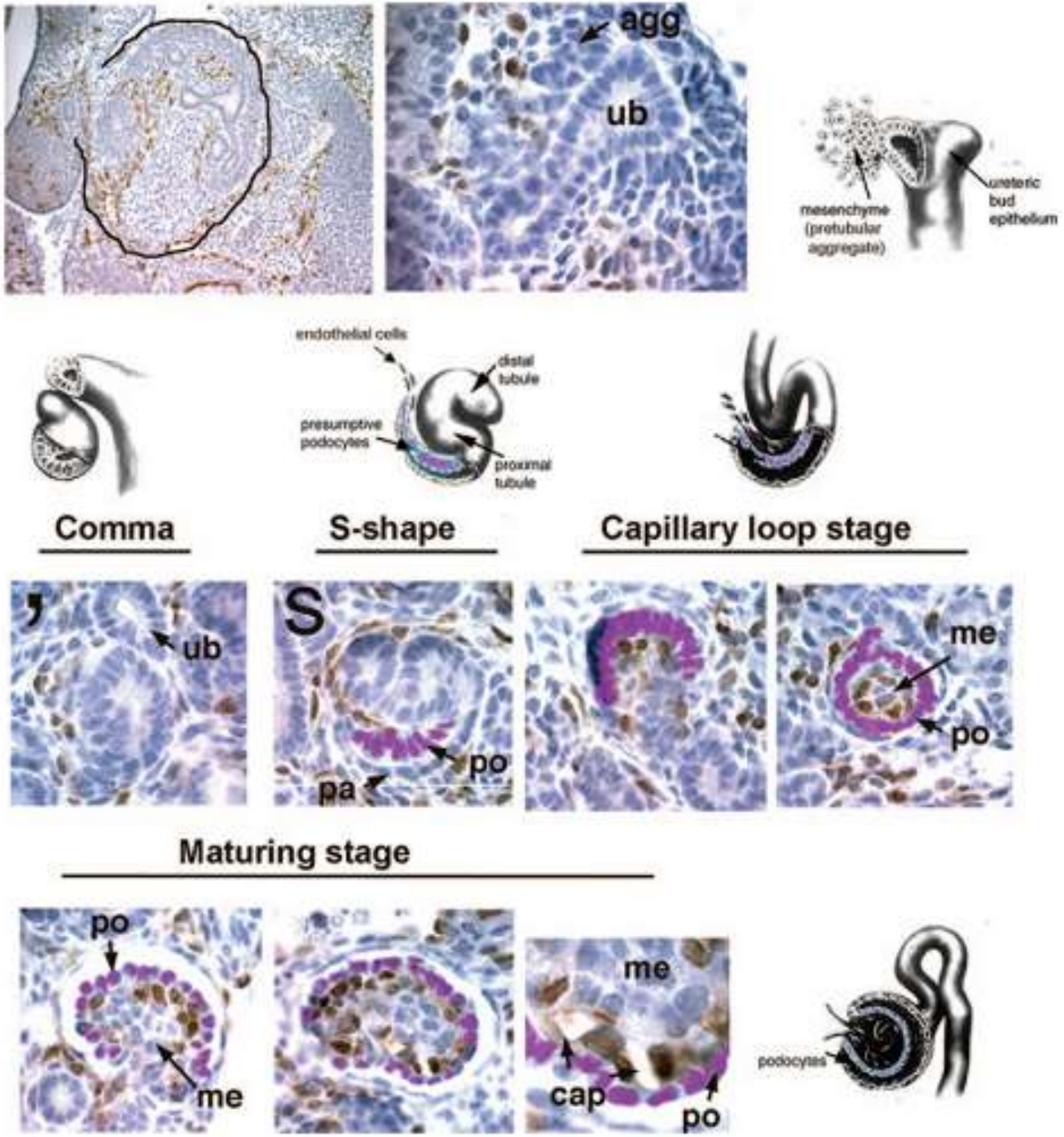
Zemin Cao, Mark E. Cooper, Leonard L. Wu, Alison J. Cox, Karin Jandeleit-Dahm, Darren J. Kelly, Richard E. Gilbert

By Homing to the Kidney, Activated Macrophages Potently Exacerbate Renal Injury

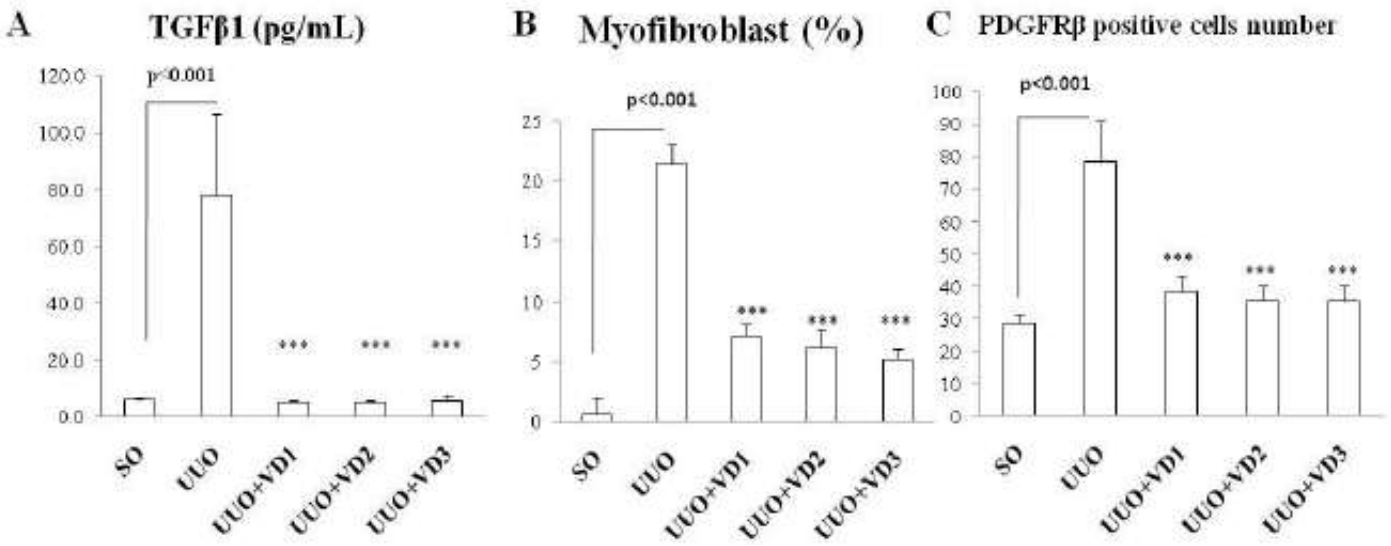
The American Journal of Pathology, Vol. 172, No. 6, June 2008

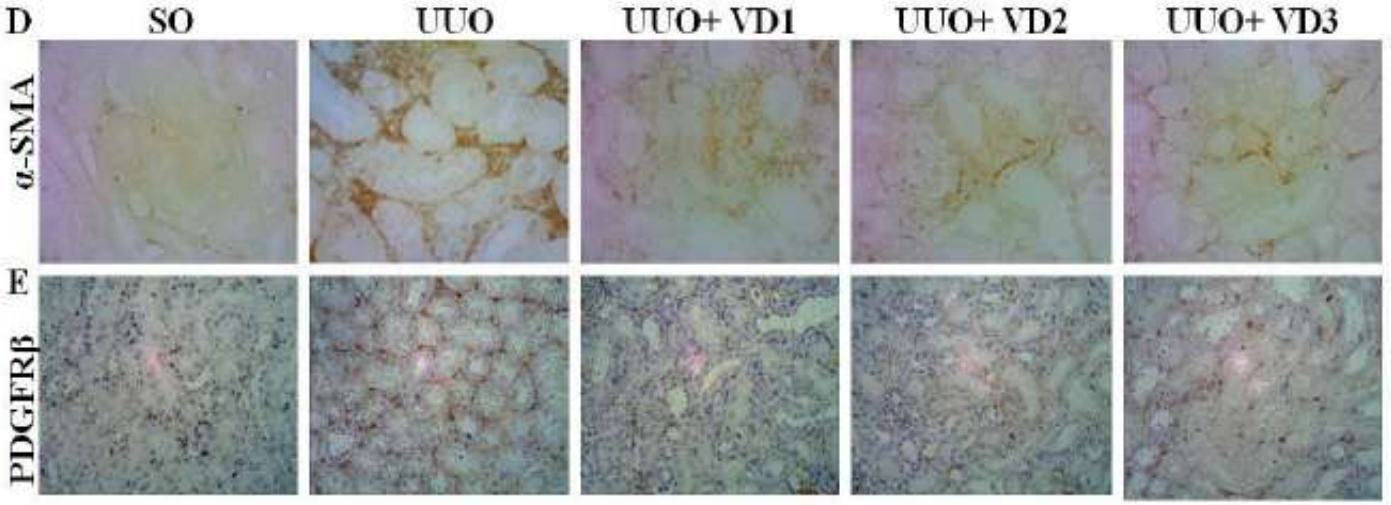
DOI: 10.2353/ajpath.2008.070625

Kidney Developmental / Nephrogenesis



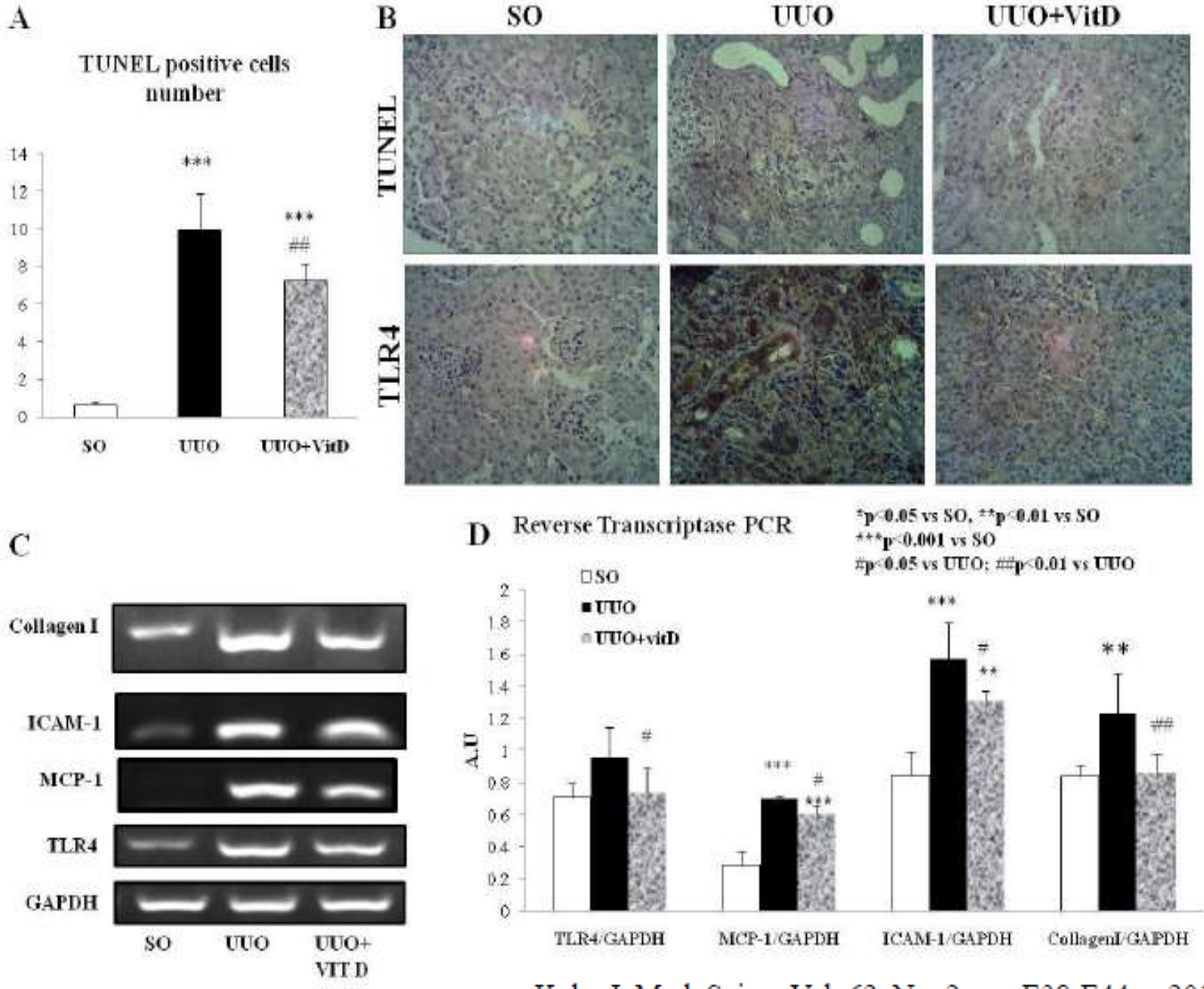
Vitamin D
renoprotective effects in kidney fibrosis-





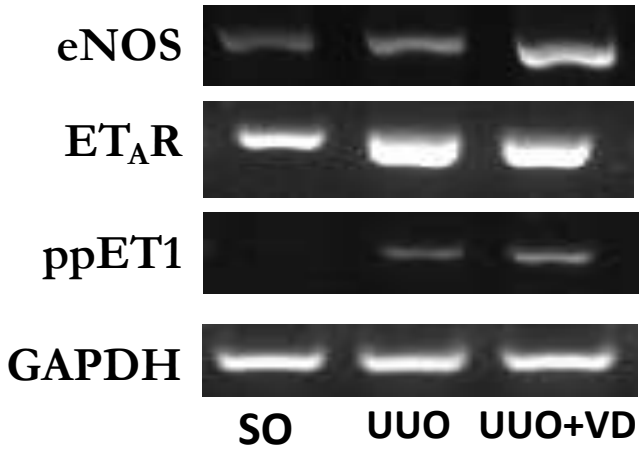
3 doses Vit D 0.125; 0.25, 0.5 µg/Kg BB

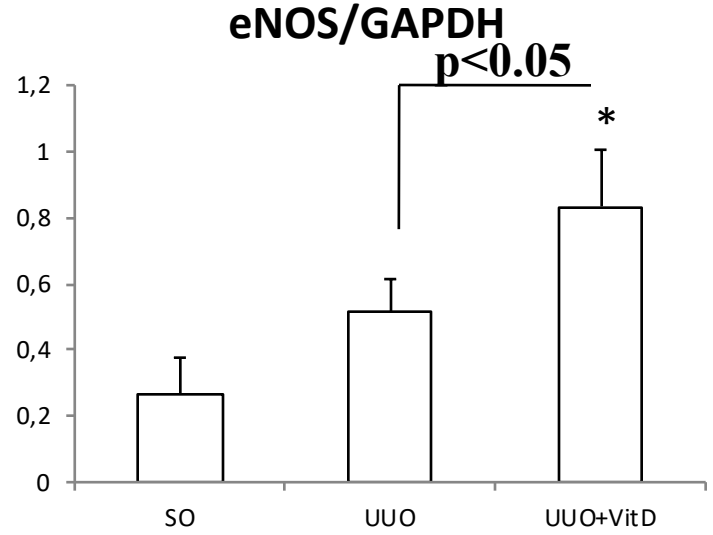
Arfian, et al 2016 Kobe J. Med. Sci., Vol. 62, No. 2, pp. E38-E44, 2016

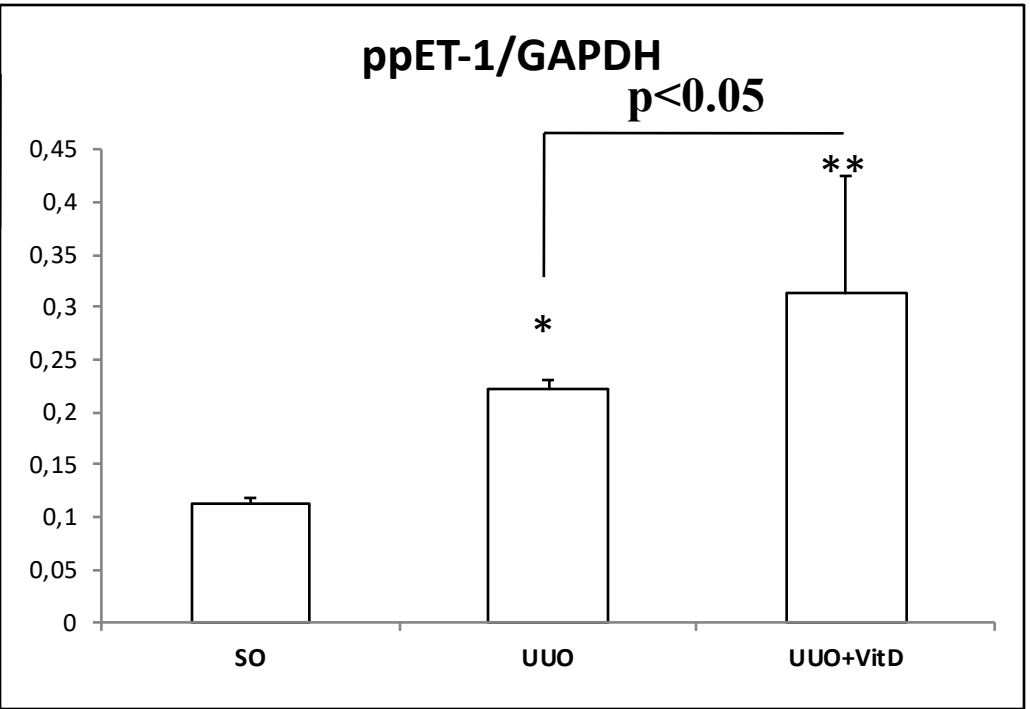


Kobe J. Med. Sci., Vol. 62, No. 2, pp. E38-E44, 2016

Upregulation of ET-1 and eNOS after Vitamin D treatment



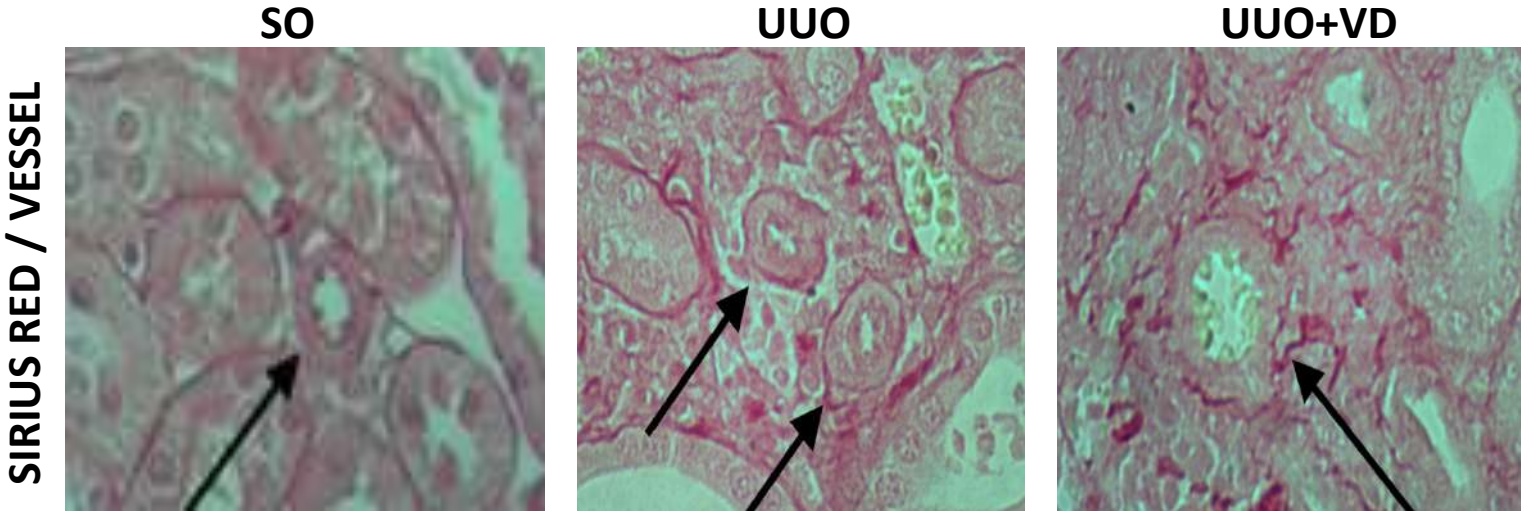




*p<0.05 VS SO
**p<0.01 vs SO

Arfian,et al unpublished data

Attenuation of Vascular remodelling in Vitamin D treated mice



group	*Wall Area*	*Lumen Area*	WLAR
SO	481,77±72,68	242,63±59,9	2,45±0,48
UUO	322,94±24,22	87.98±12,81*	4,68±0,58*
UUO-D	487,90±145,62	251.22±96,12†	2,51±0,80†

*p<0,05 VS SO
†p<0,05VS UUO

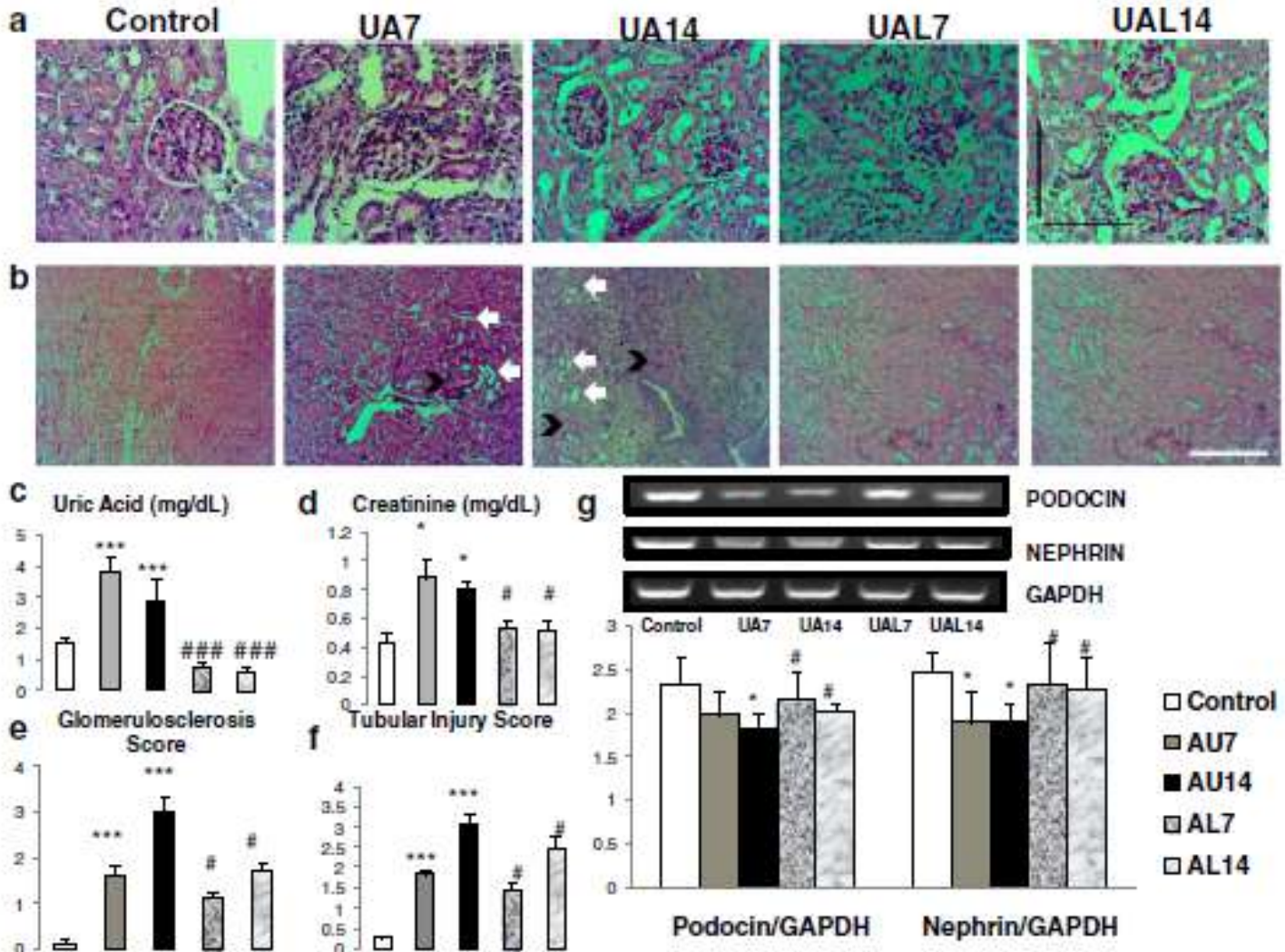
Uric acid in renal diseases

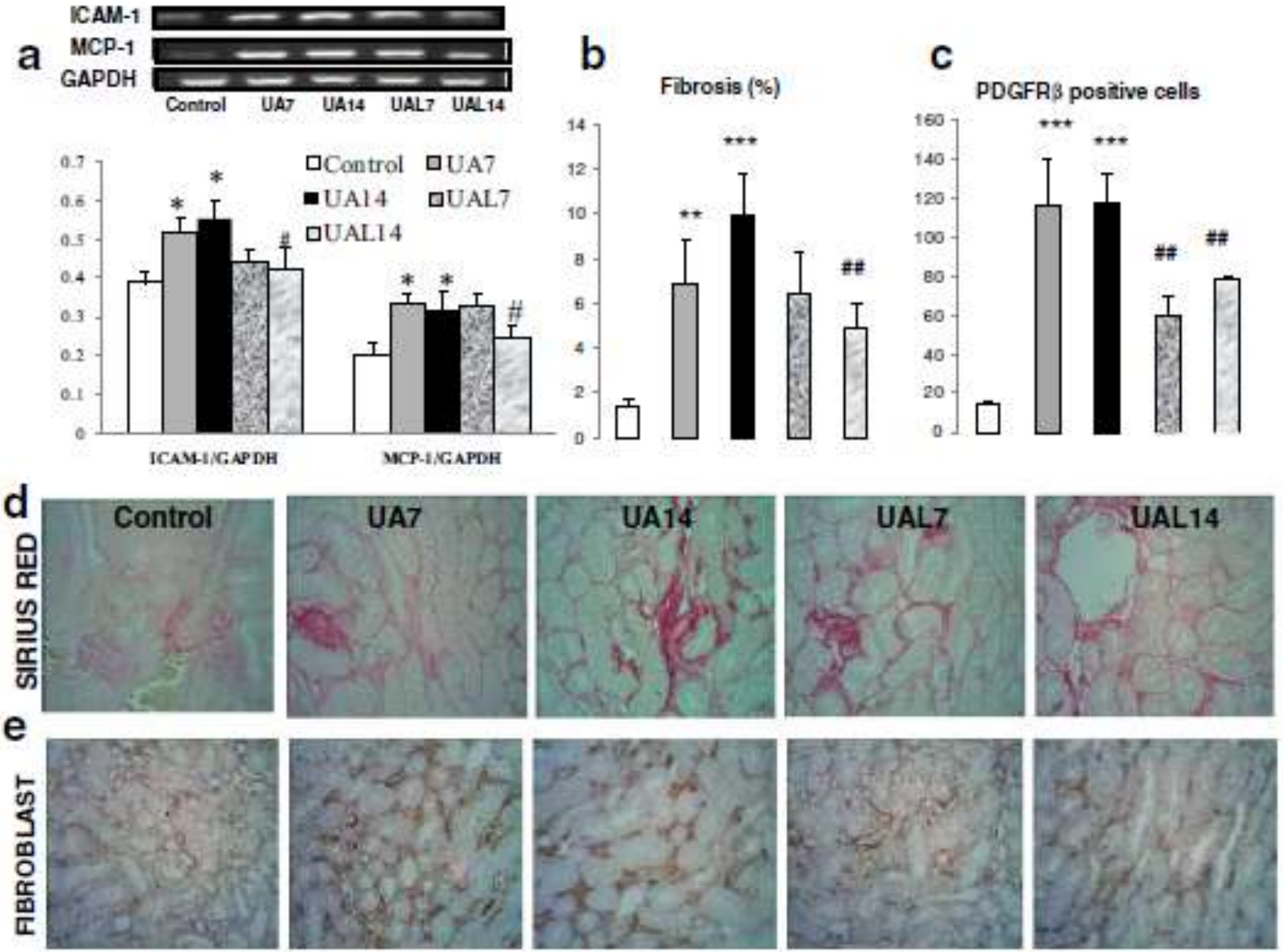
- In renal diseases, hyperuricemia had been already known contributed to the diseases progression and known as a marker of renal diseases (Berger and Yu 1975).
- Hyperuricemia nephropathy might induce renal failure due to high level of uric acid.
- Hyperuricemia model in mice

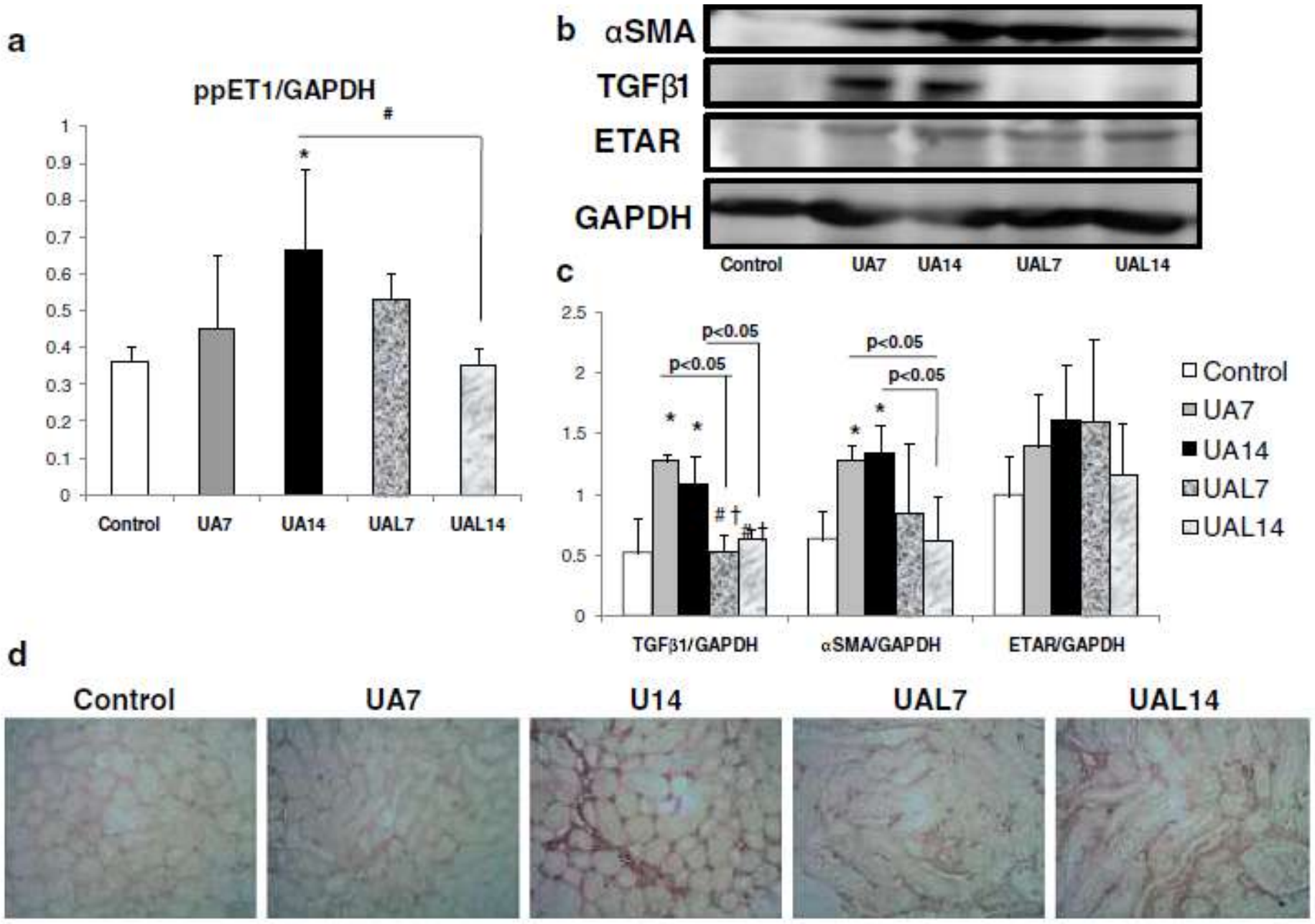
Uric acid causes kidney injury through inducing fibroblast expansion, Endothelin-1 expression, and inflammation

Muhammad Mansyur Romi[1], Nur Arfian[1*], Untung Tranggono[2], Wiwit Ananda Wahyu Setyaningsih[1] and Dwi Cahyani Ratna Sari[1]

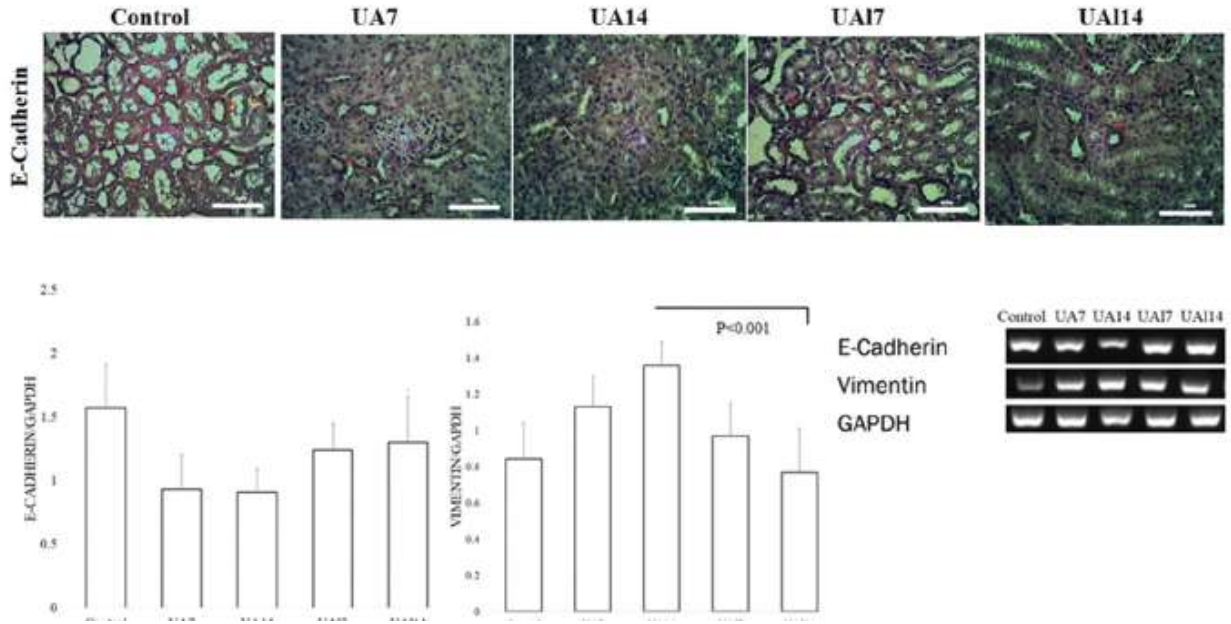
Romi *et al. BMC Nephrology*
DOI 10.1186/s12882-017-0736-x

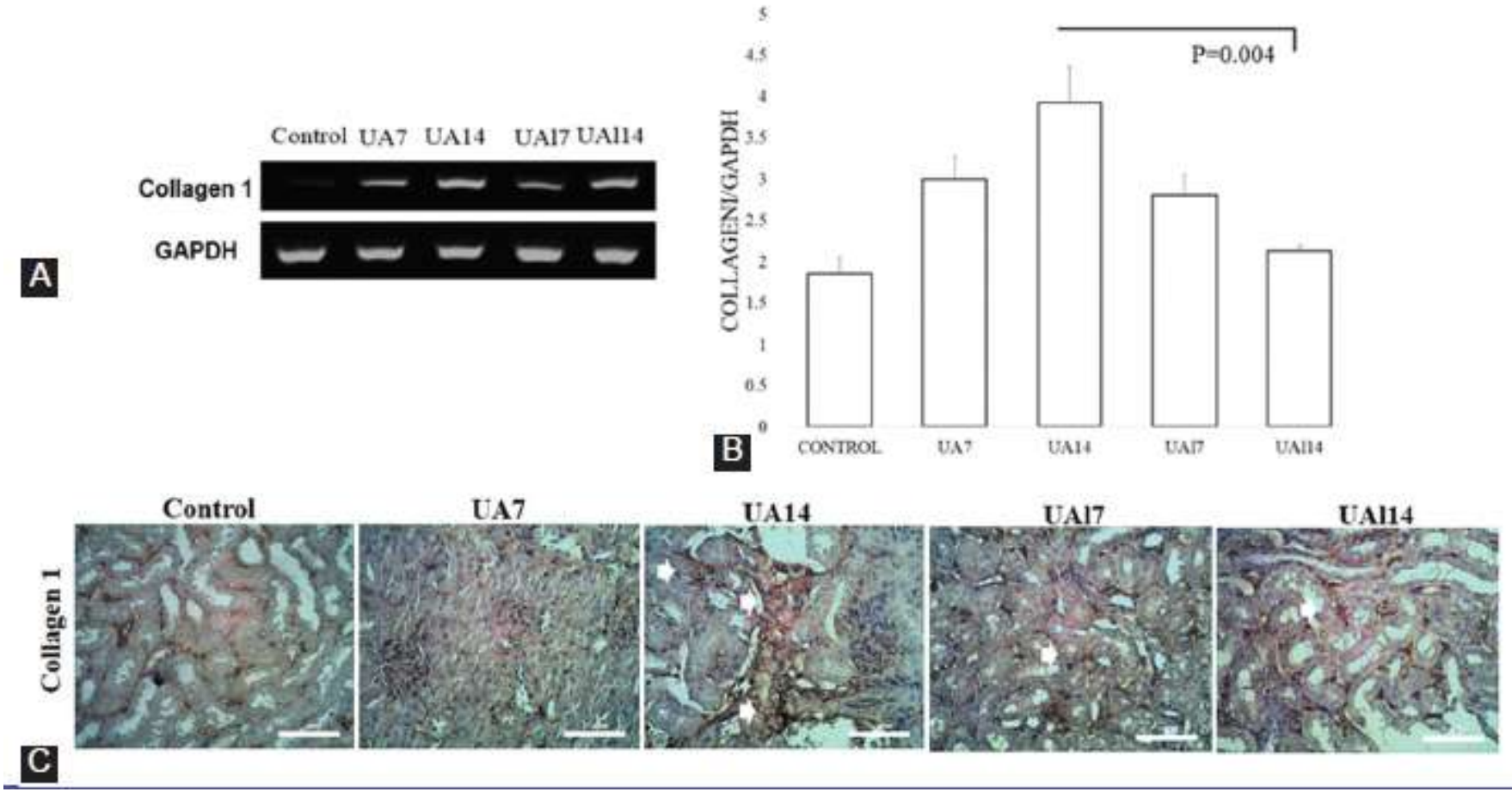



a
ICAM-1
MCP-1
GAPDH
Control
UA7
UA14
UAL7
UAL14
Control
UA7
UA14
UAL7
UAL14
ICAM-1/GAPDH
MCP-1/GAPDH
b
Fibrosis (%)
c
PDGFRβ positive cells
d
SIRIUS RED
Control
UA7
UA14
UAL7
UAL14
e
FIBROBLAST

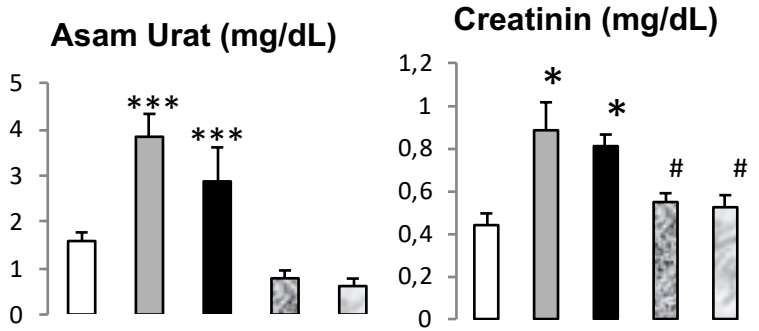

a
ppET1/GAPDH
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
#
Control
UA7
UA14
UAL7
UAL14
b
αSMA
TGFβ1
ETAR
GAPDH
Control
UA7
UA14
UAL7
UAL14
c
2.5
2
1.5
1
0.5
0
p<0.05
p<0.05
p<0.05
p<0.05
TGFβ1/GAPDH
αSMA/GAPDH
ETAR/GAPDH
Control
UA7
UA14
UAL7
UAL14
d
Control
UA7
U14
UAL7
UAL14

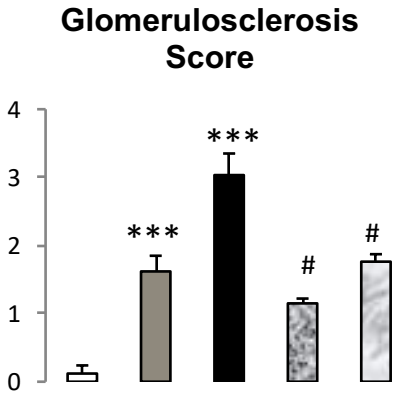
Hyperuricemia Induces Wnt5a/Ror2 Gene Expression, Epithelial–Mesenchymal Transition (EMT), and Kidney Tubular Injury in Mice

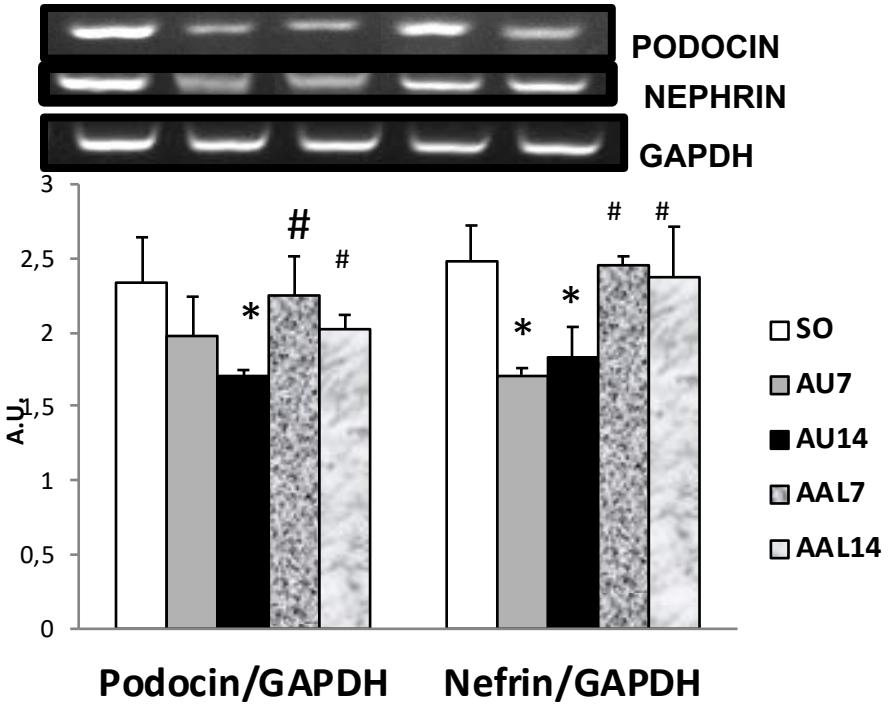


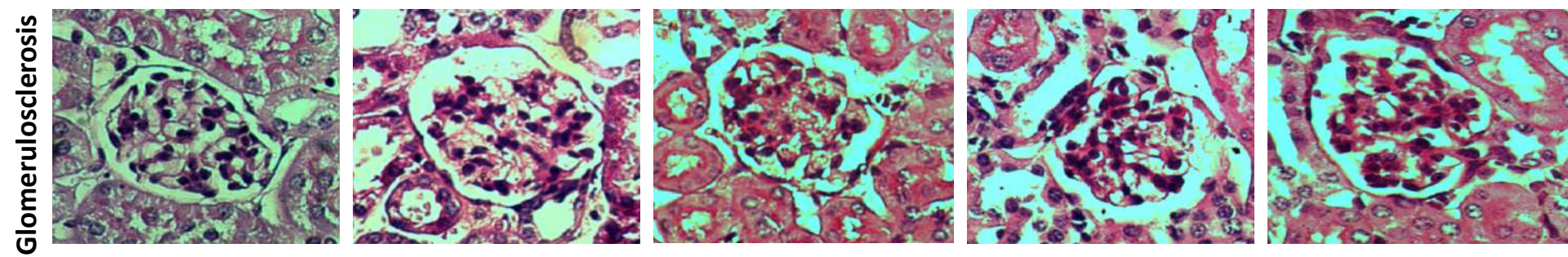

A
Control
UA7
UA14
UAI7
UAII14
Collagen 1
GAPDH
B
P=0.004
COLLAGEN1/GAPDH
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
CONTROL
UA7
UA14
UAI7
UAII14
C
Control
UA7
UA14
UAI7
UAII14
Collagen 1

Hyperuricemia induced glomerulosclerosis with podocyte injury









Uric acid induces Epithelial to Mesenchymal transition in kidney