Delivery of proteins
(Routes of administration)
Lecture 8

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Notes :

1) These regions characterized with:

a) Little lysosomal degradation capacity

b) Mucous producing goblet cell density is reduced (less mucus production).

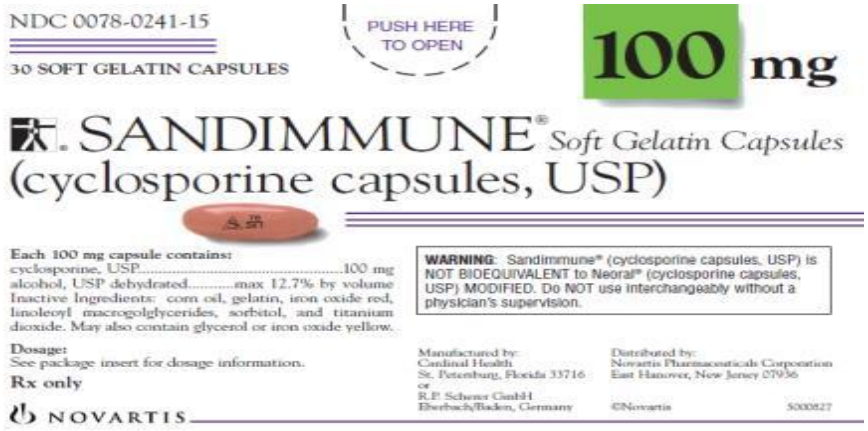
2) As attempts to improve the antigens delivery here, microspheres, liposomes or modified live vectors (like attenuated bacteria and viruses) are used.

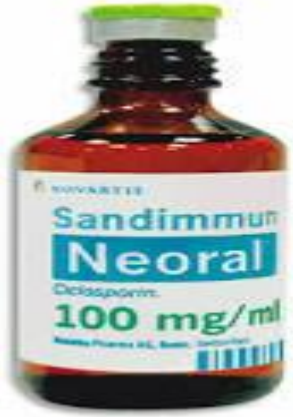
Commercially available-orally administered peptide drugs

- Desmopressin acetate

(Minirin®) (9 a.a.),1183 D

- Cyclosporine (11 a.a.), 1203 D





Alternative routes of administration :(for systemic effect)

- Includes nasal, pulmonary, rectal, buccal and transdermal routes.
- There is (no need of needles, no sterility and injection skills).
- **Without an absorption enhancing technology, they are characterized with too low bioavailability except the pulmonary route may be out this rule.**

Nasal route:

Advantages:

Easily accessible, probably lower proteolytic activity than in the GIT, use of absorption enhancers is possible**?**

Disadvantages:

Reproducibility (in pathological conditions like rhinitis, safety (e.g. ciliary movement), low bioavailability for proteins.

Examples:

Desmpressin acetate (Minrin®),

Synarel® (LHRH) agonist=nafarelin (9 a.a.)= 1322 D
Miacalcin® = Calcitonin, 32 a.a., 3432 D

Pulmonary route:

Advantages:

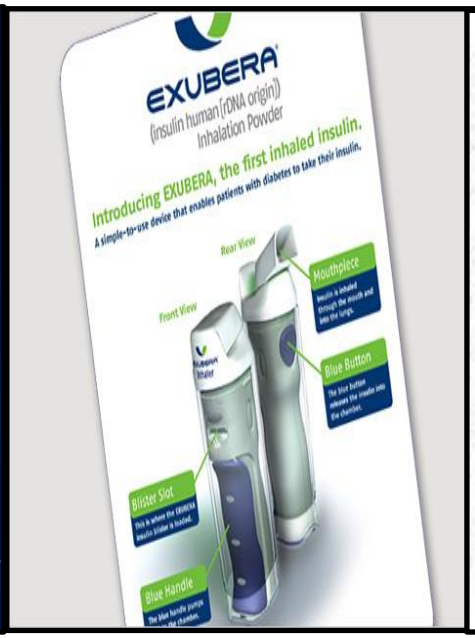
Relatively easy to access, substantial fractions of insulin are absorbed, possible use of absorption enhancers.

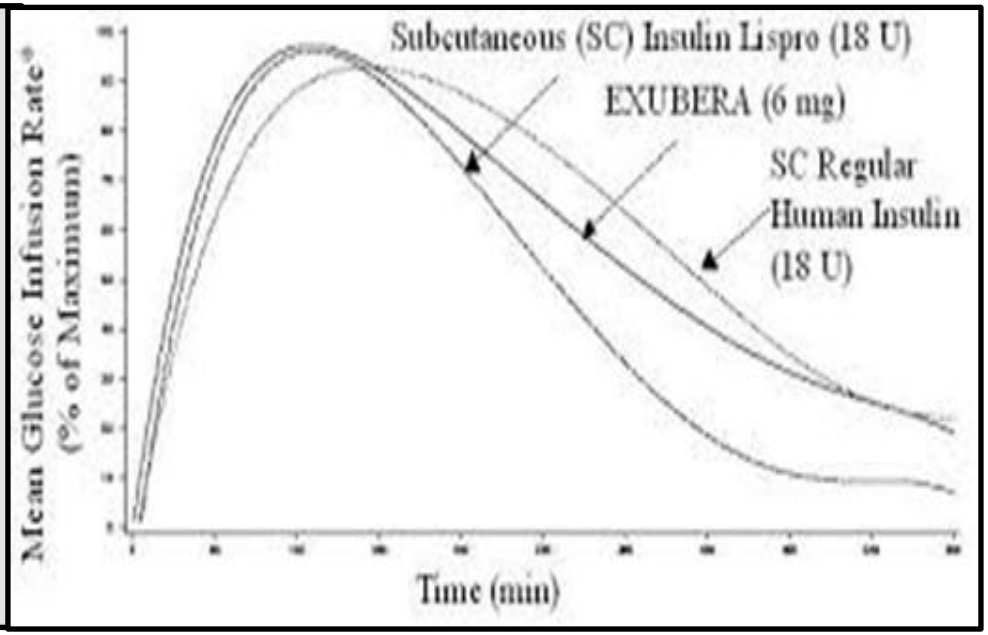
Disadvantages:

Reproducibility (in pathological conditions like smokers/non-smokers, safety (e.g. immunogenicity), presence of macrophages in the lung with high affinity for particulates.

- In humans, the drug should be inhaled instead of intra-tracheally administered (as in rats as example)
- The delivery of insulin by this route has been extensively studied and clinical phase III trials evaluating efficacy and safety have been performed or are ongoing.

(the first pulmonary insulin formulation was approved by FDA in January 2006 (Exubera®).(at 1980, was protected by soybean seeds??)





- Pulmonary inhalation of insulin is specifically tested for meal time glucose control. Uptake of insulin is faster than after a regular SC insulin injection (peak 5-60 minutes versus 60-180 minutes).
- The reproducibility of the blood glucose response to inhaled insulin was equivalent to SC injected insulin, but patients preferred inhalation over SC injection.
- **The fraction of insulin that is ultimately absorbed depends on :**

(i) **the fraction of the inhaled/nebulized dose that is actually leaving the device.**

(ii) **The fraction that is actually deposited in the lung.**

(iii) **The fraction that is being absorbed.**

- This means, total relative uptake (TO%):

(TO%) = % uptake from device X % deposited in the lung X % actually absorbed from the lung.

- ✓ **TO% for insulin is estimated to be about 10%.**
- ✓ **The fraction of insulin that is absorbed from the lung is estimated to be around 20% (small fraction).**

Absorption enhancement approaches:

- Increase the permeability of the absorption barrier:
  - Addition of fatty acids/phospholipids, bile salts, enamine derivatives of phenylglycine, ester and ether type (non)-ionic detergents, saponins, salicylate derivatives, derivatives of fusidic acid or glycyrrhizinic acid, or methylated β cyclodextrins
  - Through iontophoresis
  - By using liposomes
- Decrease peptidase activity at the site of absorption and along the "absorption route": aprotinin, bacitracin, soybean tyrosine inhibitor, boroleucin, borovaline
- Enhance resistance against degradation by modification of the molecular structure
- Prolongation of exposure time (e.g., bio-adhesion technologies)

- In iontophoresis, a transdermal electrical current is induced by positioning two electrodes on different places on the skin.
- This current induces a migration of **ionized** molecules through the skin.
- Delivery depends on:

1) The current (on/off, pulsed/direct, wave shape). 2) pH 3) Ionic strength

4) M.wt. 5) Charge on the protein

6) Temperature.

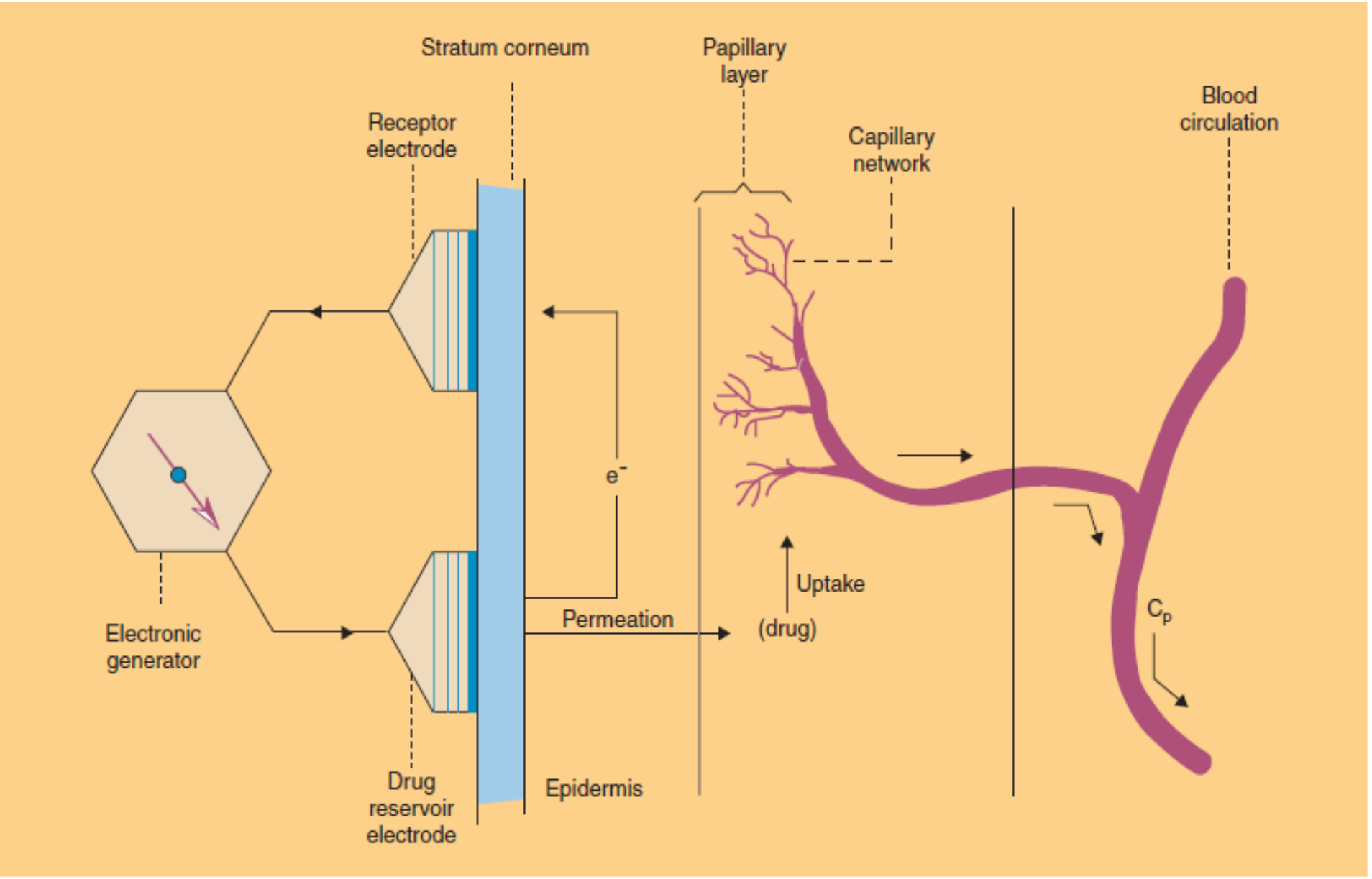


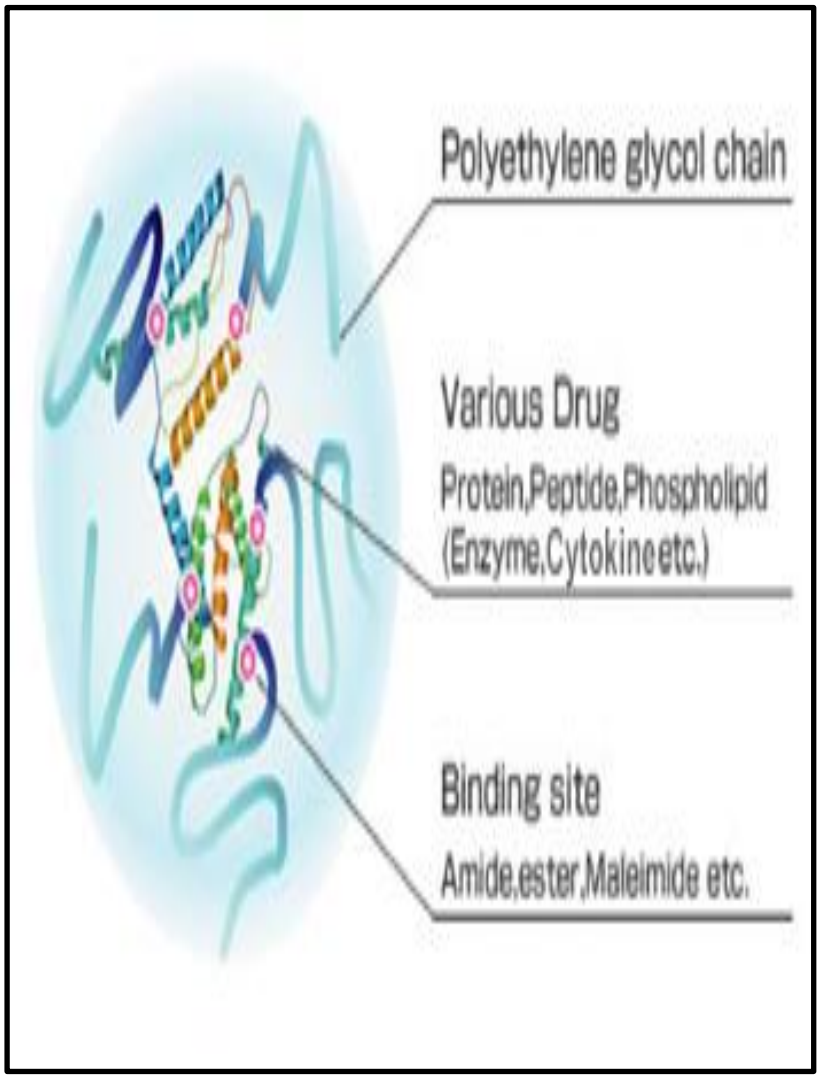
Figure 15 Schematic illustration of the transdermal iontophoretic delivery of peptide and protein drugs across the skin. *Source*:

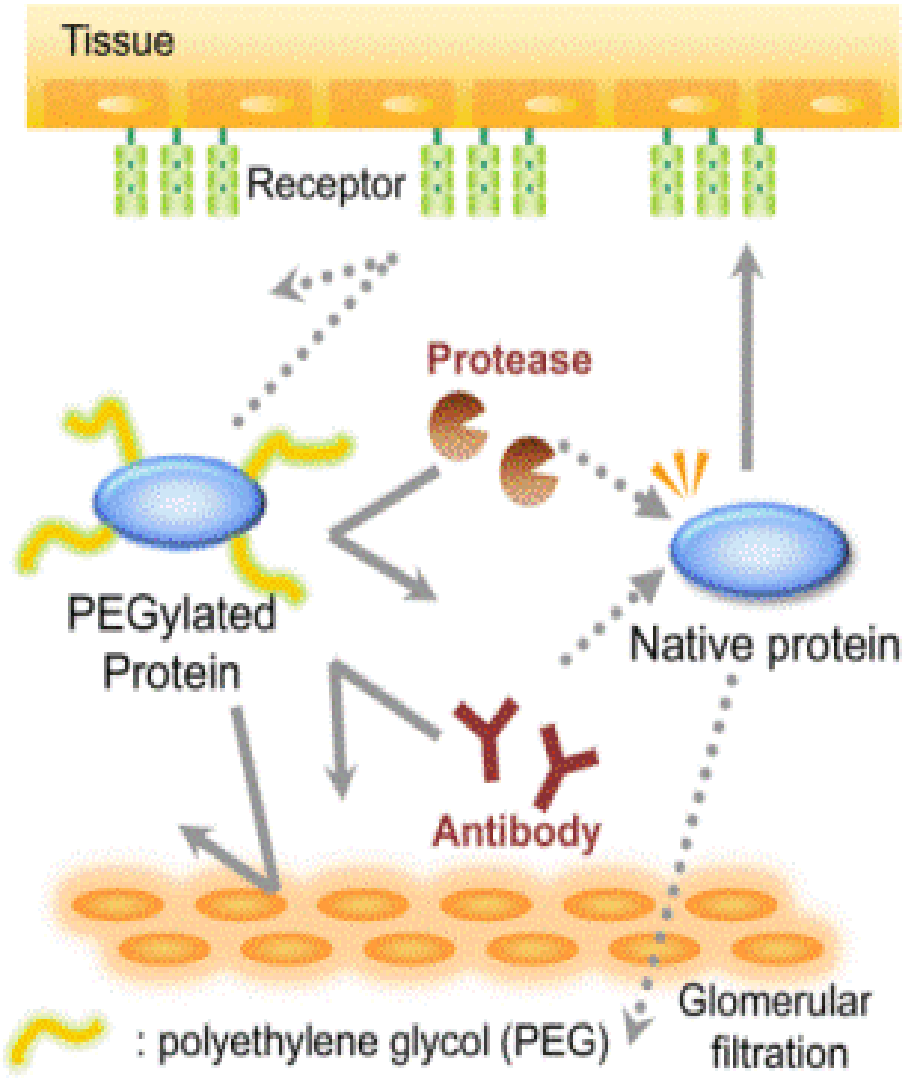
Note:

As approaches used with success, include the chemical alterations of protein molecules in order to **extend their activity or perhaps hasten their onset of action**.

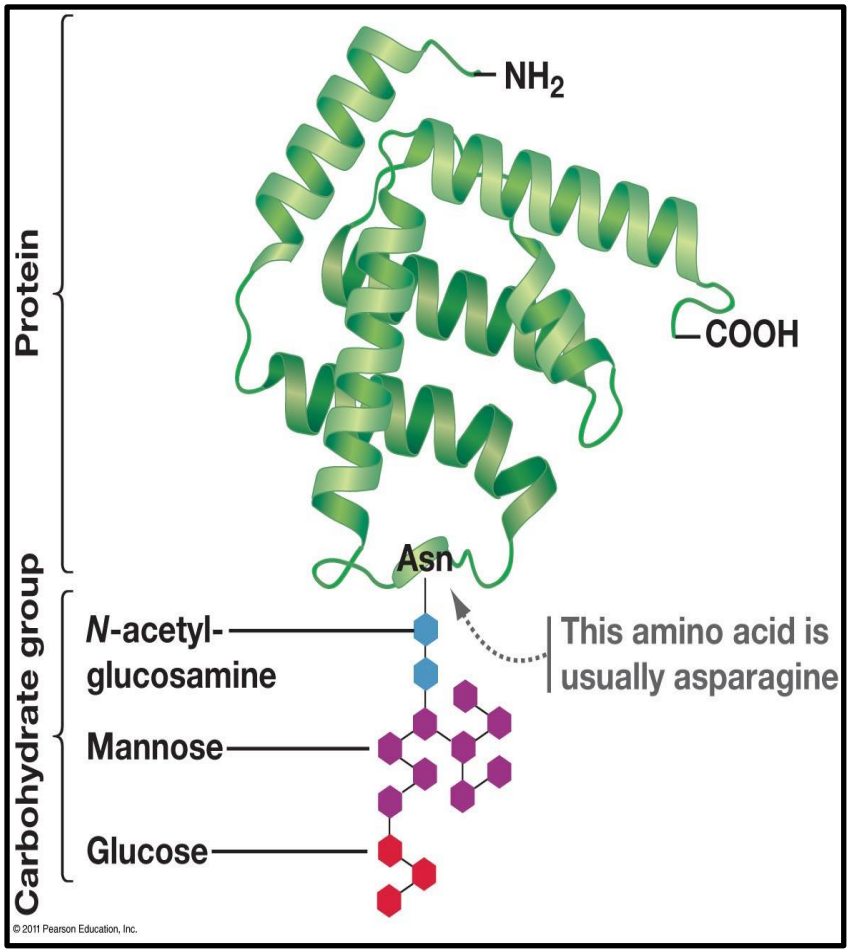
Are :**Pegylation, glycosylation and amino acids substitution**

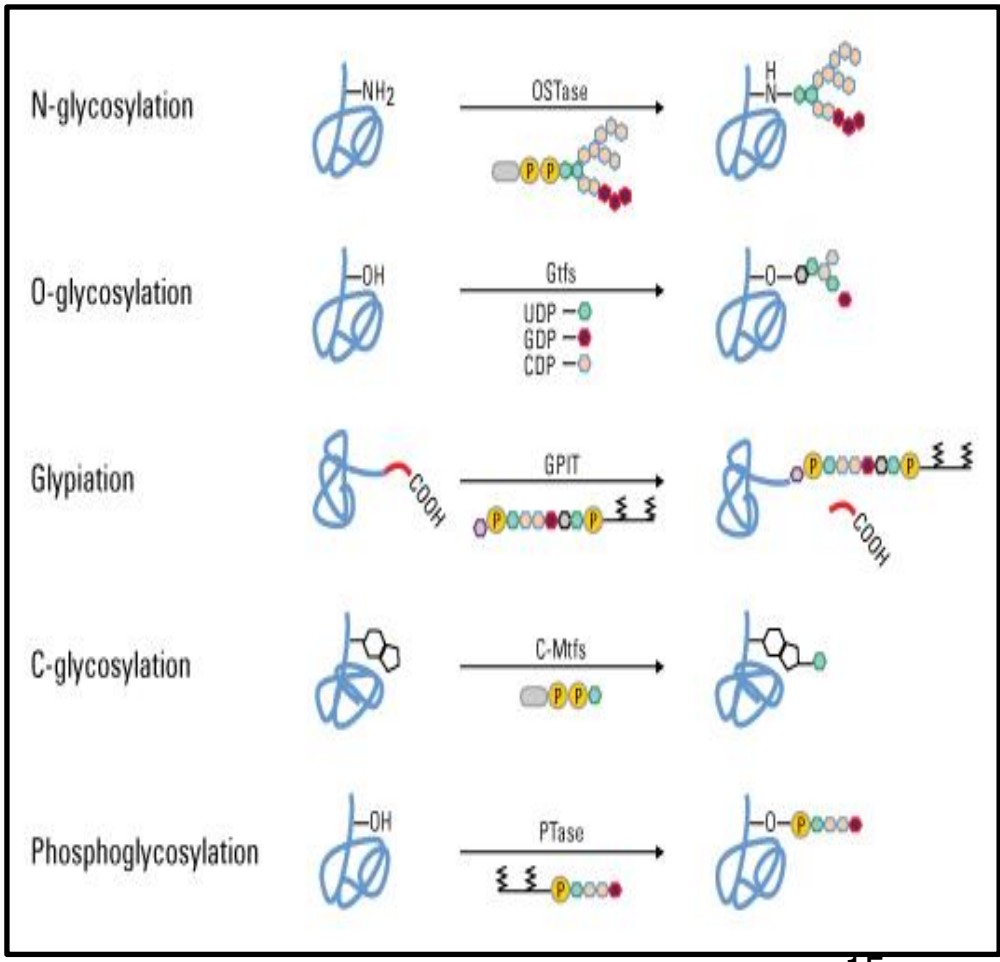
1) Pegylation: covalent attachment of proteins with a flexible strand of PEG, which generally masks the protein's surface, effectively increase the protein's molecular size, reduces renal ultrafiltration, inhibits antibodies or antigen presenting cells, and reduces degradation by proteolytic enzymes. Therefore, the protein's distribution is significantly altered.


Polyethylene glycol chain
Various Drug
Protein,Peptide,Phospholipid
(Enzyme,Cytokine etc.)
Binding site
Amide,ester,Maleimide etc.


Tissue
Receptor
Protease
PEGylated
Protein
Native protein
Antibody
: polyethylene glycol (PEG)
Glomerular
filtration

2) Glycosylation: may be occurred after protein production depending on the host or added later. In which oligosaccharide chains are attached to proteins, so there is increase in M.wt and longer half life.





3) Amino acid substitutions:

Recently, several novel insulin formulations have been developed that dramatically affect insulin's pharmacokinetic properties. This has been accomplished by substituting some amino acids in the primary structure of the protein in a manner that does not change the biological activity of the molecule.

Insulin type	Onset of Action (h)	Duration (h)	Position A-21	Position B-28	Position B-29	Position B31-32
Regular	0.5	2-4	Asp.n	Proline	Lysine	-
LysPro	0.25	0.5-1.5	=	Lysine	Proline	-
Aspart	0.25	0.5-1.5	=	Asp. acid	Lysine	-
Glargine	2-4	24	Glycine	Proline	=	Arg.-Arg.

Analogue	Modification	Mechanism
A		
Lispro (Humalog®) Eli Lilly and Co	$Pro^{B28} \rightarrow Lys$ $Lys^{B29} \rightarrow Pro$	IGF-I-related motif impairs dimerization
Aspart (NovoLog®) Novo-Nordisk	$Pro^{B28} \rightarrow Asp$	Charge repulsion at dimer interface
Glulisine (Apidra®) Sanofi-Aventis	$Asn^{B3} \rightarrow Lys$ $Lys^{B29} \rightarrow Glu$	Decreased zinc-free self-association
B		
Glargine (Lantus®) Sanofi-Aventis	Arg^{B31}-Arg^{B32} tag $Asp^{A21} \rightarrow Gly$	Shift in pI to pH 7 leads to isoelectric precipitation on injection
Detemir (Levemir®) Novo-Nordisk	Modification of Lys^{B29} by a tethered fatty acid	Stabilization of hexamer and binding to serum albumin

[a]Panel A describes rapid-acting analogues employed in prandial regimens and in insulin

Insulin types	Onset	Peak	Duration of action
Conventional insulin			
Short-acting regular	30–60 minutes	2 hours	6 hours
Intermediate-acting	2–4 hours	4–6 hours	18 hours
Analogs			
Ultra-short-acting			
Lispro	5–15 minutes	60 minutes	2 hours
Aspart	5–15 minutes	60 minutes	2 hours
Glulisine	5–15 minutes	60 minutes	2 hours
Long-acting			
Glargine	2–4 hours	Not significant	24 hours
Detemir	2–4 hours	Not significant	20 hours