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Experimentally induced renal insufficiency in laboratory animal

Measurement of GFR based on kinetics of renal inulin excretion

Ústav patologické fyziologie LF MU

Kidney functions

- regulation of
  - extracellular volume and blood pressure
  - ion balance
  - acid-base balance
  - excretion of waste
  - production of hormones

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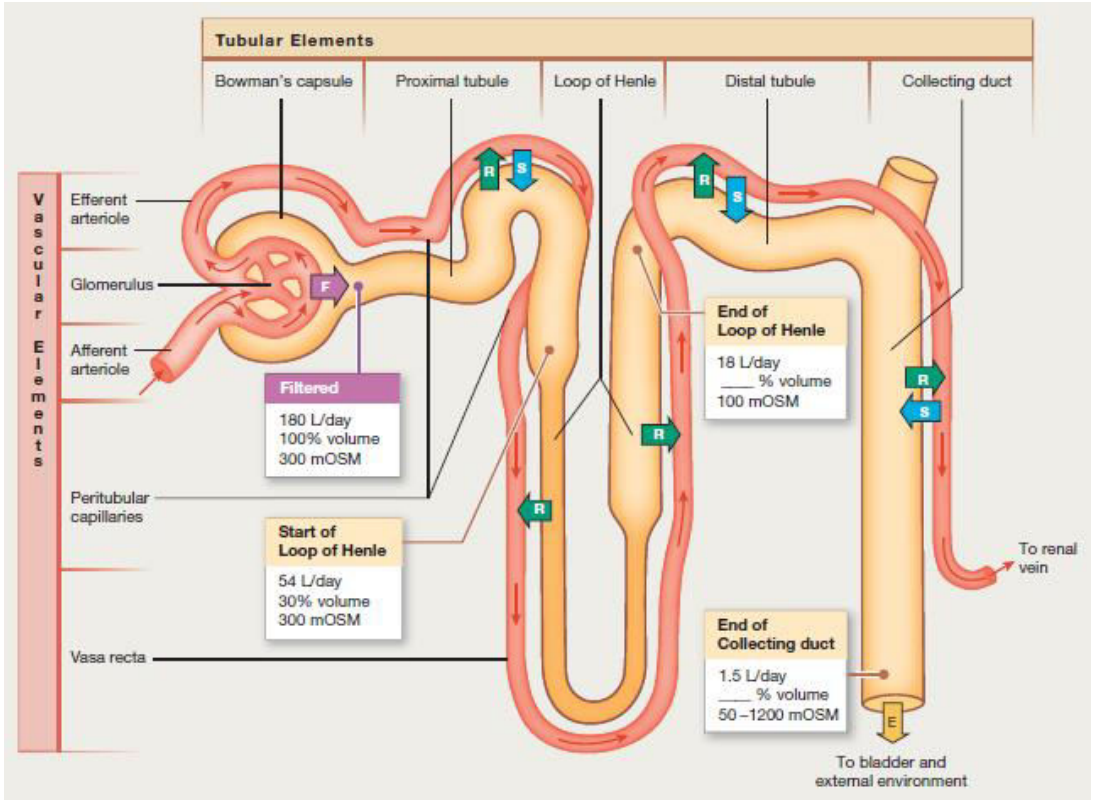
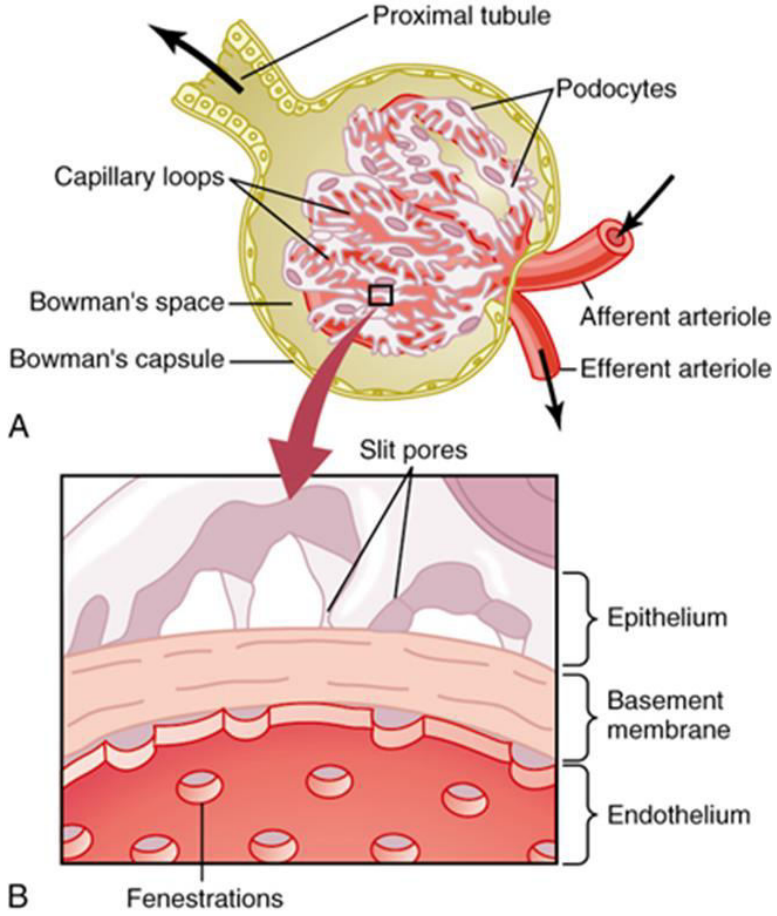
Nephron – basic processes

- nephron is the functional unit of the kidney
- filtration
  - movement of fluid from blood into the lumen of nephron
  - only in the renal corpuscule
  - filtrate is produced
- reabsorption
  - moving of substances from the filtrate into the blood
  - typically symport
    - Na/Glu, Na/AK…
  - transport maximum
    - renal threshold for glucose
- secretion
  - adding selected molecules from the blood to the filtrate
  - active proces, more selective then filtration

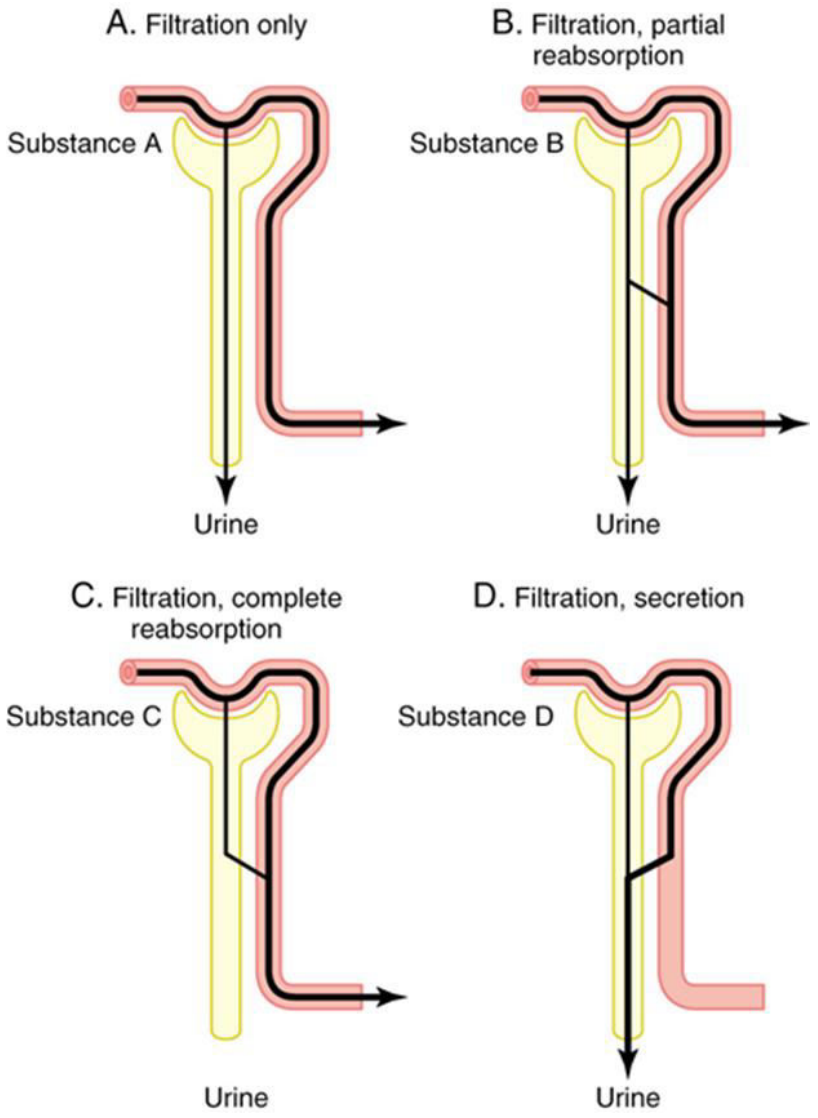


Filtration barrier

- filtration takes place in the renal corpuscule
- filtration barrier
  - glomerular capillary endothelium
    - fenestrated
    - negatively charged proteins on the surface
  - basement membrane
    - layer of extracellular matrix
    - separates endothelium from epitelial cells of Bowman‘s capsule
    - glycoproteins, collagen, …
  - epithelium of Bowman‘s capsule
    - podocytes
    - foot processes – long cytoplasmic extensions – leave narrow filtration slit closed by semiporous membrane
      - several unique proteins
- mesangial cells
  - between and around glomerular capillaries
  - contraction
    - actin-like filaments
  - production of cytokines



Renal handling of different substances



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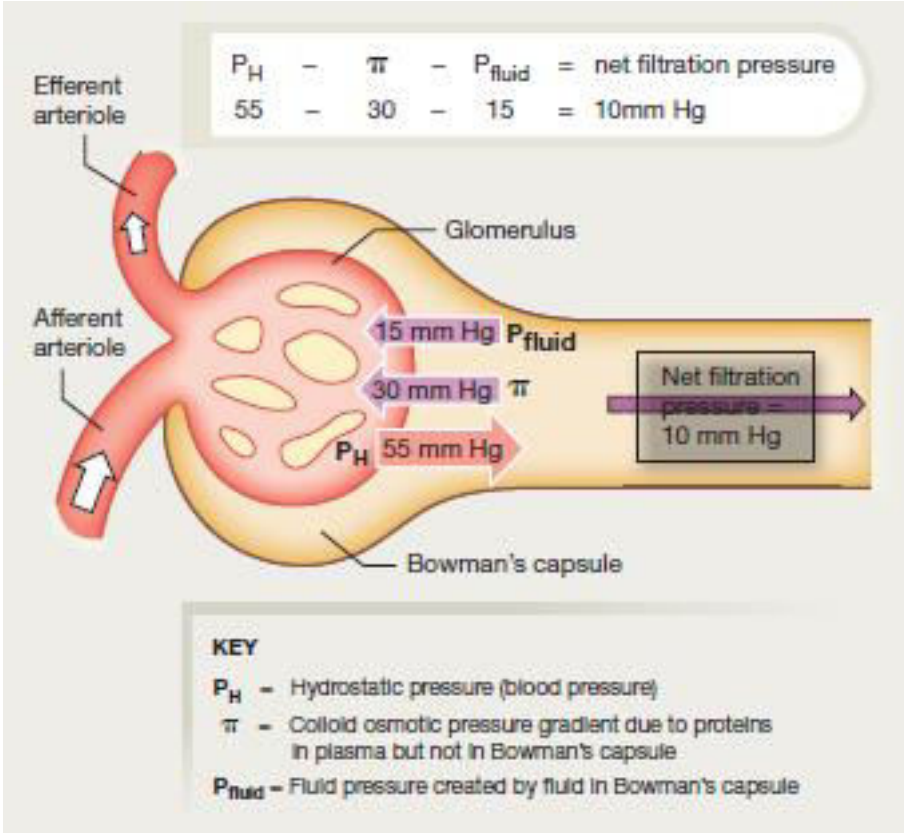
Glomerular filtration

- determined by 3 pressures
  - hydrostatic pressure
    - blood flowing through glomerular capillaries
    - 55 mm Hg, higher than opposite pressures
  - colloid osmotic pressure
    - inside glomerular capillaries
    - 30 mm Hg, higher than that of fluid in Bowman‘s capsule
    - favors fluid movement back into capillaries
  - fluid pressure
    - pressure of fluids in Bowman‘s capsule
    - 15 mm Hg, opposes filtration
- net driving force
  - 10 mm Hg
- GFR is influenced by 2 factors
  - net filtration pressure
  - filtration coefficient
    - surface area of glomerular capillaries
    - permeability



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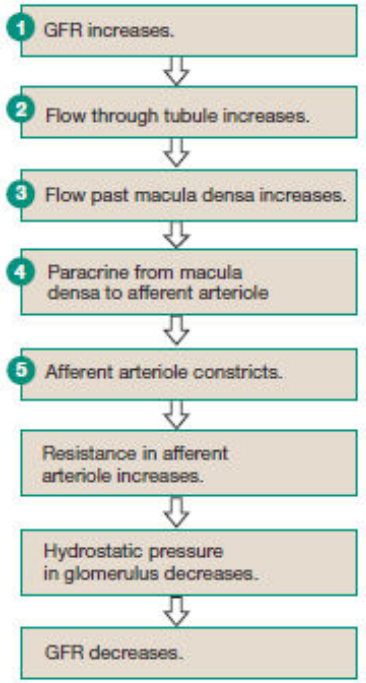
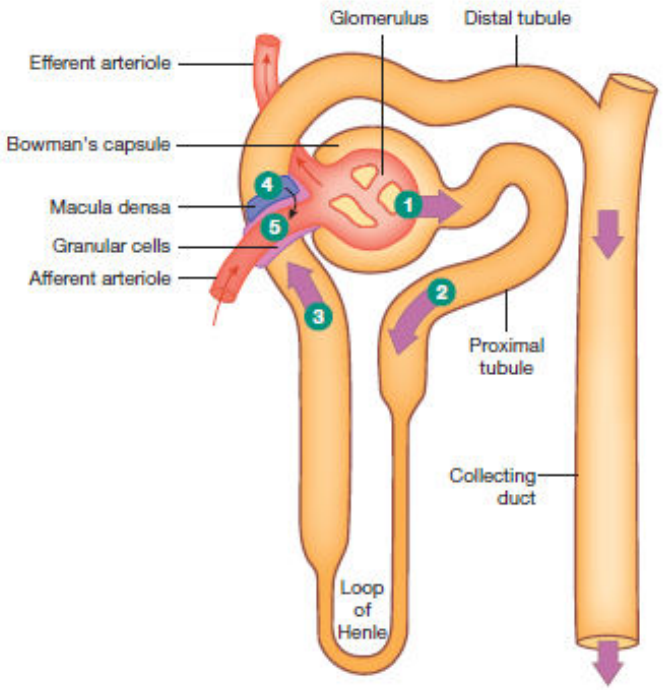
GFR is subject to autoregulation

- autoregulation
  - local control proces
  - kidney maintains a relatively constant GFR in the face of blood pressure fluctuations
    - 80 – 180 mm Hg
- mechanisms
  - myogenic response
    - intrinsic ability of vascular smooth muscle to respond to pressure changes
  - tubuloglomerular feedback
    - fluid flow through tubule influences GFR





Laboratory assessment of kidney disease

- glomerular filtration rate (GFR)
  - the best overal index of kidney function
  - approx. 125 ml/min/1.73 m^2 in young adult healthy individuals
    - indexing to body surface area reduces variation among healthy individuals
- proteinuria
  - diagnostic and prognostic information
    - CKD, AKI, preeclampsia
    - often earliest marker of glomerular diseases
- urinalysis
  - color
  - odor
  - relative density
  - pH
  - glucose
  - ketones

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Detection and diagnosis of kidney disease

- lack of signs in early stages of chronic kidney disease (CKD)
  - tests for screening and diagnosis are critical in nephrology
    - measurement of kidney function
    - early detection of abnormalities
    - specific diagnosis
    - determine prognosis
    - measurement of disease progression
    - effectiveness of therapy
  - CKD – independent risk factor for cardiovascular disease
    - identification of patients for cardiovascular disease risk factor management
- acute and chronic diseases are common
  - acute kidney injury occurs in 10 – 20 % of hospitalised patients
  - chronic kidney disease is present in 10 – 15 % of adults in the general population

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KDIGO classification of CKD

GFR Categories (mL/min/1.73 m²) Stage, Description, and Range				Persistent Albuminuria Categories, Description and Range		
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g (<3 mg/mmol)	30-300 mg/g (3-30 mg/mmol)	>300 mg/g (>30 mg/mmol)
	1	Normal or high	≥90	1 if CKD	1	2
	2	Mildly decreased	60–89	1 if CKD	1	2
	3a	Mildly to moderately decreased	45–59	1	2	3
	3b	Moderately to severely decreased	30–44	2	3	3
	4	Severely decreased	15–29	3	3	4+
	5	Kidney failure	<15	4+	4+	4+

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Kidney clearance

- the volume of plasma from which a substance is completely removed by the kidneys per time unit
- unit: volume/time
- indirect measure of glomerular filtration rate (GFR) and renal blood flow
  - GFR
    - clearance of filtered substance
  - perfusion
    - clearance of a substance that is filtered and also secreted (PAHA)

- 2 approaches to determine GFR
  - endogenous marker with stable serum concentration
  - exogenous marker
    - plasma concentration decreases
    - not routinely available because of the complexity of the protocol

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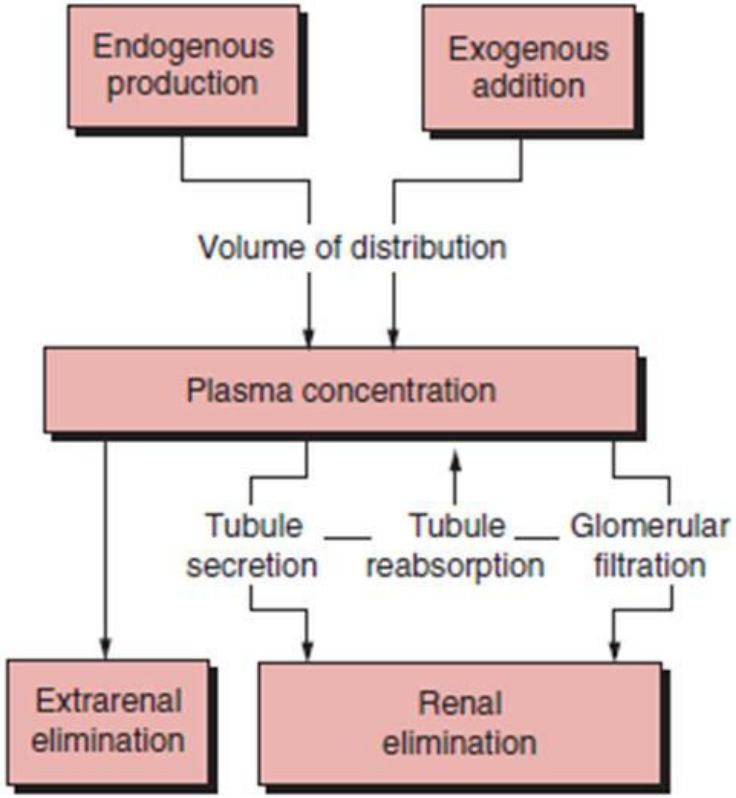
Marker of GFR

- requirements
  - stable plasma concentration
  - not protein bound
  - freely filtered by glomerulus
  - not secreted, reabsorbed, or metabolized by the tubules
  - not altering GFR *per se*
  - easy measurement
  - no interference



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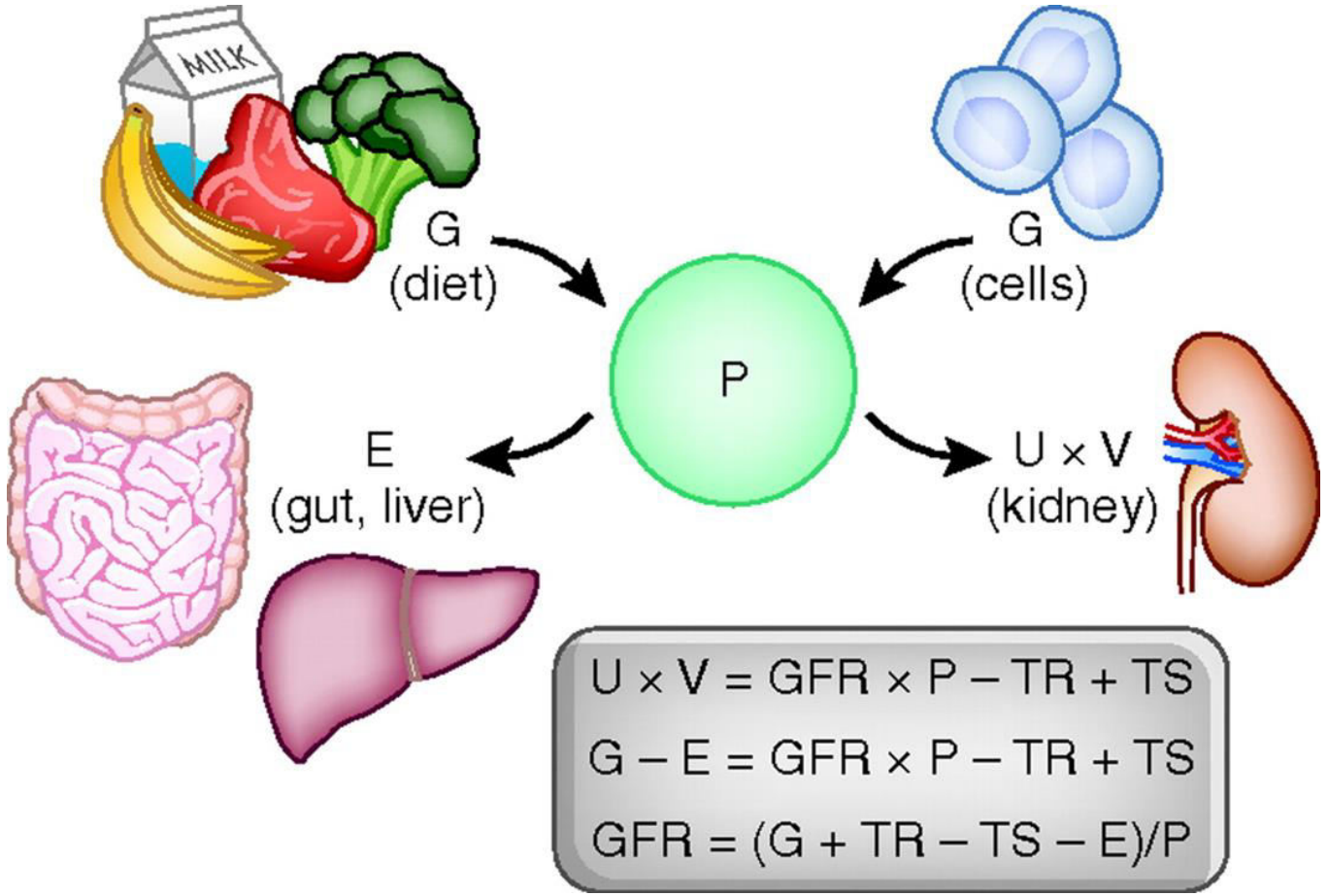
Determinants of the serum level of endogenous filtration markers



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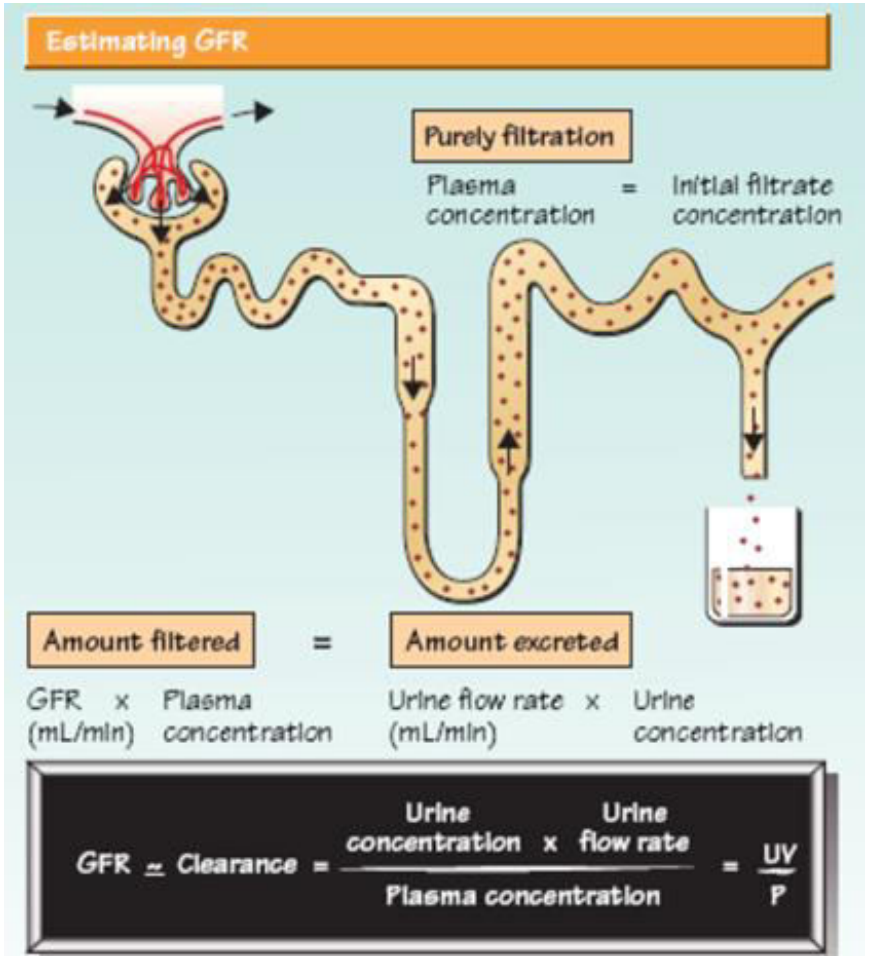
Relationship between clearance and GFR

- in the steady state, for a substance handled only be the kidneys that is neither reabsorbed nor secreted
  - amount filtered = amount excreted
  - GFR x P_x = U_x x V
  - GFR = U x V/Px
    - cX = volume of plasma filtered (~ GFR)
    - U_X = urine concentration of X
    - V = urine output (ml/min)
    - P_X = plasma concentration of X
- clearance of the substance corresponds to GFR
- reabsorption of a substance X by tubules
  - clearance <GFR
- secretion of substance X by tubules
  - clearance > GFR



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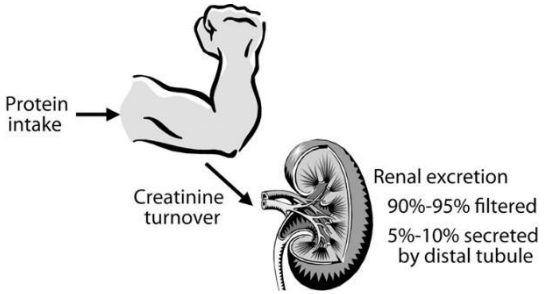
Creatinine

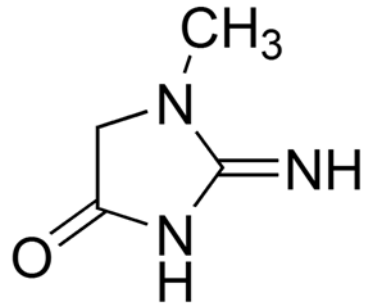
- proposed as a filtration marker in 1926
- produced by non-enzymatic degradation of phosphocreatine in the muscle
- relatively constant production
  - depends on age, gender and muscle mass
- possible GIT elimination at higher serum levels
- kidney handling
  - free filtration
  - no reabsorption
  - secretion up to 10%
- advantages
  - easy measurement
  - low cost
  - widespread availability of assays

Box 1 Factors associated with changes in serum creatinine, without change in glomerular filtration rate[3]

Increased serum creatinine
- Increased muscle mass
  - Male gender
  - Black/African race
- Diet
  - Cooked meat
  - Creatine supplementation
- Drugs
  - Inhibitors of tubular secretion: cimetidine, trimethoprim, fibrates
  - Increased creatinine production/release: corticosteroids, vitamin D metabolites

Decreased serum creatinine
- Decreased muscle mass
  - Female gender
  - Asian/Hispanic race
  - Muscle wasting/chronic disease
  - Limb amputation
- Vegetarian diet
- Critical illness





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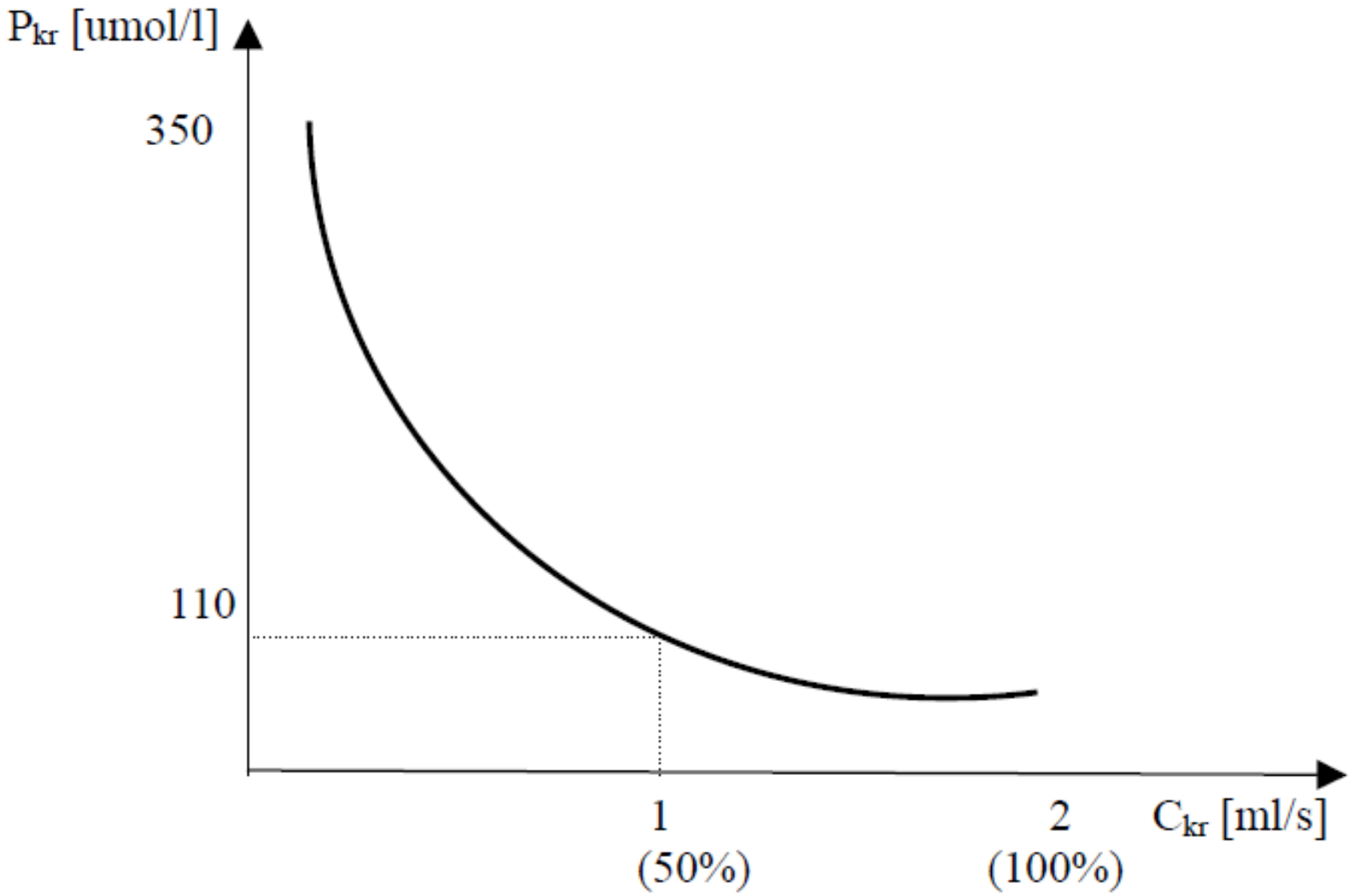
Serum creatinine vs. GFR



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Estimation of GFR from serum creatinine concentration

- CKD-EPI
- formerly
  - Cockroft-Gault
  - MDRD

Gender	Creatinine concentration	Formula
Woman	≤ 0.7	$GFR = 144 \times (Cr/0.7)^{-0.329} \times (0.993)^{age}$
	> 0.7	$GFR = 144 \times (Cr/0.7)^{-1.209} \times (0.993)^{age}$
Man	≤ 0.9	$GFR = 141 \times (Cr/0.9)^{-0.411} \times (0.993)^{age}$
	> 0.9	$GFR = 141 \times (Cr/0.9)^{-1.209} \times (0.993)^{age}$

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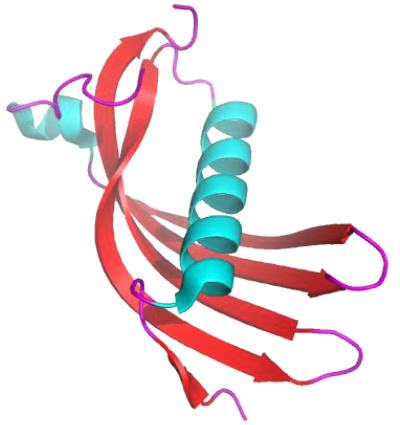
Cystatin C

- cysteine protease inhibitor
- produced by all nucleated cells
- freely filtered at glomerulus
- not secreted by tubules
- reabsorbed and metabolized by tubules
  - urine levels are low
- GFR is estimated from it‘s serum concentration
- serum concentration
  - depends on age and sex
  - further affected by
    - inflammation, treatment with corticoids, smoking
    - hyperthyreosis, CRP
  - factors that influence cystatin C levels are less defined

- possible combination of cystatin C and creatinine for the estimation of GFR



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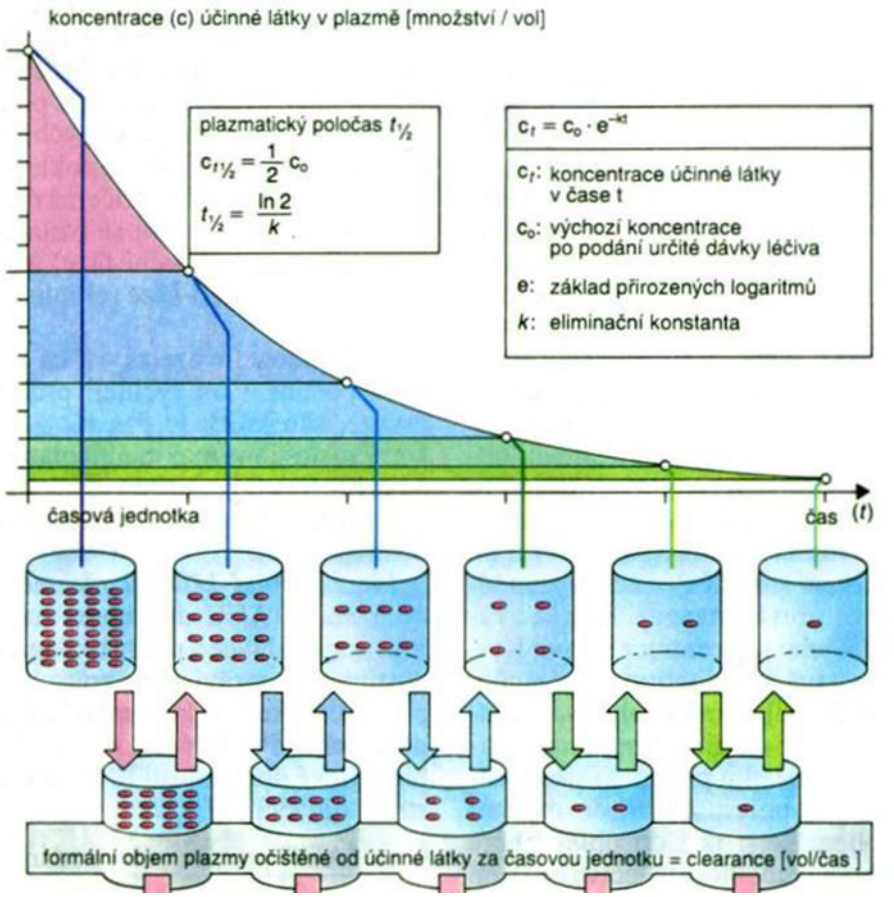
Measurement of glomerular filtration without collection of urine

- intravenous application of a substance excreted only by glomerular filtration
  - plasma concentration measurement in corresponding intervals
- plasma concentration decreases according to the exponential relation
- concentrations will be plotted with corresponding intervals on semi-logarithmic paper
  - we acquire line which we can use to identify biological half-life ($t_{1/2}$)
  - express the time it takes for the blood plasma concentration of a substance to halve its initial concentration (P_o)



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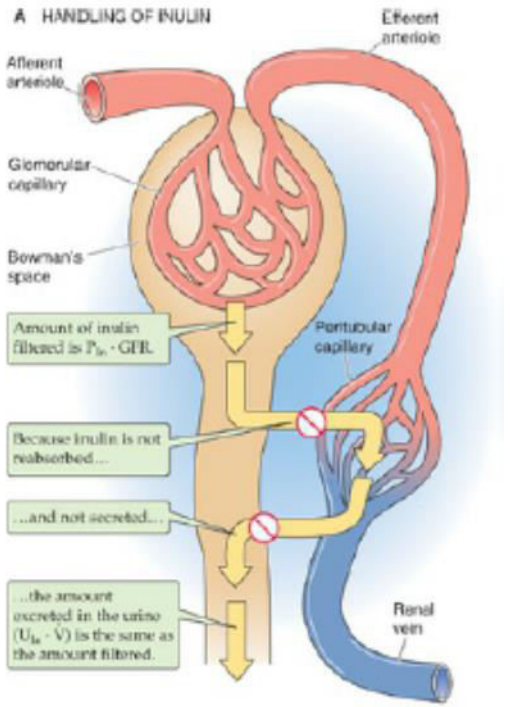
Inulin

- polysaccharide isolated from roots of a variety of plants
- meets all of the criteria for the ideal substance to measure GFR
- not practical for routine clinical applications
  - does not occur naturally in the body
- inulin clearance can be measured based on it‘s kinetics in extracellular fluid



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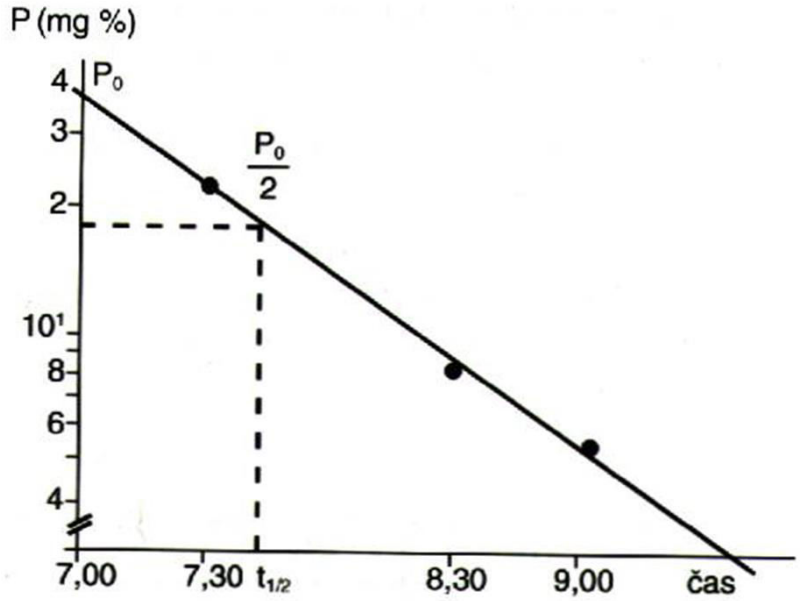
GFR calculation

- D = amount of inulin applied
  - 25 mg
- P_0 will be deduced from the curve
  - concentration in time when substance was injected = projection of line with axis Y
  - extrapolated value in relation to time when the dose was administered
- $t_{1/2}$ will be deduced from the curve



$$GFR = \frac{D \cdot \ln 2}{P_0 \cdot t_{1/2}}$$

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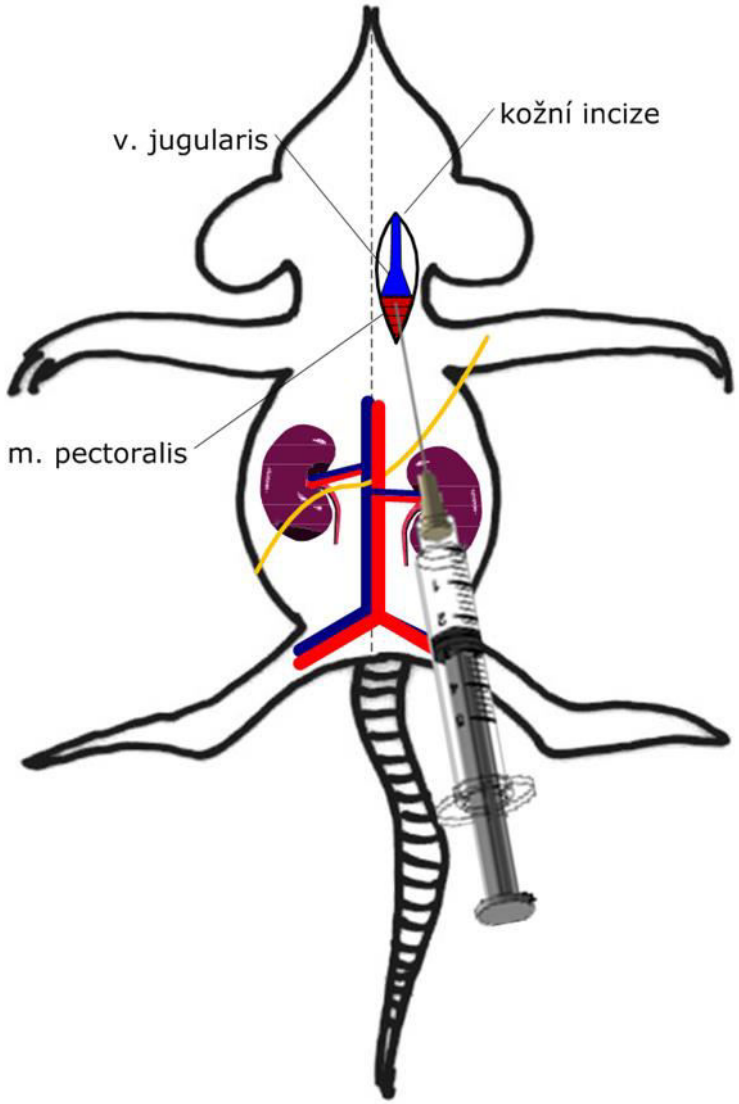
Experimental procedure

- general anesthesia
- middle laparotomy
- experimental procedure
  - ligation of a. renalis sinistra (**n = 6**)
  - control group – without ligature (**n = 6**)
- accessing v. jugularis and application of inulin
  - 25 mg/1,5 ml of physiologic solution)
- blood sampling
  - thoracotomy after 5, 10, 15, 20, 25 and 30 min
  - heart puncture - 1,5 ml of blood
- sample analysis
  - spectrophotometric determination of inulin concentration
- calculation of P_0 and $t_{1/2}$



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