Lecture-9
Pharmaceutical Biotechnology

Elimination of Protein Therapeutics

- Protein-based therapeutics are generally subject to the same catabolic pathways as endogenous or dietetic proteins.
- The end products of protein metabolism are thus amino acids that are reutilized in the endogenous amino acid pool for the de novo biosynthesis of structural or functional proteins in the human body.

- Non-metabolic elimination pathways such as renal or biliary excretion are negligible for most proteins.
- If biliary excretion occurs, however, it is generally followed by subsequent metabolic degradation of the compound in the gastrointestinal tract.

Proteolysis

- The metabolic rate for protein degradation generally increases with decreasing molecular weight from large to small proteins and peptides (Table 1),
- But is also dependent on other factors such as size, charge, lipophilicity, functional groups, and glycosylation pattern as well as secondary and tertiary structure.

Molecular weight	Elimination site	Predominant elimination mechanisms	Major determinant
<500	Blood, liver	Extracellular hydrolysis Passive lipoid diffusion	Structure, lipophilicity
500–1,000	Liver	Carrier-mediated uptake Passive lipoid diffusion	Structure, lipophilicity
1,000–50,000	Kidney	Glomerular filtration and subsequent degradation processes (see Fig. 4)	Molecular weight
50,000–200,000	Kidney, liver	Receptor-mediated endocytosis	Sugar, charge
200,000–400,000		Opsonization	α_2-macroglobulin, IgG
>400,000		Phagocytosis	Particle aggregation

Note: Other determining factors are size, charge, lipophilicity, functional groups, sugar recognition, vulnerability for proteases, aggregation to particles, formation of complexes with opsonization factors, etc. Mechanisms may overlap and endocytosis may occur at any molecular weight range.
Source: After Meijer and Ziegler, 1993.

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About Table 1

Notes:

- The molecular weight is the major determinant of the elimination mechanisms of peptides and proteins.
- Other determining factors are size, charge, lipophilicity, functional groups, sugar recognition, vulnerability or susceptibility for proteases, aggregation to particles, formation of complexes with opsonization factors, etc.
- Mechanisms may overlap and endocytosis may occur at any molecular weight range.

- The clearance of a peptide or protein describes the irreversible removal of active substance from the vascular space, which includes besides metabolism also cellular uptake.
- Proteolytic degradation of proteins can occur unspecifically nearly everywhere in the body or can be limited to a specific organ or tissue.

- Due to this unspecific proteolysis of some proteins already in blood as well as potential active cellular uptake, the clearance of protein drugs can exceed cardiac output, i.e., > 5L/min for blood clearance and > 3L/min for plasma clearance.

- Molecular weight determines the major metabolism site as well as the predominant degradation process.
- Proteolytic enzymes such as proteases and peptidases are ubiquitous (everywhere) throughout the body.
- Sites capable of extensive peptide and protein metabolism are not only limited to the liver, kidneys, and gastrointestinal tissue, but also include blood and vascular endothelium as well as other organs and tissues.

- As proteases and peptidases are also located within cells, intracellular uptake is per se more an elimination rather than a distribution process.
- While peptidases and proteases in the gastrointestinal tract and in lysosomes are relatively unspecific, soluble peptidases in the interstitial space and exopeptidases on the cell surface have a higher selectivity and determine the specific metabolism pattern of an organ.
- The proteolytic activity of SC tissue, for example, results in a partial loss of activity of SC compared to IV administrated interferon-γ

Gastrointestinal Protein Metabolism

- The gastrointestinal tract is a major site of protein metabolism with high proteolytic enzyme activity due to its primary function to digest dietary protein.
- ➢ Thus, gastrointestinal metabolism of protein drugs is one of the major factors limiting systemic bioavailability of orally administered protein drugs.

- The metabolic activity of the gastrointestinal tract, however, is not limited to orally administered peptidase and proteins.

➢ Parenterally administered peptides and proteins may also be metabolised in the intestinal mucosa following intestinal secretion. At least 20% of the degradation of endogenous albumin, for example has been reported to take place in the gastrointestinal tract.

Renal Protein Metabolism and Excretion

1. The kidneys are a major site of protein metabolism for smaller sized proteins that undergo glomerular filtration.

- The size-selective cut-off for glomerular filtration is approximately 60 kDa, although the effective molecule radius based on molecular weight and conformation is probably the limiting factor.
- Glomerular filtration is most efficient, however, for proteins smaller than 30 kDa.

- Peptides and small proteins (<5kDa) are filtered very efficiently, and their glomerular filtration clearance approaches the glomerular filtration rate (GFR,~ 120 mL/ min in humans).
- For molecular weight exceeding 30 kDa, the filtration rate falls off sharply.

2. In addition to size selectivity, **charge selectivity** has also been observed for glomerular filtration where anionic macromolecules pass through the capillary wall less readily than neutral macromolecules, which in turn pass through less readily than cationic macromolecules.

- Renal metabolism of peptides and small proteins is mediated through **three highly effective process**.

➢ As a result, only minuscule amounts of intact protein are detected in urine. (i.e., very small amount)

1. **The first mechanism** involves glomerular filtration of larger, complex peptides and proteins followed by reabsorption into endocytic vesicles in the proximal tubule and subsequent hydrolysis into small peptide fragments and amino acids.

- This mechanism of elimination has been described for IL-2, IL-11, growth hormone, and insulin.

- **The second mechanism** entails (requires) glomerular filtration followed by intraluminal metabolism, predominantly by exopeptidases in the luminal brush border membrane of the proximal tubule.
- The resulting peptide fragments and amino acids are reabsorbed into systemic circulation.
- This route of disposition applies to small linear peptides such as glucagon and LH-RH.

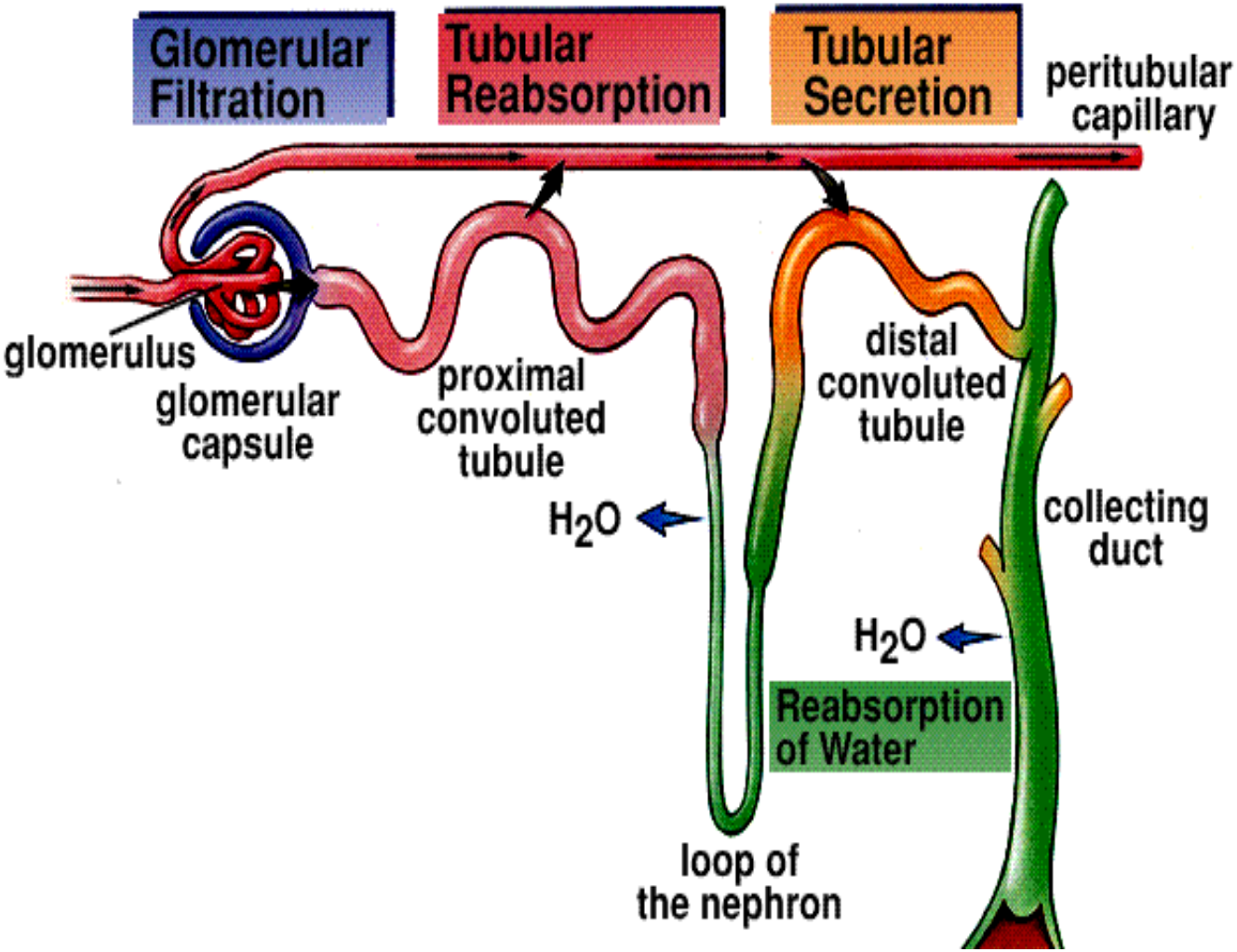
- Recent studies implicate the proton driven peptide transporters PEPT1 and especially PEPT2 as the main route of cellular uptake of small peptides and peptide-like drugs from the glomerular filtrates.
- These high-affinity transport proteins seem to exhibit selective uptake of di- and tripeptides, which implicates their role in renal amino acid homeostasis.

- For both mechanisms, glomerular filtration is the dominant, rate-limiting step as subsequent degradation process are not saturable under physiologic conditions.
- Due to this limitation of renal elimination, the renal contribution to the overall elimination of proteins is dependent on the proteolytic activity for these proteins in other body organs.

1. If metabolic activity for these proteins is high in other body regions, there is only minor renal contribution to total clearance, and it becomes negligible in the presence of unspecific degradation throughout the body.
2. If metabolic activity is low in other tissues or if distribution to the extravascular space is limited, however, the renal contribution to total clearance may approach 100%.

- This is for instance the case for recombinant human interleukin-10 (rhIL-10), for which clearance correlation closely with GFR, making dose adjustments necessary in patient with impaired renal function.

3. **The third mechanism** of renal metabolism in peritubular extraction of peptides and proteins from post-glomerular capillaries with subsequent intracellular metabolism.


Glomerular Filtration
Tubular Reabsorption
Tubular Secretion
peritubular capillary
glomerulus
glomerular capsule
proximal convoluted tubule
distal convoluted tubule
collecting duct
H_2O
H_2O
Reabsorption of Water
loop of the nephron

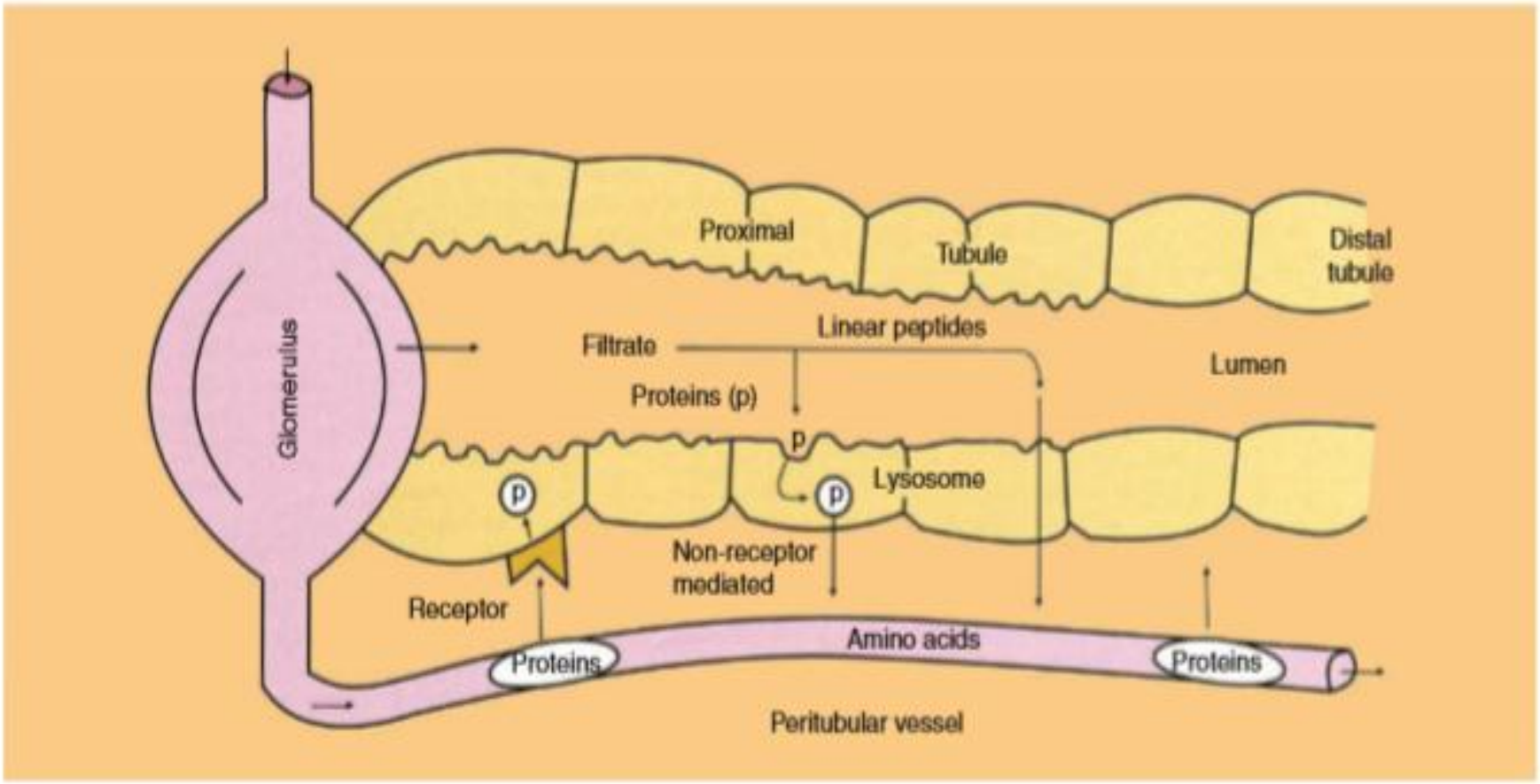
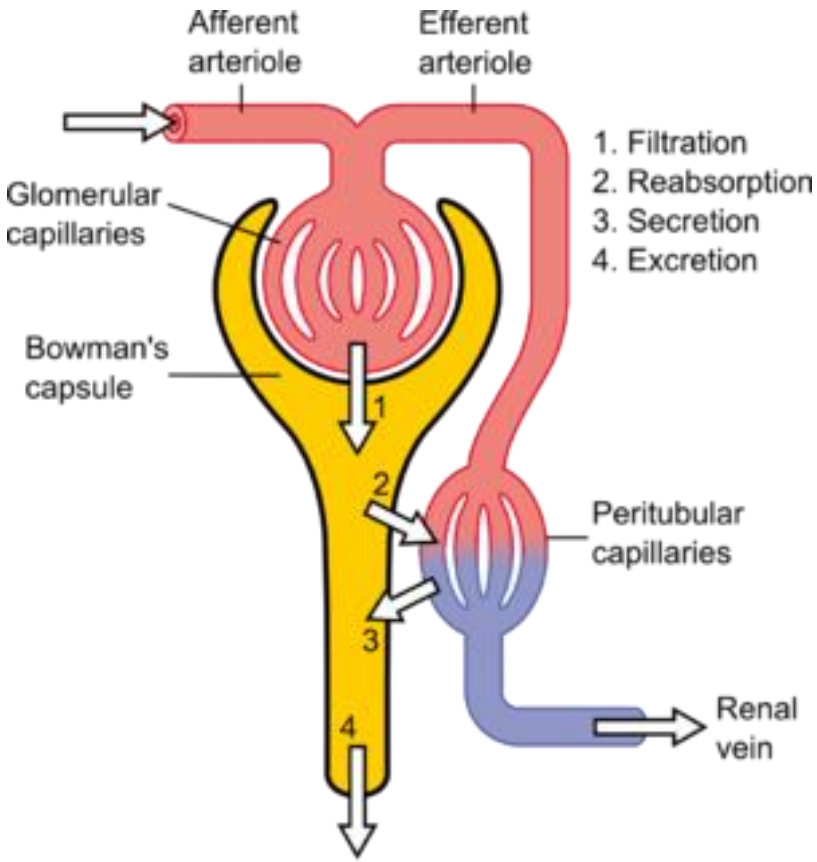


Figure 4 Pathways of renal metabolism of peptides and proteins: Glomerular filtration followed by either (a) intraluminal metabolism or (b) tubular reabsorption with intracellular lysosomal metabolism, and (c) peritubular extraction with intracellular lysosomal metabolism. *Source*: Modified from Maack et al., 1985.

- Figure shows pathways of renal elimination of proteins, include:

1. Glomerular filtration
2. Catabolism at the luminal membrane
3. Tubular absorption followed by intracellular degradation, and
4. Postglomerular peritubular uptake followed by intracellular degradation.



Excretion = Filtration – Reabsorption + Secretion