

2<sup>nd</sup> Year Pharm D  
Pharmacognosy and Phytopharmaceuticals

# Proteins & Enzymes

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# **Proteins & Enzymes**

## **Gelatin & Caesin**

### **Enzymes**

**(Proteolytic, Amylolytic and Digestive)**

**Papain, Pepsin, Bromelain, Pancreatin,**

**Serratiopeptidase, Hyaluronidase,**

**Urokinase, Streptokinase, Asparaginase,**

**Somatropine)**

# PROTEIN

- It is a complex, high mol. Wt. Organic compound, consists of amino acids joined by peptide bonds.
- Word protein derived greek: '*protos*' = '*of primary importance*'.
- Essential for structure and function of all living cells, for growth and repair
- Are enzymes or subunits of enzymes.
- Large molecules, having molecular masses of up to 3,000,000 (the muscle protein titin has a single amino-acid chain 27,000 subunits long).
- Long chains of amino acids are called proteins, shorter chain lengths are polypeptides', 'peptides', or rarely, 'oligopeptides
- 8 essential amino acids required by humans are: leucine, isoleucine, valine, threonine, methionine, phenylalanine, tryptophan, and lysine.

# Gelatin

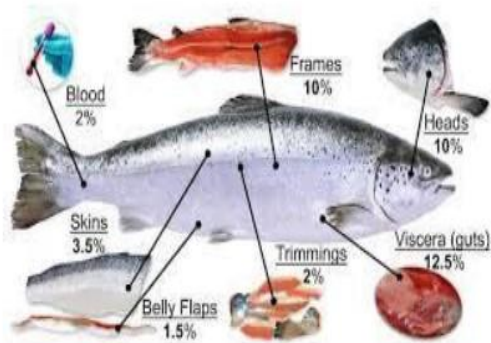


**Gelatin Synonym: Gelatinum**

**Biological Source:** Gelatin is a protein derivative obtain by evaporating an aqueous extract made from bones, skins and tendons of various domestic animals Some important sources are, Ox, and Sheep, *Ovis aries* belonging to family *Bovidae*.







1-Washing and pretreatment  
 2-Extraction (warm water)  
 3-Filtration and drying of gelatin, grinding into solid powder



Cosmetical application



Pharmaceutical and drug delivery application



HGC



SGC

Biomedical application

Tissue Engineering

Intrinsic Activity

Wound dressing /healing

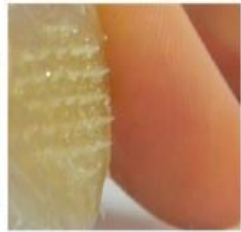
Bone substitutes

Gene Therapy



G GC GCA GCCA

Scaffolds



microneedles

**Preparation:** The process of manufacture of gelatin vary from factory to factory..

**Raw material:** Bones, skins and tendons of *Bovideans* is collected and subjected to liming operation.

**Liming Process:**

- The raw material is first subjected to the treatment known as “liming”. In this process, the skins and tendons soaked for 15 to 20 and sometimes for 40 days in a dilute milk of lime.
- During this, fleshy matter gets dissolved, chondroproteins of connective tissues gets removed and fatty matter is saponified.
- The animal skin is further thoroughly washed in running water.

➤ **Defatting:** In case of bones, the material is properly ground and defatted in close iron cylinders by treatment with organic solvents such as benzene.

➤ The mineral and inorganic part of the bone is removed by treatment with hydrochloric acid.

➤ **Extraction:** The treated material from bones, skins and tendons is boiled with water in open pans with perforated false bottom. This process can also be carried out under reduced pressure.

➤ The clear liquid runs off again and again and is evaporated until it reaches to above 45 % gelatin content.



**Setting:** The concentrated gelatin extract is transferred to shallow metal trays or trays with glass bottom. It is allowed to set as a semisolid gelly.

**Drying:** The gelly is transferred to trays with a perforated wire netting bottom and passed through series of drying compartments of 300 to 600°C increasing each time with 100° C. About a month is taken for complete drying.

**Bleaching:** In case of darker colour, finished product is subjected to bleaching by sulphur dioxide. Bleaching affords a light coloured gelatin

## **Characteristics:**

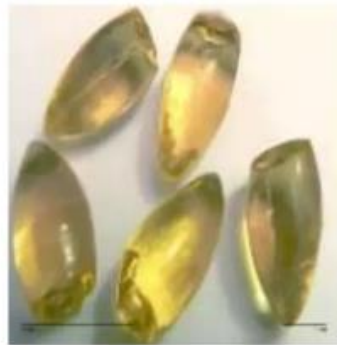
- **Gelatin occurs in the form of thin sheets or as shredded flakes or powder.**
- **It is nearly colourless or pale yellow devoid of odour and taste.**
- **Gelatin is hard and brittle but breaks with short fracture after preliminary bending.**
- **It swells in cold water and completely dissolves when heated.**
- **It is soluble in acetic acid and glycerin but insoluble in alcohol and organic solvents.**
- **A 2% hot aqueous solution gelatinizes on cooling.**
- **Gelatin reacts with hydrochloric acid to obtain glutin-peptone**

# **GELATIN: Syn: Gelatina, Gel foam, puragel**

- **Chemical Test**
- ***Biuret reaction*** : 2ml alkaline solution of a protein+ dil. Sol.  $\text{CuSO}_4 \rightarrow$  red or violet color (if peptides have at least two peptide linkages).
- ***Xanthoproteic reaction***: Sample + Conc.  $\text{HNO}_3$  + warm  $\rightarrow$  yellow + alkali  $\rightarrow$  orange color
- ***Millon's reaction***: Millon's reagent (mercuric nitrate in  $\text{HNO}_3$  + trace of  $\text{HNO}_2$ ) + Sample solution  $\rightarrow$  white precipitate + Heat  $\rightarrow$  Red
- ***Ninhydrin test***: Aq. Sample sol. + alcoholic sol. of ninhydrin + heat  $\rightarrow$  Red to violet colour
- 1g Gelatin + soda lime + heat  $\rightarrow$  smell of ammonia
- 0.5g Sample+ 10ml  $\text{H}_2\text{O}$ + 10% tannic acid  $\rightarrow$  buff col. Ppt
- Sample solution + picric acid solution  $\rightarrow$  yellow ppt

## Uses:

- Gelatin is used as a nutrient and as a styptic.
- It is largely used for the manufacture of hard and soft gelatin capsules.
- It is also used for the preparation of suppositories, pesseries, pastilles and pastes.
- It is a component in the bacteriological culture media.
- Gelatin is also employed in the micro encapsulation of drugs, in injections and perfumes.
- It is used for the production of absorbable gelatin sponge and gelatin films.



# CASEIN

- Proteolytic enzyme obtained from the stomachs of calves. It is extracted from the proteins of the milk; in the milk, casein is structured in voluminous globules.
- It comprises about 80 per cent total protein content of milk. There are two types of casein in the market.
- Acid Casein: Warm skimmed milk is acidified with dilute acid, the whey is separated, curd is washed several times, dried and pulverized.
- Rennet Casein: Skimmed milk is treated with an enzyme, rennet extract; product is separated, and purified.
- Principal casein fractions are alpha (s1) and alpha (s2)-caseins,  $\beta$ -casein and  $\kappa$ -casein. All have low solubility at pH 4.6.

## CASEINS

Caseins, it is part of a group called phosphoproteins, collections of proteins bound to something containing phosphoric acid. Casein includes four individual gene product components denoted  $\alpha$ 1-,  $\alpha$ 2-,  $\beta$ - and  $\kappa$ -casein, which differ in primary structure and type and degree of post-translational modification.

These four casein types are essentially different in their

**molecular weights as follows:**

$\alpha$ 1-casein (MW 23 KD, ~38.49%),

$\alpha$ 2-casein (MW 25 KD, ~10.06%),

$\beta$ -casein (MW 24 KD, ~38.74%),

$\kappa$ -casein (MW 19 KD, ~12.57%).



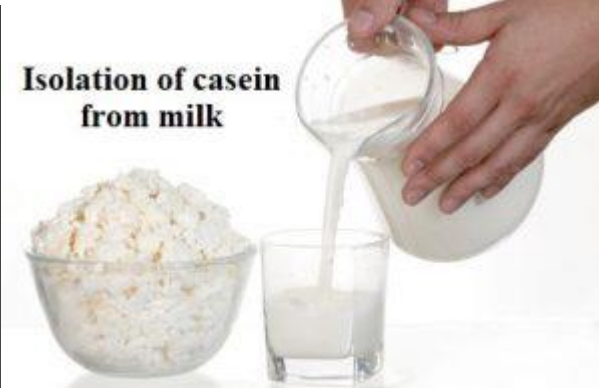
# PROCEDURE

1. Place 20 ml (20 g) of milk into a 125 ml flask and heat at 40 °C in a water bath.
2. Add 5 drops of **glacial acetic acid** and stir for about 1 min.
3. Filter the resulting mixture through filter paper held in a funnel and gently squeeze out most of liquid.
4. Remove the solid (**casein and fat**) from the cheesecloth, place it into a 100 ml beaker and add **10 ml of 95% ethanol**.
5. Stir well to break up the product. Pour off the liquid and add 10 ml of 1:1 ether-ethanol mixture to the solid.
6. Stir well and filter through filter paper
7. Let the solid drain well, then scrape it into a weighed filter paper and let it dry in the air.
8. Calculate the casein percentage in milk as follows:

$$\% \text{ Casein} = (\text{grams of casein} \div \text{grams of milk}) \times 100$$

Normal Range 3-5 %

Isolation of casein from milk



# CASEIN

- **Description:** It is white, slightly yellow, tasteless, odourless, amorphous solid, hygroscopic, stable when dry but deteriorates rapidly when damp.
- **Solubility :** insoluble in H<sub>2</sub>O, sol. in dil. alkalies, conc. acids, precipitates from dil. acid solutions.
- **Chemistry of Casein:** Casein is a phosphoprotein, contain about 0.85% P & 0.75% S. Contains about 15 amino acids also rich in essential amino acids. Molecular weight 75000 - 3,70,000, Isoelectric point 4.7, Nitrogen content 15 – 16%.
- **Standards of Quality:**
- **Loss on drying:** Not more than 6.0 %
- **Sulphated ash:** Not more than 1.5%
- **Specific gravity:** 1.25 - 1.31.



# CASEIN

- **Chemical Constituents**
- Milk consists of 80% of milk proteins (casein). The major constituents of casein are alpha (s1) and alpha (s2)-caseins,  $\beta$ -casein and kappa-casein. These caseins are conjugated proteins with phosphate group(s) which are esterified into serine residues they have a low solubility at pH 4.6.
- **Uses:** Useful dietary supplement source of protein in pre and post operative care; as a base in standardisation of proteolytic enzymes and as emulsifying agent. Industrially, used in sizing of textile and paper, as an adhesive, in preparation of casein plastic and casein paints. used by bodybuilders as a slow-digesting source of amino acids.

## ENZYMES

- Enzymes are organic catalysts produced in the body by living organisms. They perform many complex chemical reactions that make up life processes. Enzymes are lifeless and when isolated, they still exert their characteristic catalytic effect
- Enzymes are found in combination with inorganic or organic substances that have an important part in the catalytic action.
- Enzymes are obtained from plant and animal cells and many have been purified. They are used as therapeutic agents and as controlling factors in certain chemical reactions in industry.
- Pepsin, pancreatin, and papain are used therapeutically as digestants. Hyaluronidase facilitates the diffusion of injected fluids. Streptokinase and streptodornase dissolve clotted blood.

## **Properties of Enzymes**

1. Enzymes are sensitive to heat and are denatured by excess heat or cold.
2. Enzymes are sensitive to pH, the rate at which they can conduct reaction is dependent upon the pH of where the reaction is taking place
3. Enzymes are reusable and some enzymes are capable of catalyzing many hundreds or thousands of reactions

# Pepsin

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- **Biological Source** : It is the enzyme prepared from the mucous membrane of fresh stomach pig *Sus scrofa* Linn, belonging to family Suidae.

## METHOD OF PREPARATION

- Mucous lining of stomach scrapped off
  - Cut into small pieces
  - Pulp placed in water
  - Add HCL for digestion
    - Kept at 37°C

- Autolysis takes place
- Clear liquid is obtained
- Contain peptones and pepsin
- Add NaCl
- NaCl precipitates pepsin and peptone remain as such
- Collection of ppts.
- Suspended in the water in dialyser
- Removal of salt by dialysis
- To the aqueous solution, add alcohol to bring ppt of pepsin
- Collection of ppt
- Drying and packing

## **Description:**

1. Colour: pale yellow
2. Odor: odourless or with very faint odour, translucent grains
3. Taste: slightly bitter
4. It is best active at a temperature of 40°C with pH 2–4.
5. Pepsin is unstable above pH 6.
6. The enzyme gets denatured at a temperature of 70°C and in the presence of alcohol and sodium chloride.
7. Pepsin can be stored for 1–2 years at 2–8°C.

## **Uses:**

1. It is used in the deficiency of gastric secretion.
2. Pepsin is also used in the laboratory analysis of various proteins; in the preparation of cheese, and other protein-containing foods.



# Proteolytic Enzymes

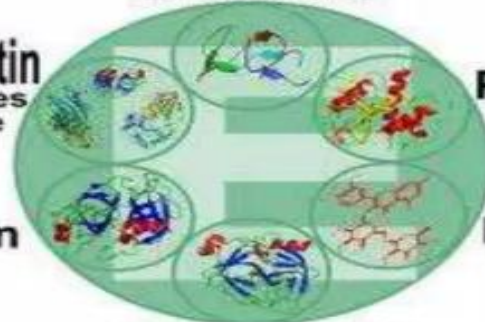


**Bromelain**



**Papain**

**Pancreatin**  
Proteases  
Amylase  
Lipase



**Trypsin**

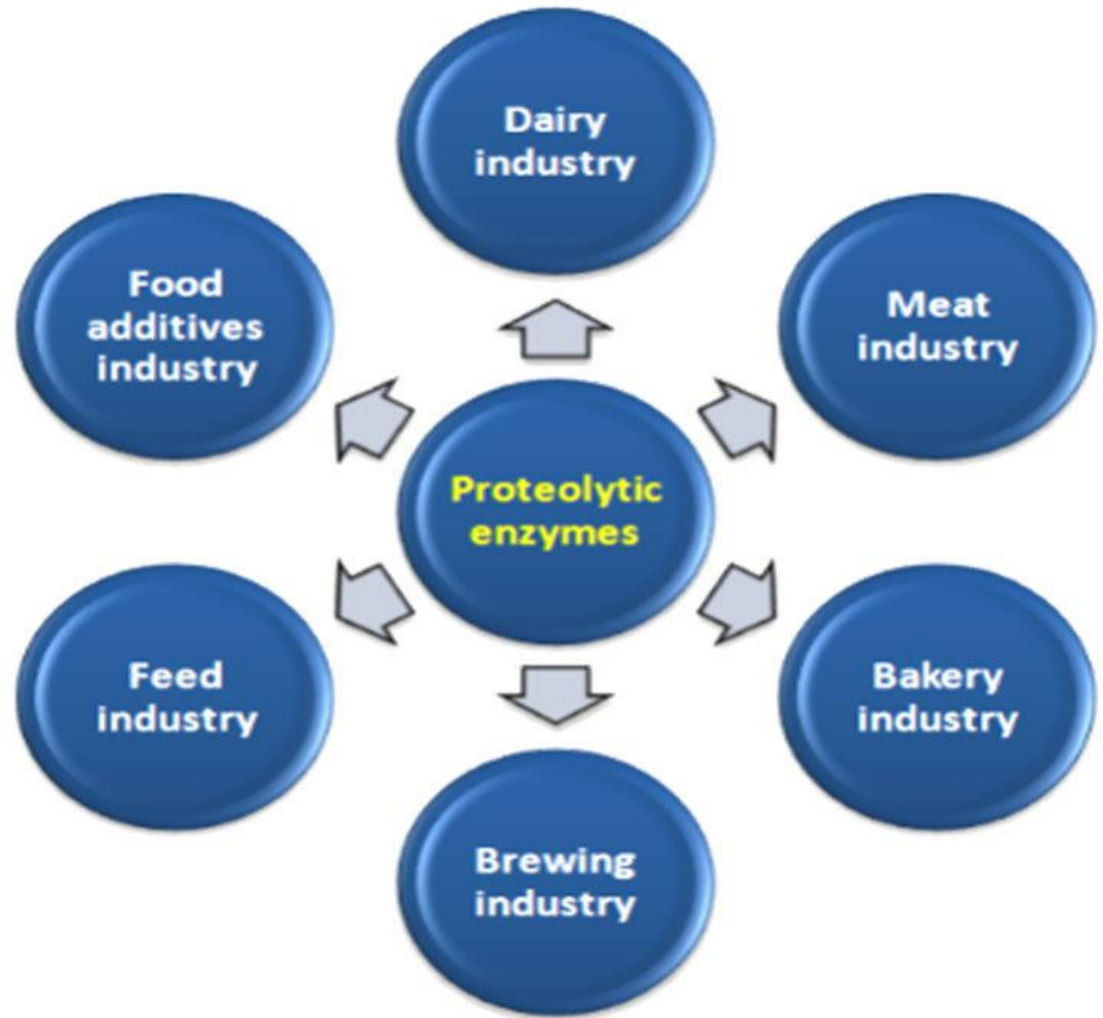
**Rutosid**

**Chymotrypsin**



# Proteolytic Enzymes

- Enzymes involved in degradation of proteins are called as **proteases** or **proteolytic enzymes**





# List of proteolytic enzymes

- a. **Papain**
- b. Ficin
- c. **Bromelain**
- d. **Pepsin**
- e. Rennin
- f. Cathepsin
- g. Trypsin

# Papain

- Found in papaya
  - **papain**, also known as **papaya proteinase**
  - Broad pH (3-11) and temperature stability
    - For this reason very popular for a variety of food applications.
1. Used as a meat tenderizer on inferior meat cuts (can also use slice of pineapple on meat)
    - The enzyme makes its way into the muscle and hydrolyzes primarily connective tissue proteins (collagen etc.) and softens muscle
    - Have to use low amount to prevent liquefaction of muscle
    - If you mix raw papaya into Jell-O it will not form a gel



# Preparation

- It is distributed throughout the plant, but mostly concentrated in the latex of the fruit.
- The latex is obtained by making two to four longitudinal incisions, about 1/8 inch deep, on the surface on four sides of nearly mature but green fruits while still on the tree.
- The incisions are made early in the morning, at intervals of three to seven days.
- The latex flows freely for a few seconds but soon coagulates.
- The exudate is collected in nonmetallic containers.
- The latex is dried as soon as possible after collection.



- Rapid drying or exposure to sun or higher temperature above 38°C produce dark colour product with weak proteolytic activity.
- The use of artificial heat yields the better grade of crude papain.
- The final product should be creamy white and friable.
- It is sealed in air-tight containers to prevent loss of activity.
- If 10% common salt or 1% solution of formaldehyde is added before drying, the product retains its activity for many months.
- Fully grown fruits give more latex of high enzyme potency than smaller or immature fruits.
- The yield of Papain varies from 20 to 250 g per tree.
- The yield of commercial Papain from latex is about 20%.

## Phytochemicals

- Caffeic acid
- Myricetin
- Quercetin
- Papain
- $\alpha$ -tocopherol
- Benzyl isothiocyanate
- Kaempferol



## Possible Antioxidative MOAs

- Free radical scavenging action
- $\downarrow$  Lipid peroxidation
- $\downarrow$  Overall ROS production

### Inflammation and Chronic conditions

(Diabetes, Alzheimer's Disease, Periodontal disease)

- $\downarrow$  MAPK/ERK, JNK
- $\downarrow$  NF- $\kappa$ B, iNOS, IL-4, IL-5, eotaxin, TNF $\alpha$
- $\downarrow$  Leucocyte counts, inflammatory cell infiltration
- Ion-reducing capacity,  $\downarrow$  Fenton reaction
- $\uparrow$  Antioxidative enzymes (GSH, GPx, SOD and CAT)
- $\uparrow$  eNOS, mass of  $\beta$  cells,  $\downarrow$  post-prandial BGL
- $\downarrow$  Mitochondrial dysfunction, cell apoptosis

### Cancer

- Chelation of ROS generating agents
- $\downarrow$  IL-6, IL-8 ( $\downarrow$  glioma cell metastasis)
- $\downarrow$  ROS thus  $\uparrow$  tumour suppressor genes
- $\uparrow$  ROS thus  $\uparrow$  cancer cells autophagy and apoptosis

### Skin

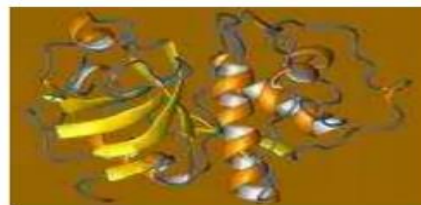
(Aging, Wound Healing)

- Act as exfoliant
- $\uparrow$  Type I collagen
- $\downarrow$  Myeloperoxidase, proteinases
- $\downarrow$  AP-1, MAPK, Nrf2
- $\uparrow$  SOD, CAT, GSH

- **Chemical test:**
  1. It decolorizes aqueous potassium permanganate solution.
  2. It causes curdling of milk. (Proteolytic activity)

## Application of papain

- **Meat Tenderizer- Papain** ( a protease found in papaya) is used as meat tenderizer to soften meat for cooking



## Fruits



- Anticancer activity
- Antidiarrheal responses
- Antioxidant activity
- Processed foods
- Source of enzyme

## Seeds



- Wound-healing activity
- Anticancer activity
- Insecticidal and repellent responses
- Biodiesel production

## Commercial products



## Leaves



- Anti-dengue activity
- Anti-malarial activity
- Anticancer activity
- Antibacterial activity
- Antifungal activity

## *Carica papaya*



## Papaya peel

- In cosmetics
- In waste water treatment
- As animal feed
- In toothpaste
- As a binder in ceramics



## Nanoparticle synthesis



Extract + Metal Solution



Nanoparticles in various shape and size

Applications



# Bromelain

- **Bromelain** is an enzyme generally found in the stems and fruits of pineapples.
- According their sources enzymes are divided into
  - A. **Stem bromelain.**
  - B. **Fruit bromelain.**
- The proteolytic enzymes are sulfhydryl proteases, since a free sulfhydryl group of a cysteine side chain is required for function



# Bromalein



- **Syn:** Stem Bromelain, Pineapple stem bromelain
- **B.S.:** it is proteolytic enzyme obtained from the stem & ripened fruit of Pineapple plant. *Ananas comosus*
- **Family:** Bromiliaceae
- **Description:**
- **Odour:** odourless
- **Color:** Buff colored powder
- **Taste:** Acrid

- **Cultivation, Collection, and Preparation**

- 1. Bromelin is found in pineapple fruit juice and stem.
- 2. It is propagated through suckers, slips, and crowns.
- 3. Plantation month is august.
- 4. Flowering month – February-march
- 5. fruit ripens during July–October
- 6. When fruits turns orange then they are cut off.
- 7. The enzyme bromelin does not disappear as the fruit ripens. The enzyme from fruit and stem are known as fruit bromelin and stem bromelin, respectively.
- 8. It is isolated from pineapple juice by precipitation with acetone and also with ammonium sulphide.



- **Identification test:**
- 0.2 g of sample add 1g of anhydrous sodium carbonate, & heat gently to carbonize.
- Cool, add 5 ml of H<sub>2</sub>O stir & filter.
- Acidify slightly the filter with dil.HNO<sub>3</sub>, heat in water bath for 5 min. & cool.
- Now add silver nitrate which yields light yellow ppts. Which insoluble in dil. HNO<sub>3</sub> or ammonia.
- Separate ppts & strong ammonia with shaking.
- Separate liquid & acidified with Dil. HNO<sub>3</sub>, yield white turbidity.

# Uses



- Angina, Dysmenorrhea & other CVS disorder.
- Arthritis
- Athletics injuries
- Bronchitis
- Burn debridement
- Cancer
- Dermatological condition
- Digestive disorder
- Pancreatic insufficiency
- Thrombophlebitis

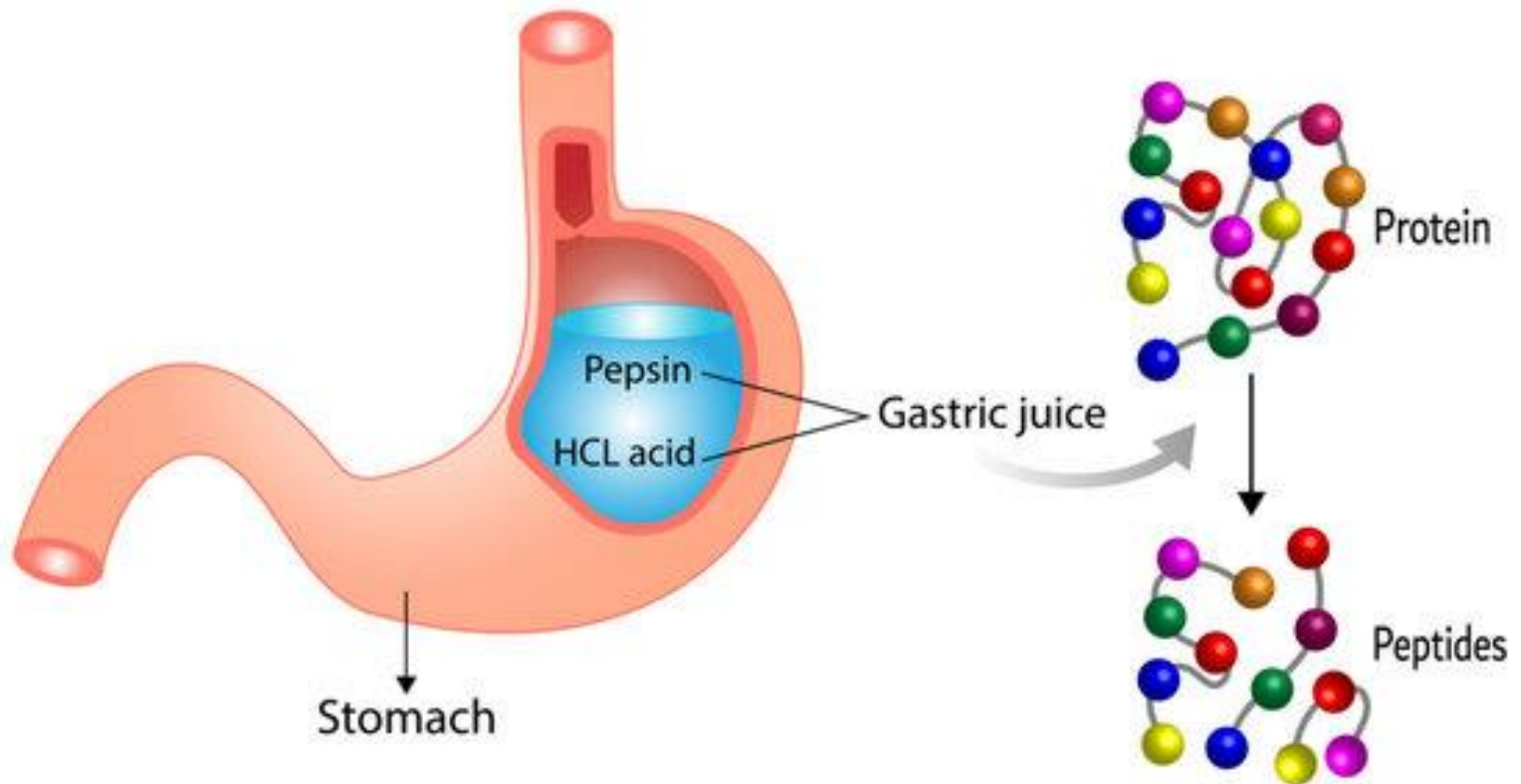
- Bromelain is present in all parts of the pineapple plant but the stem is the most common commercial source

## Applications of bromelain

- Along with papain, bromelain is one of the most popular proteases to use for meat tenderizing
- Cooked pineapple does not have a tenderizing effect, as the enzymes are heat-labile
- Preparation of meat balls
- Systemic enzyme therapy (combinations of proteolytic enzymes such as bromelain, trypsin and papain) has been used in the treatment of breast cancer

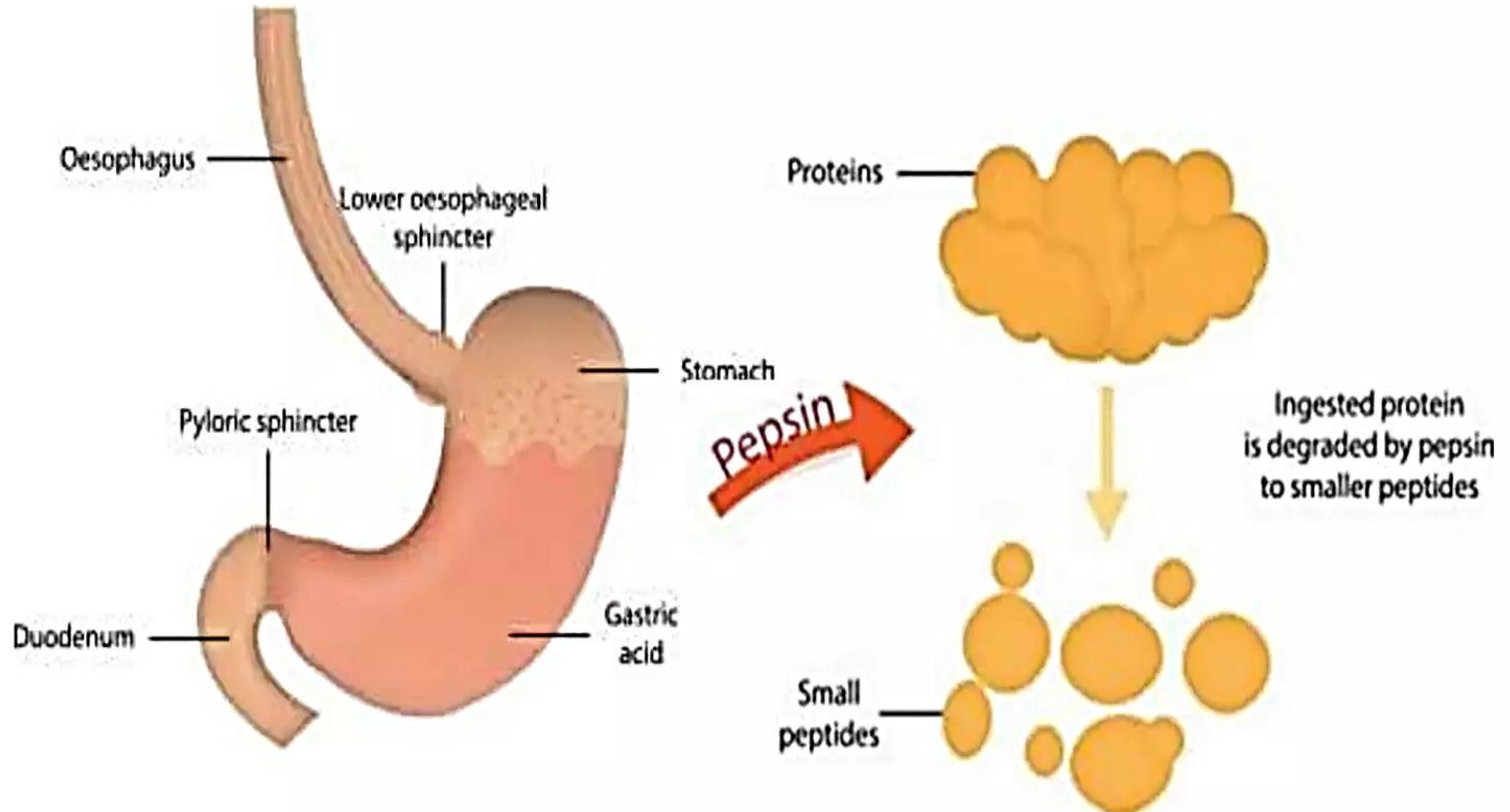


# Pepsin



# SCHEMATIC PRESENTATION OF WORKING OF PEPSIN

## *Digestion*



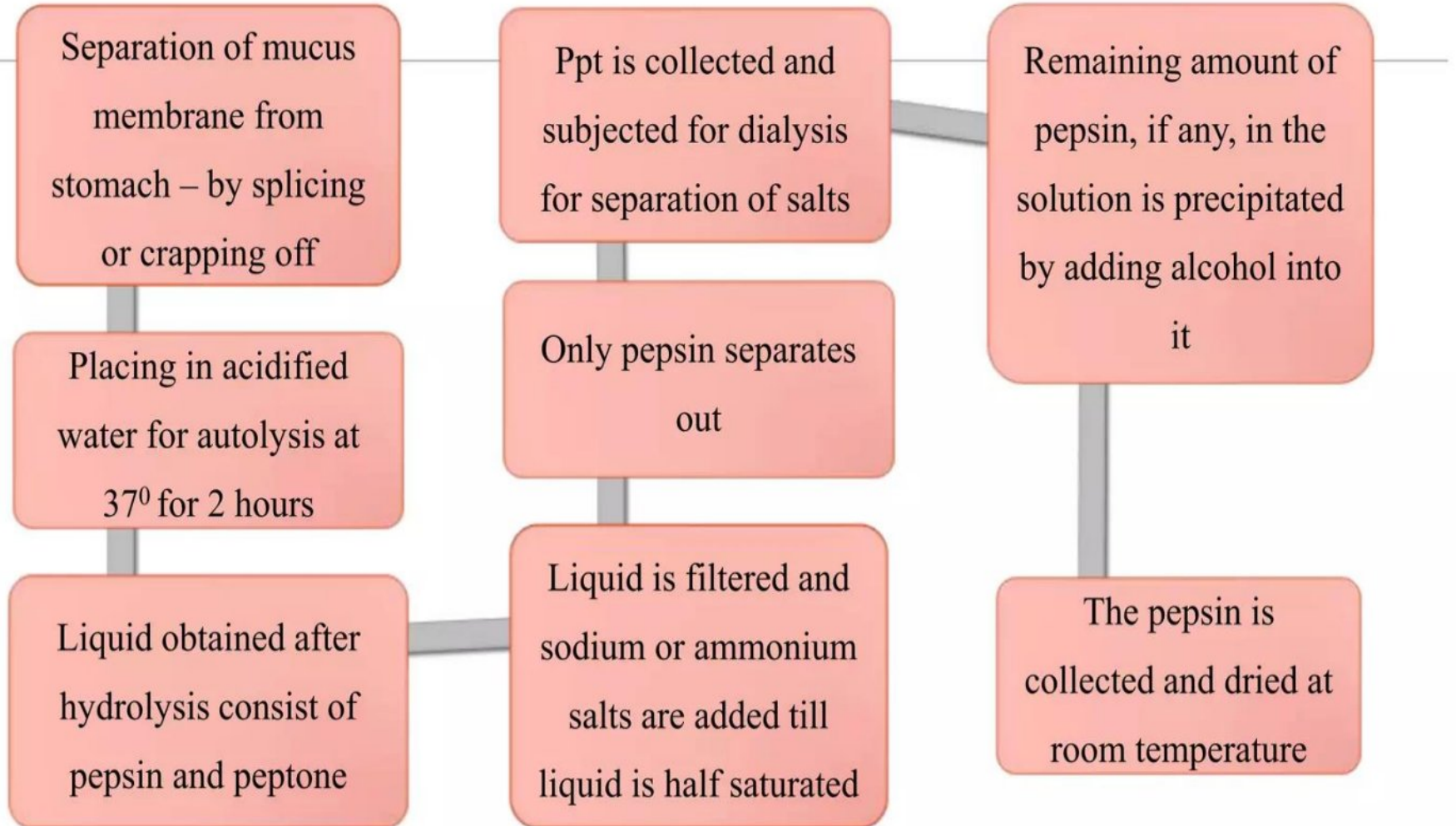
# Pepsin



- **B.S.:** Pepsin is protolytic enzyme obtained from the glandular layer of the fresh stomach of the hog(Pig), ***Sus scrofa* Linne var. domesticus Gray.** (Sheep, calf)
- **Family:** Suidae
- The generic name *Sus* is from the Greek, meaning Hog; *scrofa* is latin and means breeding ; and *domesticus* is latin and means the house hold.
- **Preparation:**
- **Mucous memb.** Is scraped from stomach, and placed in **acidified** water at **37°C. for 2 hrs.** (Pepsin & Peptone)
- Filtered & add **Sodium** or **ammonium** salt till it become half saturated.(pepsin separated, pepton remain)
- Ppts by addition of **alcohol**, collect it, dried it.



# METHOD OF PREPARATION OF PEPSIN



- Powder of pepsin is often used as a dietary supplement to aid digestion



- **Use:**
- **1)** it is component of rennet used to curdle milk during the mfg. of Cheese.
- **2)**Used to modify & provide whipping qualities to soy protein and gelatin.
- **3)** to make precooked cereals into instant hot cereals.
- **4)** for flavoring food & beverages.
- **5)** in leather industry to remove hair & residual tissue from hide.
- **6)** in preparation of F2 fragment from antibodies.

# Rennin

- **Rennin or Chymosin** is a protease produced by newborn ruminant in the fourth stomach to curdle the milk they ingest
- The native substrate of Chymosin is K-casein cleaves at amino acids like phenylalanine and methionine
- Bovine chymosin is now produced recombinantly in *E. coli*, *Aspergillus niger* alternative resource.

**# Renin is secreted by:**

**A. Liver**

**B. Kidney**

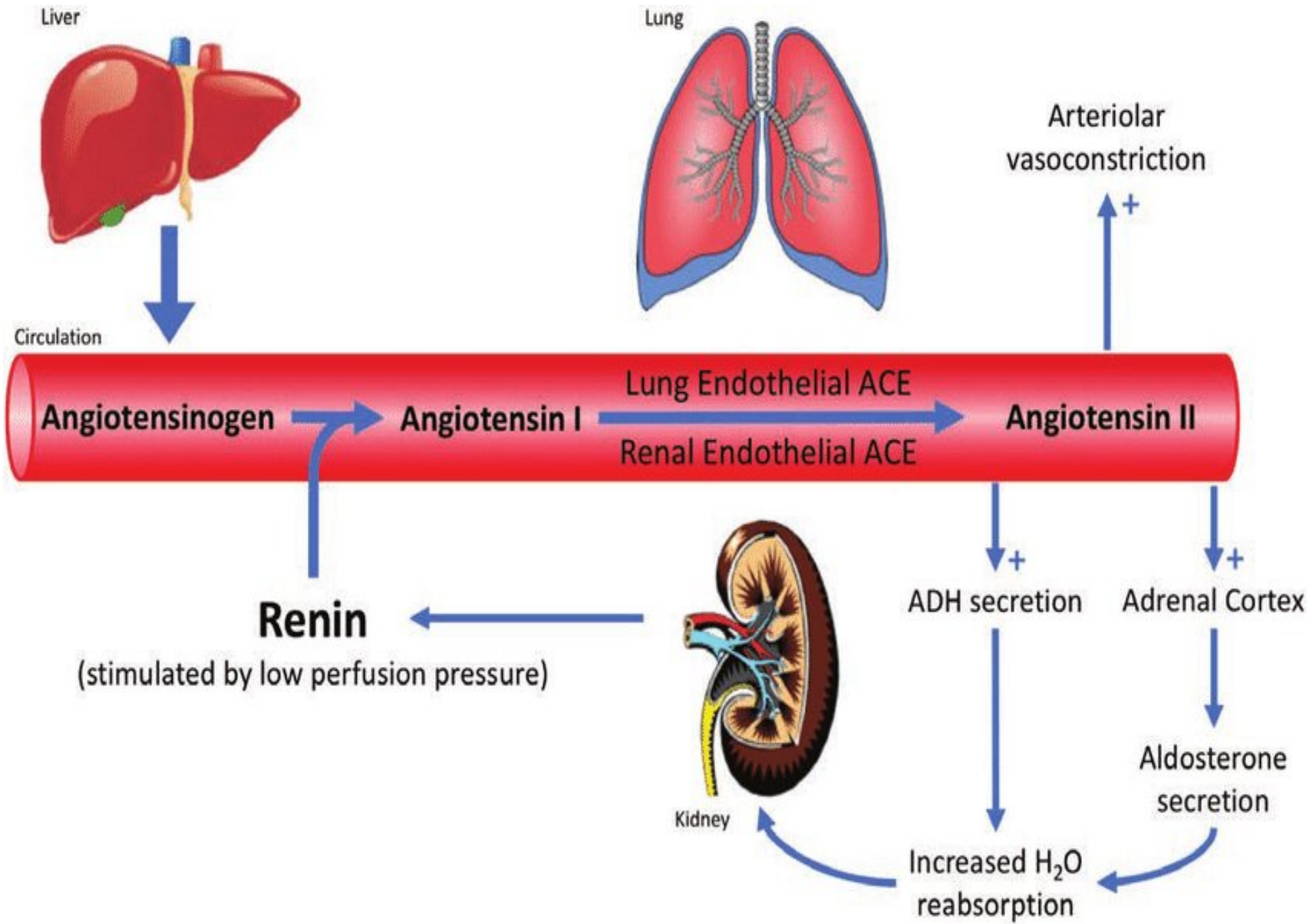
**C. Intestine**

**D. Stomach**

**Application of rennin**

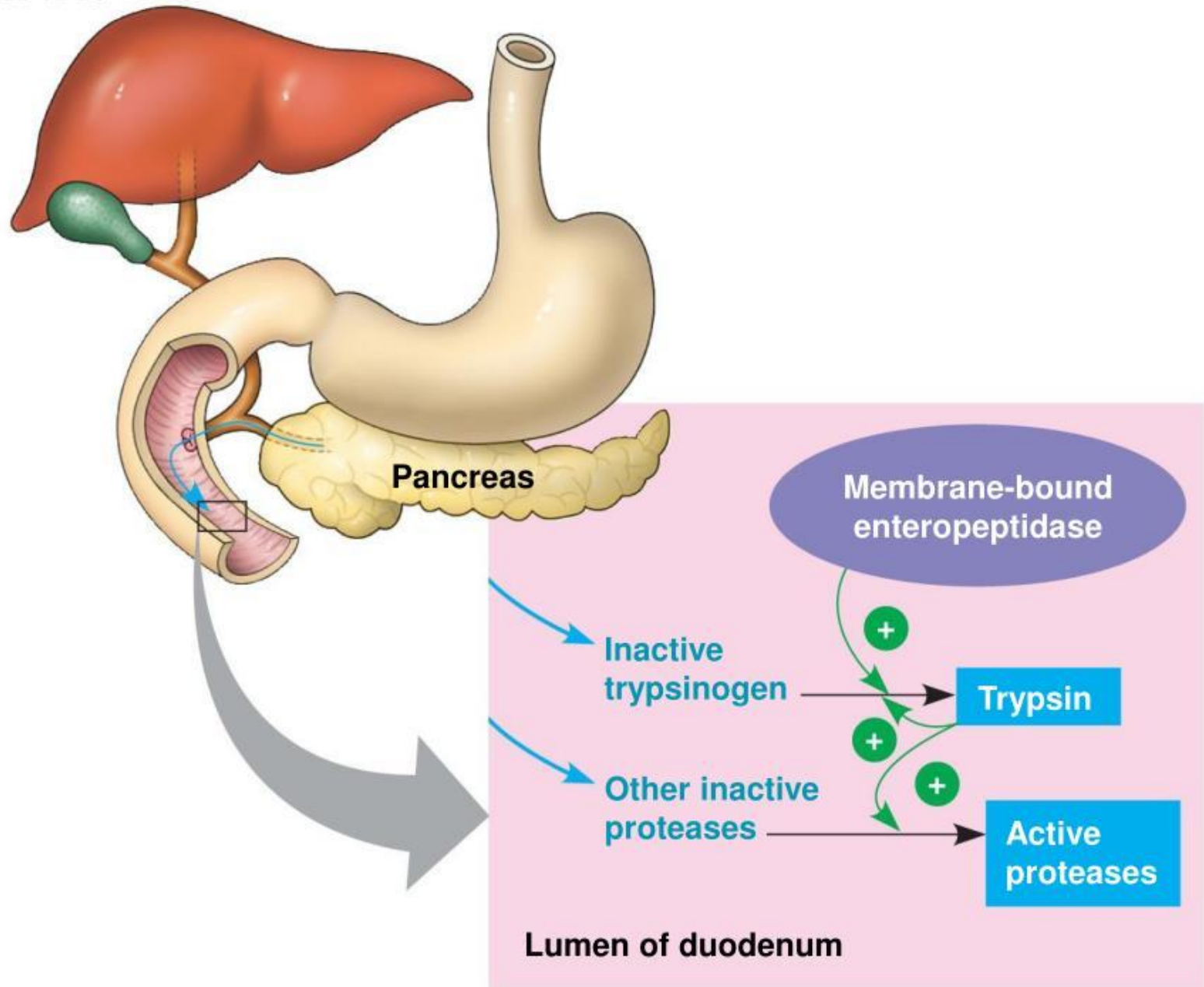
- It is widely used in the production of cheese



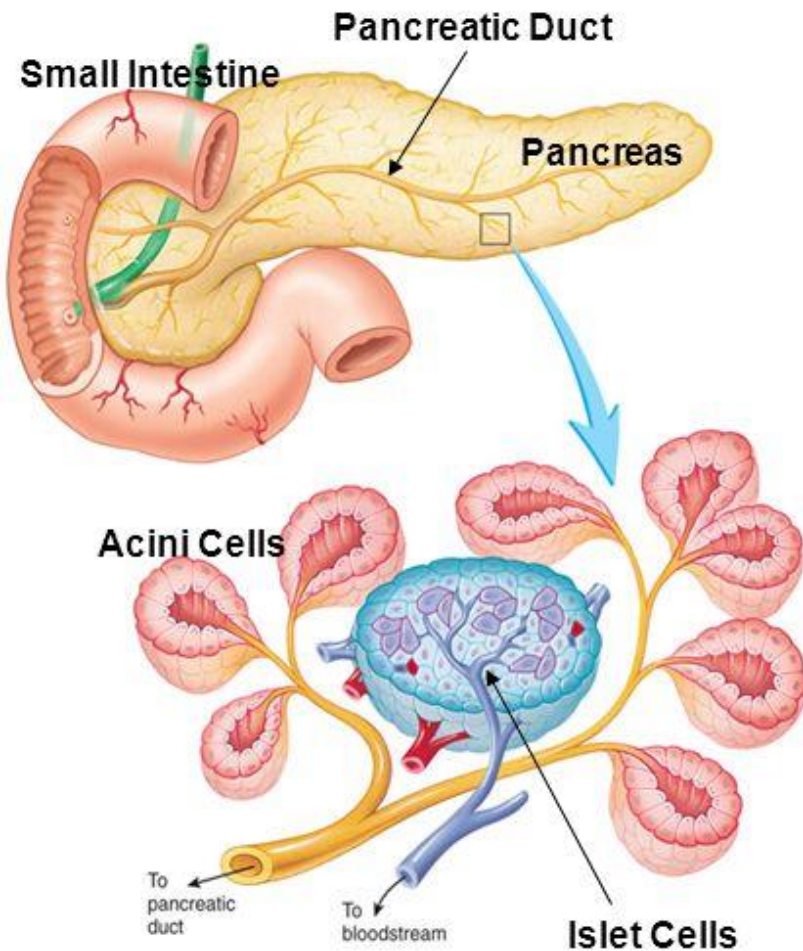


# Trypsin

- **Trypsin** is a protease found in the digestive system of many vertebrates, where it hydrolyses proteins.
- Trypsin is produced in the pancreas as the inactive protease trypsinogen.
- Trypsin cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine



# Secretion: Pancreas



Pancreas secretes

Trypsin & Trypsinogen  
Exopeptidases

Amylase

Lipase

Bicarbonate Ions (raise pH)

**Pancreatic Enzymes work best at higher pH**

# Application of trypsin

- In a Animal tissue culture lab, trypsin is used to re-suspend cells adherent to the cell culture dish wall during the process of harvesting cells
- Used in proteomics experiments to digest proteins into peptides for mass spectrometry analysis
- As a baking enzyme to improve the workability of dough;
- in the extraction of seasonings and flavourings from vegetable or animal proteins and in the manufacture of sauces
- In the production of hypoallergenic food where trypsin break down specific allergenic proteins into non allergenic peptides
- To improve the texture of fish products
- To tenderize meat along with papain
- For cold stabilization of bee





## Serratiopeptidase

### Biological source:

- Serratiopeptidase is a proteolytic enzyme excreted by nonpathogenic *Enterobacteria serratia* isolated from intestine of silkworms *Bombyx mori L.*
- It has a distinctive ability to digest the dead tissue of the Cocoon of the silkworm.

### Macroscopical characters:

- Colour: A grayish white to pale brown colour powder with
- Odour: characteristic odour.



## Serratiopeptidase

- In human body it breaks down protein deposits such as fibrin.
- This is used as a natural alternative to steroids and NSAIDs without serious side effects.
- The enzyme causes proteolysis of all non-vital tissues including blood clots, cysts, tissue plaques and cellular debris and reduces the inflammatory response.

# Preparation of Serratiopeptidase from *Enterobacteria serratia*

## Domestic Silkworm *Bombyx mori*



### Isolation of gut bacteria



\*Enumeration of gut bacteria: Viable count

\*Identification of gut bacteria: Morphological and biochemical characterization and Molecular identification

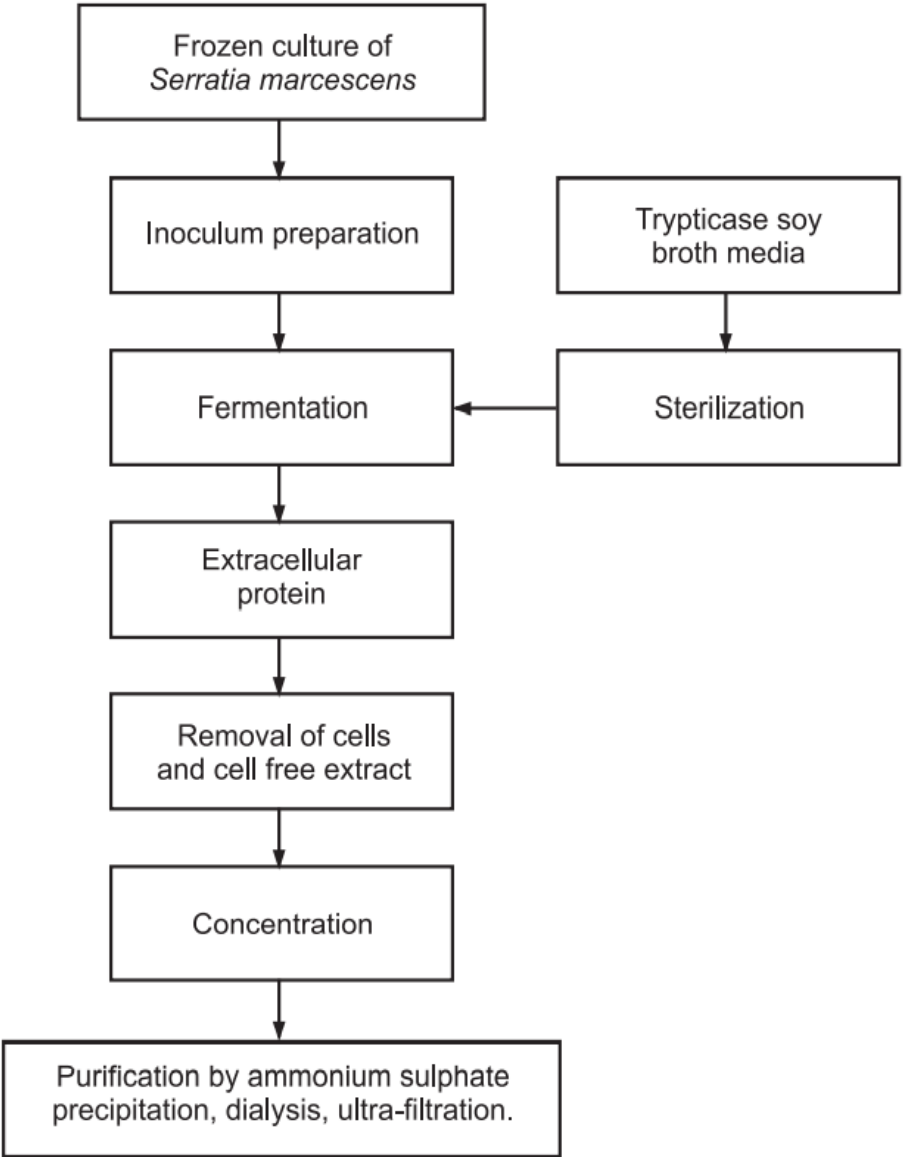


### Isolation of gut fungi



\*Enumeration of gut fungi: Viable count

\*Identification of gut fungi: Morphological characterization and Molecular identification



## Uses:

- ☞ The most widely prescribed **anti-inflammatory enzyme**.
- ☞ It eliminates inflammatory **oedema and swelling**, accelerate liquefaction of pus and sputum.
- ☞ As a fast wound **healing agent**.
- ☞ It has **applications** in *trauma surgery, plastic surgery, respiratory medicine, obstetrics* and *gynaecology*.

## Uses

- Pain, inflammation due to
  - Arthritis,
  - Trauma,
  - Surgery,
  - Sinusitis,
  - Bronchitis,
  - Carpal tunnel and painful swelling of the breasts
  - The recommended dosage for Serratiopeptidase is 10 mg to 30 mg a day.
  - Serratiopeptidase is well known for its ability to reduce pain by blocking the release of paininducing molecules from inflamed tissues

# Urokinase

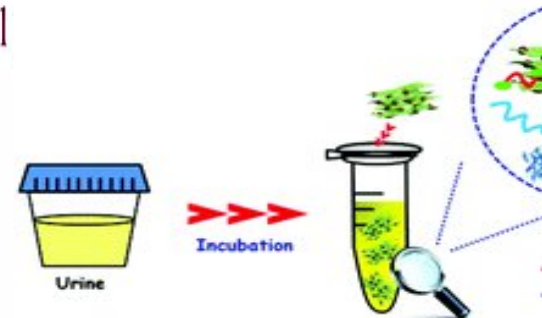


## Biological source:

- Urokinase was first discovered and isolated from human urine [3].
- The urokinase activity in urine is elaborated from the cells lining the kidney tubules [2].
- The urokinase is secreted by vascular endothelial, smooth muscle cells (SMC), epithelial cells, fibroblasts, monocytes/macrophages, and also by cells of malignant tumors of different origin [4–6].
- Cultured cells have been reported to secrete larger quantities of urokinase (50–100 ng/ml) as compared to that present in urine (10–15 ng/ml) [7]. Human plasma also contains at least 10 g/L of urokinase-reactive material

## Macroscopical characters:

- Colour: A white powder



# Urokinase



- **Syn:** U-plasminogen, Urinary plasminogen activator.
- **B.S.:** obtain from human urine. Now a days also obtained from *E.Coli* by rDNA technique.
- **Description:**
- Available in the form of lyophilised white powder which is soluble in water.
- It convert plasminogen in to plasmin.
- Degrade fibrin & other plasma protines.

## Preparation:

- 1.** Urokinase is a fibrinolytic enzyme produced by recombinant DNA using genetically manipulated *E. coli* cells.
- 2.** It is produced firstly as prourokinase and then converted to active form by plasmin or kallikrein.
- 3.** Urokinase used medicinally is also purified directly from human urine.
- 4.** It binds to a range of adsorbents such as silica gel or kaolin which can be use to initially concentrate and purify the product.
- 5.** It can be further purified by precipitation with sodium chloride or ethanol or by chromatography.
- 6.** Human urokinase needs sterile filtration, aseptic filling and freeze drying.

## **Chemical Constituents:**

1. Urokinase enzymes are serine proteases that occur as a single low molecular weight (33 kDa) and double, high molecular weight (54 kDa) polypeptide chain forms. They differ in molecular weight considerably.

## **Uses:**

Urokinase is used in the treatment of pulmonary embolism, coronary artery thrombosis and for restoring the potency of intravenous catheters



# Use



- Coronary thrombosis
- Myocardial Infraction
- Pulmonary embolism
- Venous thrombosis

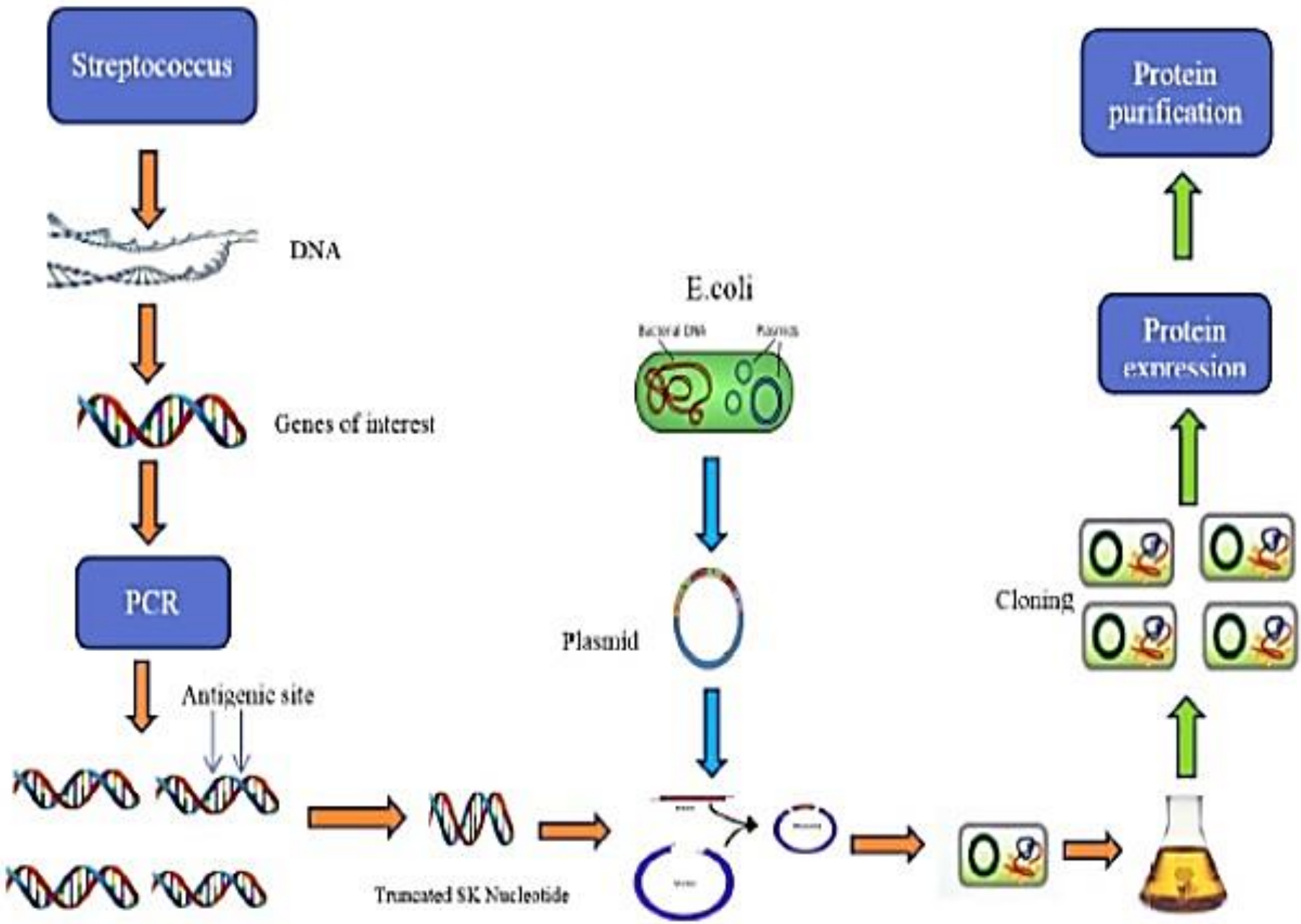
# Streptokinase

- **Synonym:** Estreptokinase, plasminokinase.
- **Biological Source:** It is a purified bacterial protein produced from the strains of  $\beta$ -haemolytic Streptococci.

## **Method of Preparation:**

1. Streptokinase is a bacterial derived enzyme of serine protease group.
2. Streptokinase is produced by fermentation using streptococcal culture and is isolated from the culture filtrate. It is produced in the form of a lyophilized powder in sterile vials.

Streptokinase



- **Description:** Streptokinase is a bacterial protein with half-life of 23 minutes.

## **Chemical Constituents:**

- Streptokinase is the purified bacterial protein with about 484 amino-acid residues.

## **Uses:**

1. Streptokinase is the first available agent for dissolving blood clots.
2. Streptokinase and anistreptase are both used in the treatment of pulmonary embolism, venous, and arterial thrombosis and coronary artery thrombosis.

# Hyaluronidase



- **Syn:** Spreading factor
- **B.S.:** it is observed in the testes & semen.
- It specifically depolymerizes hyaluronic acid, thereby enhancing the permeability of the connective tissues.
- It also act the disperse the cells of corona radiate about the newly ovulated ovum, thus largely facilitating entry of the sperm.
- **Description:**
- It reduce viscosity of tissue cement & increase rate of IM & SC injectables in humans.
- It is grp of enzymes like 4-lykanohydrolase, hayaluronate 3-3 glycano hydrolase & hyluronate lyase.
- They are mucopeptides composed of alternating N-acetyl glucosamine & glucuronic acid.

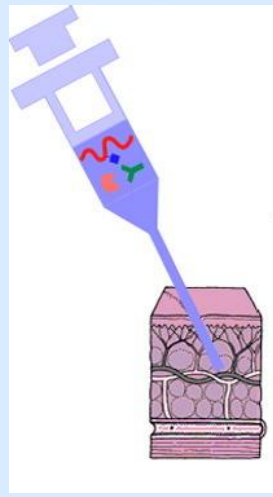
# Preparation



- It is sterile, dry, soluble enzyme preparation, which is prepared from testes & semen by fractional precipitation of aq. Extract & further by dialysis.
- Sterilization by filtration & lyophilization.
- It is odourless, white to yellow in color & highly soluble in water, but insoluble in organic solvent.
- **Identification test**
- Acid mucopolysaccharide, histochemically using colloidal iron stain.
- Determine by digestion of a serial section with hyaluronidase prior to staining.



- **Use:**
- Enhance absorption rate
- Reduce discomfort caused by intramuscular or subcutaneous injections.
- Also used in hypodermolysis (in SC & parental cases)



# Hyaluronidase Market

The global market is dominated by players such as Baxter International Inc., Halozyme Therapeutics Inc., Pfizer Inc., Shire Plc, Quidel Corporation, CSL Limited, IBSA Institut Biochimique SA, Johnson & Johnson, Nordic Group BV, Bausch Health Companies Inc., AstraZeneca, Merck KGaA, Teva Pharmaceutical Industries Ltd., Santen Pharmaceutical Co. Ltd.



## Regional Analysis

North America | Europe | Asia Pacific  
Latin America | Middle East and Africa

2023-2030

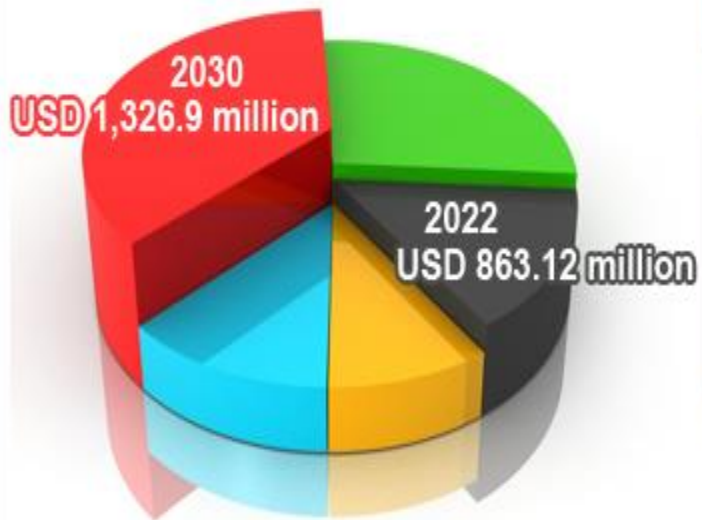
**CAGR**  
**9.01%**

## By Type

- Animal-Derived Hyaluronidase
- Synthetic Hyaluronidase

## By Application

- Chemotherapy
- Dermatology
- Ophthalmology
- Plastic Surgery



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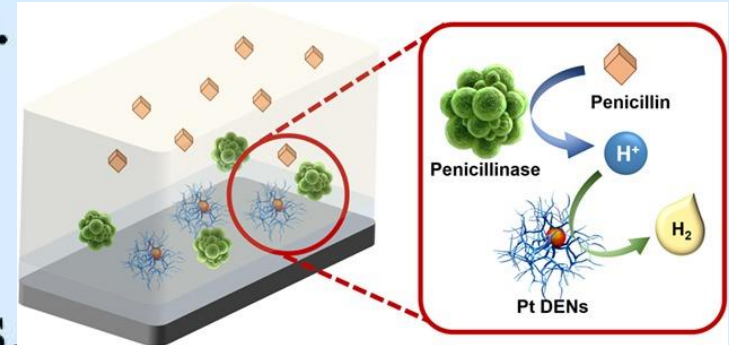




# Penicillinase



- **Syn:**  $\beta$ -lactamase
- **B.S.:** it is bacterial enzyme, *bacillus* species & certain strain of staphylococcus.
- **Description:**
  - It contain  $\beta$ -lactam ring .
  - It includes penicillin derivatives.
  - It inhibit bacterial cell wall synthesis
- **Preparation:**
  - Obtained from *B.subtilis* & *B.cereus*





- It is divided into 2 classes
- 1) Penicillin amidase or acylase
- 2)  $\beta$ -lactamase
- Amidase attacks the acyl group attached to the nucleus, that's why it's also called acylase
- This enzyme is more specific with Pen-V & K
- $\beta$ -lactamase acts on the nucleus itself,
- This enzyme is more specific with Pen-G & X & less specific with Pen-V.



- **Identification Test:**

- Each strip emarginated with benzylpenicillin & pH indicator, bromocresol purple.
- Positive produce- penicilloic acid
- These cause fall in pH
- Purple to yellow
- **Use:**
- Inactivation of penicillin
- Antigen-antibody reaction.

THANK YOU

