Delivery of proteins
(Routes of administration and absorption enhancement)

1

The parenteral route of administration:

Parenteral administration is here defined as administration via those routes where a needle is used, including IV., IM., SC. and IP. Injections.

These routes with different residence times and dispositions, so there is a significant effect on the therapeutic performance of the drug.

2

- 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)
2) Differences in disposition

3

- Regarding point 1:

As factors involved:

1) Diabetics can become insulin resistant through high tissue peptidase activity.
2) Exercise level of the muscle at the injection site
3) Message
4) Heat
5) The state of the tissue

- Regarding point 2:

As factor involved is (Molecular weight of protein)

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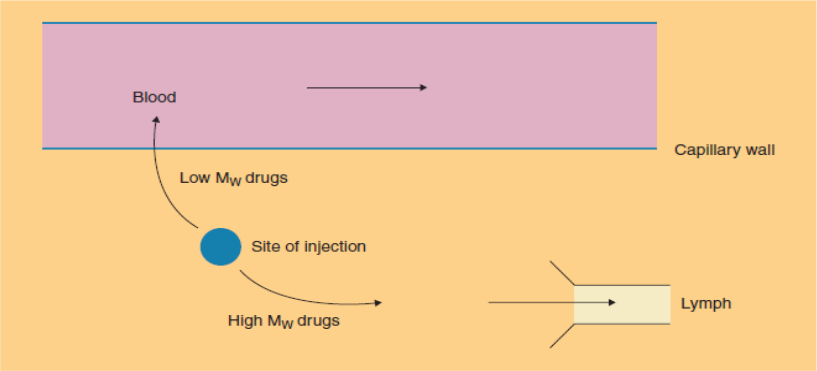
- Upon administration, the protein may be transported to the blood through the lymphatics or may enter the blood circulation through the capillary wall at the site of injection.



Routes of uptake of SC or IM injected drugs

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- The fraction of the administered dose taken by this lymphatic route is molecular weight dependent.
- 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.

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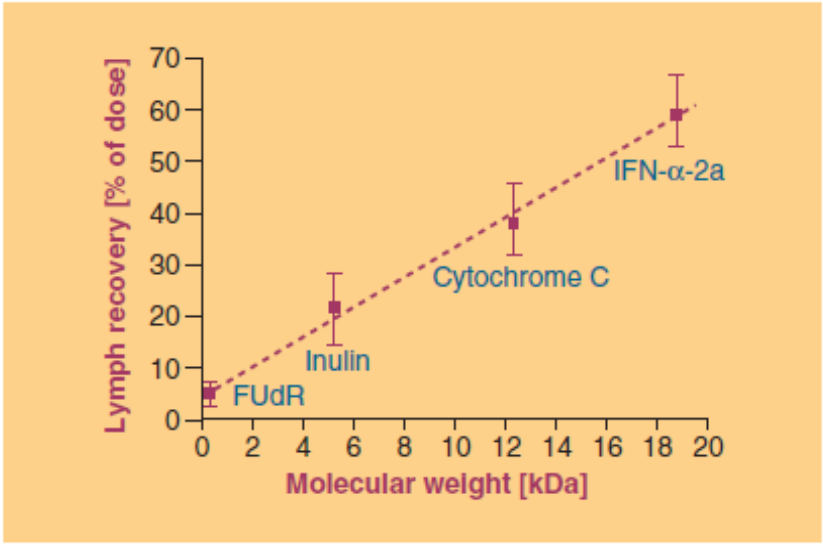

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The oral route of administration:

- It is preferred ? because it is patient friendly and no intervention by a healthcare professional is necessary to administer the drug.
- Oral bioavailability is usually very low?

Because of:

(1) protein degradation in the GIT.

(2) poor permeation within GIT in case of a passive transport process

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- For degradation, occurred due to presence of proteolytic enzymes (proteases) for breaking down protein in our food to amino acids, or di-tri-peptides to be absorbed.
- In the stomach, pepsins , a family of aspartic proteases are secreted, active between pH(3-5) and lose activity at higher pH values.
- Pepsins are endo-peptidases capable of cleaving peptide bonds distant from the ends of the peptide chain, selectively cleave peptide bonds between two hydrophobic amino acids.

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- Other endo-peptidases are active in the GIT at neutral pH values, e.g., **trypsin, chymotrypsin, and elastase** (with different peptide bond cleavage properties that more or less complement each other).
- In addition, exo-peptidases, proteases degrading peptide chains from their ends. Examples, **carboxy-peptidase A and B**.
- 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 and cytoplasmic proteases of the enterocytes.

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- For permeation, high molecular weight molecules do not readily penetrate the intact and mature epithelial barrier depending on diffusion principle. Where diffusion coefficient decreases with increasing molecular size.
- For oral immunization (vaccination), 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 Payer's patches

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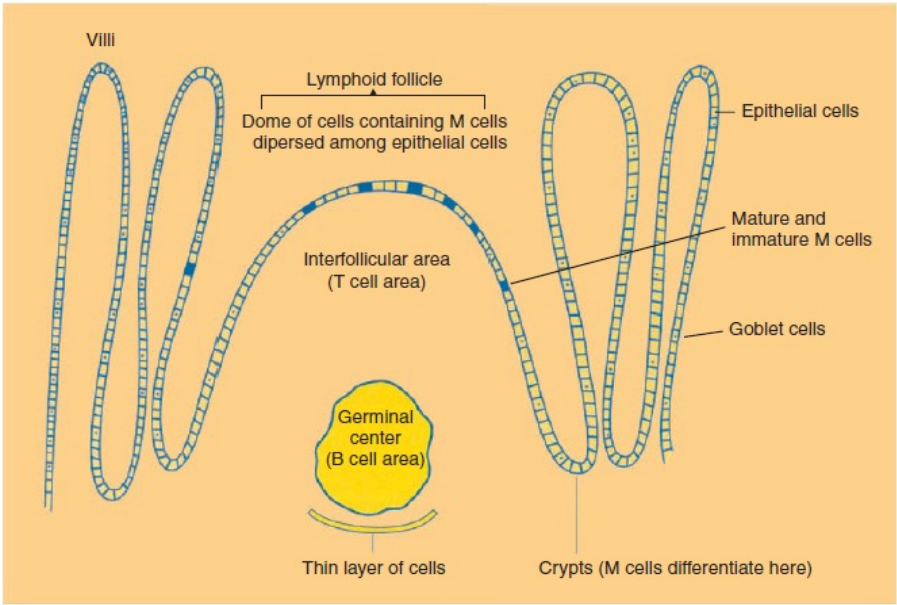

The intestinal Payer's patches

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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.

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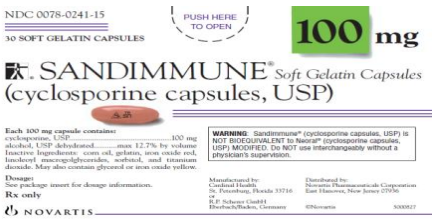
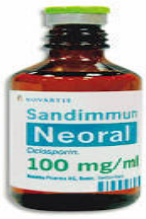
Commercially available-orally administered peptide drugs

- Desmopressin acetate (Minirin®) (9 a.a.),1183 D
- Cyclosporine (11 a.a.), 1203 D





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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.

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Nasal route:

Advantages:

Easily accessible, fast uptake, proven track record with a number of conventional drugs, probably lower proteolytic activity than in the GIT, avoidance of first pass effect, spatial containment 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

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Rectal route:

Advantages:

Easily accessible, proven track record with a number of conventional drugs, probably lower proteolytic activity than in the upper parts of the GIT, Partial avoidance of first pass effect, spatial containment of absorption enhancers is possible.

Disadvantages:

Low bioavailability for proteins.

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Buccal route:

Advantages:

Easily accessible, probably lower proteolytic activity than in the lower parts of the GIT, avoidance of first pass effect, spatial containment of absorption enhancers is possible, option to remove formulation if necessary.

Disadvantages:

Low bioavailability for proteins, no proven track record yet.

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Transdermal route:

Advantages:

Easily accessible, avoidance of first pass effect, spatial containment of absorption enhancers is possible, removal of formulation if necessary is possible,

proven track record with conventional drugs, sustained/ controlled release possible.

Disadvantages:

Low bioavailability for proteins.

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Pulmonary route:

Advantages:

Relatively easy to access, fast uptake, substantial fractions of insulin are absorbed, avoidance of first pass effect, spatial containment of absorption enhancers (?), proven track record with conventional drugs.

Disadvantages:

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

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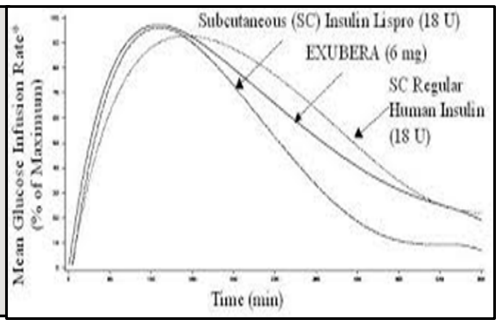
- In humans, the drug should be inhaled instead of intra-tracheally administered (as in rats as example)
- The delivery of insulin by this route has been extensively studied and clinical phase III trials evaluating efficacy and safety have been performed or are ongoing.(the first pulmonary insulin formulation was approved by FDA in January 2006 (Exubera®).(at 1980, was protected by soybean seeds??)



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- 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.**

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- 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).**

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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)

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Molecule	No. of AA	Bioavailability (%)	
		Without glycocholate	With glycocholate
Glucagon	29	<1	70–90
Calcitonin	32	<1	15–20
Insulin	51	<1	10–30
Met-hGH[a]	191	<1	7–8

[a] See Chapter 13, Growth Hormones. *Abbreviation*: AA, amino acids. *Source*: Adapted from Zhou and Li Wan Po, 1991b.

Table 7 Effect of glycocholate (absorption enhancer) on nasal bioavailability of some proteins and peptides.

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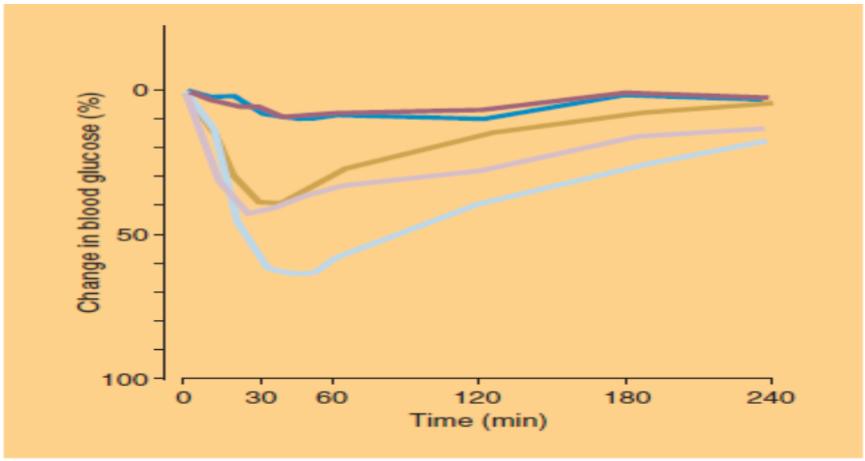

Figure 14 Change in blood glucose in rats after intranasal administration of insulin. *Source*: Discussed by Edman and Björk, 1992.

Key:
- Soluble insulin 2.0 IU/kg i.n.
- Soluble insulin 0.25 IU/kg IV
- Degradable starch microspheres-insulin 0.75 IU/kg i.n.
- Degradable starch microspheres-insulin 1.70 IU/kg i.n.
- Empty degradable starch microspheres 0.5 mg/kg i.n.

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- 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.

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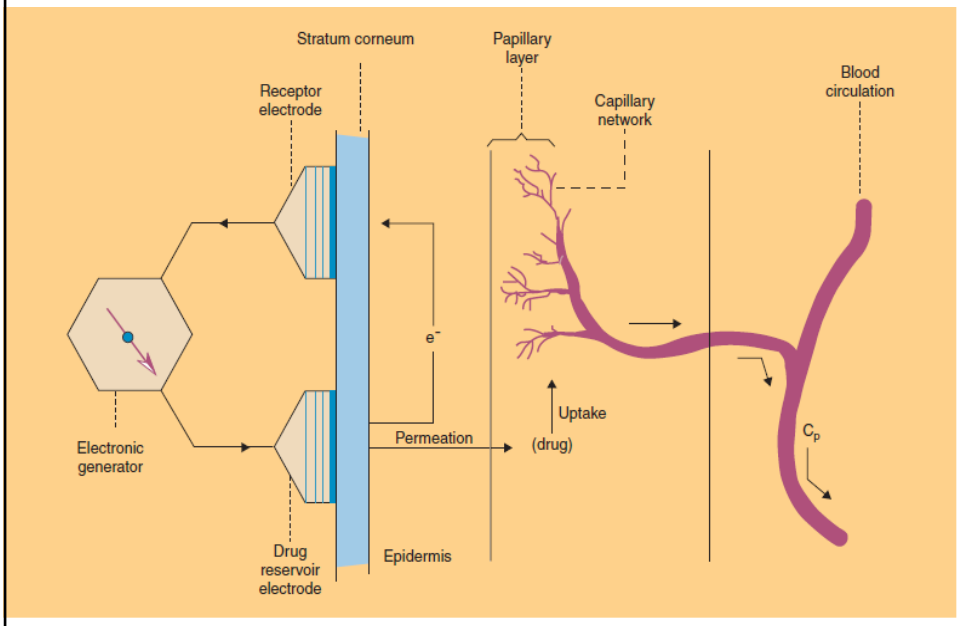

Figure 15 Schematic illustration of the transdermal iontophoretic delivery of peptide and protein drugs across the skin. *Source:*

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Note:

Other approaches that have been 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 alterations**

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.

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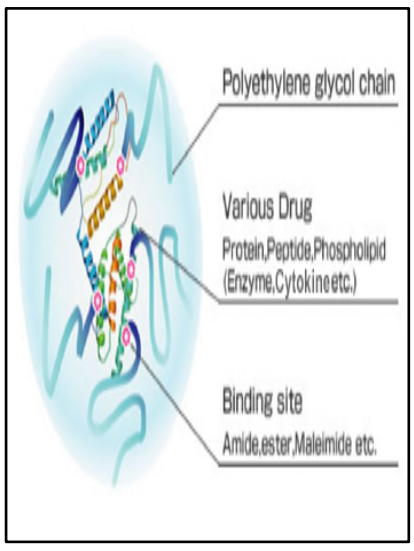

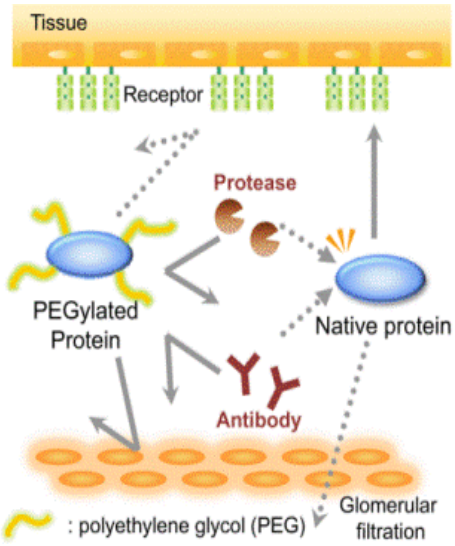

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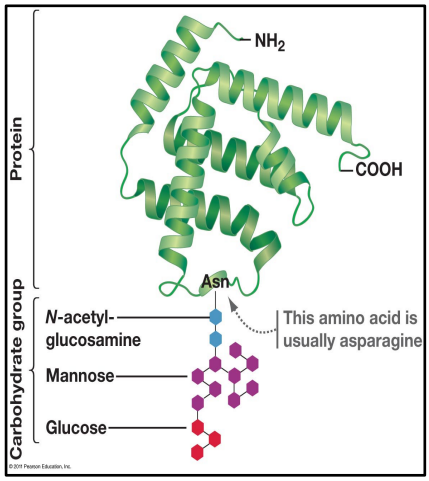
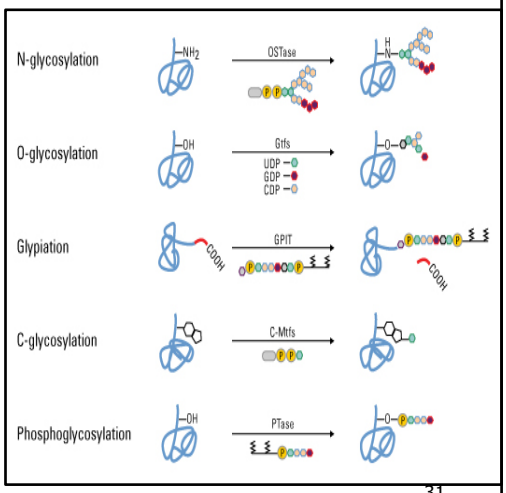
2) Glycosylation: may be occurred after protein production depending on the host. In which oligosaccharide chains are attached to proteins, so there is increase in M.wt and longer half life





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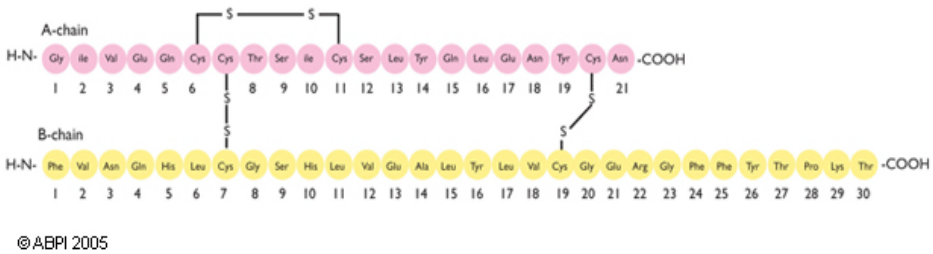
3) Amino acid substitutions:

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.