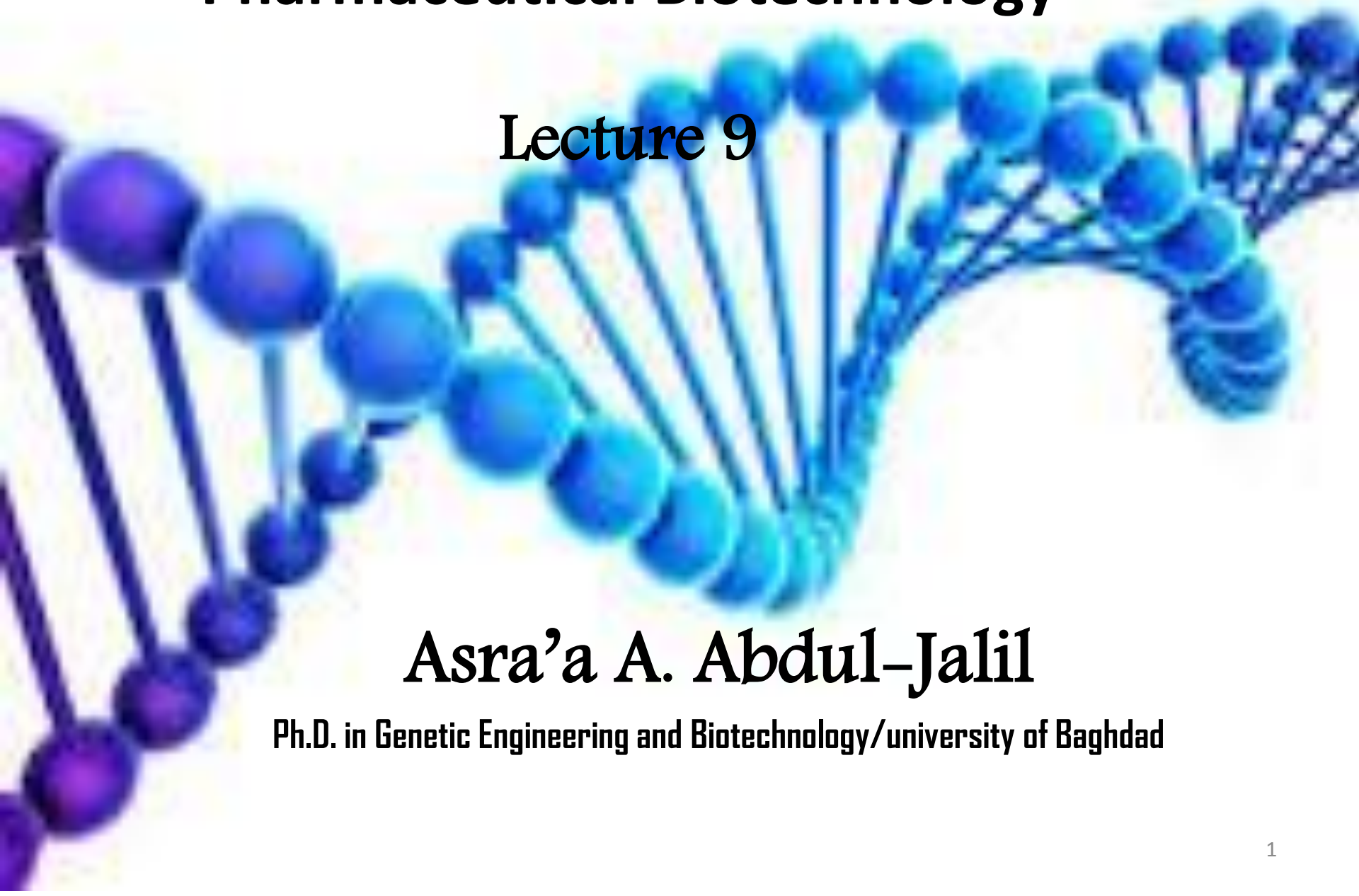
Pharmaceutical Biotechnology

Lecture 9



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The characterization of the pharmacokinetics and pharmacodynamics of peptide and protein therapeutics, however, arise from some of their specific properties:

a. Their structural similarity to endogenous structural or functional proteins and nutrients.

b. Their intimate involvement in physiologic processes on the molecular level, often including regulatory feedback mechanisms.

c. The analytical challenges to identify and quantify them in the presence of a myriad of similar molecules.

d. Their large molecular weight and macromolecule character (for proteins)

PHARMACOKINETICS OF PROTEIN THERAPEUTICS

Peptides which frequently have hormone activity, usually have short elimination half-lives, which is desirable for a close regulation of their endogenous levels and thus function. **Insulin**, for example shows dose-dependent elimination with a relatively short half-life of **26 and 52 minutes** at 0.1 and0.2 U/kg, respectively. Contrary to that, proteins that have transport tasks such as albumin or long-term immunity functions such as **immunoglobulins** have elimination half-lives of several days, which enables and ensures the continuous maintenance of physiologically necessary concentrations in the bloodstream. This is for example reflected by the elimination half-life of antibody drugs such as the anti-epidermal growth factor receptor antibody cetuximab, an IgG1 chimeric antibody for which a half-life of approximately 7 days has been reported.

Absorption of Protein Therapeutics

❑ Enteral Administration

- Peptides and proteins, unlike conventional small molecule drugs, are generally not therapeutically active upon oral administration

- The lack of systemic bioavailability is mainly caused by two factors:

(1) high gastrointestinal enzyme activity and

(2) Low permeability through the gastrointestinal mucosa

- Since **oral administration is still a highly desirable route of delivery for protein drugs due to** its:

❑ **Convenience.**

❑ **Cost-effectiveness.**

❑ **Painlessness.**

❖ Suggested approaches to increase the oral bioavailability of protein drugs include :

➢ encapsulation into micro- or nanoparticles thereby protecting proteins from intestinal degradation

➢ **Other strategies are chemical modifications such as amino acid backbone modifications**

➢ **chemical conjugations to improve the resistance to degradation and the permeability of the protein drug.**

➢ **Coadministration of protease inhibitors has also been suggested for the inhibition of enzymatic degradation**

❑ Parenteral Administration

- Most peptide and protein drugs are currently formulated as parenteral formulations because of their poor oral bioavailability. Major routes of administration include intravenous (**IV**), subcutaneous (**SC**), and intramuscular (**IM**) administration.

In addition, other non-oral administration pathways are utilized, including **nasal, buccal, rectal, vaginal, transdermal, ocular and pulmonary drug delivery.**

❖ IV administration of peptides and proteins offers the **advantage** of **circumventing presystemic degradation**, thereby achieving the highest concentration in the biological system.

❖ Protein therapeutics given by the **IV route** include, among many others:**The tissue plasminogen activator (t-PA) analogs alteplase and tenecteplase. The recombinant human erythropoietin α.**

❖ One of the potential limitations of SC and IM administration, however, are the **presystemic degradation processes** frequently associated with these administration routes, resulting in a **reduced bioavailability compared to IV administration.**

Several peptide and protein therapeutics including insulin are administered as SC injections. Following an SC injection, peptide and protein **therapeutics may enter the systemic circulation either via blood capillaries or through lymphatic vessels.**

In general, macromolecules larger than 16 kDa are predominantly absorbed into the lymphatics whereas those under 1 kDa are mostly absorbed into the blood circulation. There appears to be a linear relationship between the molecular weight of the protein and the proportion of the dose absorbed by the lymphatics. This is of particular importance for those agents whose therapeutic targets are lymphoid cells (i.e., interferons and interleukins).

Q/ Which pathway of absorption is rather unique for proteins after SC injection?
Biodistribution from the injection site into the lymphatic system.(diagram)

Distribution of Protein Therapeutics

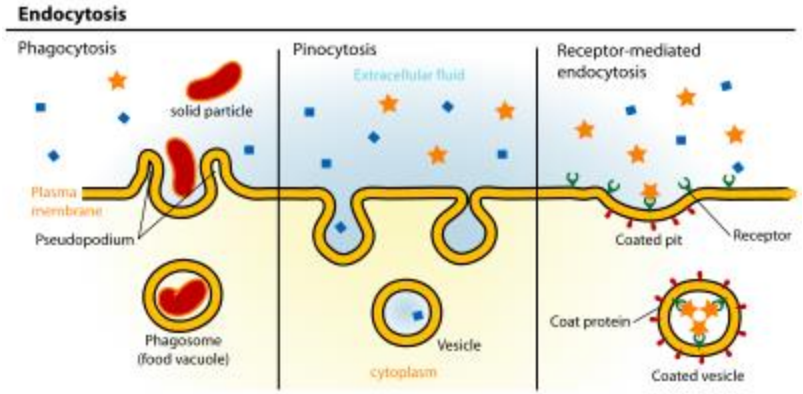
The rate and extent of protein distribution is determined largely by their:

- **Size and molecular weight.**
- **Physiochemical properties (e.g., charge, lipophilicity).**
- **Protein binding.**
- **Their dependency on active transport processes**

Since **most therapeutic proteins have high molecular weights** and are thus **large in size, their apparent volume of distribution is usually small** and limited **to the volume of the extracellular space** due to their limited mobility secondary to impaired passage through biomembranes.

In contrast to small molecule drugs, protein transport from the vascular space into the interstitial space of tissues is largely mediated by convection rather than diffusion, following the unidirectional fluid flux from the **vascular space through paracellular pores into the interstitial tissue space.** The subsequent removal from the tissues is accomplished by lymph drainage back into the systemic circulation. This underlines the unique role.

Another, but much less prominent pathway for the movement of protein molecules from the vascular to the interstitial space is transcellular migration via endocytosis.



Besides the size-dependent sieving of macromolecules through the capillary walls, **charge** may also play an important role in the biodistribution of proteins. **It has been suggested that the electrostatic attraction between positively charged proteins and negatively charged cell membranes might increase the rate and extent of tissue distribution.**

- ✓ Most cell surfaces are negatively charged because of their abundance of glycosaminoglycans in the extracellular matrix.

Protein Binding of Protein Therapeutics

- Another factor that can influence the distribution of therapeutic peptides and proteins is **binding to endogenous protein structures. Physiologically active endogenous peptides and proteins** frequently interact **with specific binding proteins** involved in their transport and regulation.

Furthermore, interaction with binding proteins may enable or facilitate cellular uptake processes and thus affect the drug's pharmacodynamics.

Similarly, therapeutically administered proteins may interact with endogenous binding proteins

Specific binding proteins have been identified for numerous protein drugs, including **recombinant human DNase** for use as mucolytic in cystic fibrosis, **growth hormone**, and **recombinant human vascular endothelial growth factor (rhVEGF).**

Elimination of Protein Therapeutics

- Protein-based therapeutics is generally subject to the same **catabolic pathways as endogenous proteins.** The **end products** of protein metabolism are thus **amino acids.**

- **Non-metabolic elimination pathways such as renal or biliary excretion** are negligible for most proteins.

Proteolysis

The **metabolic rate for protein degradation generally increases with decreasing molecular weight** from large to small proteins to peptides (**Table below**), but is also dependent on other factors such as:

- Size.
- Charge.
- Lipophilicity.
- Functional groups.
- Glycosylation.
- Pattern.
- 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.

Table Molecular weight as major determinant of the elimination mechanisms of peptides and proteins.

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.

The proteolytic activity of SC tissue, for example, results in a partial loss of activity of SC compared to IV administrated interferon-g.

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 proteins.** 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 proteins.

Parenterally administered peptides and proteins may also be metabolized 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 GIT.

Renal Protein Metabolism and Excretion

- 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 kD, Glomerular filtration is most efficient, however, for proteins smaller than 30 kDa.

Renal metabolism of peptides and small proteins is mediated through three highly effective processes (Fig. 8). As a result, only minuscule amounts of intact protein are detectable in urine.

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

2. **The second mechanism** entails 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 the systemic circulation.

3. The third mechanism of renal metabolism is peritubular extraction of peptides and proteins from post-glomerular capillaries with subsequent intracellular metabolism.
Peritubular transport of proteins and peptides from the basolateral membrane has also been shown for insulin.

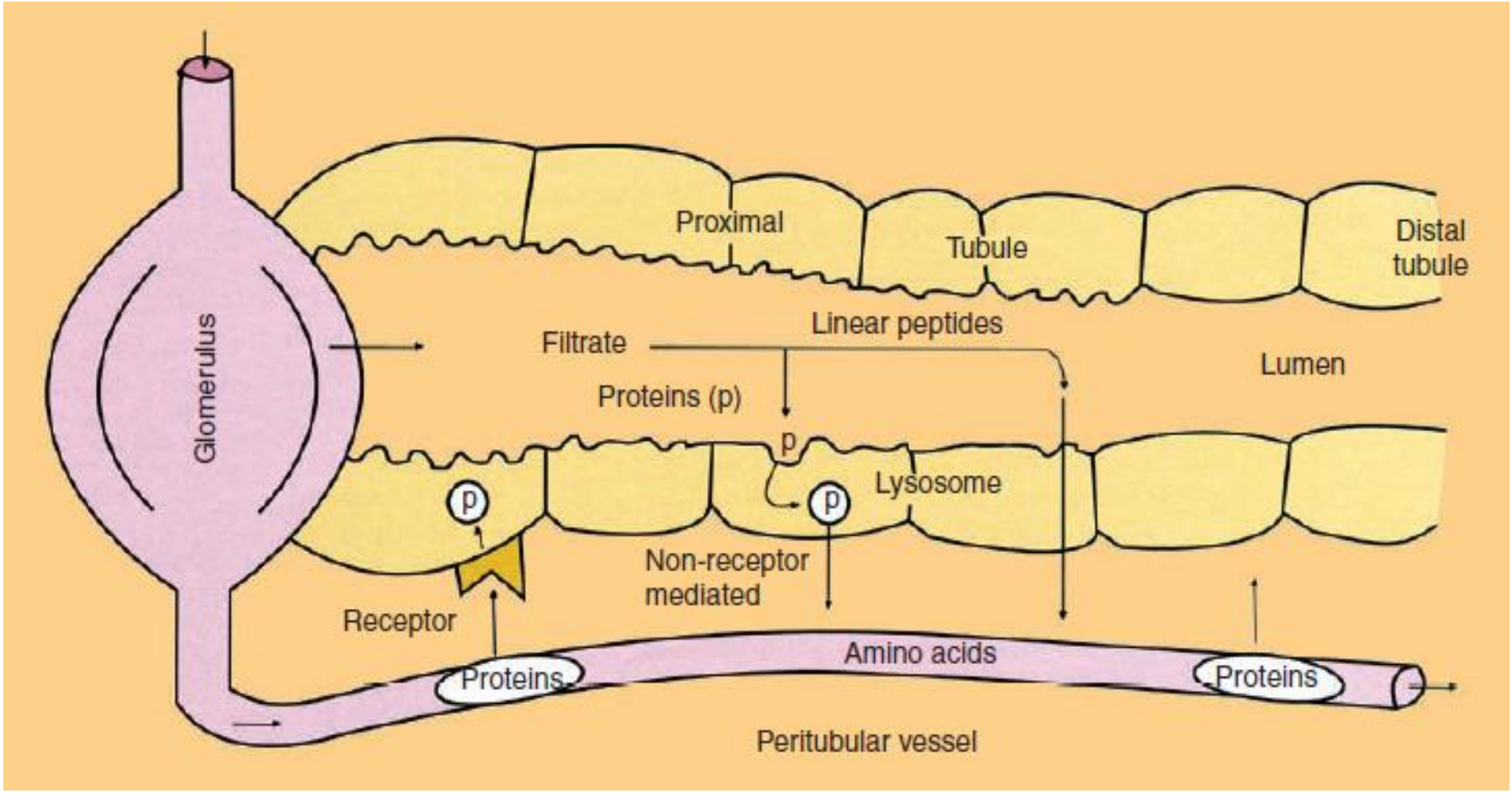


Figure : Pathways of renal metabolism of peptides and proteins: Glomerular filtration followed by either

(a) intraluminal metabolism or

(b) tubular reabsorption with intracellular lysosomal metabolism, and

(c) peritubular extraction with intracellular lysosomal metabolism

Hepatic Protein Metabolism

Aside from renal and gastrointestinal metabolism, the liver may also play a major role in the metabolism of protein therapeutics.

Exogenous as well as endogenous proteins undergo proteolytic degradation to dipeptides and amino acids that are reused for endogenous protein synthesis.

Proteolysis usually **starts with endopeptidases** that attack in the middle part of the protein, and **the resulting oligopeptides are then further degraded by exopeptidases**