Enzymes

Thousands of chemical reactions proceed very rapidly at any given instant within all living cells of an organism. Virtually all of these reactions are mediated by remarkable molecular devices called enzymes. That is, the enzymes are central to every biochemical reaction and are called the catalysts of biological systems (biocatalysts).

Enzymes catalyse an enormous diversity of biochemical reactions due to their capacity to specifically bind a very wide range of molecules. By utilizing the full repertoire of intermolecular forces, enzymes bring substrates together in an optimal orientation, the prelude to making and breaking chemical bonds.

Origin of Enzymes:

Enzymes are commonly proteinaceous substances which are capable of catalysing chemical reactions of biological origin without themselves undergoing any change. Therefore, they are called biocatalysts. Enzymes are synthesised by living cells.

The term 'enzyme' was coined by Kuhne (1878) for catalytically active substances previously called ferments. Enzymes were actually found out by Buchner (1897) with the accidental discovery that fermentation of sugar is not only caused by living yeast cells but also yeast extract.

The extract obviously possessed biocatalysts required for the process. Buchner (1903) also isolated the first enzyme. He was awarded Nobel Prize in the same year, 1903. There are numerous enzymes as every biochemical reaction is catalysed by a separate enzyme. It is estimated that a cell contains over 5000 chemicals. The number of chemical reactions is many times more**.**

Meaning of Enzyme:

An enzyme is a protein that is synthesised in a living cell and catalyses or speeds up a thermodynamically possible reaction so that the rate of the reaction is compatible with the biochemical process essential for the maintenance of the cell. It is sometimes called as organic catalyst or biocatalyst.

Over 90% of enzymes are simple globular proteins , The remainder is conjugated proteins, which have a non-protein fraction called the prosthetic group. Many enzymes have relative molecular mass of between 10,000 and 50,000da.

Catalysts and Enzymes: Catalysts are inorganic substances which increase the rate of chemical reactions without themselves undergoing any change and without modifying the equilibrium of the reactions. Enzymes are similar chemicals which are biological in origin and operate in the biochemical world.

Types of Enzymes:

Enzymes are of three types:

i. Pro-Enzyme or Zymogen,

ii. Allosteric Enzymes,

iii. Isoenzymes (Isozymes)

i. Pro-Enzyme or Zymogen:

Pro-enzyme is the inactive precursor of an enzyme. The term zymogen is often used for inactive precursor of proteolysis enzyme, e.g., pepsinogen for enzyme pepsin. Many enzymes are initially produced in the pro-enzyme or zymogen state.

They become reactive or active enzymes only at a particular pH, in the presence of substrate or some special treatment. For example, pepsinogen is changed to active enzyme pepsin in the presence of hydrochloric acid of gastric juice. Thereafter, pepsin has autocatalytic effect on further conversion of pepsinogen.

ii. Allosteric Enzymes:

They are enzymes which have separate areas for different types of modulators that alter the conformation of the active site so as to make it effective or ineffective (Fig. 9.36). The areas are called allosteric sites. The substances which cause change in allosteric sites are known as modulators, allosteric substances or effectors.

The latter are of two types— activators and inhibitors. Allosteric activator binds with an allosteric site in such a way as to make active site operational. Allosteric inhibitor, on the other hand, brings about such a change in the active site that it becomes unable to combine with substrate molecules. For example, the enzyme phosphofructokinase is activated by ADP and inhibited by ATP.

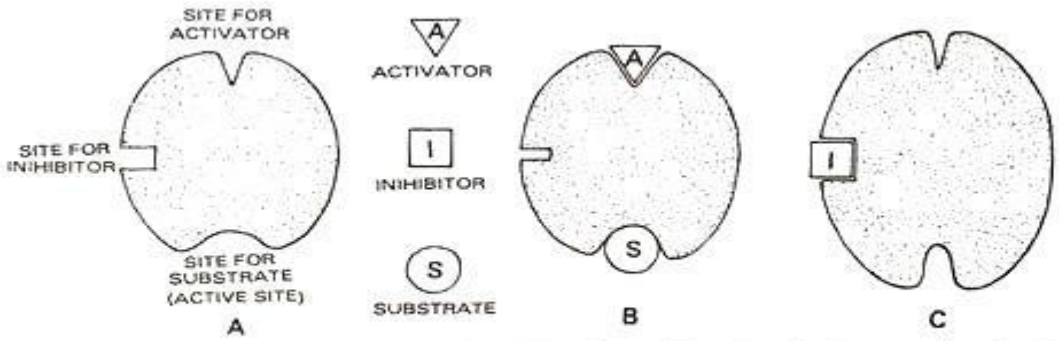


Fig. 9.36. Allosteric enzyme. A, an enzyme with various factors. B, active site becomes functional in the presence of activator. C, inhibitor distorts the enzyme molecule in such a way that the substrate cannot bind to the active site.

Isoenzymes (Isozymes):

At one time it was believed that an organism has only a single enzyme for a given step of a metabolic reaction. It was later discovered that a substrate may be acted upon by a number of variants of an enzyme producing the same product.

The multiple molecular forms of an enzyme occurring in the same organism and having a similar substrate activity are called isoenzymes or isozymes. Over 100 enzymes are known to have isoenzymes.

Thus a- amylase of wheat endosperm has 16 isozymes, lactic dehydrogenase has 5 isoenzymes in man, while alcohol dehydrogenase has 4 isozymes in maize. Isoenzymes differ in activity optima and inhibition. They are thus useful to organism in adapting to varied environmental conditions.

Nomenclature of Enzymes:

All enzyme names should end in suffixase. Exceptions are some old names, e.g., ptyalin, pepsin, trypsin. Some old names indicate the source but not the action, e.g., papain from Papaya, bromelain from Pineapple of family Bromeliaceous.

Classification of Enzymes:

In older times enzymes were classified into two broad categories:

(i) Hydrolysing:

Catalysing hydrolysis of larger molecules into smaller ones, e.g., carbohydrates or amylases, proteases, lipases, esterases, phosphorylases, amidases. Digestive enzymes are hydrolysing in nature. They are often grouped into three types— proteolytic, amylolytic and lipolytic,

(ii) Desmolysing:

Catalysing reactions other than hyrolysis, e.g., aldolases, dehydrogenases, oxidases, peroxidases, catalases, carboxylases, etc. The modem system of enzyme classification was introduced by International Union of Biochemistry (IUB) in 1961. It groups enzymes into the following six categories.

a. Oxidoreductases:

They take part in oxidation and reduction reactions or transfer of electrons.

$$\text{S reduced} + \text{S}' \text{ oxidised} \xrightarrow{\text{oxido reductase}} \text{S oxidised} + \text{S}' \text{ reduced}$$

Oxidoreductases are of three types— oxidases, dehydrogenases and reductases, e.g., cytochrome oxidase (oxidises cytochrome), succinate dehydrogenase, nitrate reductase.

$$2AH_2 + O_2 \xrightarrow{\text{oxidase}} 2A + 2H_2O$$

$$AH_2 \xrightarrow{\text{dehydrogenase}} A + 2\,(H)$$

$$A + 2(H) \xrightarrow{\text{reductase}} AH_2$$

b. Transferases:

They transfer a group from one molecule to another e.g., glutamate- pyruvate transaminase (transfers amino group from glutamate to pyruvate during synthesis of alanine). The chemical group transfer does not occur in the Free State.

$$S - G + S' \xrightarrow{\text{transferase}} S + S' - G$$

$$\underset{\text{(amino acid)}}{\text{Glutamic Acid}} + \underset{\text{(organic acid)}}{\text{Oxaloacetic Acid}} \xrightarrow{\text{transferase}} \underset{\text{(organic acid)}}{\alpha\text{-ketoglutaric Acid}} + \underset{\text{(amino acid}}{\text{Aspartic A}}$$

c. Hydrolases:

They catalyse hydrolysis of bonds like ester, ether, peptide, glycosidic, C-C, C halide, P—N, etc. which are formed by dehydration condensation. Hydrolases break up large molecules into smaller ones with the help of hydrogen and hydroxyl groups of water molecules. The phenomenon is called hydrolysis. Digestive enzymes belong to this group, e.g., amylase (hydrolysis of starch), sucrase, lactase.

$$\underset{\text{maltose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{maltase}} \underset{\text{glucose}}{2\,C_6H_{12}O_6}$$

d. Lyases:

The enzymes cause cleavage, removal of groups without hydrolysis, addition of groups to double bonds or removal of a group producing double bond, e.g., histidine decarboxylase (breaks histidine to histamine and CO_2), aldolase (fructose-1, 6-diphosphate to dihydroxy acetone phosphate and glyceraldehyde phosphate).

$$\begin{array}{c} X \quad Y \\ | \quad \| \\ C - C \end{array} \xrightarrow{\text{lyase}} X - Y + C = C$$

Fructose 1, 6-diphosphate – aldolase → Dihydroxy acetone phosphate + Glyceraldehyde phosphate.

e. Isomerases: The enzymes cause rearrangement of molecular structure to effect isomeric changes. They are of three types, isomerases (aldose to ketose group or vice-versa like glucose 6-phosphate to fructose 6-phosphate), epimerases (change in position of one constituent or carbon group like xylulose phosphate to ribulose phosphate) and mutases (shifting the position of side group like glucose-6-phosphate to glucose-1- phosphate).

$$\text{Glucose 6-phosphate} \xrightarrow{\text{isomerase}} \text{Fructose 6-phosphate}$$

$$\text{Glucose 6-phosphate} \xrightarrow{\text{mutase}} \text{Glucose 1-phosphate}$$

f. Ligases (Synthetizes):

The enzymes catalyse bonding of two chemicals with the help of energy obtained from ATP resulting in formation of such bonds as C-O, C-S, C-N and P-O, e.g., pyruvate carboxylase. It combines pyruvic acid with CO_2 to produce oxaloacetic acid.

Properties of Enzyme:

Some of the properties of enzyme are as follows:

i. Protein Nature:

Enzymes are generally globular proteins. They may have additional inorganic or organic substances for their activity. However, two types of RNA enzymes are known, ribozyme and ribonuclease-P. Peptidyl transferase has also been found to be part of rRNA by Noller (1992).

ii. Molecular Weight:

Being proteinaceous, the enzymes are giant molecules with a molecular weight of 6000 (bacterial ferredoxin) to 4,600,000 (pyruvate dehydrogenase complex).

iii. Colloidal Nature:

They are hydrophilic and form hydrosol in the Free State.

iv. Chemical Reaction:

Enzymes do not start a chemical reaction but increase the rate of chemical reaction. They do not change the equilibrium but bring about equilibrium very soon.

v. Efficiency:

The number of substrate molecules changed per minute by a molecule or enzyme is called turn over number (kcat). The higher the turn-over number, the more efficient an enzyme is. It depends upon the number of active points present over an enzyme, precise collisions between reactants and the rate of removal of end products.

The optimum turn-over number for enzyme carbonic anhydrase (enzyme present in RBCs) is 36 million, catalase 5 million, enzyme sucrase or invertase 10,000 and flavoprotein 50. Enzyme efficiency is usually much more than that of inorganic catalysts.

Vi.Reversibility:

Theoretically, all enzyme controlled reactions are reversible. Reversibility is, however, dependent upon energy requirements, availability of reactants, concentration of end products and pH.

vii. Enzyme Specificity:

Enzymes are highly specific in their action. For example, enzyme maltase acts on sugar maltose but not on lactose or sucrose. Different enzymes may act on the same substrate but give rise to different products.

For example, raffinose gives rise to rnelibiose and fructose in the presence of enzyme sucrase while in the presence of enzyme melibiase it produces lactose and sucrose. Similarly an enzyme

may act on different substrates, e.g., sucrase can act on both sucrose and raffinose producing different end products.

Viii. Heat Sensitivity:

All enzymes are heat sensitive or thermolabile. Most enzymes operate optimally between 25°-35°C. They become inactive at freezing temperatures and denatured at 50°-55°C. However, thermal algae and bacteria are an exception. Their enzymes remain functional even at 80°C. Enzymes of seeds and spores are also not denatured at 60°— 70°C.

ix. Protein Poisons:

Being made of proteins, enzymes are inactivated or denatured by all those substances and forces which destroy protein structure, e.g., heavy metals, high energy radiations.

x. pH:

Each enzyme functions at a particular pH e.g., pepsin (2 pH), sucrase (4.5 pH), salivary amylase (6.8 pH), trypsin (8.5 pH). A change in pH makes the enzymes ineffective.

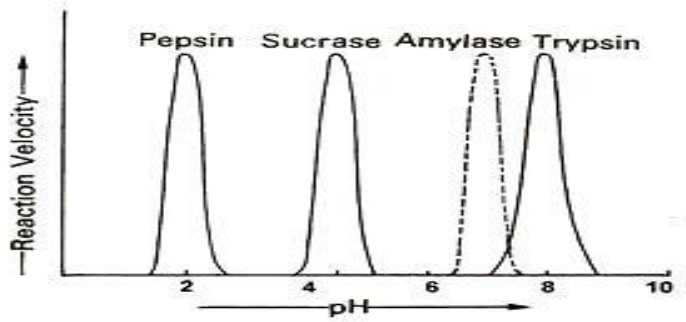


Fig. 9.28. Relation between *p*H and activity of four enzymes.

Specificity of pH for enzyme activity is useful in regulating enzymes, e.g., salivary amylase stops its activity in stomach where hydrochloric acid is secreted. The same acid activates another enzyme pepsin from its precursor called pepsinogen. Pepsinogen can also be changed into pepsin by catalytic activity of the latter.

i. Lock and Key Hypothesis:

It was put forward by Emil Fischer in 1894. According to this hypothesis, both enzyme and substrate molecules have specific geometrical shapes. 'In the region of active sites the surface configuration of the enzyme is such as to allow the particular substrate molecules to be held over it. The active sites also contain special groups having —NH2, —COOH, —SH for establishing contact with the substrate molecules.

The contact is such that the substrate molecules or reactants come together causing the chemical change. It is similar to the system or lock and key. Just as a lock can be opened by its specific key, a substrate molecule can be acted upon by a particular enzyme. This also explains the specificity of enzyme action.

After coming in contact with the active site of the enzyme, the substrate molecules or reactants form a complex called enzyme-substrate complex. In the complexed state the molecules of the substrate undergo chemical change.

The products remain attached to the enzyme for some time so that an enzyme-product complex is also formed. However, the products are soon released (Fig. 9.34) and the freed enzyme is able to bind more substrate molecules.

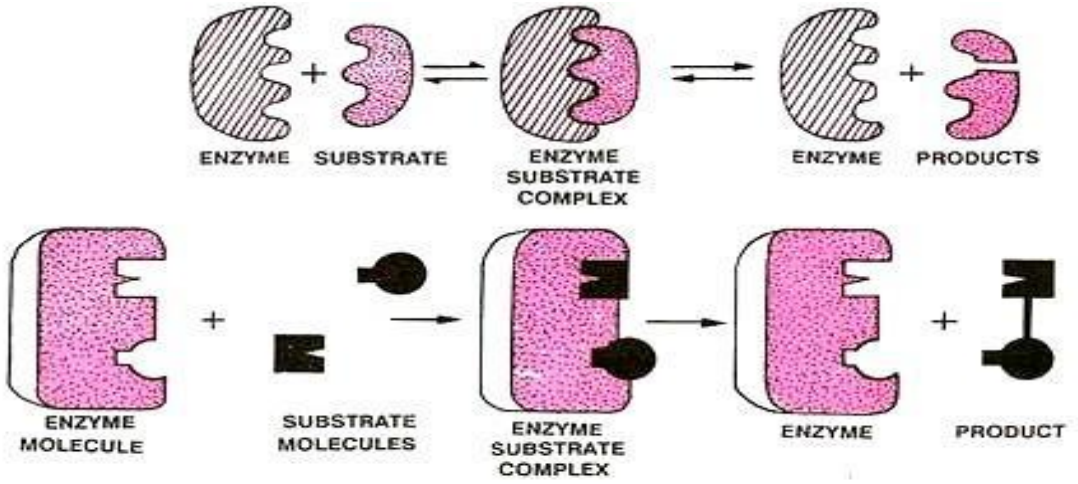


Fig. 9.33. Lock and key theory of enzyme action.
Upper Series – Breakdown Reaction Lower Series – Biosynthetic Reaction

Enzyme + Substrate ⇋ Enzyme – Substrate Complex

Enzyme – Substrate Complex ⇋ Enzyme – Products Complex

Enzyme – Products Complex ⇋ Enzyme + Products

Thus we see that the chemical reactants do not cause any alteration in the composition or physiology of the enzyme. The same enzyme molecule can be used again and again (Fig. 9.35). Hence, enzymes are required in very small concentrations.

Importance of Enzyme:

Biological Importance of Enzymes:

(i) Thousands of chemical reactions are taking place in the body of a living organism. All of them are mediated by enzymes,

(ii) Enzymes are specialised catalysts that operate at biological temperatures,

(iii) Enzyme mediated reactions do not require harsh treatment,

(iv) They are pH specific so that reactions requiring different pH operate in different parts of the body,

(v) As they operate under favourable conditions, enzymes force the organisms to live under favourable environment,

(vi) Enzymes are highly regulated. Their formation is controlled by separate genes. Activation and repression of genes allow certain enzymes to be functional or non-functional in cells.

Homeostasis

- **Homeostasis** is the tendency to resist change in order to maintain a stable, relatively constant internal environment.
- Homeostasis typically involves **negative feedback loops** that counteract changes of various properties from their target values, known as **set points**.
- In contrast to negative feedback loops, **positive feedback loops** amplify their initiating stimuli, in other words, they move the system *away* from its starting state.
- Osmoregulation : All animals balance the osmolarity by the gain and loss of water and dissolved solutes.
- Osmolarity: osmotic pressure Two factors needed for osmolarity difference
- Osmolarity: osmotic pressure osmoles per liter (osm/L) milliosmoles per liter (mosm/L) 1 mosm/L = total solute concentration of 10−3 M.
- Two basic solutions to solve the water balance against environments z Osmoconformer
- Internal Two basic solutions to solve the water balance against environments
- osmolarity in bodies is the same as that of its environment •No tendency to gain or lose water eg. most marine invertebrates.

Maintaining homeostasis

Biological systems like those of your body are constantly being pushed away from their balance points.

For instance, when you exercise, your muscles increase heat production, nudging your body temperature upward. Similarly, when you drink a glass of fruit juice, your blood glucose goes up. Homeostasis depends on the ability of your body to detect and oppose these changes.

Maintenance of homeostasis usually involves **negative feedback loops**. These loops act to oppose the **stimulus**, or cue, that triggers them. For example, if your body temperature is too high, a negative feedback loop will act to bring it back down towards the **set point**, or target value, of 98.6\,^\circ\text F98.6∘F98, point, 6, space, degree, F/ 37.0\,^\circ\text C37.0∘C37, point, 0, space, degree, C.

How does this work? First, high temperature will be detected by **sensors**—primarily nerve cells with endings in your skin and brain—and relayed to a temperature-regulatory **control center** in your brain. The control center will process the information and activate **effectors**—such as the sweat glands—whose job is to oppose the stimulus by bringing body temperature down.

Of course, body temperature doesn't just swing above its target value—it can also drop below this value. In general, homeostatic circuits usually involve at least two negative feedback loops:

- One is activated when a parameter—like body temperature—is *above* the set point and is designed to bring it back down.
- One is activated when the parameter is *below* the set point and is designed to bring it back up.

Salt regulation in freshwater and seawater fishes

Osmosis – passive regulation

Cells of living organisms contain a lot of water and different solutes (ions, proteins, polysaccharides), creating a specific concentration inside the cell membrane. This membrane is semi-permeable, meaning that it only allows the solvent (water) to move across, but not the solutes. When cells are submerged into a solution of a different concentration, the law of **osmosis** comes into play. **Osmotic pressure** is a tendency of water to move into one solution from another by osmosis – passing a membrane from a high water concentration to a low water concentration. The higher the osmotic pressure (difference between the solutions on either side of a membrane), the more water tends to move across in order to balance the concentration.

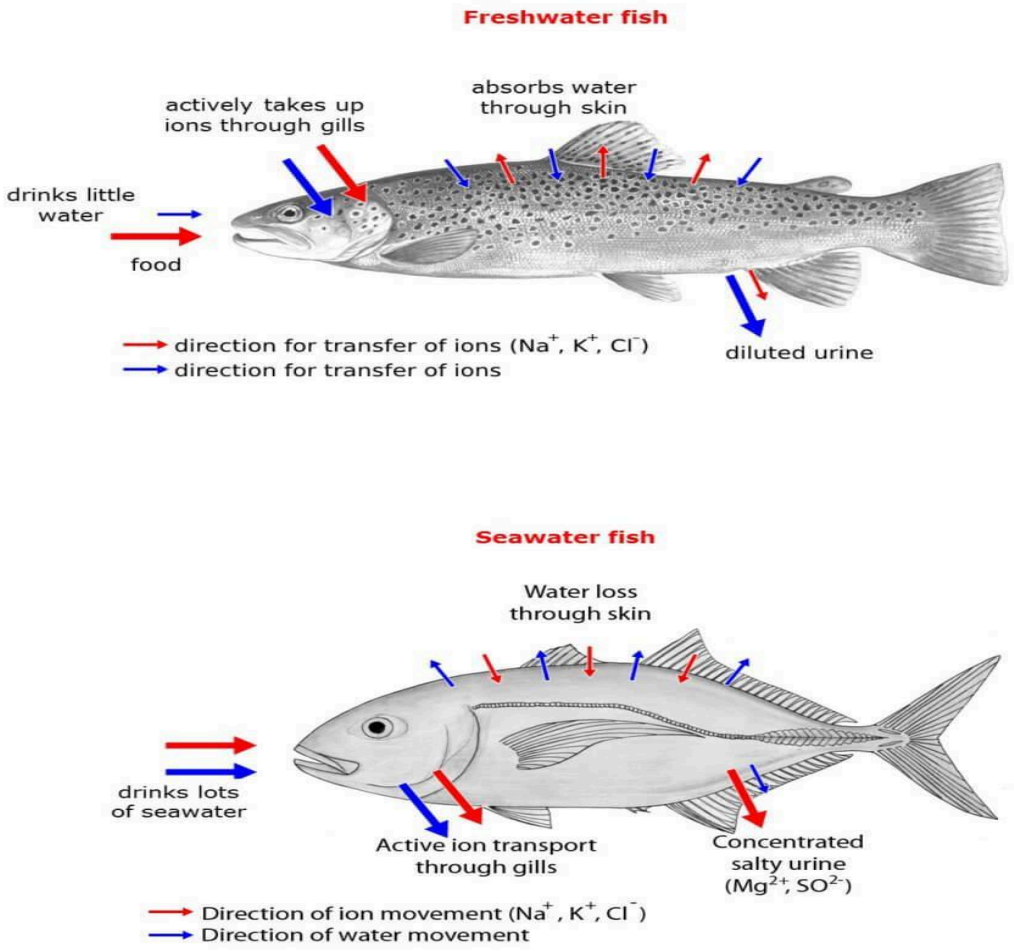
Osmoregulation – active regulation

Keeping the homeostasis in balance is a big challenge for freshwater and marine fishes, because metabolic processes can only take place in very specific physical and chemical environment. In order to keep the "internal environment" constant, continuous adaptations with regard to temperature, pH and the concentrations of Na^+, K^+, Ca^{2+}, glucose, CO_2 and O_2, take place. The key to their problem is **osmoregulation** – active regulation of the osmotic pressure to maintain the fluid balance and concentration of salts [1].

Let first take a look at freshwater fishes. Because the salt concentration inside their body is higher as in the surrounding water, water enters the body due to osmosis. Without any active regulation of this process, fishes would swell and get bigger and bigger. To compensate, the kidney produces a large amount of urine, which at the same time means loss of salts. In order to maintain a sufficient salt level, special cells in the gills (chloride cells) take up ions from the water, which are then directly transported into the blood (see Figure 1) [2, 3, 4].

In contrast, marine fishes face the opposite challenge – since the salt content in their blood is much lower than that of seawater, they constantly tend to lose water and build up salt. To replace the water loss, they continually need to drink seawater. Since their small kidney can

only excrete relatively small amount of urine, the excretion of salt additionally takes place in the gills where chloride cells work in reverse as in freshwater fishes (see Figure 2) [2].



What about fishes living in both, fresh- and seawater?

If you like to watch documentary films, you have probably come across one that presents a life cycle of salmons. A young salmon start its life in a freshwater river where it has to prepare for a life in a salty ocean. Three important changes must occur before this happens. Firstly, it has to start drinking lots of water. Secondly, the kidneys must drop their urine production dramatically. And thirdly, the chloride cells in the gills (also called molecular pumps) must shift into reverse, meaning pumping sodium out instead of in [5]. At some point, adult salmons return to their place of birth to spawn (in the documentary this is a moment of feast for grizzly bears). By re-entering the freshwater river, the above-mentioned processes have to change back. They stay a few days in the estuarial zone as these changes happen automatically.

There are also species living in estuaries – environments, where freshwater meets the sea and salt concentration changes gradually. Some species of sharks can swim very far upwards the freshwater stream. Their osmoregulating process is quite different that the one of salmons. They are able to convert ammonia to urea and retain it in the blood to such an extent that the blood is slightly more concentrated than seawater. In this way, the loss of water via osmosis is prevented and animals do not need to drink seawater and excessive salt is excreted through

the rectal gland [6]. These switches are controlled in the brain and regulated by hormones. The cortisol and thyroid hormone are the main regulators of osmotic process – influencing the rate and direction of ions pumped through the chloride cells

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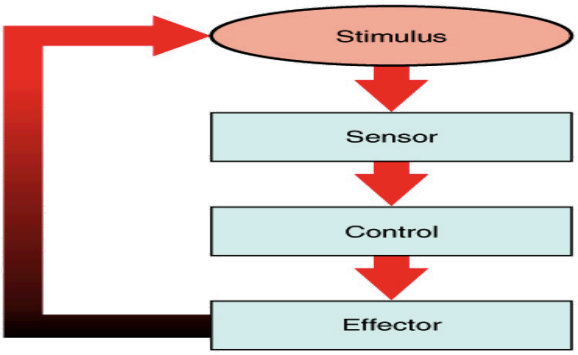
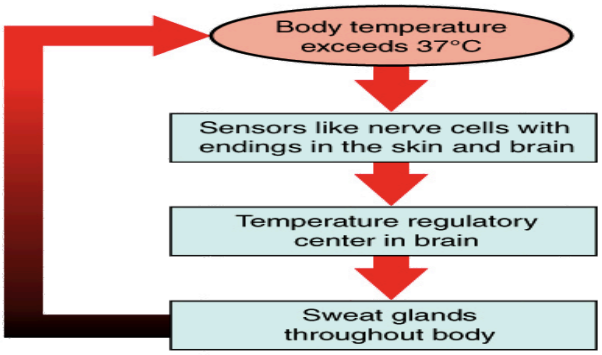


Negative feedback loop



Body temperature regulation

Disruptions to feedback disrupt homeostasis.

Homeostasis depends on negative feedback loops. So, anything that interferes with the feedback mechanisms can—and usually will!—disrupt homeostasis. In the case of the human body, this may lead to disease.

Diabetes, for example, is a disease caused by a broken feedback loop involving the hormone insulin. The broken feedback loop makes it difficult or impossible for the body to bring high blood sugar down to a healthy level.

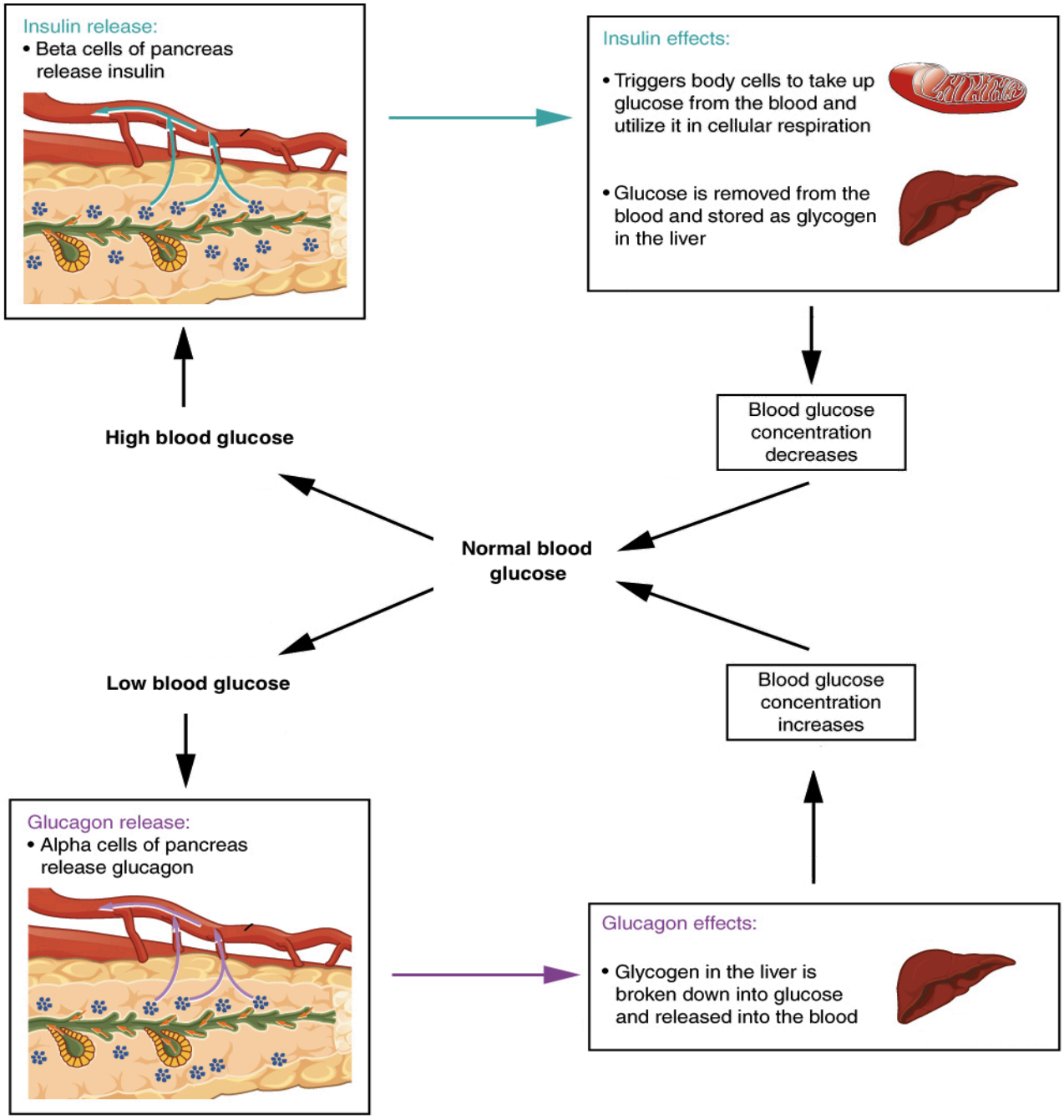
To appreciate how diabetes occurs, let's take a quick look at the basics of blood sugar regulation. In a healthy person, blood sugar levels are controlled by two hormones: insulin and glucagon.

Insulin decreases the concentration of glucose in the blood. After you eat a meal, your blood glucose levels rise, triggering the secretion of insulin from β cells in the pancreas. Insulin acts as a signal that triggers cells of the body, such as fat and muscle cells, to take up glucose for use as fuel. Insulin also causes glucose to be converted into glycogen—a storage molecule—in the liver. Both processes pull sugar out of the blood, bringing blood sugar levels down, reducing insulin secretion, and returning the whole system to homeostasis.

If blood glucose concentration rises above the normal range, insulin is released, which stimulates body cells to remove glucose from the blood. If blood glucose concentration drops below this range, glucagon is released, which stimulates body cells to release glucose into the blood.

Glucagon does the opposite: it increases the concentration of glucose in the blood. If you haven't eaten for a while, your blood glucose levels fall, triggering the release of glucagon from another group of pancreatic cells, the α cells. Glucagon acts on the liver, causing glycogen to be broken down into glucose and released into the bloodstream, causing blood sugar levels to go back up. This reduces glucagon secretion and brings the system back to homeostasis.

Diabetes happens when a person's pancreas can't make enough insulin, or when cells in the body stop responding to insulin, or both. Under these conditions, body cells don't take up glucose readily, so blood sugar levels remain high for a long period of time after a meal. This is for two reasons:



- Muscle and fat cells don't get enough glucose, or fuel. This can make people feel tired and even cause muscle and fat tissues to waste away.
- High blood sugar causes symptoms like increased urination, thirst, and even dehydration. Over time, it can lead to more serious complications.^{4,5}start superscript, 4, comma, 5, end superscript

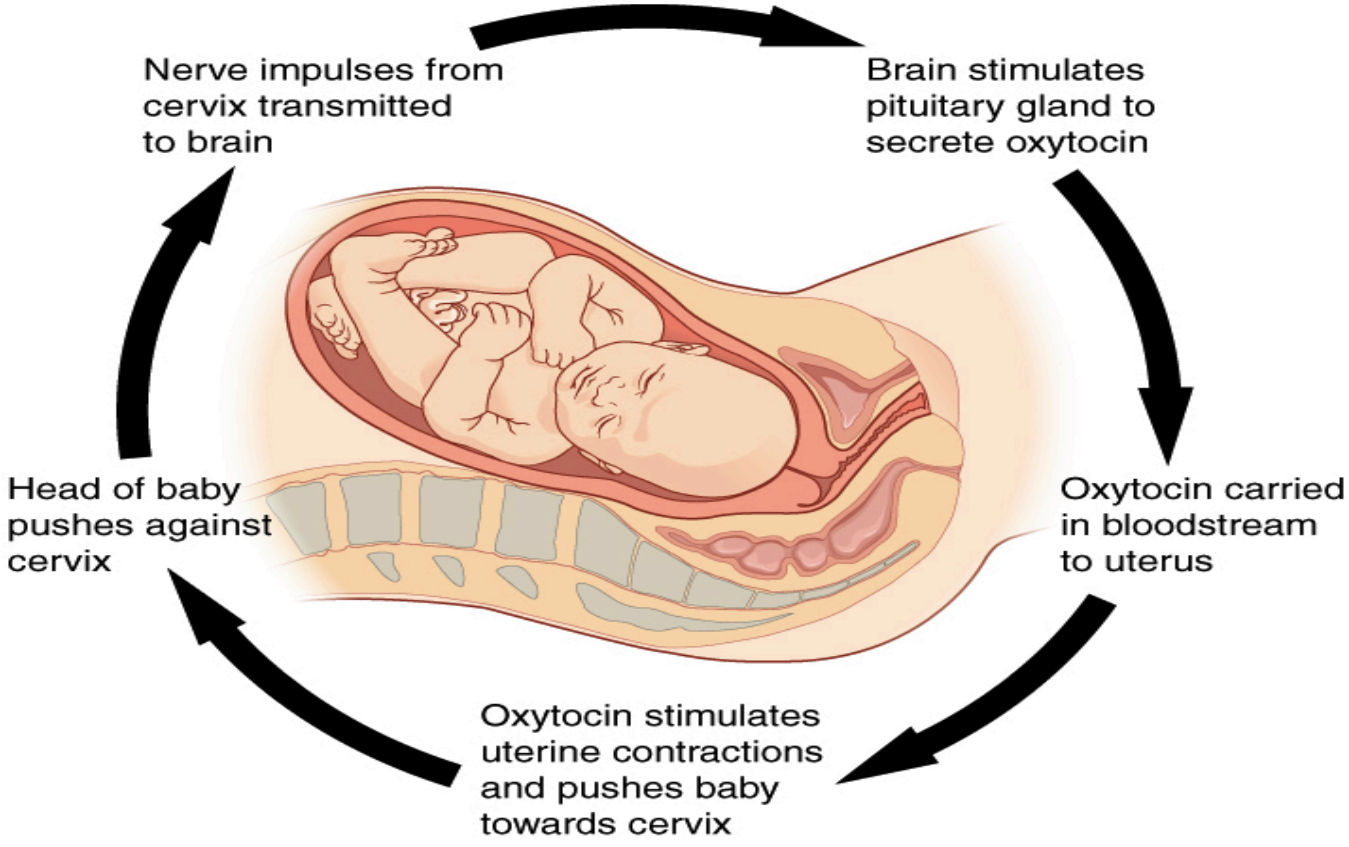
Positive feedback loops

Homeostatic circuits usually involve negative feedback loops. The hallmark of a negative feedback loop is that it counteracts a change, bringing the value of a parameter—such as temperature or blood sugar—back towards it set point.

Some biological systems, however, use positive feedback loops. Unlike negative feedback loops, **positive feedback loops** amplify the starting signal. Positive feedback loops are usually found in processes that need to be pushed to completion, not when the status quo needs to be maintained.

A positive feedback loop comes into play during childbirth. In childbirth, the baby's head presses on the cervix—the bottom of the uterus, through which the baby must emerge—and activates neurons to the brain. The neurons send a signal that leads to release of the hormone oxytocin from the pituitary gland.

Oxytocin increases uterine contractions, and thus pressure on the cervix. This causes the release of even more oxytocin and produces even stronger contractions. This positive feedback loop continues until the baby is born.



TROPISMS

Movement is usually considered as symbol of life. Movent is the charcteristic feature of both plants and animals. Large plants are fixed in position, but many of their parts of organs carry on several movements.

The parts of an organism shows the movements by responding to stimulus towards the directions of stimulus is called "tropism" The term "tropism" is restricated to movements in the plants. The concept of tropism was introduced by a botanist called "Augustin De condole".

Types of tropism:

The types of tropism are as follows

1) Thigmotropism: The movements in response to touch is called "thigmotropism".

2) Phototropism: If the movements of plants are in response to light is called "Phototropism" the stem grows towards the source of light.

3) Geo tropism: The movements occur in relation or response to the stimulus of earth's gravity is called "geotropism" Roots grow directly towards the centre of gravity". This is called as "Positive geotropism" stems grow away from the earth's gravity. This is called as "negative geotropism".

4) Thermo tropism: Some of the plant organs makedly respond to temperature. This is called as "thermo tropism".

5) Chemotropism: Some of the plant organs show the movements to some chemicals. This is called "Chemotropism".

6) Hydro tropism: If the parts of plants respond to water stimulus is called "hydrotropism".