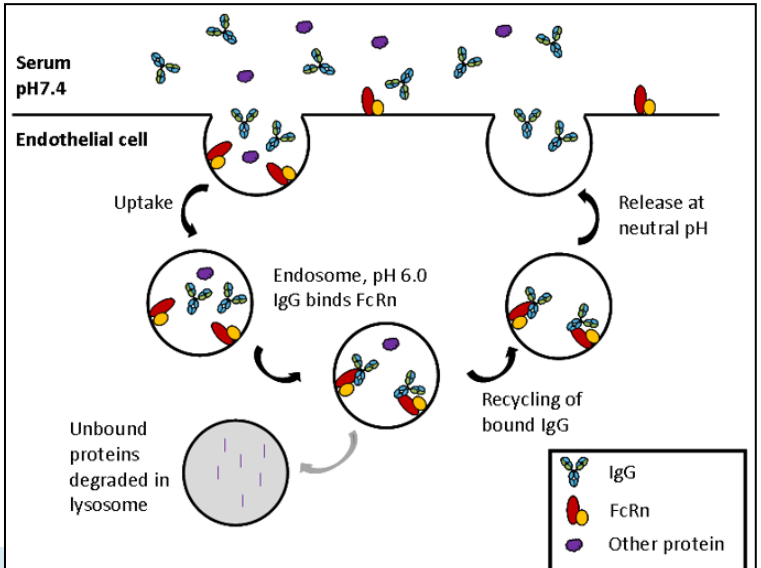
Pharmaceutical Biotechnology

DELIVERY OF PROTEINS: ROUTES OF ADMINISTRATION AND ABSORPTION ENHANCEMENT

A) The Parenteral Route of Administration

- Needle is required,
- Includes: intravenous (IV), intramuscular (IM), subcutaneous (SC), and intraperitoneal (IP) injections
- blood half-life of biotech products can vary over a wide range.
  - For example, the circulation half-life of:
    - tissue plasminogen activator (t-PA) is **a few minutes,**
    - while monoclonal antibodies (IgG) have half- lives of **a few days**

FcRn recycling



- One reason to develop modified proteins through site directed mutagenesis. How?

To enhance circulation half-life.

By expanding the mean residence time for short half-life proteins (switch from IV to IM or SC administration).

changes in disposition which

have a significant impact on the therapeutic performance of the drug.

These changes are
related to **(1)** the prolonged residence time at the IM or SC site of injection compared to IV administration and
the **enhanced exposure to degradation reactions** (peptidases)
and **(2) differences in disposition**.

Regarding point 1: Prolonged residence time at the IM or SC site of injection and the *enhanced exposure to degradation reactions.*

- For instance, diabetics can become “insulin resistant” through **high tissue peptidase activity**
- Other factors that can contribute to absorption variation are related to differences in **exercise level** of the muscle at the injection site and also massage and heat at the injection site.
- The state of the tissue, for instance, the **occurrence of pathological conditions**, may be important as well.

Regarding point 2: Differences in disposition

- Upon administration, the protein may be transported to the blood through the **lymphatics** or may enter the **blood circulation** through the capillary wall at the site of injection.

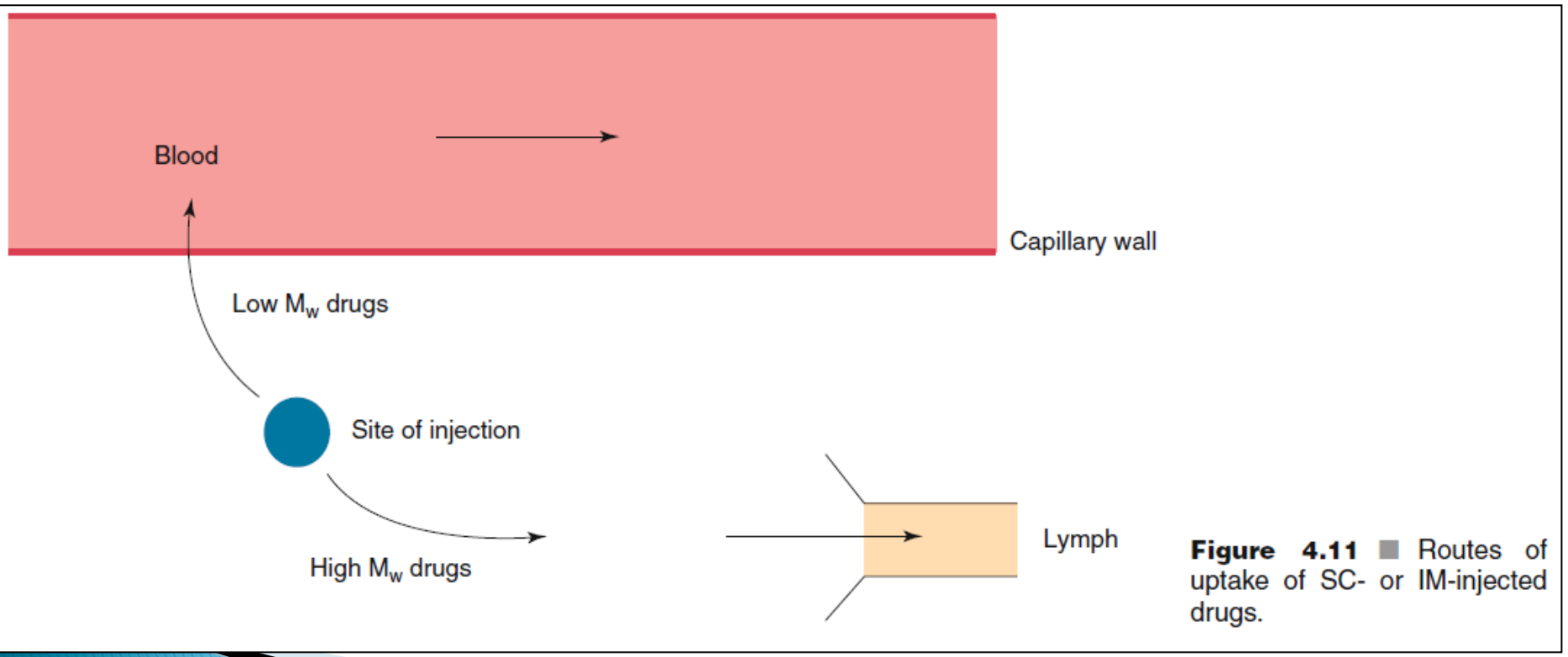


Figure 4.11 Routes of uptake of SC- or IM-injected drugs.

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- The fraction of the administered dose taking this lymphatic route is **molecular weight dependent..**

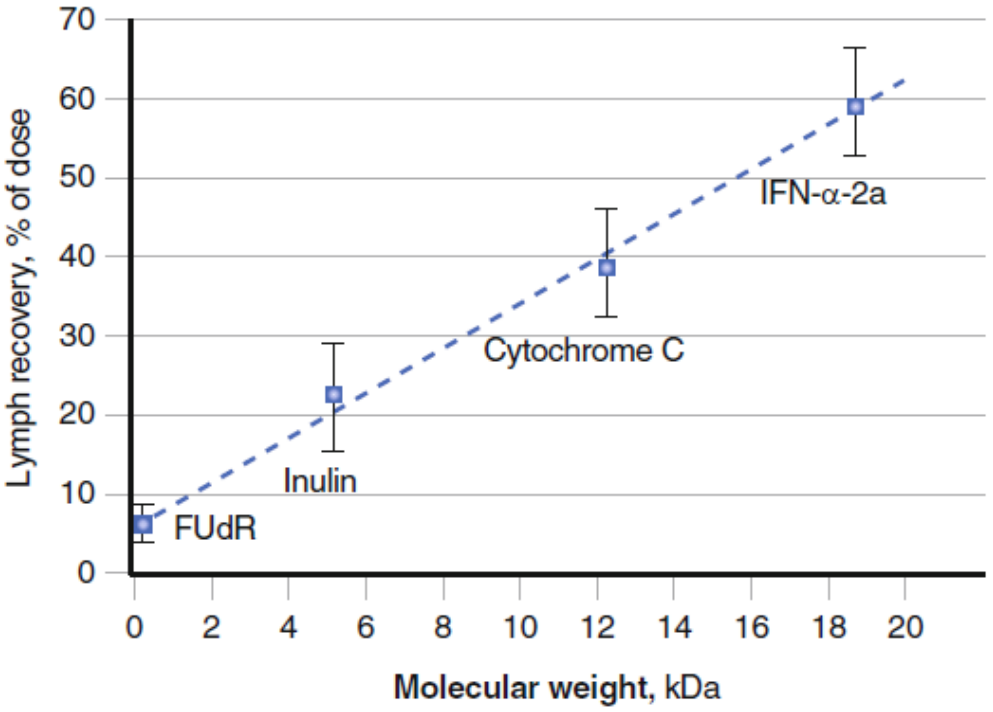


Figure 4.12 ■ Correlation between the molecular weight and the cumulative recovery of rIFN alpha-2a (M_w 19 kDa), cytochrome c (M_w 12.3 kDa), insulin (M_w 5.2 kDa), and FUDR (M_w 256.2 Da) in the efferent lymph from the right popliteal lymph node following SC administration into the lower part of the right hind leg of sheep. Each point and bar shows the mean and stan-

Continued..

- Lymphatic transport takes time (hours), and uptake in the blood circulation is highly **dependent on the injection site**.
- On its way to the blood, the lymph passes through draining lymph nodes, and **contact is possible between lymph contents and cells of the immune system such as macrophages** and B and T lymphocytes residing in the lymph nodes.

B) The Oral Route of Administration

- Oral delivery of protein drugs **would be** preferable, because
  - it is patient **friendly**
  - and **no intervention** by a healthcare professional is necessary to administer the drug.
- Oral bioavailability, however, is usually very low, why?
  - (1) protein **degradation** in the gastrointestinal (GI) tract
  - (2) poor **permeability** of the wall of the GI tract in case of a passive transport process

Regarding point 1: Protein degradation in the GI tract

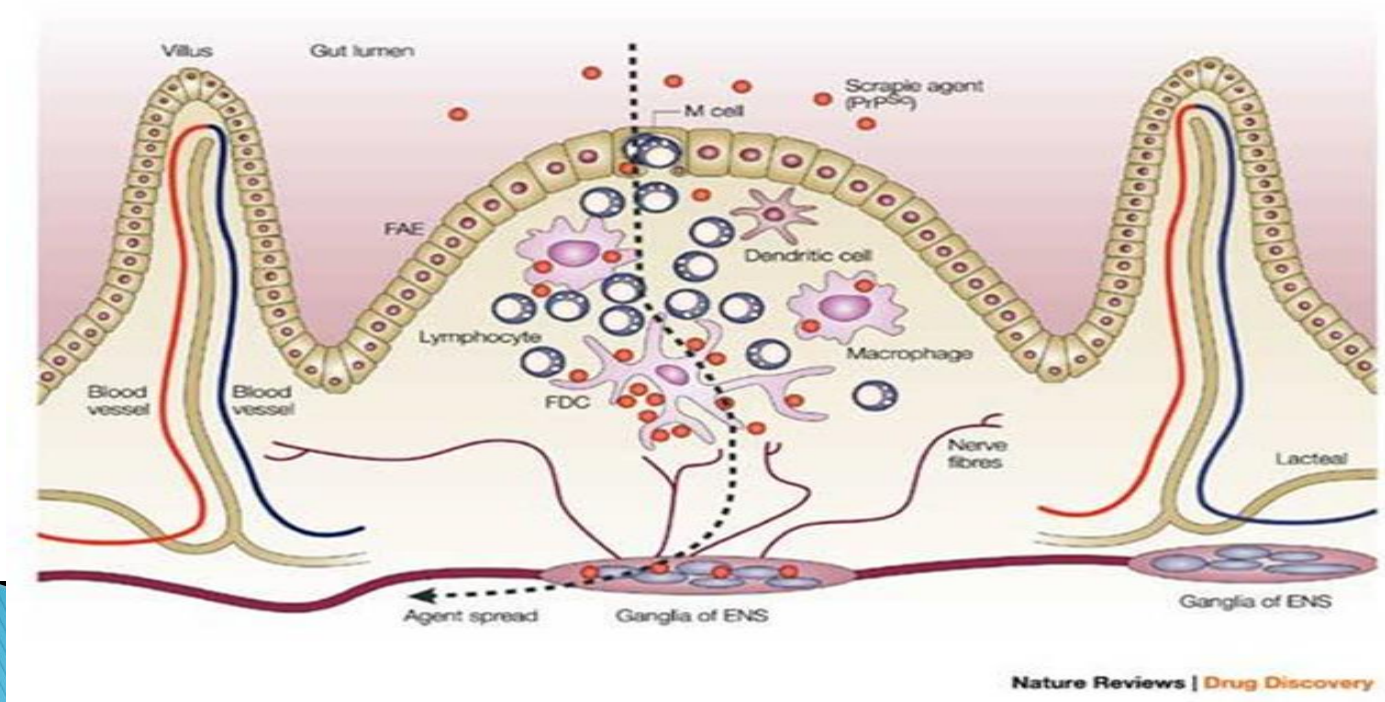
- In the stomach, **pepsins** are endopeptidases capable of cleaving peptide bonds distant from the ends of the peptide chain.
- Pepsins are particularly active between **pH 3 and 5** and lose activity at higher pH values
- **trypsin**, **chymotrypsin**, and **elastase** represent other endopeptidases that are active in the GIT at **neutral pH** values.
- **Exopeptidases**, proteases degrading peptide chains from their ends, are present as well. Examples are **carboxypeptidase A and B.**
- In the GI lumen the proteins are cut into fragments that effectively further break down to amino acids, di-and tripeptides by brush border, and **cytoplasmic proteases of the enterocytes**.

Regarding point 2: Permeability

- **High M.W. molecules do not readily penetrate** the intact and mature epithelial barrier if diffusion is the only driving force for mass transfer.
- Their diffusion coefficient **decreases** with **increasing** molecule size.
- **Proteins** (being large molecules) are **no exception** to this rule.
- **Active transport** of intact therapeutic recombinant proteins over the GI-epithelium has **not** been described yet.

What about oral vaccines?

- For oral vaccines, the aforementioned obstacles of degradation and permeation are **not necessarily prohibitive.** Why?
  - Because, only **a (small)** fraction of the antigen (protein) has to reach its target site to elicit an immune response. The target cells are lymphocytes and antigen presenting accessory cells located in **Peyer's patches**. The B-lymphocyte population includes cells that produce secretory IgA antibodies.



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- **Peyer's patches** are macroscopically identifiable follicular structures located in the wall of the GIT.
- Peyer's patches are overlaid with **microfold (M)** cells that separate the luminal contents from the lymphocytes.
- These M cells have **little lysosomal degradation capacity** and allow for antigen sampling by the underlying lymphocytes.
- Moreover, **mucus**-producing goblet cell **density** is **reduced** over Peyer's patches. This reduces mucus production and facilitates access to the M cell surface for luminal contents.
- Attempts to **improve antigen delivery via the Peyer's patches** and to enhance the immune response are made by using:
  - **microspheres**,
  - **liposomes**, or
  - modified **live vectors**, such as attenuated bacteria and viruses.

Summary, oral vaccines:
1- only small fraction of antigen is required
2- little lys. degradation through M cells
3- reduced mucus density

C) Alternative Routes of Administration

- Parenteral administration has **disadvantages** (needles, sterility, injection skills) compared to other possible routes.
- Therefore, systemic delivery of recombinant proteins by **alternative routes** of administration has been studied extensively.
- The **nose**, **lungs**, **rectum**, **oral cavity**, and **skin** have been selected as potential sites of application.

Pros and cons of other routes

Route +=relative advantage, −=relative disadvantage
Nasal
+ easily accessible, fast uptake, proven track record with a number of "conventional" drugs, probably lower proteolytic activity than in the GI tract, avoidance of first pass effect, spatial containment of absorption enhancers is possible
– reproducibility (in particular under pathological conditions), safety (e.g., ciliary movement), low bioavailability for proteins
Pulmonary
+ relatively easy to access, fast uptake, proven track record with "conventional" drugs, substantial fractions of insulin are absorbed, lower proteolytic activity than in the GI tract, avoidance of hepatic first pass effect, spatial containment of absorption enhancers (?)
– reproducibility (in particular under pathological conditions, smokers/nonsmokers), safety (e.g., immunogenicity), presence of macrophages in the lung with high affinity for particulates
Rectal
+ easily accessible, partial avoidance of hepatic first pass, probably lower proteolytic activity than in the upper parts of the GI tract, spatial containment of absorption enhancers is possible, proven track record with a number of "conventional" drugs
– low bioavailability for proteins
Buccal
+ easily accessible, avoidance of hepatic first pass, probably lower proteolytic activity than in the lower parts of the GI tract, spatial containment of absorption enhancers is possible, option to remove formulation if necessary
– low bioavailability of proteins, no proven track record yet (?)
Transdermal
+ easily accessible, avoidance of hepatic first pass effect, removal of formulation if necessary is possible, spatial containment of absorption enhancers, proven track record with "conventional" drugs, sustained/controlled release possible
– low bioavailability of proteins

Table 4.4 ■ Alternative routes of administration to the oral route for biopharmaceuticals.

The potential pros and cons for different relevant routes

I- Nasal route

Advantages:

1. Easily accessible
2. Fast uptake
3. Proven track record with a number of “conventional” drugs
4. Probably lower proteolytic activity than in the GI tract
5. Avoidance of first pass effect
6. Spatial containment of absorption enhancers is possible

Disadvantages:

1. reproducibility (in particular under pathological conditions),
2. safety (e.g., ciliary movement),
3. low bioavailability for proteins

II- Pulmonary route

Advantages:

1. relatively easy to access,
2. fast uptake,
3. proven track record with “conventional” drugs,
4. substantial fractions of insulin are absorbed,
5. lower proteolytic activity than in the G tract,
6. avoidance of hepatic first pass effect,
7. spatial containment of absorption enhancers

Disadvantages:

1. reproducibility (in particular under pathological conditions, smokers/nonsmokers),
2. safety (e.g., immunogenicity),
3. presence of macrophages in the lung with high affinity for particulates

III- Rectal route

Advantages:

1. easily accessible,
2. partial avoidance of hepatic first pass,
3. probably lower proteolytic activity than in the upper parts of the GI tract,
4. spatial containment of absorption enhancers is possible,
5. proven track record with a number of “conventional drugs

Disadvantages:

1. low bioavailability for proteins

IV- Buccal route

Advantages:

1. easily accessible,
2. avoidance of hepatic first pass,
3. probably lower proteolytic activity than in the lower parts of the GI tract
4. spatial containment of absorption enhancers is possible,
5. option to remove formulation if necessary

Disadvantages:

1. low bioavailability of proteins,
2. no proven track record yet

V- Transdermal route

Advantages:

1. easily accessible,
2. avoidance of hepatic first pass effect,
3. removal of formulation if necessary is possible,
4. spatial containment of absorption enhancers,
5. proven track record with “conventional” drugs,
6. sustained/controlled release possible

Disadvantages:

1. low bioavailability of proteins

Summary

- The **nasal, buccal, rectal, and transdermal** routes all have been shown to be of **little clinical relevance** if systemic action is required and if simple protein formulations without an absorption-enhancing technology are used.
- In general, **bioavailability is too low** and varies too much!

- The **pulmonary route** may be the exception to this rule.

Molecule	*M*w kDa	#AA	Absolute Bioavailability (%)
α-Interferon	20	165	>56
PTH-84	9	84	>20
PTH-34	4.2	34	40
Calcitonin (human)	3.4	32	17
Calcitonin (salmon)	3.4	32	17
Glucagons	3.4	29	<1
Somatostatin	3.1	28	<1

Adapted from Patton et al. (1994)

PTH recombinant human parathyroid hormone, *#AA* number of amino acids

Table 4.5 ■ Absolute bioavailability of a number of proteins (intratracheal vs. intravenous) in rats.

Intratracheal in rats!

In humans the drug should be inhaled instead of intratracheally administered.

- Absorption was **strongly protein dependent,** with no clear relationship with its molecular weight.

Related to pulmonary route

- The **first pulmonary insulin** formulation was approved by FDA in January 2006 (Exubera®) (taken off the market in 2008 because of poor market penetration).
- Pulmonary inhalation of insulin is specifically indicated for mealtime glucose control.
- Uptake of insulin is **faster** than after a regular SC insulin injection (**peak 5–60 min** vs. 60–180 min).
- The **reproducibility** of the blood glucose response to inhaled insulin was **equivalent** to SC-injected insulin.