BIOLOGY

FOR TTCs

STUDENT'S BOOK

OPTION: Sciences and Mathematics Education (**SME**)

FOREWORD

Dear Student- teacher,

Rwanda Basic Education Board is honoured to present to you this Biology Textbook for Year Three of Science and Mathematics Education (**SME**) Option which serves as a guide to competence-based teaching and learning to ensure consistency and coherence in the learning of Biology subject. The Rwandan educational philosophy is to ensure that you achieve full potential at every level of education which will prepare you to be well integrated in society and exploit employment opportunities.

The government of Rwanda emphasizes the importance of aligning teaching and learning materials with the syllabus to facilitate your learning process. Many factors influence what you learn, how well you learn and the competences you acquire. Those factors include the instructional materials available among others. Special attention was paid to the activities that facilitate the learning process in which you can develop your ideas and make new discoveries during concrete activities carried out individually or with peers.

In competence-based curriculum, learning is considered as a process of active building and developing knowledge and meanings by the learner where concepts are mainly introduced by an activity, a situation or a scenario that helps the learner to construct knowledge, develop skills and acquire positive attitudes and values. For effective use of this textbook, your role is to:

- Work on given activities including laboratory experiments which lead to the development of skills;
- Share relevant information with other learners through presentations, discussions, group work and other active learning techniques such as role play, case studies, investigation and research in the library, from the internet or from your community;
- Participate and take responsibility for your own learning;
- Draw conclusions based on the findings from the learning activities.

I wish to sincerely extend my appreciation to the people who contributed towards the development of this book, particularly REB staff who organized the whole process from its inception. Special gratitude goes to teachers, illustrators and designers who diligently worked to successful completion of this book.

Dr. MBARUSHIMANA Nelson

Director General of Rwanda Basic Education Board

ACKNOWLEDGEMENT

I wish to express my appreciation to all the people who played a major role in development of this Biology book for Year Three of TTC, Science and Mathematics Education (**SME**) Option. It would not have been successful without active participation of different education stakeholders.

I owe gratitude to different Universities and schools in Rwanda that allowed their staff to work with REB in the in-house textbooks production project. I wish to extend my sincere gratitude to lecturers, teachers, tutors, illustrators, designers and all other individuals whose efforts in one way or the other contributed to the success of writing of this textbook.

Finally, my word of gratitude goes to the Rwanda Basic Education Board staff particularly those from the Curriculum, Teaching and Learning Resources Department who were involved in the whole process of in-house textbook writing.

Joan MURUNGI,

Head of Curriculum, Teaching and Learning Resources Department

TABLE OF CONTENTS

UNIT 1

POPULATION AND NATURAL RESOURCES

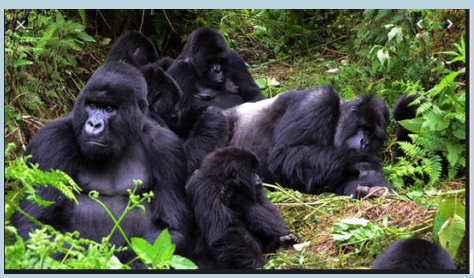
Key unit competence

Describe the factors affecting population size and the importance of natural resources

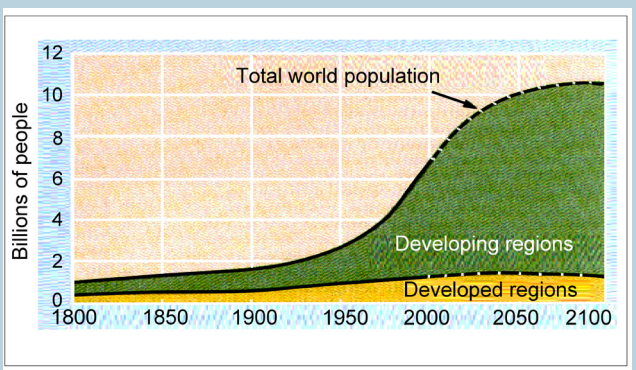
Introductory activity 1

Living organisms in their natural habitat are different in number where by some are still represented by a significant number (figure A) While others can disappear when they are not protected (Figure B). The change in number of organisms does not happen abruptly without any reasons behind. Refer to the figures and do activity below :

(A)



(B)



(C)

a) Referring to **figure B** above, identify the reasons that were behind their decrease?

b) Referring to **figure A,** why does others species still represented by a significant number?

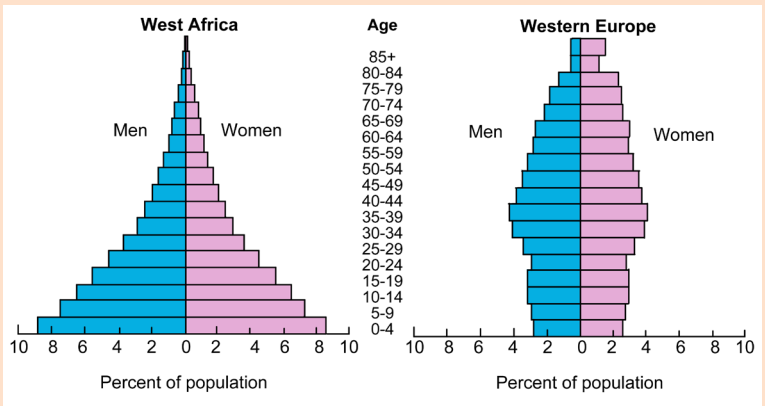
c) Observe the graph **C** and identify what it indicates in terms of population growth, especially in developing countries. What do you think would be the effect on the nature and what measures would be taken to maintain the nature?

1.1 Population characteristics

Activity 1.1

The human population size in some areas increases yet their habitat does not increase. **The pyramid of age structure** in that area shows there are more young people than adults.

The **growth pattern below** shows that there is an increase in population of these areas which is a result of high **birth rate** compared to **death rate**.



(A) (B)

d) Among bolded terms (**human population size, pyramid of age structure, growth pattern, birth rate and death rate),** one of them is better applied to the above figures. Find out the corresponding term based on the parameters presented on both figure A and B.

e) Based on the shapes of figure A and B. Find out the figure that corresponds to the description done in above text. Explain how you have arrived to your choice.

f) Using the school library and additional information from the internet, Explain bolded terms found in the text at the start of this activity.

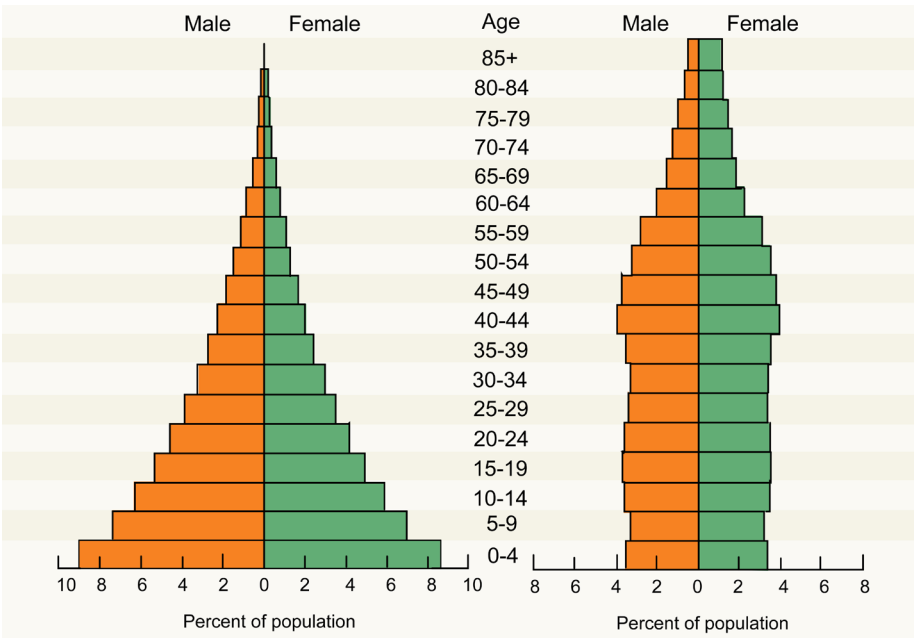
Populations are dynamic, constantly changing components of ecosystem. They are commonly described using the following characteristics:

1.1.1 Population density

Population density is defined as the numbers of individuals per unit area or per unit volume of environment. Larger organisms as trees may be expressed as 100 trees per square kilometer. For example, the number of Acacia tree species per square kilometer in the Akagera National park, whereas smaller ones like phytoplanktons (as algae) as 1 million cells per cubic meter of water. In terms of weight it may be 50 kilograms of fish per hectare of water surface.

1.1.2 Population age structure

One important demographic variable in present and future growth trends is a country's age structure, the relative number of individuals of each age in the population. The **age structure** of a **population** is the **distribution** of people of various **ages.** Age structure is commonly graphed as "pyramids" like those in figure below.



(a) **(b)**

Figure 1.1: Age- sex structure pyramids of developing (a) and developed countries (b)

The shapes of the age-sex structure pyramids shown above show the age sex-structure of a developing and developed country. The main characteristics of developing countries including some of the African countries in terms of population growth include high death rate; high birth rate and low life expectancy, while the main characteristics of developed countries such as most European

countries in terms of population growth have low death rate, low birth rate and longer life expectancy. The age structure of a population affects a nation's key socioeconomic issues. For example, countries with young populations (high percentage under age 15) need to invest more in schools while countries with older populations (high percentage ages 65 and over) need to invest more in the health sector.

1.1.3 Population explosion

The human population increased relatively slowly until about 1950, at which time approximately 500 million people inhabited Earth. Our population doubled to 1 billion within the next two centuries, doubled again to 2 billion between 1850 and 1930, and doubled still again by 1975 to more than 4 billion. The global population is now more than 6.6 billion people and is increasing by about 75 million each year. Population ecologists predict a population of 7.8-10.8 billion people on Earth by the year 2050.

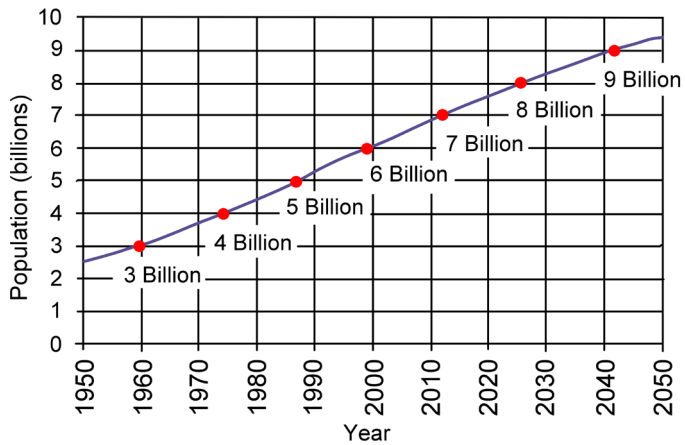


Figure 1.2: World population growth from 1950 to 2050.

Such human population increase impacts negatively the environment. For instance, human population explosion contributes to pollution leading to; **ozone depletion, eutrophication, acid rain, global deforestation, soil erosion and desertification.** A population explosion is a sudden increase in the number of individuals in a particular species. Human population explosions is sometimes cited as a cause of resource scarcity and a lack of opportunity for individuals.

One of the best way of regulating human population increase in different countries including Rwanda is practicing the family planning. Family planning is the practice of controlling the number of children in a family and the intervals between their births. If married couples are sexually active, they have to adopt at least one family planning technique such as contraception and timing of reproduction. Other techniques commonly used include; sexuality education,

prevention and management of sexually transmitted infections, pre-conception counselling and management, and infertility management.

1.1.4 Birth and death rates

Birth rate is the ratio of live births in a specified area to the adults in population of that area. It is usually expressed per one thousand individuals per year. It is estimated from this calculation:

$$\text{Birth rate} = \frac{\text{Number of births}}{\text{Number of adults in the population}} \text{X } 1000$$

Death rate is the ratio of deaths to the adults in population of a particular area during a particular period of time. It is usually calculated as the number of deaths per one thousand individuals per year and it is estimated from this calculation:

$$\text{Death rate} = \frac{\text{Number of deaths}}{\text{Number of adults in the population}} \text{X } 1000$$

1.1.5 Population growth patterns

Population growth patterns are graphs (population growth curves) in which increases in size are plotted per unit time. When a population size increases, the growth rate also increases. The factors that contribute to the population growth are immigration of new species as well as the birth rate. Population growth is also influenced negatively by emigration and death rate.

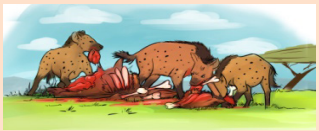
Application activity 1.1

1) In a habitat, there are 200 adult lions. Each year, 20 lions are produced while 5 lions die.
   a) Calculate the birth rate of this population.
   b) Calculate the death rate of that population
2) A population of 820 insects occupies a surface area of 1.2 km^2. These insects gather nectar from a population of 560 flowering plants which occupy a surface area of 0.2km^2. Which population has greater density?

1.2 Factors affecting population density

Activity 1.2

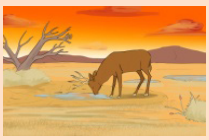
Observe the figures below and respond to the following questions:



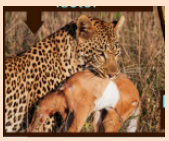
(A)

(B)

(C)



(D)



(E)

(F)

a) Observe the figures above and identify what is taking place in each figure.

b) Based on what is happening as a result of interaction between organisms or not, make two Groups from the above figures and find the names that correspond to those two groups.

c) By use of books or search engine describe how identified factors in (a) affect the population density.

Populations are differently distributed. The distribution and the density are controlled by environmental factors, which can either increase or decrease the population size by affecting birthrate, death rate, immigration and emigration. These factors are grouped into two major categories: Density -dependent factors and Density- independent factors.

1.2.1 Density-dependent factors

Without some type of negative feedback between population density and the vital rates of birth and death, a population would never stop growing. Density dependent factors are factors whose effects on the size or growth of the population vary with the population density. The density dependent factors include the following: availability of food or resources, predation, disease and migration.

a) Competition for resources

In a crowded population, increasing population density intensifies competition for declining nutrients and other resources, resulting in a lower birth rate. Crowding can reduce reproduction by plants and many animal populations also experience internal competition for food and other resources.

Figure 1.3: Interaspecific and interspecific competions

b) Diseases

Population density can also influence the health and thus the survival of organisms. If the transmission rate of a particular disease depends on a certain level of crowding in a population, the disease would impact more the population with high density. Among plants, the severity of infection by fungal pathogens is often greater in locations where the density of the host plant population is higher. Animals, too, can experience an increased rate of infection by pathogens at high population densities.

c) Predation

Predation is also an important cause of density-dependent mortality if a predator encounters and captures more food as the population density of the prey increases. As a prey population builds up, predators may feed preferentially on that species, consuming a higher percentage of them which affects directly population density.

1.2.2 Density-independent factors

Density independent factors can affect the population regardless of their density.

Most density independent factors are abiotic factors, such as volcanic eruptions, temperature, storms, floods, draught, chemical pesticides and major habitat disruption. Even if all population can be affected by these factors, the most vulnerable appear to be on small organisms with large population such as insects.

Figure 1.3: Natural disaster is a perfect example of a density independent factor.

Application activity 1.2

1) A population of field mice increases after a farmer leaves his field unharvest for a season. Which of the following categories does this factor fall into? Explain your choice.
   a) Density Independent Factors,
   b) Density Dependent Factors,
   c) Increased death rate
2) Compare the density -dependent and density independent factors. In your comparison highlight examples of those factors.

1.3 Methods or techniques of measuring and estimating population density

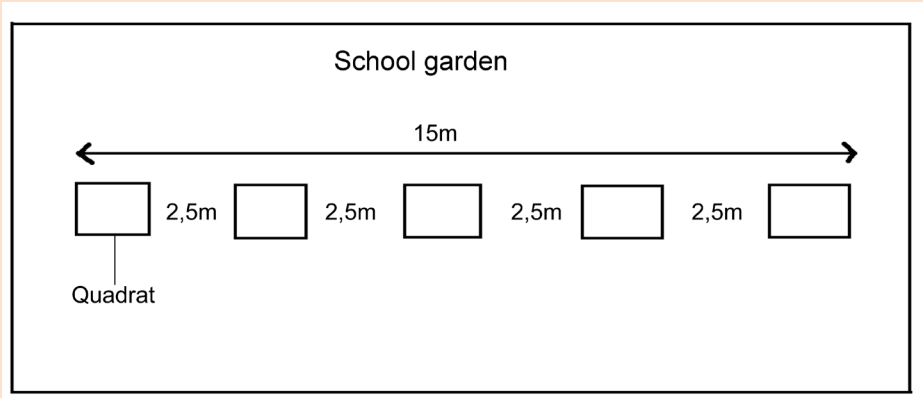
Activity 1.3

Using strings/ropes, a decameter and quadrats in your school garden, carry out the following field work:

a) Move in the school garden and make a line transect of 15 meters by the use of a decameter and rope or a string.

b) Count all plants species found at each five meters across transect.

c) On the ground, apply five different quadrats of one square meter separated by 2.5 meters and count different plants species within each quadrat. The sketch that show the disposition of quadrats upon 15 meters is as follow:



Record your samples in the following table with respect to each quadrat:

Species	Number of individuals in each quadrat					Total
	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 4	Quadrat 5	

a) Calculate the population density and species frequency for each studied quadrat.

b) Compare the results of different quadrats.

1.3.1 Quadrat method

A quadrat is a square frame that marks off an area of ground, or water, where you can identify different species present and/or take a measurement of their abundance. Before any experiment, the decision on a suitable size for the quadrat and the number of samples to use is taken. Samples must be selected randomly to avoid any bias, such as choosing to take all of samples from the place with fewest species simply because it is the easiest to do. This would not represent the whole area you are surveying. A quadrat method enables the calculations of 3 aspects of species distribution including; species frequency, species density and species percentage cover.

1.3.2 Species frequency

Species frequency is a measure of the chance (probability) of a particular species being found within any one of the quadrat, and it is found simply by recording whether the species was present in each analyzed quadrat. For example, if a quadrat is placed 40 times, and a given plant was identified in 20 samples, then the species frequency for this plants equals $\frac{20}{40} X 100 = 50\%$

1.3.3 Species density

Species density is a quantity of how many individuals there are per unit area, it can also be the number of species in a sampled area for example, per square meter. To achieve this, one takes the total number of counted individuals and then divide it by the number of quadrats done. An example is:

Total number of individuals= 250

Total area of quadrats =500m^2

$$\text{Species density} = \frac{250}{500} = 0.5 \text{ individuals} / m^2$$

1.3.4 Species cover

Species cover is a measure of the proportion of ground occupied by the species and gives an estimate of the area covered by the species as the percentage of the total area. For example, if there are 100 small squares in one quadrat, then the squares in which the plant species are present are counted. If plants are found in 25 squares within that quadrat, the conclusion is that the plant covers 25% of the area.

1.3.5 Line transect method

Line transect is a tape or string laid along the ground in a straight line between two poles as a guide to a sampling method used to measure the distribution of organisms. For example, the investigation on change at the edge of a field where it becomes very marshy is done by randomly selecting a starting point in the field and lay out a measuring tape in a straight line to the marshy area, and then sample the organisms that are present along the line, which is called a transect. The simplest way to do this is to record the identity of the organisms that touch the line at set distances for example, every two meters.

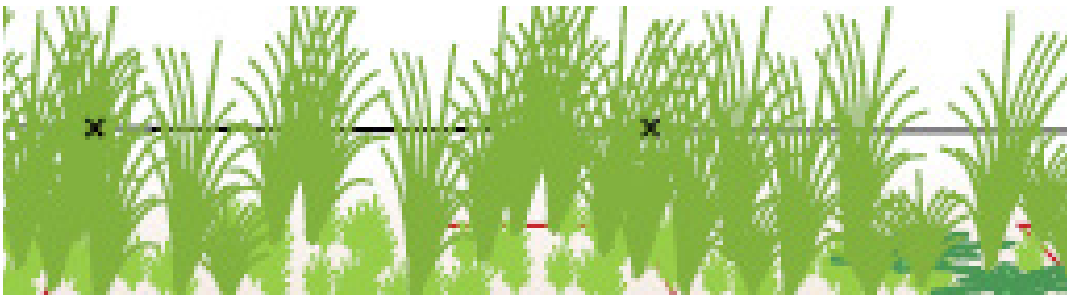


Figure 1.4: Line transect

1.3.6 Capture-recapture method

Capture-recapture method involves capturing the organism, marking it without any harm, and release it in the same area so that it can resume a normal role in the population. For example, fish can be netted and their opercula is netted with aluminium discs, birds can be netted and rings can be attached to their legs, small animals may be tagged by dyes, or by clipping the fur in distinctive pattern, while arthropods can be marked with paint. In all cases, some form of coding may be adopted so that individual organisms are identified. Having trapped, counted and marked are representative sample of the population.

At a later stage, the population is trapped again and counted, and the population size is estimated using the Lincoln index as follows:

$$\text{Estimated total population (n)} = \frac{N_1 \text{ X } N_2}{N}$$

Where:

N_1: the number of organisms in initial sample,

N_2: the number of organism in a second sample,

N: the number of marked organisms recaptured.

Application activity 1.3

1) Conduct a survey using a quadrat of 0.5m^2 and found the following statistics for a couch grass by quadrat:

Quadrats	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Number of couch grass	0	3	4	1	0	0	5	2

a) Calculate **the** species frequency, and the species density of couch grass from the results of this survey.

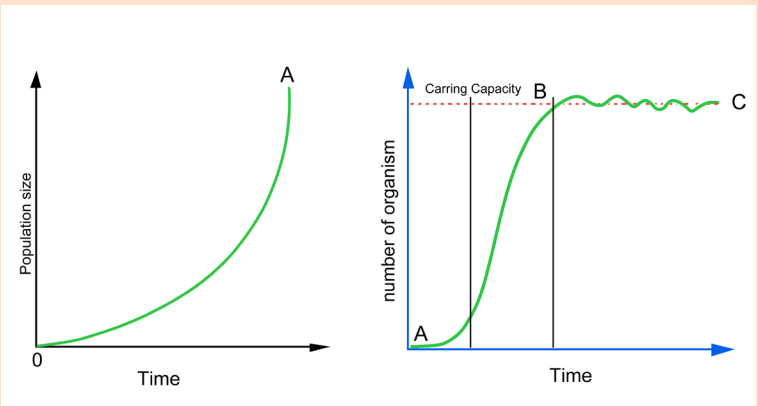
b) Given that the total surface area of the school ground is 200 m^2 and couch grasses were found on 50 m^2. Calculate the percentage cover occupied by couch grasses.

A fish farmer wanted to know the total population in her fish pond. She netted 240 fish and tagged (marked) their opercula with aluminium discs. She released those fish into the pond. After one week, she netted again 250 fish among which 15 had the aluminium discs. Calculate the estimated population from marked individuals.

1.4 Population growth patterns and Environmental resistance

Activity 1.4

The following graphs are of insect's growth in separate conditions, study it and answer the following questions:



(W) (Z)

a) What does these graphs represent based on parameters presented on horizontal and vertical axis?

b) Based on the shape of the graph**W** from **0** to **A** and the shape of **Z** from **A** up to **C** findout their similarities and their differences.

c) Explain how does food supply brings fluctuation which is the result of the shape **B** to **C** on graph Z.

1.4.1 Population growth patterns

Population growth patterns are graphs also called population growth curves in which the increases in size are plotted per unit time. Two types of population growth patterns may occur depending on specific environmental conditions:

a) Exponential growth pattern / J-shaped curve

Exponential growth is a pattern of population growth in which a population starts out growing slowly but grows faster as population size increases. An exponential growth pattern also called J shapes curve occurs in an ideal, and unlimited environmental resources. In such an environment there will be no competition. Initially population growth is slow as there is a shortage of reproducing individuals that may be widely dispersed. As population numbers increase, the rate of growth similarly increases, resulting in an exponential J-shaped curve. Exponential population growth can be seen in populations that are very small or in regions that are newly colonized by a species.

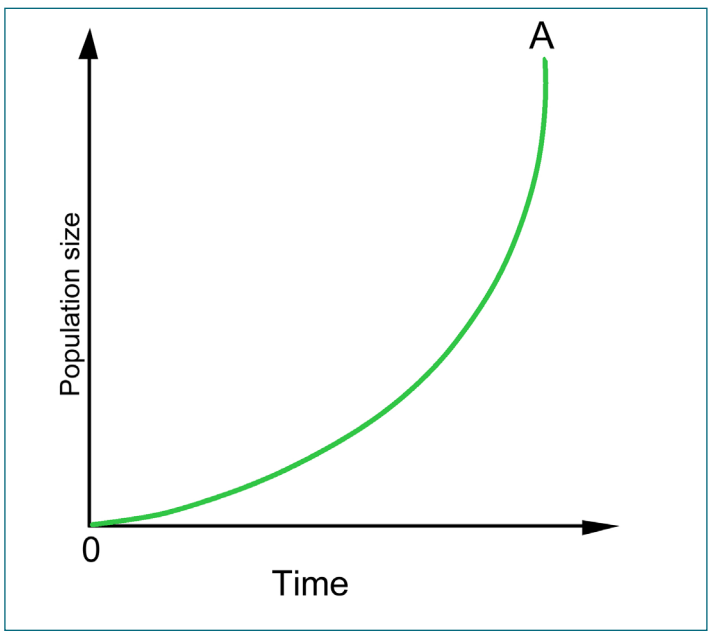


Figure 1.4: Exponential growth curve

b. Logistic growth pattern / sigmoid growth curve

Logistic growth is a pattern of population growth in which growth slows and population size levels off as the population approaches the carrying capacity. A logistic growth pattern also called S-shaped curve occurs when environmental factors slow the rate of growth.

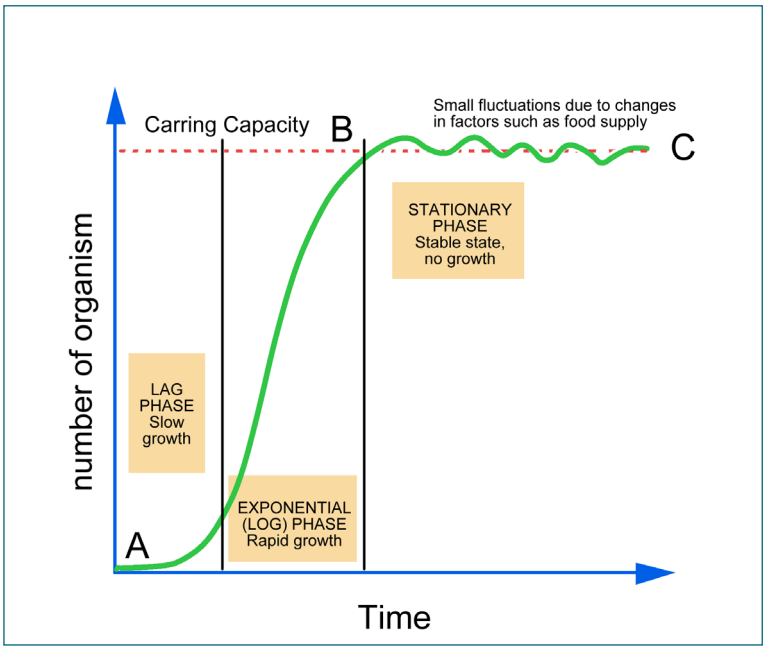


Figure 1.5: Logistic growth curve

The sigmoid or S- shaped curve represented by the figure 1.8 shows three main stages in population growth: The **lag phase** where there is a slow growth, the **log phase or exponential** growth phase, also called **logarithmic phase**, in which the number of individuals increases at a faster rate and the **plateau phase or stationary phase**, in which the number of individuals are stabilized.

Causes of the exponential phase are various and include the plentiful of resources such as; food, space or light, little or no competition from other organisms, and favourable abiotic factors such as; temperature or oxygen and reduced of lack of predation or diseases. The stationary phase, however is caused by a balanced number of; births plus the number of immigrations and the number of deaths plus the number of emigration. Other causes may include; the increase of mortality caused by predators and diseases, excess of wastes and competition for available resources such as food, space, shelter and minerals. Some of these causes may include the carrying capacity explained as is the maximum number of individuals that a particular habitat can support.

1.4.2 Environmental resistance

Environmental resistance is the total sum of limiting factors, both biotic and abiotic, which act together to prevent the maximum reproductive potential also called biotic potential from being realized. It includes external factors such as predation, food supply, heat, light and space, and internal regulatory mechanisms such as intraspecific competition and behavioral adaptations.

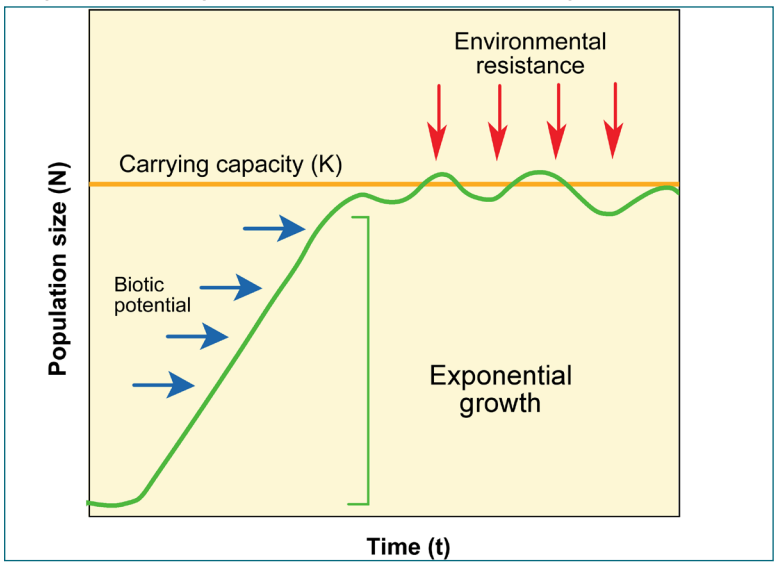


Figure 1.6: Effect of environmental to population growth population growth

1.4.3 Environmental balance

A balance of nature is the stable state in which natural communities of animals and plants exist, and are maintained by competition, adaptation and other interactions between members of the communities and their non-living environment. Every biotic factor, **affects** or **causes a change** in the natural environment. For example, when a living organism interacts with the environment, this causes a change in the environment. The following are some of the examples of biotic factors are include animals, plants, fungi, bacteria, and protists and their effects on balance of nature can be seen through the following phenomena:

- **Respiration:** when animals are respiring, they take in oxygen and give out carbon dioxide (CO_2) from respiration. The CO_2 can be taken in by plant leaves and be used in the process of photosynthesis to make food and give out oxygen.
- **Predation:** when animals, for example, predate on other animals, this reduces the numbers of prey, which in turn affects the ecosystem.
- **Parasitism:** cause diseases that may slow down the growth rate of a population and/or reduces the number of organisms.
- **Competition:** when organisms compete over nutritional resources, this could reduce the growth of a population.

Application activity 1.4

A small group of mice invaded a new habitat with unlimited resources and their population grew rapidly. A flood then swept through the habitat and three quarters of the mice died. Two months later, the population was increasing again.

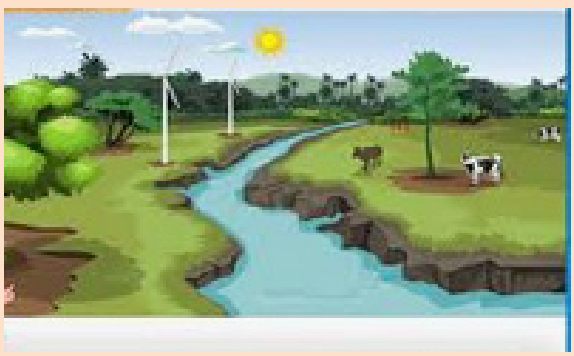
a) What role did the flood play for the mouse population?

b) Draw a graph depicting the population history of this mouse.

1.5 Renewable natural resources

Activity 1.5

Observe the figures below carefully and respond to the following questions:



(A)

(B)

(C)

Natural resources refer to materials or substances occurring in environment and which can be exploited for economic gain. They are also resources that exist without actions of humankind Natural resources such as; solar energy,

wind, air, water, soil and biomass (plants and animals) are **renewable natural resources**. Below are the examples of renewable natural resource:

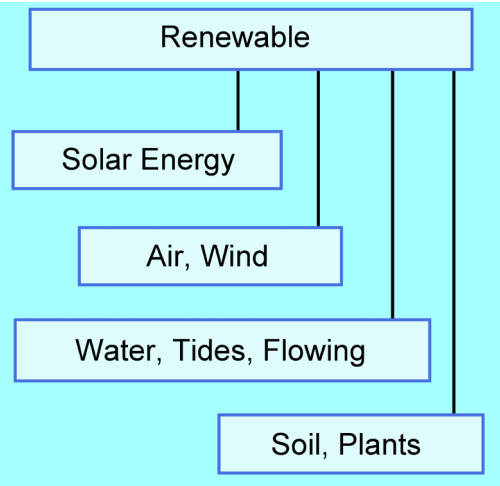


Figure 1.7: Examples of renewable natural resources

1.5.1 Importance of renewable natural resources in economic growth of Rwanda

- Water is used for irrigation, domestic activities, industrial use, and mining.
- Lakes and rivers are source of food (fish) for humans and contribute for recreation (tourism).
- Land serves as the storehouse of water, minerals, livestock, and home for wild animals which generate an income in different ways.
- Soil contributes to agricultural crop production, and supports forest and food crops.
- Trees are the major sources of timber, construction materials and firewood and contribute to fight against erosion, water and air purification and wind protection.
- Some plants are source of food and money for humans and other animals.
- National Parks contribute to economic development of the country through tourism.

1.5.2 Methods of conserving renewable natural resources

They are various methods used for conservation of renewable natural resources and they include:

- **Planting trees to prevent soil erosion**. The vegetation prevents soil erosion but also is a home for most insects, birds and some symbiotic plants. This creates a habitat for wildlife hence conserving wild organisms

- **Practicing of judicious ways to conserve water in our homes:** This entails simple practices like ensuring that taps are closed when they are not in use. Using less water during domestic activities aids to conserve lots of water in our homes.
- **Growing vegetation in catchment areas:** Catchment areas act as a source of water that flows in; streams, rivers and oceans. Vegetation in the catchment areas allows sufficient infiltration of water into deeper soil layers thus leading to formation of ground water
- **Prior treatment of human sewage and Industrial wastes:** Water flowing from industries comes with many toxic wastes that must be treated before getting to the natural water bodies. This reduces harm inform of pollutants e.g. chemical and thermal forms.
- **Practice of in-situ and ex-situ conservation of wild plants which** involves conservation of flora in their natural habitats and outside the natural habitats respectively. This requires setting up measures that protect areas such as national parks and game reserves. The ex-situ conservation of plants uses the areas such as; pollen banks, DNA banks, seed banks, botanical gardens, tissue culture banks among others.
- **Ensure the recycling of wastes:** These wastes include; plastics, paper bags that have resulted to tones of garbage. Recycling entails re-manufacturing of already used materials. This reduces the amount of waste available reducing soil and water pollution.
- **Practice crop rotation:** Planting the same crops for a long period of time reduces soil fertility. The practice of crop rotation will restore and maintain soil fertility thus conserving the soil.
- **Construction of terraces in sloping land:** This will prevent soil erosion as water tends to run downhill.

Application activity 1.5

1. You live in place which is dominated by sloping lands and bare soil then your parents complain about their soil that is washed away by the rainfall. What can you do to help your parent to prevent that sloping land?

2. The water bill at your home is always high and you are given a responsibility to reduce it as some who attended secondary school. Implement the measures that will reduce that water bill at your home.

1.6 Non-renewable natural resources

Activity 1.6

Observe the figures and respond to the following questions:

(A)

(B)

a) Based on the on figures above identify the activities that are taking place on both A and B.

b) Identify the effects of activity taking place on figure B.

c) Find out the purpose of activity taking place on figure A.

Non-renewable natural resource are resources of economic value that cannot be readily replaced by natural means on a level equal to its consumption. They include fossil fuels, oil, coal natural gas cited among many others as it is indicated below:

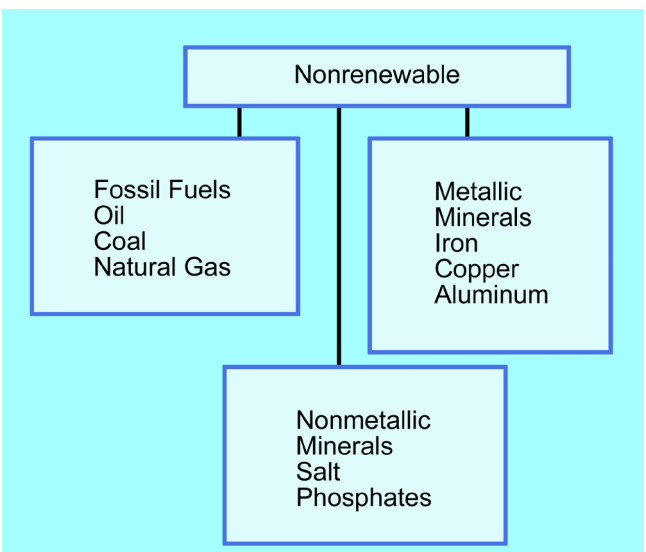


Figure 1.8. Examples of non-renewable natural resources

1.6.1 Importance of nonrenewable natural resources in economic growth of Rwanda

- Minerals including gravel, metals, sand, and stones are used for construction and for income generation for the country.
- Imported fossil fuels derivatives such as gas oil and asphalts are used as source of energy and construction of asphaltic roads to easy the transportation.
- Natural gas e.g. gas methane from Kivu is used as source energy.
- Some animals including; mountain gorillas in Volcanoes National Park, lions in Akagera National Park and many other wild animals contribute to economic development of the country through tourism.

1.6.2 Methods of conserving nonrenewable natural resources

There are various a methods used for conservation of nonrenewable natural resources and they include:

▪ The use of alternative sources of energy such as solar and wind energy because they do not produce harmful gases that damage the ozone layer compared to the burning of fossils fuels such as coal and charcoal.

▪ **Use pipelines to transport oil:** During oil transportation on ships, spills can happen which will negatively affect both plant and animal life. Therefore, use of pipelines is more recommended

▪ **Putting in place of policies and regulations to prevent poaching** because poachers continue to kill many animals such as; elephants and rhinos, for their tusks and skins which are sold off in the black market. Poachers are a major threat to our biodiversity as they are slowly making some species extinct.

▪ **Use of bio-fuels and biogas:** For more than a century, fossil fuels have been a major source of energy. However, they are depleting rapidly, this calls for alternative sources of fuel such as bio-fuels and biogas which mainly reduce the occurrence of air pollution.

▪ **Establish special schemes to preserve endangered plant and animal species:** This includes; botanical gardens, sanctuaries that may be established to protect the endangered species so that they can be available for future generation

Application activity 1.6

1) Different industries are making cars and motorcycles that use electricity instead of fuel. What is the contribution of that method compared to the one that uses fuel?
2) Why does mining companies that extract minerals legally, are obliged to restore the mining site after the completion of extraction of minerals at that mining company.

Skills lab 1

Biogas is a type of biofuel that is naturally produced from the decomposition of organic waste. When organic matter, such as food scraps and animal waste, break down in an anaerobic environment (an environment absent of oxygen) they release a blend of gases, primarily methane and carbon dioxide**.** People are encouraged to use biogas in their home as alternative source of energy. This can reduce the rate of deforestation which can result in maintenance of plant and animal species as well as soil protection against erosion. Sensitization can be a tool to help people to have these alternative sources of energy in large number.

Procedure

- Select 10 families at your village.
- Record the families that have biogas.
- Select other ten families which use woods in cooking.
- Record the money spent by each family while cooking either using biogas or woods
- Compare the money spent by each family
- Prepare action of sensitizing people on using biogas based on recorded data.

Evaluation sheet

Items	Number	None
Families that had biogas before sensitization.		
Families that did have biogas before sensitization.		
Families that possess biogas after sensitization		
Families that do not possess biogas after sensitization.		

End unit assessment 1

I. Choose the letter of the answer that best complete each statement

1. During population growth

   a) Birth rate increases

   b) Death rate increases

   c) Birth rate decreases.

   d) Birth rate and death rate decreases.

2. Population that reaches the carrying capacity of its environment is said to have reached

   a) logistic growth

   b) exponential growth

   c) density dependence

   d) a steady state

3. On a logistic growth curve, the portion of the curve in which the population grows rapidly is called

   a) logistic growth

   b) a steady state

   c) exponential growth

   d) carrying capacity

4. Which of the following is a characteristic of developing countries?

   a) A fast population growth due to a high death rate but higher birth rate.

   b) A fast population growth due to a high birth rate but falling death rate.

   c) A slow population growth due to a low birth rate and falling death rate.

   d) A slow population growth due to a low birth rate and low death rate.

5. Which of the following would be an example of population density?
   a) 100 caterpillars
   b) 100 caterpillars per mango tree
   c) 100 caterpillars clumped into 5 specific areas

II. Open questions

1. How can a density dependent factor, such as a food supply affect the carrying capacity of a habitat?

2. Describe how density dependent and density dependent factors regulate population growth.

3. Suggest the reasons why the luck of available clean water could be a limiting factor for a country's population.

4. a) Distinguish between carrying capacity and biotic potential.

   b) Explain how environmental resistance affects the population growth.

5. Students made a survey of blackjack (Bidens pilosa) growing their school environment. Ten quadrats of 1.0 m2 were placed randomly in the garden and the number of blackjack plants in each quadrat was counted.

The results are summarized in the following table:

Quadrats	1	2	3	4	5	6	7	8	9	10
Number of blackjack first garden	0	0	4	3	0	1	2	4	0	3

Calculate: a. the species frequency of blackjack in this gardens.

b. The species density of blackjack plants in that area.

c. Explain why it is important to use randomly placed quadrats.

6. Describe how has the growth of Earth's human population has changed in 2 recent centuries? Give your answer in terms of growth rate and the number of people added each year?

7. Construct a bar graph showing the age structure of a given country using the following data: Pre-reproductive years (0-14) are 42 percent; reproductive years (15-44) are 39 percent; post-reproductive years (45-85+) are 19 percent. Interpret obtained graph.

8. Explain the relationship between a growing population and the environment

9. Observe the pictures below and respond to the following questions.

a) Identify the human activities shown above that harm the natural resources.

b) Describe all effects of the identified activities on the environment.

c) Suggest the possible measures to solve the above problems.

UNIT 2

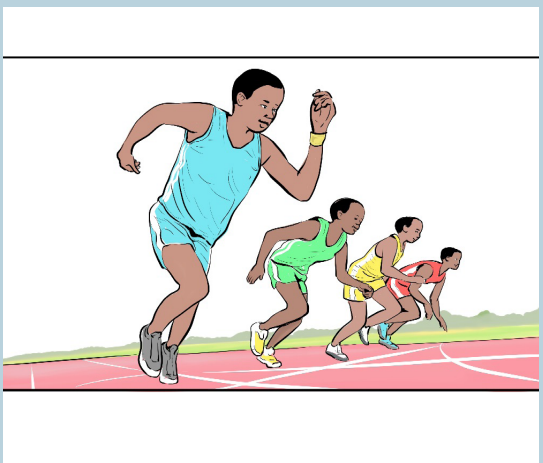
ENERGY AND CELLULAR RESPIRATION

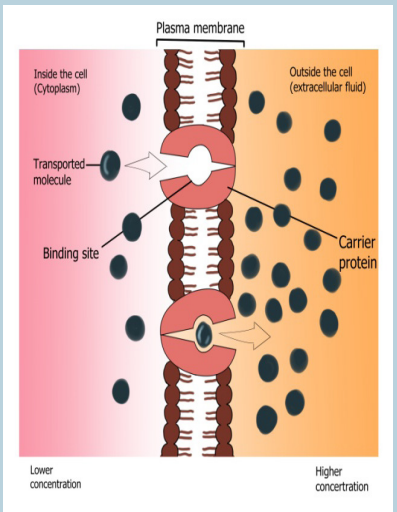
Key unit competence

Describe the structure and importance of ATP, and outline the roles of the coenzymes NAD, FAD and coenzyme A during cellular respiration and the process of cellular respiration

Introductory activity 2.1

Living organisms perform different tasks like running, moving and pumping substances across cell membranes as shown on the figures below:





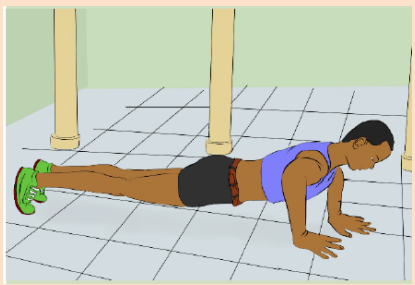
a) What is the requirement to perform such activities and others that seem like these?

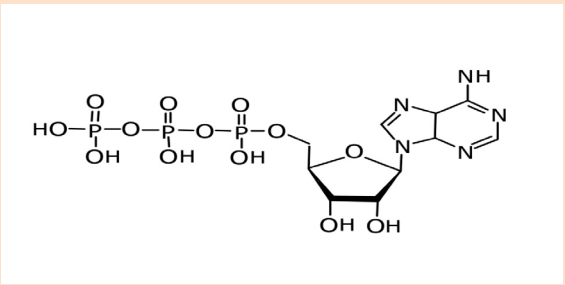
b) By which mechanism do you think is taking place in organism cells to obtain such requirement? In which form this requirement would appear?

2.1 Energy of living organisms

Activity 2.1

Observe the figures below and answer to the following questions





(A) **(B)**

a) The figures A represents the activity that requires energy, based on figure A above identify other more activities that requires energy.

b) What could be the name of figure B, its main chemical parts and its roles for living organisms?

2.1.1 *Need for energy by organisms*

Without some input of energy, natural processes tend to break down in randomness and disorder. Living organisms have high ordered systems that require a constant input of energy to prevent them becoming disordered which would lead to their death. This energy comes from the breakdown of organic molecules to make **adenosine triphosphate** (ATP) which is a source of energy needed to carry out processes that are essential to life.

More precisely energy is needed for:

- **Metabolism** which involves specifically the **anabolism** process in which simple substances are build up into complex ones e.g. monosaccharides are built up into polysaccharides and amino acids are built up into polypeptides
- **Active transport** of ions and different molecules against a concentration gradient across cell membranes. The transport of sodium (Na^+), potassium (K^+) magnesium (Mg^+), calcium (Ca^+) and chloride (Cl^-) across the plasma membrane cannot be possible without the use of energy. The transport proteins that move solutes against their concentration gradients are all carrier proteins rather than channel proteins. Active transport enables a cell to maintain internal concentrations of small solutes that differ from concentrations in its environment. Some transport proteins act as pumps,

moving substances across a membrane against their concentration or electrochemical gradients. Energy is usually supplied by adenosine triphosphate (ATP) hydrolysis

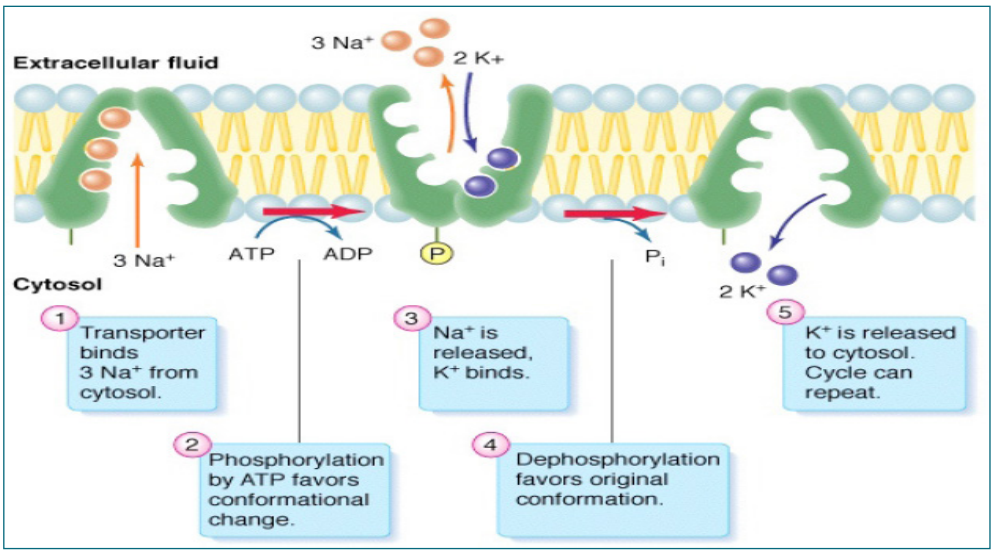


Figure 2.1: Active transport of chemical ions/anions across the cell membrane

- **Movement** within an organism when substances move in the body e.g. circulation of blood and of the orgasm it's self during locomotion due to muscular contraction or movement of cilia and flagella.
- **Maintenance, repair and division of cell and organelles** within them.
- **Maintenance of body temperature in endothermic organisms** e.g. birds and mammals that need energy to replace that lost as heat to the surrounding environment.
- **Production of substances** used within organism e.g. enzymes and hormones.

2.1.2 *Structure of adenosine triphosphate (ATP)*

The special carrier of energy is the molecule of adenosine triphosphate (ATP). The ATP molecule is a phosphorylated nucleotide and it has three parts:

- **Adenine**: is a nitrogen containing organic base belongs to the group called purines
- **Ribose:** is a pentose sugar molecule means it has 5-carbon ring structure that act as the backbone where the other parts are attached**.**
- **Phosphates:** that are chain of three phosphate groups.

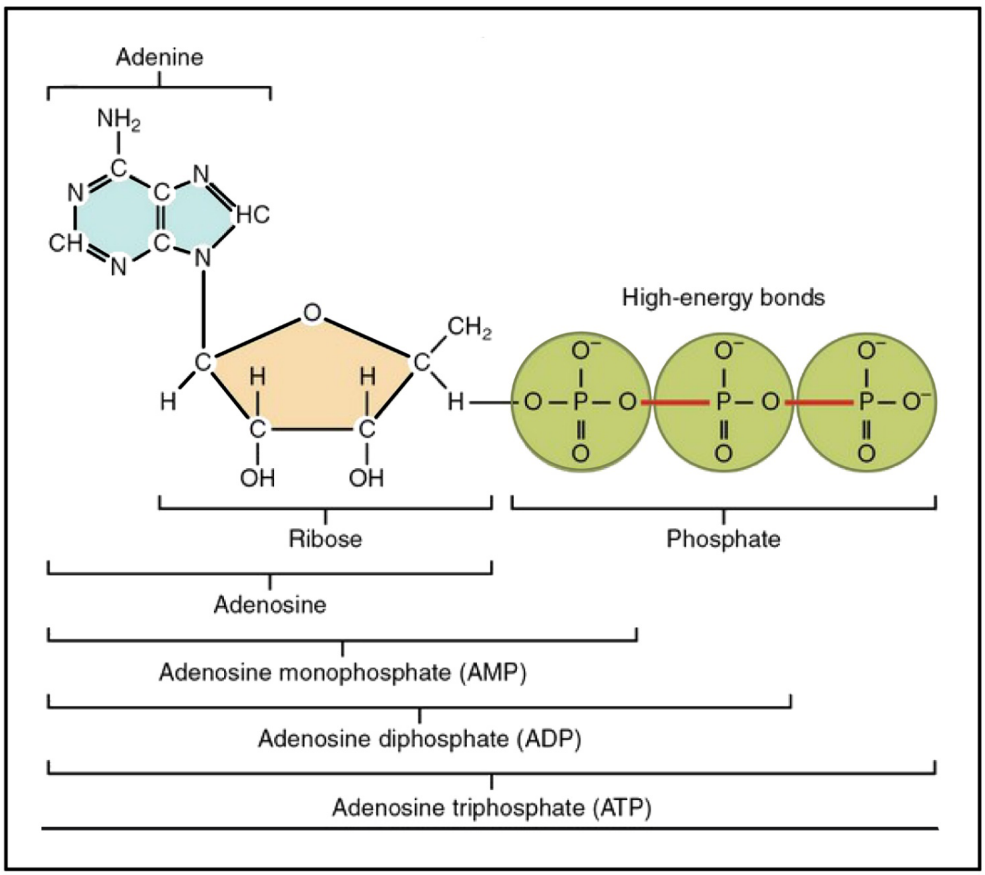


Figure 2.2: Structure of Adenosine Triphosphate (ATP)

ATP has the following biological functions in the cell:

a) Active transport

ATP plays a critical role in the transport of macromolecules such as proteins and lipids into and out of the cell membrane. It provides the required energy for active transport mechanisms to carry such molecules against a concentration gradient.

b) Cell signaling

ATP has key functions of both intracellular and extracellular signaling. In nervous system, adenosine triphosphate modulates the neural development, the control of immune systems, and of neuron signaling.

c) Structural maintenance

ATP plays a very important role in preserving the structure of the cell by helping the assembly of the cytoskeletal elements. It also supplies energy to the flagella and chromosomes to maintain their appropriate functioning.

d) Muscle contraction

ATP is critical for the contraction of muscles. It binds to myosin to provide energy and facilitate its binding to actin to form a cross-bridge. Adenosine diphosphate (ADP) and phosphate group (Pi) are then released and a new ATP molecule binds to myosin. This breaks the cross-bridge between myosin and actin filaments, thereby releasing myosin for the next contraction.

e) Synthesis of DNA and RNA

The adenosine from ATP is a building block of RNA and is directly added to RNA molecules during RNA synthesis by RNA polymerases. The removal of pyrophosphate provides the energy required for this reaction. It is also a component of DNA.

Application activity 2.1

1) Energy is contained within ATP, draw and label its structure. On diagram show the names that result from the combination of different parts of ATP.
2) The person faints on playground as a result of doing vigorous physical exercise for long time. What can you do to save the life of that person?

2.2 Adenosine triphosphate (ATP) and coenzyme in respiration

Activity 2.2

Based on the structure of ATP molecule, explain how the synthesis and breakdown of ATP is done.

2.2.1 Synthesis and breakdown of ATP

a) Breakdown of ATP

Adenosine triphosphate (ATP) is the energy currency for cellular processes. It provides the energy for both energy-consuming **endergonic reactions** and energy-releasing **exergonic reactions**. The three phosphate groups in ATP structure are the main key to how ATP stores energy. Each phosphate group is very negatively charged so they repel one another which makes the covalent bonds that link to be unstable. These unstable covalent bonds are broken easily because they have low activation energy. When the first two phosphates are removed 30.5Kjmol^{-1} are released for each phosphate group and 14.2 KJ mol^{-1} are released for the removal of the final phosphate group. In living cells, usually only the terminal phosphate group is removed as follow:

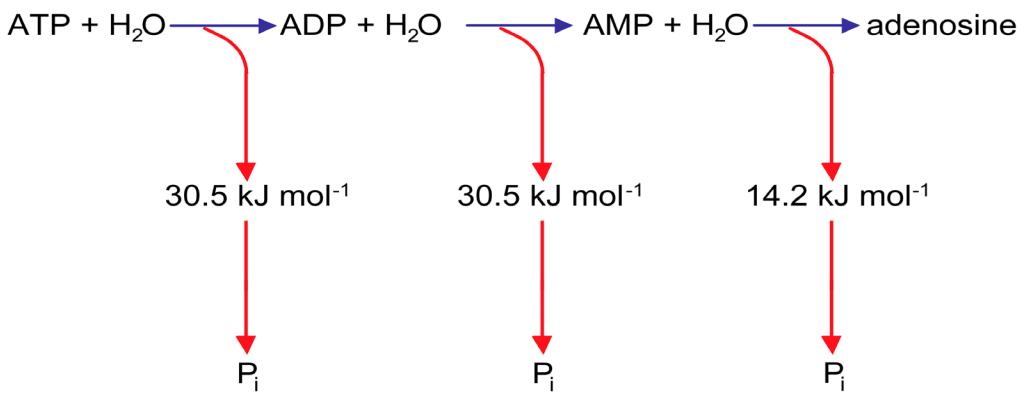


Figure 2.3 Hydrolysis of ATP (Pi is inorganic phosphate, H_3PO_4).

These reactions are all reversible. It is the interconversion of ATP and ADP that is all-important in providing energy for the cell:

$$ATP + H_2O \rightleftharpoons ADP + H_3PO_4 \pm 30.5\,kJ$$

The calculated ΔG for the hydrolysis of one mole of ATP into ADP and P_i is estimated at −7.3 kcal/mole equivalent to −30.5 kJ/mol. However, this is only true under standard conditions, and the ΔG for the hydrolysis of one mole of ATP in a living cell is almost double the value at standard conditions and equals -14 kcal/mol or −57 kJ/mol. ATP is a highly unstable molecule. Unless quickly used to perform work, ATP spontaneously dissociates into ADP + P_i, and the free energy released during this process is lost as heat. To harness the energy within the bounds of ATP, cells use a strategy called energy coupling.

The hydrolysis of ATP to ADP and Pi is a reversible reaction, where the reverse reaction combines ADP + P_i to regenerate ATP from ADP as it is shown in the equation above.

b) Synthesis of ATP

Energy for ATP synthesis can become available in two ways. In respiration, energy released by reorganizing chemical bonds (chemical potential energy) during making some ATP. However, most ATP in cells is generated using electrical potential energy. This energy is from the transfer of electrons by electron carriers in mitochondria and chloroplasts. It is stored as a difference in proton (hydrogen ion) concentration across some phospholipid membranes in mitochondria and chloroplasts, which are essentially impermeable to protons. Protons are then allowed to flow down their concentration gradient (by facilitated diffusion) through a protein that spans the phospholipid bilayer. Part of this protein acts as an enzyme that synthesizes ATP and is called **ATP synthase**. The transfer of three protons allows the production of one ATP molecule, provided that ADP and an inorganic phosphate group (Pi) are available inside the organelle. This process occurs in both mitochondria and chloroplasts and it was first proposed by Peter Mitchell in 1961 and is called **chemiosmosis**.

Since the hydrolysis of ATP releases energy, ATP synthesis must require an input of free energy. Recall that free energy is the portion of system's energy that can perform work when temperature and pressure are uniform throughout the system. The synthesis of ATP from ADP involves the addition of a phosphate molecule, which is called **phosphorylation** reaction. This Phosphorylation is catalyzed by the enzyme ATP synthase (sometimes called ATP synthetase or ATPase).

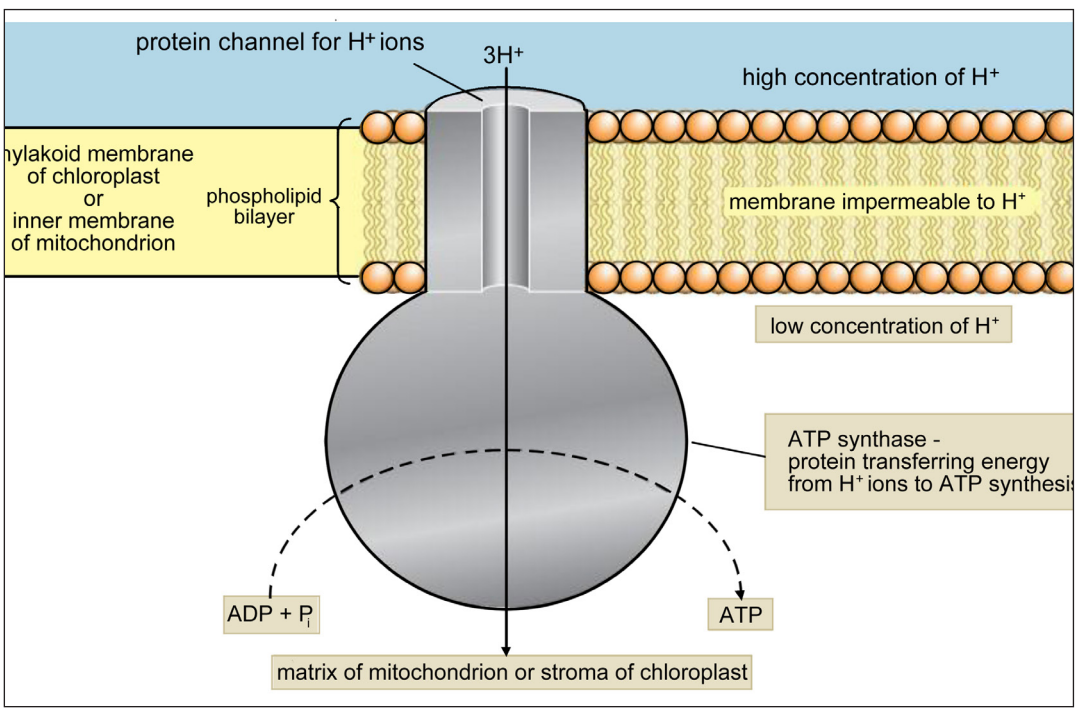


Figure 2.4: ATP synthesis (chemiosmosis)

2.2.2 Roles of coenzymes in respiration

The transformation of succinate to fumarate, the sub-products of the breakdown of glucose during glycolysis process, two hydrogens are transferred to flavin adenine dinucleotide (FAD), forming $FADH_2$. The reduced coenzymes NADH and $FADH_2$ transfer higher energy electrons to the electron transport chain. Finally, another coenzyme called coenzyme A sometimes abbreviated by CoA, a sulfur-containing compound is attached via its sulfur atom to the two-carbon intermediate, forming acetyl CoA. The Acetyl CoA has a high potential energy, which is used to transfer the acetyl group to a molecule in the citric acid cycle (Krebs cycle), a reaction that is therefore highly exergonic producing great number of energy in the form of ATP.

Application activity 2.2

Application activity 2.2

1) Using the chemical equations explain the synthesis and the hydrolysis of ATP in a living cell.
2) The hydrolysis and synthesis of ATP are reversible reactions. Estimate the amount of energy for each process.
3) Calculate the amount of energy produced by 5 moles of ATP

a) Under standard conditions

b) In a living cell

2.3 Respiratory substrates and their relative energy values

Activity 2.3

Activity 2.3: Simple combustion experiments to determine the relative energy values of different food substances.

- Cut up a range of dried foods into small pieces around 1 cm square or 0.5 cm cubed.
- Use the measuring cylinder to measure 20 cm^3 of water into the boiling tube.
- Clamp the boiling tube to the clamp stand.
- Measure the temperature of the water with the thermometer. Record the temperature in a suitable table.
- Impale the piece of food carefully on a mounted needle.

- Light the Bunsen burner and hold the food in the flame until it catches a light.
- As soon as the food is alight, put it under the boiling tube of water as shown on figure and keep the flame under the tube.
- Hold the food in place until the food has burnt completely.
- As soon as the food has burned away completely and the flame has gone out, stir the water carefully with the thermometer and measure the temperature of the water again. Note down the highest temperature reached.
- Repeat the procedure for other foods.
- Calculate the rise in temperature each time and Calculate the energy released from each food by using this formula.

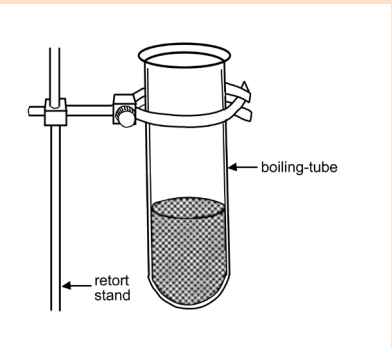
$$\text{Energy released from food per gram (J)} = \frac{\text{Mass of water (g) x temperature rise } (^0\text{C}) \text{ x } 4.2}{\text{Mass of food sample (g)}}$$

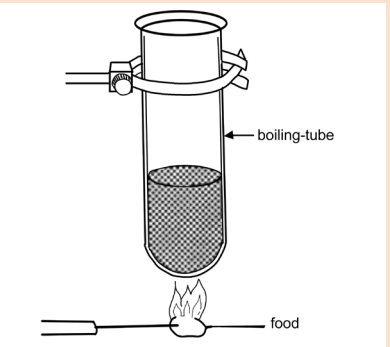
Where 4.2 represents the value of the specific heat capacity of water, in joules per gram per degree Celsius. If the number is more than 1000 J/g, express it as kilojoules (kJ):

1 kilojoule = 1000 joules

Compare obtained results.

Follow the set up below:





A respiratory substrate refers to the substance required for cellular respiration to derive energy through oxidation. They include carbohydrates, lipids and proteins.

Carbohydrates include any of the group of organic compounds consisting of carbon, hydrogen and oxygen, usually in the ratio 1:2:1. The examples of carbohydrates include sugars, starch and cellulose. Carbohydrates are the most abundant of all classes of biomolecules, and glucose whose chemical

formula is $C_6H_{12}O_6$ is the most known and the most abundant. Its breakdown produces energy in the following way: $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$+Energy (ATP + heat).

This breakdown is exergonic metabolic reaction, having a free-energy change of -686 kcal (-2,870 kJ) per mole of glucose decomposed.

Lipids include diverse group of compounds which are insoluble in water but dissolved readily in other lipids and in organic solvents such as ethanol (alcohol). Lipids mainly fats and oils contain carbon, hydrogen and oxygen, though the proportion of oxygen is lower than in carbohydrates. Fats and oils have a higher proportion of hydrogen than either carbohydrates or proteins. This property makes them a more concentrated source of energy, where each gram of fat or oil yields about 38kJ (38 kJ/g) more than twice the energy yield of a gram of carbohydrate.

Proteins are other respiratory substrate. They are large and complex biological molecules which play many and diverse roles during respiration. They mainly work as enzymes. Enzyme is a biological catalyst that controls biochemical reactions in living organisms.

$$\text{Ethanal} + NADH^{+} \xrightarrow{\textit{Alcaohol dehydrogenase}} H^{+}\ \text{Ethanol} + NAD^{+}$$

Back to glucose when it is broken down during the process called **glycolysis**, the dehydrogenases enzymes transfer electrons from substrates, here glucose, to NAD^+ which in turn forms NADH. At this stage the electron transport chain accepts electrons from NADH and passes these electrons from one molecule to another in electron chain transfer leading to a controlled release of energy for the synthesis of ATP. At the end of the chain, the electrons are combined with molecular oxygen and hydrogen ions (H^+) to form one molecule of water. When NAD is oxidized, its oxidized form NAD^+ is converted into its reduced from NADH, and two molecules of ATP are produced.

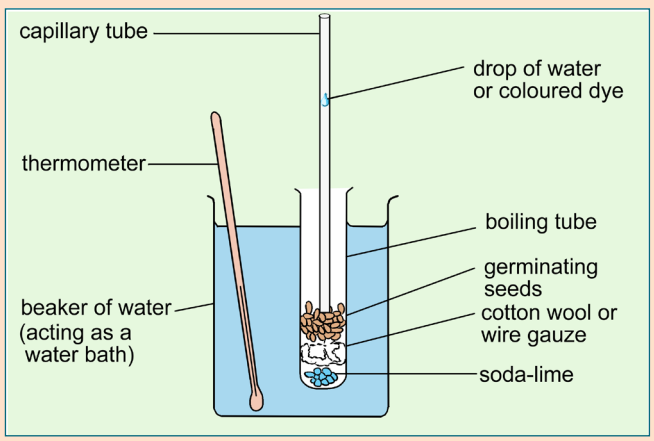
Application activity 2.2

1) Calculate the amount of energy produced by 5moles of glucose in kcal and kJ if one mole of glucose produce -686 kcal and 2,870 kJ per mole of glucose.
2) Specify the number of ATP produced by glycolysis during respiration process.

2.4 Measurement of respiration and respiratory quotients

Activity 2.3

- Set up the boiling tube so it is vertical and supported in a water bath such as a beaker.
- Use pea seeds that have been soaked for 24 hours and rinsed in 1% formaldehyde for 5 minutes.
- Kill an equal quantity of soaked seeds by boiling them for 5 minutes.
- Cool the boiled seeds in cold tap water; rinse them in bleach or formaldehyde for 5 minutes as before.
- Start with a water bath at about 20 °C and allow the seeds to adapt to that temperature for a few minutes before taking any readings.
- Record the initial and final positions of the water drop with a permanent marker with small label onto the glass.
- Measure the distance travelled by colored dye (or drop of water) with a ruler.
- Repeat the procedure (introducing a new bubble each time) at a range of different temperatures, remembering to allow time for the seeds to adapt to the new conditions before taking further readings.
- Interpret your observation. Follow the set up below:



The rate of respiration is measured by the use of respirometer device, typically by measuring oxygen consumed and the carbon dioxide given out. It can also be used to measure the depth and frequency of breathing, and allows the investigation on how factors such as; age, or chemicals can affect the rate of respiration. Currently, the computer technology is also used to automatically measure the volume of gases exchanged and drawing off small samples to analyze the proportions of oxygen and carbon dioxide in the gases.

The respiratory quotient (RQ) is the ratio of the volume of carbon dioxide produced to the volume of oxygen used in respiration during the same period of time. The RQ is often assumed to equal the ratio of carbon dioxide expired: oxygen inspired during a given time as it is summarized in the following formula:

$$RQ = \frac{\text{Volume of carbon dioxide given out}}{\text{Volume of oxygen taken in}}$$

The RQ is important as it can indicate whether the respiration is aerobic or anaerobic.

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O +$ Energy (ATP + heat).

As each molecule of gas occupies the same volume, this would give RQ = 1.0, and this is common for all carbohydrates. Further studies indicated the respiratory quotient to be 0.9 for proteins and 0.7 for fats, and concluded that an, RQ greater than 1.0 indicates anaerobic respiration, while RQ equals or less than 1.0 indicates aerobic respiration.

Note that respiration during germination, especially in early stages was also studied. Results indicated that it is difficult for oxygen to penetrate the seed coat, so that at this stage, the RQ is about 3 to 4. Later when the seed coat is shed, it becomes easier for oxygen to reach respiration tissues and the levels of RQ falls. Results indicated that eventually seeds with large carbohydrate stores have an RQ around 1.0 and those with large lipid stores have RQs of 0.7 to 0.8.

a. Measuring and obtaining the RQ values in invertebrate (e.g. woodlice)

In this particular respirometer, woodlice have been placed in a boiling tube which is connected to a U-tube. The U-tube acts as a manometer (a device for measuring pressure changes). The other end of the U-tube is connected to a control tube which is treated in exactly the same way as the first tube, except that it has no woodlice but instead glass beads which take up the same volume as the woodlice. The two boiling tubes (but not the manometer) are kept in water bath at constant temperature. The U-tube contains a colored liquid which moves according to the pressure exerted on it by the gases in the two boiling tubes. Both tubes contain potassium hydroxide solution which absorbs any carbon dioxide produced. The setup is summarized below:

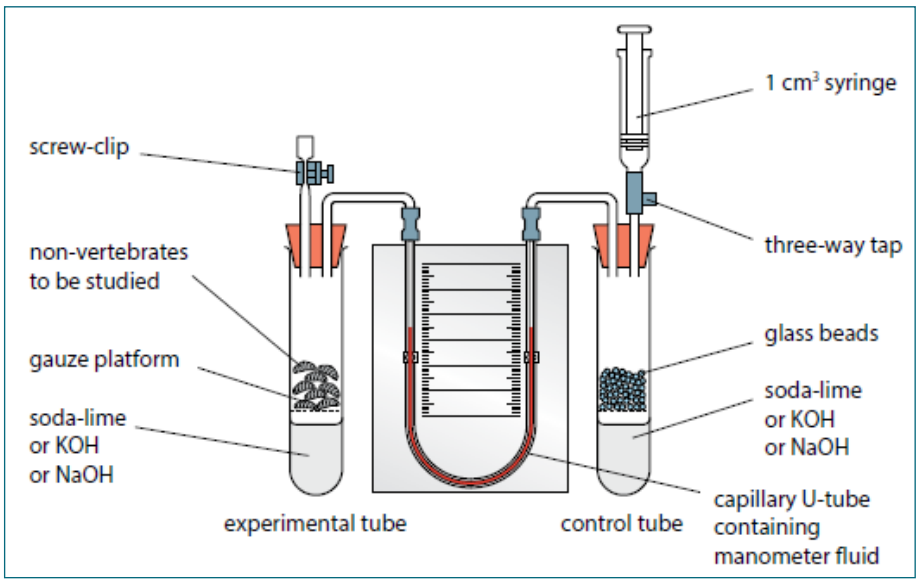


Figure 2.5: Simple experiment using respirometer to determine the RQ in germinating seeds

When the woodlice respire aerobically, they consume oxygen, which causes the liquid to move in the U- tube in the direction of arrows. The rate of oxygen consumption can be estimated by timing how long it takes for the liquid to rise through a certain height. The experiment can be repeated by replacing the potassium hydroxide solution with water. Comparing the changes in manometer liquid level with and without potassium hydroxide solution gives an estimate of carbon dioxide production can be used to measure the respiratory quotient.

If the internal radius of the manometer tube is known, the volumes of gases can be calculated using the equation:

Volume of gases = $\varpi r^2 h$,

Where ϖ is equal to 3.14**, r** is the internal radius of the tube and **h** is the distance moved by the liquid.

b. Measuring and obtaining the RQ values during seed germination process

During seed germination, CO_2 is released. To test its presence, chemicals including Sodium hydroxide or Potassium hydroxide are used due to their ability to absorb CO_2. As the germinating seeds use oxygen, pressure reduces in tube A so the manometer level nearest to the seeds rises (figure 2.8). The syringe is used to return the manometer fluid levels to normal. The volume of oxygen used is calculated by measuring the volume of gas needed from the syringe to return the levels to the original values. If water replaces the sodium hydroxide, then the carbon dioxide evolved can be measured. The setup is summarized below:

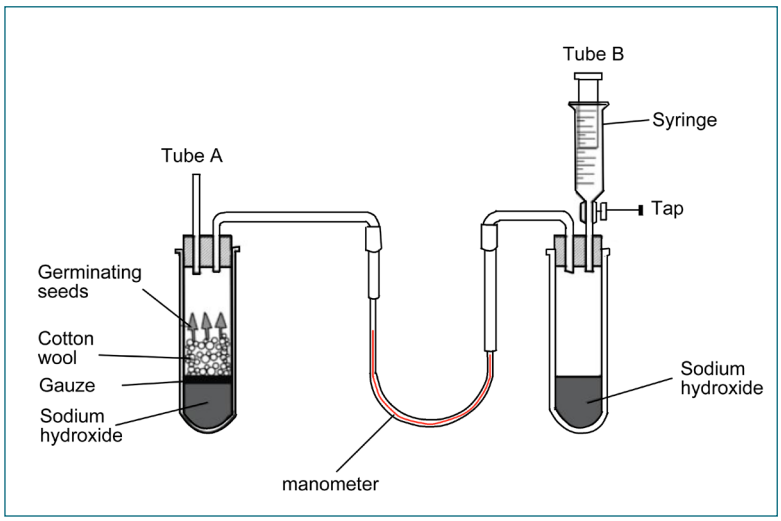


Figure 2.6: Simple experiment using respirometer to determine the RQ in germinating seeds

This graph suggests that the seed begins with carbohydrate as a metabolite, changes to fat/oil then returns to mainly using carbohydrate.

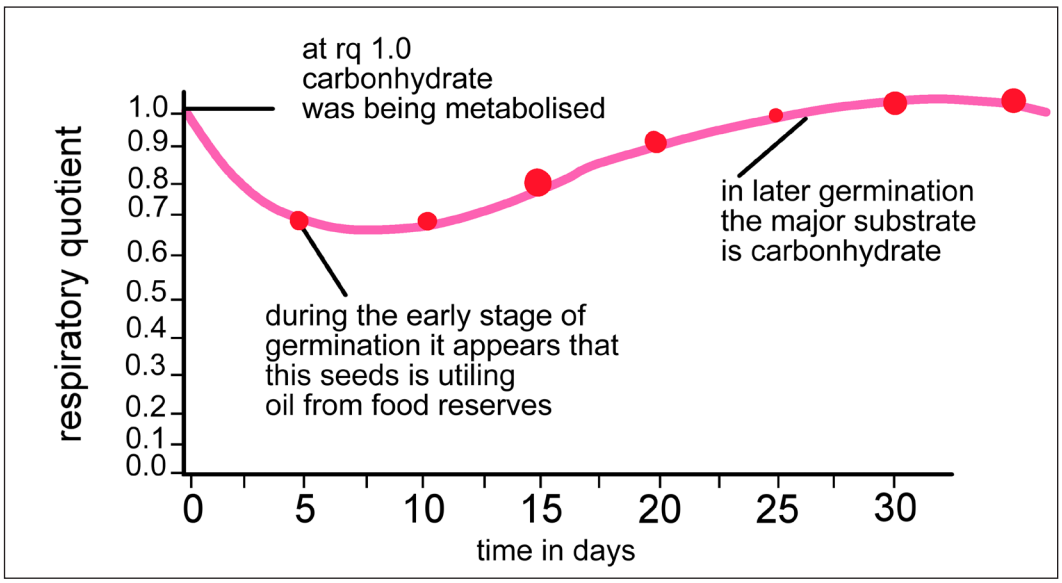


Figure 2.7: Graph showing the RQ values during seed germination

Application activity 2.4

1) Using the following equation of oleic acid (a fatty acid found in olive oil):

$$2C_{18}H_{34}O_2 + 51O2 \rightarrow 36CO_2 + 34H_2O$$

a) Calculate the RQ for the complete aerobic respiration.

b) Based on your findings, state which substrate is being respired

2) Measurements of oxygen uptake and carbon dioxide production by germinating seeds in a respirometer showed that 25 cm^3 of oxygen was used and 17.5 cm^3 of carbon dioxide was produced over the same time period.

i) Calculate the RQ for these seeds.

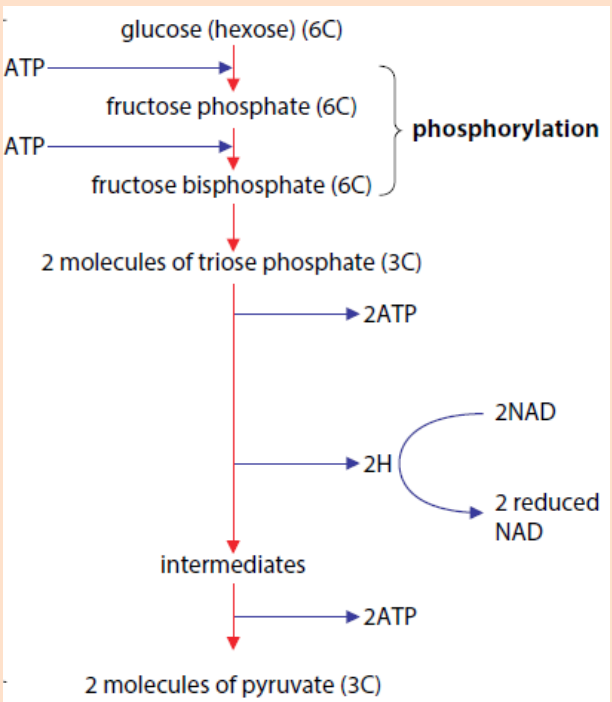
ii) Identify the respiratory substrate used by the seeds.

2.5 Aerobic respiration and Glycolysis

Activity 2.5

Glycolysis process

Observe the figure below and do the following activities



a) If this representation on figure above (→ATP) shows energy used and this (ATP→) represent energy produced during this process. Identify the energy used and energy produced then calculate net energy produced during this process.

b) According to your observation, what are the end products of this process above?

Cellular respiration is the complex process in which cells make adenosine triphosphate (ATP) by breaking down organic molecules. The energy stored in ATP can then be used to drive processes requiring energy, including biosynthesis, locomotion or transportation of molecules across cell membranes. The main fuel for most cells is carbohydrate, usually glucose which is used by most of the cells as respiratory substrate. Some other cells are able to break down fatty acids, glycerol and amino acids.

Glucose breakdown can be divided into four stages: **glycolysis, the link reaction, the Krebs cycle and oxidative phosphorylation.**

Glycolysis is the splitting or lysis of a glucose molecule. It is a multi-step process in which a glucose molecule with six carbon atoms is eventually split into two molecules of pyruvate, each with three carbon atoms. Energy from ATP is needed in the first steps, and it is released in the later steps to synthesize ATP. There is a net gain of two ATP molecules per molecule of glucose broken down.

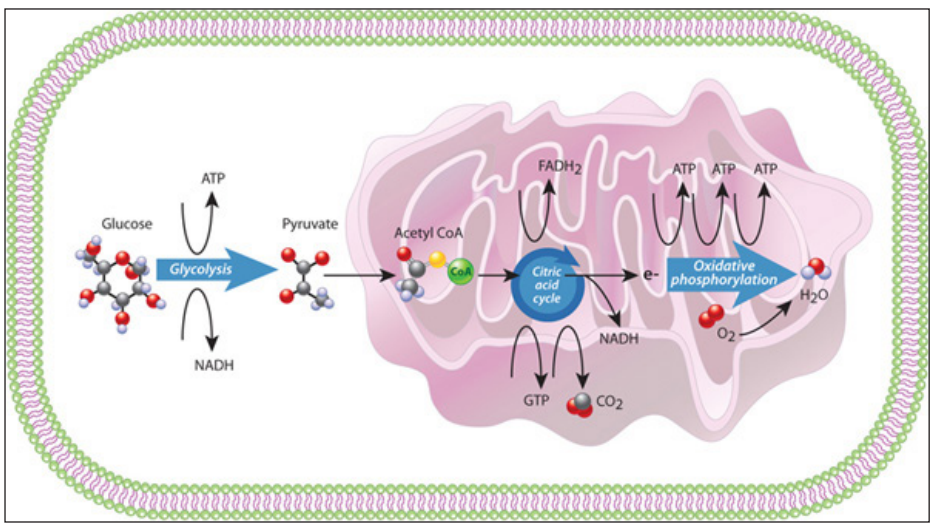


Figure 2.8: Metabolism in a eukaryotic cell: Glycolysis, the citric acid cycle, and oxidative phosphorylation

Glycolysis takes place in the cytoplasm of a cell. Within the mitochondrion, the citric acid cycle occurs in the mitochondrial matrix, and oxidative metabolism occurs at the internal folded mitochondrial membranes (cristae). Glucose enters

the cell and is phosphorylated by the enzyme called hexokinase, which transfers a phosphate group from ATP to the sugar. The ATP used in this process has 2 advantages: the charge of the phosphate group traps the sugar in the cell because the plasma membrane is impermeable to large ions. Phosphorylation also makes glucose more chemically reactive. Even though glycolysis consumes two ATP molecules, it produces a gross of four ATP molecules (4 ATP), and a net gain of two ATP (2 ATP) molecules for each glucose molecule that is oxidized. Glycolysis results in a net gain of two ATP (2ATP), two NADH and two pyruvate molecules

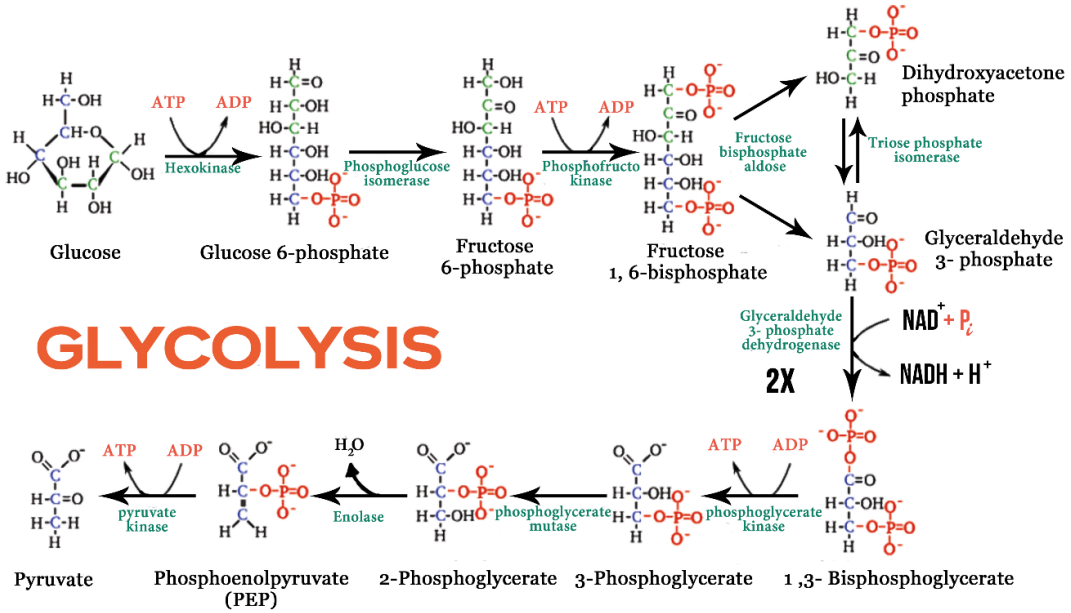


Figure 2.9: Reactions of glycolysis

Application activity 2.5

1) Why is ATP needed for glycolysis?
2) How many gross ATP molecules are produced during glycolysis from one glucose molecule?
3) How many NADH are made during glycolysis?
4) The following flowchart summarizes the reactions that take place in glycolysis

   Glucose → 2 × glyceraldehydes 3-phoshate → 2 × pyruvate

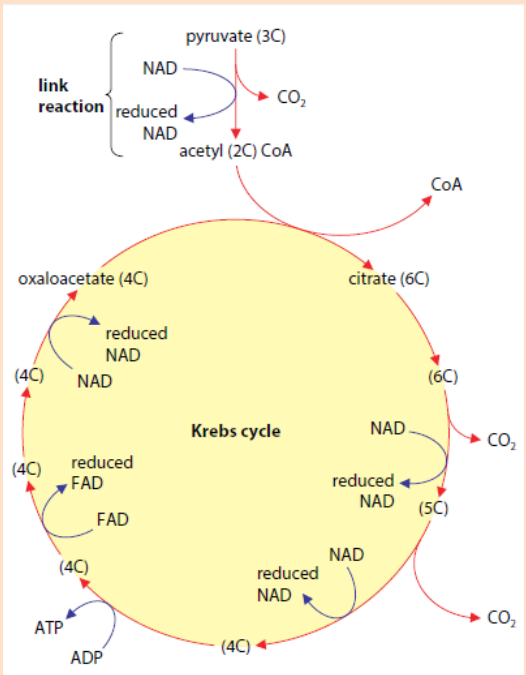
   a) How many carbon atoms are there in glucose, glyceraldehydes 3-phoshate and pyruvate?

   b) What is the net gain of ATP in glycolysis?

2.6 Link reaction and Krebs cycle (TCA cycle)

Activity 2.6

Use the figure below and do the following activities:



a) The above figure summarizes two stages that take place during respiration, observe it and identify the number of CO_2, ATP, reduced FAD and reduced NAD.

b) Knowing that the above stages involve two molecule of pyruvates calculate the total number of CO_2, ATP, reduced FAD and reduced NAD.

2.6.1 Link reaction

Pyruvate, the end product of glycolysis is oxidized to Acetyl-CoA by enzymes located in the mitochondrion of eukaryotic cells as well as in the cytoplasm of prokaryotes. In the conversion of pyruvate to Acetyl-CoA, one molecule of NADH and one molecule of CO_2 are formed (Figure 2.10). This step is also known as the link reaction or transition step, as it links glycolysis to the Krebs cycle.

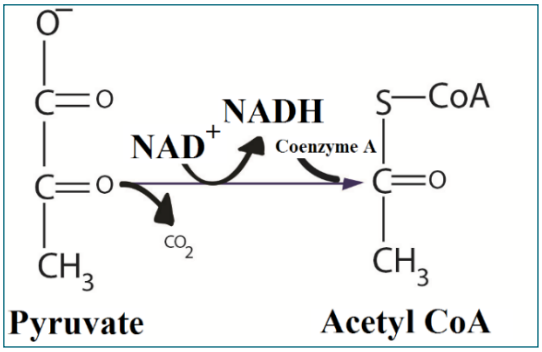


Figure 2.10: Link reaction between glycolysis and Krebs cycle

Krebs cycle (Citric acid cycle)

The coenzyme has a sulphur atom, which attaches the acetyl fragment by an unstable bond. This activates the acetyl group for the first reaction of the Krebs cycle also called citric acid cycle or Tricarboxylic Acid Cycle (TCA). It is also known as the citric acid cycle, because the first molecule formed when an acetyl group joins the cycle. When oxygen is present, the mitochondria will undergo aerobic respiration which leads to the Krebs cycle.

In the presence of oxygen, when acetyl-CoA is produced, the molecule then enters the citric acid cycle inside the mitochondrial matrix, and gets oxidized to CO_2 while at the same time reducing NAD+ to NADH. NADH can then be used by the electron transport chain to create more ATP as part of oxidative phosphorylation. For the complete oxidation of one glucose molecule, two Acetyl-CoA must be metabolized by the Krebs cycle. Two waste products namely H_2O and CO_2, are released during this cycle.

The citric acid cycle is an 8-step process involving different enzymes and co-enzymes. Throughout the entire cycle, Acetyl-CoA (2 carbons) combines with oxaloacetate (4 carbons) to produce citrate. Citrate (6 carbons) is rearranged to a more reactive form called isocitrate (6 carbons). Isocitrate (6 carbons) is modified to α-Ketoglutarate (5 carbons), Succinyl-CoA, Succinate, Fumarate, Malate, and finally to Oxaloacetate. The net energy gain from one cycle is 3 NADH, 1 $FADH_2$, and 1 Guanosine Triphosphate (GTP). The GTP may subsequently be used to produce ATP. Thus, the total energy yield from one whole glucose molecule (2 pyruvate molecules) is 6 NADH, 2 $FADH_2$, and 2 ATP. 2 molecules of carbon dioxide are also produced in one cycle (for a total of 4 molecules of carbon dioxide from one glucose molecule).

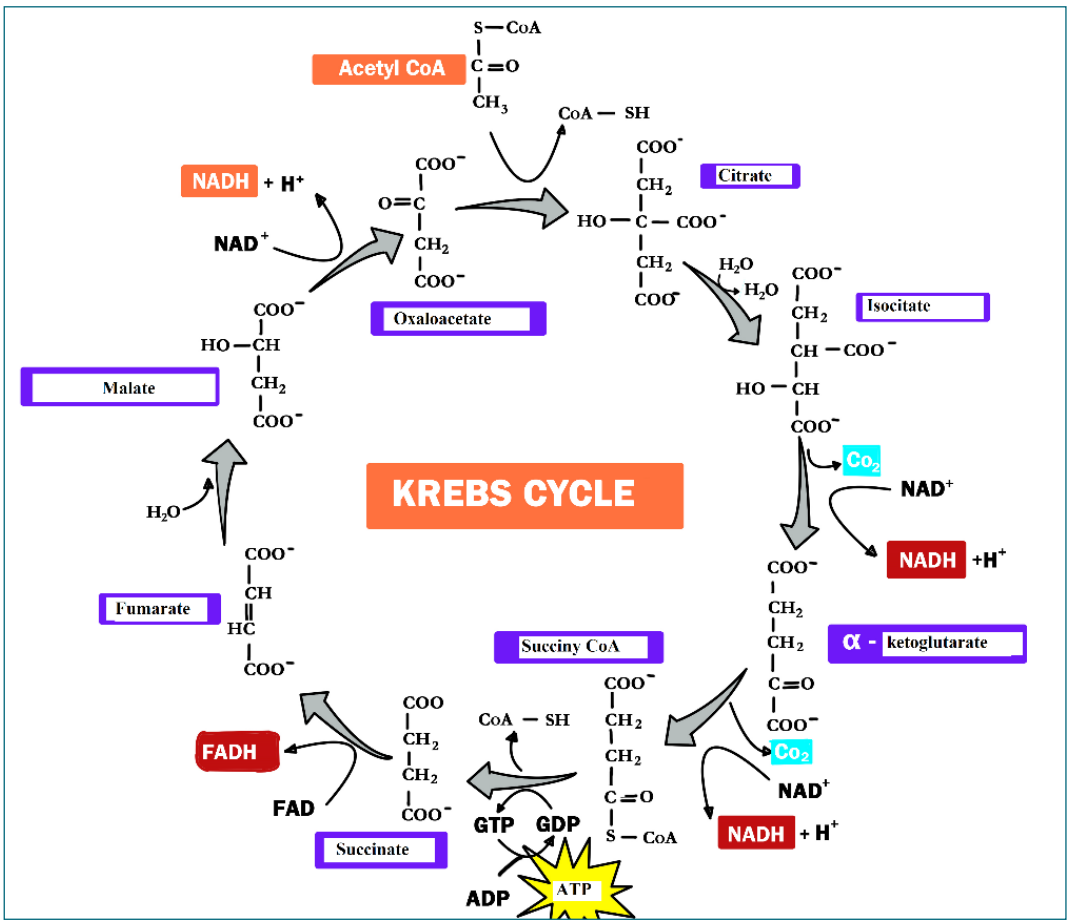


Figure 2.11: Krebs cycle

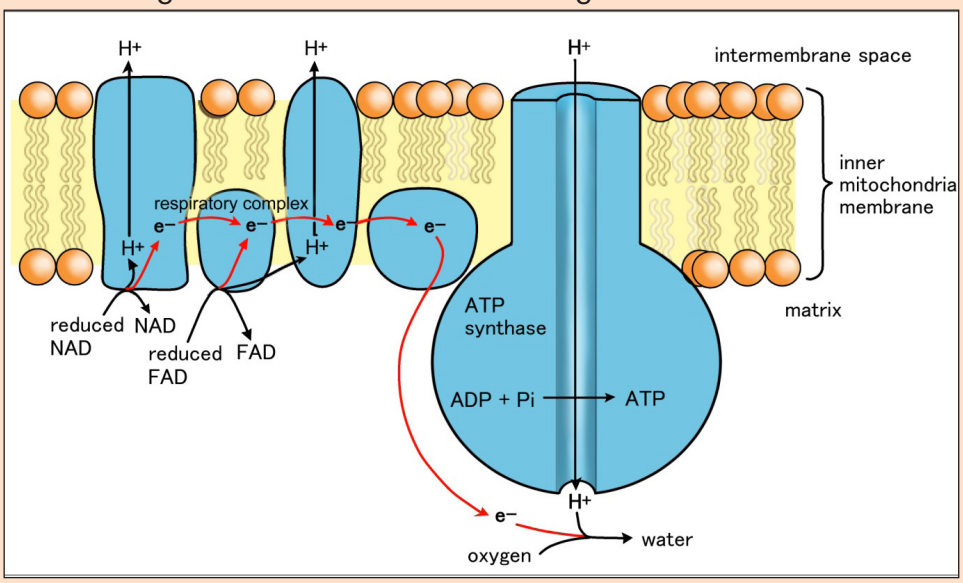
Application activity 2.6

1) Use the chemical equation to show the conversion of pyruvate into acetyl-coA.
2) Identify and note the main products of the Krebs cycle from one glucose molecule

2.7 Oxidative phosphorylation

Activity 2.7

Observe the figure below and do the following activities



a) This figure summarizes last stage that take place during cellular respiration, observe it and identify the role of reduced NAD, reduced FAD and oxygen in this stage.

b) Give the explanation of the above figure.

In the final stage of aerobic respiration known as the **oxidative phosphorylation**, the energy for the phosphorylation of ADP to ATP comes from the activity of the electron transport chain. Oxidative Phosphorylation is the production of ATP using energy derived from the redox reactions of an electron transport chain.

In eukaryotes, oxidative phosphorylation occurs in the mitochondrial cristae. It comprises the electron transport chain that establishes a proton gradient across the inner membrane by oxidizing the NADH produced from the Krebs cycle. ATP is synthesized by the ATP synthase enzyme when the chemiosmosis gradient is used to drive the phosphorylation of ADP. Chemiosmosis is the production of ATP from ADP using the energy of hydrogen ion gradients. The electrons are finally transferred to oxygen and, with the addition of two protons, water is formed. The average ATP yield per NADH is probably 3 and for $FADH_2$ of this electron carrier is worth a maximum of only two molecules of ATP.

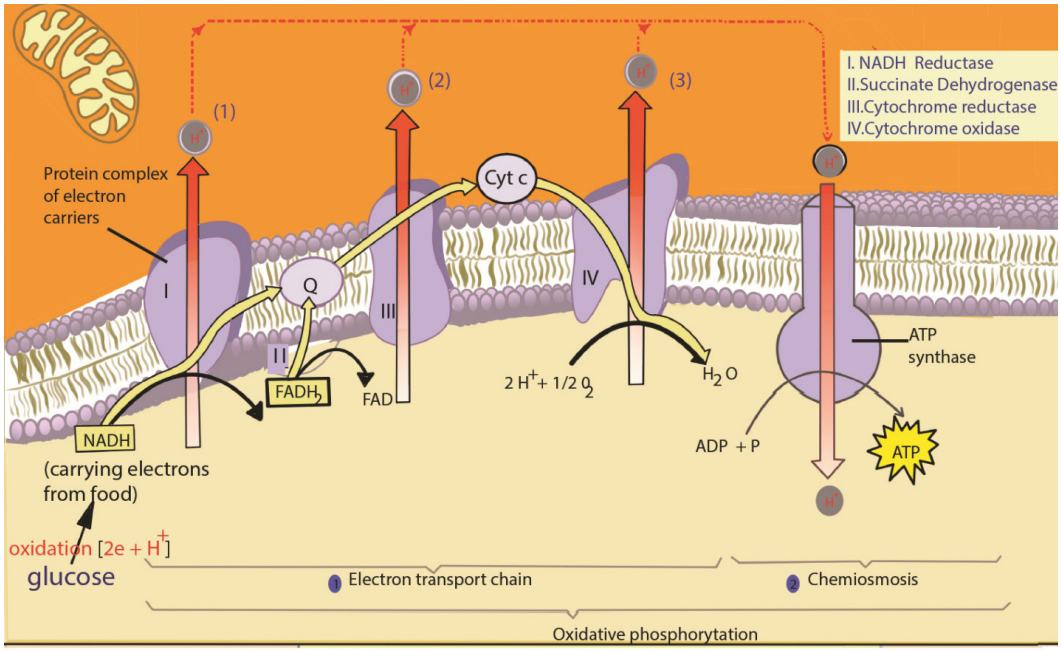


Figure 2.12: Electron transport chain

Role of oxygen in chemiosmosis

ATP can be synthesized by chemiosmosis only if electrons continue to move from molecule to molecule in the electron transport chain. Oxygen serves as the final acceptor of electrons. By accepting electrons from the last molecule in the electron transport chain, and allows additional electrons to pass along the chain. As a result, ATP can continue to be synthesized. Oxygen also accepts the protons that were once part of the hydrogen atoms supplied by NADH and $FADH_2$. By combining with both electrons and protons, oxygen forms water as shown in the following equation:

$$O_2 + 4e^- + 4H^+ = \rightarrow 2H_2O$$

Overview of aerobic respiration

A considerable number of ATP is produced during oxidative phosphorylation and it is estimated between 32 and 34 ATPs. These are added to 2 ATP produced during glycolysis and 2 ATP produced during citric cycle. The total number of ATP produced during a complete respiration process for one molecule of glucose is then estimated between 36 and 38 ATPs.

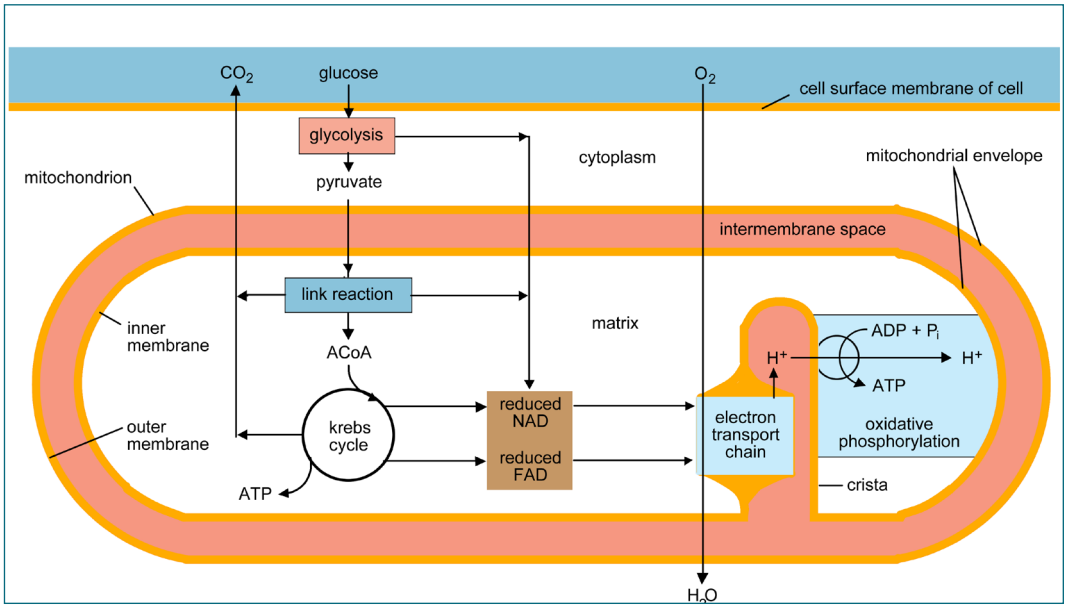


Figure 2.13: Summary of aerobic respiration

Note that the amount of ATP produced from glucose is usually less than 38 ATP for the following reasons: some ATP is used to transport pyruvate from the cytoplasm into the mitochondria and some energy is used to transport NADH produced in glycolysis from the cytoplasm into the cristae of mitochondria.

Overall net gain of energy from glucose

Step in aerobic respiration	Location	Net gain of ATP
1. Glycolysis	Cytoplasm	2 ATP
2.Link stage or decarboxylation	Cytoplasm	0 ATP
3.Tricarboxilic acid cycle or Krebs cycle	Matrix of mitochondrion	2 ATP
4. Electron transfer chain or Respiratory chain	Cristae of mitochondrion	34 ATP
Total number of ATP gained	_____	38 ATP

Application activity 2.7

1) a) How many ATP are formed from 1 NADH?

   b) How many ATP are formed from 1 FADH?

2) How many ATP are formed after a complete oxidation of one glucose molecule.

2.8 Efficiency of aerobic respiration

Activity 2.8

During the complete oxidation of a molecule of glucose it is estimated to produce 686Kcal. Knowing that inside the cell each ATP produced is equivalent to 7.3 Kcal,

Considering all the amount of ATP produced, find out the percentage of energy that is equivalent to amount of total ATP produced during aerobic respiration. Use below formula for your calculations:

$$\text{Efficient of aerobic respiration} = \frac{\text{Energy required to make ATP X 100}}{\text{Energy released by oxidation of glucose}}$$

The complete oxidation of glucose produces the energy estimated at 686 Kcal. Under the condition that exists inside most of the cells, the production of a standard amount of ATP from ADP absorbs about 7.3 Kcal. Glucose molecule can generate up to 38 ATP molecules in aerobic respiration. The efficiency of aerobic.

$$\text{Efficient of aerobic respiration} = \frac{\text{Energy required to make ATP X 100}}{\text{Energy released by oxidation of glucose}}$$

$$= \frac{38 \text{ ATP X } 7.3 \text{ Kcal X } 100}{687 \text{ Kcal}} = 40\%$$

This result indicates that the efficiency of aerobic respiration equals 40%. The remained energy (around 60%) is lost from the cell as heat.

Application activity 2.8

1) 1. Under which conditions can aerobic respiration take place in animal cells?
2) 2. Calculate the efficiency aerobic respiration, when a complete oxidation of glucose produce the energy estimated at 500Kcal under a production of a standard amount of ATP from ADP absorbed is about 7.3 Kcal.

2.9 Efficiency of anaerobic respiration

Activity 2.9

Anaerobic respiration in yeast

a) Boil some water to expel all the dissolved oxygen.

b) When cool, use the boiled water to make up a 5% solution of glucose and a 10% suspension of dried yeast.

c) Place 5 Cm^3 of the glucose solution and 1 Cm^3 of the yeast suspension in a test-tube and cover the mixture with a thin layer of liquid paraffin to exclude atmospheric oxygen

d) Fit a delivery tube as shown in figure below and allow it to dip into clear limewater.

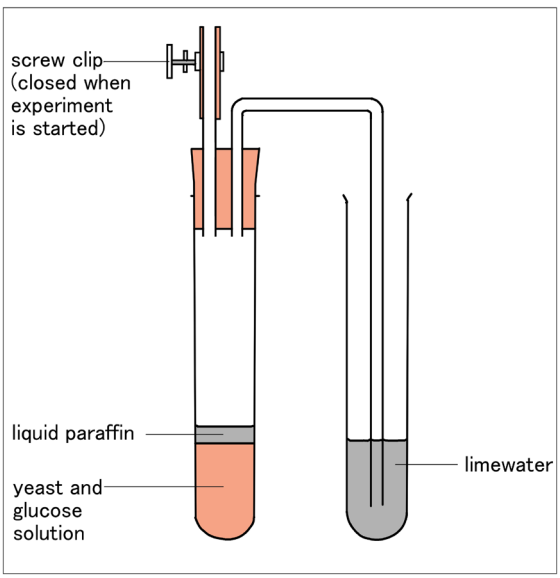


Figure 12.6 Experiment to show anaerobic respiration in yeast

Observe the change that takes place in test tube containing, then explain the bases of such change.

Without oxygen, pyruvate (pyruvic acid) is not metabolized by cellular respiration but undergoes a process of fermentation. The pyruvate is not transported into the mitochondrion, but it remains in the cytoplasm, where it is converted to waste products like alcohol or lactic acid or other compounds depending on the kind of cells that are active which may be removed from the cell. This serves the purpose of oxidizing the electron carriers so that they can perform glycolysis again and removing the excess pyruvate. Fermentation oxidizes NADH to NAD^+ so it can be re-used in glycolysis.

In the absence of oxygen, fermentation prevents the build-up of NADH in the cytoplasm and provides NAD^+ for glycolysis. This waste product varies depending on the organism. In skeletal muscles, the waste product is lactic acid. This type of fermentation is called lactic acid fermentation. In yeast and plants, the waste products are ethanol and carbon dioxide. This type of fermentation is known as alcoholic or ethanol fermentation. The ATP generated in this process is made by substrate-level phosphorylation, which does not require oxygen.

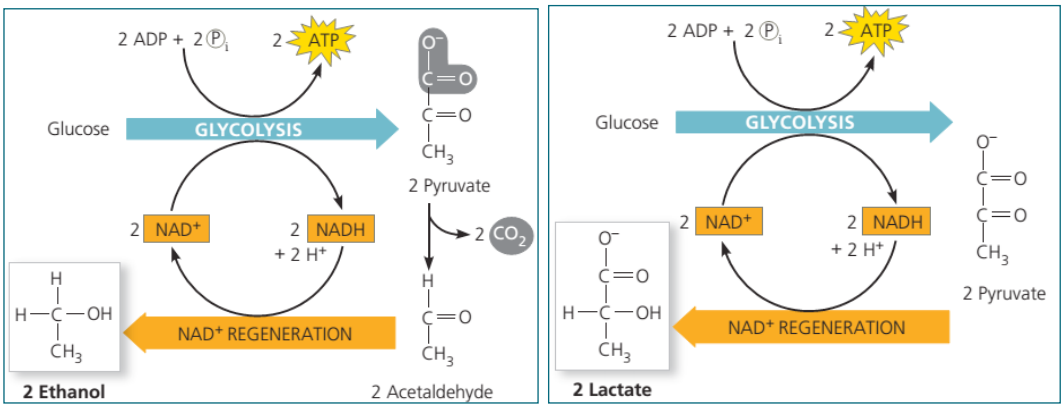


Figure 2.14: Alcoholic and lactic fermentation

Fermentation is less efficient at using the energy from glucose since only 2 ATP are produced per glucose, compared to the 38 ATP per glucose produced by aerobic respiration. This is because the waste products of fermentation still contain plenty of energy. Glycolytic ATP, however, is created more quickly.

Due to the fact that anaerobic respiration produces only 2 ATP, the efficiency of anaerobic respiration is less than that of aerobic respiration. It is calculated as follows:

Efficiency of aerobic respiration = Energy required to make ATP x 100 Energy released by oxidation of glucose 2 ATP x 7.3 Kcal x 100 687 Kcal =2%.

The production of a small yield of ATP from anaerobic respiration in yeast and mammalian muscle tissue, including the concept of oxygen debt.

Standing still, the person absorbs oxygen at the resting rate of 0.2 $dm^3\ min^{-1}$. (This is a measure of the person's metabolic rate.) When exercise begins, more oxygen is needed to support aerobic respiration in the person's muscles, increasing the overall demand to 2.5 $dm^3\ min^{-1}$. However, it takes four minutes for the heart and lungs to meet this demand, and during this time lactic fermentation occurs in the muscles. Thus the person builds up an oxygen deficit. For the next three minutes, enough oxygen is supplied. When exercise stops, the person continues to breathe deeply and absorb oxygen at a higher rate than when at rest. This post-exercise uptake of extra oxygen, which is 'paying back' the oxygen deficit, is called the oxygen debt.

The oxygen is needed for:

- Conversion of lactate to glycogen in the liver
- Re-oxygenation of haemoglobin in the blood
- A high metabolic rate, as many organs are operating at above resting levels.

The presence of the lactic acid is sometimes described as an "**oxygen debt"**. This is because significant quantities of lactic acid can only be removed reasonably quickly by combining with oxygen. However, the lactic acid was only formed due to lack of sufficient oxygen to release the required energy to the muscle tissue via aerobic respiration. Lactic acid can accumulate in muscle tissue that continues to be over-worked. Eventually, so much lactic acid can build-up that the muscle ceases working until the oxygen supply that it needs has been replenished, this is called muscle cramps

To repay such an oxygen debt, the body must take in more oxygen in order to get rid of the additional unwanted waste product lactic acid. Mineral depletion, inadequate blood supply and Nerve compression can be the causes of muscle cramps.

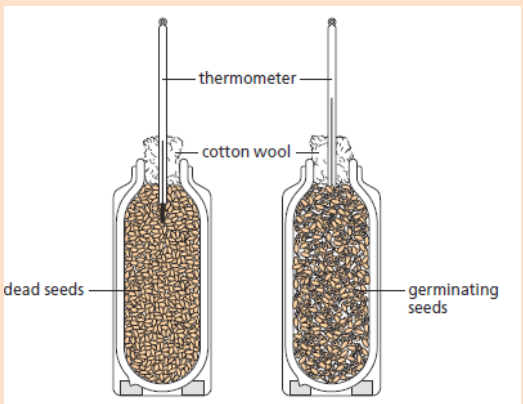
Application activity 2.9

1) Under which conditions can anaerobic respiration take place in animal cells?
2) Calculate the efficiency of anaerobic, when a complete oxidation of glucose produce the energy estimated at 200 Kcal under a production of a standard amount of ATP from ADP absorbed is about 7.3 Kcal

2.10 Factors which affect the rate of respiration

Activity 2.10

- Fill a small vacuum flask with beans grains or pea seeds that have been soaked for 24 hours and rinsed in 1% formaldehyde for 5 minutes.
- Kill an equal quantity of soaked seeds by boiling them for 5 minutes.
- Cool the boiled seeds in cold tap water, rinse them formaldehyde for 5 minutes as before and then put them in a vacuum flask of the same size as the first one.
- Place a thermometer in each flask so that its bulb is in the middle of the seeds.
- Plug the mouth of each flask with cotton wool and leave both flasks for 2 days, noting the thermometer readings whenever possible. Set it as follow:



a) What is the purpose of soaking seeds for 24 hours and in formaldehyde for 5 minutes.

b) Why do you need flask containing dead seeds?

c) Compare the temperature change in those two flasks and explain those changes.

Cellular respiration is the process of conversion of chemical energy stored in the food to ATP or higher energy compounds. The factors that affect the cellular respiration are:

a. Amount of nutrients

If the amount of nutrients is high, then the energy is high in the cellular respiration. The nutrients which can go through cellular respiration and transform into

energy are fat, proteins and carbohydrates. The amount of nutrients available to transform into energy depend upon the diet of the person.

b. Temperature

The rate of the cellular respiration increases if the body temperature is warmer. The lower the temperature, the slower the rate of cellular respiration. The reason for this is enzymes which are present in cellular respiration process. Enzyme reactions require optimum temperatures.

c. State of the cell

Metabolically active cells such as neurons, root of human hair have higher respiration rate than the dormant cells such as skin cells and bone cells. This is because metabolically active cells can store energy in the body because of the many metabolic reactions that take place in them.

d. Water

It is the medium where the reaction happens. When a cell is dehydrated the respiration and other metabolism decreases.

e. Cellular activity

Some cells need more energy than others. For example, growing cells or very active cells such as neurons need a lot of energy.

f. O_2 /CO_2 content

When there is high mount of O_2 and lower amount of CO_2 there is increase of the rate of respiration. This is because oxygen is needed during aerobic respiration.

g. ATP/ADP range

When there is more ATP than ADP, respiration rate slows down to avoid excess of ATP.

Application activity 2.10

1) Explain how proteins and lipids are metabolized for energy during respiration
2) Explain why the body does not use fats to produce energy as carbohydrates given that they produce much energy than carbohydrates.

2.11 Use of other substrates in respiration.

Activity 2.11

When someone has eaten carbohydrates such as cassava and sweet potatoes you do not feel hungry in the same time as another one who has consumed milk or cheese.

1) Can you suggest the reason for this?
2) Which one can take a short time for digestion and why?

Carbohydrates are the first nutrients that most organisms can catabolize for energy. In some cases, living things must be able to metabolize other energy-rich nutrients to obtain energy in times of starvation. Most organisms possess metabolic pathways that, when necessary, metabolize proteins, lipids. In each case, the larger molecules are first digested into their component parts, which the cell may reassemble into macromolecules for its own use. Otherwise, they may be metabolized for energy by feeding into various parts of glycolysis or the Krebs cycle

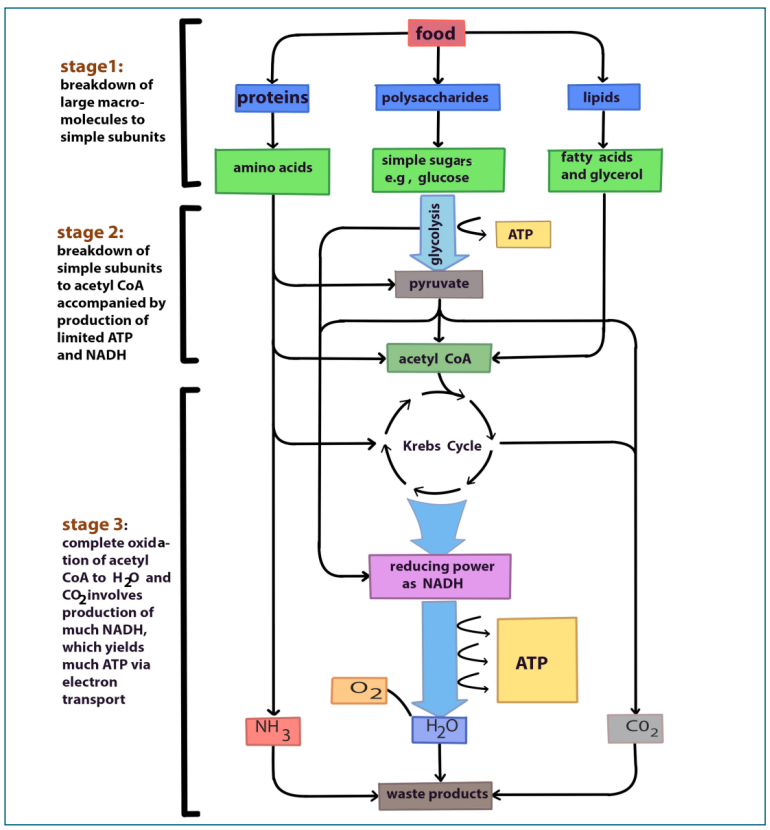


Figure 2.15: Oxidation of different organic substrates

Carbohydrates, fats and proteins can all be used for cellular respiration. Monomers of these foods enter glycolysis or the Krebs cycle at various points. Glycolysis and the Krebs cycle are catabolic pathways through which all kinds of food molecules are channeled to oxygen as their final acceptor of electrons.

Application activity 2.11

1) Explain how proteins and lipids are metabolized for energy during respiration
2) Explain why the body does not use fats to produce energy as carbohydrates given that they produce much energy than carbohydrates.

Skill lab 2

Fried breads are slices of bread that have been fried in oil or butter.

1) On a sheet of paper write down the ingredients used to make fried bread.
2) Write down all requirement to make fried bread.
3) Investigate the procedures and make your own fried bread according to that procedures investigated.
4) Compare your fried bread with the one sold in shops.
5) Present some samples to your teacher.

End unit assessment 2

1. Explain the reasons why chemical energy is the most important type of energy for living organisms.

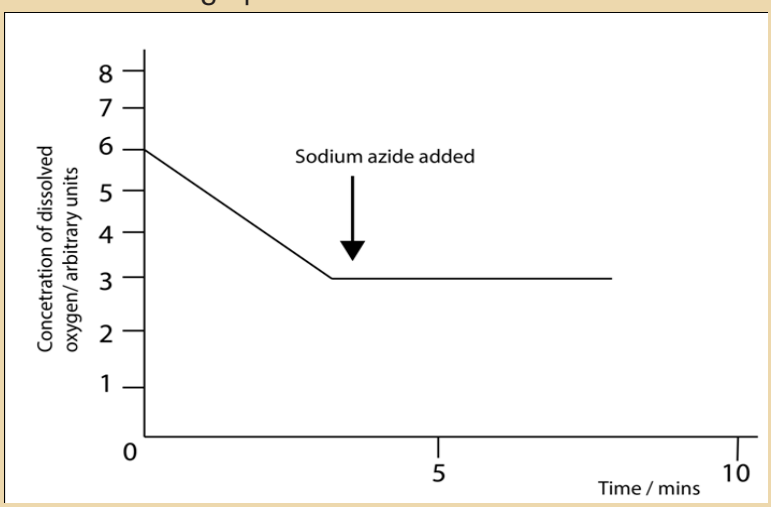
2. Why do all organisms need energy and where does this energy come from?

3. Give the structure of ATP and specify its importance to living organisms?

4. The equation $\mathbf{C_{57}H_{104}O_6 + 80O_2 \rightarrow 57CO_2 + 52H_2O + Energy}$ represents oxidation of lipids. Calculate RQ for this equation.

5. Calculate the total amount of energy produced for:
   a) 3 moles of hydrolysed ATP
   b) moles of synthesized ATP
   c) 5 moles of decomposed glucose

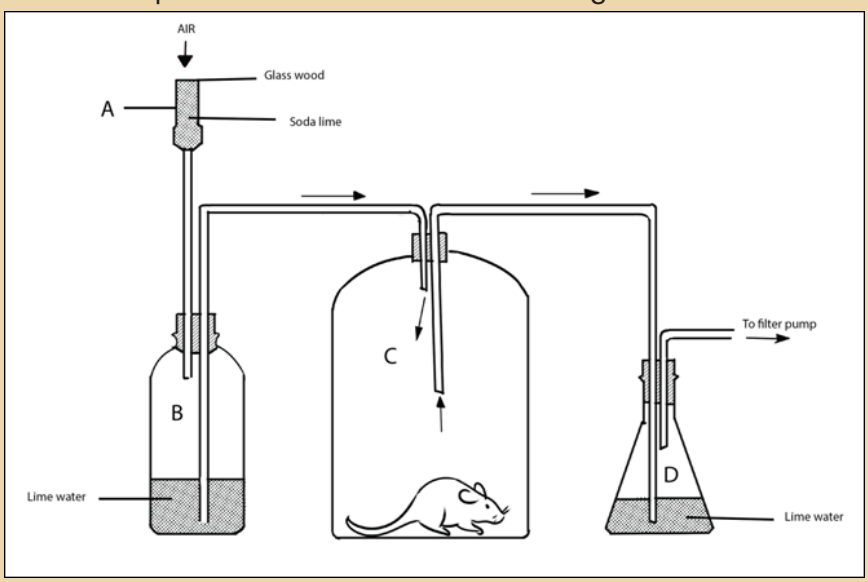
6. Active mitochondria can be isolated from liver cells. If these mitochondria are then incubated in a buffer solution containing a substrate, such as succinate, dissolved oxygen will be used by mitochondria. The concentration of dissolved oxygen in the buffer solution can be measured using an electrode. When this experiment was done, the concentration of dissolved oxygen was measured every minute for five minutes. Sodium azide (NaN_3) which combines with cytochromes and prevents electron transport was added thereafter. The results are shown in the graph below.



   a) Suggest what effect the addition of sodium azide will have on the production of ATP and give an explanation for your answer.
   b) Explain why the concentration of oxygen decreased during the first five minutes.

c) Suggest what effect the addition of sodium azide will have on the production of ATP and give an explanation for your answer

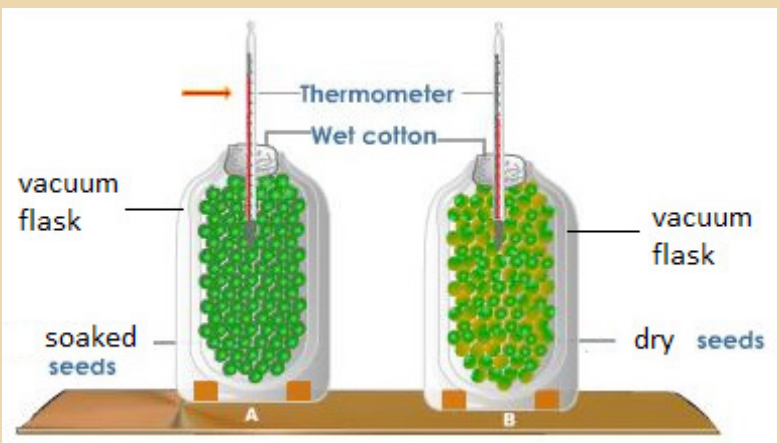
7. During an experiment, the mouse was inside the bell jar. The air pipe from the bell jar was connected to the first beaker containing lime water and filter pump. The glass wool containing soda lime covered by a piece of paper was connected to the second beaker by air pipe. Another air pipe was connected from the second beaker containing lime water to the belly jar in the first step. The set of the experiment looked like the following:



8. What are the major differences between cellular respiration and photosynthesis?

9. Compare aerobic respiration with anaerobic respiration or fermentation.

10. A student set up an experiment using germinating seeds and boiled seeds as shown in the diagram below:



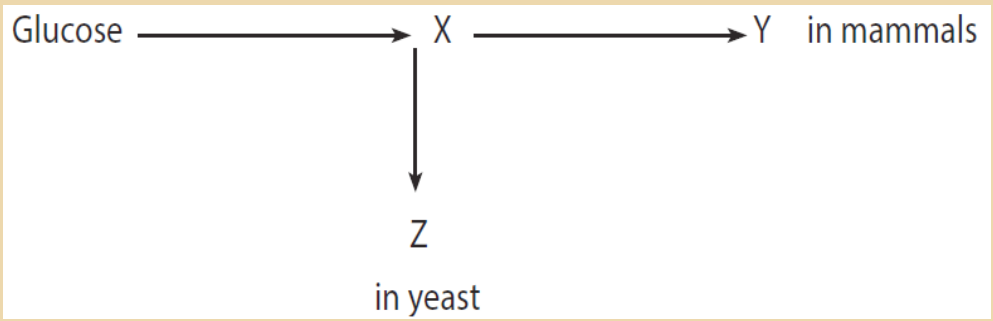
a) State the objective of this experiment and the observation made after 24 hours?

b) Account for the observation made in (a) above?

c) Suggest why vacuum flasks were used in the experiment?

d) What was the purpose of the set-up B?

11) The diagram summarizes how glucose can be used to produce ATP, without the use of oxygen



Which compounds are represented by the letters X, Y and Z?

12) Complete the table below:

	Input(s)	Output(s)	Location in cell/organelle
Glycolysis			
Fermentation			
Citric acid cycle			
Respiratory chain			

UNIT 3

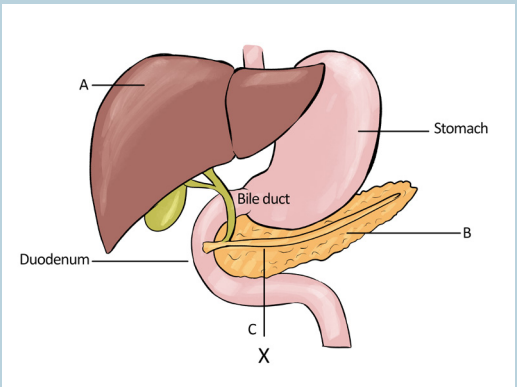
REGULATION OF GLUCOSE LEVEL AND TEMPERATURE

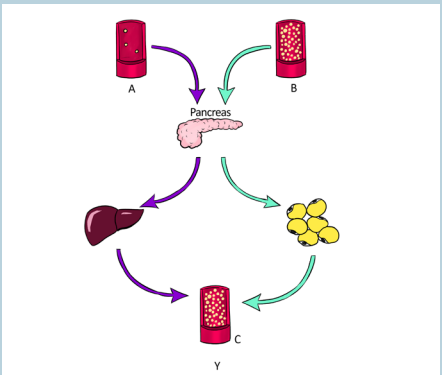
Key unit competence

Explain the mechanism of the regulation of blood glucose levels and regulation of temperature in living organisms

Introductory activity 3

The human body maintains constant different substances in the blood, a process called homeostasis. The figures below show different organs involved in the regulation of blood glucose level in the body.





Observe the illustrations X and Y above and answer to the questions that follow:

a) What are the parts represented by the letters A, B and C on the illustration X?

b) All the organs shown in the illustration X are involved in the digestion of food. What are the functions of A and B in the digestion?

c) What are the organs involved in the regulation of blood glucose level on the illustration X? In which way does each organ state help in this regulation?

d) The illustration Y shows the regulation of blood glucose level. What does the letters A, B and C show in this regulation?

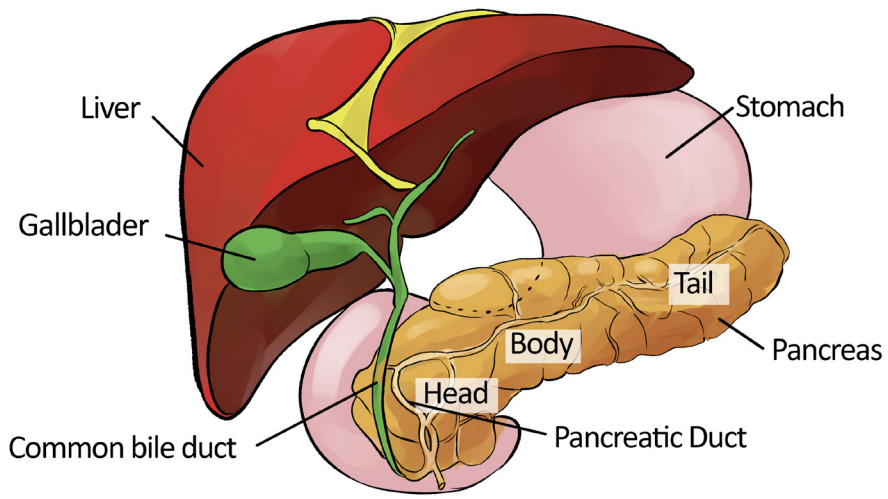
e) Alpha and beta cells are responsible for producing the hormones that are involved in the regulation of blood glucose level. Which organ on the illustration Y produces these hormones?

f) Compare the mechanism of working of the organs A and B in the regulation of blood glucose level.

3.1 Structure and functions of the liver and the pancreas

Activity 3.1

Each organ of our body is made of different tissues which are also composed of cells. These cells carry out different functions that help in the functioning of the organ. Refer to the image below to answer the questions that follow:



a) Observe the liver and the pancreas and make short notes on their structures.

b) What are the functions of the liver and the pancreas?

c) Which hormones are produced by the pancreas and what are their functions?

d) Compare the modes of action of insulin and glucagon.

e) Examine what happens when the blood glucose regulation fails?

3.1.1 Importance of glucose

Glucose is one of the most important carbohydrates molecules in our body. Body requires glucose to carry out some of its most important functions. Glucose is synthesized in green plants, from carbon dioxide, CO_2 and water, H_2O with the help of energy from sunlight. This process is known as **photosynthesis**. The reverse of the photosynthesis reaction i.e., breakdown of glucose in the presence of oxygen to form carbon dioxide and water releasing the energy, is the main source of power for all the living organisms. The excess glucose in plants is stored in the form of starch which serves as foods for various animals.

Glucose as an energy source

Almost 80 per cent of carbohydrates in our food are converted to glucose during digestion in the alimentary canal. Fructose and galactose is the other main product of carbohydrates digestion. After absorption from the alimentary tract, fructose and galactose are converted into glucose in the liver. And therefore, glucose constitutes more than 95 per cent of all the carbohydrates circulating in the blood.

Body cells require glucose continuously for its various metabolic activities. These cells directly absorbed glucose from the blood. Once inside the cells, glucose combines with a phosphate moiety to form **Glucose-6-phosphate** with the help of enzyme **glucokinase** in liver and **hexokinase** in most other cells. This phosphorylation reaction is irreversible and helps to retain the glucose inside the cells. However, in liver cells, renal tubular epithelial cells and intestinal epithelial cells, an enzyme **glucose phosphatase** converts the glucose-6-phosphate back to glucose.

The complete oxidation of one molecule of glucose into carbon dioxide and water inside the cells produces as many as **38 molecules of ATP** (2 from glycolysis, 2 from the Krebs cycle and 34 from the oxidative phosphorylation).

3.1.2 Role of liver and pancreas in glucose regulation

Our body maintains a narrow range of glucose concentration in the blood between 80 mg/dL to 120 mg/dL which may increase up to 180 mg/dL after a meal containing high amount of carbohydrates. The hormones responsible for the regulation of blood sugar level— insulin and glucagon are secreted by the pancreas. The excess glucose in our blood is converted into glycogen in the liver. Therefore, pancreas and liver play a vital role in the regulation of blood sugar concentration.

Role of liver in glucose regulation

The liver is the largest internal solid organ in the body second to the skin as the largest organ overall. It performs various functions in our body, including synthesis and storage of proteins and fats, carbohydrates metabolism, formation and secretion of bile, detoxification and excretion of potentially harmful compounds. Liver contains two main cell types: Kupffer cells and Hepatocytes.

1) **Kupffer cells** are a type of macrophage that capture and break down old, worn out red blood cells passing through liver sinusoids.
2) **Hepatocytes** are cuboidal epithelial cells that line the sinusoids and make up the majority of cells in the liver. Hepatocytes perform most of the liver's functions—metabolism, storage, digestion, and bile production.

Hepatocytes cells contain various enzymes which help in the regulation of blood glucose.

These are:

1) **Glycogen synthase**; responsible for glycogen biosynthesis (Glycogenesis). When the concentration of glucose in the blood increases beyond the normal value, the excess glucose is converted to glycogen in liver with the help of enzyme glycogen synthase.
2) **Glycogen phosphorylase**; responsible for breaking down of glycogen (Glycogenolysis). When the blood glucose level drops, the enzyme glycogen phosphorylase convert glycogen to glucose-6-phosphate. Other two enzymes, glucan transferase and glucosidase also help in glycogenolysis.
3) **Glucose phosphatase**; responsible for conversion of glucose-6-phosphate to glucose in the liver. Glucose is then released into the blood stream, thereby increasing the blood glucose level.

Role of the pancreas in glucose regulation

Pancreas is the most important endocrine organ for the regulation of blood glucose. It secretes insulin and glucagon, the two main hormones responsible for the regulation of blood glucose.

1) **Insulin:** When the blood glucose concentration increases rapidly, for example after a meal with high carbohydrates content, pancreas secretes insulin hormone into the blood stream. Insulin binds to its receptors and increases the rate of glucose uptake, storage and utilization by almost all tissues of the body resulting in lowering of blood glucose level. Besides, insulin also stimulates glycogenesis, lipid and proteins biosynthesis which helps in decreasing blood glucose concentration.

2) **Glucagon:** In response to decrease in blood glucose concentration, pancreas secretes glucagon which activates the enzyme glycogen phosphorylase responsible for degradation of glycogen to glucose-6-phosphate. Glucose-6-phosphate is then dephosphorylated to form glucose and finally released into the blood stream thereby increasing the blood glucose level. Glucagon also stimulates gluconeogenesis i.e., biosynthesis of glucose from non-carbohydrate compounds like pyruvate and amino acids.

3.1.3 Detailed structure of liver lobule and islet of langerhans

Liver and liver lobules

The liver is a roughly triangular in shape and extends across the entire abdominal cavity under the diaphragm. Most of the liver's mass is located on the right hypochondrium (i.e., upper part of the abdomen) as well as part of the abdomen (Figure 3.3). The liver is made of very soft, pinkish-brown tissues encapsulated by a connective tissue capsule. This capsule is further covered and reinforced by the peritoneum of the abdominal cavity, which protects and holds the liver.

The liver consists of 4 distinct lobes: the left, right, caudate, and quadrate lobes. The falciform ligament divides the liver into two main lobes, right and left. The larger right lobe is again sub-divided into three lobes, the right lobe proper, the caudate lobe and the quadrate lobe (Figure 3.1). Each liver lobe is made up of about 100,000 small hexagonal functional units known as lobules. A typical liver lobule comprises rows of liver cells, hepatocytes, radiating out from a central vein. The six angles of the hexagon are occupied by a portal triad comprising a hepatic portal vein, a hepatic artery and a bile duct. The portal veins and arteries are connected to the central vein through a network of capillary-like tubes called sinusoids (Figure 3.2). Blood flows out of the sinusoids into the central vein and is transported out of the liver.

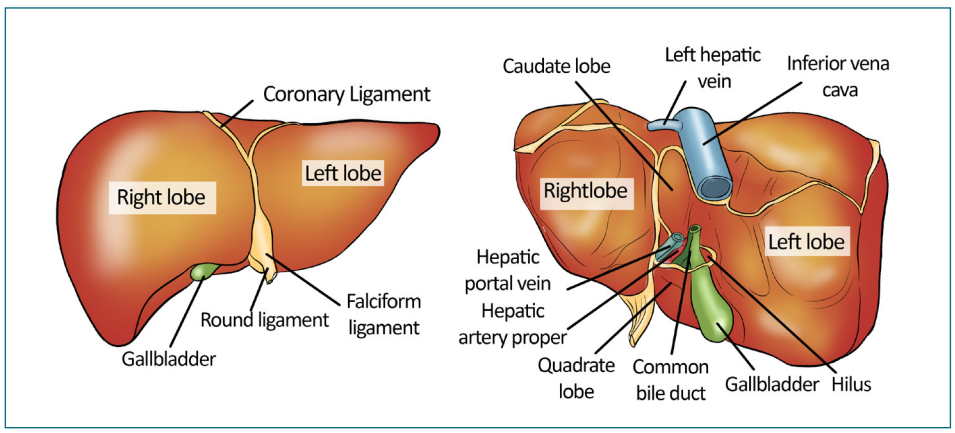


Figure 3.1: Anatomy of Liver (Anterior view and Posterior view)

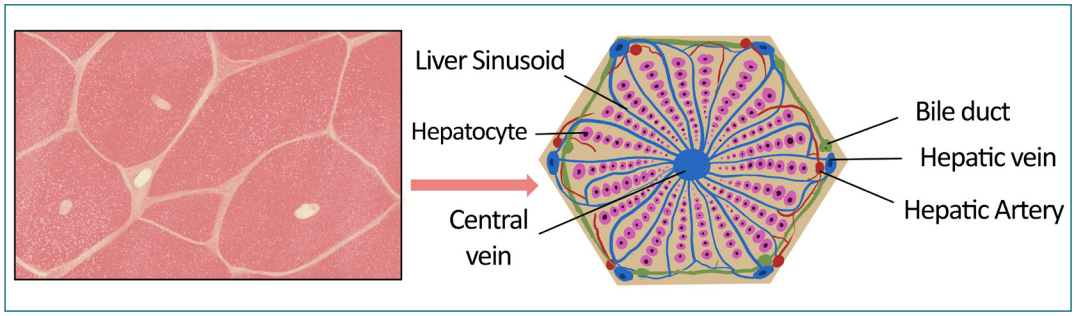


Figure 3.2: Transverse section of liver showing liver lobules and diagrammatic representation of a typical liver lobule

Pancreas

The pancreas is an elongated, tapered organ, located in the abdominal region, behind the stomach and next to the duodenum—the first part of the small intestine (Figure 3.3). The right side of the organ, called the head, is the widest part of the organ and lies in the curve of the duodenum. The tapered left side which extends slightly upward is the body of the pancreas.

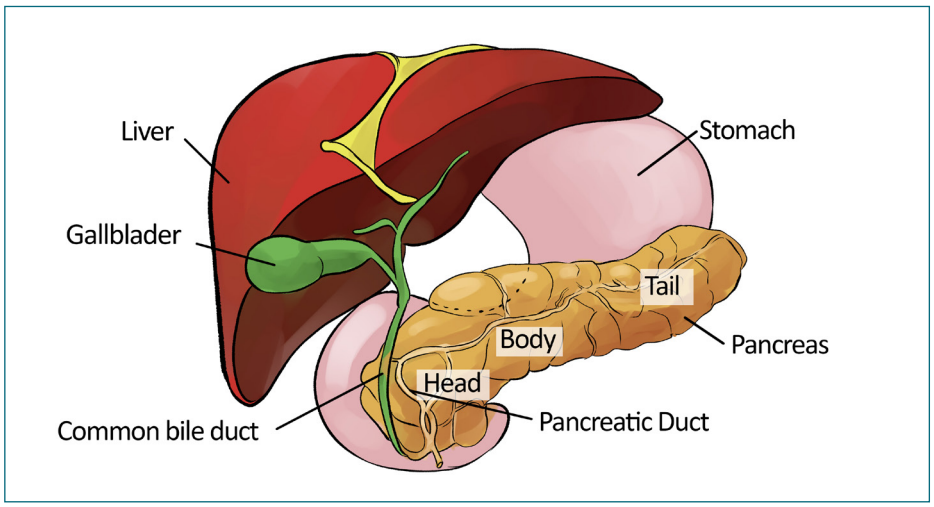


Figure 3.3: Diagrammatic representation of the location of pancreas and liver in the body

Structure and function of pancreas

Pancreas has two main functional components:

1) **Exocrine cells, the acini**—Cells that release digestive enzymes into the gut via the pancreatic duct. These enzymes include trypsin and chymotrypsin to digest proteins; amylase for the digestion of carbohydrates; and lipase to break down fats. The pancreatic duct joins the common bile duct to form the **ampulla of Vater** in the duodenum. The pancreatic juices and bile (from gallbladder) released into the duodenum help the body to digest fats, carbohydrates as well as proteins.

2) **Endocrine pancreas**: Highly vascularized groups of cells known as the **Islets of Langerhans** within the exocrine tissue constitute the endocrine pancreas (Figure 3.4). The human pancreas has 1–2 million islets of Langerhans. It contains four different types of cells which are distinguished from one another by their morphology and staining characteristics:

i) **Alpha cells:** Which secrete **glucagon**, constitute about 25 per cent of all the cells of islet of Langerhans.

ii) **Beta cells:** The most abundant of the islet cells constitute about 60% of the cells. They release **insulin**, a hormone involved in decreasing the blood glucose level.

iii) **Delta cells:** Constitute about 10 per cent of total cells and secrete **somatostatin** which regulates both the alpha and beta cells.

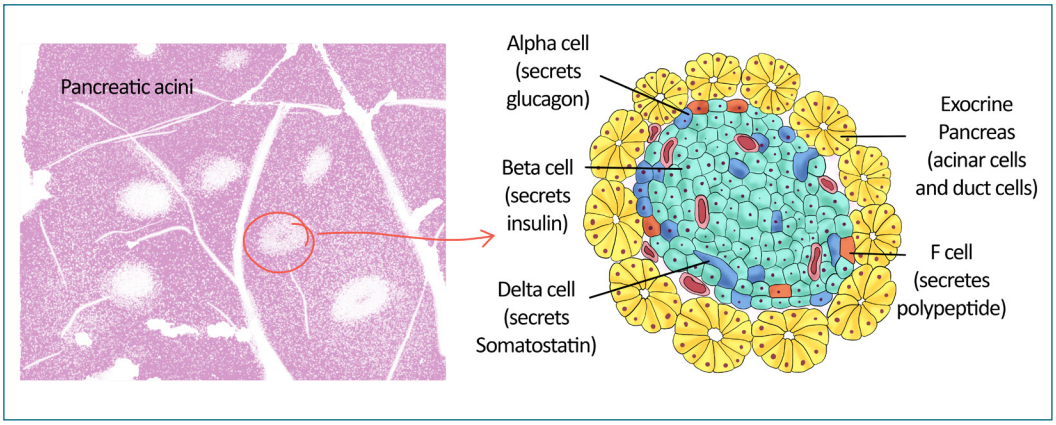


Figure 3.4: Transverse section of pancreas showing the acini and islet of langerhans and diagrammatic representation of an islet of langerhans

Application activity 3.1

1) The homeostatic level of blood glucose is around 90 mg per 100 ml of blood. Three person have taken their blood glucose levels using a glucometer and their results are:

Peter: 85 mg per 100 cm^3 of blood

Mary: 130 mg per 100 cm^3 of blood

John: 65 mg per 100 cm^3 of blood

Interpret these results obtained by using a glucometer?

3.2 Control mechanisms by hormones

Activity 3.2

Different hormones are involved in the regulation of blood glucose level. List and explain those hormones and their functions.

3.2.1 Homeostatic control of blood glucose concentration by insulin and glucagon

Insulin and glucagon are the major hormones responsible for the regulation of blood glucose. Both insulin and glucagon are secreted by the pancreas, and are referred to as pancreatic endocrine hormones.

Insulin

Insulin was first discovered in **1922 by Banting and Best**. Although there is always a low level of insulin secreted by beta cells of pancreas, the amount secreted into the blood increases as the blood glucose level rises. In the blood, it circulates entirely in an unbound form with plasma half-life of about 6 minutes. Only a small portion of the insulin binds with the insulin receptors of the target cells while the rest is degraded by the enzyme insulinase, mainly in liver and to a lesser extends in kidney and muscles.

Functions of insulin

Binding of insulin to the receptors stimulates the rate of glucose uptake, storage and utilization by almost all tissues of the body mainly in muscles, adipose tissue and liver. Other important functions of insulin include:

i) Insulin promotes **glycogenesis** by activating enzyme glycogen synthase.

ii) Insulin inactivates liver phosphorylase, the key enzyme of glycogenolysis.

iii) Insulin promotes **lipid synthesis** by increasing the conversion of **excess glucose into fatty acids in the liver**. These fatty acids are transported as triglycerides to the adipose tissue where it is deposited as fat.

iv) Insulin inhibits the enzymes responsible for gluconeogenesis in liver.

v) Insulin promotes protein synthesis by increasing the rate of transcription and translation. It also stimulates transport of many amino acids into the cells.

vi) Insulin inhibits breakdown of lipids and proteins.

Regulation of insulin secretion

The secretion of insulin by beta cells of islet of Langerhans depends on the following factors:

i) **Blood glucose level:** Increased in the blood glucose level stimulates the insulin secretion while decreased in the blood glucose concentration inhibits the secretion.

ii) **Blood fatty acids and amino acids concentration:** Insulin secretion is also stimulated by increased in the concentration of blood's fatty acids and amino acids concentration and inhibited when its concentration decreased.

iii) **Gastrointestinal hormones:** Insulin secretion increases moderately in response to several gastrointestinal hormones—gastrin, secretin, cholecystokinin and gastric inhibitory peptide.

iv) These hormones are released after the person takes meal and the increased in insulin secretion can be regarded as preparation for the glucose and amino acids uptake by cells.

v) **Other hormones:** Other hormones that are associated with the increase in the insulin secretion are glucagon, growth hormone, cortisol, progesterone and estrogen.

Glucagon

Glucagon is secreted by the alpha cells of the pancreatic islets in response to low blood glucose levels and to events whereby the body needs additional glucose, such as in response to vigorous exercise.

Functions of glucagon

The effect of glucagon in regulating blood glucose level is exactly opposite to insulin:

i) The most important function of glucagon is activation of glycogen phosphorylase enzyme responsible for degradation of glycogen to glucose-6-phosphates. The glucose-6-phosphate is then dephosphorylated to glucose and finally released into the blood stream resulting in increase in blood glucose concentration.

ii) Glucagon also stimulates the increase in rate of amino acid uptake and its conversion into glucose, i.e., gluconeogenesis.

iii) Glucagon activates adipose cell lipase enzyme which stimulates lipids metabolism.

iv) Glucagon also inhibits the storage of triglycerides in the liver by preventing the liver from removing fatty acids from the blood.

v) Glucagon also enhances the strength of the heart; increases blood flow in some tissues, especially the kidneys; enhances bile secretion; and inhibits gastric acid secretion.

Regulation of glucagon secretion

Glucagon secretion increases with the decrease in the concentration of blood glucose level while the increasing concentration of glucose inhibits its secretion. Other factors which stimulate glucagon secretion are, increase in the concentration of amino acids in blood and vigorous physical exercise.

Negative-positive feedback mechanism

A positive feedback mechanism is the exact opposite of a negative feedback mechanism. With negative feedback, the output reduces the original effect of the stimulus. In a positive feedback system, the output enhances the original stimulus. Negative feedback is an important regulatory mechanism for physiological function in all living cells. It occurs when a reaction is inhibited by increase concentration of the product. Regulation of blood glucose level is an excellent example of homeostatic control through negative feedback mechanism (Figure 3.5).

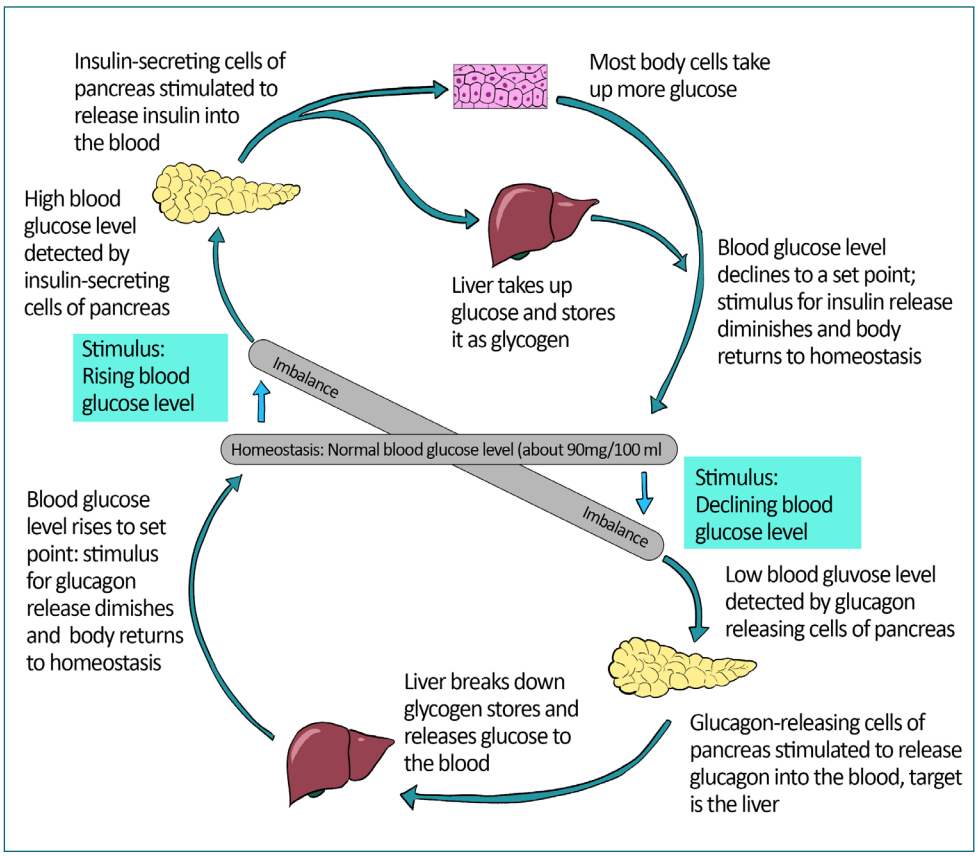


Figure 3.5: Negative feedback regulation of blood glucose level by insulin and glucagon

Response to an increase in blood glucose level

When there is increase in blood glucose level, the beta cells of the pancreatic islets of Langerhans increase the release of insulin into the blood. Insulin binds to receptors on the cell membrane and stimulates the cells to increase glucose uptake. This led to decrease in blood glucose level. Besides, insulin also stimulates glycogenesis and glycolysis while inhibiting glycogenolysis, gluconeogenesis, lipolysis etc. which all contributes in reducing blood glucose levels.

Response to a decrease in blood glucose level

Decreased in blood glucose level stimulates the alpha cells of pancreas islets to increase the secretion of glucagon. Glucagon activates enzyme glycogen phosphorylase in the liver and muscle cells which start glycogenolysis. It also promotes gluconeogenesis, lipid metabolism etc. The overall effect of glucagon is increase in the concentration of blood glucose.

3.2.2 Other hormones involved in glucose regulation

Other than insulin and glucagon, there are many hormones which contribute to the regulation of blood glucose level (Figure 3.6). They are:

1) **Somatostatin:** It is secreted by delta cells of pancreatic islet of Langerhans in response to many factors related to ingestion of food like increased concentration of glucose, amino acids, fatty acids and several gastrointestinal hormones released from the upper gastrointestinal tract. Somatostatin acts locally within the islets of Langerhans and inhibits the secretion of both insulin and glucagon. It also reduces the motility of the stomach, duodenum, and gallbladder and decreases the secretion and absorption in the gastrointestinal tract. Hence, lowers overall blood glucose level.
2) **Epinephrine:** Commonly known as Adrenaline, it is secreted by the medulla of the adrenal glands in response to strong emotions such as fear or anger. It causes increases in the heart rate, muscle strength, blood pressure and sugar metabolism. In response, it enhances the process of glycogenolysis, increasing the overall blood glucose concentration.
3) **Cortisol:** It is also known as stress hormone and is secreted by the adrenal cortex of the adrenal gland in response to stress. Cortisol enhances gluconeogenesis and increases the concentration of glucose in the blood.
4) **Adrenocorticotropic Hormone (ACTH):** In response to various stresses, hypothalamus secretes corticotropin-releasing hormone which stimulates anterior pituitary to secrete ACTH. It stimulates adrenal cortex to release the cortisol hormones.

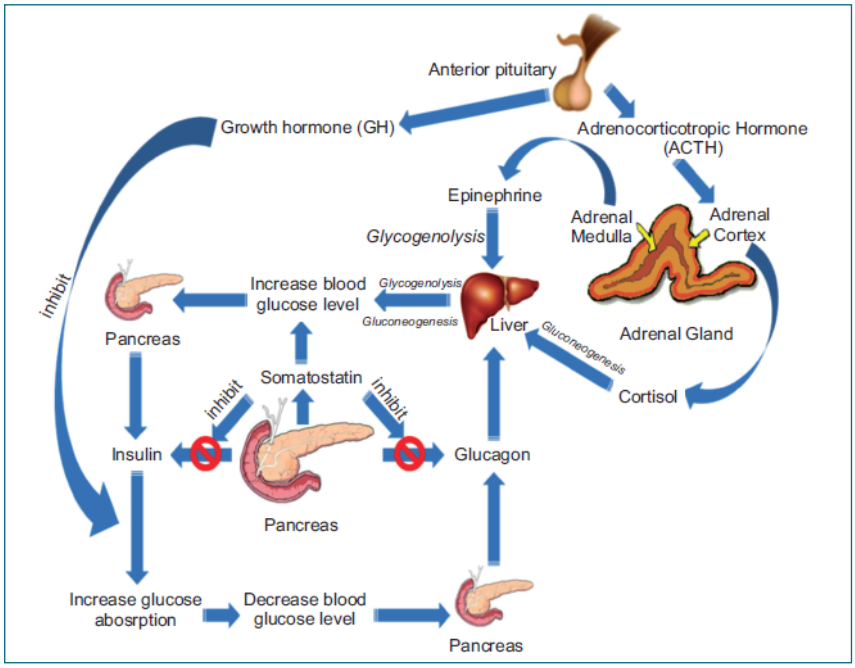


Figure 3.6: Hormonal regulation of blood glucose level

5) **Growth hormone (GH):** It is another anterior pituitary hormone which antagonizes the action of insulin by inhibiting the glucose uptake by cells and increasing the blood glucose level.

6) **Gastrointestinal hormones:** The hormones released by gastrointestinal tract such as gastrin, secretin, cholecystokinin and gastric inhibitory peptide etc. increase the digestion and absorption of nutrients in the gastrointestinal tracts. These hormones stimulate the pancreas to secrete insulin in anticipation of the increase in blood glucose level.

3.2.3 Mechanism of hormonal regulation

Our body maintains certain variables like temperature, pH etc. within a safe range so that it does not cause any harm to the body and the internal environment remains stable and relatively constant. This is known as **homeostasis**. Hormones are chemical messenger that are directly released into the blood stream. They play very important role in maintaining the homeostasis.

Steps of hormonal signaling

Hormonal signal transduction is a complex process which involves the following steps:

i) Hormones are first synthesis in particular cells of an organ and stored for secretion in response to certain stimulus.

ii) When the organ receives the stimulus; hormones are secreted directly into the blood stream.

iii) Blood carries the hormone to the target cell(s).

iv) The hormone is recognized by the specific receptor in the cell membrane or by the intracellular receptor protein.

v) The hormonal signal is relayed and amplified through a series of signal transduction process in the target cells which lead to cellular response.

3.2.4 Cause of blood sugar imbalances in the body

Our body obtains glucose from various food sources or synthesis in the liver and muscles from other compounds like pyruvate, lactate, glycerol, and glucogenic amino acids. The blood carries glucose to all the cells in the body where it is metabolized to produce energy.

Blood sugar levels keep on fluctuating throughout the day increasing after meals and decreasing in between the meals. When the blood glucose level rises beyond the normal value, the condition is known as **hyperglycaemia**. On the other hand, hypoglycaemia or low blood sugar is the condition in which the blood glucose level is below normal (~80 mg/dL).

Hyperglycaemia

High blood glucose level can be caused due to various reasons like:

i) **Carbohydrates:** Eating food containing too much of carbohydrates. The body of a person cannot process high levels of carbohydrates fast enough to convert it into energy.

ii) **Insulin control:** The pancreas of the individual are unable to produce enough insulin.

iii) **Stress:** Stress stimulates the secretion of certain hormones like cortisol and epinephrine etc., which increases the blood glucose level.

iv) **Low levels of exercise:** Daily exercise is a critical contributor to regulating blood sugar levels.

v) **Infection, illness, or surgery:** With illness, blood sugar levels tend to rise quickly over several hours.

vi) **Other medications:** Certain drugs, especially steroids, can affect blood sugar levels.

A high blood sugar level can be a symptom of diabetes. If hyperglycaemia persists for several hours, it can leads to dehydration. Other symptoms of hyperglycaemia include dry mouth, thirst, frequent urination, blurry vision, dry, itchy skin, fatigue or drowsiness, weight loss, increased appetite, difficulty breathing, dizziness upon standing, rapid weight loss, increased drowsiness and confusion, unconsciousness or coma.

Hypoglycaemia

Hypoglycaemia is generally defined as a serum glucose level below 80 mg/dL. Symptoms typically appear when the blood glucose levels reach below 70 mg/dL and levels below 60 mg/dL can be fatal.

Common causes of low blood sugar include the following:

i. Overmedication with insulin or antidiabetic pills

ii. Use of alcohol

iii. Skipped meals

iv. Severe infection

v. Adrenal insufficiency

vi. Kidney failure

vii. Liver failure, etc.

Common symptoms of hypoglycaemia include trembling, clammy skin, palpitations (pounding or fast heart beats), anxiety, sweating, hunger, and irritability. If the brain remains deprived of glucose for longer period, a later set of symptoms can follows like difficulty in thinking, confusion, headache, seizures, and coma. And ultimately, after significant coma or loss of consciousness, death can occur.

3.2.5 Diabetes mellitus

Diabetes mellitus (commonly referred to as diabetes) is a chronic condition associated with abnormally high levels of sugar in the blood due to impaired carbohydrate, fat, and protein metabolism. It can be due to absence or insufficient production of insulin by the pancreas, or inability of the body to properly use insulin. Hence, there are two types of diabetes mellitus – **Type I** causes by lack of insulin secretion and **Type II**, caused by reduced sensitivity of target cells to insulin.

Type I diabetes

It is known as **insulin dependent diabetes mellitus** (IDDM) and it is due to insufficient insulin production by the beta cells of pancreatic islet of Langerhans or due to absence of the beta cells itself. Since the pancreas makes very little or no insulin at all, glucose cannot get into the body's cells and remain in the blood leading to hyperglycemia. The concentration of blood glucose level can be as high as 300 – 1,200 mg/dL. The symptoms of Type I diabetes include:

i) **Loss of glucose in urine**; due to increase in blood glucose, concentration goes beyond 180 mg/dL.

ii) **Dehydration**; due to osmotic loss of water from cells and inability to reabsorb water in kidney.

iii) **Tissue injury**; due to damages blood vessels in many tissues.

iv) **Metabolic** acidosis; due to increased fat metabolism.

v) **Depletion of body's protein**; due to increase protein metabolism.

Treatment of Type I Diabetes

Persons with Type I diabetes require treatment to keep blood sugar levels within a target range which includes:

i) Taking insulin from external source everyday either through injections or using an insulin pump.

ii) Monitoring blood sugar levels several times a day.

iii) Eating a healthy diet that spreads carbohydrate throughout the day.

iv) Regular physical activity or exercise. Exercise helps the body to use glucose more efficiently.

v) It may also lower your risk for heart and blood vessel disease.

vi) Not smoking.

vii) Not drinking alcohol if you are at risk for periods of low blood sugar.

Type II diabetes

Also known as **non-insulin dependent diabetes mellitus (NIDDM)**, it is due to the inability of cells to take up glucose from the blood. It can be either due to defective insulin receptors over cell surfaces or abnormality of blood plasma protein, amylin. Due to decrease sensitivity of cells to insulin, a condition known as **insulin resistance**, the beta cells secrete large amount of insulin into the blood stream resulting in increased concentration of insulin in blood. This condition is known as **hyperinsulinaemia**. Type II diabetes are more common and account for almost 80–90 per cent of the total diabetes mellitus cases.

The symptoms of type II diabetes include:

i) Obesity, especially accumulation of abdominal fat;

ii) Fasting hyperglycaemia;

iii) Lipid abnormalities such as increased blood triglycerides and decreased blood high density lipoprotein-cholesterol; and

iv) Hypertension.

Treatment of Type II Diabetes

There's no cure for diabetes, so the treatment aims to keep the blood glucose levels as normal as possible and to control the symptoms and prevent health problems developing later in life. In type II diabetes, the pancreas is still working but our body develops insulin resistance and is unable to effectively convert glucose into energy leaving too much glucose in the blood. Therefore, Type II diabetes can be managed through lifestyle modification including:

i) Healthy diet as eating well helps manage our blood glucose levels and body weight.

ii) Regular exercise helps the insulin work more effectively, lowers your blood pressure and reduces the risk of heart disease.

iii) Regular monitoring of blood glucose levels to test whether the treatment being followed is adequately controlling blood glucose levels or we need to adjust the treatment.

Importance of controlled diet in diabetes

Controlled diet is very important for diabetic patients because blood sugar is mostly affected by the food one eats. The glycaemic index of a food measures how the food affects the blood glucose level. The higher the **glycaemic index** of the food, the greater the potential of increasing blood glucose. Therefore, in order to control glucose levels in the blood, it is important that diabetic primarily chooses low glycaemic index carbohydrates like dried beans and legumes such as lentils and pintos, non-starchy vegetables, fruits, whole grain bread and cereals, sweet potatoes etc. Foods like white bread, white rice, cornflakes, white potatoes, popcorn, pineapple, and melons are high glycaemic index foods and should be eaten moderately.

Because people with diabetes are at risk of high blood pressure, it makes sense to also choose foods that are heart healthy (i.e., lean, low-fat) and the ones that are low in salt. Increasing the amount of fibre in diet and reducing fat intake, particularly saturated fat, can help prevent diabetes or manage the diabetic condition from developing any complications. Therefore, one should:

i) Increase the consumption of high-fibre foods, such as wholegrain bread and cereals, beans and lentils, and fruits and vegetables.

ii) Choose foods that are low in fat for example, replace butter, ghee and coconut oil with low-fat spreads and vegetable oil.

iii) Choose skimmed and semi-skimmed milk, and low-fat yoghurts.

iv) Eat fish and lean meat rather than fatty or processed meat, such as sausages and burgers.

v) Grill, bake, poach or steam food instead of frying or roasting it.

vi) Avoid high-fat foods, such as mayonnaise, chips, crisps, pasties, poppadums and samosas.

vii) Eat fruit, unsalted nuts and low-fat yoghurts as snacks instead of cakes, biscuits, bombay mix or crisps etc.

Coping with situation of diabetics and hypertension

Blood pressure is the measure of the force of blood pushing against blood vessel walls. The heart pumps blood into the arteries, which carry the blood throughout the body. The normal blood pressure is less than 120 (systolic) over 80 (diastolic). High blood pressure, also called **hypertension**, is dangerous because it makes the heart work harder to pump blood out to the body and contributes to hardening of the arteries, or **atherosclerosis**, to stroke, kidney disease, and to the development of heart failure. Diabetics are more likely to develop high blood pressure and other heart and circulation related problems, because diabetes damages arteries and makes them targets for hardening (atherosclerosis). Obesity is another main factor which is responsible for hypertension.

When it comes to preventing diabetes complications, normal blood pressure is as important as good control of blood glucose levels. Therefore, to treat and help prevent high blood pressure, one should control their blood glucose, stop smoking, eat healthy, maintain a healthy body weight, limit alcohol and salt consumption and exercise regularly.

3.2.6 Monitoring of blood glucose levels

Blood glucose monitoring is a way of testing the **concentration of glucose in the blood (glycaemia).** As mentioned earlier, the concentration of blood glucose is fluctuating throughout the day. Under certain physiological disorders, especially when the person is suffering from diabetes mellitus, the blood glucose concentration can increase well above the normal concentration. Most people with type II diabetes need to monitor their blood sugar levels at home. A blood glucose test is generally performed by piercing the skin (typically, on the finger) to draw blood, then applying the blood to a chemically active disposable 'test-strip' or to a biosensors.

1. Dipstick test

A **dipstick** or the **reagent strips** is a narrow strip of plastic with small pads attached to it. Each pad contains specific reagents for a different reaction, thus allowing for the simultaneous determination of several compounds. The blood glucose test use enzymes **glucose oxidase** and **hexokinase** which are specific to glucose, embedded on a **test strip** or a **dipstick**. When the blood sample is applied onto the strip, the enzymes catalyzed glucose specific

reaction which changes the colour. The chemical reaction involved in the glucose oxidase test is as follows:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Chromogen} \xrightarrow{\text{Peroxidase}} \text{Oxidized Chromogen}$$

Numbers of chromogen like potassium iodide, tetramethylbenzine, O-tolidinehydrochloride, 4-aminoantipyrine etc. are used in the dipstick. The colour reaction of the dipsticks is kinetic and will continue to react after the prescribed time. Therefore, reading taken after the prescribed time can give false result.

2. *Biosensors*

A biosensor is a device which is composed of two elements; a **bio-receptor** that is an immobilized sensitive biological element (e.g. enzyme, DNA probe, antibody) recognizing the analyte (e.g. enzyme substrate, complementary DNA, antigen) and a **transducer**, used to convert biochemical signal resulting from the interaction of the analyte with the bioreceptor into an electronic signal. The intensity of generated signal is directly or inversely proportional to the analyte concentration. For example, the glucose biosensor is based on the fact that the immobilized Glucose oxidase enzyme which catalyzes the oxidation of β-D-glucose by molecular oxygen producing gluconic acid and hydrogen peroxide. An electrochemical transducer converts this reaction into electronic signal which appears on the screen of the glucose meter.

3. Continuous glucose monitoring

Continuous glucose monitoring systems (CGMS) use a glucose sensor inserted under the skinin the form of a small needle. The signal from the sensor is transmitted wirelessly and the result is recorded in a small recording device. The monitor of the device updates and displays the blood sugar level every few minutes. The glucose sensor needs to be removed and replaced at least once per week.

Advantages of continuous glucose monitoring:

i) The monitor displays blood sugar level every few minutes, allowing one to see whether the level is increasing, decreasing, or is stable.

ii) The receiver can also be set to alarm if the blood sugar level is above or below a pre-set level.

iii) The blood sugar results from the continuous monitor can be downloaded to a computer, allowing you to check blood sugar trends over time.

The only disadvantage of continuous monitor other than the cost is its inaccuracy compared to more traditional accurate dipstick method. Therefore, most experts recommend continuous glucose monitoring along with several finger sticks daily to calibrate the CGMS device and to verify that the sensor readings are accurate.

Roles of adrenaline in the control of blood sugar level

Adrenaline, a natural stimulant created in the kidney's adrenal gland, travels through the bloodstream and controls functions of the autonomous nervous system, including the secretion of saliva and sweat, heart rate and pupil dilation. The substance also plays a key role in the human flight-or-flight response.

The "fight or flight" hormone that gives us a quick boost of extra energy to cope with danger — including the danger of low blood glucose. When blood glucose levels drop too low, the adrenal glands secrete epinephrine (also called adrenaline), causing the liver to convert stored glycogen to glucose and release it, raising blood glucose levels. Epinephrine also causes many of the symptoms associated with low blood glucose, including rapid heart rate, sweating, and shakiness. The epinephrine response spurs the liver to correct low blood glucose or at least raise blood glucose levels long enough for a person to consume carbohydrate.

3.2.7. Detection of glucose in urine

Urine analysis can be used to test pH, protein, glucose, ketones, occult blood, bilirubin, urobilinogen, nitrite, leukocyte esterase etc. in the urine sample. Simple test for glucose in urine can be used to diagnose diabetes mellitus. Generally, healthy person do not loss glucose in their urine whereas a person with diabetes mellitus loses small to large quantities of glucose in their urine.

Detection of glucose in urine

The presence of glucose in the urine is called **glycosuria** (or **glucosuria**). The urine analysis of glucose is based on enzyme **glucose oxidase** which is impregnated in a dipstick (reaction described in previous section).

Detection of protein in urine

The glomerular filtrate of a normal kidney contains little amount of low–molecular weight protein. Most of these proteins get reabsorbed in the tubules with less than 150 mg being excreted through urine per day. Therefore, the abnormal increase in the amounts of protein in the urine, **Proteinurea**, can be an important indicator of renal diseases. There are certain physiologic conditions such as exercise and fever that can lead to increased protein excretion in the urine in the absence of renal disease.

Proteinuria is a symptom of **chronic kidney disease (CKD)**, which can be due to **diabetes, high blood pressure**, and **diseases that cause inflammation** in the kidneys. Therefore, urine analysis for protein is part of a routine medical assessment for everyone. If CKD is not checked in time, it can lead to **end-stage renal disease (ESRD)**, when the kidneys completely stop functioning. A person with ESRD requires a kidney transplant or regular blood-cleansing treatments called **dialysis** to further sustain.

The tests for proteinuria are based either on the "**protein error of indicators**" principle (ability of protein to alter the colour of some acid-base indicators without altering the pH) or on the ability of protein to be precipitated by acid or heat. According to "protein error of indicators" principle, a protein-free solution of **tetrabromphenol blue** at pH 3 is yellow in colour and its colour changes from yellow to blue (or green) when the pH increases from pH 3 to pH 4. However, in the presence of protein (albumin), the colour changes occur between pH 2 and 3 i.e., an "error" occurs in the behaviour of the indicator. The method is more sensitive to albumin than to other proteins, whereas the heat and acid tests are sensitive to all proteins.

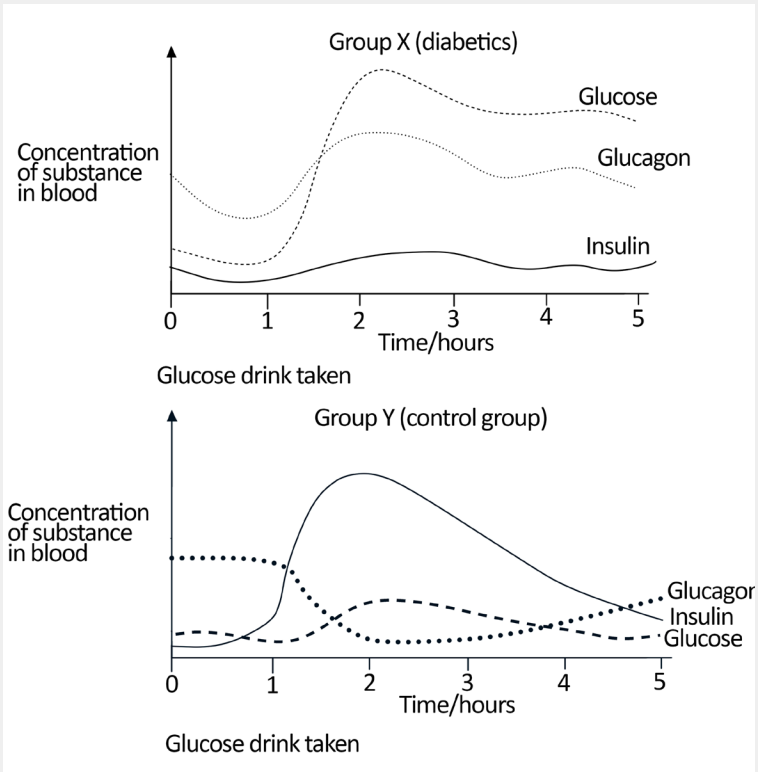
The test result may show false-positive results in a highly buffered alkaline urine, which may result from alkaline medication or stale urine. Also, if the dipstick is left in the urine for too long, the buffer could be washed out of the reagent resulting in increased pH and the strip may turn blue or green even if protein is not present. On the other hand, false-negative results can occur in dilute urines or when the urine contains proteins other than albumin in higher concentrations.

Detection of ketones in urine

As discussed earlier, **ketones, or ketone bodies** are formed during lipid metabolism. One of the intermediate products of fatty acid breakdown is acetyl CoA. If the lipid metabolism and carbohydrate metabolism are in balanced, Acetyl-CoA enters the citric acid cycle (Krebs cycle) where it reacts with oxaloacetate to form citrate. When carbohydrate is not available in the cells, all available oxaloacetate get converted to glucose and so none is available for condensation with Acetyl- CoA. As such, Acetyl-CoA cannot enter the Krebs cycle and is diverted to form ketone bodies.

Application activity 3.2

An experiment was carried out with two groups of people. Group X has type I diabetes mellitus while group Y did not (control group). Every 15 minutes' blood samples were taken from all members of both groups and the mean of levels of insulin, glucagon, and glucose were calculated. After an hour, every person was given a glucose drink. The results are shown in the figure below:



a) Name a hormone other than insulin and glucagon that is involved in regulating blood glucose levels.

b) State two differences between groups X and Y in the way insulin secretion responds to the drinking of glucose.

c) Suggest a reason why the glucose level falls in both groups during the first hour.

d) Using information from the graphs, explain the changes in the blood glucose level in group Y after the glucose drink.

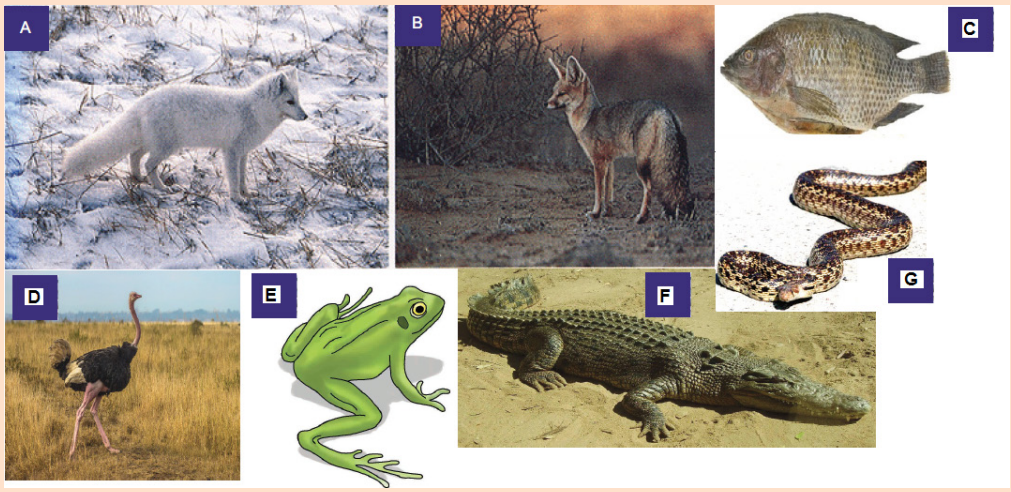
e) Explain the difference in blood glucose level in group X compared to group Y.

f) Suggest what might happen to the blood glucose level of group X if they had no food intake over the next 24 hours.

3.3 Adaptations of animals to temperature changes in the environment

Activity 3.3

Observe the photo below and answer the questions that follow:



a) Show 2 main differences between individual A and individual E.

b) How is individual C different from individual D?

c) The individual A is adapted to live in cold environments. Analyze it carefully to identify any two characteristics that this animal has.

d) Which among the animals on the photo is adapted to live in hot climates? Justify your answer.

Thermoregulation is the ability of an organism to keep its body temperature within certain boundaries, even when the surrounding temperature is very different. This process is one aspect of homeostasis: a dynamic state of stability between an animal's internal environment and its *external* environment.

One of the most important examples of homeostasis is the regulation of body temperature. Not all animals can do this physiologically. Animals that maintain a fairly constant body temperature (birds and mammals) are called **endotherms**, while those that have a variable body temperature (all others) are called **ectotherms**. Endotherms normally maintain their body temperatures at around 35 - 40°C, so are sometimes called **warm-blooded animals**, but in fact ectothermic animals can also have very warm blood during the day by basking in the sun, or by extended muscle activity. The difference between the two groups is thus that endothermic animals use **internal** corrective mechanisms, whilst ectotherms use **behavioral** mechanisms (e.g. lying in the sun when cold, moving

into shade when hot). Such mechanisms can be very effective, particularly when coupled with **internal** mechanisms to ensure that the temperature of the blood going to vital organs (brain, heart) is kept constant.

3.3.1 Importance of temperature regulation

Besides water, our body consists of many inorganic and organic compounds including proteins, lipids, carbohydrates etc. Among these, proteins are the most important compounds and are regarded as "workhorse" molecules of life, taking part in essentially every structure and activity of life. Proteins make up about 75 per cent of the dry weight of our bodies and serve four important functions:

i) They are nutrients.

ii) They also form the structural components of our body including skin, hair etc. They are building materials for living cells, appearing in the structures inside the cell and within the cell membrane.

iii) As haemoglobin, Hb they carry oxygen to all the body organs and

iv) They function as biological catalysts as **enzymes** facilitating and controlling various chemical reactions of our body.

Protein molecules are often very large and are made up of hundreds to thousands of amino acid units. They are of varying shape and size. For examples, keratins, a protein in hair and collagen in tendons and ligaments linear chains of amino acids. Other proteins called globular proteins, fold up into specific shapes and often more than one globular unit are bound together. Enzymes are globular proteins. Though large, enzymes typically have a small working region, known as active site which acts as the binding site of ligands. The shape of globular proteins is held together by many forces, including highly resistant strong covalent bonds. However, there are also many weak forces, like hydrogen bonds, which are susceptible to pH, osmolality and temperature changes.

Since the function of enzymes is attributed to its shape, small changes in the shape can greatly reduce its function. Every enzyme has an optimal temperature at which it works best and this temperature is approximately the normal body temperature of the body**.** Therefore, in order to ensure the optimal function of the enzymes within, the core body temperature need to be maintained more or less constant. If the body temperature falls below the normal value, the enzymes catalyzed reactions of the animal will be slowed. Similarly, too much rise in body temperature might result in enzyme denaturation and hence reduced catalytic activities. *Rise in body temperature also reduces the oxygen carrying capacity of haemoglobin*. Increasing temperature weakens and denatures the bond between oxygen and haemoglobin which in turn decreases the concentration of the oxyhaemoglobin. This can lead to **hypoxia** – a condition in which tissues receive insufficient oxygen supply from the blood.

3.3.2 Adaptations of animals to temperature changes in the environment

From deepest corner of the sea to high mountains, living organisms have colonized almost everywhere. However, they are not distributed evenly with different species found in different areas. Many abiotic factors including temperature, humidity, soil chemistry, pH, salinity, oxygen levels etc., influence the availability of species in certain area. Each species has certain set of environmental conditions within which it can best survive and reproduce to which they are best adapted. This is known as limits of tolerance (i.e., the upper and lower limits to the range of particular environmental factors within which an organism can survive). No organism can survive if the environmental factor is below its lower limits of tolerance or above the higher limits. Therefore, organisms having a wide range of tolerance are usually distributed widely, while those with a narrow range have a more restricted distribution. For examples, **euryhaline fishes** (like salmon) **can survive wide range of salt concentration** and therefore are found both in freshwater and salt water environment while **stenohaline** fishes are found **only in saltwater or freshwater**.

Temperature is one of the most important factors which directly or indirectly influence the distribution of organisms to a large extend. For example**,** polar bears can survive very well in low temperatures ranges, but would die from overheating in the tropics**.** On the other hand, a giraffe does very well in the heat of the African savanna, but would quickly freeze to death in the Arctic**.** Compared to ectotherms or cold blooded animals, endotherms due to their ability to generate their own body heat, are generally more widely distributed. Besides, all the organisms have varying degree of morphological, physiological or behavioral adaptations that helps them to survive the extreme temperature conditions of their habitat.

Effect of temperature

As discussed above, all the living organisms have a particular range of temperature within which they can best survive and reproduce. Temperature below or above this temperature ranges are harmful to the organism in various ways. Some of the well-known effects of temperature on living organisms are given below.

1. **Effect of temperature on cells:** If the temperature is too cold, the cell proteins could be destroyed due to the formation of ice, or as the water is lost, the cytoplasm can become highly concentrated. Conversely, extreme heat can coagulate cell proteins.
2. **Effect on metabolism:** Most of metabolic activities of microbes, plants and animals are regulated by enzymes and the functions of enzymes are

greatly affected by temperature. Therefore, increase or decrease in the body temperature will greatly affect the various metabolic activities. For example, the activity of liver arginase enzyme upon arginine increases gradually with increase in the temperature from 17°C to 48°C. With the increase in temperature beyond 48°C, the enzymatic activity decreased sharply.

3. **Effect on reproduction:** Changes in temperature affect both the maturation of gonads i.e., gametogenesis and fecundity of animals. For example, some animal species can breed throughout the year, some only in summer or in winter, while some species have two breeding periods, spring and autumn. Therefore, temperature determines the breeding seasons of most organisms. Also, it was observed that female *Chrotogonus trachyplerus* an acridid insect lays highest number of eggs per female at the temperature of 30°C and decreases with increase in temperature from 30°C to 35°C.

4. **Effect on sex ratio:** In certain animals like copepod ***Maerocyclops albidu***, rises in temperature significantly increase the number of male offspring. Similarly, in plague flea, ***Xenopsyll acheopis***, males' population outnumbered females when the mean temperature is between 21°C to 25°C. However, further decreases in temperature reverse the conditions with the considerable increases in female population.

5. **Effect on growth and development:** In general growth and development of eggs and larvae is more rapid in warm temperatures. For example, Trout eggs develop four times faster at 15°C than at 5°C. On the other hand, seeds of many plants will not germinate and the eggs and pupae of some insects will not hatch until chilled.

6. **Effect on colouration:** Animals generally have a darker pigmentation in warm and humid climates than those found in cool and dry climates. This phenomenon is known as *Gioger* rule. In the frog *Hyla*and the horned toad *Phrynosoma*, low temperatures have been known to induce darkening. Some prawn turn light coloured with increasing temperature.

7. **Effect on morphology:** Temperatures have profound effects on the size of animals and various body parts. Endotherms generally attain a larger body size (reduced surface-mass ratio) in colder temperatures than in warmer temperatures. As such the colder regions harbour larger species. Conversely, the poikilotherms (ectotherms) tend to be smaller in colder regions. We will discuss the various morphological modifications due to extreme climates in the later sections.

8. **Effect on animal behaviour:** Temperature certainly has profound effect on the behavioural pattern of animals. The advantage gained by certain cold blooded animals through thermotaxis or orientation towards a source of heat are quite interesting. Ticks locate their warm blood hosts by a turning

reaction to the heat of their bodies. Certain snakes such as rattle snakes, copper heads, and pit vipers are able to detect mammals and birds by their body heat which remains slightly warmer than the surroundings.

9. **Effect on animal distribution:** Since the optimum temperature for many organisms varies, temperature imposes a restriction on the distribution of species. The diversity of animals and plants gradually decrease as we move from equator towards the pole.

Morphological Adaptations

1. Body size and shape: Ectotherms or cold-blooded animals whose body temperature depends on the temperature of external environments are usually smaller in size compared to endotherms or warm blooded animals. For instance, compare the size of elephant, blue whales and crocodiles or snakes. Within the same species, individuals living in the colder climates tend to be larger than those living in warmer climates. This is known as **Bergmann's rule**. For example, whitetail deer in the southern part of the United States have a smaller body size than white tail deer in the northern states the far northern states.

2. Body Extremities: According to Allen's rule, animals living in the colder climates have more rounded and compact form. This is achieved by reducing the size of the body extremities i.e., ears, limbs, tails etc. On the other hand, animals living in the warmer climates have longer body extremities. For instance, compare the size of the ear of Arctic fox with that of the Desert fox (Figure 13.2). Longer body extremities increase the surface to volume ratio of the desert fox which enable them to lose heat more easily.

Most cold-blooded organisms have either an elongated or a flat body shape. For example, fishes, snakes, lizards, and worms have long and slender body form which ensures rapid heat up and cool down processes.

*Figure 3.7: Allen's rule: Body extremities and temperature. Arctic fox (**Alopax lagopus**) with its short tail, ear and legs and Desert fox (**Vulpes chama**) with longer tail, ear and legs*

Both Bergmann's rule and Allen's rule depend on simple principle that "*the ratio of surface area to volume of an object is inversely proportional to the volume of the object*". In other words, the smaller an animal is, the higher the surface area-to-volume ratio. Higher surface area-to-volume ratio ensures these animals to lose heat relatively quickly and cool down faster, so they are more likely to be found in warmer climates. Larger animals, on the other hand, have lower surface area-to-volume ratios and lose heat more slowly, so and they are more likely to be found in colder climates.

3. Insulation: All the marine mammals have **a thick insulating layer of fat** known as **Blubber**, just beneath the skin. It covers the entire body of animals such as seals, whales, and walruses (except for their fins, flippers, and flukes) and serves to stores energy, insulates heat, and increases buoyancy. Thickness of blubber can range from a couple of inches in dolphins and smaller whales, to 4.3 inches in polar bears to more than 12 inches in some bigger whales. To insulate the body, blood vessels in blubber constrict in cold water. Constriction of the blood vessels reduces the flow of blood to the skin and minimizes the heat loss. In such animals, skin surface temperature is nearly identical to the surrounding water, though at a depth of around 50 mm beneath the skin, the temperature is the same as their core temperature.

Some marine mammals, such as polar bears and sea otters, **have a thick fur coat**, as well as **blubber, to insulate them**. The blubber insulates in water while fur insulates in air or terrestrial environment. The feathers of the birds also function in insulating the body from cold temperature.

Physiological Adaptations

1. Evaporation: In a cold region, i.e., when the surrounding environment of the animal is cold than the body temperature, **conduction** and **radiation** are the main ways an animal will dissipate heat. However, in warmer region, the air temperature is often higher than the animal's body temperatures, so the only physiological thermoregulatory mechanism available is **evaporation**. Animals use three evaporative cooling techniques that include **sweating, panting**, and **saliva spreading**.

(a) Sweating: It is the loss of water through sweat glands found in the skin of mammals. The number of sweat glands can vary from none in whales, few in dogs to numerous in humans. Most small mammals do not sweat because they would lose too much body mass if they did. For example, in a hot desert the amount of water a mouse would lose through sweating to maintain a constant body temperature would be more than 20% of its body weight per hour, which could be lethal for the animal. Therefore, smaller mammals use other techniques to cool down their body. On the other hand, sweating is an important thermoregulatory mechanism for primates including humans. An adult human can loss as much as 10–12 litres of water per day through sweating.

(b) Panting: It is rapid, shallow respiration that cools an animal by increased evaporation from the respiratory surfaces. It is a common thermoregulatory technique used by small animals like dogs and rodents to loss heat.

(c) Saliva spreading: It is a means of thermoregulation used by marsupials. Under extreme heat, saliva will drip from animal's mouth and is then wiped on its fore and hind legs. This technique induces the cooling effect of evaporation by wetting the fur. However, since the animal cannot spread saliva while moving, they need to adapt other evaporative techniques during such situation.

2. Counter current mechanism: As mentioned above, in addition to its role in the transport of oxygen and food, circulatory system of our body is responsible for distribution of heat throughout the body. This is true in case of both endotherms and ectotherms. In endotherms, most of the body heat is generated in brain, liver, heart and skeletal muscles. This heat is transported to other parts of the body through blood. On the other hand, in ectotherms, the circulatory system help in transporting heat from skin to others body parts. The counter current heat exchanger is generally located in body extremities like limbs, neck, gills, which are directly in contact to the external environment.

In cold region, when the warm blood flows through the arteries, the blood gives up some of its heat to the colder blood returning from the extremities in the veins running parallel to the arteries. Such veins are located in the deeper side of the body and carry the warm blood to the heart and most of the body heat is retained. Such mechanism can operate with remarkable efficiency. For instance, a seagull can maintain a normal temperature in its torso while standing with its unprotected feet in freezing water (*Figure 3.8*).

When the external temperature is higher than the body temperature and heat loss is not a problem, most of the venous blood from the extremities returns through veins located near the surface. If the core body temperature becomes too high, the blood supply to the surface and extremities of the body is increased enabling heat to be released to the surroundings.

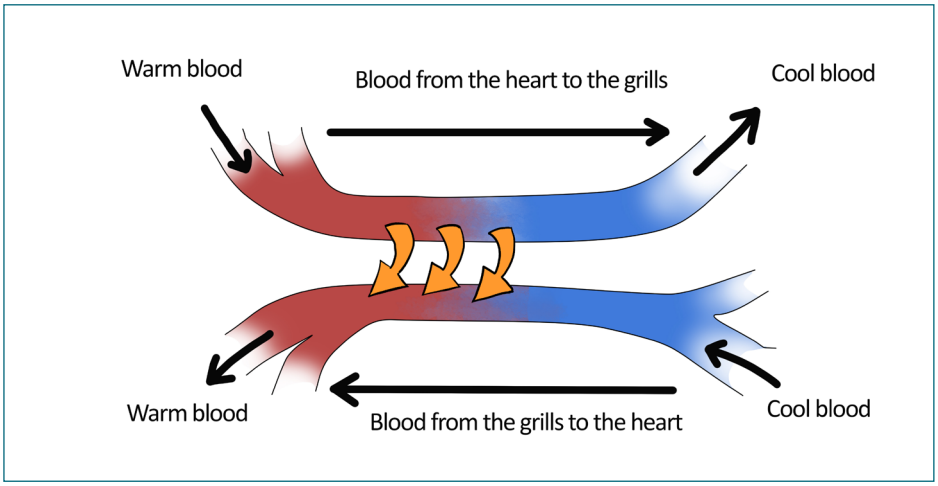


Figure 3.8: Counter current heat exchange mechanism

3. Hyperthermia: Hyperthermia is a condition of having the body temperature greatly above the normal. Although all the endotherms can maintain a constant body temperature, some are able to raise their body temperature as a way to decrease the amount of water and energy used for thermoregulation. For example, camels and gazelles can increase their body temperature by 5–7°C during the day when the animal is dehydrated. Hyperthermia helps in saving water by letting their body temperature increase instead of using evaporative cooling to keep it at a constant temperature.

4. Water retention: Human body obtains about 60 per cent of the water they need from ingested liquid, 30 per cent from ingested food, and 10 per cent from metabolism. While rodent adapted to arid conditions obtains approximately 90 per cent from metabolism and 10 per cent from ingested food. The predaceous marsupial Mulgara species can go its whole life without ingesting water but by obtaining water from the food they eat and from metabolism. The fawn hopping mouse eats seed, small insects, and green leaves for moisture, and Kowaris eat insects and small mammals to obtain water. These animals have specialized kidneys with extra microscopic tubules to extract most of the water from their urine and return it to the blood stream. And much of the moisture that would be exhaled in breathing is recaptured in the nasal cavities by specialized organs.

Many desert dwelling insects tap plant fluids such as nectar or sap from stems, while others extract water from the plant parts they eat, such as leaves and fruit. The abundance of insects permits insectivorous birds, bats and lizards to thrive in the desert. Elf owls survive on katydids and scorpions. Pronghorns can survive on the water in cholla fruits. Kit foxes can satisfy their water needs with the water in their diet of kangaroo rats, mice, and rabbits, along with small amounts of vegetable material.

5. Excretion: As mentioned above, desert dwelling mammals and birds have specialized kidneys with long loops of Henle compared to animals that live in aquatic environments and less arid regions. A longer tubules help in reabsorbing most of the water from their urine and return it to blood stream. As a result, the urine becomes highly concentrated. In these animals, most of the water in the faeces gets reabsorbed in the alimentary canals and colon. Camels produce dryer faeces than other ruminants. For example, sheep produce faeces with 45 per cent water after 5 days of water deprivation, while camels produce faeces with 38 per cent water even after 10 days of water deprivation. The ability to excrete concentrate urine and dry faeces is an important adaptation to arid conditions. Desert rodents can have urine five times as concentrated as that of humans.

Behavioural adaptations

Behavioural adaptations are used to reduce the amount of heat gained or lost by animals, and, thereby, reducing the amount of energy and water to maintain the body temperature. Ectoderms or cold blooded animals rely on their behaviour to maintain a favourable body temperature.

1. Nocturnality: It is the simplest form of behavioural adaptation characterized by activity during the night and sleeping during the day. As such, nocturnal animals avoid direct exposure to heat of the day, thereby preventing loss of water needed for evaporative cooling. The night temperatures are generally 15–20°C colder than the daytime, so require much less energy and water to regulate body temperature. Most of the desert animals like quoll, bilby, and the spinifex hopping mouse, are nocturnal. Other large animals like lions prefer to hunt at night are to conserve water.

Crepuscular animals are those animals that are mainly active during twilight (i.e., the period before dawn and that after dusk). Examples include hamsters, rabbits, jaguars, ocelots, red pandas, bears, deer, moose, spotted hyenas etc. Many moths, beetles, flies, and other insects are also crepuscular in habit. These crepuscular animals take advantage of the slightly cooler mornings and evenings to escape the daytime heat, and to evaporate less water.

2. **Microhabitat:** Among the diurnal animals (animals which are mainly active during the day and rest during night), the use of microhabitat like burrows, shade is another type of behavioural adaptation to avoid the daytime heat. Fossorial animals (digging animals), such as mulgaras, spent much of their time below ground eating stored food. Lizards and snakes seek a sunny spot in the morning to warm up their body temperatures more quickly and remain in shade when the temperature rises.

3. Migration: It is the physical movement of animals over a long distance from one area to another**.** It is found in all major animal groups, including birds,

mammals, fish, reptiles, amphibians, insects, and crustaceans. Many factors like climate, food, the season of the year or mating could lead to migration. It helps the animals in avoiding the extreme environmental conditions by moving to more favourable places. For example, many migratory birds like arctic tern (*Sterna paradisaea*) migrate north-south, with species feeding and breeding in high northern latitudes in the summer, and moving some hundreds of miles south during the winter to escape the extreme cold of north. Monarch butterflies spend the summer in Canada and the Northern America and migrate as far south as Mexico for the winter.

4. Hibernation and Aestivation: Warm blooded animals which do not migrate generally survive the extreme cold condition of winter by sleeping. **Hibernation** is the state of dormancy during the cold conditions, i.e., winter. During hibernation, body temperature drops, breathing and heart rate slows, and most of the body's metabolic functions are put on hold in a state of quasi-suspended animation. This allows them to conserve energy, and survive the winter with little or no food.

Many insects spend the winter in different stages of their lives in a dormant state. Such phenomenon is known as **diapause**. During diapauses, insect's heartbeat, breathing and temperature drop. Some insects spend the winter as worm-like larvae, while others spend as pupae. Some adult insects die after laying their eggs in the fall and eggs hatch into new insects in the spring when the food supply and temperature become favorable.

Aestivation or summer dormancy on the other hand, is a state of animal dormancy, characterized by inactivity and a lowered metabolic rate, in response to high temperatures and arid conditions**.** It allows an animal to survive the scarcity of water or food as aestivating animal can live longer off its energy reserves due to the lowered metabolism, and reduced water loss though lowered breathing rates. Lung fishes, toad, salamander, desert tortoise, swamp turtles are some of the other non-mammalian animals which undergo aestivation.

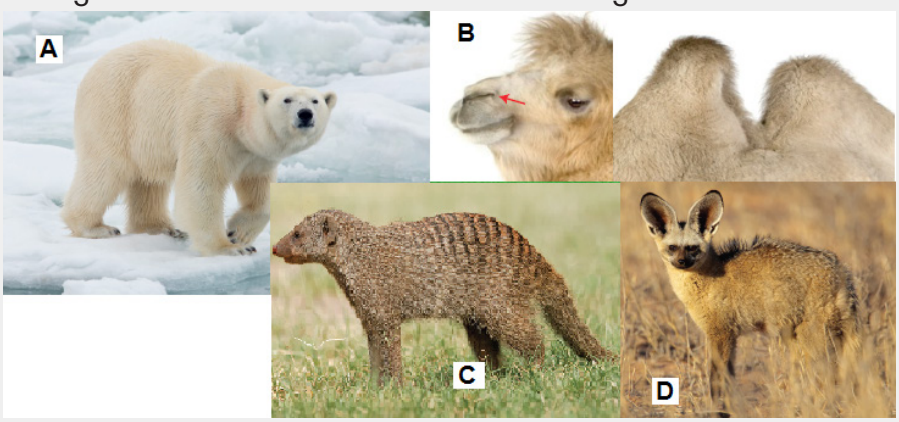
5. Social behavior: Among all the adaptations, living together is one of the most important adaptations of the animal kingdom. Animals can derive a lot of benefit from spending time with other members of the same species like finding food, defense against predators and care for their young**.** For example, emperor penguins can survive the harsh Antarctica winter **huddling** together in groups that may comprise several thousand penguins. Huddling greatly reduces the surface area of the group compared to individuals and a great deal of warmth and body fat is conserved. Many social mammals, including many rodents, pigs and primates survive extreme cold by huddling together in groups.

6. Locomotion: Different types of locomotion require varying amount of energy. Many mammals like kangaroo, hares hop, which is an energy efficient type of

locomotion. When animals go from walking to running, there is an increasing energy cost; however, once kangaroos start moving, there is no additional energy cost. This is because when a kangaroo lands, energy is stored in the tendons of its hind legs which is used to power the next hop.

Application activity 3.3

1) The figure below shows different animals living in different climates



a) Which animal(s) on the photo appears to be adapted to live in cold climates? Why?

b) Which animal(s) on the photo appears to be adapted to live in hot climates? Why?

c) What are the adaptations of the animal A that help it to survive in its environment?

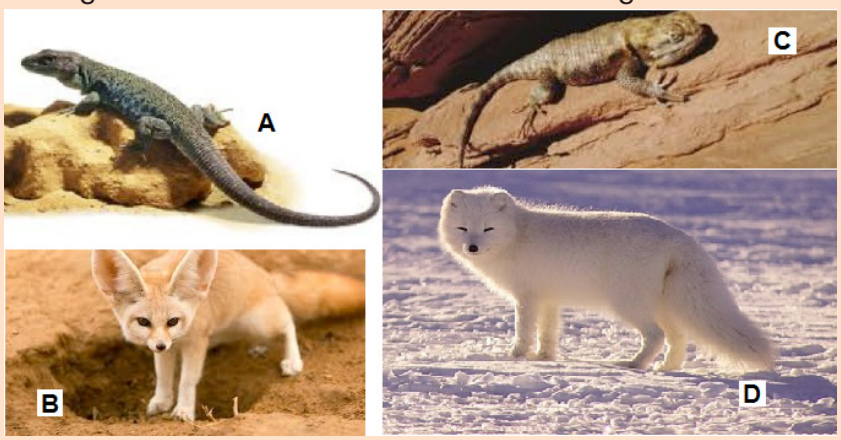
d) What is the functions of the humps on the animal B?

e) Some animals such as the animal A hibernate during the winter. Explain the importance of hibernation to these animals.

3.4 Response to cold and hot conditions by endothermic and ectothermic animals

Activity 3.4

1) The figure below shows different animals living in different climates



a) The animals A and B are reptiles under different environmental conditions. Compare their behaviors in regards to how they regulate their temperature.

b) The animals' C and D are mammals under different environmental conditions. Compare their behaviors in regards to how they regulate their temperature.

c) What are the adaptations of the animal D that help it to survive in its environment?

d) How is the animal A different to animal D according to how they regulate their body temperature.

3.4.1 Endotherms' response to temperature changes

Endothermic organism can maintain relatively high body temperatures within a narrow range. Since most of the body heat is produced as a result of various metabolic activities, thermoregulation in endotherms depends on food and water availability. For example, bear undergoes hibernation during the winter because there is no sufficient food during the cold season. On the other hand, in arid environment like deserts, many deserts animals are nocturnal to avoid the extreme daytime heat to avoid loss of water through evaporation.

Response to hot temperature

When the body temperature increases in response to the external temperature, the body's temperature control system uses three important mechanisms to reduce the body heat. These are:

1. Vasodilation of blood vessels in the skin: The blood vessels in skin become intensely dilated due to the inhibition of the sympathetic centres in the posterior hypothalamus that cause vasoconstriction. Vasodilation increases the rate blood flow to the skin and as a result, the amount of heat transfer from the core of the body increases tremendously.

2. Sweating: As discussed in the previous section, sweating is an important adaptation to lose body heat through evaporative cooling. An increase in 1°C in body temperature causes enough sweating to remove ten times the basal rate of body heat production.

3. Decrease in heat production**:** As mentioned above, metabolic activities of the body are the main source of body heat. The mechanisms that cause excess heat production, such as shivering and chemical thermogenesis, are strongly inhibited when exposed to hot temperature.

Response to cold temperature

In response to cold temperature, the temperatures control system performs exactly opposite mechanism to that performs in hot temperature. These are:

1. Vasoconstriction of blood vessels in the skin**:** The blood vessels in the skin constrict under the influence of posterior hypothalamic sympathetic centres which reduce the blood flow to the skin.

2. Piloerection: Piloerection means hairs "standing on end". Sympathetic stimulation causes the arrector pili muscles attached to the hair follicles to contract, which brings the hairs to an upright stance. The upright projection of the hairs allows them to entrap a thick layer of air next to the skin which acts as insulator, so that transfer of heat to the surroundings is greatly depressed.

3. Increase in heat production **(thermogenesis):** Endothermic metabolic rates are several times higher than those of ectotherms. The metabolic heat production of endotherms is regulated in response to fluctuations in the environment temperature. This phenomenon is known as adaptive thermogenesis or facultative thermogenesis. It can be defined as *"Heat production by metabolic processes in response to environmental temperature with the purpose of protecting the organism from cold exposure and buffering body temperature from environmental temperature fluctuations". Under cold temperature stress, heat production by the metabolic activities increased tremendously by promoting shivering, sympathetic excitation of heat production, and thyroxine secretion.*

These mechanisms will be discussed later. Extreme shivering can increase the temperature four to five times the normal production.

3.4.2 Ectotherms' response to temperature changes

Ectotherms cannot maintain stable body temperature and their body temperature relies on the external temperature. They depend more on energy assimilation rather than utilizing it for temperature regulation. Therefore, ectotherms regulate their body temperature behaviourally and by cardiovascular modulation of heating and cooling rates. At the same time, metabolism and other essential rate functions are regulated so that reaction rates remain relatively constant even when body temperatures vary. This process is known as acclimatization or temperature compensation. For example, many fish adjust metabolic capacities to compensate for seasonal variation in water temperature with the result that metabolic performance remains relatively stable throughout the year. Reptiles often regulate their body temperature to different levels in different seasons to minimize the behavioural cost of thermoregulation. At the same time, tissue metabolic capacities are adjusted to counteract thermodynamically-induced changes in rate functions.

Response to hot temperature

When the external temperature increases, ectotherms protect their bodies from overheating using various mechanisms. These are:

1. **Use of microhabitat:** Under extreme heat conditions, many ectotherms like lizards and snakes prefer to stay in shade, either beneath the rocks, crevices or underground burrows.

Amphibians and fishes enter cold water when their body temperature increases.

2. **Acclimatization:** If a salamander living at 10°C is exposed to 20°C, its metabolic rate increases rapidly. But if the exposure to the higher temperature lasts for several days, the animal experiences a compensating decrease in the metabolic rate. This decrease in the metabolic rate is due to acclimatization. The higher metabolic rate is due to the increase in the enzymes activity with temperature. However, with prolonged exposure to the condition, the metabolic rates decrease to prevent excessive energy loss. Ectotherms also exhibit acclimatization of temperature tolerance range with animal acclimated to high temperature are able to tolerate higher temperature than those exposed only to low temperature. Similarly, cold acclimated animals have better tolerance to low temperature than high temperature acclimated animal.

Response to cold temperature

Ectotherms response to cold temperature is exactly opposite to the response shown when exposed to hot temperature. That is:

1. Basking to sun: When the body temperature of the ectotherms becomes colder than the normal, the animals either bask to sunlight to warm up the body or move to a warmer place. Under extreme cold conditions, all the metabolic activities may cease and the animals enter the state of torpor (reduced metabolic activities).

2. Cold Acclimatization: Decrease in the temperature result in reduced metabolic rate. Therefore, as a compensatory measure to meet the require body metabolism, the cold acclimatization of ectotherms is characterized by increase in concentration of various metabolic enzymes. There is also significant increase in the mitochondria and capillaries concentration in the skeletal muscle. This increase the ATP production through aerobic respiration in these tissues. Therefore, in those animals which have prolonged exposure to cold temperature, there may be increase in the locomotion, though the basal rates of metabolism remain below the warm acclimatized animals.

Application activity 3.4

1. a) Describe the importance of hibernation to animals.

b) The camel is one of the animals adapted to live in deserts. Explain three of its adaptations that help it to survive in arid conditions.

c) State three adaptations of animals to living in cold climates.

3.5 Role of the brain

Activity 3.5

Find information about the role of hypothalamus and different thermoreceptors in temperature regulation. Make short notes and present them in front of the class.

So far we have discussed that on the basis of types of thermoregulation, all the living organisms can be classified into two groups – ectotherms and endotherms. Endotherms can regulate their body temperature within a narrow range through various physiological mechanisms while ectotherms being depended on external temperature mostly rely on their behaviour to maintain body temperature. But how do these animals sense and counter the changing temperature of their body will be discussed in the section.

Thermoreceptors

A **thermoreceptor** is a sensory receptor which is basically the receptive portion of a sensory neuron that converts the absolute and relative changes in temperature, primarily within the innocuous range to nerves impulses. **Thermoreception** is the sense by which an organism perceives the temperature of the external and internal environment from the information supply by thermoreceptors. In vertebrates, most of the thermoreceptors are found in skins which are actually free nerve endings. Deep body thermoreceptors are also found mainly in the spinal cord, in the abdominal viscera, and in or around the great veins in the upper abdomen and thorax region.

Mammals have at least two types of thermoreceptors: the **warm receptors**, those that detect heat or temperatures above normal body temperature and **cold receptors**, those that detect cold or temperatures below body temperature. The warm receptors are generally unmyelinated nerves fibres, while cold receptors have thinly myelinated axons and hence faster conduction velocity. Increasing body temperature results in an increase in the action potential discharge rate of warm receptors while cooling results in decrease. On the other hand, cold receptors' firing rate increases during cooling and decreases during warming. Another types of receptor called **nociceptors**, detect pain due to extreme cold or heat which is beyond certain threshold limits.

A specialized form of thermoception known as distance thermoreception is found in some snakes like pit viper and boa, use a specialized type of thermoreceptor which can sense the infrared radiation emitted by hot objects. The snake's face has a pair of holes, or pits, lined with temperature sensors. These sensors indirectly detect infrared radiation by its heating effect on the skin inside the pit which helps them to locate their warm blooded prey. The common vampire bat may also have specialized infrared sensors on its nose.

Hypothalamus

The hypothalamus is a very small, but extremely important part of the brain that acts as the **link** between the **endocrine and nervous systems of the body**. The hypothalamus plays a significant role in the endocrine system and is responsible for maintaining the body's **homeostasis** by stimulating or inhibiting many key processes, including **body temperature, fluid and electrolyte balance, appetite and body weight, glandular secretions** of the stomach and intestines, production of substances that influence the pituitary gland to release hormones and sleep cycles.

Role of Hypothalamus in thermoregulation

Thermoregulation is carried out almost entirely by nervous feedback mechanisms, and almost all these operate through temperature-regulating centres located in

the hypothalamus (Figure 3.7). The hypothalamus contains large numbers of heat-sensitive as well as cold sensitive neurons which acts as thermoreceptor, sensing the temperature of the brain. The posterior hypothalamus region contains the thermoregulatory centre which integrate the signals from of all the thermoreceptors found in skin, deep organs and skeletal muscles, as well as from the anterior hypothalamus and control the heat-producing and heat-conserving reactions of the body.

Cooling Mechanism

When the body temperature increases beyond the set-point, the anterior hypothalamus is heated. The posterior hypothalamus senses the heat and inhibits the adrenergic activity of the sympathetic nervous system, which control vasoconstriction and metabolic rate. This causes cutaneous vasodilation and increase heat loss through skin. It also reduces the body metabolic rate resulting in decreasing heat production through metabolic reactions. Under intense heating, the cholinergic sympathetic fibres innervating the sweat glands release acetylcholine, stimulating the secretion of sweat. Many behavioural responses to heat, such as lethargy, resting in shade, lying down with limbs spread out, etc., decreases heat production and increases heat loss.

Heating Mechanism

When the body temperature falls below the set-point, the body regulating mechanism tries to reduce heat loss and increase heat production. The immediate response to cold is vasoconstriction throughout the skin. The result is vasoconstriction of the skin blood vessels, reducing the blood flow and subsequent heat loss through skin. Sympathetic stimulation also causes piloerection and reduces the heat loss from the body by trapping heat within the body hair.

The primary motor centre for shivering is excited by the cold signals from skin and spinal cord which cause shivering of the skeletal muscles. Intense shivering can increase the body heat production four to five times normal. Cooling the anterior hypothalamic due to decrease in body temperature stimulates hypothalamus to increases the production of the neurosecretory hormone **thyrotropin-releasing hormone**. This hormone in turn stimulates the anterior pituitary gland, to secrete **thyroid-stimulating hormone**. Thyroid-stimulating hormone then stimulates thyroid glands to increased output of thyroxine. The increased thyroxine level in the blood increases the rate of cellular metabolism throughout the body and hence increases heat production.

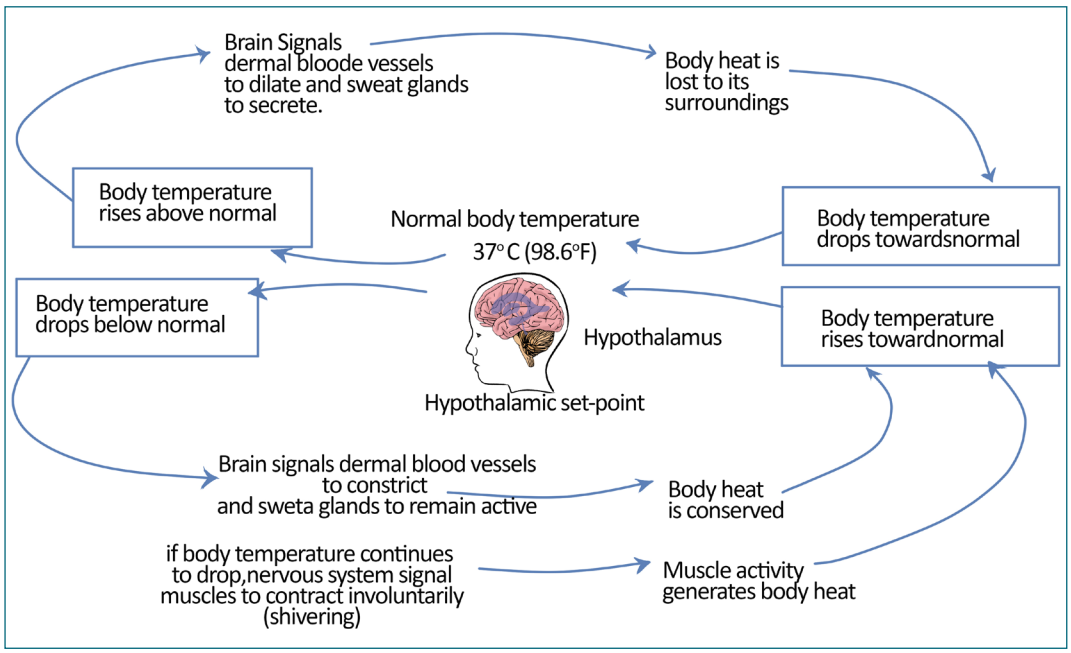
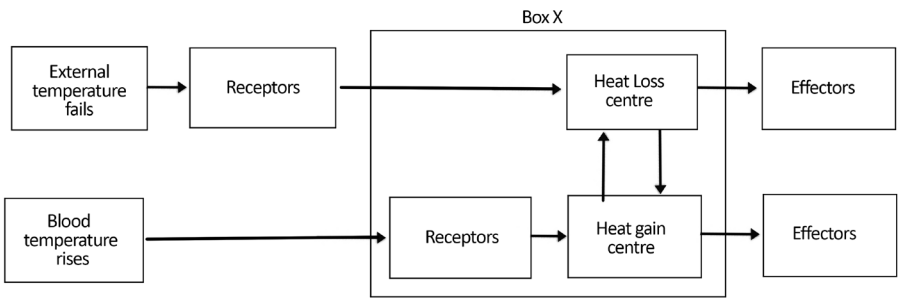


Figure 3.9: Nervous feedback mechanism for regulation of body temperature

Application activity 3.5

1) The diagram shows the way in which temperature is regulated in body of a mammal.



a) Which part of the brain is represented by box X?

b) i) How does the heat loss center control the effectors which lower the body temperature?

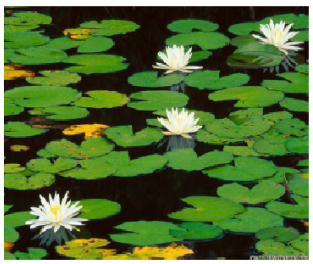
ii) Explain how blood vessels can act as effectors and lower the body temperature?

3.6 Temperature controls in plants

Activity 3.6

Observe carefully the photos below and answer to the questions that follow:

A



B

C

a) In which habitat do these plants live?

b) What are the adaptations of plant A that help it to survive in its environment?

c) Make a comparison between plant A and plant B.

Like all the other living organisms, plants depend on enzymes catalyzed chemical reactions for their growth and development. For example, plants synthesize their own food from water and carbon dioxide using sunlight through photosynthesis. The process of photosynthesis involves a series of complex enzyme system and other proteins. Therefore, along with carbon dioxide, water, light, nutrients and humidity, temperature is also one of the limiting factors for growth and development of plants.

Unlike animals, plants remain fixed in a particular site and absorb heat from the sunlight. The excess heat from the body is released to the surrounding through radiation and evaporation. The process of evaporation of water from the leaves and stem of plants to the surrounding environment is known as **transpiration**. It occurs through **stomata**, small opening located on the underside of the leaves. The stomata are specialized cells in the leaves which can open or close, limiting the amount of water vapour that can evaporate. Higher temperature causes the opening of stomata whereas colder temperature causes the opening to close. The opening of the stomata and hence the transpiration rate of plants depends on environmental conditions such as light, temperature, the level of atmospheric CO_2 and relative humidity. Higher relative humidity leads to more opening, while higher CO_2 levels lead to closing of stomata. Under high environmental temperature, the plant body gets heat up. In order to cool down, the plant increases its transpiration rate. The evaporative loss of water from the plant's body lowers the temperature.

Besides transpiration, many plants have different adaptations that help them survive in extreme temperature conditions ranging from hot and arid deserts to cold and snow covered mountains. These adaptations make it difficult for the plant to survive in a different place other than the one they are adapted to. This explains why certain plants are found in one area, but not in another. For example, cactus plants, adapted to desert conditions can't survive in the Arctic.

These adaptations will be discussed later in this unit.

3.6.1 Effect of temperature changes on plants

The most obvious effect of temperature on plants is changes in the rate of photosynthesis and respiration. Both processes increase with rise in the temperature up to a certain limit. However, increase in temperature beyond the limits, the rate of respiration exceeds the rate of photosynthesis and the plants productivity decreases.

Another important effect of temperature is during the process of **germination** of seeds. Like most other processes it also depends on various factors including air, water, light, and, of course, temperature. In many plant species, germination is triggered by either a high or low temperature period that destroys germination inhibitors. This allows the plant to measure the end of winter season for spring germination or end of summer for fall germination. For example, winter adapted plant seeds remain dormant until they experience cooler temperatures. Temperature of 4°C is cool enough to end dormancy for most cool dormant seeds, but some groups, especially within the family Ranunculaceae and others, need conditions cooler than −5°C. On the other hand, some plants like Fire poppy (*Papaver californicum*) seeds will only germinate after hot temperatures during a forest fire which cracks their seed coats. The fire does not cause direct germination, rather weakens the seed coat to allow hydration of the embryo.

Pollination is another phenological stage of plants sensitive to temperature extremes across all species. Since pollination is carried out by pollinators like honey bees, butterflies etc., any factors including temperature that affect these pollinators will certainly affect the process.

Heat adapted plants

In extremely hot and dry desert region with annual rainfall averages less than 10 inches per year, and there is a lot of direct sunlight shining on the plants, the main strategy for the survival of the plants is to avoid extensive water loss through transpiration. Therefore, in such region many plants called **succulents**, like cactus can store water in their stems or leaves. Some plants are leafless or have small seasonal leaves that only grow after rains. These leafless plants conduct photosynthesis in their green stems. Leaves are often modified into spines to discourage animals from eating plants for water. Also waxy coating

on stems and leaves help reduce water loss. Other plants have very long root systems that spread out wide or go deep into the ground to absorb water.

On the other hand, in hot and humid tropical rainforest, the abundance of water can cause problems such as promoting the growth of bacteria and fungi which could be harmful to plants. Heavy rainfall also increases the risk of flooding, soil erosion, and rapid leaching of nutrients from the soil. Plants grow rapidly and quickly use up any organic material left from decomposing plants and animals. The tropical rainforest is very thick, and not much sunlight is able to penetrate to the forest floor. However, the plants at the top of the rainforest in the canopy must be able to survive the intense sunlight. Therefore, the plants in the tropical rainforest usually have large leaves with drip tips and waxy surfaces allow water to run off easily. Some plants grow on other plants to reach the sunlight.

Similarly, in aquatic plants adapted for life in water, the leaves are very large, fleshy and waxy coated. Increase surface area allows plants to lose excess water while the shiny wax coating discourages the growth of microbes. The roots and stems are highly reduced since water, nutrients, and dissolved gases are absorbed from the water directly through the leaves.

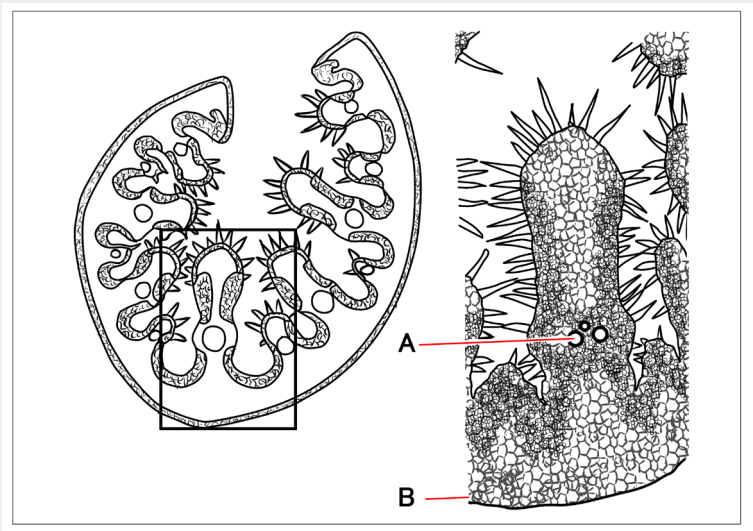
Cold adapted plants

In extremely cold region like tundra which is characterized by a permanently frozen sub-layer of soil called **permafrost**, the drainage is poor and evaporation slow. With the region receiving very little precipitation, about 4 to 10 inches per year usually in the form of snow or ice, plant life is dominated by small, low growing mosses, grasses, and sedges. Plants are darker in colour, some even red which helps them absorb solar heat. Some plants are covered with hair which helps keep them warm while others grow in clumps to protect one another from the wind and cold.

In a slightly warmer temperate forest, with temperature varies from hot in the summer to below freezing point in the winter, many trees are deciduous that is they drop their leaves in the autumn to avoid cold winter, and grow new ones in spring. These trees have thin, broad, light-weight leaves that can capture a lot of sunlight to make a lot of food during the warm weather and when the weather gets cooler, the broad leaves cause too much water loss and can be weighed down by too much snow, so the tree drops its leaves. They usually have thick bark to protect against cold winters.

Application activity 3.6

1) The diagram below shows a transverse section of a leaf *Ammophila arenaria*, which is a xerophyte. The photomicrograph shows the details of the area indicated by the box in the diagram.



a) Name the parts labelled A and B.

b) Describe two xeromorphic features shown in this leaf and, in each case, indicate how the feature helps to reduce transpiration.

Skills Lab 3

Aim: To perform blood glucose quantification test.

Materials Required:

- Glucose meter
- Test strips
- Lancets (small needles used to prick the skin) and lancet device that holds the lancet.

Procedure:

1) Wash your hands with soap and water and dry them properly.
2) Prepare the blood glucose meter with the test strip according to the manufacturer's instructions.
3) Use the lancet device to prick the side of your fingertip with a lancet.
4) Place a drop of blood onto the correct part of the test strip.
5) The strip will draw up the blood into the meter and show a digital reading of the blood glucose level within seconds.
6) Note the reading.
7) Use a clean cotton ball to apply pressure to the fingertip for a few moments until the bleeding stops.
8) Similarly, measure the blood glucose level of your friends.
9) Compare your blood glucose level with that of your friends.

Discussion:

In general, a fasting blood glucose reading (taken before a meal) should be between 72 mg/dL to 126 mg/dL. And a blood glucose reading 2 hours after a meal should be between 90 mg/dL to 180 mg/dL.

Precautions:

1) Make sure the lancelet is properly sterilized.
2) Insert the test strip properly.

End unit assessment 3

I. Multiple Choice Questions

1) Which of the following monosaccharides is not a product of carbohydrate metabolism in our body?

(a) Glucose (b) Fructose (c) Ribose (d) Galactose

2) Which of the following is not a part of portal triad?

(a) Central vein (b) Hepatic artery

(c) Hepatic portal vein (d) Bile duct.

3) Somatostatin is secreted by

(a) Alpha cells (b) Beta cells

(c) Delta cells (d) F cells

The process of formation of glucose from non-carbohydrates source in the body is known as

(a) Glycogenesis (b) Gluconeogenesis

(c) Glycolysis (d) Glycogenolysis

5) Which of the following hormone is responsible for decreasing blood glucose level?

(a) Glucagon (b) Insulin (c) Somatostastin (d) Adrenaline

6) The enzyme used in the dipstick for testing concentration of glucose is

(a) Glucose oxidase (b) Glycogen phosphorylase

(c) Glucose phosphatase (d) Glucosidase

II. State whether the following statements are True (T) or False (F)

1) Excess glucose in the body is stored in the form of glycogen.

2) Trypsin is an enzyme used for carbohydrate digestion.

3) Bile salt is secreted by exocrine liver.

4) Glucagon is secreted by pancreas in response to high blood glucose concentration.

5) Insulin administration is recommended for person with type II diabetes mellitus.

6) Type I diabetes mellitus is cause due to insufficient secretion of insulin by beta cells.

7) Ketone bodies are formed when our body have excessive fat metabolism.

8) Hyperinsulinaemia is associated with type II diabetes mellitus.

9) All the living organisms have a particular range of temperature within which they can best survive and reproduce.

10) Nocturnality is the simplest form of behavioral adaptation characterized by activity during the day and sleeping during the night.

11) Crepuscular animals take advantage of the slightly cooler mornings and evenings to escape the daytime heat, and to evaporate less water.

12) Body temperature of Ectotherms rely on the external temperature.

13) Thermoregulation in endotherms depends on food and water availability.

14) Glycogenolysis is the breakdown of glucose to form pyruvate.

III Long Answer Type Questions

1) List few adaptive features shown by plants inhabiting extreme cold and hot environments.

2) Explain the role of the brain and thermoreceptors in temperature regulation.

3) In your own words, explain the importance of maintaining fairly constant temperatures for efficient metabolism.

4) Describe the functions of liver and pancreas in regulating blood glucose level.

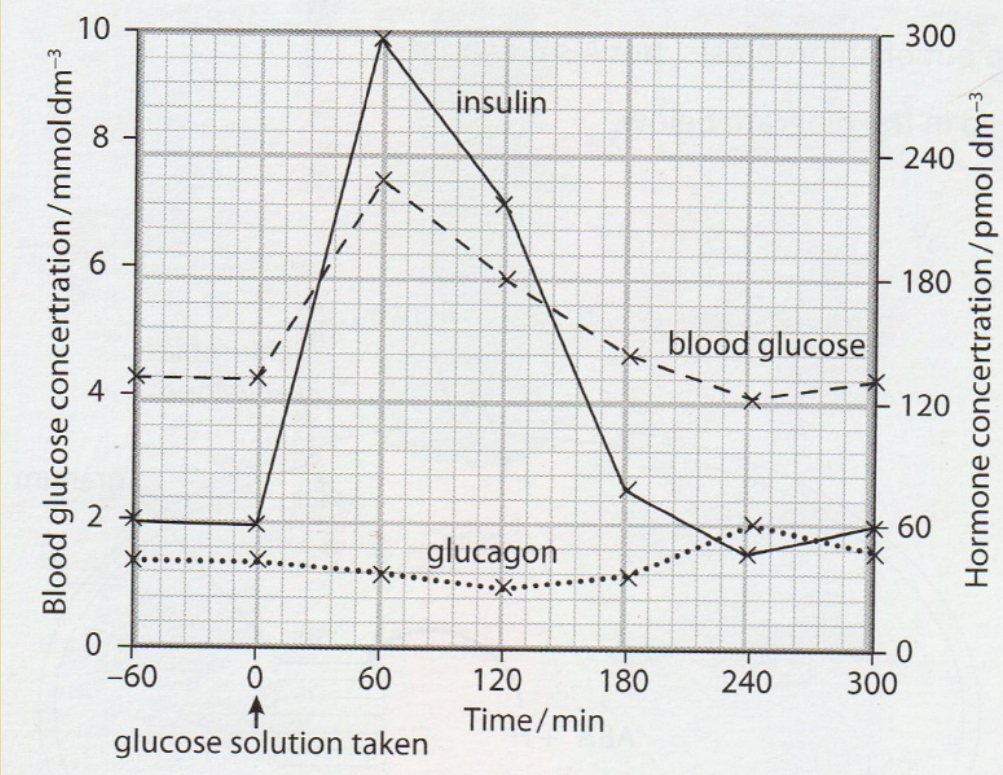
5) Discuss in brief the importance of urine analysis in diagnosis diabetes mellitus.

6) The control of blood glucose concentration involves a negative feedback mechanism.

a) What are the stimuli, receptors and effectors in this control mechanism?

b) Explain how negative feedback is involved in this homeostatic mechanism.

7) An investigation was carried out to determine the response of pancreatic cells to an increase in the glucose concentration of the blood. A person who had been told not to eat or drink anything other than water for 12 hours then took a drink of a glucose solution. Blood samples were taken from the person at one hour intervals for five hours, and the concentration of glucose, insulin and glucagon in the blood and the concentration of glucose, insulin and glucagon in the blood were determined. The results are shown in the graph below:



a) Explain why the person was told not to eat or drink anything other than water for 12 hours before having the glucose drink.

b) Use the information in the figure to describe the response of the pancreatic cells to an increase in the glucose concentration.

c) Outline the role of insulin when the glucose concentration in the blood increases.

d) Suggest how the results will change if the investigation continued longer than five hours without the person taking any food.

e) Outline the sequence of events that follows the binding of glucagon to its membrane receptor on a liver cell.

UNIT 4

PRINCIPLES OF GENE TECHNOLOGY AND ITS APPLICATIONS

Key unit competence

Explain the principles of gene technology and evaluate how gene technology is applied in areas of medicine, forensic science and agriculture

Introductory activity 4

You hear about them all the time. They are often depicted in cartoons, comic books, movies, and science fiction as mad scientists. These are the scientists who take a gene from one organism and place it into an unrelated organism. These are the scientists who make hormones that farmers inject into the cows that produce the milk we drink.

These are the scientists who modify the crops we eat, creating what some people call “Franken foods” or genetically modified organisms. The figure below shows a GMO tomato and a GMO rice also called golden rice.

You may have wondered if it might soon be possible to replace a beloved family member or pet, or bring back extinct species through cloning, or even clone yourself. You might worry about a future where parents unwilling to fix their children’s “genetic defects” face discrimination.

Use the image above and your own knowledge to answer the questions that follow:

a) Who are these scientists who make such manipulations?

b) What do they do?

c) What are the basic tools that these scientists use?

d) What are the possible products that they do?

e) Is anyone trying to determine if it is unhealthy to eat these modified foods, whether genetically modified plants will cause environmental problems, or if genetically modified animals are less healthy than their counterparts?

4.1 Recombinant DNA technology

Activity 4.1

Carpenters require tools such as hammers, screwdrivers, and saws; surgeons require scalpels, forceps, and stitching needles; and mechanics require hoists, wrenches, and pumps. These individuals use their implements to modify, deconstruct, or build a system that they are working with. Just like any other technicians, molecular biologists use tools to complete a project. The tools in their laboratories may aid them in investigating genetic disorders, altering the genetic makeup of organisms so that they produce useful products such as insulin, or analysing DNA evidence in a criminal investigation. Find out the possible tools used in recombinant DNA technology and their functions.

Genetic engineering, also known as **recombinant DNA (rDNA) technology**, means altering the genes in a living organism to produce a Genetically Modified Organism (GMO) with a new genotype. Various kinds of genetic modification are possible: inserting a foreign gene from one species into another, forming a transgenic organism; altering an existing gene so that its product is changed; or changing gene expression so that it is translated more often or not at all.

4.1.1 Techniques of genetic engineering

Genetic engineering is a very young discipline, and is only possible due to the development of techniques from the 1960s onwards. These techniques have been made possible from our greater understanding of DNA and how it functions following the discovery of its structure by Watson and Crick in 1953. Although the final goal of genetic engineering is usually the expression of a gene

in a host, in fact most of the techniques and time in genetic engineering are spent isolating a gene and then cloning it.

An overview of gene transfer

There are many different ways in which a GMO may be produced, but these steps are essential.

- The gene that is required is identified. It may be cut from a chromosome, made from mRNA by reverse transcription or synthesized from nucleotides.
- Multiple copies of the gene are made using the technique known as the polymerase chain reaction (PCR).
- The gene is inserted into a **vector** which delivers the gene to the cells of the organism. Examples of vectors are **plasmids**, viruses and liposomes.
- The vector takes the gene into the cells.
- The cells that have the new gene are identified and cloned.

To perform these steps, the genetic engineer needs a 'tool kit' consisting of:

- **Enzymes**, such as **restriction endonucleases**, **DNA ligase** and **reverse transcriptase**
- **Vectors,** including **plasmids** and **viruses**
- **Host cell,** a living system (microbial, plant, animal) in which the vector can be propagated.
- **Genes** coding for easily identifiable substances that can be used as **markers**.

4.1.2 Plasmids

A plasmid is a genetic structure, in some cells, that can replicate independently of the chromosomes; it is typically a small circular DNA strand in the cytoplasm of a bacterium or protozoan. Plasmids are much used in the laboratory during manipulation of genes as vectors.

The properties of plasmids are:

- It is big enough to hold the desired gene.
- It is circular (or more accurately a closed loop), so that it is less likely to be broken down.
- It contains control sequences, such as a transcription promoter, so that the gene will be replicated or expressed.
- It contains marker genes, so that cells containing the vector can be identified.

Plasmids are not the only type of vector that can be used. **Viruses** can also be used as vectors. Another group of vectors are **liposomes**, which are tiny spheres of lipid containing the DNA.

The production of genetically modified organisms (GMO), also called **transgenic organisms**, is a multistage process which can be generally summarized as follows:

- Identification of the gene of interest.
- Isolation of the gene of interest.
- Cutting of gene of interest and opening of plasmid with restriction enzymes in order to have sticky ends.
- Associating the gene with an appropriate promoter and poly -A sequence and insertion into plasmids.
- Multiplying the plasmid in bacteria and recovering the cloned construct for injection.
- Transference of the construct into the recipient tissue, usually fertilized eggs.
- Integration of gene into recipient genome.
- Expression of gene in recipient genome.
- Inheritance of gene through further generations.

Application activity 4.1

1) Explain briefly the terms below:
   a) Recombinant DNA
   b) Transgenic organism
   c) Enzyme
2) State the main tools of a genetic engineer and their functions.

4.2 Roles of enzymes in genetic engineering

Activity 4.2

Enzymes are biological molecules which speed up the rates of chemical reactions in our body. There are different enzymes with different functions used in genetic engineering. Find the information about these enzymes and their possible functions.

The enzymes involved in gene manipulation include; **restriction endonucleases** (restriction enzymes), **methylases**, **ligase** and **reverse transcriptase**.

4.2.1 Restriction enzymes

These are enzymes that cut DNA at specific sites. They are properly called restriction endonucleases because they cut the bonds in the middle of the polynucleotide chain. Each type of restriction enzyme recognizes a characteristic sequence of nucleotides that is known as its **recognition site**. A recognition site is a specific sequence within double-stranded DNA, usually palindromic and consisting of four to eight nucleotides, that a restriction endonuclease recognizes and cleaves. Molecular biologists can use these enzymes to cut DNA in a predictable and precise manner.

Most restriction enzymes make a staggered cut in the two strands, forming sticky ends.

The cut ends are "sticky" because they have short stretches of single-stranded DNA. These sticky ends will stick (or anneal) to another piece of DNA by complementary base pairing, but only if they have both been cut with the same restriction enzyme. Restriction enzymes are highly specific, and will only cut DNA at specific base sequences, 4-8 base pairs long.

Restriction enzymes are produced naturally by bacteria as a defense against viruses (they "restrict" viral growth), but they are enormously useful in genetic engineering for cutting DNA at precise places **("molecular scissors")**. Short lengths of DNA cut out by restriction enzymes are called **restriction fragments**. There are thousands of different restriction enzymes known, with over a hundred different recognition sequences. Restriction enzymes are named after the bacteria species they came from, so Eco R1 is from E. coli strain R.

Table 4.1: List of some restriction enzymes and their respective recognition sites

Microorganism of origin	Enzyme	Recognition site	After restriction enzyme digestion
Escherichia coli	EcoRI	5′-GAATTC-3′ 3′-CTTAAG-5′	5′-G AATTC-3′ 3′-CTTAA G-5′
Serratiamarcescens	SmaI	5′-GGGCCC-3′ 3′-CCCGGG-5′	5′-GGG CCC-3′ 3′-CCC GGG-5′
Arthrobacterluteus	AluI	5′-AGCT-3′ 3′-TCGA-5′	5′-AG CT-3′ 3′-TC GA-5′

Streptomyces albus	*Sal* I	5′-GTCGAC-3′ 3′-CAGGTG-5′	5′-G TCGAC-3′ 3′-CAGGT G-5′
Haemophilus parainfluenzae	*Hind*III	5′-AAGCTT-3′ 3′-TTCGAA-5′	5′-A AGCTT-3′ 3′-TTCGA A-5′

The ends of DNA fragments produced from a cut by different restriction endonucleases differ, depending on where the phosphodiester bonds are broken in the recognition site. In the example in **Table 4.1**, *Eco*RI produces **sticky ends**; that is, both fragments have DNA nucleotides that are now lacking their respective complementary bases. These overhangs are produced because *Eco*RI cleaves between the guanine and the adenine nucleotide on each strand. Since A and G are at opposite ends of the recognition site on each of the complementary strands, the result is the overhang. Another restriction endonuclease, *Sma*I, produces **blunt ends**, which means that the ends of the DNA molecule fragments are fully base paired (**Table 4.1**).

Sticky ends are fragment end of a DNA molecule with **short single stranded overhangs**, resulting from cleavage by a restriction enzyme. **Blunt ends** are fragment ends of a DNA molecule that are **fully base paired**, resulting from cleavage by a restriction enzyme.

Restriction enzymes are named according to the bacteria from which they originate.

For example, the restriction enzyme ***Bam*HI** is named as follows:

- ***B*** represents the genus *Bacillus*
- ***am*** represents the species *amyloliquefaciens*
- **H** represents the strain
- **I** mean that it was the first endonuclease isolated from this strain
- Following the same pattern, the rationale for the name of the restriction enzyme ***Hin*d II** is the following:
- ***H*** represents the genus *Haemophilus*
- ***in*** represents the species *influenzae*
- **d** represents the strain Rd
- **II** means that it was the second endonuclease isolated from this strain.

Generally speaking, the first letter is the initial of the genus name of the organism from which the enzyme is isolated. The second and third letters are usually the initial letters of the species name**.** The fourth letter indicates the **strain**, while the numerals indicate the order of discovery of that particular enzyme from that strain of bacteria.

4.2.2 Methylases

Restriction endonucleases must be able to distinguish between foreign DNA and the genetic material of their own cells; otherwise a bacterium's DNA would be in danger of being cleaved by its own immune system. **Methylases** are enzymes that add a methyl group to one of the nucleotides found in a restriction endonuclease recognition site, altering its chemical composition. In prokaryotes, they modify the recognition site of a respective restriction endonuclease by placing a methyl group on one of the bases, preventing the restriction endonuclease from cutting the DNA into fragments. When foreign DNA is introduced into the bacterium, it is not methylated, rendering it defenceless against the bacterium's restriction enzymes. Methylases are important tools for a molecular biologist when working with prokaryotic organisms. They allow the molecular biologist to protect a gene fragment from being cleaved in an undesired location.

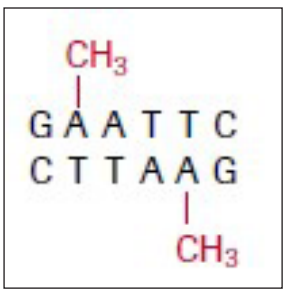


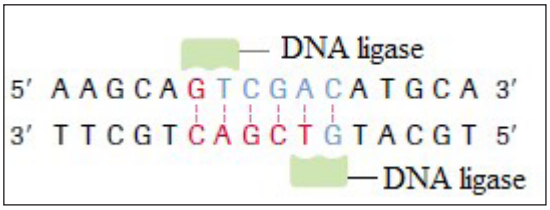
Figure 4.1: Methylated EcoRI site

4.2.3 DNA ligase

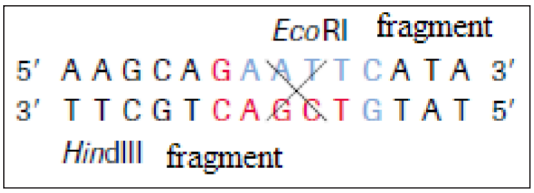
This enzyme repairs broken DNA by joining two nucleotides in a DNA strand. It is commonly used in genetic engineering to do the reverse of a restriction enzyme that is to join together complementary restriction fragments. The sticky ends allow two complementary restriction fragments to harden, but only by weak hydrogen bonds, which can quite easily be broken by gentle heating. The backbone is still incomplete. DNA ligase completes the DNA backbone by forming covalent bonds. **T_4 DNA ligase** is an enzyme that originated from the T4 bacteriophage and which is used to join together DNA blunt or sticky ends. So, DNA ligase is able to join complementary sticky ends produced by the same restriction enzyme via a condensation reaction:

iv) Complementary sticky ends produced by **Hind III**

v) Hydrogen bonds form between complementary bases. DNA ligase reconstitutes the phosphodiester bond in DNA backbones.



vi) If fragments are not complementary, then hydrogen bonds will not form.

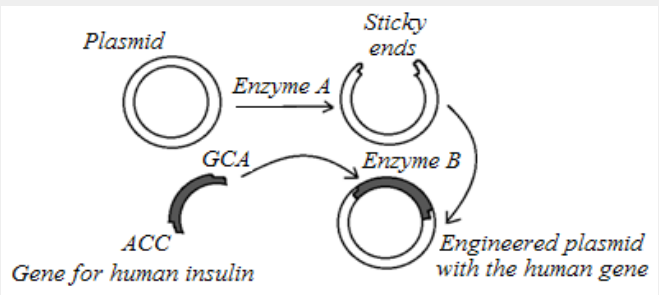


4.2.4 Reverse transcriptase

Reverse transcription is a process whereby a mRNA is converted into cDNA (complementary DNA, also called copy of DNA). It requires the enzymes called reverse transcriptase. It is shown by this reaction:

Application activity 4.2

1) Which of the following tools of recombinant DNA technology is *incorrectly* paired with its use?
   a) Restriction enzyme: cut DNA into smaller segments of various sizes.
   b) DNA ligase: enzyme that cuts DNA, creating the sticky ends of restriction fragments
   c) DNA polymerase: used to make many copies of DNA
   d) Reverse transcriptase: production of cDNA from mRNA
2) The diagram below shows the stages in the insertion of the gene for insulin into a bacterium.



   a) Name the substance that makes up the plasmid.

b) Identify the enzyme labelled A. what is its role?

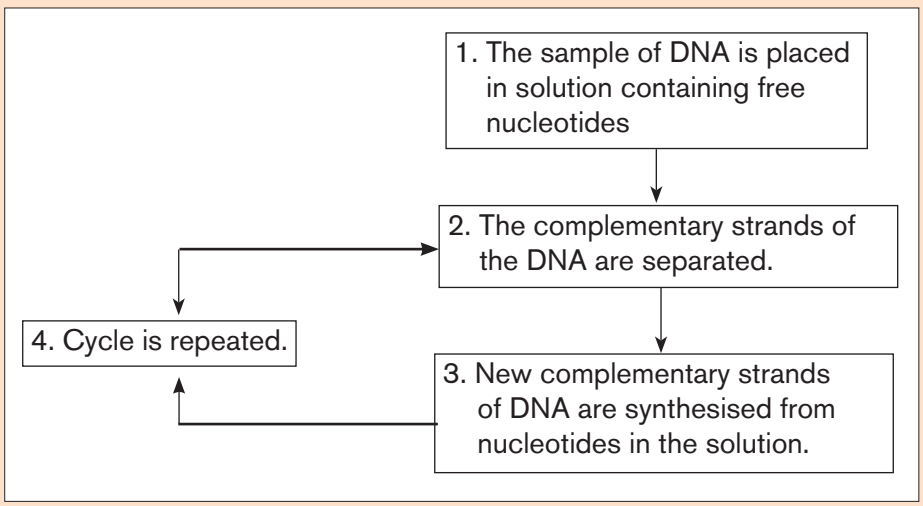
c) Identify enzyme B on the diagram. What is its role?

d) What term is given to a length of DNA formed from different sources?

4.3 Polymerase chain reaction (PCR)

Activity 4.3

The polymerase chain reaction is a process which can be carried out in a laboratory to make large quantities of identical DNA from very small samples. The process is summarized in the flowchart.



a) At the end of one cycle, two molecules of DNA have been produced from each original molecule. How many DNA molecules will have been produced from one molecule of DNA after 5 complete cycles?

b) Suggest one practical use to which this technique might be applied.

c) Give two ways in which the polymerase chain reaction differs from the process of transcription.

d) The polymerase chain reaction involves semi-conservative replication. Explain what is meant by semi-conservative replication.

The **Polymerase Chain reaction (PCR)** is a method widely used in molecular biology to make several copies of a specific DNA segment**.** Using PCR, copies of DNA sequences are exponentially amplified to generate thousands to millions of more copies of that particular DNA segment. DNA can clone (or amplify) DNA samples as small as a single DNA molecule. It is a newer technique, having

been developed in 1983 by **KARY Mullis**, for which discovery he won the Nobel Prize in 1993. The polymerase chain reaction is simply DNA replication in a test tube. If a length of DNA is mixed with the four nucleotides (A, T, C and G) and the enzyme DNA polymerase in a test tube, then the DNA will be replicated many times.

The polymerase chain reaction (PCR) is an automated process, making it both rapid and efficient. It requires the following:

- The DNA fragment to be copied.
- **Taq polymerase** – DNA polymerase obtained from the bacterium *Thermus aquaticus*, after which it is named. The bacterium lives in hot springs, and so the remarkable feature of *Taq polymerase* is that it is very tolerant to heat (it is thermostable) and does not denature at the high temperatures of the polymerase chain reaction so that take place. DNA polymerase is an enzyme is an enzyme capable of joining together tens of thousands of nucleotides in a matter of minutes.
- **Primers:** short sequences of nucleotides that have a set of bases complementary to those at one end of each of the two DNA fragments.
- **Nucleotides:** which contain each of the four bases found in DNA. They are nucleotide triphosphate (dNTPs) as energy is required for the synthesis of the phosphodiester bonds.
- **Thermocycler:** a computer-controlled machine that varies temperatures precisely over a period of time.

The polymerase chain reaction is illustrated in the figure 4.2 and is carried out in three stages:

- **Separation** of the DNA double helix**:** the mixture containing DNA fragments, primers, dNTPs and *Taq polymerase* is placed in a vessel in the thermocycler. The temperature is increases to 95°C causing the two strands of the DNA fragments to separate as hydrogen bonds are broken.
- **Annealing** of the primers: the mixture is cooled to 55°C causing the primers to join (anneal) to their complementary bases at the end of the DNA fragment. The primers provide the starting sequences for *Taq polymerase* to begin DNA copying because *Taq polymerase* can only attach nucleotides to the end of an existing chain. Primers also prevent the two separate strands from simply rejoining.
- **Synthesis of DNA:** the temperature is increased to 72°C. This is the optimum temperature for the *Taq polymerase* to add complementary nucleotides along each of the separated DNA strands. It begins at the primer on both strands and adds the nucleotides in sequence until it reaches the end of the chain.

Each original DNA molecule has now been replicated to form two molecules. The cycle is repeated from step 2 and each time the number of DNA molecules doubles. This is why it is called a chain reaction, since the number of molecules increases exponentially, like an explosive chain reaction. Typically, PCR is run for 20-30 cycles. This is known as **DNA amplification**. The complete cycle takes around two minutes. After only 25 cycles over a million copies of the DNA can be made and 100 billion copies can be manufactured in just a few hours. The PCR has revolutionized many aspects of science and medicine. Even the minutest sample of DNA from a single hair or a speck of blood can now be multiplied to allow forensic examination and accurate cross-matching.

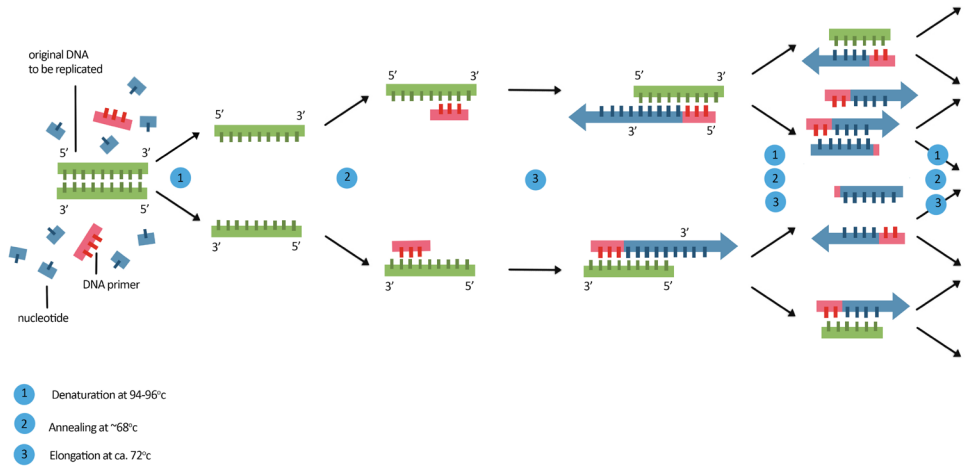


Fig 4.2: PCR technique

Applications of the PCR technique

PCR is useful in forensic criminal investigations, medical diagnosis, paternity testing and genetic research, and only requires a small amount of DNA to work. In criminal investigations, forensic scientists can find enough DNA in a hair follicle or one cell to use as a starting point for PCR. Therefore, only a small amount of DNA evidence is needed because it can be copied over and over again. PCR can also improve medical diagnoses, such as confirming the presence of the AIDS-causing virus. HIV cannot be detected immediately by looking for antibodies, because it takes time for the body to build antibodies against it. Traditional testing relies on the detection of these antibodies. With PCR, primers can be designed to complement short regions of the DNA of HIV. The DNA can be amplified and then examined for the presence of the HIV genome. Another application of PCR is that researchers can use it to determine, from fossil remains, whether or not two species are closely related.

Application activity 4.3

1) Which of the following are required in a polymerase chain reaction?

a) DNA polymerase, template strand and primers.

b) RNA polymerase, template strand and primers

c) RNA polymerase, template strand and ligase

d) RNA polymerase, ligase and primers.

2) Each cycle of a polymerase chain reaction (PCR) takes 5 minutes. If there are 1000 DNA molecules at the start of the reaction, how long will it take for the number of fragments produced by the reaction to be greater than 1 million?

a) 15 minutes

b) 35 minutes

c) 50 minutes

d) 55 minutes

4.4 Gel electrophoresis

Activity 4.4

Explain the process of gel electrophoresis, the process by which electrophoresis takes place and the possible importance of this technique.

Gel electrophoresis is a laboratory technique used to separate mixtures of DNA, RNA or proteins according to molecular size. In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores**.**

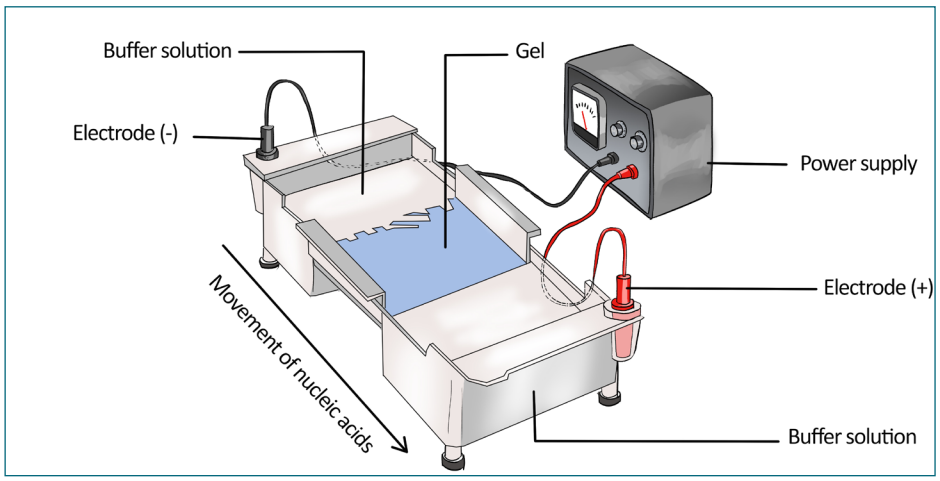


Figure 4.3: Setup of gel electrophoresis

In a common gel electrophoresis setup, a nucleic acid such as DNA is loaded into wells at one end of the gel and then migrates toward the positive electrode at the opposite end. The rate of migration of fragments varies with size. The steps of gel electrophoresis are shown below.

- The DNA samples are cut with a restriction enzyme into smaller segments of various sizes. The DNA is then placed in wells made on a thick gel.
- An electric current runs through the gel for a given period of time. Negatively charged DNA fragments migrate toward the positively charged end of the porous gel. Smaller DNA fragments migrate faster and farther than longer fragments, and this separates the fragments by size. The gel floats in a buffer solution within a chamber between two electrodes.
- The DNA is transferred to a nylon membrane and radioactive probes are added. The probes bind to complementary DNA.
- The X-ray film is exposed to the radiolabeled membrane. The resulting pattern of bands is called a **DNA fingerprint**.

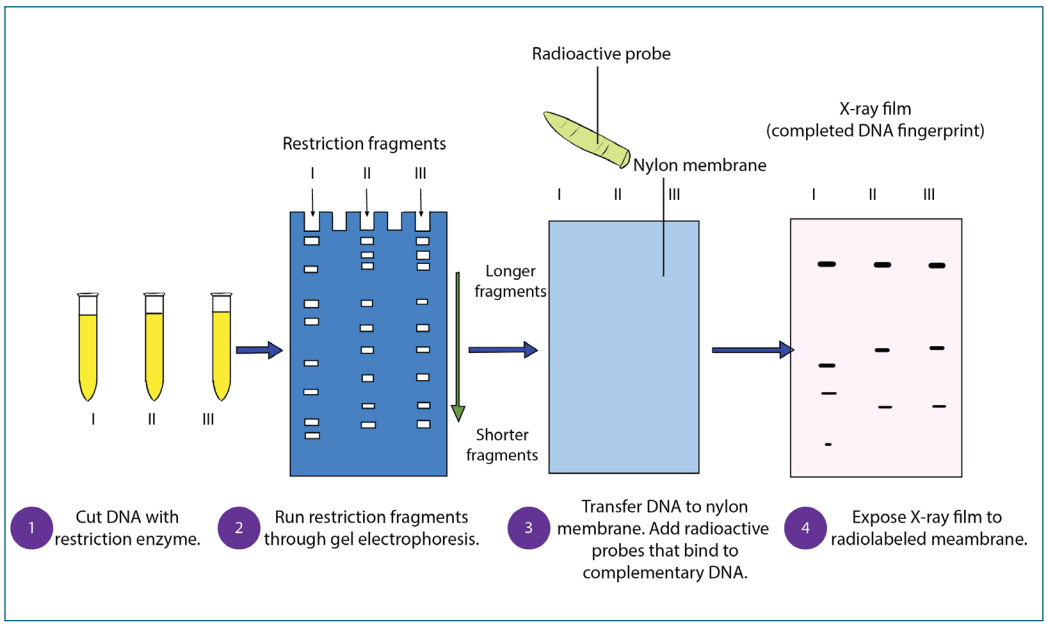


Figure 4.4: Steps of gel electrophoresis

During electrophoresis, DNA fragments migrate through the gel at a rate that is inversely proportional to the logarithm of their size. The shorter the fragment is, the faster it will travel because of its ability to navigate through the pores in the gel more easily than a large fragment can. Larger fragments are hampered by their size. Hence, the longer a nucleotide chain, the longer it takes for the migration.

Gel electrophoresis takes advantage of DNA's negative charge. A solution containing different-size fragments to be separated is placed in a well. A well is a depression at one end of the gel. The gel itself is usually a square or rectangular slab and consists of a buffer containing electrolytes and **agarose**, or possibly **polyacrylamide**. Agarose is a gel-forming polysaccharide found in some types of seaweed that is used to form a gel meshwork for electrophoresis. Polyacrylamide is an artificial polymer used to form a gel meshwork for electrophoresis.

The gel is loaded while it is submerged in a tray containing an electrolytic solution called the buffer. Using direct current, a negative charge is placed at one end of the gel where the wells are, and a positive charge is placed at the opposite end of the gel. The electrolyte solution conveys the current through the gel. The negatively charged DNA will migrate toward the positively charged electrode, with the shorter fragments migrating faster than the longer fragments, achieving separation. Small molecules found within the loading dye migrate ahead of all the DNA fragments. Since the small molecules can be visualized, the electrical current can be turned off before they reach the end of the gel.

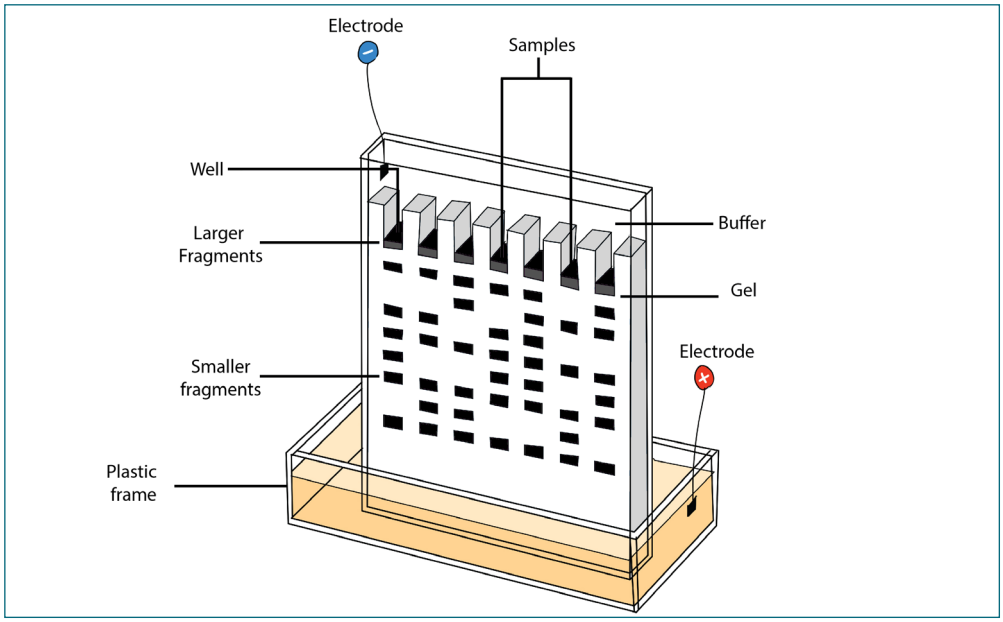


Figure 4.5: Fragments arrangement in gel electrophoresis

Once gel electrophoresis is complete, the DNA fragments are made visible by staining the gel. The set of fragments generated with a particular restriction enzyme produces a banding pattern characteristic for that DNA. The most commonly used stain is ethidium bromide. Ethidium bromide is a flat molecule that fluoresces under ultraviolet (UV) light and is able to insert itself among the rungs of the ladder of DNA. When the gel is subjected to UV light, the bands of DNA are visualized because the ethidium bromide is inserted among the nucleotides**.** The size of the fragments is then determined using a molecular marker as a standard. The molecular marker, which contains fragments of known size, is run under the same conditions (in the same gel) as the digested DNA.

Gel electrophoresis is not limited to the separation of nucleic acids but is also commonly applied to **proteins**. Proteins are usually run on polyacrylamide gels, which have smaller pores, because proteins are generally smaller in size than nucleic acids. Proteins, however, are not negatively charged; thus, when researchers want to separate proteins using gel electrophoresis, they must first mix the proteins with a **detergent** called **sodium dodecyl sulfate**. This treatment makes the proteins unfold into a linear shape and coats them with a negative charge, which allows them to migrate toward the positive end of the gel and be separated. Finally, after the **DNA**, **RNA**, or **protein** molecules have been separated using gel electrophoresis, bands representing molecules of different sizes can be detected. The gel electrophoresis is used for different purposes such as DNA analysis, protein and antibody interactions, testing antibiotics and testing vaccines.

Application activity 4.4

The gel shown in the figure below was run after bacterial DNA was digested using restriction enzymes where A, B, C and D are the comb lane of the gel. In your notebook, indicate on the gel

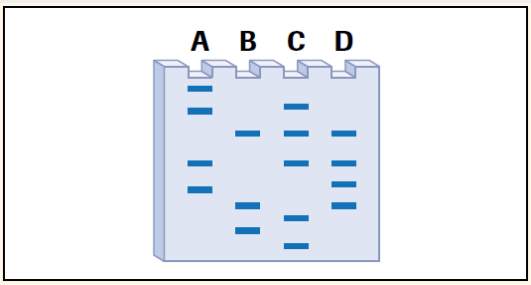
a) Where the positive electrode was located;

b) Where the negative electrode was located;

c) The location of the largest band;

d) The location of the smallest band;

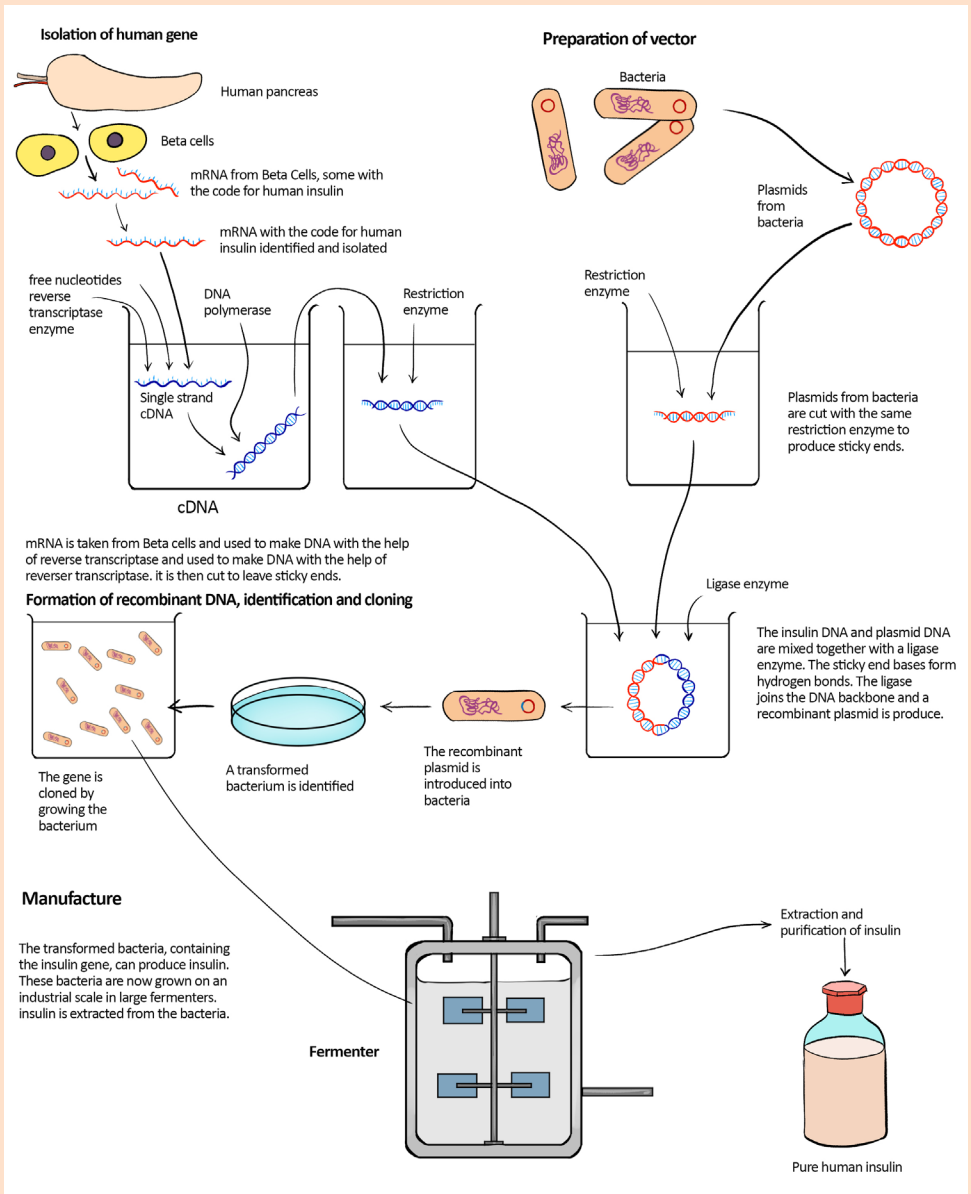
e) The number of cuts that were made on the linear fragment of DNA to produce this number of bands.



4.5 Production of human proteins by recombinant DNA technology

Activity 4.4

The figure below shows the process by which bacteria can be bioengineered to produce human insulin. Follow each of the steps used to produce GMO bacteria. Use the figure to make a list of steps followed when producing a genetically modified organism bacterium.



Production of insulin

One form of diabetes mellitus is caused by the inability of the pancreas to produce insulin. Before insulin from GM bacteria became available, people with this form of diabetes were treated with insulin extracted from the pancreases of pigs or cattle. In the 1970s, biotechnology companies began to work on the idea of inserting the gene for human insulin into a bacterium and then using this bacterium to make insulin. They tried several different approaches, finally succeeding in the early1980s. This form of human insulin became available in1983.

The procedure involved in the production of insulin is shown in the figure 4.6. There were problems in locating and isolating the gene coding for human insulin from all of the rest of the DNA in a human cell. Instead of cutting out the gene from the DNA in the relevant chromosome, researchers extracted mRNA for insulin from pancreatic β cells, which are the only cells to express the insulin gene. These cells contain large quantities of mRNA for insulin as they are its only source in the body. The mRNA was then incubated with the enzyme **reverse transcriptase** which comes from the group of viruses called **retroviruses**. As its name suggests**,** this enzyme reverses transcription, using mRNA as a template to make single-stranded DNA**.** These single-stranded DNA molecules were then converted to double-stranded DNA molecules using DNA polymerase to assemble nucleotides to make the complementary strand. The genetic engineers now had insulin genes that they could insert into plasmids to transform the bacterium ***Escherichia coli*.**

The main advantage of this form of insulin is that there is now a reliable supply available to meet the increasing demand. Supplies are not dependent on factors such as availability through the meat trade.

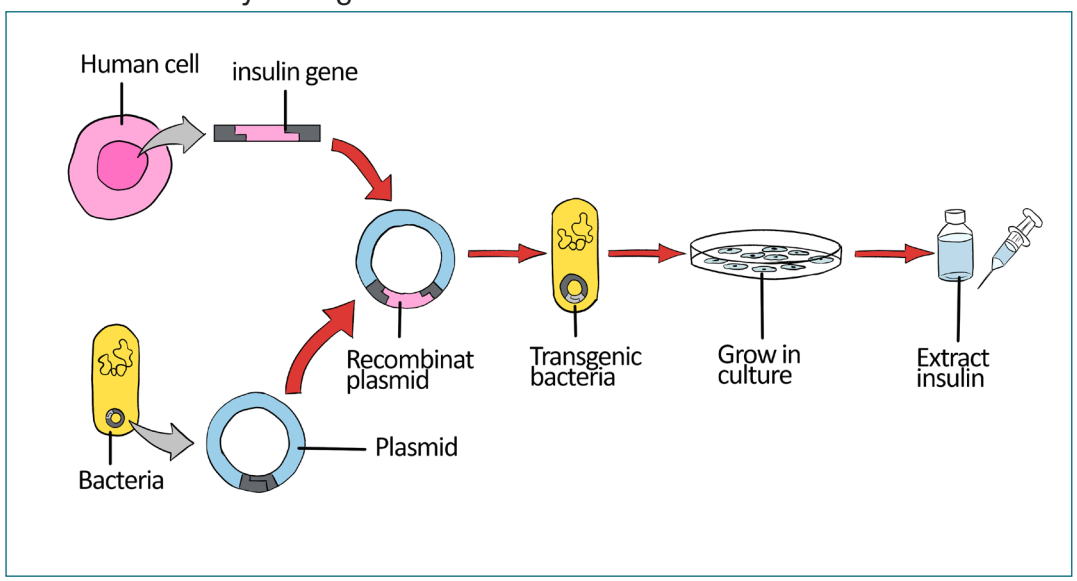


Fig 4.6: Production of human insulin by using bacteria

There were problems in locating and isolating the gene coding for human insulin from all of the rest of the DNA in a human cell. Instead of cutting out the gene from the DNA in the relevant chromosome, these are steps involved in human insulin production:

- Researchers extracted mRNA for insulin from pancreatic β cells, which are the only cells to express the insulin gene. These cells contain large quantities of mRNA for insulin as they are its only source in the body.
- The mRNA was then incubated with the enzyme reverse transcriptase which comes from the group of viruses called retroviruses. As its name suggests, this enzyme reverses transcription, using mRNA as a template to make single stranded DNA.
- These single-stranded DNA molecules were then converted to double stranded DNA molecules using DNA polymerase to assemble nucleotides to make the complementary strand.
- The genetic engineers now had insulin genes that they could insert into plasmids to transform the bacterium *Escherichia coli*.
- When the bacterial cells copy their own DNA, they also copy the plasmids and the donor genes that plasmids carry. After the cells have grown into colonies, on an industrial scale in large fermenters insulin is extracted from the bacteria.

Production of bovine growth hormone

Recombinant (*r*) bovine growth hormone is a protein that has been made by manipulating the **DNA** sequence (gene) that carries the instructions for, or encodes, the growth hormone protein so it can be produced in the laboratory. Hormones are substances secreted from specialized glands. Hormones travel through the bloodstream to affect their target organs. Growth hormone acts on many different organs to increase the overall size of the body. Before the advent of genetic technologies, growth hormone was procured from the pituitary glands of slaughtered cows and then injected into live cows.

The same technique has been used to obtain human growth hormone from the pituitary glands of human cadavers. When the human growth hormone is injected into humans who have a condition called *pituitary dwarfism*, their size increases. However, harvesting the growth hormone from the pituitary glands of cows and humans is laborious, and many cadavers are necessary to obtain small amounts of the protein.

Producing *r*BGH

The first step in the production of the *r*BGH protein is to transfer the BGH gene from the nucleus of a cow cell into a bacterial cell. Bacteria with the BGH gene will then serve as factories to produce millions of copies of this gene and its protein product; making many copies of a gene is called **cloning** the gene.

Cloning a gene using bacterial cells

The following steps are involved in moving a BGH gene into a bacterial cell:

a) BGH gene is cut from the cow chromosome using restriction enzymes that leave "sticky ends" with specific base sequences.

b) A plasmid from a bacterium is cut with the same restriction enzymes, creating the same "sticky ends" as the cow gene.

c) The cleaved gene and plasmid are placed together in a test tube. Complementary "sticky ends" fit together, resulting in a recombinant plasmid.

d) The recombinant plasmid is reinserted into a bacterial cell.

e) The plasmids and the bacterial cells replicate, making millions of copies of the *r*BGH gene.

f) The *r*BGH genes produce large quantities of *r*BGH proteins that are harvested, purified, and injected into cows to increase milk production.

Application activity 4.4

1) Rearrange the statements below to produce a flow diagram showing the steps involved in producing bacteria capable of synthesizing a human protein such as human growth hormone (hGH).
   1. Insert the plasmid into a host bacterium.
   2. Isolate mRNA for hGH.
   3. Insert the DNA into a plasmid and use ligase to seal the 'nicks' in the sugar–phosphate chains.
   4. Use DNA polymerase to clone the DNA.
   5. Clone the modified bacteria and harvest hGH.
   6. Use reverse transcriptase to produce cDNA.
   7. Use a restriction enzyme to cut a plasmid vector.

4.6 Use of microarrays in the analysis of genomes and in detecting mRNA

Activity 4.6.

Indicate and explain the use and applications of the microarray DNA technology

A DNA microarray consists of tiny amounts of a large number of single-stranded DNA fragments representing different genes fixed to a glass slide in a tightly

spaced array, or grid. (The microarray is also called a *DNA chip* by analogy to a computer chip.) Ideally, these fragments represent all the genes of an organism.

The mRNA from the organism or the cell to be tested is labelled with a fluorescent dye and added to the chip. When the mRNAs bind to the microarray, a fluorescent pattern results that is recorded by a computer. Now the investigator knows what DNA is active in that cell or organism. A researcher can use this method to determine the difference in gene expression between two different cell types, such as between liver cells and muscle cells.

A mutation microarray, the most common type, can be used to generate a person's genetic profile. The microarray contains hundreds to thousands of known disease-associated mutant gene alleles. Genomic DNA from the individual to be tested is labelled with a fluorescent dye, and then added to the microarray. The spots on the microarray fluoresce if the individual's DNA binds to the mutant genes on the chip, indicating that the individual may have a particular disorder or is at risk for developing it later in life. This technique can generate a genetic profile much more quickly and inexpensively than older methods involving DNA sequencing.

Microarrays have proved a valuable tool to identify the genes present in an organism's genome and to find out which genes are expressed within cells. They have allowed researchers to study very large numbers of genes in a short period of time, increasing the information available. A microarray is based on a small piece of glass or plastic usually 2 cm^2 (Figure 4.7). Short lengths of single-stranded DNA are attached to this support in a regular two-dimensional pattern, with 10 000 or more different positions per cm^2. Each individual position has multiple copies of the same DNA probe. It is possible to search databases to find DNA probes for a huge range of genes. Having selected the gene probes required, an automated process applies those probes to the positions on the microarray.

Figure 4.7: A microarray, also known as a DNA chip (Source:https://pharmaceuticalintelligence.com/2016/01/07/gene-editing-the-role-of-oligonucleotide-chips/dna-chip-3/)

When microarrays are used to analyze genomic DNA, the probes are from known locations across the chromosomes of the organism involved and are 500 or more base pairs in length. A single microarray can even hold probes from the entire human genome.

Microarrays can be used to compare the genes present in two different species**.** DNA is collected from each species and cut up into fragments and denatured to give lengths of single-stranded DNA. The DNA is labelled with fluorescent tags so that – for example – DNA from one species may be labelled with green tags and DNA from the other species labelled with red tags. The labelled DNA samples are mixed together and allowed to hybridize with the probes on the microarray. Any DNA that does not bind to probes on the microarray is washed off. The microarray is then inspected using ultraviolet light, which causes the tags to fluoresce. Where this happens, we know that hybridization has taken place because the DNA fragments are complementary to the probes. Green and red fluorescent spots indicate where DNA from one species only has hybridized with the probes. Where DNA from both species hybridize with a probe, a yellow colour is seen. Yellow spots indicate that the two species have DNA with exactly the same base sequence. This suggests that they have the same genes **(Figure 4.8).** The microarray is then scanned so that the data can be read by a computer. Data stored by the computer indicate which genes are present in both species, which genes are only found in one of the species and which genes are not present in either species.

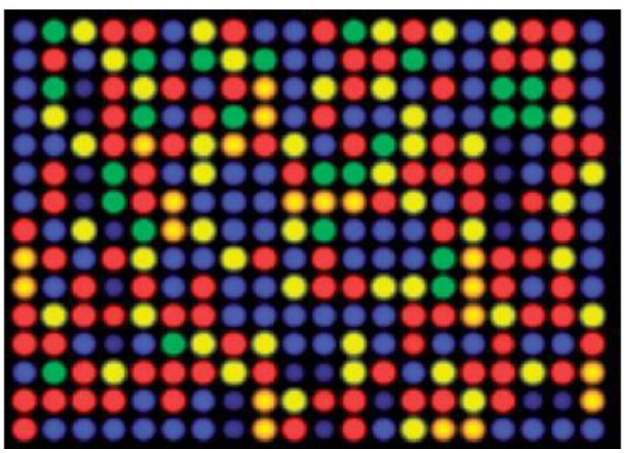


Figure 4.8: A DNA microarray as viewed with a laser scanner. The colours are analysed to show which genes or alleles are present (Source: https://www.sciencephoto.com/media/211400/view/dna-microarray)

Using microarray analysis, researchers can quickly compare gene expression in different samples, such as those obtained from normal and cancerous tissues**.** The knowledge gained from such gene expression studies is making a significant contribution to the study of cancer and other diseases.

Application activity 4.6.

Using your knowledge of the microarray DNA technology, explain three uses of this technique.

4.7 Gene therapy and genetic screening

Activity 4.7

Gene technology can be involved in the detection and treatment of genetic disorders. Discuss on different cases of genetic disorders that are treated by using gene therapy.

4.7.1 Genetic screening

Genetic screening is the analysis of a person's DNA to check for the presence of a particular allele. This can be done in adults, in a foetus or embryo in the uterus, or in a newly formed embryo produced by in vitro fertilisation. An adult woman with a family history of breast cancer may choose to be screened for the faulty alleles of the genes Brca-1 and Brca-2, which considerably increase an individual's chance of developing breast cancer. Should the results be positive, the woman may elect to have her breasts removed (elective mastectomy) before such cancer appears.

In 1989, the first 'designer baby' was created. Officially known as pre-implantation genetic diagnosis (PGD), the technique involved mixing the father's sperm with the mother's eggs (oocytes) in a dish – that is, a 'normal' IVF procedure. It was the next step that was new. At the eight-cell stage, one of the cells from the tiny embryo was removed. The DNA in the cell was analysed and used to predict whether or not the embryo would have a genetic disease for which both parents were carriers. An embryo that was not carrying the allele that would cause the disease was chosen for implantation, and embryos that did have this allele were discarded.

Since then, many babies have been born using this technique. It has been used to avoid pregnancies in which the baby would have had Duchenne muscular dystrophy, thalassaemia, haemophilia, Huntington's disease and others. In 2004, it was first used in the UK to produce a baby that was a tissue match with an elder sibling, with a view to using cells from the umbilical cord as a transplant into the sick child.

For some time, genetic testing of embryos has been leaving prospective parents with very difficult choices to make if the embryo is found to have a genetic condition such as Down's syndrome or cystic fibrosis. The decision about whether or not to have a termination is very difficult to make. Now, though, advances in medical technology have provided us with even more ethical issues to consider.

4.7.2 Gene therapy

Gene therapy is the alteration of a genetic sequence in an organism to prevent or treat a genetic disorder.

Gene technology and our rapidly increasing knowledge of the positions of particular genes on our chromosomes have given us the opportunity to identify many genes that are responsible for genetic disorders such as sickle cell anaemia and cystic fibrosis. When genetic engineering really began to get going in the 1990s, it was envisaged that it would not be long before gene technology could cure these disorders by inserting 'normal' alleles of these genes into the cells.

Gene therapy has proved to be far more difficult than was originally thought. The problems lie in getting normal alleles of the genes into a person's cells and then making them work properly when they get there. In theory, a normal allele of the defective gene could be inserted into the somatic cells of the tissue affected by the disorder. For gene therapy of somatic cells to be permanent, the cells that receive the normal allele must be ones that multiply throughout the patient's life. Bone marrow cells, which include the stem cells that give rise to all the cells of the blood and immune system, are prime candidates. One type of severe combined immunodeficiency (SCID) is caused by a single defective gene. If the treatment is successful, the patient's bone marrow cells will begin producing the missing protein, and the patient will be cured.

The most common vectors that are used to carry the normal alleles into host cells are **viruses** (often retroviruses or lentiviruses) or small spheres of phospholipid called **liposomes.** Occasionally **'naked' DNA is used.** The first successful gene therapy was performed in 1990 on a four-year-old girl from Cleveland, Ohio. She suffered from the rare genetic disorder known as severe combined immunodeficiency (SCID). In this disorder, the immune system is crippled and sufferers die in infancy from common infections. Children showing the condition are often isolated inside plastic 'bubbles' to protect them from infections.

Application activity 4.7

DNA technology is increasingly being used in the diagnosis of genetic and other diseases and offers potential for better treatment of genetic disorders or even permanent cures. Suggest the advantages of genetic screening and therapy over the normal methods of treating diseases.

4.8 Genetically modified organisms in agriculture

Activity 4.8

Discuss on the different GMO used in agriculture and the possible advantages of these plants over the natural plants.

4.8.1 Gene technology and agriculture

Many new products have been developed using this technology. Crops have been genetically engineered to increase yield, hardiness, uniformity, insect and virus resistance, and herbicide tolerance. The vast bulk of genetically modified plants grown around the world are crop plants modified to be resistant to herbicides or crops that are resistant to insect pests. These modifications increase crop yield. A few crops, such as vitamin A, enhanced rice, provide improved nutrition.

4.8.2 Use of Agrobacterium tumefaciens to transfer genes in plants

Agrobacterium is a bacterium that uses a horizontal gene transfer (HGT). HGT is the transfer of DNA between different genomes. HGT can occur in bacteria through transformation, conjugation and transduction. However, it is also possible for HGT to occur between eukaryotes and bacteria. Bacteria have three ways of transferring bacteria DNA between cells:

1) **Transformation**: The uptake and incorporation of external DNA into the cell thereby resulting in the alteration of the genome.
2) **Conjugation**: The exchange of genetic material through cell-to-cell contact of two bacterial cells. A strand of plasmid DNA is transferred to the recipient cell and the donor cell then synthesis DNA to replace the strand that was transferred to the recipient cell.

3) **Transduction**: A segment of bacterial DNA is carried from one bacterial cell to another by a bacteriophage. The bacteriophage infects a bacterial cell and takes up bacterial DNA. When this phage infects another cell, it transfers the bacterial DNA to the new cell. The bacteria can then become a part of the new host cell.

Agrobacterium has the ability to transfer DNA between itself and plants and is therefore commonly used in genetic engineering. The process of using ***Agrobacterium*** for genetic engineering is illustrated in the diagram below.

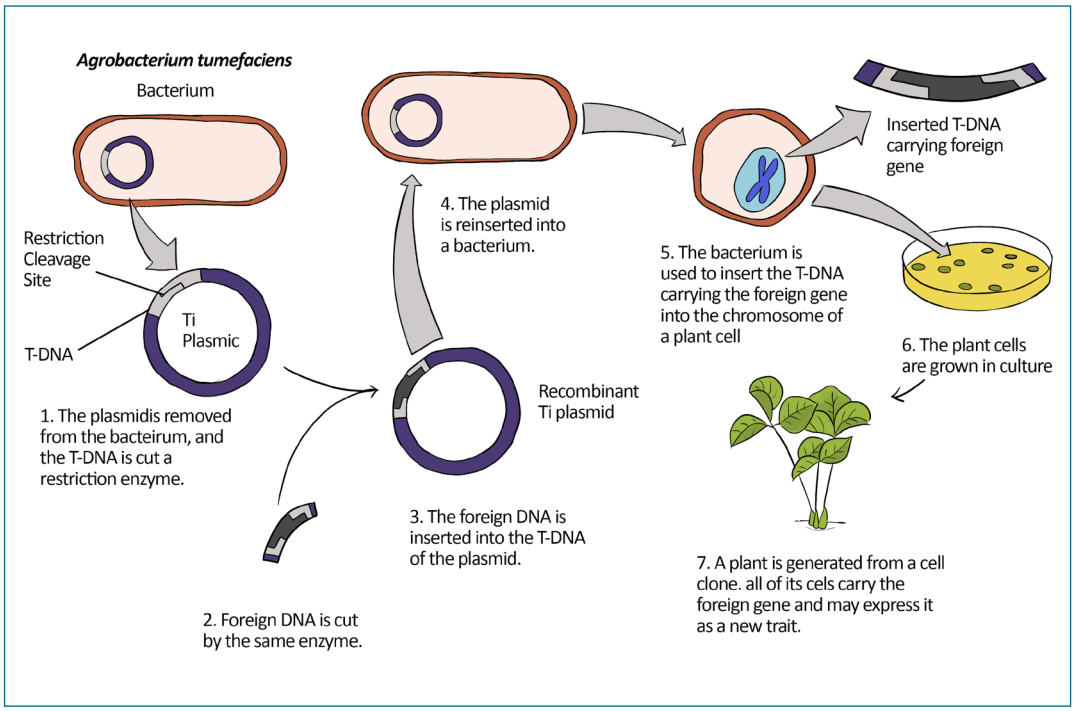


Figure 4.9: Process of formation of a transgenic plant

Summary of formation of a transgenic plant:

- The *Agrobacterium* cell contains a bacterial chromosome and a Tumor inducing plasmid (Ti Plasmid).
- The Ti plasmid is removed from the agrobacterium cell and a restriction enzyme cleaves the T-DNA restriction site.
- The foreign gene of interest is inserted into the middle of the TDNA.
- Recombinant plasmids can be introduced into cultured plant cells by electroporation. Or plasmids can be returned to *Agrobacterium,* which is then applied as a liquid suspension to the leaves of susceptible plants, infecting them. Once a plasmid is taken into a plant cell, its TDNA integrates into the cell's chromosomal DNA.

a. Golden rice

Golden rice is a staple food in many parts of the world, where people are poor and rice forms the major part of their diet. Deficiency of vitamin A is a common and serious problem; its deficiency can cause blindness. In the 1990s, a project was undertaken to produce a variety of rice that contained carotene in its endosperm. Genes for the production of carotene were extracted from maize and the bacterium *Pantonoea ananatis*. These genes, together with promoters, were inserted into plasmids. The plasmids were inserted into bacteria called *Agrobacterium tumefaciens*. These bacteria naturally infect plants and so could introduce the genetically modified plasmid into rice cells. The rice embryos, now containing the carotene genes, were grown into adult plants.

This genetically modified rice is called **golden rice**, because it contains a lot of yellow pigment carotene. The genetically modified rice is being bred into other varieties of rice to produce varieties that grow well in the conditions in different parts of the world, with the same yield, pest resistance and eating qualities as the original varieties.

Figure 4.10: Normal rice (white) compared with golden rice (yellow)
(https://www.google.com/search?q=white+rice+and+yellow+rice)

b. Herbicide-resistant crops

Herbicide-resistant crops called oil seed rape or Brassica napus, is grown in many parts of the world as a source of vegetable oil which is used as biodiesel fuel, as a lubricant and in human and animal foods. Natural rape seed oil contains substances that are undesirable in oil that is to be used in human or animal food. A hybrid, was made to produce low concentrations of these undesirable substances, called **canola** (**Canadian oilseed low acid**), and this name is now often used to mean any variety of oil seed rape. Gene technology has been used to produce herbicide-resistant strains. Growing an herbicide-resistant crop allows fields to be sprayed with herbicide after the crop has germinated, killing any weeds that would otherwise compete with the crop for space, light, water or ions. This increases the yield of the crop.

c. Insect pests-resistant plants

Another important agricultural development is that of genetically modified plants protected against attack by insect pests. Bt maize is genetically engineered **(GE)** plant that produces crystal (Cry) proteins or toxins derived from the soil bacterium, *Bacillus thuringiensis* (Bt), hence the common name "Bt maize". Bt maize plant has revolutionized pest control in a number of countries, but there still are questions about its use and impact.

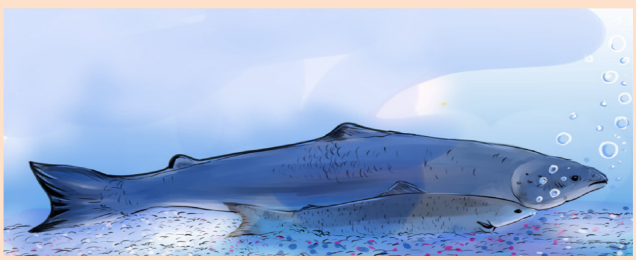
Application activity 4.8

Explain why are Ti plasmids are used to insert genes into plant cells?

4.9 Significance of genetic engineering in improving the quality and yield of crop plants and livestock

Activity 4.9

The figure below shows an Atlantic salmon and a GMO salmon. A GM salmon and non-GM salmon are of the same age.



Suggest any advantage of growing GM salmon over non-GM salmon and discuss how the GM salmons are produced.

4.9.1 Why are animals genetically modified?

Genetically modified animals are animals that have been **genetically modified** for a variety of purposes including producing drugs, enhancing yields, increase resistance to disease, etc. The vast majority of genetically modified animals are at the research stage with the number close to entering the market remains small. The process of genetically engineering mammals is a slow, tedious, and expensive process. Researchers have genetically engineered a number of mammals, from laboratory animals to farm animals, as well as birds, fish and insects.

The most widely used genetically modified animals are laboratory animals, such as the fruitfly (*Drosophila*) and mice. Genetically engineered animals enable scientists to gain an insight into basic biological processes and the relationships between mutations and disease. However, farm animals, such as sheep, goats and cows, can also be genetically modified to enhance specific characteristics. These can include milk production and disease resistance, as well as improving the nutritional value of the products they are farmed for. For example, cows, goats and sheep have been genetically engineered to express specific proteins in their milk.

The majority of work on genetically modified farm animals is still in the research phase and is yet to be used commercially. The advantages and disadvantages associated with genetically modifying animals for agriculture, divided up into four key areas:

Advantages	Disadvantages
▪ Genetic engineering holds great potential in many fields, including agriculture, medicine and industry. ▪ Genetic modification can increase the yield from farm animals, for example cows can be engineered to produce more milk for the same size of herd. ▪ Genetically modified farm animals are being used to produce important medicinal products, such as antibodies, in large quantities. These products can be used for the treatment of many different human conditions. The current production system for such products is donated human blood, which is in limited supply due to a lack of donors. ▪ Sheep and goats can be modified to produce medicinal products in their milk. This has no negative impact on the animal but the product can help to treat human diseases.	The transfer of genetic material from one species to another raises potentially serious health issues for animals and humans. There is a risk that new diseases from genetically engineered animals could be spread to non-genetically engineered animals, and even humans. In many cases, selective breeding? is just as effective as genetic engineering and doesn't carry the same risks. We don't yet know if eating the products of genetically modified animals could potentially harm us.

4.9.2 Why are crop plants genetically modified?

Crop plants are genetically modified to increase their shelf life, yield and nutritive value.

- To increase the shelf life: to increase the time of ripening and the time of storage.
- Improving the yield of crop plants has been the driving force behind the vast majority of genetic engineering. Yield can be increased when plants are engineered to be resistant to pesticides and herbicides, drought, and freezing. For example, a gene from an Arctic fish has been transferred into a strawberry to help prevent frost damage.

Many people believe that improving farmers' yields may help decrease world hunger problems. Others argue that, since there is already enough food being produced to feed the entire population, it might make more sense to use less technological approaches to feeding the hungry. Significant numbers of people around the world are malnourished, hungry, or starving, not due to a shortage of food but because access to food is tied to access to money or land.

- Genetic engineers may also be able to increase the nutritive value of crops. Some genetic engineers have increased the amount of **β-carotene** in rice, a staple food for many of the world's people. Scientists hope the engineered rice will help decrease the number of people who become blind in underdeveloped nations because cells require β-carotene in order to synthesize vitamin A, a vitamin required for vision. Therefore, eating this genetically modified rice, called Golden Rice, increases a person's ability to synthesize vitamin A.

Application activity 4.9

a) What is the importance of using golden rice in developing countries?

b) Suggest any three importance of using GM organism?

4.10 Ethical and social implications of using genetically modified organisms (GMOs).

Activity 4.10

From your daily life experience, discuss the ethical and social implications of using genetically modified crops in food production.

Ethics includes **moral principles** that control or influence a person's **behaviour**. It includes a set of standards by which a community regulates its behaviour and decides as to which activity is legitimate and which is not. Bioethics may

be viewed as a set of standards that may be used to regulate our activities in relation to the biological world. Biotechnology, particularly recombinant DNA technology, is used for exploitation of the biological world by various ways.

Some genetically modified plants are grown in strict containment of glasshouses, but a totally different set of problems emerges when genetically engineered organisms such as crop plants and organisms for the biological control of pests are intended for use in the general environment. Few countries would object to the growth of genetically modified crops that produce vaccines for human or animal use, yet there are people who object to the growth of pro-vitamin A enhanced rice. The major bioethical concerns pertaining to biotechnology are summarized below:

- When animals are used for production of certain pharmaceutical proteins, they are treated as factory machines.
- Introduction of a transgene from one species into another species violates the integrity of species.
- The transfer of human genes into animals or vice-versa is great ethic threat to humanity.
- Biotechnology is disrespectful to living beings, and only exploits them for the benefit of humans.
- Genetic modification of organism can have unpredictable/ undesirable effects when such organisms are introduced into the ecosystem.

Moreover, most objections are raised against the growth of herbicide-resistant or insect-resistant crops as follow:

- The modified crop plants may become agricultural weeds or invade natural habitats.
- The introduced gene may be transferred by pollen to wild relatives whose hybrid offspring may become more invasive.
- The introduced gene may be transferred by pollen to unmodified plants growing on a farm with organic certification.
- The modified plants may be a direct hazard to humans, domestic animals or other beneficial animals, by being toxic or producing allergies.
- The herbicide that can now be used on the crop will leave toxic residues in the crop.
- Genetically modified seeds are expensive, as is herbicide, and their cost may remove any advantage of growing a resistant crop.
- Growers mostly need to buy seed each season, keeping costs high, unlike for traditional varieties, where the grower kept seed from one crop to sow for the next

– In parts of the world where a lot of genetically modified crops are grown, there is a danger of losing traditional varieties with their desirable background genes for particular localities This requires a program of growing and harvesting traditional varieties and setting up a seed bank to preserve them.

Table 4.2: Beneficial and harmful effects of genetic engineering

Some reasons why the work of genetic engineers is important	Some reasons why the work of genetic engineers is controversial
GM animals and crops may make farms more productive	GM crops encourage agribusiness, which may close down some small farms.
GM crops may be made to taste better, last longer, or contain more nutrients	GM animals and crops may cause health problems in consumers.
Genetic engineers hope to cure diseases and save lives	GM crops might have unexpected adverse effects on the environment.
	Present research might lead to the unethical genetic modification of humans.

Application activity 4.10

Discuss ethical and social implications raised against insect-resistant crops.

4.11 Bioinformatics

Activity 4.11

Dicuss on the importance of Bioinformatics and its importance. Thereafter; Explain how the bioinformatics has contributed to the progress in DNA sequence analysis.

Bioinformatics is the collection, processing and analysis of biological information and data using computer software. In other words, it is the branch of biology that is concerned with the acquisition, storage, and analysis of the information found in nucleic acid and protein sequence data. Bioinformatics combines biological data with computer technology and statistics. It builds up databases and allows links to be made between them. The databases hold gene sequences of complete genomes, amino acid sequences of proteins and protein structures.

4.11.1 Scientists use bioinformatics to analyze genomes and their functions

Different government agencies carried out their mandate to establish databases and provide software with which scientists could analyse the sequence data. For example, in the United States, a joint endeavour between the National Library of Medicine and the **National Institutes of Health (NIH)** created the **National Center for Biotechnology Information (NCBI)**, which maintains a website (www.ncbi.nlm.nih.gov) with extensive bioinformatics resources. On this site are links to databases, software, and a wealth of information about genomics and related topics. Similar websites have also been established by the **European Molecular Biology Laboratory (EMBL)** and the **DNA Data Bank of Japan**, two genome centers with which the NCBI collaborates.

These large, comprehensive websites are complemented by others maintained by individual or small groups of laboratories. Smaller websites often provide databases and software designed for a narrower purpose, such as studying genetic and genomic changes in one particular type of cancer.

The NCBI database of sequences is called Genbank. As of August 2007, it included the sequences of 76 million fragments of genomic DNA, totaling 80 billion base pairs! Genbank is constantly updated, and the amount of data it contains is estimated to double approximately every 18 months. Any sequence in the database can be retrieved and analyzed using software from the NCBI website or elsewhere.

UniProt (universal protein resource) holds information on the primary sequences of proteins and the functions of many proteins, such as enzymes. The search tool **BLAST** (basic local alignment search tool) is an algorithm for comparing primary biological sequence information, such as the primary sequences of different proteins or the nucleotide sequences of genes. Researchers use BLAST to find similarities between sequences that they are studying and those already saved in databases. When a genome has been sequenced, comparisons can be made with other known genomes. For example, the human genome can be compared to the genomes of the fruit fly, *Drosophila,* the nematode worm, or the malarial parasite, *Plasmodium*. All the information about the genome of *Plasmodium* is now available in databases. This information is being used to find new methods to control the parasite. For example, being able to read gene sequences is providing valuable information in the development of vaccines for malaria.

4.11.2 Applications of bioinformatics

Bioinformatics has various applications in human genetics. For example, researchers found the function of the protein that causes cystic fibrosis by using the computer to search for genes in model organisms that have the

same sequence. Because they knew the function of this same gene in model organisms, they could deduce the function in humans. This was a necessary step toward possibly developing specific treatments for cystic fibrosis. The human genome has 3 billion known base pairs, and without the computer it would be almost impossible to make sense of these data. For example, it is now known that an individual's genome often contains multiple copies of a gene. But individuals may differ as to the number of copies called copy number variations. Now it seems that the number of copies in a genome can be associated with specific diseases. The computer can help make correlations between genomic differences among large numbers of people and disease. It is safe to say that without bioinformatics, our progress in determining the function of DNA sequences; in comparing our genome to model organisms; in knowing how genes and proteins interact in cells; and so forth, would be extremely slow. Instead, with the help of bioinformatics, progress should proceed rapidly in these and other areas.

Application activity 4.11

a) Describe 2 applications of bioinformatics

b) Explain the role of bioinformatics following the sequencing of genome of Plasmodium in the control and prevention of malaria.

Skills lab 4

Sensitize people about the use of DNA in crime investigation

In violent crimes, body fluids or small pieces of tissue may be left at the scene or on the clothes or other possessions of the victim or assailant. If enough blood, semen, or tissue is available, forensic laboratories can determine the blood type or tissue type by using antibodies to detect specific cell-surface proteins. However, such tests require fairly fresh samples in relatively large amounts. Also, because many people have the same blood or tissue type, this approach can only exclude a suspect; it cannot provide strong evidence of guilt.

DNA testing, on the other hand, can identify the guilty individual with a high degree of certainty, because the **DNA sequence of every person is unique** (except for identical twins).

Genetic markers that vary in the population can be analyzed for a given person to determine that individual's unique set of genetic markers, or genetic profile.

(This term is preferred over "DNA fingerprint" by forensic scientists, who want to emphasize the heritable aspect of these markers rather than simply the fact that they produce a pattern on a gel that, like a fingerprint, is visually recognizable). The Rwanda Forensic Laboratory can now use DNA test to convict criminals and to help to solve different problems such as paternity testing.

Student-teachers will have to sensitize people about the behavior that they need to take for example if there is someone who has been murdered. They will have to avoid to touching him because they can be taken as guilty or they can make the police unable to find the murderer due to the fact that the murdered person has been touched by a lot number of people.

End unit assessment 4

I. Choose the letter corresponding to the best answer.

1) Different enzymes are used in the various steps involved in the production of bacteria capable of synthesizing a human protein. Which step is catalyzed by a restriction enzyme?
   a) Cloning DNA
   b) Cutting open a plasmid vector
   c) Producing cDNA from mRNA
   d) Reforming the DNA double helix

2) What describes a promoter?
   a) A length of DNA that controls the expression of a gene.
   b) A piece of RNA that binds to DNA to switch off a gene.
   c) A polypeptide that binds to DNA to switch on a gene
   d) A triplet code of three DNA nucleotides that codes for 'stop'

3) Which statement correctly describes the electrophoresis of DNA fragments?
   a) Larger fragments of DNA move more rapidly to the anode than smaller fragments.
   b) Positively charged fragments of DNA move to the anode.
   c) Small negatively charged fragments of DNA move rapidly to the cathode.

d) Smaller fragments of DNA move more rapidly than larger fragments.

II. OPEN ENDED QUESTIONS

1) The table shows enzymes that are used in gene technology. Copy and complete the table to show the role of each enzyme.

Enzyme	Role
DNA ligase	
DNA polymerase	
Restriction enzymes	
Reverse transcriptase	

a) Explain what is meant by:

i) Gene therapy

ii) Genetic screening.

b) Explain why it is easier to devise a gene therapy for condition caused by a recessive allele than for one caused by a dominant allele.

2) Refer to what you have studied on DNA and PCR,

a) How many molecules of DNA are produced from one double-stranded starting molecule, after eight cycles of PCR?

b) Explain why it is not possible to use PCR to increase the number of RNA molecules in the same way as it is used to increase the number of DNA molecules.

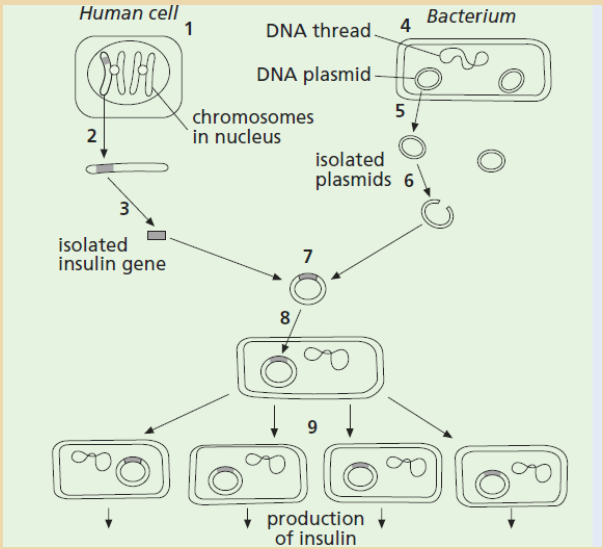
3) The latest estimate of the number of genes in the human genome is 21 000. Before the invention of microarrays, it was very time consuming to find out which genes were expressed in any particular cell.

a) Explain how it is possible to find out which genes are active in a cell at a particular time in its development.

b) Why is it not possible to use the same technique to find out which genes are active in red blood cells?

III. Long Answer Type Questions

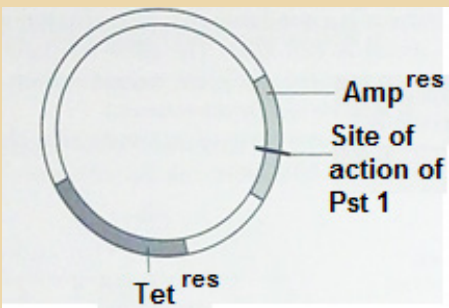
1) Bacteria are used in genetic engineering. The diagram outlines the process of inserting human insulin genes into bacteria using genetic engineering.



Complete the table below by identifying **one** of the stages shown in the diagram that matches **each** description.

Description of stage	Number of stage
The plasmids are removed from the bacterial cell	
A chromosome is removed from a healthy cell	
Plasmids are returned to the bacterial cell	
Restriction enzyme is used	
Bacterial cells are allowed to reproduce in a fermenter	

The diagram below shows a map of pBr322, a small piece of double-stranded, circular DNA found in a bacterium in addition to the bacterial chromosome. The genes for ampicillin resistance (Amp^{res}) and tetracycline resistance (Tet^{res}) are indicated.



Pst 1 is a restriction endonuclease (enzyme) that has its effect at the site shown. Pst 1 recognizes the base sequence 5′ C–T–G–C–A–G / G–A–C–G–T–C 5′ and acts on the DNA between guanine and adenine bases.

a) State the name given to such a piece of circular DNA.

b) Explain the use of such DNA in genetic engineering

c) Using the information given:

i) Explain what is meant by the term restriction endonuclease.

ii) Explain what is meant by the term sticky ends.

UNIT 5

VARIATION AND ARTIFICIAL AND NATURAL SELECTION

Key unit competence

Explain variation and mutation as a source of biodiversity, the role of artificial and natural selection in the production of varieties of animals and plants with increased economic importance

Introductory activity 5

Human population is classified as a single species "*homo sapiens*" .Small group of individuals with different skin colour do not look the same ,here is a representation of human population observe it carefully then answer to the questions below..

a) Some individuals in the figure above look like they are an intermediate of other skin colour, from your observation is there any cause of this?

b) All of us we are human being but we do not look the same, why?

5.1 Variation

All living organisms on the earth are unique, individuals of different species are easy to differentiate and even those of the same species present differences (morphological, physiological, cytological and behaviouristic). Such differences among individuals of the same species are referred to as "**Variation**". These differences between cells, individual organisms, or groups of organisms of any species are caused either by genetic differences (genotypic variation) or by the effect of environmental factors on the expression of the genetic potentials (phenotypic variation). So, organisms that have helpful variations tend to survive better, and reproduce more. As they reproduce, their genes (including the helpful genes) become more common in the gene pool, and these variations spread out more and more.

5.1.1 Genetic variation

Activity 5.1.1

A mutation in one gene causes the shell of the Japanese land snail (*Euhadra*) to spiral in the opposite direction from others. Snails with opposite spirals cannot mate, resulting in reproductive isolation

Using the knowledge acquired in genetics, what types of variation is indicated by the Japanese Land snail?

Genetic variation result from the differences in DNA sequences of individuals (gene make up), those variations can be inherited by the transfer of genes.

There are three primary sources of genetic variation:

- **Variation from mutations** are changes in the DNA. A single mutation can have a large effect, but in many cases, evolutionary change is based on the accumulation of many mutations. Ex, base substitution (Glu →Val), deletion and insertion
- **Variation from gene flow/gene migration/allele flow** is any movement of genes from one population to another and is an important source of genetic variation. Ex, a bee carrying pollen from one flower population to another
- **Variation from recombination/ reproduction** can introduce new gene combinations into a population. This genetic shuffling/ genetic recombination (meiosis & crossing over) is another important source of genetic variation. At meiosis, the process that generates a haploid product of meiosis whose genotype is different from either of the two haploid genotypes that constituted the meiotic diploid. The creation of genetic variation by recombination can be a much faster process than its creation by mutation. For example, when just two chromosomes with "normal" survival, taken from a natural population of *Drosophila,* are allowed to recombine for a single generation, they produce an array of chromosomes with 25 to 75 percent as much genetic variation in survival as was present in the entire natural population from which the parent chromosomes were sampled. This outcome is simply a consequence of the very large number of different recombinant chromosomes that can be produced even if we take into account only single crossovers.

• Why is genetic variation important for evolution?

Variation is one of the main things that drive evolution. First, there are limited resources available, and there is just not enough; food, water, shelter, etc. available for all organisms. Second, to make matters worse, most species have many offspring that can possibly survive. Just think of how many insect eggs are laid compared to the number that make it to adulthood. This leads to competition for the limited resources.

Not all individuals in a species are the same. There are variations in; size, speed, coloration, etc. These small variations can help or hinder individuals in their survival. These variations are caused by small differences in genes. **Organisms that have helpful variations are more likely to survive.** On average, they get more food, get better shelter, etc. Coloration can help a predator get closer to prey and eat better. Or, for the prey species, coloration can make it harder for predators to find and eat it. So, organisms that have helpful variations tend to survive better, and reproduce more. As they reproduce, their genes (including the helpful genes) become more common in the gene pool, and these variations spread out more and more.

Variation can be influenced by numerous factors including:

i. Independent assortment of chromosomes.

Due to the law of independent assortment, traits are transmitted from parents to offspring independently of one another.

This occurs at the time of gamete formation. At the time of gamete formation during meiosis, the parental chromosomes separate randomly hence forming different gametes with different chromosomes. This independent assortment gives a wide variety of different gametes and hence individuals.

ii. Crossing over

Crossing over allows the alleles on DNA molecules to change positions from one homologous chromosome segment to another in other word is the transfer or exchanges of genetic material from one homologous chromosome to another during gamete formation known as meiosis. The new formed chromosomes are known as recombinant chromosomes; Genetic recombination is responsible for genetic diversity in species or population.

iii. Random mating

Random mating involves individuals pairing by chance, not according to their genotypes or phenotypes. Random mating is a source of variation in a population. For example, a population in which mating only occur between organisms of similar phenotypes as red beetles mating with red beetles and yellow beetles mating with yellow beetles, will tend to show less variation than a population where crosses are random.

iv. Random fertilization of gametes

Random fertilization means that the collection of genes within one gametes, each gametes contain a unique set of gene combination, and the ova is fertilized randomly by the male gamete as a result each zygote is unique hence the variation among individuals**.**

v. Mutations

Mutation is a random change in the sequence of DNA, either due to errors during DNA replication or by the influence of environmental factors. Mutations in gametes cell can be inherited while somatic mutations are not transmitted from generation to generation (not inherited).

vi. Environmental factors

These variations caused by environmental factors are not inherited, environmental variation are not prominent in animals as in plants, and this is due to the environmental effect on the meristems of various parts. Some environmental factors that can induce variation include, availability of food, light intensity, Temperature, water, minerals etc…

Application activity 5.1.1

1) Which of the following give rise to genetic variation in a population?
   a) Crossing over and independent assortment in meiosis
   b) Different environmental conditions
   c) Random mating and fertilization.
   d) Mutation.
      i) a, b, c and d
      ii) a, b and c only
      iii) a, c and d only
      iv) b, c and d only
2) Variation caused by environmental factors are not inherited. Why?
3) What is random mating?

5.1.2 Phenotypic variation

Activity 5.1.2

1) Observe the following figures of students and make analysis on their size (weight and height). These students live together in the same school which means that the type of food they consume is the same.

Figure of students

a) Write down your observation

b) Try to form 3 groups according to their height

c) By looking on their size can you try to make 3 groups according to their weight?

d) By considering weight and height, why are not the same to those soldiers

Phenotypic variations can be brought about by genes or environmental factors or a combination of both genes and environment they are not inherited. So, there are characteristics that are not inherited but influenced by the environmental factors, a child that get insufficient food will not grow to the size expected, a cat with a skin disease may have bald patches in its coat. Those conditions are not inherited. Such Phenotypic variations can be divided into two types such as continuous variations or quantitative and Discontinuous variations or Qualitative variations.

a) Continuous variation

Continuous variation is variation which does not show clear cut differences i.e. it shows a gradual change from one extreme to another. Characteristics such as; human height and weight show continuous variation, and are usually determined by a large number of genes (i.e. polygenic) and/ or considerable environmental influence. Some examples of continuous variation are: Height, weight, heart rate, finger length, and leaf length. They are also called **fluctuating variations**

In continuous variations/quantitative:

- Different alleles at a single gene locus have a small effect on the phenotype
- Different genes have the same, often additive, effect on the phenotype
- A large number of genes may have a combined effect on a particular phenotypic trait, these genes are known as polygenes

A typical example of continuous variation is height. There are no distinct categories of height; people are not either tall or short. There are all possible intermediates between very short and very tall *(Figure 5.1).*

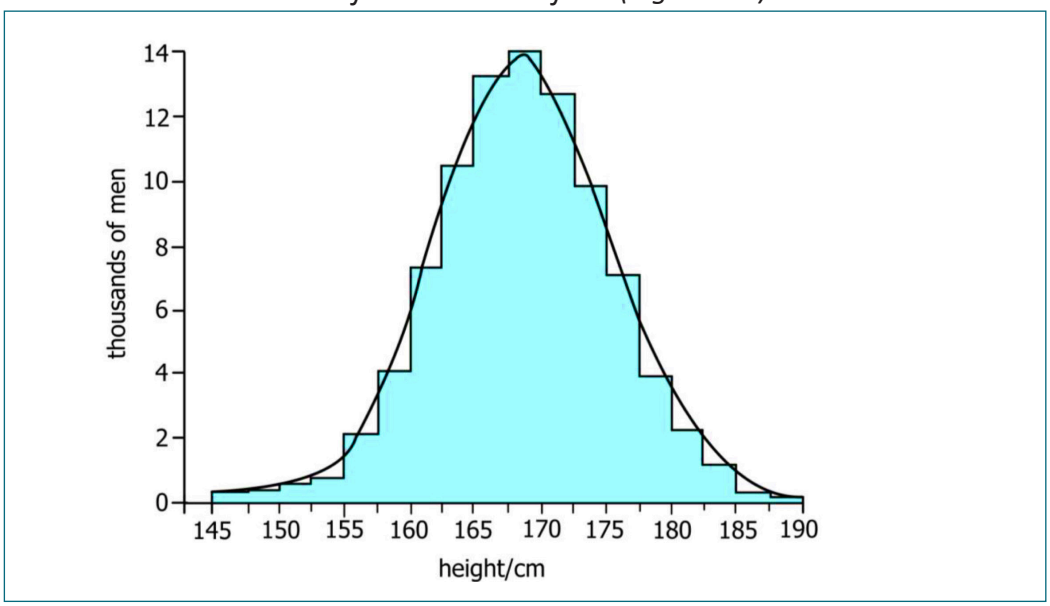


Fig 5.1: Continuous variation height of students

Continuously variable characteristic is greatly influenced by environment. A person may inherit tallness trait and yet not get enough food to grow tall. A plant may have a gene for large fruits but not get enough water, minerals and sunlight to grow large fruits.

b) Discontinuous variation.

Discontinuous variation is indicated as a variation where there is a clear difference among individuals there is no intermediates, in human you are male or female apart from abnormalities, Sex are inherited in a discontinuous way, some people are able to roll they tongue in a tube other can't do it.

There are many characteristics that are difficult to classify as continuous or non-discontinuous such as human eye colour people can be classified roughly as having blue eyes or brown eyes, but there are also categories described as grey, Hazel or green.

A typical example of discontinuous variation is human blood group; discontinuous variations is controlled by a single pair of alleles or small number of genes.

A person is one of four blood group: A, B, AB and O there is no blood group between

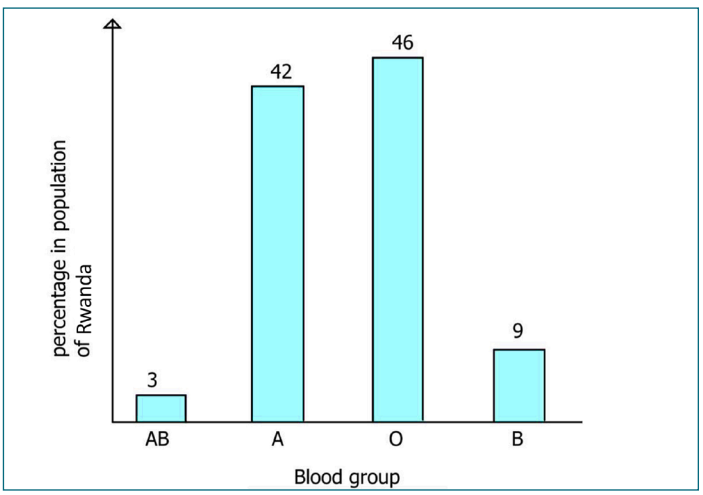


Fig 5.2: Discontinuous variation in blood group

The major distinctions between continuous and discontinuous variations in inheritance are as follows:

Continuous variations have the following characteristics:

- The variations fluctuate around an average or mean of species.
- Direction of continuous variations is predictable.
- They are already present in the population.

- Continuous variations are formed due to chance segregation of chromosomes during gamete formation, crossing over and chance pairing during fertilization.
- They can increase adaptability of the race but cannot form new species.
- Continuous variations are connected with the mean or average of the species by intermediate stages.
- The continuous variations are also called fluctuations.
- When represented graphically, continuous variations give a smooth bell shaped curve - They are very common
- Continuous variations do not disturb the genetic system.

Discontinuous variations have the following characteristics:

- A mean or average is absent in discontinuous variations.
- The direction of discontinuous variations is unpredictable.
- Discontinuous variations are new variations though similar variations might have occurred previously.
- Discontinuous variations are produced by changes in genome or genes.
- Discontinuous variations are the fountain head of continuous variations as well as evolution
- These variations are not connected with the parental type by intermediate stages.
- Discontinuous variations are also known as mutations or sports.
- A curve is not produced when discontinuous variations are represented graphically.
- These variations appear occasionally.
- They disturb the genetic system of the organism

Table 5.1. Comparison between Discontinuous and continuous variation

	Continuous variation	Discontinuous variation
Properties	No distinct categories No limit on the value Tend to be quantitative	distinct categories No in- between categories Tend to be qualitative
Representation	Line graph (normal distribution)	Bar graph (discrete distribution)
Caused by	Genetic/ environmental factors or both	Genetic factors only

Controlled by	A lot of genes/ many pairs of alleles and environment →range of phenotypes between 2 extremes, e.g height in humans	A few genes → limited number of phenotypes with no intermediates e.g A, B , AB, and O blood groups in humans
Examples	Height, weight, heart rate, finger length, leaf length, intelligence	Tongue rolling, finger prints, eye color , blood groups, color blindness

Application activity 5.1.2

The histogram shows the height of wheat plants in an experiment plot.

Based on the figure

a) Which type of variation is shown by the height of each of the strains of wheat plants

b) Give other example of discontinuous variation

5.2 Natural selection

Natural selection is a process that results in the adaptation of an organism to its environments by means of selectively reproducing changes in its genotype or genetic constitution. In 1858, Charles Darwin and Alfred Russel Wallace published a theory of evolution by natural selection.

Individuals with certain variants of the trait may survive and are capable to reproduce more than less successful individuals with unfavorable characters; therefore, the population evolves. Over time, this process can result in populations that specialize for particular ecological niches (microevolution) and may eventually result in speciation (the emergence of new species also known as macroevolution). In other words, natural selection is a key process to change organisms and make them suitable to different environment.

The allele responsible for the variation that help individuals to survive better is inherited by the offspring, they will survive and transmit the trait to its offspring, in time this particular variety outnumber and finally replace the original variety.

This is known as "the survival of the fittest" but this doesn't indicate good health to an organism but the one which is well fitted to the conditions of environment.

Factors of natural selection

Activity.5.2.1

The puffball can produce billions of offspring, if all offspring produced survived to maturity they would carpet the surrounding land surface.

For your observation, how can the factor of producing a high number of offspring for an organism have an impact on surviving in the environment?

Natural selection which is one of the evolution means is due to several factors in a population including **over production** and **environmental factors**.

Role of over production and variation in natural selection

Over production is the production of more offspring that can be supported by the available resources (food, light, space…). Individuals possessing genes that help them to survive in an environment and this trait is transmitted from generation to generations.

Darwin appreciated that all species have the potential to increase their numbers exponentially, he realized that, in nature, population rarely, if ever, increased in size at such rate. The reason why reproductive rate is high is because an individual cannot control the climate, availability of food, rate of predation etc. Therefore, the production of sufficient offspring ensures a sufficient large population surviving during hash conditions.

Application 5.2.1

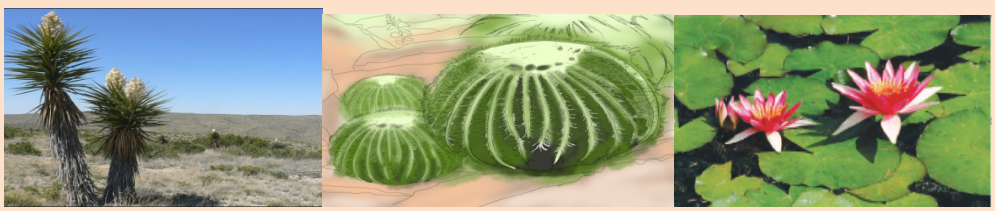
1) In the natural selection species that is able to produce a high number of offspring ensure the possibility of great number offspring to survive.

   How do we call such process?

2) How does over production lead to competition**?**

Environmental factors

Activity 5.2.2



The figure above represents plants into different environments, observe them carefully and

a) Write down your observations according their environments and their structures.

b) Mention your differential observation of the plant (a), (b), (C) according to their environment.

Environmental factors as forces of natural selection, Environment is a responsible agent of natural selection. Thus, it selects and determines individuals in different ways according to different types of natural selections.

Selection pressure

Selection pressure are environmental factors that limit the population of species it is also known as environmental resistance.

It includes:

- **Availability of resources:**
  - Competition for food
  - Competition for a space in which to live, breed and rear young
  - Competition for mating etc.
- **Environmental conditions:**
  - Need for light, water, oxygen,
  - Climate changes temperature, whether conditions or geographical access
- **Biological factors:** Predators and diseases(pathogens)

Selection pressure can be density dependent or independent density factor, and it extent varies from time to time and place to place.

The selection is of three main types:

- **Stabilizing selection**
- **Direction selection**
- **Disruptive selection**

a) Stabilizing selection

Stabilizing selection is a type of natural selection in which a population mean stabilizes on a particular non-extreme trait value as result of genetic diversity decreases as illustrated in the figure below.

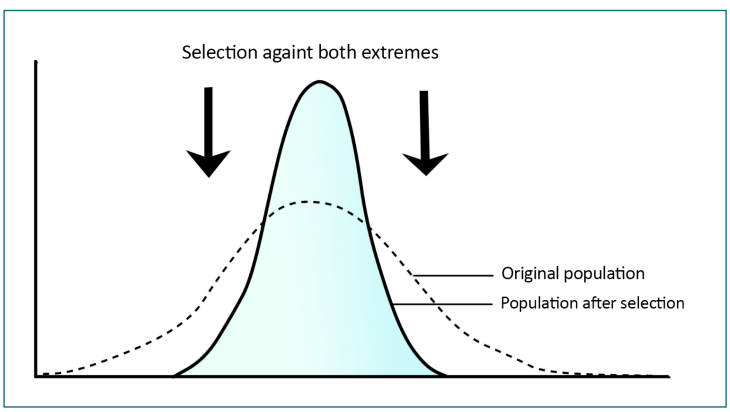


Figure.5.3: Illustration of stabilizing selection

As illustrated in the above figure (Fig. 5.3), in stabilizing selection, natural selection favors the individuals in the population with the intermediate phenotypes. These individuals have greater survival and reproductive success. Individuals with extreme phenotypes are less adaptive and are therefore eliminated.

b) Directional selection

Directional selection is a mode of natural selection in which a single or new fit phenotype is favored when exposed to environmental changes, causing a population genetic variance or allele frequency to continuously shift in one direction or one end of the spectrum of existing variation.

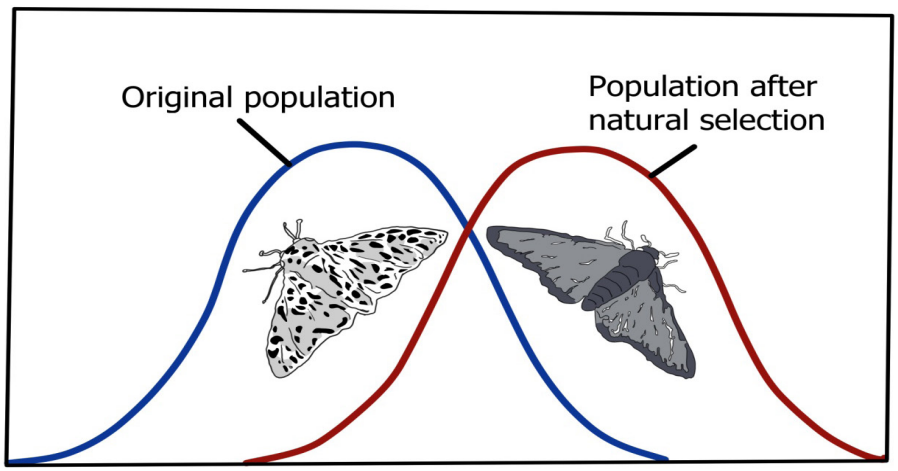


Figure.5.4: Illustration of directional selection

c) Disruptive or diversifying selection

In disruptive selection, both the extreme phenotypes in the population are selected and become more prevalent. The individuals with extreme phenotypes or end- phenotypic spectrum have greater survival and reproductive success. The disruptive selection pressure increases the chances of the advantageous alleles to be passed on to the next generation. By disruptive selection, the intermediate phenotype is selected against and gradually decreases in number from generation to generation, and may become extinct.

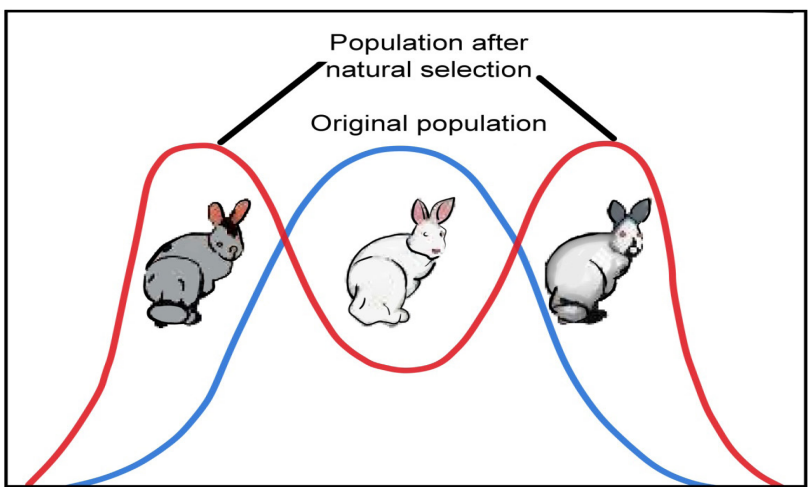


Figure5.5: Illustration of disruptive selection

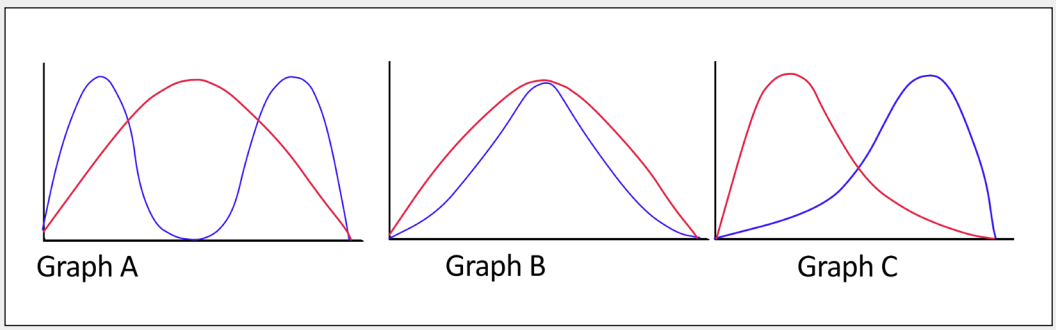
From the above figure, disruptive selection many generations may cause the formation of two separate gene pools and the formation of new species.

From the factors of natural selection some examples that indicate natural selection today are known and include:

- Antibiotic resistance,
- industrial melanism
- pesticides resistance in insect and mammals

Application activity 5.2.2

1) Classify the following figures according to the types of natural selection



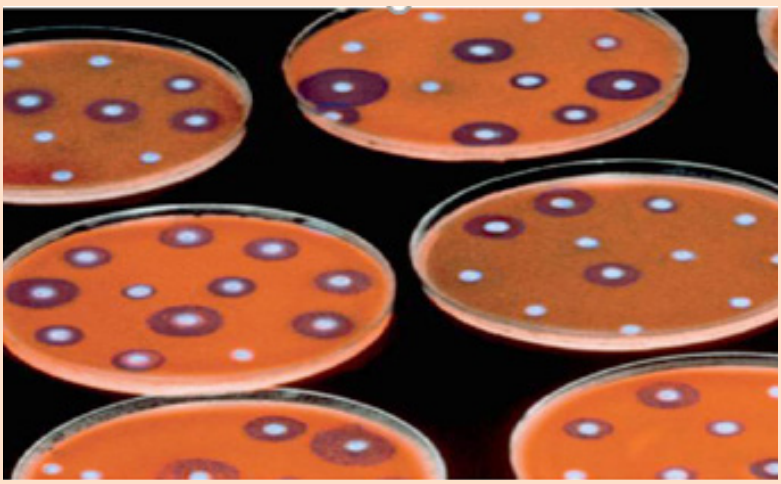
Key: Blue line indicates a given population after natural selection while red line indicates a given population before natural selection.

2) Give 2 examples of natural selection due to environment**?**

A. Antibiotic resistance in bacteria

Activity 5.2.2.A

Those Petri dishes contain gel that are nutrient for the growth of bacteria, the red gel in each of these petri dishes has been inoculated with bacteria. The small blue circles are discs impregnated with antibiotics but some bacteria can grow around those antibiotics that are supposed to kill them.



From the observation above what can you say on those bacteria that are able to grow in the presence of Antibiotics?

Antibiotic resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth; in other words, the bacteria are “resistant” and continue to multiply in the presence of therapeutic levels of an antibiotic.

When the antibiotic is used the bacteria can develop the ability to defeat the drugs designed to kill them. These bacteria survive to this antibiotic continue to live and produce offspring that are resistant to this antibiotic.

Antibiotic resistance is a natural process even though a number of bacteria drug resistance is attributed to human being by overuse and misuse of antibiotics. In some countries and over the Internet, antibiotics can be purchased without a doctor’s prescription. Patients sometimes take antibiotics unnecessarily, to treat viral illnesses like the common cold.

How do bacteria become resistant?

Some bacteria are naturally resistant to certain types of antibiotics. However, bacteria may also become resistant in two ways: by a genetic mutation or by acquiring resistance from another bacterium.

How does antibiotic resistance spread?

Genetically, antibiotic resistance spreads through bacteria populations both "vertically," when new generations inherit antibiotic resistance genes, and "horizontally," when bacteria share or exchange sections of genetic material with other bacteria. Horizontal gene transfer can even occur between different bacterial species. Environmentally, antibiotic resistance spreads as bacteria themselves move from place to place; bacteria can travel via air, water and wind.

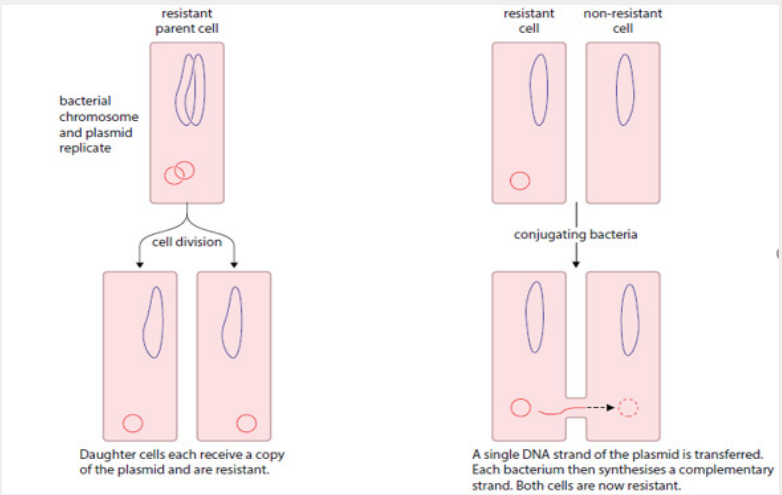
People can pass the resistant bacteria to others; for example, by coughing or contact with unwashed hands.

Can bacteria lose their antibiotic resistance?

Yes, antibiotic resistance traits can be lost, but this reverse process occurs more slowly. If the selective pressure that is applied by the presence of an antibiotic is removed, the bacterial population can potentially revert to a population of bacteria that responds to antibiotics.

Application activity 5.2.2.A

1) Compare the two diagram below and differentiate them in the two categories of resistance transmission in bacteria.



B. Pesticides resistance in insect and mammals

Activity 5.2.2.B

Discuss and explain the pesticides resistance in insect and mammals and present your findings.

Pesticide resistance means a decreased ability of pesticides to kill pest, Pest species evolve pesticide resistance via natural selection and it can be passed from one generation to the other trough reproduction.

a) Pesticide resistance in insect

The intensive use of insecticide and genetics are the fundamental factors of insecticide resistance. By natural selection insect with the genes that confer the resistance to the insect survive and transmit the trait to the next generation, most of pest species including insect produce large broods which increase the probability of mutations and ensures large resistant populations.

Resistance can be for a single insecticide, but it is more common that insect resistance can be developed to the pesticide with the same mode of action.

This is known as **cross resistance** while **multiple resistance** is when insects resist to two or more pesticides. In addition to resistance there is **Tolerance**, it is not a result of selection pressure but a natural tendency, for example mature caterpillar are tolerant to many insecticides than their young one.

b) Pesticide resistance in mammals

Resistant weed species have now been reported and about 10 species of small mammals and plants attacking nematodes are known to be resistant. Resistance in mammals has affected the control of rat populations. A chemical called warfarin has been used to kill rats since the 1950s.It is given to rats in the form of food baited with the chemical. Once ingested, warfarin prevents blood clotting, causing hemorrhaging and death of the rats. A warfarin –resistant allele arose in the rat population that allow them to survive when warfarin was ingested.

Application 5.2.2.B

Answer by true or false

1) Due to the development of exoskeleton in some insect like caterpillar they become more susceptible to pesticides than their young ones?
2) In our house there is a lot of mice, we use to kill them using 'sumu ya Panya"but it can't kill them nowadays, they are resistant to it.
3) To fight against malaria we can use a mosquito net which kills Anopheles, Anopheles do not resist on it.

C. Industrial melanism

Activity 5.2.2.C

1) Observe the following diagram of moth and answer to the question below

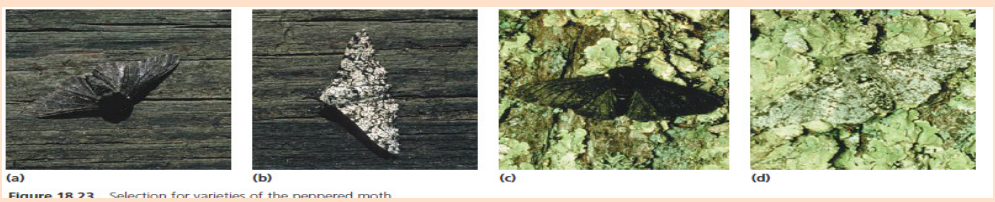


Figure 18.23 Selection for varieties of the peppered moth

a) Write down your observation about the colour of moth and its back ground.
b) Why those moth do not have the same colour?

Industrial melanism is an effect of evolution prominent in several arthropods where melanism has evolved in an environment where the air is greatly affected by Sulphur dioxide and dark soot deposits. Darker pigmented individuals are better fitted into those polluted environments which favors their camouflage. Other explanation links this industrial melanism with immunization (strengthening of immunity), absorption of heat at high rate in reduced sunlight and ability to excrete trace element into melanic scales and feathers.

Industrial melanism can be observed in more than 70 species of *Lepidoptera* (butterflies and moths*).*

Application 5.2.2.C

In the light forest, after the end of the simulation 76% light moths and 24% dark moths were observed. Relate this to the industrial melanism and find an explanation to those number.

5.3 Artificial selection

Activity 5.3

1. The original Rwandan cattle (a) from which individuals were first domesticated use to have long horns, but now days many cattle (b) do not look the same as the original one.

With the daily life experience and the observation on the figures above, why does the number of traditional cows (a) are decreasing in Rwandan societies.

Artificial selection or selective breeding involves the selection of trait of interest done by human being not environment and use them as the parent of the next generation Artificial selection has been practiced by humans for several centuries. It has played an important role in the evolution of modern crop plants, farm animals and domestic pets from the wild ancestors. This led to a population with desired traits. Artificial selection is used by humans to produce varieties of

animals and plants that have an increased economic importance. It is considered as a safe way of developing new strains of organisms, compared with genetic engineering, and is a much faster process than natural selection. However, artificial selection removes variation from a population, leaving it susceptible to disease and unable to cope with changes in environmental conditions. Potentially, therefore, artificial selection puts a species at risk of extinction.

Comparison between natural selection and artificial selection

Artificial selection	Natural selection
Selection is done on the basis of require character	Selection is totally based on the adaptable character and the one who is able to cop up in all types of natural conditions
Does not lead to new species	May lead to new species
Man-made selection	Nature/natural/environmental selection
Genetic diversity is lowered	Genetic diversity remains high
Proportion of heterozygous in the population is reduced.	Proportion of heterozygous remains high.

Two methods of carrying out selective breeding are known as **in breeding** and **out breeding**

Application 5.3

1) At break time one of the student who come from Mamba sector in Gisagara district told us about the agriculture activity, she said that before sowing ground nuts their parents select seeds that look good. I was asking myself why they can't saw any seed.

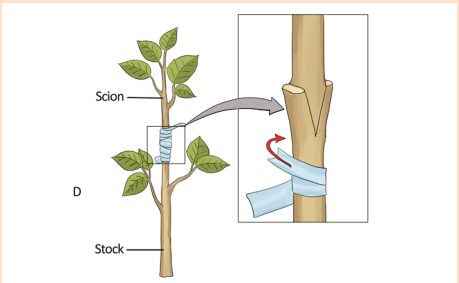
a) From your knowledge is this selection, artificial or natural selection

b) Give other examples of artificial selection that you know

Methods of Artificial selection

Activity 5.3.1

1. Carefully observe the following diagram and write down what are your observation of the activity that take place.



a) Inbreeding

In breeding methods, is a method of artificial selection in which there is a breeding of closely relative's individuals with a desired trait, in this case the chances to obtain offspring showing the desired characteristics are greater. This characteristic of interest is retained as far as possible and the origin of the desirable trait is spontaneous mutation. The inbreeding presents some negative consequences like the loss of vigour, with the population being weakened by lack of diversity, the increase of expression of recessive allele that is why there is a need of introducing new genes from outside to make the population healthier and stronger.

b) Outbreeding

This method of artificial selection involves crossing unrelated individuals showing two different characteristics in order to obtain an individual which combine both characteristics for example by crossing a crop plant that gives an excellent yield with the one which resist to disease in the expectation of a plant with a high yield and disease resistance. It frequently produces tougher individuals with a better chance of survival. This is called **hybrid vigour**

Selective breeding in cattle: Nowadays milk dairy industry are interested in modern-day cattle for milk production and farmers involved in it use selective breeding by artificial insemination.

In the selection farmers follows some factors including:

- Volume of milk produced each day
- Length of milking(lactation) period
- Protein and fat content of milk

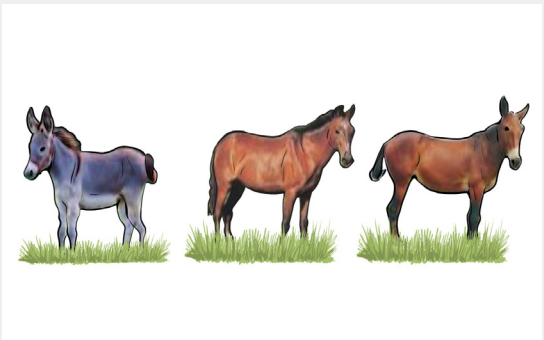
- Disease resistance,

The selective breeding process

- Selecting a suitable cow and bull by consulting the pedigree records of each and trough progeny testing.
- Collection of sperm from the selected bull and storing them by freezing
- Detection of when the cow is in oestrus by observing changes in her behavior, e.g. Increase restlessness, feeding less.
- Artificially inseminating the defrosted semen into the cow.
- Checking that fertilization has occurred and the calf in cow.

Both artificial insemination and embryo transplantation can be used.

Application 5.3.1



Before leading the following passage observe carefully the fig of animals above.

The hybrid offspring of donkey (I) and horse (J) is a mule (k), which is robust but sterile, animals can be crossed according to the characteristic needed in artificial selection.

a) Which type of artificial selection indicated above?

b) What can you do, to prevent the sterility of your hybrid(mule)

Skills lab

This exercise illustrates the effect of natural selection on populations of predators and prey. Students-teacher, in groups of four, will represent predators, each with a different adaptation for capturing their prey. The prey will consist of different species represented by different colored beans.

Procedure.

1) Each team of 4 students will count out exactly 100 dried beans of each color.
2) Thoroughly mix the beans and spread them evenly over your'habitat.'your habitat depends on the weather.
   i) If the weather is poor, it is dark outside, or your instructor would rather, your habitat will be a tray of sediment in the classroom.
   ii) If the weather is lovely,or your instructor is adventurous,you will do this about lab outside.Each team will mark off 1m*1m ''habitat'' in the grass using yarn, a meter stick,and wood stakes.
   iii) All 'prey' are confined to the habitat,wherever it is!
3) Each student (predotor) will have a different feeding apparatus:A fork,spoon,Knife or forceps.
4) When everyone is ready, predators will spend 60 seconds capturing prey with their devices and depositing them into a cup while obeying the following rules:
   i) Predators must only use their capture device to capture prey
   ii) Predators may not scoop prey up with their cup.
   iii) If predators 'eat' too much of the environment, they will become constipated and die.
5) Each predator determines the number of prey captured and records results in data

   Sheet: Generation1.

4) Calculate and fill the statistics on the data sheet (see example below).

Data sheet: Generation1

Prey type	Black bean	Pinto bean	Red bean	White bean	total	Captured%
Population size	100	100	100	100	400	
Forceps						
Spoon						
Fork						
knife						

Prey type	Black bean	Pinto bean	Red bean	White bean	total	Captured%
Total kills						
#survived						
% survived						
fork						
%Total population						

Draw your conclusion about the natural selection and present it to your facilitator

End unit assessment 5

1) Suggest how each of the following might decrease the chances of an antibiotic resistant strain of bacteria developing
   a) Limiting the use of antibiotics to cases where there is a real need
   b) Regularly changing the type of antibiotic that is prescribed for a particular disease
   c) Using two or more antibiotics together to treat a bacterial infection.
2) Differentiate between natural selection from artificial selection
3) Some individuals of the swallowtail butterfly scientifically known as *Papilio machaon* of the family papilionidae pupate on brown stems or leaves; others pupate on green stems or leaves. Two distinct colour forms of the pupae are found, namely brown and green, with very few intermediates.
   a) What type of natural selection does this example show?
   b) Explain why the intermediate colour formed would be at selective disadvantage.
4) Copy and complete the table to compare artificial selection with natural selection

Natural selection	Artificial selection
The selecting agents is total environment of the organism	
Adaptations to the prevailing conditions are selected	
Many different traits contributing to fitness are selected	

Distinguish inbreeding and outbreeding

6) Explain why artificial breeding is beneficial to man?

UNIT 6

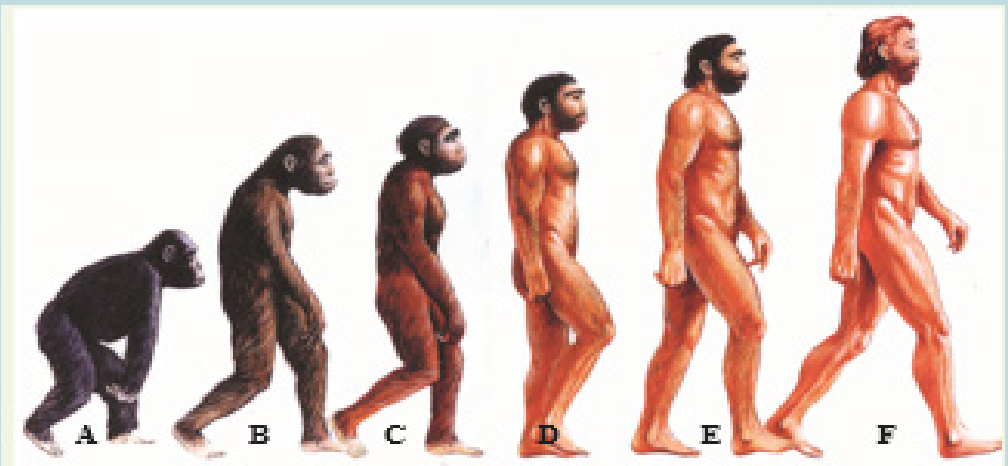
EVOLUTION AND SPECIATION

Key unit competence

Analyze the relevance of theories of evolution and explain the process of speciation

Introductory activity 1

During Kwita Izina Ceremony (naming a newborn Gorilla) in Rwanda. On Rwanda television I saw an image of mountain gorilla, it was closely related to human being. Later while I was reading biology book I found this image which shows human being and their ancestors



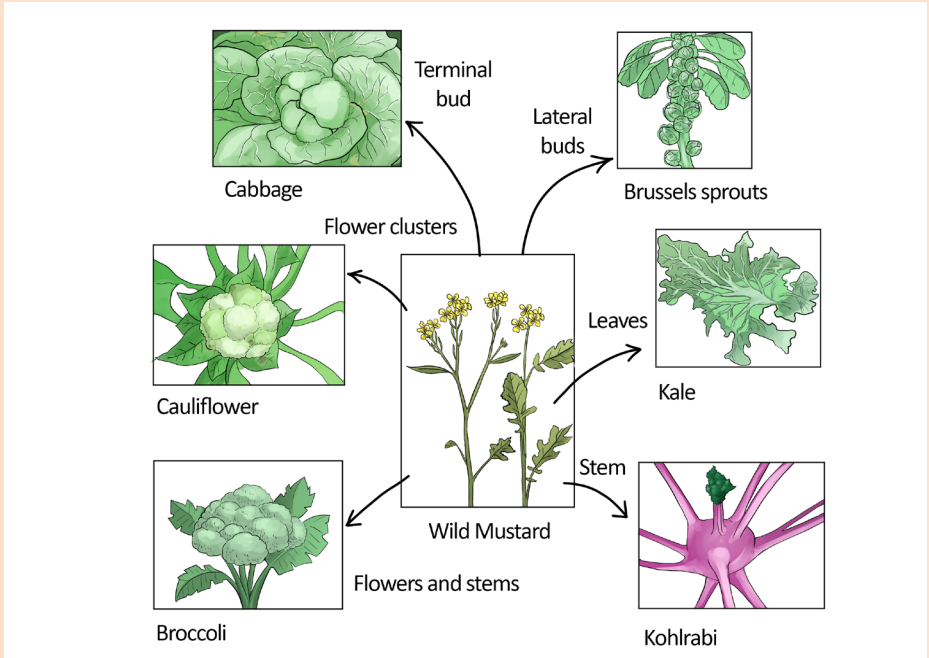
a) Observe carefully the image above and record the similarities among A and D, and D and F.

b) Write a short note of your observation about the image.

6.1 Theories of evolution

Activity 6.1

1) Observe the diagram below of plant (vegetables), do you see any relationship among those types of vegetables



According to the most biologists the principal questions in biology is "where do all living things come from?" but we know that life comes from the pre-existing life means that every species descends from other species, it is what we call "**evolution**"

Evolution is a changeover successive generation of inheritable trait of a population or it is the process by which new species are formed from pre-existing ones over a period of time. As there is emergence of new species others are disappearing, the species that disappear are said to become extinct. An enormous fossil, such as those of early birds, provides evidence of evolution. Genetics studies of populations of bacteria, protists, plants, insects, and even humans provide further evidence of the history of the change among organisms that live or have lived on earth.

Theory of evolution is a short term for theory of evolution by natural selection which was proposed by Charles Darwin and Alfred Russel Wallace in the nineteenth century.

Four main theories of evolution are known**:**

- Lamarckism or theory of inheritance of acquired characters
- Darwinism or theory of natural selection.
- Neo-Darwinism or modern concept or Synthetic theory of evolution and
- Creation Theory.

a) Lamarckism

Lamarckism or theory of inheritance of acquired characters developed by Jean Baptist Lamarck (1744-1829) French Biologist. His theory is based on the inheritance of acquired characteristic (variations) in the body of organism in the response to the environment conditions

i) Assumptions of Lamarck's theory

- Organisms tend to increase in size as they become more complex to a predetermined limit.
- When influenced by the environment, body changes can be induced in organisms.
- Organisms acquire new features because of need.
- Development of an organ and its effectiveness is promoted by its use whereas its disuse brings about decline.
- Acquired features are inherited by future generations.

ii) Merits/Advantages

- Lamarck was able to show that the environment influences the course of evolution.
- He observed that features are passed down from parents to their offspring.
- He was able to recognize that as organism increase in size, they become more complex to a predetermined limit. (Predetermine: to determine or decide in advance)

iii) Demerits /disadvantages

- Acquired changes are not heritable as they are influenced by genes.
- Somatic changes are not heritable as they are not passed through reproduction.
- The process of gametogenesis is not related to occupation or their activity.
- Use or disuse of somatic cells does not affect gamete formation.

b) Darwinism

The evolution is not a modern concept, since the ancient time, philosophers, Aristotle, Socrates, Confucius and others have suggested that complex species evolved from simple pre-existing ones by a process of continuous and gradual change. In nineteenth century Charles Darwin an A. Wallace published the paper describing their theory of evolution by natural selection later on 24 November 1859 Darwin published the book *"The origin of species by means of Natural selection or the preservation of favoured races in the struggle for life"* containing many evidence to support the theory.

According to Darwin's theory:

- Each species living today arose from a pre-existing species.
- All species have evolved from one ancestral type.
- Natural selection provides the mechanism for one species to change into another.

The main evidence for his first suggestion, which has been called descent with modification, comes from fossils.

Darwin use different observations in his research including the following:

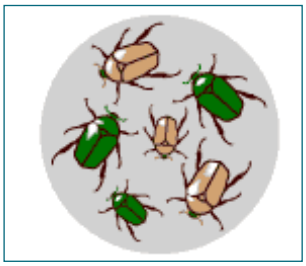
a) **Reproductive powers of living organism/over production/biotic potential**: Over production is the production of more offspring that can be supported by the available resources this ensures the surviving of a high number of offspring and the geometric or exponential growth of the population.

b) **Scarcity of resources**: Darwinism states that, the increase of the population geometrically is not directly proportional to the increase of resources (food, space...) which increase in arithmetic way.

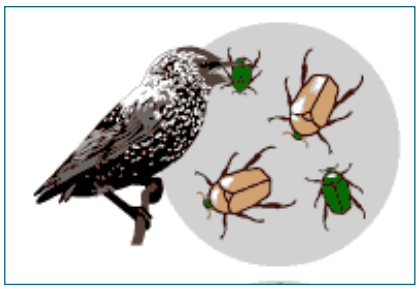
c) **Struggle for existence:** Darwin deduced on the basis of 1 and 2 that members of the species were constantly competing with each other in an effort to survive. In this struggle for existence only a few would live long enough to breed

d) **Survival of the fittest by natural selection:** Among the offspring there will be some better able to withstand the prevailing conditions. That is, some will be better adapted (fitter) to survive in the struggle for existence. These types are more likely to survive long enough to breed. Darwin's idea of evolution by natural selection is relatively simple but misunderstood. To find out how it works, imagine a population of beetles:

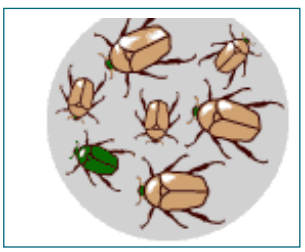
i) Variation in the beetles' population some are green and some brown



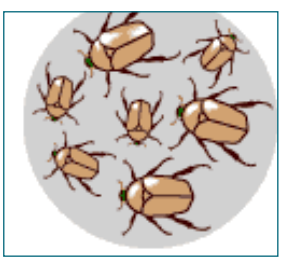
ii) Green beetles tend to reproduce less as they are eaten by predators than brown one



iii) Surviving beetles pass they brown genes to their offspring



iv) The brown coloration the important trait which allows the beetles to have more offspring and to survive, will dominate the population and eventually all beetles will be brown.



e) Inheritance of useful trait/like produce like: The selected individuals produce offspring with the useful trait so that they can fit into the environment.

Darwin's theory was based on three main observations:

i) Within a population are organisms with varying characteristics, and these variations are inherited (at least in part) by their offspring.

ii) Organisms produce more offspring than are required to replace their parents

iii) On average, population numbers remain relatively constant and no population gets bigger indefinitely.

After his observations Darwin concluded that within a population many individuals do not survive and fail to reproduce.

Assumptions of Darwinism

- Most organisms have the potential to produce large number of offspring or progeny than the environment can support. This leads to still competition as the numbers of organisms are fairly stable.
- All organisms, even of the same species vary in a few characteristics,
- Only those organisms of a given species with variations that adapt them to the environment, survive the competition and live. There is survival for the fittest by natural selection.
- The features favored/selected by nature survive and are inherited. Therefore, new species may develop by natural selection, which is one of the forces of evolution.

- **Merits of Darwin's theory of natural selection**
  - Species always change as the environment changes.
  - Species are compared with their ancestors due to presence of similarities in characteristics.
  - Enough data are / can be collected for explaining variation in a population that may result into formation of a new species.

- **Demerits of Darwin's theory of natural selection**
  - Not all variations inherited, except for only genetic variations.
  - It provides inadequate explanation of existence of many vestigial structures in organisms.
  - Explanation on deleterious mutations that are retained in a population is not adequate.

c) Neo-Darwinism

Neo-Darwinism is the modern theory of evolution that incorporates scientific evidence particularly from genetics and molecular biology, the Neo-Darwinism combine the work of Mendel genetics and Darwin, for example, we now know that

the variations that are so important in natural selection come about by random and spontaneous changes in genes, particularly from mutations in reproductive cells. According to Neo-Darwinism, nature selects those individuals with beneficial mutations and allows them to be passed to their offspring through reproduction from generation to generation. The mutations are transmitted within the population and if selected by nature, they may form a new species.

d) Special creation

It is believed that a **special being**, God created the universe and all living organisms (bible Genesis 1:1-2; Psalm 139:13-14). In this theory, heavens and earth were first created. Light, day and night were created next and subsequently, all living things with human beings the last in the creation. It shows that there was direct creation of organism with no precursor to life.

6.1. 1 Evidence for evolution

a) Fossils

The evidence for evolution are provided mainly by the study of fossil (paleontology). Fossils come into different forms, such as imprints, the burrow of worm, or mineralized bone preserved by natural process in rocks, ice etc. The study of fossils show how the organisms have changed over time.

Relative and radiometric dating are the method used by scientist to determine the age of fossils and rocks.

b) Anatomy and Embryology

Anatomy or comparative anatomic structures is the study of biological different organisms. Structures in different species that have similar internal frame, work, position and embryonic development are said to be **homologous.** For example, bones in the appendages of a human, dog, bird, and whale all share the same overall construction resulting from their origin in the appendages of a common ancestor. Overtime, evolution led to changes in the shapes and sizes of these bones in different species, but they have maintained the same overall layout**.**

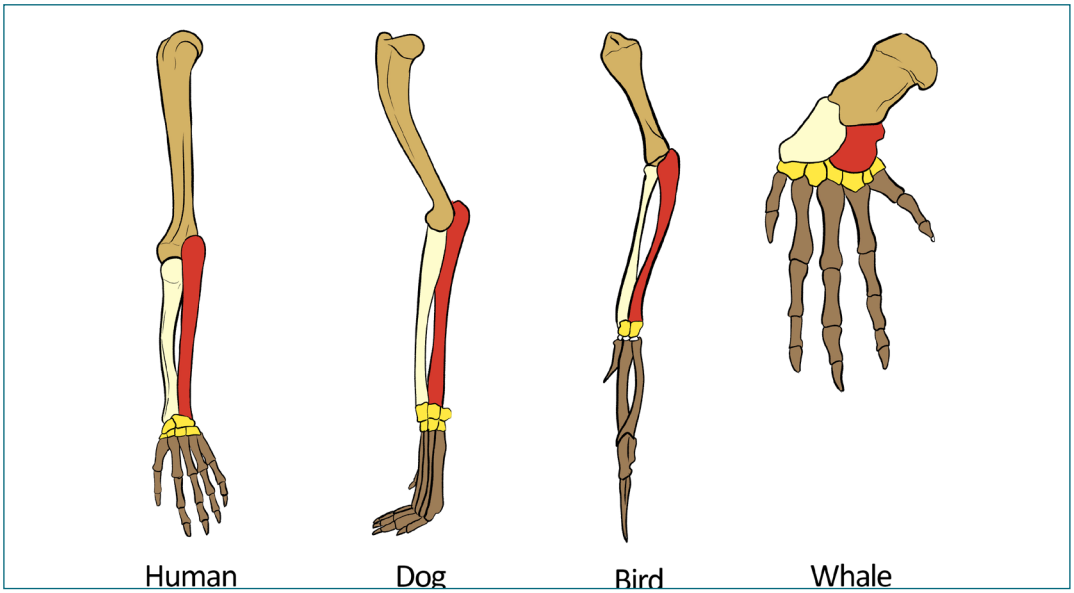


Fig 6.1: Homologous structure in the fore limb of vertebrates

Paleontologists have found fossils showing how the bones of lizard-like ancestor evolved into the ear bones of modern mammals.

The fact that two different organisms look alike does not always suggest a close evolutionary relationship. Structures of unrelated species can evolve to look alike because structures are adapted of similar functions. These are called **analogous structures.** Another evidence is **vestigial structures** body structure with no function or which do not serve their original purpose but probably useful in the ancestor**s.**

c) Comparative embryology

The study for embryo it is called embryology, an embryo is an unborn or unhatched animal or human young in its earliest phases. Therefore, species that show a similar embryonic development are assumed to be closely related, even if the adult stages are very different. For example, echinoderms (the phylum containing starfish and sea urchins) are believed to be related to chordates (the phylum including vertebrates) because of similarities in their early embryonic development.

d) Comparative biochemistry and cell biology

The most persuasive evidence that organisms have evolved from a common ancestor comes from studies comparing the cell biology and biochemistry of different organisms, which reveal that:

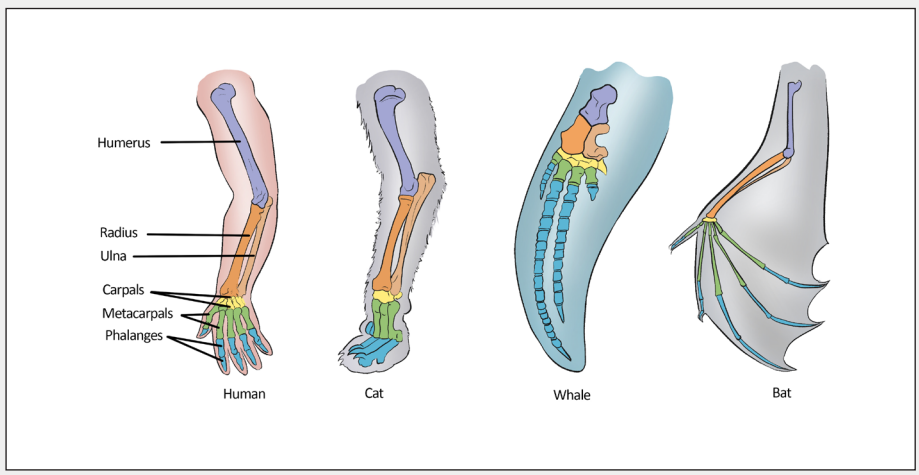
- The genetic code contained within nucleic acids is almost universal
- Physiological processes vital to all organisms, such as respiration, follow very similar metabolic pathways.

– ATP is the universal energy currency

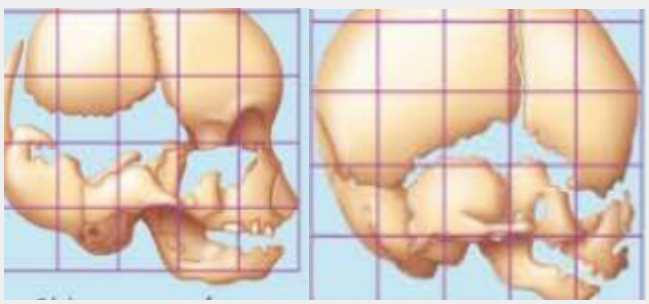
The cellular and biochemical details of organisms are quite similar, but any differences can give an idea of how closely different species are related. Species that are closely related would be expected to differ only slightly from each other. Detailed comparisons of DNA, metabolic pathways, key proteins, and organelles such as ribosomes have been used to work out the evolutionary relationships of organisms. For example, ribosomes inside mitochondria and chloroplast are similar to those in bacteria, suggesting that these organelles may have evolved from bacteria. Mammalian blood proteins can be tested to see how similar they are to human blood proteins: blood serum from the mammal in question is added to rabbit serum containing anti-human antibodies.

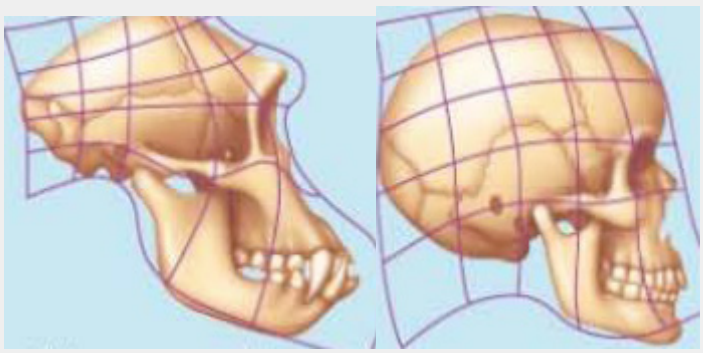
Application Activities 6.1

1) The diagram below indicates the part of front limb of different animals, if the labeled diagram is an arm of human being label other diagram by corresponding them, according to how they have evolve



2) The skull of chimpanzee and that of human being are shown her below





The above diagram corresponds to the adult skull, relate them with their fetus

3) Correct this statement

Mitochondrial DNA differences are inconsistent with the existence of a recent human common ancestor for all ethnic groups.

6.2 Cause of evolution

Activity 6.2

Find out the cause of evolution and discuss it among your classmates.

It is difficult to meet **Hardy-Weinberg equilibrium** in real populations. The Hardy-Weinberg Theorem describes populations in which allele frequencies are not changing means that it does not evolve.

The force behind evolution are mainly summarized in four factors:

- Competition changes in the environment.
- Sexual reproduction.
- Mutations.
- Gene recombination.
- Industrialization.
- Effect of drugs or chemical resistance.
- Artificial selection.

a) Competition changes in the environment

Imagine that we are plunged into a new ice age. The climate becomes much colder, so that snow covers the ground for almost all of the year. Assuming that rabbits can cope with these conditions, white rabbits now have a selective advantage during seasons when snow lies on the ground, as they are better camouflaged (like the hare in *figure 6.3.).* Rabbits with white fur are more likely to survive and reproduce, passing on their alleles for white fur to their offspring. The frequency of the allele for white coat increases at the expense of the allele for agouti. Over many generations, almost all rabbits will come to have white coats rather than agouti

Fig 6.2: Excellent camouflage from predators when viewed against snow

b) Sexual reproduction

Sexual reproduction is a reproduction using gametes (male gametes and female gametes) each gamete contain a unique set of gene combination, and the ova is fertilized randomly by the male gamete as a result each zygote is unique hence the variation among individuals**.**

c) Mutation

Mutation creates new genetic variation in a gene pool. It is how all new alleles first arise. In sexually reproducing species, the mutations that matter for evolution are those that occur in gametes. Only these mutations can be passed to offspring. For any given gene, the chance of a mutation occurring in a given gamete is very low. Thus mutations alone do not have much effect on allele frequencies. However, mutations provide the genetic variation needed for other forces of evolution to act

d) Gene recombination

Natural selection is usually the most powerful mechanism or process causing evolution to occur, however, it only selects among the existing variation already in a population. It does not create new genetic varieties or new combinations or varieties. One of the sources of those new combinations of genes is recombination during meiosis. It is responsible for producing genetic combinations not found in earlier generations

e) Industrialization

Many species of organisms, especially insect species, have two or more adult body forms that are genetically distinct from one another, but which are contained within the same interbreeding population. This condition is known as polymorphism (another type of natural selection). The peppered moth (*Biston betularia),* for example, has two main forms with different wing colours. One form has pale wings with dark markings; the other form is called melanic because the wings contain large amounts of melanin (a black pigment), so they are almost black.

f) Effect of drugs or chemical resistance

Drug resistance is a reduction in effectiveness of medication such as antimicrobial in treating a disease or condition**.** Antibiotic resistance is a severe problem throughout the world. For example, some strains of the common bacterium *Staphylococcus aureus* are resistant to antibiotics such as penicillin and methicillin. Penicillin resistance has probably evolved in the following way:

- By chance, a mutation produces an individual bacterium with an allele that allows it to produce an enzyme, penicillinase, which deactivates penicillin.
- This bacterium is immediately resistant to penicillin. (As bacteria have only one strand of DNA and one copy of each gene, the mutant allele is expressed immediately and is not masked by a dominant allele.)
- If the population to which the mutant belongs is exposed to penicillin, the mutant will survive and reproduce whereas those without the mutant will be killed.

g) Artificial selection

Over the years, humans have used artificial selection to create dog breeds over the past 150 years or so, humans have been specifically mating dogs that look a certain way to create the animals we now keep as pests via a process known as breeding. This is artificial selection, where one species (humans) directs the traits that get passed down to future generations of another species (dogs).

Application activity 6.2

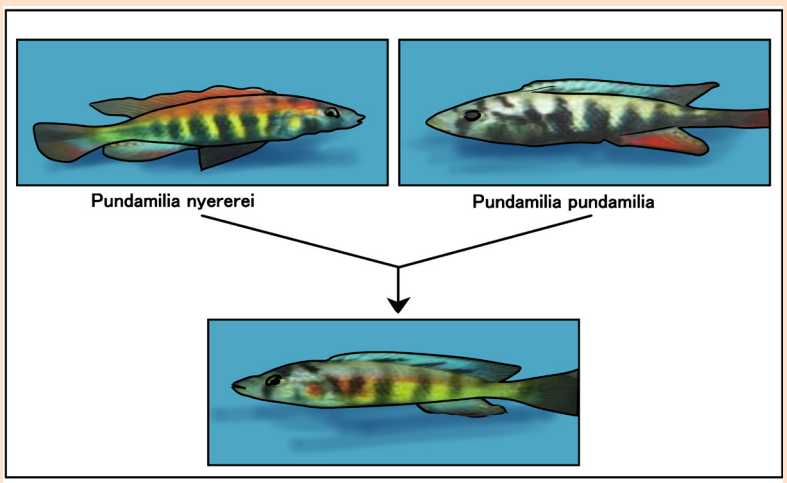
1) Numerous factors can induce the evolution of species, observe the diagram below then suggest the cause of the loss of hair.

2) After understanding the evolution, give the factors that are inducing today's evolution.

6.3 Speciation

Activity 6.3

1. Observe the diagram below, and write a short notes for your observation



Speciation is the evolution of new species from the existing ones. A species is a group of organisms with similar features which can interbreed to produce fertile offspring, and which are reproductively isolated from other species. Organisms which do not interbreed under normal circumstances to produce fertile offspring are regarded as reproductively isolated. Mechanisms that prevent the formation

of hybrids are called prezygotic isolating mechanisms, Prezygotic (before a zygote is formed) isolating. Mechanisms include:

- Individuals not recognizing one another as potential mates or not responding to mating behavior
- Animals being physically unable to mate
- Incompatibility of pollen and stigma in plants
- Inability of a male gamete to fuse with a female gamete.

The mechanisms that affect the ability of hybrids to produce fertile offspring are called postzygotic isolating mechanisms. Postzygotic isolating mechanisms include:

- Failure of cell division in the zygote
- Non-viable offspring (offspring that soon die)
- Viable, but sterile offspring.

The most important isolating mechanism is thought to be geographical isolation, in which two populations originally of the same species are separated from each other by a physical barrier such as a mountain, river, or ocean.

a) Allopatric speciation

Allopatric means 'different countries' and describes the form of speciation where two populations become **geographically isolated**. Geographical isolation may be the result of any physical barrier between two populations which prevents them interbreeding. These barriers include oceans, rivers, mountains ranges and deserts. Which proves a barrier to one species may be no problem to another. The isolated populations then undergo phenotypic divergence as:

- They independently undergo genetic drift
- Different mutations arise in two populations
- They become subjected to dissimilar selective pressure

b) Sympatric speciation

Sympatric literally means. ('Same country') Sympatric speciation occurs when organisms inhabiting the same area become reproductively isolated into two groups for reasons other than geographical barriers. Such reasons might include:

1) The genitalia of two groups may be incompatible (mechanical isolation): It may be physically impossible for the penis of a male mammal to enter the female's vagina
2) The gametes may be prevented from meeting: In animals, the sperm may not survive in the female's reproductive tract or, in plants; the pollen tube may fail to grow.

3) Fusion of the gametes may not take place: Despite the sperm reaching the ovum, or the pollen tube entering the micropyle, the gametes may be incompatible and so will not fuse.

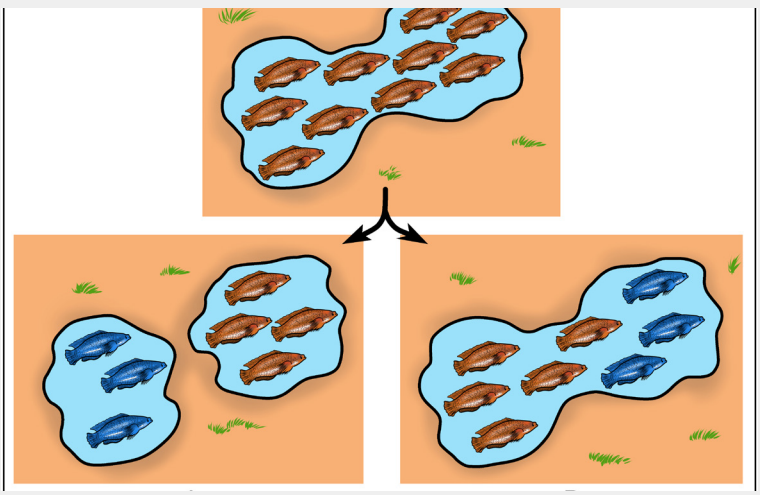
4) Development of the embryo may not occur (hybrid inevitability): Despite fertilization taking place, further development may not occur, or fatal abnormalities may arise during early growth

5) Polyploidy (hybrid sterility): When individuals of different species breed, the sets of chromosomes from each parent are obviously different. These sets are unable to pair up during meiosis and so the offspring cannot produce gametes.

6) Behavioral isolation: Before copulation can take place, many animals undergo elaborate courtship behavior. This behavior is often stimulated by the colour and markings on the members of the opposite sex, the call of a mate or particular actions of a partner.

Application activity 6.3

1) Which type of speciation is indicated by the diagram below



A and B

2) Which of the following is a correct definition of speciation?

a) When one species has a genetic mutation, allowing it to breed with another species

b) When a species has a genetic defect, making it a brand new species

c) The process by which a species goes extinct, allowing a new species a chance to live in anew habitat

d) An evolutionary process that leads to the formation of a new species.

3) Which of the following is not true in the formation of a new species?

a) If an isolated population has a new environmental conditions new traits can be favored eventually leading to the inability to reproduce with the original population.

b) A mutation causes a population to breed with a different species.

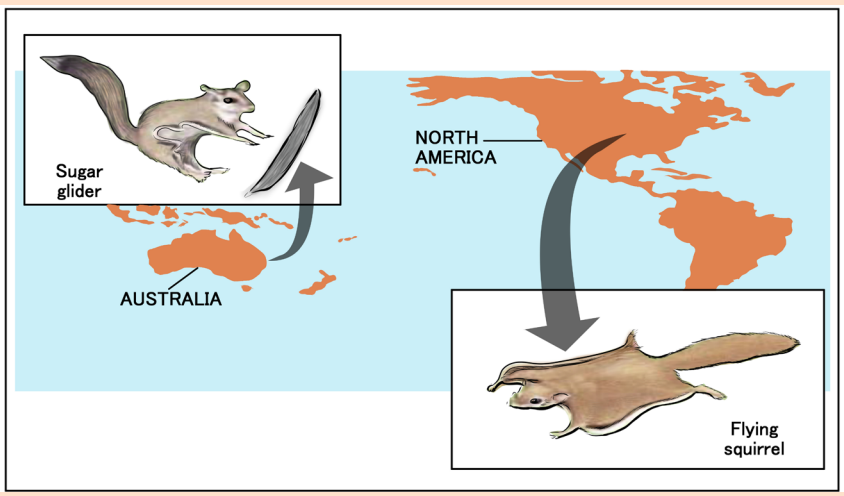
c) Reproductive isolation can occur by the formation of a mountain range.

d) A population needs to become reproductively isolated.

6.4 Mechanisms of speciation

Activity 6.4

The following image are for two different animals.



a) Write down the similarities and differences in these animals on the above image.

b) Can you consider them as a single species?

a) Continental drift

The continents which now exist have not always appeared as they do today. At one time, the earth had a single large land mass called Pangaea. This is thought to have broken up into two parts, a northern Laurasia and a southern Gondwanaland. Over millions of years, the two great land masses split up and moved by a process called continental drift to form our present continents. The

theory that these land masses were once joined is supported by the discovery in Australia, South Africa, South America, and Antarctica of fossils belonging to the same extinct species. Fossils in North and South America show differences between the species, suggesting that these two continents have only joined together relatively recently. Before this, their fauna (animals) and flora (plants) were geographically isolated and evolved independently.

Australia shows many excellent examples of species that evolved independently following its geographical isolation. It is thought that Australia became isolated about 120 million years ago, when marsupials (mammals without a placenta but with a pouch in which the young develop) and eutherian mammals (mammals with a true placenta) diverged from a common ancestor

b) Migration

Migration also called gene flow is any movement of individuals, and/or the genetic material they carry, from one population to another. Gene flow includes lots of different kinds of events, such as pollen being blown to a new destination or people moving to new cities or countries. If gene versions are carried to a population where those gene versions previously did not exist, gene flow can be a very important source of genetic variation. In the graphic below, the gene version for brown coloration moves from one population to another.

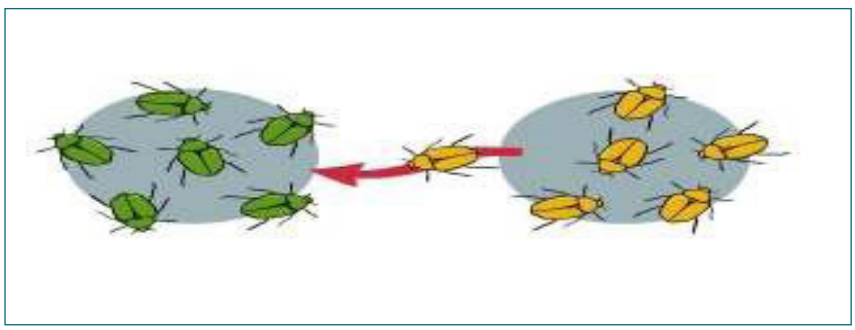


Figure: Illustration of migration

c) Divergent evolution

A single species evolves into several new species that live in different ways. The **five of Darwin's finches are a good example.** There are separate species of finch in the group, all of which probably evolved from individuals belonging to ne mainland species.

The islands have few other bird species. In the absence of competition, the finches became adapted to fill all the available niches. In particular, they evolved a wide range of beak sizes and shapes so that they could take advantage of the food sources on the different islands. The evolution of an ancestral species into different species to fill different niches is called adaptive radiation

d) Convergent evolution

Unrelated species independently evolve similarities when adapting to similar environments

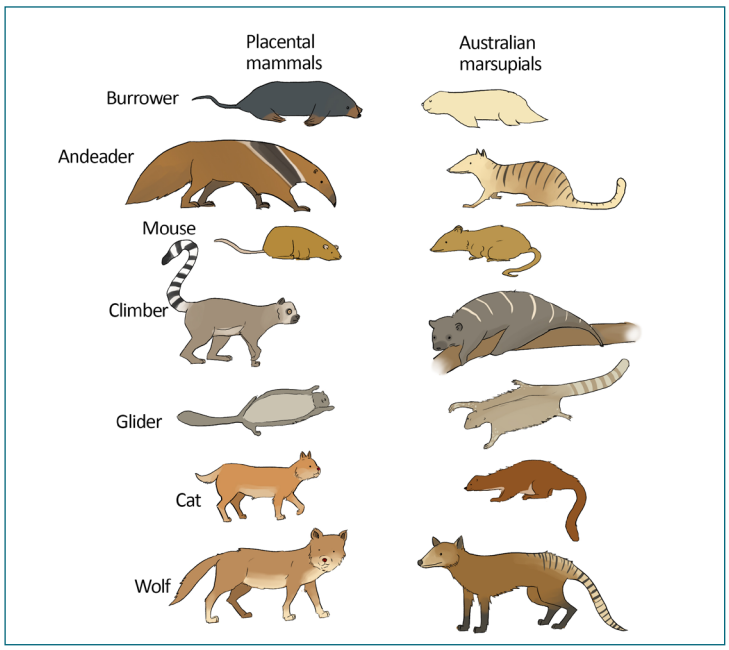


Figure 6.3: Convergent evolution

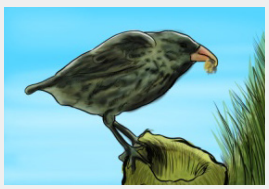
6.1: Table isolating mechanisms

Type of isolation	Reason for the isolation
Geographical isolation	Organisms isolated by a physical barrier, such as a mountain, river, or ocean
Temporal isolation	Organisms breed at different times of year
Ecological isolation	Organisms live in different habitats within the same area
Behavioral isolation	Organisms have different behavior patterns, e.g. they use different behavior to attract a mate. In the fruit fly drosophila, for example, normal mating involves males performing a ritualized 'dance' that has a definite sequence of wing and body movements. Closely related species will not normally mate because the courtship dances of males are different. But experiments have shown that, in some cases, mating will occur if the antennae of the female are removed. Presumably, the female is unable to detect the wrong courtship dance and permits mating.

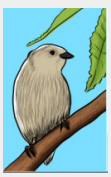
Mechanical isolation	Organisms cannot mate because of anatomical differences which make it impossible for gametes to come together
Gametic isolation	Genetic or physiological incompatibility between different organisms prevents hybrids forming, e.g. pollen may fail to grow on a particular stigma with incompatible genes
Hybrid isolation	Different organisms interbreed but offspring do not survive or are infertile

Application activity 6.4

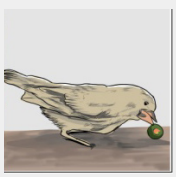
1) Observe the following birds



Cactus eating bird



Insect-eating bird



Seed-eating bird

Observe those figures, what is the type of speciation?

2) Which effect of natural selection is likely to lead to speciation?

a) Differences between populations are increased.

b) The range of genetic variation is reduced.

c) The range of phenotypic variation is reduced.

d)Favorable alleles are maintained in the population.

Skills lab

Formulate models

Camouflage provides an adaptive advantage Camouflage is a structural adaptation that allows organisms to blend with their surroundings. In this activity, you'll discover how natural selection can result in camouflage adaptations in organisms.

Procedure

Working with a partner, punch 100 dots from a sheet of white paper with a paper hole punch. Repeat with a sheet of black paper. These dots will represent black paper.

1) Scatter both white and black dots on a sheet of black paper.
2) Decide whether you or your partner will role-play a bird.
3) The ''bird ''looks away from the paper, then turns back and immediately picks up the first dot he or she sees.
4) Repeat step 4 for one minute

Analysis

1) Observe what color dots were most often collected?
2) Infer how does color affect the survival rate of insects?

Hypothesize what might happen over many generations to a similar population in nature?

End unit assessment 6

1) Name two examples of adaptive radiation.
2) What effect did industrial pollution have on:
   a) The frequency of the C (melanic) allele within a population of peppered moths.
   b) The rate of mutation of the c allele to the C allele
3) Explain what is meant by heterozygous advantage, using the sickle-cell allele as an example.
4) Answer the following questions:
   a) Distinguish between homologous structures and analogous structures with specific examples.
   b) Name the type of evolution exhibited by comparing:
      i) Flipper of whale and forelimb of desert rat.
      ii) Wing of a bat and wing of butterfly
      iii) Wing of a flamingo and wing of an insect

Essay questions

1) Explain the various evidences of organic evolution.
2) Explain Darwin's theory of natural selection. The environment or nature selects the individual with variations that are favored by the environment. These compete with the others and able to reach sexual maturity, reproduce and pass over the favorable characteristics to their offspring.
3) What do you understand by Lamarckism? How does it differ from Darwinism?
4) How can you convince that evolution progress?
5) A Darwin and Lamarck contribution to science is unparalleled. Discuss.

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