LECTURE 10:

CLINICAL APPLICATIONS OF ENZYMES AND ISOENZYMES

- Enzymes and Medicine
- Isoenzymes
- Marker Enzymes of Tissue Damage used in diagnosis
- Therapeutic enzymes

Enzymes and Medicine

- **Diagnostic indicators –** the activities of many enzymes are routinely determined in plasma (rarely in tissue biopsies) for diagnostic purposes in diseases of the heart, liver, skeletal muscle, pancreas and other tissues - **enzyme diagnostics**

- **Diagnostic tools** – use as chemicals in clinical laboratory assays

- **Therapeutic agents** – several enzymes are used as drugs; new approach - **enzymotherapy**

Why Enzymes are important in Medicine

1. Key to understanding inborn errors of metabolism.
2. Important in detoxification reactions.
3. Targets of chemotherapy.
4. Essential to rationale drug design.
5. Aid in diagnosis and monitoring therapy.
6. Key to many therapeutic and treatment strategies.
7. Key to metabolic control and balance.

Isoenzymes

• Enzymes that have similar but not identical amino acid sequences but catalysing the same biochemical reaction

Results from gene duplication

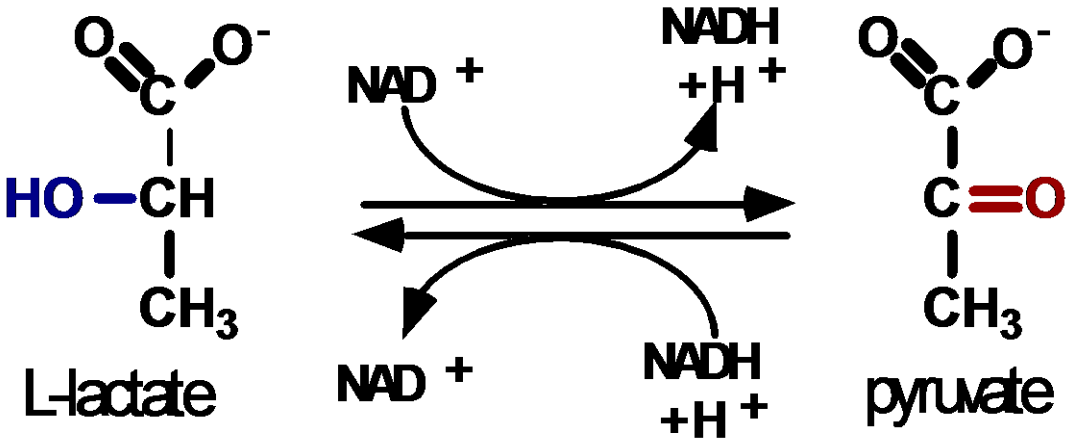
• They differ in kinetics - different K_M and V_{max} values

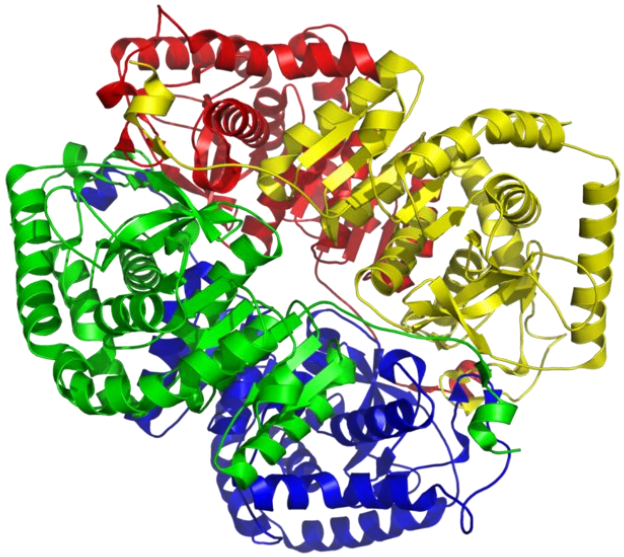
• Use different effectors and forms of coenzymes

• Cellular distribution of each form will vary

Examples: Hexokinase – Muscle
Glucokinase - Liver

Lactate dehydrogenase (LDH)



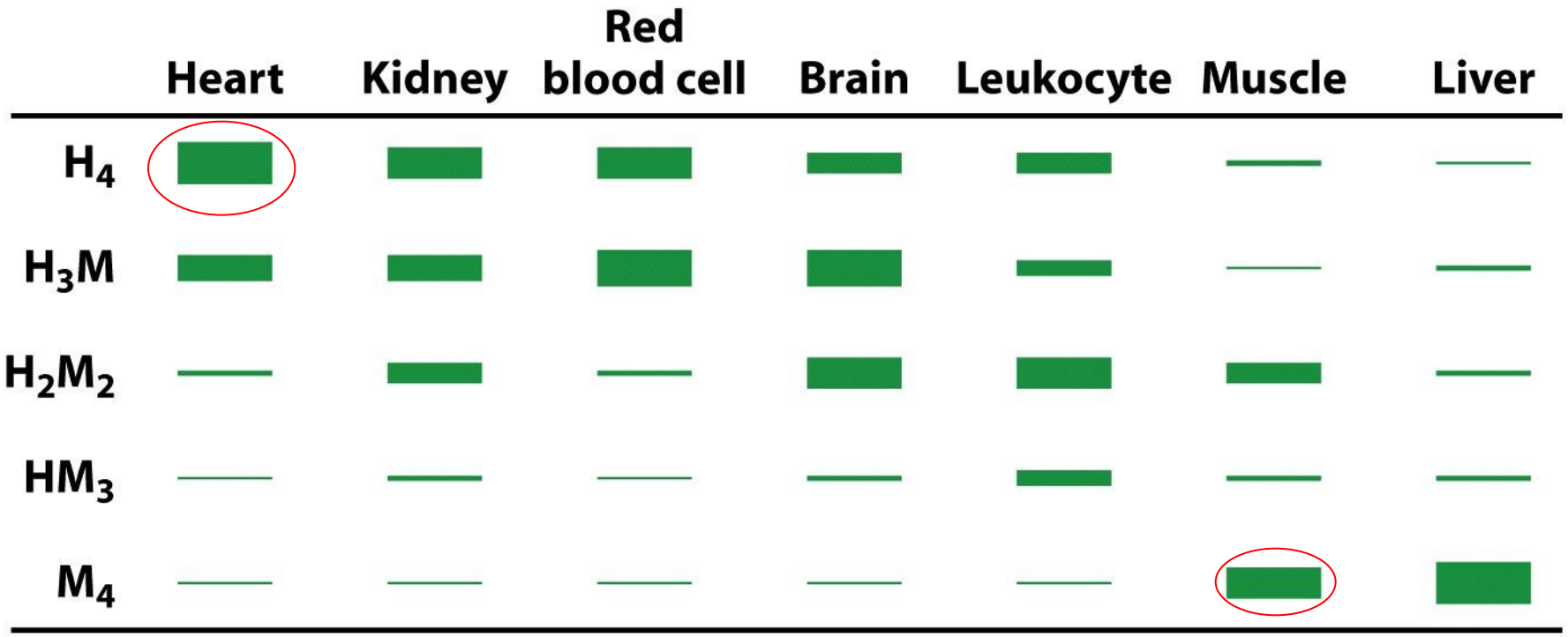


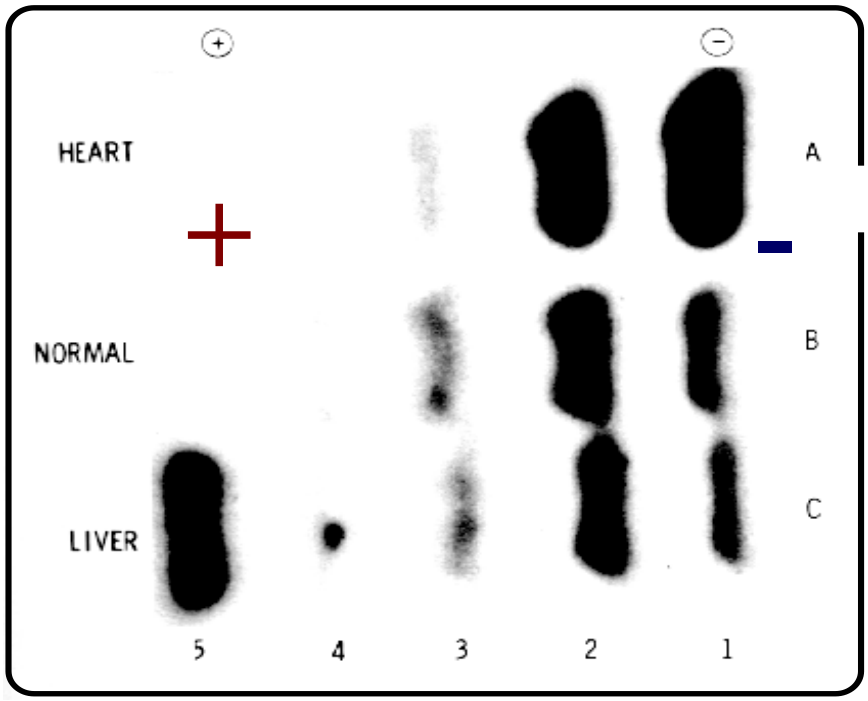
- LDH is a tetramer of two different types of subunits,called H and M, which have small differences in amino acid sequence.

- The two subunits can combine randomly with each other,forming 5 isoenzymes that have the compositions H_4, H_3M, H_2M_2, HM_3, M_4.

Isoenzymes of LDH

- LDH exists in 5 forms
  - LDH-1(H_4), LDH-2(H_3M):heart and red blood cells
  - LDH-3(H_2M_2):brain and kidney
  - LDH-4(HM_3), LDH-5(M_4): liver and skeletal muscle
- They can be resolved electrophoretically.





- Measurement of LDH isoenzymes helps determine the location of tissue damage.

- **"HEART": the serum of a patient with a myocardial infarction (heart attack)**
- **"NORMAL": normal serum**
- **"LIVER": the serum of a patient with liver disease.**

Identification of Isoenzymes

1. In Agar gel or polyacrylamide gel **electrophoresis**, the Isoenzymes have different mobility. LDH, CK and ALP Isoenzymes can be separated by electrophoresis.
2. **Heat stability**: one of the Isoenzymes may be easily denatured by heat, e.g. bone Isoenzyme of ALP (BALP).
3. **Inhibitors**: one of the Isoenzymes may be sensitive to one inhibitor, e.g. tartrate labile ACP.
4. K_M value or **substrate specificity** may be different for Isoenzymes, e.g. glucokinase has high Km and hexokinase has low Km for glucose.
5. **Cofactor** requirements may be different for isoenzymes. Mitochondrial isocitrate dehydrogenase is NAD+ dependent and the cytoplasmic Isoenzyme is NADP+ dependent.
6. Tissue **localization** may be different for Isoenzymes. H4 form of LDH is present in heart, while M4 variety is seen in skeletal muscle.
7. Specific **antibodies** may identify different types of isoenzymes. For example, CK Isoenzymes are separated by antibodies.

List of isoenzymes

1. Lactate dehydrogenase (LDH)
2. Creatine kinase (CK or CPK)
3. Cytochrome P450s
4. Phosphodiesterases

5. Add more examples to this list

Marker Enzymes of Tissue Damage used in diagnosis

Pancreatic enzymes:

α-Amylase

- Amylases normally occurring in human plasma are small molecules with molecular weights varying from 54 to 62 kDa. The enzyme is thus small enough to pass the glomeruli of the kidneys making it the only plasma enzyme physiologically found in urine

- Marked increase (five to 10 times the upper reference limit): is a pointer to acute pancreatitis or severe glomerular impairment

Lipase

Lipase is a small molecule and is filtered through the glomerulus. It is totally reabsorbed by the renal tubules, and it is not normally detected in urine

Plasma lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas

- **Trypsin**
- **Chymotrypsin**
- **Elastase**

Liver enzymes

1. Markers of hepatocellular damage

Aspartate aminotransferase (AST) is present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. Half- life = 17 hours.

Aminotransferases (ALT) is present in high concentrations in liver and to a
lesser extent, in skeletal muscle, kidney and heart. Half- life = 47 hours

NOTE: In liver damage, both enzymes are increased but ALT increases more. In myocardial infarction AST is increased with little or no increase in ALT

2. Markers of cholestasis

Alkaline phosphatase (ALP) elevated in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta

- Causes of increased ALP: Aging, rickets and osteomalacia, liver disease, malignancy

Gamma-glutamyl-transferase (GGT): catalyzes the transfer of the γ –glutamyl group from peptides and compounds that contain it to an acceptor

Causes of raised plasma GGT activity

• Induction of enzyme synthesis, without cell damage, by drugs or alcohol

• Hepatocellular damage, such as that due to infectious hepatitis

Other liver enzymes of clinical significance

Cholinesterase

Glutamate dehydrogenase

Muscle enzymes

Creatine Kinase (CK)

- Serum CK activity is greatly elevated in all types of muscular dystrophy
- CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal persons

Lactate Dehydrogenase (LDH)

Other clinically important enzymes

Acid Phosphatase (ACP)

Glucose -6-phosphate Dehydrogenase

What are the functions of ACP and G6PD above?

Clinically Important Enzymes

Enzyme	Principle Sources of Enzyme in blood	Clinical applications
Alanine aminotransferase	Liver	Hepatic parenchymal diseases
Alkaline phosphetase	Liver, bone, intestinal mucosa, placenta	Bone diseases, hepatobiliry diseases
Amylase	Salivary glands, pancreae	Pancreatic diseases
Aspartate aminotrasferase	Liver, skeletal muscle, heart erythrocytes	Hepatic parenchymal disease, muscle disease
Cholinesterase	Liver	Organophosphorus insecticide poisoning, hepatic parenchymal disease
Creatine kinase	Skeletal muscle, heart	Muscle diseases(M.I.)

Enzyme	Principle Sources of Enzyme in blood	Clinical applications
γ-glutamyl transferase	Liver	Hepatobiliary diseases, marker of alcohol abuse
Lactate dehydrogenase	Heart, liver, skeletal muscle, erythroctes, platelets, lymph nodes	Hemolysis, hepatic parenchymal diseases, tumor marker
lipase	Pancreas	Pancreatic diseases
5'-nucleotidase	Liver	Hepatbiliary diseases
Trypsin	pancreas	Pancreatic diseases

This list is not conclusive

Therapeutic enzymes have a broad variety of specific uses

- Oncolytics
- Anticoagulants
- Thrombolytics
- Replacements for metabolic deficiencies
  - Digestive aids
  - Metabolic storage disorders, etc
- Miscellaneous enzymes of diverse function

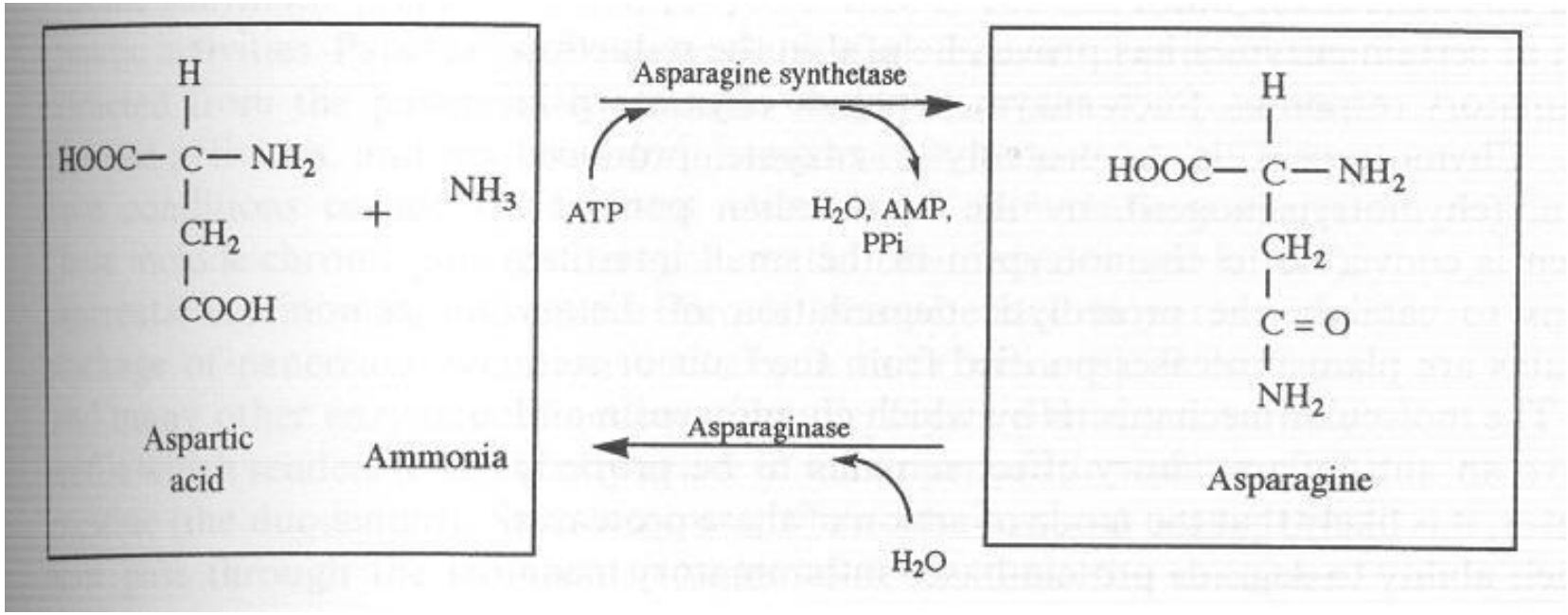
Table 6.5 Some enzymes that may be used for therapeutic purposes

Enzyme	Therapeutic application
Ancrod (serine protease)	Anticoagulant
Tissue plasminogen activator	Thrombolytic agent
Urokinase	Thrombolytic agent
(Activated) Factors IIV and IX	Treatment of clotting disorders
Asparaginase	Treatment of some types of cancer
DNase	Treatment of cystic fibrosis
Glucocerebrosidase	Treatment of Gaucher's disease
Trypsin Papain Collagenase	Debriding/anti-inflammatory agents
Lactase Pepsin Pancrelipase Papain	Digestive aids
Superoxide dismutase	Prevention of oxygen toxicity

Oncolytic enzymes

Asparaginase

- A tetrameric enzyme that catalyses the hydrolysis of the amino acid asparagine



Asparaginase…

- It may be purified from a wide variety of microorganisms (yeast, fungi, bacteria such as *E. coli*)
- Asn is required for normal metabolic activity
- Most human cells are capable of synthesizing Asn but certain malignant cells are not

➢ This can be used in the destruction of malignant cells....

Asparaginase…

- Source of clinically used asparaginase:
  - *E. coli*: two isozymes of which only one is effective
  - *Erwinia chrysanthemi*
- Treatment of childhood leukaemia
  - Side effects: severe allergic reaction, nausea, vomiting, fever, compromised kidney and liver function
  - Allergic reaction is greatly reduced by coupling the asparaginase with PEG
- Asparaginase production by a recombinant *Pichia pastoris* strain harbouring *S. cerevisiae* ASP3 gene
  (Ferrera et al, Enzyme and Microbial Technology 39(7) 2006)

Other oncolytic enzymes

- **Diphtheria toxin** (an oncolytic enzyme still in the experimental stage), catalyzes transfer of the adenosine diphosphate ribose (ADP-ribose) moiety of nicotinamide adenine dinucleotide (NAD) to elongation factor 2
- This enzyme halts protein synthesis
- The protein synthesis in tumor cells is 100 to 10,000 time more sensitive to this toxin than the analogous process in normal cells

- Enzymes that degrade macromolecules: neuraminidase, ribonuclease, and a diverse group of proteases
  - **Neuraminidase** removes sialic acid residues from the surface of (neoplastic) cells, thereby altering their immunogenicity, and rendering them sensitive to immune response
  - 2000 -- The FDA has approved the Orphan Drug application of Wobe-Mugos as an adjunct therapy for multiple myeloma. Wobe-Mugos (vitamins + proteolytic enzymes), used successfully in Europe in conjunction with chemotherapy since 1977

Debriding agents

- Debriding agents effectively clean open wounds by removal of foreign matter and any surrounding dead tissue
- Trypsin, papain and collagenase (all proteolytic enzymes) have often be used
  - **Trypsin**: from mammalian pancreas, hydrolyse peptide bonds involving arg and lys
  - **Papain**: from the leaves and the unripe fruit of the papaya tree, hydrolyse peptide bonds involving basic amino acids (e.g. lys, arg, his)
  - **Collagenase**: from culture extracts of various animal cells or normally from various *Clostridium* species (pathogenic)

Anti-inflammatory agents

- Administration of some enzymes is shown to be effective in the reduction of various inflammatory responses
  - **Chymotrypsin:** chymotrypsinogen (the zymogen form produced in pancreas) is converted to active form in small intestine
  - **Bromelains:** plant proteases purified from the stem or the fruit of pineapple
- Their anti-inflammatory action is not known in detail. Probably their ability to degrade protein-based inflammatory mediators play a role in their action

Enzymes as digestive aids-1

- Most digestive aid preparations are based on polymerases responsible for breakdown of polysaccharides, proteins and lipids
- Such preparations may include
  - a single enzyme or
  - multiple enzymes
- **α-amylase**: hydrolyse α1-4 glycosidic bonds
  - Amylase from *B. subtilis* or species of *Aspergillus* have various industrial applications
  - Oral amylase administration is used to aid digestion
- **Lactase:** hydrolysis of lactose
  - In many geographical regions, adults has greatly reduced lactase activity

Enzymes as digestive aids-2

- Various proteolytic enzymes, e.g. papain, pepsin
- **Pancreatin**: a preparation extracted from pancreas containing various enzymes
  - Used in deficiencies related with secretion of pancreatic enzymes (e.g. chronic pancreatitis, pancriatic carcinomas, cyctic fibrosis)
- One problem associated with oral administration is gastric inactivation
  - Co-administration of inhibitors of garstric acid secretion
  - Enteric coated tablet or capsules
  - Use of microbial proteases, amylases and lipases

Superoxide dismutase

- It is an important enzyme in all aerobic organisms

$$2O_2^- + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

- Two forms are found in eukaryotes: cytoplasmic (zinc and copper) and mitocondrial (manganese)

- Isolates from bovine liver and erytrocytes clinically used as anti-inflammatory agent (injection into patients with osteoarthritis of the knee)

Nuclease treatment of cistic fibrosis

- Cystic fibrosis (CF) is one of the most commonly occuring genetic diseases (1 in 2500 in northern Europe)
- Underlying cause is identified to the mulfunction of ion transport
- Major clinical symptom is the production of viscous mucus in the respiratory track
- Change in lung physiology $\Rightarrow$ bacterial infections $\Rightarrow$ immune response $\Rightarrow$ bacterial destruction $\Rightarrow$ liberation of DNA $\Rightarrow$ highly viscous mucus
- Therapy:
  - Percussion therapy is used to help the ejection of mucus
  - **Bovine DNAse** treatment was approved in USA in 1950s but prolonged usage caused adverse reactions
  - DNAse I produced by expression of cDNA in CHO cell lines (Pulmozyme) has been approved for medical use.

Enzyme-replacement therapy (ERT)

Brady and Schiffmann, The Lancet Neurology, 2004

- Metabolic storage disorders → insufficient activity of housekeeping enzymes
  - Gaucher's disease ($40 000–320 000/year)

    →**Glucocerebrosidase** absence (glycolipid accumulation in cells, espacially in macrophages)

    → Enzyme from human placentae

    → Recombinant enzyme in CHO cell line (Cerezyme, 1994)
  - Fabry's disease, in which the heart, kidney, gastrointestinal tract, and peripheral nerves are damaged ($160 000/year)
  - Pompe's disease, in which the heart, skeletal muscles, and brain are involved
  - Hurler's disease and Maroteaux-Lamy syndrome in which the eyes, liver, joints, and skeleton are usually affected

Nerve agent scavengers

Rochu et al., Toxicology, 2006

- The requirements:

  (a) a high reaction rate with organophosphate molecules

  (b) a long half-life *in vivo* to be effective over a prolonged time

  (c) immunotolerance

  (d) no adverse effects on physiological processes

- Stoichiometric scavengers or
- Catalytic scavengers → **paraoxanase**

Topical enzyme therapy for skin diseases

Klein et al, The Lancet, 357(9260), 2001

- Xeroderma pigmentosum → the frequency of all forms of skin cancer is higher (a genetic defect in DNA repair)
- Bacterial DNA repair enzyme, **T4 endonuclease V**, delivered intracellularly, increases the rate of repair of sunlight-induced DNA damage in human cells
- Topical administration of this enzyme in a liposomal delivery vehicle was tested

➢ Promising results with no adverse effect.

What are the practical challenges of using enzymes as drug molecules?

END TERM EXAM

TUESDAY 28TH MARCH 2023

TIME 8.00 - 10.00 AM

Venue: AUDITORIUM