DELIVERY OF PROTEINS: ROUTES OF ADMINISTRATION AND ABSORPTION ENHANCEMENT

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THE PARENTERAL ROUTE OF ADMINISTRATION

- **Parenteral administration** is defined as administration via those routes where a needle is used, including intravenous **(IV)**, intramuscular **(IM)**, subcutaneous **(SC)** and intraperitoneal **(IP)** injections.
- **The blood half-life of biotech products can vary over a wide range**.

For example, the circulation half-life of **t-PA** is a **few minutes**, while monoclonal antibodies **(MAB)** have **half-lives of a few days**

- **One reason to develop modified proteins through site directed mutagenesis**

To enhance circulation half-life.

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

1- changes in disposition,

2- with a significant impact on the therapeutic performance of the drug.

- The term **site-directed mutagenesis or protein engineering :**
- facilitates the generation of engineered therapeutic proteins displaying some clinical advantage over the native protein product.
- Techniques such as site-directed mutagenesis facilitate the **logical introduction of predefined changes in a protein's amino acid sequence**. Such changes can be as minimal as the insertion, **deletion** or **alteration** of a single amino acid residue, or can be more substantial (e.g. the alteration/deletion of an entire domain, or the generation of a novel hybrid protein).
- This is made by controlled **alteration of the nucleotide sequence coding for the polypeptide of interest** such that specific, predetermined changes in amino acid sequence are introduced.. Site-directed mutagenesis is now most often undertaken by using a variant of the basic PCR method, known as **'overlap PCR'**, in which primers of altered nucleotide sequences are used for the PCR reactions.

THESE CHANGES ARE RELATED TO:

i. The prolonged residence time at the IM or SC site of injection compared to IV administration

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enhanced exposure to degradation reactions (peptidases).

ii. Differences in disposition.

REGARDING POINT 1 (PROLONGED RESIDENCE TIME AT IM OR SC SITE OF INJECTION AND THE ENHANCED EXPOSURE TO DEGRADATION REACTIONS.)

A- For instance, **diabetics** can become **"insulin resistant"** through high tissue dipeptidyl peptidase {DPP-IV} activity .

B- Other factors that can contribute to **absorption variation** are related to **differences in exercise level of the muscle at the injection site**.

C- The **state of the tissue**, for instance the **occurrence of pathological conditions**, may be important as well.

REGARDING POINT 2 (DIFFERENCES IN DISPOSITION).

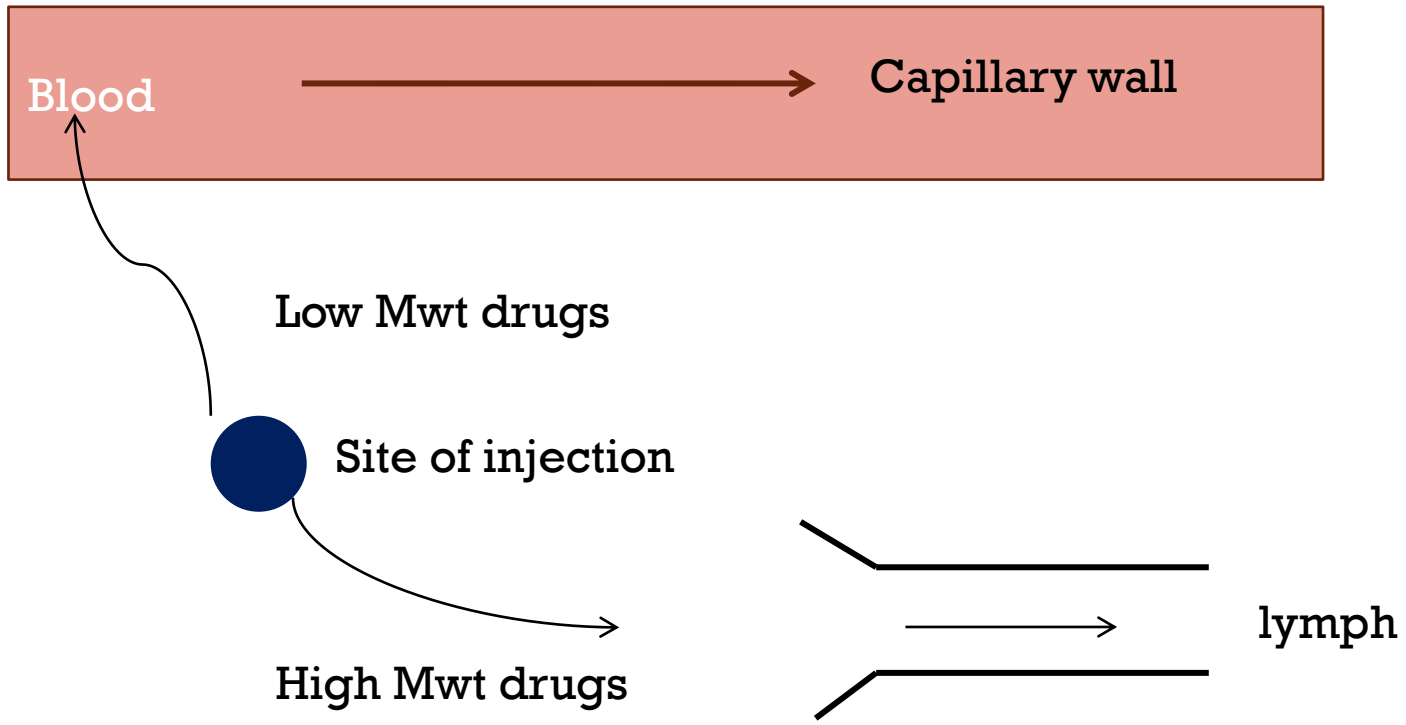
Upon administration, the protein may be transported to the blood circulation

or

- through the lymphatics
- through the capillary wall at the site of injection.

Note: The fraction of the administered dose taking this lymphatic route is molecular weight dependent.

ROUTES OF UPTAKE OF SC OR IM INJECTED DRUGS



- 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, B- and T-lymphocytes residing in the lymph nodes.

THE ORAL ROUTE OF ADMINISTRATION

- **Oral delivery of protein drugs would be preferable because:**

1. It is patient friendly
2. No intervention by a healthcare professional is necessary to administer the drug.

- **Not Preferable:**

Oral bioavailability is usually very low.

THE TWO MEAN REASONS FOR FAILURE OF UPTAKE AFTER ORAL ADMINISTRATION

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).

i. The human body has developed a very efficient system to break down proteins in our food to amino acids, or di- or tri-peptides.

ii. These building stones for body proteins are actively absorbed for use wherever necessary in the body.

iii. **In the stomach pepsins** (a family of aspartic proteases) are secreted. They are particularly active between pH 3 and 5 and lose activity at higher pH values.

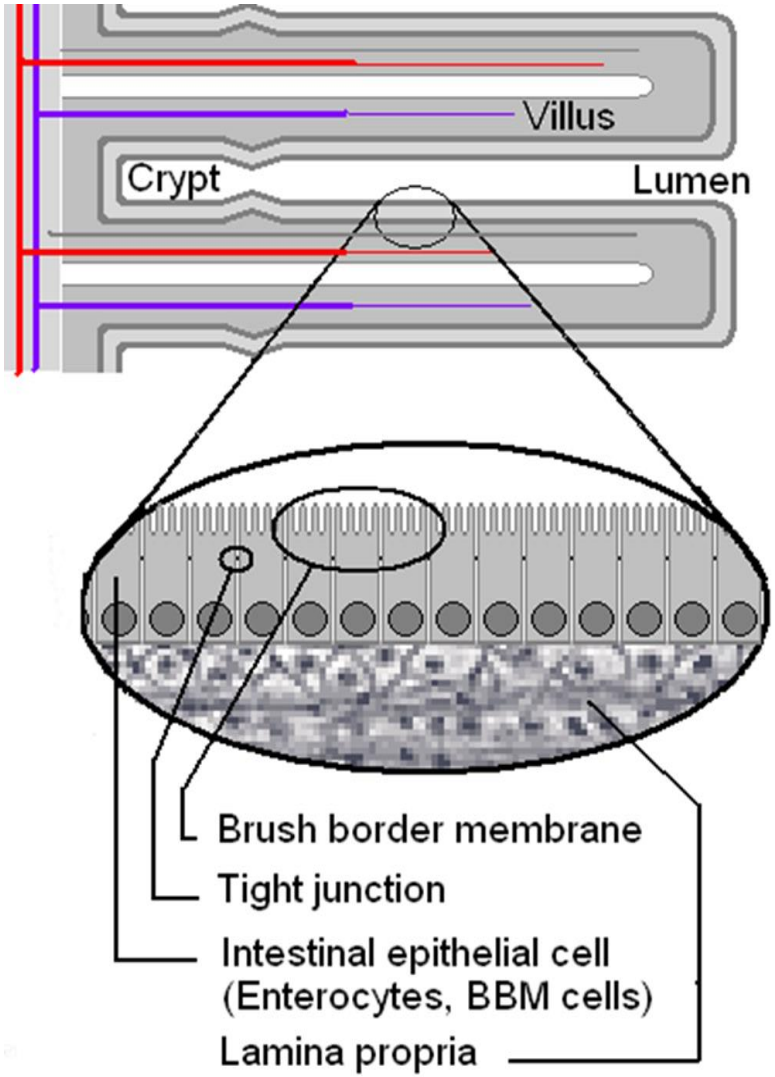
iv. **Pepsins are endopeptidases** capable of cleaving peptide bonds distant from the ends of the peptide chain. They preferentially **(cleave peptide bonds between two hydrophobic amino acids).**

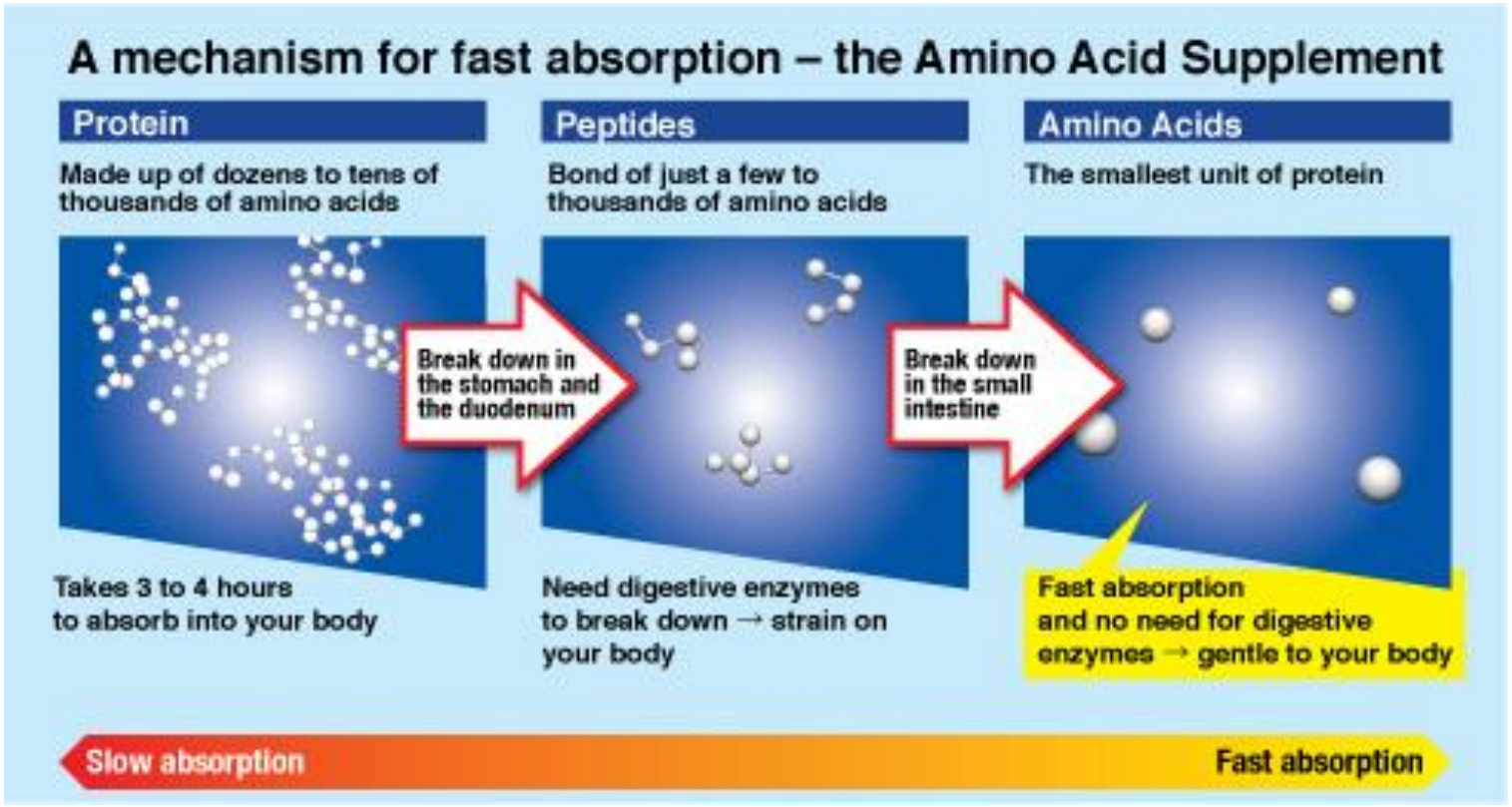
v. **Other endopeptidases** are **active in the GI tract at neutral pH** values, e.g., **trypsin**, **chymotrypsin**, **and elastase**.

They have different peptide bond cleavage characteristics that more or less complement each other.

vi. **Exopeptidases**, proteases **degrading peptide chains from their ends**, are present as well. Examples are **carboxypeptidase A and B**.

viii. In the GI lumen the **proteins are cut into fragments** that effectively **further break down to amino acids, di- and tri-peptides by brush border (microvillus) and cytoplasmic proteases of the enterocytes (intestinal absorptive cells).**




A mechanism for fast absorption – the Amino Acid Supplement
Protein
Made up of dozens to tens of thousands of amino acids
Peptides
Bond of just a few to thousands of amino acids
Amino Acids
The smallest unit of protein
Break down in the stomach and the duodenum
Break down in the small intestine
Takes 3 to 4 hours to absorb into your body
Need digestive enzymes to break down → strain on your body
Fast absorption and no need for digestive enzymes → gentle to your body
Slow absorption
Fast absorption

REGARDING POINT 2 (PERMEABILITY).

i. High molecular weight molecules **do not readily penetrate the intact and mature epithelial barrier** if diffusion is the sole driving force for mass transfer.

ii. **Their diffusion coefficient decreases with increasing molecule size.**

iii. Protein are no exception to this rule.

iv. **Active transport** of intact therapeutic recombinant proteins over the GI-epithelium **has not been described yet.**

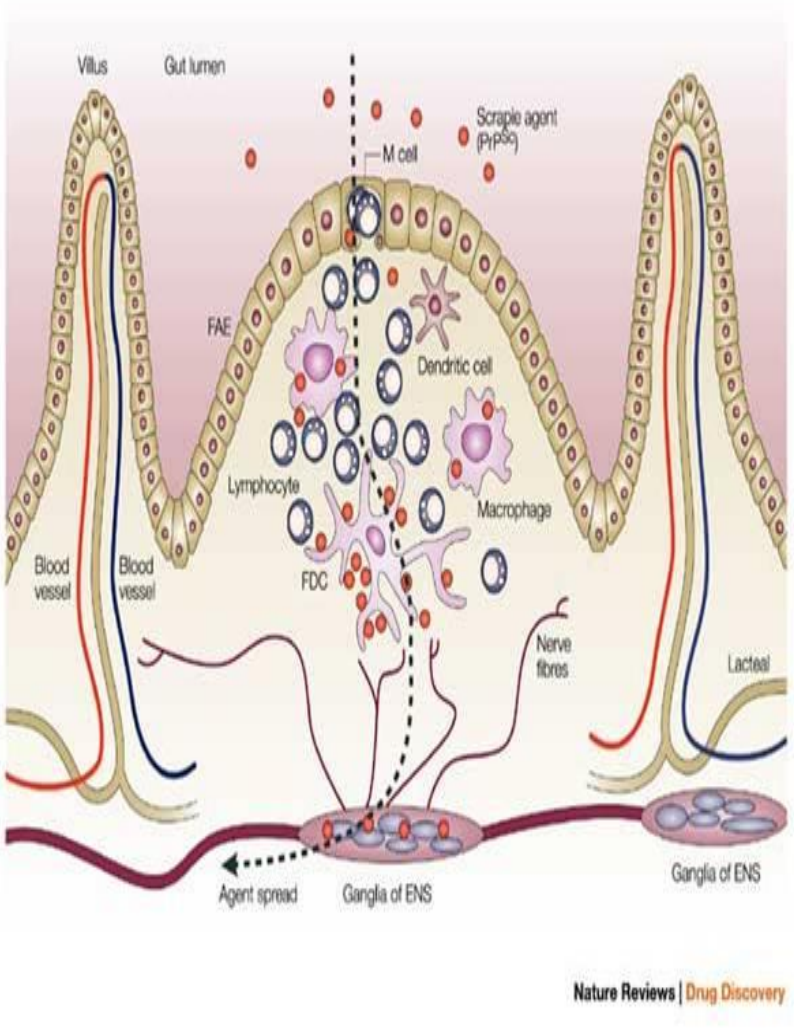
CONCLUSION

The above analysis leads to the conclusion that nature, unfortunately, does **not allow us to use the oral route of administration for therapeutic protein if high** (or at least constant) **bioavailability is required**.

- However, for the category of **oral vaccines** the above-mentioned hurdles (walls) of degradation and permeation are not necessarily prohibitive.

Ex: For oral immunization, only a (small) fraction of the antigen (protein) has to reach its target site to elicit an immune response.

- The target cells are B-lymphocyte cells that produce secretory IgA antibodies.
- and antigen presenting accessory cells located in Peyer's patches (macroscopically identifiable follicular structures located in the wall of the GI tract).



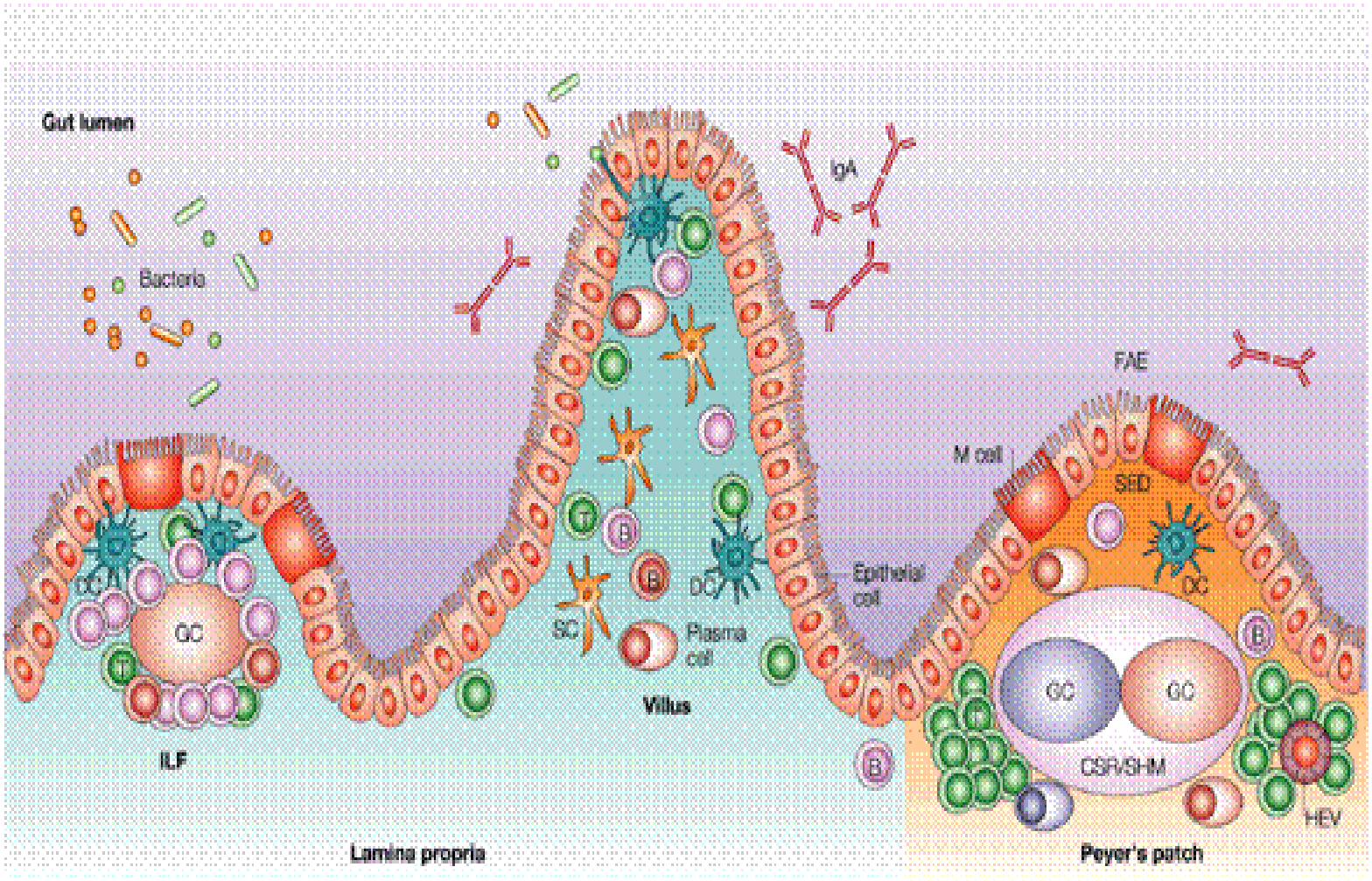
- **Peyer's patches are overlaid with microfold (M) cells** (separate the luminal contents from the lymphocytes).

- 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 antigene 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**.



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