Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

SEMESTER-IV

HARD CORE THEORY PAPER

ANIMAL BEHAVIOUR AND MICROBIOLOGY

ZCORT-411

SELF LEARNING MATERIAL

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI, NADIA,
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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani, a dedicated team under the able guidance of the Hon'ble Vice-Chancellorhas invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Amalendu Bhunia, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every member of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are self-writing and collected from e-book, journals and websites.

Director
Directorate of Open and Distance Learning
University of Kalyani

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HARD CORE THEORY PAPER (ZCORT- 411)

Animal Behaviour and Microbiology

Group A (Animal Behaviour)				
Module	**Unit**	**Content**	**Credit**	**Page No.**
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	V	Kinship: selfishness, altruism		
	VI	Conflict: Sexual selection, aggression, competition dominance, Infanticide		
	VII	Game theory - Models and strategies.		
	VIII	Communications: Channels, functions		
	IX	Communications: origin and modification of signal, signal receiving mechanism.		
	X	Evolution of feeding behavior: optimal foraging theory.		
	Group B (Microbiology)			
	XI	History of microbiology.		
	XII	Bacteriology: Structure and function of capsule, pili, flagella, cell wall, cell membrane, outer-membrane, chromosome and plasmid		
	XIII	Virology: Structural organization of viruses, Prions and viroids, Lytic cycle of bacteriophages, Lysogeny and lysogeny control, lysogenic conversion, induction and significance.		

ZCORT- 411 (Animal Behaviour and Microbiology)	XIV	Animal and Veterinary Microbiology: Microbial interactions with animals (marine and freshwater invertebrates, ruminants), symbiotic light production, sulfide-based mutualism.	3	
	XV	Disease causing microbes: *Escherichia coli* and *Streptococcus* spp.		
	XVI	Culture techniques: Microbial nutrition and growth; types of culture media, sterilization of culture media; culture techniques: pure cultures.		
	XVII	Industrial microbiology: Microbial fermentation; production and commercialization.		
	XVIII	Mode of transmission, pathogenicity and prevention of microbial diseases: Air-borne (Tuberculosis)		
	XIX	Mode of transmission, pathogenicity and prevention of microbial diseases: Arthropod borne (JE and Yellow fever)		
	XX	Mode of transmission, pathogenicity and prevention of microbial diseases: SARS-COV 2 (infection and concept of herd immunity).		
	Total counselling session 18hrs.			

Unit-I

Introduction to animal behaviour: History, foundation, approaches and methods

Objective: In this unit you will know about history, foundation, approaches and methods of animal behaviour.

Introduction

Do the squirrels in your neighbourhood bury acorns underground? Does your cat start meowing around the time you usually feed her? Do you start hanging around the kitchen when it's close to dinner time? If you've noticed any of these things, congratulations—you've made your first observations in behavioural biology! These are all examples of animal behaviours. Yep, you and I count as animals too. In fact, these behaviours are just a tiny sampling of the amazing and diverse behaviours we can see in nature.

We could ask what behaviour is used for, but it might be better to ask, what isn't it used for? Animals have behaviours for almost every imaginable aspect of life, from finding food to wooing mates, from fighting off rivals to raising offspring. Some of these behaviours are innate, or hardwired, in an organism's genes. For instance, this is true of the squirrel and its acorn. Other behaviours are learned, such as your tendency to hang around the kitchen at dinnertime or your ability to read the words on this screen.

In this section, we'll take a closer look at animal behaviour—how it's studied, how it can run the gamut from hardwired to learn, what are its approaches and methods.

Animal behaviour is the scientific study of the wild and wonderful ways in which animals interact with each other, with other living beings, and with the environment. It explores how animals relate to their physical environment as well as to other organisms, and includes topics such as how animals find and defend resources, avoid predators, choose mates, reproduce, and care for their young-ones.

What is behaviour?

Animal behaviour includes all the ways animals interact with other members of their species, with organisms of other species, and with their environment. Behaviour can also be defined more narrowly as a change in the activity of an organism in response to a stimulus, an external or internal cue or combination of cues. e.g., your dog might start drooling—a change in activity—in response to the sight of food—a stimulus.

Ethology is a field of basic biology, like ecology or genetics. It focuses on the behaviours of diverse organisms in their natural environment.

How to understand a behaviour?

Nikolaas (Niko) Tinbergen was a Dutch ornithologist, or bird biologist, who studied behaviour and is now considered one of the founders of the field of ethology. Based on his own research, Tinbergen proposed four basic questions helpful in understanding any animal behaviour. Let's look at these questions, using the production of song by the zebra finch—a common songbird—as an example.

1. **Causation—what causes the behaviour?**

What triggers the behaviour, and what body parts, functions, and molecules are involved in carrying it out?Example: Singing is triggered in zebra finches by social cues, such as the proximity of a potential mate, as well as the appropriate hormonal state. The ability to produce songs is influenced by male hormones and occurs mainly in male birds. Songs are produced when air flows from air sacs in the bronchi through an organ called the syrinx. Certain parts of the brain control song production and are well-developed in male zebra finches.

2. **Development—how does the behaviour develop?**

Is the behaviour present early in life? Does it change over the course of the organism's lifetime? What experiences are necessary for its development? Example: Young male zebra finches first listen to the songs of nearby males of their species, particularly their fathers. Then, they start to practice singing. By adulthood, male zebra finches have learned to produce their own songs, which are unique but often have similarities to those of their fathers. Once a finch has perfected its song, the song remains fixed for life (Fig 1).

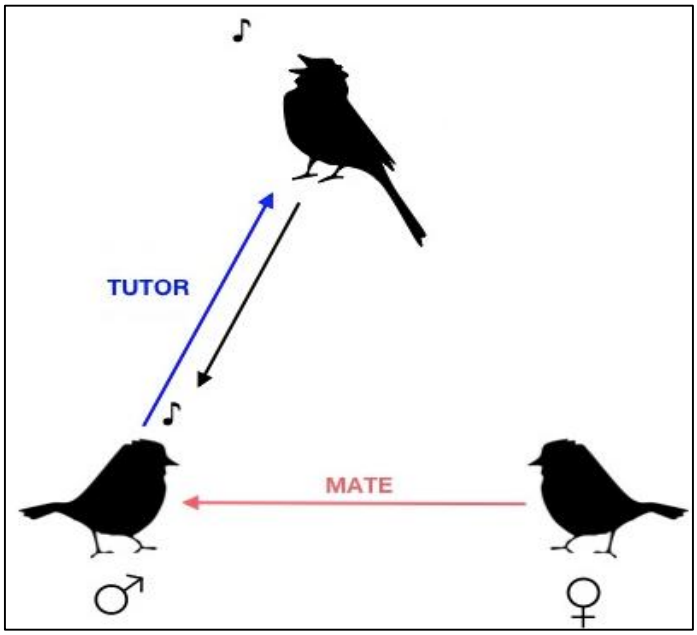

Fig 1: Learning behaviour of zebra finches

3. **Function/adaptive value—how does the behaviour affect fitness?**

How does the behaviour affect an organism's chances of survival and reproduction?

Example: Singing helps male zebra finches attract mates, increasing the chances that they will reproduce. Singing is part of an elaborate courtship ritual that entices the female to choose the male.

4. **Phylogeny—how did the behaviour evolve?**

How does the behaviour compare to those of related species? Why might it have evolved as it did?

Example: Almost all species of birds can make vocal sounds, but only those in the suborder Passeri are songbirds. Relative to the zebra finch, other songbird species differ in the timing of their listening and practicing phases, the plasticity of song over their lifetimes, the extent to which the song is similar among individuals of the species, and the way that singing is used—for example, for defence of territory vs. courtship of mates.

Innate vs. learned behaviours

When we are trying to understand how a behaviour develops and how it arose evolutionarily, one important question is whether the behaviour is genetically pre-programmed or acquired through experience.

Let's consider some vocabulary:

- Innate behaviours are genetically hardwired and are inherited by an organism from its parents.

- Learned behaviours are not inherited. They develop during an organism's lifetime as the result of experience and environmental influence.

Behavioural biologists have found that many behaviours have both an innate and a learned component. So, it's generally most accurate for us to ask to what extent a behaviour is innate or learned.

Mostly innate behaviours

There are some examples of behaviours that are really and truly hardwired. These behaviours take place in a highly predictable way in response to the right stimulus, even if the organism has never before encountered that stimulus. For example, an adult salamander will swim perfectly if it's placed in water, even if it never saw water when it was young and has never watched another salamander swim. In this case, the behaviour of swimming can only be explained as something genetically pre-programmed in the salamander. Similarly, you—or any

human—will rapidly jerk your hand away if you touch a very hot object. This response is a reflex that's hardwired in the circuits of your sensory and motor neurons and doesn't even involve your brain.

Partly innate, partly learned behaviours

In other cases, an organism is genetically programmed to develop a behaviour, but the form the behaviour takes depends on the individual's experience. One example is the learning of a song by a zebra finch or other songbird, as we saw above. All male zebra finches will begin listening to and learning song at about the same age and practicing and producing song at a slightly later age. Although this pattern is genetically determined, the exact features of the song a bird sings will depend on the songs it hears during its learning period. Another, more familiar example is language acquisition in humans. Babies are pre-programmed for language learning, but which language they learn depends on what they're exposed to during their plastic, or formative, period.

Mostly learned behaviours

In other cases, behaviours are largely dependent on experience—they're learned—and can't be fully explained by genetic pre-programming. For instance, if a rat receives a food reward each time it pushes a lever, it will quickly learn to push the lever in order to get the food. Similarly, if a cow gets an electric shock each time it brushes up against an electric fence, like the one below, it will rapidly learn to avoid the fence.Pushing a lever to get a reward and avoiding electric fences are not hardwired in rats and cows but are, instead, learned behaviours the animals develop through experience.

If a behaviour is learned rather than innate, it isn't directly inherited. But it does still depend on genes. For instance, not all types of animals could learn to push a lever to get a reward. The rat's capacity to learn this behaviour depends on how its brain is wired, and the construction, maintenance, and function of a rat brain are all determined by genes in the rat genome.

Natural selection shapes behaviour

To the extent that a behaviour is genetically determined or relies on genes, it’s subject to evolutionary forces, such as natural selection. In many cases, we can see how a behaviour gives a survival or reproduction benefit to an animal that performs it—in other words, the behaviour increases fitness.

What are natural selection and fitness?

Here are some examples of behaviours that clearly increase fitness:

- Baby birds of many species instinctively open their mouths for food when the mother returns to the nest. Birds with this heritable behaviour will tend to get fed more—and thus survive to adulthood more—than those that don't.

- Mother greylag geese (Fig 2) instinctively roll eggs back into the nest if they fall out. Geese with this heritable behaviour will tend to have more offspring that survive to hatch than geese without the behaviour.

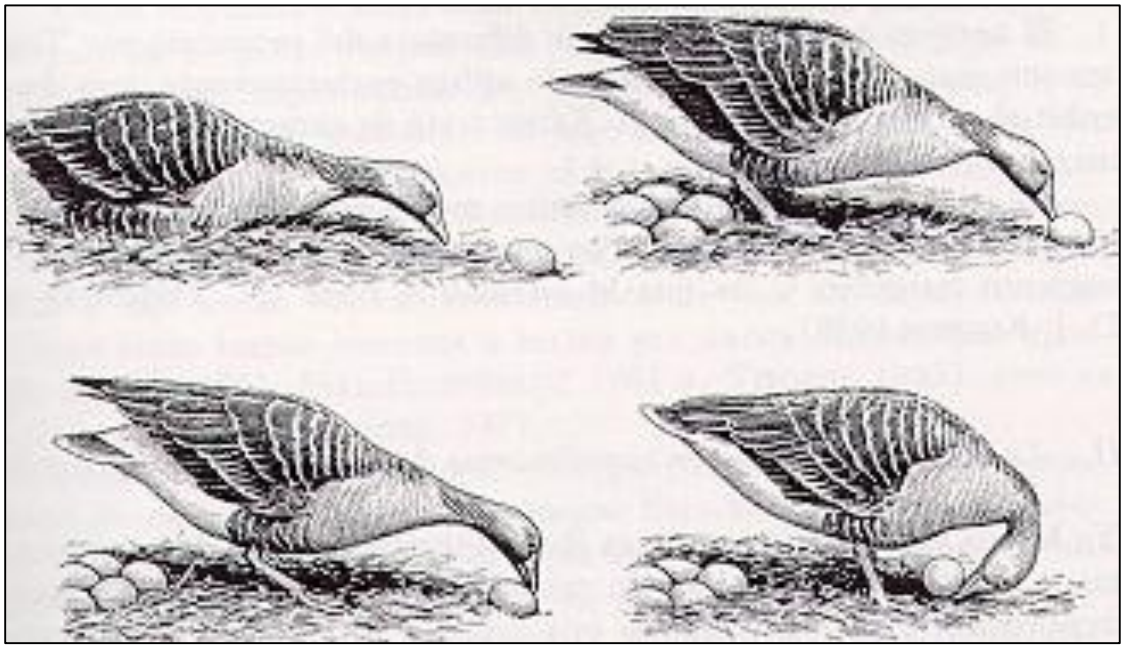

Fig 2: Greylag geese roll eggs back into the nest

- Zebra finch males learn songs while they are juveniles, young birds, and they use these songs in courtship rituals. Birds with the heritable tendency to learn a song will obtain a mate more often than those that don't.

An important point from the last example is that natural selection can act even when the behaviour itself is not inherited. A zebra finch doesn't inherit its song directly—it has to learn the song. But its capacity and tendency to learn a song are genetically determined, so they can be subject to natural selection.

Historical Perception in Animal Behaviour

During the course of history, interest in animal behaviour has stemmed from the fact that animals were a primary source of food, clothing, and materials for tools and shelter. Knowledge about their behaviour was, thus, necessary for successful hunting.

Moreover, there has always been human curiosity about the natural world. The historical perception, thus, relates from the early days of human existence to the present experimental and theoretical approaches.

A. Early Days of Human Existence:

Humans from early days were hunters and meat-eaters. The early hominids (Homo erectus) practiced crude variety of hunting. The Peking man (a form of Homo erectus), some 4, 00,000 years ago, was a better hunter. He knew the use of fire and made tools from animal bones. Early Homo sapiens were keen observers of animal behaviour and utilized this knowledge for hunting and evading predators. Hunters the cliff where they could be slaughtered with rocks or clubs. Cave paintings of prehistoric man suggest close observation of the animals at various times in their life cycles, from game to hunting, and, thus, depicting man's relationship with animals.

Later world:

After civilization, interest in animal behaviour increased due to curiosity about natural phenomenon and a desire to record and categories observations. Early scholars [Aristotle (384-322 B.C.), Pliny (23-79 A.D.) and others] attempted to record what they observed in the world around them.

However, their perception of animal behaviour was poor due to lack of full knowledge about what was taking place or due to biased religious or philosophical outlook. However, these formed the basis of later development in the field.

B. Emergence of Animal Behaviour as a Scientific Discipline:

Scientific study of animal behaviour emerged in the latter part of the nineteenth century.

Three major developments contributed significantly to the study of behaviour:

(a) Theory of evolution by natural selection,

(b) Development of a systematic comparative method, and

(c) Studies in genetics and inheritance.

(a) Darwin-Wallace theory of natural selection:

During the nineteenth century several voyages were made by Europeans to all parts of the globe for exploration and discovery of exotic fauna and flora. These scientists made observations and brought live and preserved specimens to zoos and laboratories in Europe, where scholars could observe, record their behaviour and interrelationships of these discovered species.

One such scientist was Charles Darwin (1809-1882) who made voyages to the Galapagos Islands. Darwin's thinking about the competition for survival among members of a species was influenced by Thomas Malthus' "Essay on the Principle of Population". Sir Charles Lyell (1797-1875), a geologist, made observations of rock strata and succession of fossils. This led others to believe that species were not fixed entities.

A. R. Wallace's (1823-1913) voyage to the Malay Archipelago and Darwin's travels on the Beagle to South America and South Pacific, led each man – independently – to formulate the theory of evolution by natural selection.

Behaviour, morphology and physiology of animals were thought to be subjected to the effects of natural selection. The theory of evolution by natural selection have been modified subsequently due to the recent developments in biology, particularly genetics.

(b) Systematic comparative method:

The use of comparative method in studying animal behaviour is credited to George John Romanes (1848-1894). The comparative method involves the studying of animals to get insights into the behaviour of humans.

Romanes proposed that there is a continuity of mental processes from one species to another. He put forward that although humans can know only their own thoughts, they could infer the mental process of animals, including other people, from knowledge of their own.

Romanes suggested that a sequence would be constructed for the evolution of various emotional states in animals — worms, who exhibit only surprise and fear, were placed at the lowest scale; insects were capable of various social feelings and curiosity; fishes showed play, jealousy and anger; reptiles exhibited affection; birds displayed pride and terror; and, finally, mammals were credited with hate, cruelty and shame. Romanes' theory relied largely on inferences rather than on recorded facts.

(c) Genetics and Inheritance:

Subsequently, the discovery of genetics and development of theories of inheritance by Gregor Mendel (1822-1884) greatly influenced research in animal behaviour. Present-day behavioural

biology is a combination of evolutionary theory (explains how traits change through time) and genetics (how traits are passed from generation to generation).

Any trait that evolves is passed on from generation to generation. Behaviour, thus, may change as a species evolves. Behaviour-genetic analysis from its early studies of inheritance got greatly expanded in the 1930s, and till modem times has been used as a powerful tool by many animal behaviourists.

C. Present Experimental and Theoretical Approaches:

The various theories, ideas etc. put forward in the latter half of the nineteenth century form the foundation of the present day's experimental approaches to the study of animal behaviour.

Four major approaches are given:

(a) Control mechanisms of behaviour has been sought through studies by comparative animal psychologists and physiologists. Much of the earlier psychological researches were heavily dependent on introspections and inferences. These methods have been later replaced by systematic, objective observations and replicable experiments.

Modern psychologists and physiologists explore areas such as learning processes, physiological control of behaviour, sensation and perception and behaviour genetics.

(b) The functional significance and evolution of behaviour patterns and explanations of behaviour mechanisms such as drives, innate releasing mechanisms etc. are the fields of classical ethologists. Behavioural traits are subject to natural selection.

Ethologists have traditionally made many of their research observations in a natural setting, conducted to assess the function of behaviour pattern. Ethological approach is used to determine how key stimuli trigger specific behaviour patterns. Modern ethology is concerned with four area of enquiry — causation, development, evolution, and function of behaviour.

(c) Environmental context for behaviour and the ways in which animals interact with their living and non-living environments are under the preview of behavioural ecologists. Investigations are conducted in both field and laboratory settings.

(d) The study of the social behaviour and organization in animals are the fields of socio-biologists. Sociobiology has emerged as a new approach to the study of animal behaviour. It applies principles of evolutionary biology to the study of social behaviour in animals.

These varied approaches to the study of behaviour have led to the modern synthetic view of animals living and behaving in their natural environment. Although these approaches have

been shown above as separate entities, they however, did not develop entirely independently of one another. In recent decades they have been moulded into a single discipline. The workers working in these fields may call themselves ethologists, animal behaviourists or comparative psychologists. They, however, are all pursuing the same goal using the same general theoretical frameworks and using similar experimental techniques and methods.

Aims and Objectives of Animal Behaviour:

Behaviour is recognized as one of the most important functions of animal life. The study of the behaviour of animals is the final objective of all other branches of biology. Some of the elementary actions of the animals — to avoid predators, to gather food, to reproduce etc., depend on an extraordinary complex and beautiful synchronized mechanism of nerve cells, glands and muscles, along with a supporting skeleton.

These structures are part of a complex mechanism that must combine properly through the inherited constitution of each individual. Ethology has, thus, made important contributions to other disciplines like anthropology, sociology, psychology, physiology, environmental biology, sociobiology etc. Animal behaviour has also applications to human behaviour, to neurosciences, to the study of animal welfare and to the educating of future generations.

1. Ethology and Environment:

Animal behaviour generally provides the first clue to environmental degradation. Changes in sexual behaviour affects the population size of animals allowing us to take measures to save the environment. Field studies of natural behaviour of animals are vital to provide baseline data for environmental monitoring. For example, Environmental Protection Agencies use changes in swimming behaviour of minnows as an index of possible pesticide pollution.

Animal behaviour studies have led to the understanding of insect reproduction and host plant protection, leading to the discovery of non-toxic pheromones for insect pest control. Thus, the need for toxic pesticides can be avoided. Knowledge of predator-prey relationship would lead to the maintenance of proper food web in a given ecosystem.

2. Foraging Behaviour and Habitat Preservation:

Understanding of foraging behaviour in animals has led to an understanding of forest regeneration. Many animals serve as seed dispersers resulting in propagation of plant species and are, therefore, a tool for habitat preservation. Knowledge of foraging behaviour of honey bees, when applied to mechanisms of pollination can be important for plant breeding and propagation.

3. Animal Behaviour and Conservation:

It is essential that we know about the natural behaviour (foraging, reproductive, migratory, home range etc.) of endangered species in order to develop protective measures for their conservation. Re-introduction of animals into their natural habitat, such as the Golden lion tamarin of Brazil, requires detailed knowledge about the behaviour of such species.

Reproductive behaviour studies have led to improved captive breeding methods of near-extinct species such as snow leopard, red panda, golden lion tamarin, whooping cranes etc., so as to save them from extinction. Animal behaviour research, both in captivity and in natural habitat, has become increasingly important. Many of the world's leading conservationists have a background in animal behaviour or behavioural ecology.

4. Animal Behaviour and Economic Implication:

Research on salmon migration has revealed a lot about the mechanisms of migration. This information has been valuable in preserving the salmon industry in the Pacific Northwest, which has led to the development of the salmon fishing industry in the Great Lakes, USA.

Thus, basic animal behaviour research has important economic implications. Such behavioural researches, if conducted on hilsa fish, can result in a boom of the dwindling hilsa fishing industry in India.

5. Animal Behaviour and Welfare of Animals:

Knowledge about the welfare of animals is possible only through the studies of its behaviour. The Society of Animal Welfare has placed increased emphasis on the welfare of domestic, pet and research animals. Cruelty on animals is punishable by law. It is the duty of an ethologist to look at the behaviour and well-being of animals in laboratory and field.

6. Animal Behaviour and Neuro-ethology:

Through close observation of animal behaviour, Sir Charles Sherrington (1954), a Nobel Prize winner, developed a model for the structure and function of the nervous system. This work of Sherrington has been amply supported through subsequent neurobiological research on humans.

Neuro-ethology is the science of animal behaviour and neurobiology combined together. It provides an important framework for explaining neural mechanisms which can benefit humans. Behavioural studies of poisonous and venomous animals, and the extraction of poison (neurotoxic chemicals) from such animals, is used to make various medicines which has undoubtedly benefited mankind.

7. Animal Behaviour and Science Education:

Courses on animal behaviour and behavioural ecology have been recently introduced in Indian universities. Still, the related departments like Anthropology, Zoology, Psychology and Wildlife have very little syllabus on Ethology. Students are still far away from wildlife and their behaviour.

It is disheartening that most students have very little knowledge about the commonly found birds and animals around their own residence, leave aside those found in National Parks, Sanctuaries and Reserves. It is, therefore, essential that schools, colleges and universities should give emphasis on the study of Wildlife Biology, Conservation, Management, Animal Behaviour and Animal Husbandry.

8. Ethology and Human Behaviour:

Many problems in human society are related with the interaction of environment and animal behaviour. Studies on animal behaviour have led to interpretation of the framework of human society and to understand the various society-related problems.

Many studies on child abuses and infanticides in humans, finds its bearing from observation of animal behaviour. Various ethologists document that human societies have gradually evolved from animal societies and that understanding the behaviour of animals has helped in unfolding the mysteries of our own social organizations.

For example:

1. Researches on chimpanzee and mon¬key has illustrated the importance of cooperation and reconciliation in social groups. This work provides new dimensions for understanding the aggressive behaviour of human beings. The behavioural studies of human being would have been much less today without the influence of animal research.

2. Work on social development in rhesus monkeys has been of major importance to theories of child development and to psychiatry.

3. The woolly spider monkey in Brazil displays no aggressive behaviour among group members. Studies on how this species of monkey avoids aggression can be implemented to minimise human aggression.

4. Male parental care studies on California mouse, marmosets, tamarins and others can give us insight of father's involvement in child care.

5. Researches on circadian rhythms in animals has led to research relevant to human factors such as jet-lag or changing from one shift to another in an industry.

6. It has been possible to detect stress and psychological disorders through research on animal behaviour.

7. Researches on chimpanzee using language analogues have led to new technology (computer keyboards using arbitrary symbols) that has been successfully applied to teaching languages to disabled or physically challenged humans.

Probable Questions:

1. Define ethology.
2. Describe the steps of understanding the behaviour.
3. What do you mean by innate and learned behaviour?
4. Differentiate between innate and learned behaviour.
5. What is natural selection? How natural selection shapes behaviour?
6. Discuss the Historical Perception in Animal Behaviour
7. Discuss the aims of animal behaviour.

Suggested Readings:

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2. Chattopadhyay, S. (2012). Life: Evolution, Adaptation, Ethology. 3rd Edn. Books and Allied, Kolkata.

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4. Mandal, F. (2010). A Text Book of Animal Behaviour. Pentice Hall India.

5. Mathur, R. (2005). Animal Behaviour. Rastogi Pub. Meerut.

6. Refinetti, R. (2000). Circadian Physiology. CRC Press, Boca Raton.

7. Ruhela, A. and Sinha, M. (2010). Recent Trends in Animal Behaviour. Oxford Book Co. Jaipur.

Unit-II

Learning and memory: Forms of learning and memory

Objective: In this unit you will know about the basic idea of learning and memory with basic forms of learning and memory.

Introduction

Memory is the faculty of the mind by which information is encoded, stored, and retrieved. Memory is vital to experiences and related to limbic systems, it is the retention of information over time for the purpose of influencing future action. If we could not remember past events, we could not learn or develop language, relationships, or personal identity.

Often memory is understood as an informational processing system with explicit and implicit functioning that is made up of a sensory processor, short-term (or working) memory, and long-term memory. This can be related to the neuron. The sensory processor allows information from the outside world to be sensed in the form of chemical and physical stimuli and attended to with various levels of focus and intent. Working memory serves as an encoding and retrieval processor. Information in the form of stimuli is encoded in accordance with explicit or implicit functions by the working memory processor. The working memory also retrieves information from previously stored material. Finally, the function of long-term memory is to store data through various categorical models or systems.

Explicit and implicit functions of memory are also known as declarative and non-declarative systems. These systems involve the purposeful intention of memory retrieval and storage, or lack thereof. Declarative, or explicit, memory is the conscious storage and recollection of data. Under declarative memory resides semantic and episodic memory. Semantic memory refers to memory that is encoded with specific meaning, while episodic memory refers to information that is encoded along a spatial and temporal plane. Declarative memory is usually the primary process thought of when referencing memory.

Non-declarative, or implicit, memory is the unconscious storage and recollection of information. An example of a non-declarative process would be the unconscious learning or retrieval of information by way of procedural memory, or a priming phenomenon. Priming is the process of subliminally arousing specific responses from memory and shows that not all memory is consciously activated, whereas procedural memory is the slow and gradual learning of skills that often occurs without conscious attention to learning.

Memory is not a perfect processor, and is affected by many factors. The manner information is encoded, stored, and retrieved can all be corrupted. The amount of attention given new stimuli can diminish the amount of information that becomes encoded for storage. Also, the storage process can become corrupted by physical damage to areas of the brain that are associated with memory storage, such as the hippocampus. Finally, the retrieval of information from long-term memory can be disrupted because of decay within long-term memory. Normal functioning, decay over time, and brain damage all affect the accuracy and capacity of memory. Memory loss is usually described as forgetfulness or amnesia.

Sensory memory

Sensory memory holds sensory information less than one second after an item is perceived. The ability to look at an item and remember what it looked like with just a split second of observation, or memorization, is the example of sensory memory. It is out of cognitive control and is an automatic response. With very short presentations, participants often report that they seem to "see" more than they can actually report. The first experiments exploring this form of sensory memory were precisely conducted by George Sperling (1963) using the "partial report paradigm". Subjects were presented with a grid of 12 letters, arranged into three rows of four. After a brief presentation, subjects were then played either a high, medium or low tone, cuing them which of the rows to report. Based on these partial report experiments, Sperling was able to show that the capacity of sensory memory was approximately 12 items, but that it degraded very quickly (within a few hundred milliseconds). Because this form of memory degrades so quickly, participants would see the display but be unable to report all of the items (12 in the "whole report" procedure) before they decayed. This type of memory cannot be prolonged via rehearsal.

Three types of sensory memories exist. Iconic memory is a fast-decaying store of visual information; a type of sensory memory that briefly stores an image which has been perceived for a small duration. Echoic memory is a fast-decaying store of auditory information, another type of sensory memory that briefly stores sounds that have been perceived for short durations. Haptic memory is a type of sensory memory that represents a database for touch stimuli.

- ## Short-term memory

Short-term memory is also known as working memory. Short-term memory allows recall for a period of several seconds to a minute without rehearsal. Its capacity is also very limited: George A. Miller (1956), when working at Bell Laboratories, conducted experiments showing that the store of short-term memory was 7±2 items (the title of his famous paper, "The magical number

7±2"). Modern estimates of the capacity of short-term memory are lower, typically of the order of 4–5 items; however, memory capacity can be increased through a process called chunking. For example, in recalling a ten-digit telephone number, a person could chunk the digits into three groups: first, the area code (such as 123), then a three-digit chunk (456) and lastly a four-digit chunk (7890). This method of remembering telephone numbers is far more effective than attempting to remember a string of 10 digits; this is because we are able to chunk the information into meaningful groups of numbers. This may be reflected in some countries in the tendency to display telephone numbers as several chunks of two to four numbers.

Short-term memory is believed to rely mostly on an acoustic code for storing information, and to a lesser extent a visual code. Conrad (1964) found that test subjects had more difficulty recalling collections of letters that were acoustically similar (e.g. E, P, D). Confusion with recalling acoustically similar letters rather than visually similar letters implies that the letters were encoded acoustically. Conrad's (1964) study, however, deals with the encoding of written text; thus, while memory of written language may rely on acoustic components, generalizations to all forms of memory cannot be made.

- **Long term memory**

The storage in sensory memory and short-term memory generally has a strictly limited capacity and duration, which means that information, is not retained indefinitely. By contrast, long-term memory can store much larger quantities of information for potentially unlimited duration (sometimes a whole life span). Its capacity is immeasurable. For example, given a random seven-digit number we may remember it for only a few seconds before forgetting, suggesting it was stored in our short-term memory. On the other hand, we can remember telephone numbers for many years through repetition; this information is said to be stored in long-term memory.

While short-term memory encodes information acoustically, long-term memory encodes it semantically: Baddeley (1966) discovered that, after 20 minutes, test subjects had the most difficulty recalling a collection of words that had similar meanings (e.g. big, large, great, huge) long-term. Another part of long-term memory is episodic memory, "which attempts to capture information such as 'what', 'when' and 'where'". With episodic memory, individuals are able to recall specific events such as birthday parties and weddings.

Short-term memory is supported by transient patterns of neuronal communication, dependent on regions of the frontal lobe (especially dorsolateral prefrontal cortex) and the parietal lobe. Long-term memory, on the other hand, is maintained by more stable and permanent changes in neural connections widely spread throughout the brain. The hippocampus is essential (for learning new information) to the consolidation of information from short-term to long-term

memory, although it does not seem to store information itself. It was thought that without the hippocampus new memories were unable to be stored into long-term memory and that there would be a very short attention span, as first gleaned from patient Henry Molaison after what was thought to be the full removal of both his hippocampi. More recent examination of his brain, post-mortem, shows that the hippocampus was more intact than first thought, throwing theories drawn from the initial data into question. The hippocampus may be involved in changing neural connections for a period of three months or more after the initial learning.

Research has suggested that long-term memory storage in humans may be maintained by DNA methylation, and the ‘prion' gene.

- **Learning:**

Learning is the process of acquiring new, or modifying existing, knowledge, behaviors, skills, values, or preferences. The ability to learn is possessed by humans, animals, and some machines; there is also evidence for some kind of learning in some plants. Some learning is immediate, induced by a single event (e.g. being burned by a hot stove), but much skill and knowledge accumulates from repeated experiences. The changes induced by learning often last a lifetime, and it is hard to distinguish learned material that seems to be "lost" from that which cannot be retrieved.

Human learning begins before birth and continues until death as a consequence of ongoing interactions between person and environment. The nature and processes involved in learning are studied in many fields, including educational psychology, neuropsychology, experimental, and pedagogy. Research in such fields has led to the identification of various sorts of learning. For example, learning may occur as a result of habituation, or classical conditioning, operant conditioning or as a result of more complex activities such as play, seen only in relatively intelligent animals. Learning may occur consciously or without conscious awareness. Learning that an aversive event can't be avoided nor escaped may result in a condition called learned helplessness. There is evidence for human behavioral learning prenatally, in which habituation has been observed as early as 32 weeks into gestation, indicating that the central nervous system is sufficiently developed and primed for learning and memory to occur very early on in development.

Play has been approached by several theorists as the first form of learning. Children experiment with the world, learn the rules, and learn to interact through play. Lev Vygotsky agrees that play is pivotal for children's development, since they make meaning of their environment through playing educational games.

Types

1. Non-associative learning

Non-associative learning refers to "a relatively permanent change in the strength of response to a single stimulus due to repeated exposure to that stimulus. Changes due to such factors as sensory adaptation, fatigue, or injury do not qualify as non-associative learning." Non-associative learning can be divided into habituation and sensitization.

a. Habituation

Habituation is an example of non-associative learning in which the strength or probability of a response diminishes when the stimulus is repeated. The response is typically a reflex or unconditioned response. Thus, habituation must be distinguished from extinction, which is an associative process. In operant extinction, for example, a response declines because it is no longer followed by reward. An example of habituation can be seen in small song birds—if a stuffed owl (or similar predator) is put into the cage, the birds initially react to it as though it were a real predator. Soon the birds react less, showing habituation. If another stuffed owl is introduced (or the same one removed and re-introduced), the birds react to it again as though it were a predator, demonstrating that it is only a very specific stimulus that is habituated to (namely, one particular unmoving owl in one place). Habituation has been shown in essentially every species of animal, as well as the sensitive plant *Mimosa pudica* and the large protozoan *Stentor coeruleus*.

b. Sensitization

Sensitization is an example of non-associative learning in which the progressive amplification of a response follows repeated administrations of a stimulus. An everyday example of this mechanism is the repeated tonic stimulation of peripheral nerves that occurs if a person rubs their arm continuously. After a while, this stimulation creates a warm sensation that eventually turns painful. The pain results from the progressively amplified synaptic response of the peripheral nerves warning that the stimulation is harmful. Sensitization is thought to underlie both adaptive as well as maladaptive learning processes in the organism.

2. Active learning

Experiential learning is more efficient than passive learning like reading or listening. Active learning occurs when a person takes control of his/her learning experience. Since understanding information is the key aspect of learning, it is important for learners to recognize what they understand and what they do not. By doing so, they can monitor their own mastery of subjects. Active learning encourages learners to have an internal dialogue in which they verbalize understandings. This and other meta-cognitive strategies can be taught to a child over time. Studies within metacognition have proven the value in active learning, claiming that

the learning is usually at a stronger level as a result. In addition, learners have more incentive to learn when they have control over not only how they learn but also what they learn. Active learning is a key characteristic of student-centred learning. Conversely, passive learning and direct instruction are characteristics of teacher-centred learning (or traditional education).

3. Associative learning

Associative learning is the process by which a person or animal learns an association between two stimuli. In classical conditioning a previously neutral stimulus is repeatedly paired with a reflex eliciting stimulus until eventually the neutral stimulus elicits a response on its own. In operant conditioning, a behavior that is reinforced or punished in the presence of a stimulus becomes more or less likely to occur in the presence of that stimulus.

4. Operant conditioning

In operant conditioning, a reinforcement (by reward) or instead a punishment given after a given behavior, change the frequency and/or form of that behavior. Stimulus present when the behavior/consequence occurs comes to control these behavior modifications.

5. Classical conditioning

The typical paradigm for classical conditioning involves repeatedly pairing an unconditioned stimulus (which unfailingly evokes a reflexive response) with another previously neutral stimulus (which does not normally evoke the response). Following conditioning, the response occurs both to the unconditioned stimulus and to the other, unrelated stimulus (now referred to as the "conditioned stimulus"). The response to the conditioned stimulus is termed a conditioned response. The classic example is Ivan Pavlov and his dogs. Pavlov fed his dogs meat powder, which naturally made the dogs salivate—salivating is a reflexive response to the meat powder. Meat powder is the unconditioned stimulus (US) and the salivation is the unconditioned response (UR). Pavlov rang a bell before presenting the meat powder. The first time Pavlov rang the bell, the neutral stimulus, the dogs did not salivate, but once he put the meat powder in their mouths they began to salivate. After numerous pairings of bell and food, the dogs learned that the bell signaled that food was about to come, and began to salivate when they heard the bell. Once this occurred, the bell became the conditioned stimulus (CS) and the salivation to the bell became the conditioned response (CR). Classical conditioning has been demonstrated in many species. For example, it is seen in honeybees, in the proboscis extension reflex paradigm. And recently, it was demonstrated in garden pea plants.

Another influential person in the world of classical conditioning is John B. Watson. Watson's work was very influential and paved the way for B.F. Skinner's radical behaviorism. Watson's behaviorism (and philosophy of science) stood in direct contrast to Freud and other accounts based largely on introspection. Watson's view was that the introspective method was too

subjective, and that we should limit the study of human development to directly observable behaviors. In 1913, Watson published the article "Psychology as the Behaviorist Views," in which he argued that laboratory studies should serve psychology best as a science. Watson's most famous, and controversial, experiment, "Little Albert", where he demonstrated how psychologists can account for the learning of emotion through classical conditioning principles.

6. Observational Learning

Observational learning is learning that occurs through observing the behavior of others. It is a form of social learning which takes various forms, based on various processes. In humans, this form of learning seems to not need reinforcement to occur, but instead, requires a social model such as a parent, sibling, friend, or teacher with surroundings.

Imprinting

Imprinting is a kind of learning occurring at a particular life stage that is rapid and apparently independent of the consequences of behavior. In filial imprinting, young animals, particularly birds, form an association with another individual or in some cases, an object that they respond to as they would to a parent. In 1935, the Austrian Zoologist Konrad Lorenz discovered that certain birds follow and form a bond if the object makes sounds.

Play

Play generally describes behavior with no particular end in itself, but that improves performance in similar future situations. This is seen in a wide variety of vertebrates besides humans, but is mostly limited to mammals and birds. Cats are known to play with a ball of string when young, which gives them experience with catching prey. Besides inanimate objects, animals may play with other members of their own species or other animals, such as orcas playing with seals they have caught. Play involves a significant cost to animals, such as increased vulnerability to predators and the risk of injury and possibly infection. It also consumes energy, so there must be significant benefits associated with play for it to have evolved. Play is generally seen in younger animals, suggesting a link with learning. However, it may also have other benefits not associated directly with learning, for example improving physical fitness.

Play, as it pertains to humans as a form of learning is central to a child's learning and development. Through play, children learn social skills such as sharing and collaboration. Children develop emotional skills such as learning to deal with the emotion of anger, through play activities. As a form of learning, play also facilitates the development of thinking and language skills in children.

There are five types of play:

1. sensorimotor play aka functional play, characterized by repetition of activity
2. role play occurs starting at the age of 3
3. rule-based play where authoritative prescribed codes of conduct are primary
4. construction play involves experimentation and building
5. movement play aka physical play

These five types of play are often intersecting. All types of play generate thinking and problem-solving skills in children. Children learn to think creatively when they learn through play. Specific activities involved in each type of play change over time as humans progress through the lifespan. Play as a form of learning, can occur solitarily, or involve interacting with others.

Enculturation

Enculturation is the process by which people learn values and behaviors that are appropriate or necessary in their surrounding culture. Parents, other adults, and peers shape the individual's understanding of these values. If successful, enculturation results in competence in the language, values and rituals of the culture. This is different from acculturation, where a person adopts the values and societal rules of a culture different from their native one.

Multiple examples of enculturation can be found cross-culturally. Collaborative practices in the Mazahua people have shown that participation in everyday interaction and later learning activities contributed to enculturation rooted in nonverbal social experience. As the children participated in everyday activities, they learned the cultural significance of these interactions. The collaborative and helpful behaviors exhibited by Mexican and Mexican-heritage children is a cultural practice known as being "acomedido". Chillihuani girls in Peru described themselves as weaving constantly, following behavior shown by the other adults.

- **Episodic learning**

Episodic learning is a change in behavior that occurs as a result of an event. For example, a fear of dogs that follows being bitten by a dog is episodic learning. Episodic learning is so named because events are recorded into episodic memory, which is one of the three forms of explicit learning and retrieval, along with perceptual memory and semantic memory.

- **Multimedia learning**

Multimedia learning is where a person uses both auditory and visual stimuli to learn information. This type of learning relies on dual-coding theory.

- **E-learning and augmented learning**

Electronic learning or e-learning is computer-enhanced learning. A specific and always more diffused e-learning is mobile learning (m-learning), which uses different mobile telecommunication equipment, such as cellular phones.

When a learner interacts with the e-learning environment, it's called augmented learning. By adapting to the needs of individuals, the context-driven instruction can be dynamically tailored to the learner's natural environment. Augmented digital content may include text, images, video, audio (music and voice). By personalizing instruction, augmented learning has been shown to improve learning performance for a lifetime. See also minimally invasive education.

Moore (1989) reported that three core types of interaction are necessary for quality, effective online learning:

→ Learner–learner (i.e. communication between and among peers with or without the teacher present),

→ Learner–instructor (i.e. student teacher communication), and

→ Learner–content (i.e. intellectually interacting with content that results in changes in learners' understanding, perceptions, and cognitive structures).

In his theory of transactional distance, Moore (1993) contented that structure and interaction or dialogue bridge the gap in understanding and communication that is created by geographical distances (known as transactional distance).

- **Rote learning**

Rote learning is memorizing information so that it can be recalled by the learner exactly the way it was read or heard. The major technique used for rote learning is learning by repetition, based on the idea that a learner can recall the material exactly (but not its meaning) if the information is repeatedly processed. Rote learning is used in diverse areas, from mathematics to music to religion. Although it has been criticized by some educators, rote learning is a necessary precursor to meaningful learning.

- **Meaningful learning**

Meaningful learning is the concept that learned knowledge (e.g., a fact) is fully understood to the extent that it relates to another knowledge. To this end, meaningful learning contrasts with rote learning in which information is acquired without regard to understanding. Meaningful learning, on the other hand, implies there is a comprehensive knowledge of the context of the facts learned.

- **Informal learning**

Informal learning occurs through the experience of day-to-day situations (for example, one would learn to look ahead while walking because of the danger inherent in not paying attention to where one is going). It is learning from life, during a meal at table with parents, play, exploring, etc.

- **Formal learning**

Formal learning is learning that takes place within a teacher-student relationship, such as in a school system. The term formal learning has nothing to do with the formality of the learning, but rather the way it is directed and organized. In formal learning, the learning or training departments set out the goals and objectives of the learning.

- **Non-formula learning**

Non-formal learning is organized learning outside the formal learning system. For example, learning by coming together with people with similar interests and exchanging viewpoints, in clubs or in (international) youth organizations, workshops.

Non-formal learning and combined approaches

The educational system may use a combination of formal, informal, and non-formal learning methods. The UN and EU recognize these different forms of learning. In some schools, students can get points that count in the formal-learning systems if they get work done in informal-learning circuits. They may be given time to assist international youth workshops and training courses, on the condition they prepare, contribute, share and can prove this offered valuable new insight, helped to acquire new skills, a place to get experience in organizing, teaching, etc. To learn a skill, such as solving a Rubik's Cube quickly, several factors come into play at once:

- ✓ Reading directions helps a player learn the patterns that solve the Rubik's Cube.
- ✓ Practicing the moves repeatedly helps build "muscle memory" and speed.
- ✓ Thinking critically about moves helps find shortcuts, which speeds future attempts.
- ✓ Observing the Rubik's Cube's six colours help anchor solutions in the mind.
- ✓ Revisiting the cube occasionally helps retain the skill.

- **Tangential learning**

Tangential learning is the process by which people self-educate if a topic is exposed to them in a context that they already enjoy. For example, after playing a music-based video game, some people may be motivated to learn how to play a real instrument, or after watching a TV show that references Faust and Lovecraft, some people may be inspired to read the original

work. Self-education can be improved with systematization. According to experts in natural learning, self-oriented learning training has proven an effective tool for assisting independent learners with the natural phases of learning.

Extra Credits writer and game designer James Portnow was the first to suggest games as a potential venue for "tangential learning". Mozelius et al. points out that intrinsic integration of learning content seems to be a crucial design factor, and that games that include modules for further self-studies tend to present good results. The built-in encyclopedias in the civilization games are presented as an example - by using these modules gamers can dig deeper for knowledge about historical events in the gameplay. The importance of rules that regulate learning modules and game experience is discussed by Moreno, C., in a case study about the mobile game. In this game, developed by Landka in collaboration with ESA and ESO, game progress is rewarded with educational content, as opposed to traditional education where learning activities are rewarded with gameplay.

- **Dialogic learning**

Dialogic learning is a type of learning based on dialogue.

- **Incidental learning**

In incidental teaching learning is not planned by the instructor or the student, it occurs as a byproduct of another activity — an experience, observation, self-reflection, interaction, unique event, or common routine task. This learning happens in addition to or apart from the instructor's plans and the student's expectations. An example of incidental teaching is when the instructor places a train set on top of a cabinet. If the child points or walks towards the cabinet, the instructor prompts the student to say “train.” Once the student says “train,” he gets access to the train set.

Here are some steps most commonly used in incidental teaching:

- ✓ An instructor will arrange the learning environment so that necessary materials are within the student’s sight, but not within his reach, thus impacting his motivation to seek out those materials.
- ✓ An instructor waits for the student to initiate engagement.
- ✓ An instructor prompts the student to respond if needed.
- ✓ An instructor allows access to an item/activity contingent on a correct response from the student.
- ✓ The instructor fades out the prompting process over a period of time and subsequent trials.

Incidental learning is an occurrence that is not generally accounted for using the traditional methods of instructional objectives and outcomes assessment. This type of learning occurs in part as a product of social interaction and active involvement in both online and onsite courses. Research implies that some un-assessed aspects of onsite and online learning challenge the equivalency of education between the two modalities. Both onsite and online learning have distinct advantages with traditional on-campus students experiencing higher degrees of incidental learning in three times as many areas as online students. Additional research is called for to investigate the implications of these findings both conceptually and pedagogically.

- **Machine learning**

Machine learning, a branch of artificial intelligence, concerns the construction and study of systems that can learn from data. For example, a machine learning system could be trained on email messages to learn to distinguish between spam and non-spam messages.

Domains

Benjamin Bloom has suggested three domains of learning:

→ *Cognitive*: To recall, calculate, discuss, analyse, problem solve, etc.

→ *Psychomotor*: To dance, swim, ski, dive, drive a car, ride a bike, etc.

→ *Affective*: To like something or someone, love, appreciate, fear, hate, worship, etc.

These domains are not mutually exclusive. For example, in learning to play chess, the person must learn the rules (cognitive domain)—but must also learn how to set up the chess pieces and how to properly hold and move a chess piece (psychomotor). Furthermore, later in the game the person may even learn to love the game itself, value its applications in life, and appreciate its history (affective domain).

Transfer

Transfer of learning is the application of skill, knowledge or understanding to resolve a novel problem or situation that happens when certain conditions are fulfilled. Research indicates that learning transfer is infrequent; most common when "... cued, primed, and guided..." and has sought to clarify what it is, and how it might be promoted through instruction.

Over the history of its discourse, various hypotheses and definitions have been advanced. First, it is speculated that different types of transfer exist, including: near transfer, the application of skill to solve a novel problem in a similar context; and far transfer, the application of skill to

solve novel problem presented in a different context. Furthermore, Perkins and Salomon (1992) suggest that positive transfer in cases when learning supports novel problem solving, and negative transfer occurs when prior learning inhibits performance on highly correlated tasks, such as second or third-language learning. Concepts of positive and negative transfer have a long history; researchers in the early 20th century described the possibility that "...habits or mental acts developed by a particular kind of training may inhibit rather than facilitate other mental activities". Finally, Schwarz, Bransford and Sears (2005) have proposed that transferring knowledge into a situation may differ from transferring knowledge out to a situation as a means to reconcile findings that transfer may both be frequent and challenging to promote.

A significant and long research history has also attempted to explicate the conditions under which transfer of learning might occur. Early research by Ruger, for example, found that the "level of attention", "attitudes", "method of attack" (or method for tackling a problem), a "search for new points of view", "a careful testing of hypothesis" and "generalization" were all valuable approaches for promoting transfer. To encourage transfer through teaching, Perkins and Salomon recommend aligning ("hugging") instruction with practice and assessment, and "bridging", or encouraging learners to reflect on past experiences or make connections between prior knowledge and current content.

Factors affecting learning

External factors

1. Heredity: A classroom instructor can neither change nor increase heredity, but the student can use and develop it. Some learners are rich in hereditary endowment while others are poor. Each student is unique and has different abilities. The native intelligence is different in individuals. Heredity governs or conditions our ability to learn and the rate of learning. The intelligent learners can establish and see relationship very easily and more quickly.

2. Status of students: Physical and home conditions also matter: Certain problems like malnutrition i.e.; inadequate supply of nutrients to the body, fatigue i.e.; tiredness, bodily weakness, and bad health are great obstructers in learning. These are some of the physical conditions by which a student can get affected. Home is a place where a family lives. If the home conditions are not proper, the student is affected seriously. Some of the home conditions are bad ventilation, unhygienic living, bad light, etc. These affect the student and his or her rate of learning.

3. Physical environment: The design, quality, and setting of a learning space, such as a school or classroom, can each be critical to the success of a learning environment. Size, configuration, comfort—fresh air, temperature, light, acoustics, furniture—can all affect a student's learning. The tools used by both instructors and students directly affect how information is conveyed, from display and writing surfaces (blackboards, markerboards, tack surfaces) to digital technologies. For example, if a room is too crowded, stress levels rise, student attention is reduced, and furniture arrangement is restricted. If furniture is incorrectly arranged, sight lines to the instructor or instructional material is limited and the ability to suit the learning or lesson style is restricted. Aesthetics can also play a role, for if student morale suffers, so does motivation to attend school.

- ## Internal factors

There are several internal factors that affect learning. They are

***1. Goals or purposes*:** Each and everyone has a goal. A goal should be set to each pupil according to the standard expected to him. A goal is an aim or desired result. There are 2 types of goals called immediate and distant goals. A goal that occurs or is done at once is called an immediate goal, and distant goals are those that take time to achieve. Immediate goals should be set before the young learner and distant goals for older learners. Goals should be specific and clear, so that learners understand.

2. Motivational behaviour: Motivation means to provide with a motive. Motivation learners should be motivated so that they stimulate themselves with interest. This behaviour arouses and regulates the student's internal energies.

3. Interest: This is a quality that arouses a feeling. It encourages a student to move over tasks further. During teaching, the instructor must raise interests among students for the best learning. Interest is an apparent (clearly seen or understood) behaviour.

4. Attention: Attention means consideration. It is concentration or focusing of consciousness upon one object or an idea. If effective learning should take place attention is essential. Instructors must secure the attention of the student.

5. Drill or practice: This method includes repeating the tasks "n" number of times like needs, phrases, principles, etc. This makes learning more effective.

6. Fatigue: Generally, there are three types of fatigue, i.e., muscular, sensory, and mental. Muscular and sensory fatigues are bodily fatigue. Mental fatigue is in the central nervous system. The remedy is to change teaching methods, e.g., use audio-visual aids, etc.

7. Aptitude: Aptitude is natural ability. It is a condition in which an individual’s ability to acquire certain skills, knowledge through training.

8. Attitude: It is a way of thinking. The attitude of the student must be tested to find out how much inclination he or she has for learning a subject or topic.

9. Emotional conditions: Emotions are physiological states of being. Students who answer a question properly or give good results should be praised. This encouragement increases their ability and helps them produce better results. Certain attitudes, such as always finding fault in a student's answer or provoking or embarrassing the student in front of a class are counterproductive.

***10. Speed, Accuracy and retention*:** Speed is the rapidity of movement. Retention is the act of retaining. These 3 elements depend upon aptitude, attitude, interest, attention and motivation of the students.

11. Learning activities: Learning depends upon the activities and experiences provided by the teacher, his concept of discipline, methods of teaching and above all his overall personality.

12. Testing: Various tests measure individual learner differences at the heart of effective learning. Testing helps eliminate subjective elements of measuring pupil differences and performances.

***13. Guidance*:** Everyone needs guidance in some part or some time in life. Some need it constantly and some very rarely depending on the students' conditions. Small learners need more guidance. Guidance is an advice to solve a problem. Guidance involves the art of helping boys and girls in various aspects of academics, improving vocational aspects like choosing careers and recreational aspects like choosing hobbies. Guidance covers the whole gamut of learners' problems- learning as well as non- learning.

In animal evolution

Animals gain knowledge in two ways. First is learning—in which an animal gathers information about its environment and uses this information. For example, if an animal eats something that hurts its stomach, it learns not to eat that again. The second is innate knowledge that is genetically inherited.

An example of this is when a horse is born and can immediately walk. The horse has not learned this behavior; it simply knows how to do it. In some scenarios, innate knowledge is more beneficial than learned knowledge. However, in other scenarios the opposite is true—animals must learn certain behaviors when it is disadvantageous to have a specific innate behavior. In these situations, learning evolves in the species.

Costs and benefits of learned and innate knowledge

In a changing environment, an animal must constantly gain new information to survive. However, in a stable environment, this same individual needs to gather the information it needs once, and then rely on it for the rest of its life. Therefore, different scenarios better suit either learning or innate knowledge. Essentially, the cost of obtaining certain knowledge versus the benefit of already having it determines whether an animal evolved to learn in a given situation, or whether it innately knew the information. If the cost of gaining the knowledge outweighs the benefit of having it, then the animal does not evolve to learn in this scenario—but instead, non-learning evolves. However, if the benefit of having certain information outweighs the cost of obtaining it, then the animal is far more likely to evolve to have to learn this information.

Non-learning is more likely to evolve in two scenarios. If an environment is static and change does not or rarely occur, then learning is simply unnecessary. Because there is no need for learning in this scenario—and because learning could prove disadvantageous due to the time it took to learn the information—non-learning evolves. However, if an environment is in a constant state of change, then learning is disadvantageous. Anything learned is immediately irrelevant because of the changing environment. The learned information no longer applies. Essentially, the animal would be just as successful if it took a guess as if it learned. In this situation, non-learning evolves. In fact, a study of *Drosophila melanogaster* showed that learning can actually lead to a decrease in productivity, possibly because egg-laying behaviors and decisions were impaired by interference from the memories gained from the new learned materials or because of the cost of energy in learning.

However, in environments where change occurs within an animal's lifetime but is not constant, learning is more likely to evolve. Learning is beneficial in these scenarios because an animal can adapt to the new situation, but can still apply the knowledge that it learns for a somewhat extended period of time. Therefore, learning increases the chances of success as opposed to guessing. An example of this is seen in aquatic environments with landscapes subject to change. In these environments, learning is favored because the fish are predisposed to learn the specific spatial cues where they live.

Probable questions:

1. Discuss the basic idea about memory.
2. Discuss short term memory.
3. What do you mean by long term memory?
4. What do you mean by learning?
5. Discuss different types of learning.
6. Discuss different types of play.
7. What is formal and non-formal learning?
8. Name the factors affecting learning/ Give a detailed discussion about the factors.

Suggested Readings:

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Unit III

Learning and memory: learning and habitat selection - migration, navigation and orientation

Objective: In this unit you will know about the basic idea of learning and habitat selection - migration, navigation and orientation

Habitat

In ecology, a habitat is the type of natural environment in which a particular species of organism lives. A species' habitat is those places where the species can find food, shelter, protection and mates for reproduction. It is characterized by both physical and biological features.

The physical factors may include (for example): soil, moisture, range of temperature, and light intensity. Biotic factors will include the availability of food and the presence or absence of predators. Every organism has certain habitat needs for the conditions in which it will thrive, but some are tolerant of wide variations while others are very specific in their requirements. A habitat is not necessarily a geographical area, it can be the interior of a stem, a rotten log, a rock or a clump of moss; for a parasitic organism has as its habitat the body of its host, part of the host's body (such as the digestive tract), or a single cell within the host's body.

Geographic habitat types include polar, temperate, subtropical and tropical. The terrestrial vegetation type maybe forest, steppe and grassland, semi-arid or desert. Freshwater habitats include marshes, streams, rivers, lakes, and ponds; marine habitats include salt marshes, the coast, the intertidal zone, estuaries, reefs, bays, the open sea, the sea bed, deep water and submarine vents.

Habitats may change over time. Causes of change may include a violent event (such as the eruption of a volcano, an earthquake, a tsunami, a wildfire or a change in oceanic currents); or change may occur more gradually over millennia with alterations in the climate, as ice sheets and glaciers advance and retreat, and as different weather patterns bring changes of precipitation and solar radiation. Other changes come as a direct result of human activities, such as deforestation, the ploughing of ancient grasslands, the diversion and damming of rivers, the draining of marshland and the dredging of the seabed. The introduction of alien species can have a devastating effect on native wildlife, through increased predation, through

competition for resources or through the introduction of pests and diseases to which the indigenous species have no immunity.

Habitat selection

Habitat selection is a hierarchical process involving a series of innate and learned behavioural decisions made by an animal about what habitat it would use at different scales of the environment Studies of habitat selection by deer mice (*Peromyscus maniculatus*) revealed that heredity and experience play a role in determining selection. Habitat selection was generated by foraging decisions. However, foraging is only one behaviour driving habitat selection.

Habitat may be selected for cover availability, forage quality and quantity, and resting or denning sites. Proximate factors serve as cues an animal uses to determine the suitability of a site including the specific vegetation composition within a desired habitat. Reproductive success and survival of the species are the ultimate reasons that influence a species to select a habitat. The ability to persist is governed by ultimate factors such as forage availability, shelter, and avoiding predators. Several interacting factors have an influence on habitat selection for an individual (e.g., competition, cover, and predation).

Competition is involved because each individual is involved in intraspecific and interspecific relationships that partition the available resources within an environment. Competition may result in a species failing to select a habitat suitable in all other resources or may determine spatial distribution within the habitat (Keen 1982). Predation also complicates selection of habitat. The existence of predators may prevent an individual from occupying an area. Survival of the species and its future reproductive success are the driving forces that presumably cause an individual to evaluate these biotic factors. With a high occurrence of competition and predators, an individual may choose a different site with less optimal resources. Once predators are removed, areas with necessary resources can then be inhabited. Habitat selection is therefore an active behavioural process by an animal. Each species searches for features within an environment that are directly or indirectly associated with the resources that an animal would need to reproduce, survive, and persist. Habitat selection is a compilation of innate and learned behaviours that lie on a continuum of closed to open (i.e., learning) genetic programs. A genetic program gives an individual preadaptation to behave in a certain manner. Therefore, preadaptation to certain environmental cues plays an important role in habitat selection, but the potential for learning may exist in some species.

Habitat preference

Habitat preference is the consequence of habitat selection, resulting in the disproportional use of some resources over others. Habitat preferences are most strikingly observed when animals spend a high proportion of time in habitats that are not very abundant on the landscape.

Habitat availability Habitat availability is the accessibility and pourability of physical and biological components of a habitat by animals. Availability is in contrast to the abundance of resources, which refers only to their quantity in the habitat, irrespective of the organisms present. For example, the abundance of a prey species for a particular predator could be measured, yet not all of the prey in the habitat is available to the predator because there may be factors, (e.g., ample cover) that restrict their accessibility. Vegetation beyond the reach of an animal is not available as forage, even though the vegetation may be preferred. Measuring actual resource availability is important to understand wildlife habitat, but in practice it is seldom measured because of the difficulty of determining what is and what is not available. Consequently, quantification of availability usually consists of a priori or a posteriori measure of the abundance of resources in an area used by an animal, rather than true availability.

Habitat Is Species Specific

When I hear someone state "This is great wildlife habitat", it is like walking into a brick wall and I can only guess what they mean. All the components necessary for reproduction and survival are not the same for all species and "great wildlife habitat" for one species may not even come close to serving as appropriate habitat for others. This has and will continue to be a problem because manipulations of the landscape will favour the habitats of some species but be detrimental to the habitats of others. A lot of effort has been placed on ecosystem management in the 1990s, but when considering specific organisms, the manager needs to consider their unique array of requirements for survival. With a knowledge of habitat requirements for the species of interest, the manager can make informed decisions as to how landscape alterations will influence plant and animal communities.

Migration, navigation and orientation

Birds are not perfectly adapted to all environments and sometimes a bird has to leave – *migration*. In its purest sense, migration refers to seasonal movements between a location where an individual or population breeds and a location where it survives during the non-breeding period. Long-distance migrants are those that have a complete shift between the breeding and wintering areas, such as the Blackpoll Warbler that breeds in Canada; short-distance migrants, such as the Pine Warbler, make shorter trips, such as up and down the side of a mountain. Partial migrants are those species in which some individuals of a species leave and others stay, such as American Robins. Average distances covered are about 1000-3000 km each way, but 4000-6000 km is not uncommon.

A migratory journey is usually broken into a series of short flights. Most songbirds migrate at night, singly or in loose flocks, and my cover 300-600km per night but they don't fly every night and a 3000 km journey may take 3-4 weeks.

Birds might also have more than two migratory destinations. Anna's Hummingbird, for example, breeds in the coastal chaparral and valley of California, summers in the high mountains, and winters in the deserts of Arizona and Mexico. Birds tend to migrate along four major routes in North America called flyways, depicted below (Fig 1).

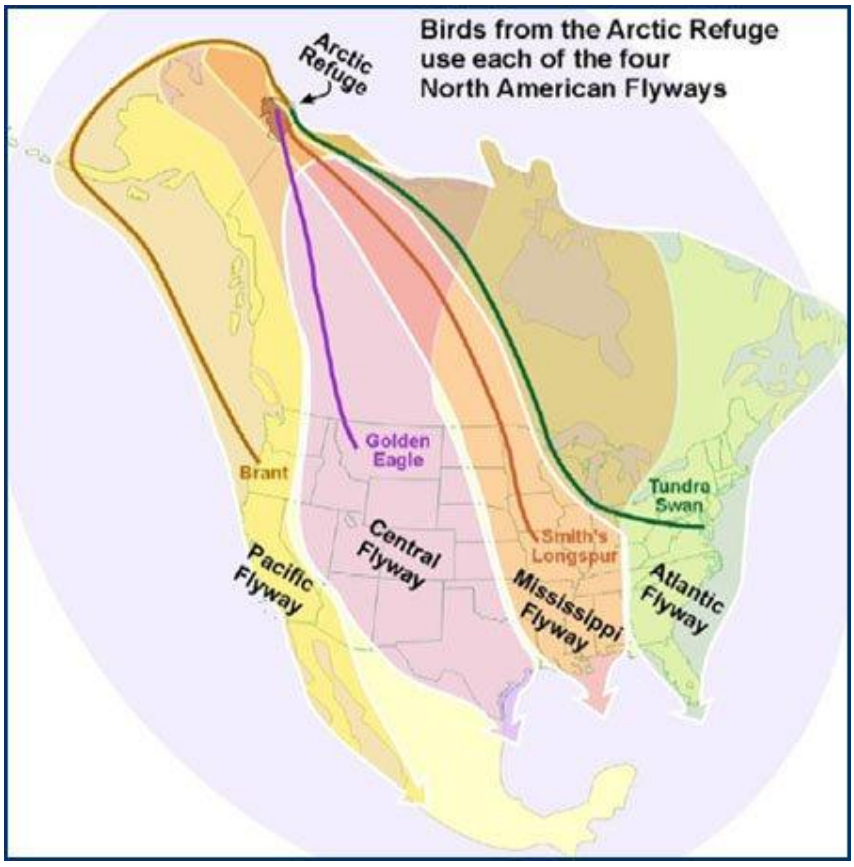

Fig 1: Migratory routes

Food Habits and Migration

There is often a relationship between food habits and migration. Insects become scarce in the winter in northern latitudes, so insectivorous birds must move closer to the equator where insects are more abundant. Insectivorous birds are the largest group of migrants. Some insect-eaters, such as chickadees, can eat seeds in the winter and thus do not tend to migrate. Fruit-eating birds may not have to migrate as far because fruits are generally available at the edge of the temperate zone. And seeds are available all year around in most areas, so seed-eaters tend not to migrate. But seeds are not produced in the winter and may be covered by snow, forcing birds southward, or at least down the mountains. By contrast, finches and crossbills that feed on pine seeds from the cones don't have that problem and are non-migratory.

Fish-eating birds may be cut off from their food supply when northern lakes freeze over, but can generally overwinter if the lake doesn't totally freeze over. Most aquatic birds' winter at the edge of the temperate zone, but some go to the tropics. The most well-known of these is the Arctic Tern which migrates from the edges of the Arctic to the Antarctic and back again each year – a 22,000-mile round trip. Large hawks which feed on mammals and birds tend to migrate because their prey either hibernates or migrates. In addition, photoperiod shortens and they have less time for hunting. Owls, on the other hand, don't migrate as much (as a group) because the shortening photoperiod is not a major factor to them.

Geography and Migratory Behaviour

There is more land area in the North Temperate Zone than there is in either the tropics or the South Temperate Zone; *i.e.* there is more potential breeding area than wintering area. There is even more contrast in habitat types; *e.g.* in the New World, the total amount of tropical forest in South and Central America is about equal to the total amount of forest in North America. But there is a limited amount of other habitat types in both areas. So a grassland species of North America must either winter in the south-western US or northern Mexico, or fly all the way to Venezuela to find grassland. Wetland species- for example, shorebirds that nest in the marshes of the Arctic tundra – have no comparable habitat in the south and thus winter along the coastlines or travel all the way to the grasslands of Argentina.

In the Old World, much of the northern land mass is forest or grassland, while most of Africa is desert and scrubland, only about 20% being tropical forest. Thus, the birds that migrate have to adapt to living in a much different environment. But there are limits to this flexibility- a sandpiper can't live in a rainforest. Australia, being relatively isolated by contrast, has relatively few migratory species – only 8% show north-south migrations. But 26% are nomadic– that is, they travel around the continent continually. Although there is a large amount of tropical forest in South America, the vast majority of birds that migrate from North America to South America winter in Central America, or the West Indies – very few actually go to northern South America or into the interior of South America.

Site Fidelity

If a bird returns to the same site each year, it must have some sort of genetically-based ability to remember the site and how to get there. For example, a young warbler may carry genetic information that causes it to remember its place of birth and then respond to cues that guide it to Puerto Rico. The young bird then spends the winter moving around, searching for a good place to stay. After overwintering, it returns north to the area of its hatching. The next year it returns to the same site in Puerto Rico. So, the bird may spend its life in only a few hectares in two sites thousands of miles apart. There must be a genetic basis to migration, because young

birds that have never migrated before migrate successfully to their breeding grounds. In many shorebirds, the adults leave for their wintering grounds before the young, so the young have to know where to go without help.

Route Selection

Evolution of a migratory route is determined by many factors. Geographic or topographic factors are important for some species. But even some small warblers and hummingbirds have the ability to fly across the Atlantic Ocean on their way from North America to Mexico. But other species, such as some hawks and Turkey Vultures, follow the land route rather than crossing the ocean. Obviously, migratory routes have changed a great deal over geologic time; the glaciers of the ice ages advancing and retreating must have forced birds to change their routes, so migratory routes must have evolved like any other behaviour to changing conditions.

Timing of Migration

The timing of migration is controlled by both proximate and ultimate factors. Ultimate factors are evolutionary and proximate factors are cues.

Resource and Breeding Factors -ultimate factor that determines the suitability of the breeding and wintering grounds. Breeding sandpipers must arrive early in the year in the Arctic as soon as the snow clears so that they have enough food and sufficient time to raise their young before fall.

Climatic Factors – ultimate factor that determines food supply and survivability on the breeding or wintering grounds, but also a proximate factor that influences the timing of migration. Bad weather, rain, and heavy winds may cause birds to fly faster or slow them down. In the Northern Hemisphere, high pressure systems have winds that blow clockwise around them and low-pressure systems have counter clockwise winds. So, if there is a high-pressure system (causing winds to blow from the north), followed by a low-pressure system (causing winds to blow from the south), the birds will wait until the low-pressure system starts to blow and get a tail wind on their way north. That's why there are often "waves" of migrants. Birds take about 24 hours to cross the Yucatan Peninsula to the US nonstop; with a tail wind this can be reduced to 20 hours. If the wind should switch direction, it could slow the birds down enough that they die enroute.

Photoperiod Clues – this is a proximal cue that tells the birds when to migrate. If the birds depended merely on the weather, they could leave too early and be caught with bad weather later or no food in their breeding site; if the weather is bad and they left too late, they would be outcompeted. So they depend on the photoperiod for timing as photoperiod is very predictable and, on the average, is most beneficial for migration.

Navigation and Orientation

How does a bird find its way? We can learn some things about the navigation and orientation behaviour of birds through observation or through banding, but the best information has come through experiments.

Species vary in their navigational abilities. Some birds have the ability to find their way back if they are displaced. Homing pigeons are pretty good at this and the most impressive was a Manx Shearwater, taken from its burrow on the Atlantic coast and flown to England by commercial aircraft, returned to the burrow in 12.5 days.

Most early studies on navigation tried to explain it by one mechanism. But now we know that birds use several mechanisms.

Landmarks. There is some indication that birds use landmarks, at least sometimes. Homing pigeons taken 200 miles off the coast of France in the middle of the Atlantic – only 2% returned home. But in other studies, pigeons were fitted with nearly opaque contact lenses – so they could see light, but nothing else, and most found their way home (but had a hard time landing). And, of course, birds that navigate at night cannot see landmarks.

Solar Cues. The position of the sun seems to be one of the prime navigational cues for both nocturnal and diurnal fliers.

In the 1950s, Kramer put Starlings in cages with opaque walls and a glass top. He found that on sunny days, they oriented in the appropriate direction, but on cloudy days they oriented randomly. Bellerose, working with Blue-winged Teal, fitted them with radio transmitters and found that on cloudy days, they would circle until they got above the clouds at which time they started to move in the appropriate direction.

Using the sun as a compass requires compensating for its movement across the sky, which birds seem to be able to do by setting their internal biological clocks. One experiment with homing pigeons kept pigeons under artificial light until their biological clock was six hours off from the actual local time. When released, they flew 90 degrees off from the proper direction. This internal clock is called a “sun compass”.

Radio- tagged Blue-winged Teal were noted to circle under a cloudy sky until they got above the clouds and then headed in the proper direction, indicating that they need to see the sun to navigate. Even nocturnal birds seem to take their cue from the sun as cloudy days either result it no migration at night or the migrants show an unusual variation of direction.

Stellar Cues. Cues provided by the stars are obviously important only to nocturnal migrants. To determine the importance of stellar cues in orientation, birds were put in cages outside with a view of the night sky to see which way they oriented.

But the most sophisticated experiments put these birds not under the normal night sky, but in a planetarium. This way, the sky could be changed and the birds' response to the moving sky could be measured. In a classic experiment by a German ornithologist, Sauer, in the 1950's, warblers were put into cages in a planetarium in the fall. The birds oriented in a certain direction and Sauer moved the night sky in that direction (at a normal speed) about 300 Km, the distance the birds would normally move in a night. The next night he did the same thing – move the sky in the direction the birds were oriented. At the end of a couple of weeks, the birds quit showing their restlessness as they had migrated to the night sky that showed middle Africa, where they would have gone if they were free. Not only that, they migrated around the Mediterranean Sea enroute. All by stars.

Geomagnetic Cues. Geomagnetism is perhaps the most controversial and least understood of the major possible navigational cues. Early studies by Merkel and Wiltschko (1964) placed birds in a large cement cage with no environmental clues at all; the birds oriented properly. But when they were put in a large steel cage, which would obviously affect magnetic lines of force around the cage, the birds oriented randomly.

The best studies have been done by Keeton in the 1970's with homing pigeons. When brass bars were attached to pigeons, they generally oriented themselves in the direction of home from an unfamiliar location, whether it was a sunny or overcast day. When a magnetic bar was attached to the pigeons, they did about the same on a sunny day, but were disoriented on an overcast day – the magnet seemed to have thrown them off when they had no sun to orient by. A more sophisticated experiment was done later with Helmholtz coils. The coils produced a magnetic current around the birds' heads. The current could either flow in a clockwise direction (normal direction of the magnetic field in the northern hemisphere), in which case the birds flew directly home on either a cloudy or sunny day; or the current could be made to go counter clockwise- in this case the birds flew directly home on a sunny day, but flew 180 degrees in the wrong direction on an overcast day. These experiments demonstrate two things: that geomagnetic lines of force do seem to be used for orientation and that the sun compass is the dominant navigation mechanism.

Thus, orientation and navigation may be due to a variety of cues. There is some evidence that odours may play a role in navigation, or even sounds (ocean waves, waterfalls).

Probable questions:

1. How can you establish that habitat is species specific?
2. Discuss in details about the migratory behaviour.
3. Discuss the factors which controls the timing of migration.
4. What are the mechanisms by which bird can control its navigation?

Suggested Readings:

1. Alcock, J. (2001). Animal Behaviour: An Evolutionary Approach. , Sinauer Associate Inc., USA.

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4. Mandal, F. (2010). A Text Book of Animal Behaviour. Pentice Hall India.

5. Mathur, R. (2005). Animal Behaviour. Rastogi Pub. Meerut.

6. Refinetti, R. (2000). Circadian Physiology. CRC Press, Boca Raton.

7. Ruhela, A. and Sinha, M. (2010). Recent Trends in Animal Behaviour. Oxford Book Co. Jaipur.

Unit IV

Kinship: Relatedness, inclusive fitness

Objective: In this unit you will know about kinship, relatedness with inclusive fitness, selfishness and altruism.

Introduction

Kinship is one of the main organizing principles of society. It is one of the basic social institutions found in every society. This institution establishes relationships between individuals and groups. People in all societies are bound together by various kinds of bonds.

The most basic bonds are those based on marriage and reproduction. Kinship refers to these bonds, and all other relationships resulting from them. Thus, the institution of kinship refers to a set of relationships and relatives formed thereof, based on blood relationships (consanguineal), or marriage (affinal).

Definition

'The social relationships deriving from blood ties (real and supposed) and marriage are collectively referred to as kinship.' – Abercrombie et al.

Kinship: The degree of coefficient of relationship (genetic or hereditary relatedness) between members of a species is called kinship.

Kin: The relatives of same species. Or kin is a group of individuals of common ancestry

Types of Kinship:

Kinship is of two types:

a. Affinal Kinship, and
b. Consanguineous Kinship.

Affinal Kinship:

The bond of marriage is called affinal kinship. When a person marries, he establishes relationship not only with the girl whom he marries but also with a number of other people in the girl's family. Moreover, it is not only the person marrying who gets bound to the family members of the girl but his family members also get bound to the family members of the girl.

Thus, a host of relations are created as soon as a marriage takes place. For example, after marriage a person becomes not only a husband, but he also becomes brother-in-law and son-

in- law. Here it may be noted that in English language a number of relations created by marriage are referred by the same term. Thus, the same term 'brother-in-law is used for bahnoi, sala, jija and sadahu. On marriage a person also becomes foofa, nandoi and mausa.

Likewise, a girl on marriage becomes not only a wife but also becomes daughter-in-law, she also becomes chachi, bhabhi, devrani, jethani, mami etc. Thus, marriage creates a host of relationships which are called affinal kin.

Consanguineous Kinship:

The bond of blood is called consanguineous kinship. The consanguineous kin are related through blood whereas the affinal kin are related through marriage. The bond between parents and their children and that between siblings is consanguineous kinship. Siblings are the children of the same parents.

Thus, son, brother, sister, uncle (chacha), elder uncle (taoo), nephew and cousin are consanguineous kin. i.e., related through blood. In this connection it may be pointed out that blood relationship may be actual as well as supposed.

Among polyandrous tribes the actual father of a child is unknown. An adopted child is treated as if it were one's own biologically produced child. Thus, blood relationship may be established not only on biological basis but also on the basis of social recognition.

Kinship and its degree

The relationship among individuals or people depends on the level of closeness and separation of its relationship. Closeness and distance are based on how these individuals are related to each other.

- **Primary kinship**

Primary kinship is based on direct relations. Individuals or people that are directly related are said to be primary in nature. Primary kinship is further divided into two:

- ***Primary consanguineal kinship*:** this kin refers to that kin that is directly related to each other by birth. For instance, association with or amongst parents and children and among siblings.
- ***Primary Affinal kinship*:** the relation that takes place with marriage is said to be Primary Affinal kinship. The direct primary affinal kinship is the husband-wife relationship

- **Secondary kinship**

Secondary kinship alludes to the primary kinship. As it were, the individuals who are specifically identified with primary kinship (i.e. primary kin of our primary kinship) become secondary kinship. In other words, it means relations that come through primary kinship are said to be secondary kinship.

There are two types of Secondary kinship:

- ***Secondary consanguineal kinship*:** This kind of kin refers to primary consanguineal kinship. The basic example of secondary consanguineal kinship would be the relationship between grandparents and grandchildren.
- ***Secondary Affinal kinship*:** This kind of kinship refers to primary affinal kinship primary kinship. For example, Anita's husband is her primary affinal kinship and for Anita's husband, her parents and siblings are his primary kin. Therefore, meaning the relationship between Anita and her sister-in-law/ brother-in-law or parents in law and more vice versa is said to be Secondary Affinal kinship. Also, your sibling's spouse and his/her parents in law will be his secondary affinal kinship.

- **Tertiary kinship**

Tertiary kinship is the secondary kinship of our primary kin or primary kin of our secondary kinship. For example, wife of our brother-in-law would be related to us as tertiary kin.

Tertiary kinship is further divided into two:

- ***Tertiary consanguineal kinship*:** An example of tertiary consanguineal kin would be our primary consanguineal kins (i.e. our parents) primary kins (i.e. our parents' parents meaning our grandparents) primary kins. (i.e. our grandparents' parents).
- ***Tertiary Affinal kinship*:** It means primary affinal kins primary kin or secondary affinal primary kin or primary affinal kins secondary kin. For example, our spouse's grandparents or grand uncles and aunties.

Importance of Kinship

Kinship has several importances in a social structure.

1. Kinship decides who can marry with whom and where marital relationships are taboo.
2. It determines the rights and obligations of the members in all the sacraments and religious practices from birth to death in family life.
3. Importance of kinship is observed on the occasion of marriage and family functions.

4. It determines family line relationships i.e. gotra and kula.

Inclusive fitness

Basic idea

Cooperation is abundant throughout the natural world and exists at all biological levels, from genes forming genomes to individuals collaborating in societies. Nature documentaries are frequently packed with stunning examples, from kamikaze bees stinging intruders to save the lives of their nest mates to meerkat helpers feeding the pups of others. However, beneath this appearance of kindness lies one of the most challenging issues for evolutionary theory. The problem is that natural selection favors genes that increase an organism's ability to survive and reproduce and so how can behavior that benefits others ever evolve? To simplify this problem the complex spectrum of social behaviors can be broken down into pair-wise interactions and classified according to the direct fitness benefits (number of offspring an individual produces stripped of social interactions) and costs to the actors and recipients involved. This leads to four types of behavior: Selfishness (benefit to actor, cost to the recipient) and mutually beneficial interactions (benefit to actor, benefit to the recipient) are easily understood as they increase the direct fitness of the actor. Altruism (cost to the actor, benefit to the recipient) and spite (cost to the actor, cost to the recipient), on the other hand, present an evolutionary paradox—how can a gene that is disadvantageous to an individual spread in a population? Darwin realized this problem, but it wasn't until 1963–1964, when William (Bill) D. Hamilton produced his benchmark papers, that it became clear how actions that decrease direct fitness can evolve through natural selection. Hamilton coined the term **"Inclusive Fitness"** to emphasize that the quantity that individuals attempt to maximize is not simply direct fitness, but also something called indirect fitness—the effect individuals have on the number of offspring everybody else in the population produces weighted by their relatedness. Inclusive fitness theory remains one of the most active areas of evolutionary research and provides an extremely important tool for understanding both the process and purpose of evolution.

Biology

Inclusive fitness, theory in evolutionary biology in which an organism's genetic success is believed to be derived from cooperation and altruistic behaviour. Inclusive fitness theory suggests that altruism among organisms who share a given percentage of genes enables those genes to be passed on to subsequent generations. In this way, an altruistic act that supports the survival of a relative or other individual theoretically enhances the genetic fitness of both the recipient of the act and the altruistic organism. The propagation of shared genes was believed to be an underlying mechanism for the evolution of eusociality (cooperative behaviour

characterized by division of labour and group integration that is found in certain species of animals, mainly social insects).

The idea of inclusive fitness was first proposed in 1932 by British geneticist J.B.S. Haldane in *The Causes of Evolution*. The theory was later named and developed by British evolutionary biologist William Donald Hamilton, who used inclusive fitness to explain direct (reproductive) and indirect (aided by a relative or a colony member) inheritance of genetic traits associated with altruism. Hamilton presented his inclusive fitness theory in 1963; the following year British evolutionary biologist John Maynard Smith coined the term *kin selection* to describe Hamilton's theory. Inclusive fitness later came to be understood as forming a general basis for kin selection theory, which attempts to interpret altruistic social behaviour in animals through genetic relatedness and benefits and costs associated with altruistic acts. Thus, in contrast to inclusive fitness, which considers genetic traits in both related and unrelated individuals, kin selection is concerned only with relatives. Hamilton's inclusive fitness theory, as well as kin selection, seemed too many biologists to reconcile the conflict between natural selection, in which "selfish" genes perpetuate their own fitness through survival of the fittest, and selfless behavior, in which eusocial genes shared by relatives and colony members influence cooperative behaviour that encourage the propagation of those genes.

Inclusive fitness theory is most commonly applied to eusocial organisms, such as bees and ants, although it has also been invoked to explain cooperative breeding in animals such as birds and the adoption of orphaned young by asocial red squirrels (*Tamiasciurus hudsonicus*). In certain bird species, such as the Florida scrub jay (*Aphelocoma coerulescens*) and the groove-billed ani (*Crotophagas ulcirostris*), some individuals will stay near nesting sites and participate in the rearing of related offspring. Individuals that do not disperse to their own territories have been thought to perceive the inclusive fitness gains of cooperative breeding as being greater than fitness gains offered by dispersal to potentially less-favorable territory. In such instances, inclusive fitness through cooperative breeding is the result of constraints on territory quality and is influenced by factors such as food, mate attraction, and predation. Indeed, in the absence of constraints, staying near relatives is less advantageous, potentially limiting breeding opportunities and thereby making kin selection and inclusive fitness less beneficial to reproductive success. The amount of labour that cooperative breeding individuals contribute to raising relatives is variable. In contrast, eusocial organisms have fixed and stereotyped divisions of labour; castes such as sterile workers presumably accumulate reproductive advantages by helping their relatives in the cooperative raising of young.

Hamilton's rule

The potential genetic return depends on the relatedness between two individuals. Therefore, closer relationship gives greater potential genetic return.

It is expressed as-

B/C >1/r or rB> C or rB-C >0

Where, B= Benefits of altruistic act

C= Costs of altruistic act

r= Coefficient of relationship

***Example*:** An altruist cannot produce one offspring of its own but its altruistic act saves five (5) nephews from perishing (accident). The value of r between non-related is 0, between uncle & nephews is 0.25 and between parent and offspring is 0.5. Is this act net gain for altruist?

Ans: According to Hamilton's rule, we know that-

B/C > 1/r

Here, B = 5 x 0.25 =1.25 & C = 1 x 0.25 = 0.25

B/C = 1.25 / 0.25 = 5

And, 1/r = 1/0.25 = 4

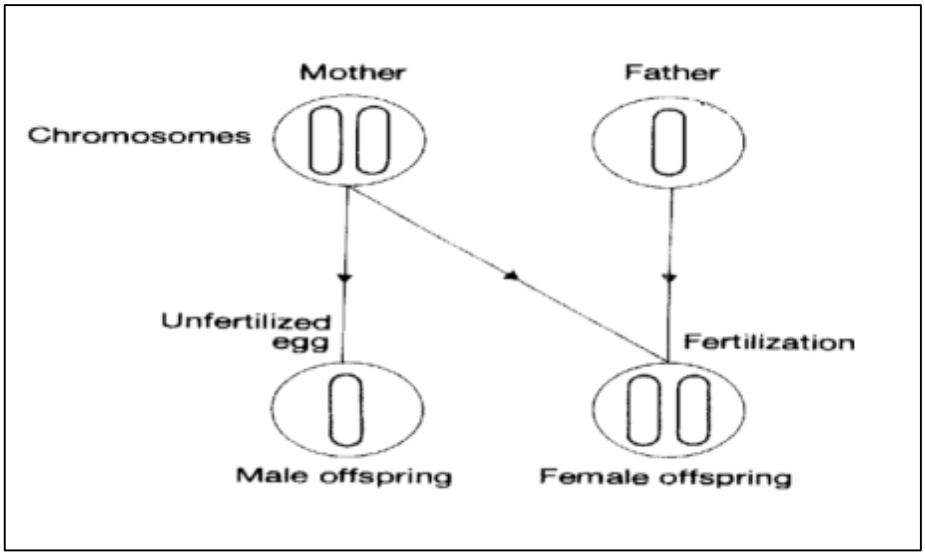
So, B/C > 1/r

Therefore, Altruistic act will make net gain for the altruist

Hamilton introduced a brave new concept, 'inclusive fitness', which basically says someone could still have a reproductive fitness, even if he/she has no direct offspring. This is while the 'traditional fitness' only count how many children one has, but inclusive fitness takes account of all others who share genes with the person (or animal). For example, I should share approximately 50% of gene with my full brother, therefore if I decide not to marry and have kids, but I help my brother to raise 4 children, it is equivalent to my self-having two children. This inclusion of anyone elses' fitness, who shares common genes by descent, factored by a coefficient of relatedness, is called inclusive fitness. Therefore, although workers do not reproduce, if they share genes with their mother (the queen) to raise more sisters (future queens) their genes would be transmitted too to the next generation. In fact, in honey bees and other hymenoptera, the relatedness among sisters is higher than among other animals. This is because of the haplo-diploidy sex determination: drone develop from unfertilized eggs and carry one copy of chromosomes (haploid) from their mother only (no father), while females

are fertilized and carry two copies of chromosomes (diploid). Haploid drones do not have the complimentary copy of genes to do exchange, so all the sperms produced by a single drone are identical. Assume the queen is mated to a drone, then all her daughters will share 50% genes from the father, but 25% of their genes from the mother. The coefficient of relatedness among the offspring is therefore 0.75. This is much higher than the 0.5 for sister-sister in a diploid organism (such as humans). The workers who share the same father and mother, are therefore also called 'super-sisters' because of this higher relatedness. This theory of one can pass genes through relatives and gain fitness is called 'kin selection'. Hamilton postulated that because supersisters share 75% of their genes, it is actually a better deal to be a worker, to whom a new queen would have 75% of genes by common descent with her, whereas from the queen's point of view, she only transmitted 50% of her genes to the new queen. In this sense, the inclusive fitness is actually higher for the sterile worker sisters, than for the fertile mother. One difficulty with the above argument is that the honey bee queen actually mates with more than one male (drone), in some cases as many as more than 30 drones, because half-sisters (workers who share the same mother, but fathered by different males) are only related to one another by 0.25 the average 68 relatedness among the workers in such a colony is close to the average between 0.75 and 0.25, which is 0.5, not different from other diploid organisms.

It is easy to see though, that eusociality can evolve easier in groups within which individuals are highly related, either due to haplodiploidy, or due to mating systems. In both termites and the naked mole rats, animals within a group are all highly related, perhaps due to inbreeding, although it is known in naked mole rats, some males would migrate to other nests to accomplish periodic outcrossing, which might be necessary to reduce the cost of inbreeding. In aphids, all colony members are 'clones' because the mother can reproduce asexually (parthenogenesis). However, the marine shrimps do not show high degrees of inbreeding.



- # Advantages of Inclusive fitness

Inclusive fitness brings with it several advantages for the study of social behaviour.

Advantage 1: predicting gene frequency change

The first advantage of inclusive fitness is that, under additivity, it correctly predicts the direction of gene frequency change. Hamilton's rule provides a simple tool for doing so (Hamilton 1964). Given a trait that has an effect, in terms of adult offspring number, on its bearer, −c, and has an effect on social interactants, *b*, that trait will spread in the population if rb−c>0, where *r* is the relatedness to the recipients affected by the trait. More generally, genes whose bearers tend to have a higher value of inclusive fitness will be favored by natural selection (Hamilton 1964, 1970). The rule easily extends to multiple recipients, although it is crucial that there is just one actor. Note that we are referring to the simple form of Hamilton's rule derived by Hamilton (1964), in which the fitness effects are absolute effects on offspring number, as this form is sufficient under nonadditivity.

Advantage 2: a design principle for individuals

Inclusive fitness provides a design principle for organisms. A fundamental question in biology (dating, in spirit if not detail, to Darwin) is how the dynamics of gene frequency change leads to the appearance of design and adaptation in organisms. Fisher's Fundamental Theorem (1930) provided such a link for non-social traits, by proving that natural selection always tends to increase mean fitness. It sometimes then follows that organisms appear designed as if trying to maximize that quantity. Hamilton established a similar result to Fisher's, but for social traits. Inclusive fitness is a quantity that, under additivity, organisms should appear designed to maximize (Hamilton 1964; Queller 1992; Grafen 2006; Gardner et al. 2011; West and Gardner 2013; Lehmann and Rousset 2014; Rousset 2015; Lehmann et al. 2016; Taylor 2017).

Inclusive fitness is particularly useful as a design principle because it is can be conceptualized as an individual level property. Although it is possible to search for design principles at the level of the gene or the group, students of behavior tend to predict and measure organismal phenotypes.

Advantage 3: interpreting behaviour

Inclusive fitness provides a simple, economic interpretation of organismal behaviour (Hamilton 1970; Grafen 1984; Frank 1998). Organisms should trade off their own offspring against those of another individual at a rate *r* (relatedness). This serves three purposes.

First, it helps generate testable predictions, even without complex mathematical models. Simple verbal reasoning can lead us to predict how many eggs a certain species of bird should

lay each year, or how much food a cub should leave for its sibling, and these predictions are then readily testable.

Second, it guides us to new study systems by suggesting what biological features might lead to problematic or interesting cases. A heuristic for generating predictions is exactly how a scientific field makes progress, as has been demonstrated in the fields of behavioral ecology and evolutionary ecology (Krebs and Davies 1978, 1987; Charnov 1982; Krebs and Davies 2009; West 2009; Westneat and Fox 2010; Davies et al. 2012).

Third, it helps us understand social behaviour by providing a way to reason about adaptations. For example, it is true that populations should be made up of genes that are associated with a higher contribution of gene copies to the next generation. But this does not tell us much about what kinds of traits and real-life observations would defy our expectations, what population structures might lead to particularly unusual phenomena, or what adaptations (underpinned by many genes) might spread. Inclusive fitness offers us all of those things, by telling us that organisms should make decisions using this simple trade-off in offspring.

Advantage 4: empirical testability

An additional benefit of this simple trade-off is that inclusive fitness predictions are testable in the laboratory and the field. Inclusive fitness, remarkably, does not require knowing the genetics of a trait (the 'phenotypic gambit'), the genotypes of various individuals in the population, or even gene frequencies (Grafen 1984; West and Gardner 2013). We only need to know the fitness effects of the trait and the relatedness to the recipients. In practice, pedigree relatedness usually suffices (because it leads to the genes in the genome pulling in the same direction), making experiments surprisingly feasible (West and Gardner 2013).

Probable questions:

1. Define kinship, kin selection and kin.
2. What do you mean by Consanguineous Kinship?
3. What do you mean by Affinal Kinship?
4. Discuss the degree of kinship on the basis of closeness and distance.
5. Discuss secondary kinship.
6. Write down the importance of kinship.
7. Write short notes on inclusive fitness.

8. What is Hamilton's rule? Elaborate this rule with an example.
9. What do you mean by inclusive fitness?
10. Mention the advantages of inclusive fitness.

Suggested Readings:

1. Alcock, J. (2001). Animal Behaviour: An Evolutionary Approach. , Sinauer Associate Inc., USA.

2. Chattopadhyay, S. (2012). Life: Evolution, Adaptation, Ethology. 3rd Edn. Books and Allied, Kolkata.

3. Dujatkin, L.A. (2014). Principles of Animal Behaviour. 3rd Edn. W.W.Norton and Co.

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Unit V

Kinship: selfishness, altruism

Objective: In this unit you will know about kinship, selfishness and altruism.

Introduction

Kin Selection and Inclusive Fitness

The basic idea of kin selection is simple. Imagine a gene which causes its bearer to behave altruistically towards other organisms, e.g. by sharing food with them. Organisms without the gene are selfish—they keep all their food for themselves, and sometimes get handouts from the altruists. Clearly the altruists will be at a fitness disadvantage, so we should expect the altruistic gene to be eliminated from the population. However, suppose that altruists are discriminating in who they share food with. They do not share with just anybody, but only with their relatives. This immediately changes things. For relatives are genetically similar—they share genes with one another. So, when an organism carrying the altruistic gene shares his food, there is a certain probability that the recipients of the food will also carry copies of that gene. (How probable depends on how closely related they are.) This means that the altruistic gene can in principle spread by natural selection. The gene causes an organism to behave in a way which reduces its own fitness but boosts the fitness of its relatives—who have a greater than average chance of carrying the gene themselves. So the overall effect of the behaviour may be to increase the number of copies of the altruistic gene found in the next generation, and thus the incidence of the altruistic behaviour itself.

Though this argument was first made explicit by William Hamilton (1964). Hamilton demonstrated rigorously that an altruistic gene will be favoured by natural selection when a certain condition, known as *Hamilton's rule*, is satisfied. In its simplest version, the rule states that $b > c/r$, where c is the cost incurred by the altruist (the donor), b is the benefit received by the recipients of the altruism, and r is the *co-efficient of relationship* between donor and recipient. The costs and benefits are measured in terms of reproductive fitness. The co-efficient of relationship depends on the genealogical relation between donor and recipient—it is defined as the probability that donor and recipient share genes at a given locus that are 'identical by descent'. (Two genes are identical by descent if they are copies of a single gene in a shared ancestor.)

Though Hamilton himself did not use the term, his idea quickly became known as 'kin selection', for obvious reasons. Kin selection theory predicts that animals are more likely to

behave altruistically towards their relatives than towards unrelated members of their species. Moreover, it predicts that the *degree* of altruism will be greater, the closer the relationship. In the years since Hamilton's theory was devised, these predictions have been amply confirmed by empirical work. For example, in various bird species, it has been found that 'helper' birds are much more likely to help relatives raise their young, than they are to help unrelated breeding pairs. Similarly, studies of Japanese macaques have shown that altruistic actions, such as defending others from attack, tend to be preferentially directed towards close kin. In most social insect species, a peculiarity of the genetic system known as 'haplodiploidy' means that females on average share more genes with their sisters than with their own offspring. So a female may well be able to get more genes into the next generation by helping the queen reproduce, hence increasing the number of sisters she will have, rather than by having offspring of her own. Kin selection theory therefore provides a neat explanation of how sterility in the social insects may have evolved by Darwinian means.

Kin selection theory is often presented as a triumph of the 'gene's-eye view of evolution', which sees organic evolution as the result of competition among genes for increased representation in the gene-pool, and individual organisms as mere 'vehicles' that genes have constructed to aid their propagation. The gene's eye-view is certainly the easiest way of understanding kin selection, and was employed by Hamilton himself in his 1964 papers. Altruism seems anomalous from the individual organism's point of view, but from the gene's point of view it makes good sense. A gene wants to maximize the number of copies of itself that are found in the next generation; one way of doing that is to cause its host organism to behave altruistically towards other bearers of the gene, so long as the costs and benefits satisfy the Hamilton inequality. But interestingly, Hamilton showed that kin selection can also be understood from the organism's point of view. Though an altruistic behaviour which spreads by kin selection reduces the organism's personal fitness (by definition), it increases what Hamilton called the organism's *inclusive* fitness. An organism's inclusive fitness is defined as its personal fitness, plus the sum of its weighted effects on the fitness of every other organism in the population, the weights determined by the coefficient of relationship r. Given this definition, natural selection will act to maximize the inclusive fitness of individuals in the population. Instead of thinking in terms of selfish genes trying to maximize their future representation in the gene-pool, we can think in terms of organisms trying to maximize their inclusive fitness. Most people find the 'gene's eye' approach to kin selection heuristically simpler than the inclusive fitness approach, but mathematically they are in fact equivalent.

Contrary to what is sometimes thought, kin selection does not require that animals must have the ability to discriminate relatives from non-relatives, less still to calculate coefficients of relationship. Many animals can in fact recognize their kin, often by smell, but kin selection can operate in the absence of such an ability. Hamilton's inequality can be satisfied so long as

an animal behaves altruistically towards others animals that are *in fact* its relatives. The animal *might* achieve this by having the ability to tell relatives from non-relatives, but this is not the only possibility. An alternative is to use some proximal indicator of kinship. For example, if an animal behaves altruistically towards those in its immediate vicinity, then the recipients of the altruism are likely to be relatives, given that relatives tend to live near each other. No ability to recognize kin is presupposed. Cuckoos exploit precisely this fact, free-riding on the innate tendency of birds to care for the young in their nests.

Another popular misconception is that kin selection theory is committed to 'genetic determinism', the idea that genes rigidly determine or control behaviour. Though some sociobiologists have made incautious remarks to this effect, evolutionary theories of behaviour, including kin selection, are not committed to it. So long as the behaviour in question have a genetical *component*, i.e. are influenced to some extent by one or more genetic factor, then the theories can apply. When Hamilton (1964) talks about a gene which 'causes' altruism, this is really shorthand for a gene which increases the probability that its bearer will behave altruistically, to some degree. This is much weaker than saying that the behaviour is genetically 'determined', and is quite compatible with the existence of strong environmental influences on the behaviour's expression. Kin selection theory does not deny the truism that all traits are affected by both genes and environment. Nor does it deny that many interesting animal behaviours are transmitted through non-genetical means, such as imitation and social learning.

The importance of kinship for the evolution of altruism is very widely accepted today, on both theoretical and empirical grounds. However, kinship is really only a way of ensuring that altruists and recipients both carry copies of the altruistic gene, which is the fundamental requirement. If altruism is to evolve, it must be the case that the recipients of altruistic actions have a greater than average probability of being altruists themselves. Kin-directed altruism is the most obvious way of satisfying this condition, but there are other possibilities too. For example, if the gene that causes altruism also causes animals to favour a particular feeding ground (for whatever reason), then the required correlation between donor and recipient may be generated. It is this correlation, however brought about, that is necessary for altruism to evolve. This point was noted by Hamilton himself in the 1970s: he stressed that the coefficient of relationship of his 1964 papers should really be replaced with a more general correlation coefficient, which reflects the probability that altruist and recipient share genes, whether because of kinship or not. This point is theoretically important, and has not always been recognized; but in practice, kinship remains the most important source of statistical associations between altruists and recipients.

Altruism

What is Altruism?

You're hanging out with your friends while studying one day, and you open up a bag of chips. Being the good-natured person that you are, you offer to share your chips with your friends, even though it means there's less available for you. Your friends compliment you and thank you for your altruistic behavior. In the human world, altruism is an act of selflessness, where an action is taken that doesn't benefit the person doing it.

The same concept applies to biology. In biology, altruism means any individual acting in a way that reduces their own fitness while increasing the fitness of the group. Today we're going to learn why that happens and which groups of animals have taken their relationships to the next level.

Examples of Altruism

Although we as humans are used to taking care of each other, not many animals do this. Most are in direct competition with each other outside of their offspring. Not all animals believe sharing is caring! However, some animals that live in more complex social organizations got the memo.

Fig: Meerkats on high alert

For example, meerkats, which are found in Africa, are small carnivorous mammals that live underground. Meerkats stand tall on the ground, and when a predator such as a hawk approaches, one meerkat will call out an alarm. The alarm draws the hawk's attention to the watchman, while the other meerkats can escape safely into the burrow.

Vampire bats have been known to regurgitate blood to bats that are sick or were unable to find food, even though it means less food for themselves. Good thing you can just give the bag of chips to your friends, right?

Even complex societies of insects are known for their altruism. Termites and bee societies are made of sterile drones. They cannot reproduce, but only forage and build the nest for the queen. The worker insects have no purpose but to serve their colony in these ways.

Altruism and Natural Selection

Altruism is the opposite of what we normally think about in evolution, which is survival of the fittest. A famous scientist named Charles Darwin came up with the idea that all life is competing to survive and reproduce, called natural selection. Individuals that survive to pass on their genes are more fit than those that don't. Altruism rubs against this line of thinking.

In some communities, certain individuals will sacrifice their well-being for the overall good of the group. Essentially, they're taking a hit for the team. In the vain of natural selection, this makes no sense. If an altruistic organism is sacrificing itself for the group, how will its genes be passed on so that there are more altruistic individuals in the future?

Altruism and Kin selection

Altruism is a phenomenon in which one individual benefit the other at its own expense. The phenomenon occurs in social animals or in closely knit populations, and is considered a paradox of natural selection theory. How can a gene that benefits other individuals at the expense of the bearer be favoured by natural selection? But kin selection favours traits that result in decreased personal fitness provided they increase the survival and reproductive fitness of the species, population or family.

Kin selection works not on individuals but on genotypes. An altruist by way of helping other individuals increases the fitness of its own genome. A honey bee worker is a sterile female and shares at least 50% of its genotype with its sisters even when its mother and father are unrelated. Workers share only 25% genes with haploid brother drones. If a worker decides to breed on its own, its diploid daughters and haploid sons will never be more than 50% related to it. So, the worker becomes sterile and ensures the survival of her genetically identical sisters because then queen can produce more offspring than workers reproducing individually.

The equation shows that a gene that favours altruism could spread when participants are related and the cost to the actor is low as compared to the benefit to the recipient. Altruism always pays when ***Br>C.*** Therefore, altruism is promoted by kin selection and close genetic relationship.

In a large number of bird species, especially those in which nesting opportunities are limited, young ones help their parents in rearing their own sisters and brothers by way of nest building, nest defense and feeding the chicks, although they are themselves capable of breeding. In such birds, as for example in bee-eaters, help is always given to their kin, and the importance of this assistance can be gauged by the fact that there is considerable mortality of chicks due to starvation if such a help is withdrawn.

Reciprocal altruism and Group selection

The theory of group selection was championed by Wynne-Edwards (1962). Altruism has evolved among the related individuals by means of kin selection. But there are instances of cooperation among the unrelated individuals. Altruistic act towards non-kin is possible only if the recipient is likely to return the favour at a later date, in a *'Tit for Tat'* manner.

Natural selection will favour altruism among unrelated individuals only if they reciprocate, and then this is called Group selection, which will select out selfish individuals from the population. Robert Trivers (1971) proposed that reciprocal altruism can develop in the following conditions:

1. If interacting individuals remain together for considerably longer time.

2. If frequency of altruistic attempts is high.

3. If the cost and benefit to both individuals are more or less equal.

4. If individuals that fail to reciprocate are punished in some way, such as withdrawing the benefits in future.

Species which have mutual dependence in defence, foraging, territoriality etc. are

Most likely to develop reciprocal altruism, as in monkeys, baboons, chimpanzees and man. Kin selection and reciprocal altruism are sometimes found to coexist in many social groups of animals and at times it is difficult to distinguish between the two or measure them independently.

Selfishness

Selfishness is the tendency to prioritize one's own desires and needs above the needs and desires of other people.

What is Selfishness?

We are all born with a drive to stay alive and healthy, and selfishness may be a misplaced manifestation of this. A certain degree of selfishness is normal. For example, many people would choose to ensure their own food needs are met before giving food to others. But selfishness can also be a pathological personality trait. Selfish people may prioritize their own petty needs above the significant needs of others. For example, a person is exhibiting selfishness when he or she steals money from their mother to buy a comic book.

Some mental health problems can contribute to the development of selfishness. Many personality disorders, particularly antisocial personality disorder and narcissistic personality disorder, cause people to be so wrapped up in their own desires that they either do not notice or do not care about the needs of others. Many other mental illnesses can cause extreme self-involvement, which can contribute to selfishness. A depressed person, for example, might be so wrapped up in his or her own feelings of suffering that he/she is unable to provide for his/his children or communicate with his/her partner.

Different Conceptions of Selfishness

Many religions decry selfishness and emphasize the virtues of compassion, empathy, and self-sacrifice. The pacifist movement, which draws on many religious traditions, is a radical answer to selfishness, and emphasizes non-violence even in the face of overwhelming hostility. Some religious gurus have advocated extreme self-sacrifice, emphasizing the primacy of others over oneself.

There is significant debate in evolutionary biology about the evolved nature of selfishness. Richard Dawkins' book *The Selfish Gene*, for example, argues that our genes have the "selfish" desire to propagate themselves and do nothing else. Some biologists argue that people are innately selfish. Others, however, emphasize that helping others can ensure the survival of the species and argue that compassion, empathy, and self-sacrifice are as innate to people as selfishness. People are sometimes more likely to show self-sacrificing behaviour for close relatives, and some biologists argue that this is an evolved trait. Many parents would give up their own lives for the lives of their children; one interpretation of this inclination is that when a child survives, the parent's genes survive with the child.

Selfishness as second-order altruism

Altruism and selfishness, like free will and determinism, seem to be polar opposites. Selfishness is seldom considered a group-beneficial strategy. In the typical evolutionary formulation, altruism benefits the group, selfishness undermines altruism, and the purpose of the model is to identify mechanisms, such as kinship or reciprocity that enable altruism to evolve. Recent

models have explored punishment as an important mechanism favouring the evolution of altruism, but punishment can be costly to the punisher, making it a form of second-order altruism. This model identifies a strategy called "selfish punisher" that involves behaving selfishly in first-order interactions and altruistically in second-order interactions by punishing other selfish individuals. Selfish punishers cause selfishness to be a self-limiting strategy, enabling altruists to coexist in a stable equilibrium. This polymorphism can be regarded as a division of labour, or mutualism, in which the benefits obtained by first-order selfishness help to "pay" for second-order altruism.

Selfishness is rarely described as a group-beneficial strategy. Selfish strategies are labelled as deviant, cheating, free-riding, egoistic, but most of all, as undermining altruism and cooperation. In contrast, altruistic and cooperative strategies, almost by definition, benefit the group, often at the expense of the individual actor. In the typical evolutionary model, the invasion of selfish strategies into a group leads to the dissolution of altruism. Examples include scroungers among foraging groups, infanticide of unrelated infants, sneaking worker reproduction in eusocial insect colonies, and failure to help in territorial defence.

Probable questions:

1. Discuss the relationship between kin selection and inclusive fitness.
2. What do you mean by altruism? Give example.
3. What is reciprocal altruism? Give example.
4. Discuss the relationship between altruism and kin selection emphasising on natural selection.
5. What is selfishness?
6. Discuss the selfishness as second order altruism.

Suggested Readings:

1. Alcock, J. (2001). Animal Behaviour: An Evolutionary Approach. , Sinauer Associate Inc., USA.

2. Chattopadhyay, S. (2012). Life: Evolution, Adaptation, Ethology. 3rd Edn. Books and Allied, Kolkata.
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6. Refinetti, R. (2000). Circadian Physiology. CRC Press, Boca Raton.

7. Ruhela, A. and Sinha, M. (2010). Recent Trends in Animal Behaviour. Oxford Book Co. Jaipur.

Unit VI

Conflict: Sexual selection, aggression, competition dominance, Infanticide

Objective: In this unit you will know about conflict; Sexual selection, aggression, competition dominance and infanticide

- ## Sexual Selection

Sexual selection is a mode of natural selection in which members of one biological sex choose mates of the other sex to mate with (intersexual selection), and compete with members of the same sex for access to members of the opposite sex (intrasexual selection). Sexual selection, theory in postulating that the evolution of certain conspicuous physical traits—such as pronounced coloration, increased size, or striking adornments—in animals may grant the possessors of these traits greater success in obtaining mates. From the perspective of natural selection, such increases in mating opportunities outweigh the risks associated with the animal's increased visibility in its environment. This concept was initially put forth by English naturalist Charles Darwin in The Descent of Man (1871).

Mutual attraction between the sexes is an important factor in reproduction. Particularly in birds and mammals, the males are often larger and stronger, more brightly coloured, or endowed with conspicuous ornamentation. These traits, however, make animals more visible to predators—the long plumage of male peacocks (*Pavocristatus*) and birds of paradise (*Paradisaea*) and the enormous antlers of aged male deer (*Odocoileus*) (Fig 1) are cumbersome loads in the best of cases. Darwin knew that natural selection could not be expected to favour the evolution of disadvantageous traits, and he was able to offer a solution to this problem. He proposed that such traits arise by "sexual selection," which "depends not on a struggle for existence in relation to other organic beings or to external conditions but on a struggle between the individuals of one sex, generally the males, for the possession of the other sex."

The presence of a particular trait among the members of one sex can make them somehow more attractive to the opposite sex. This type of "sex appeal" has been experimentally demonstrated in all sorts of animals, from vinegar flies (*Drosophila*) to pigeons, mice, dogs (*Canis familiaris*), and rhesus monkeys (*Macacca mulatta*). When, for example, *Drosophila* flies, some with yellow bodies as a result of spontaneous mutation and others with the normal yellowish grey pigmentation, are placed together, normal males are preferred over yellow males by females with either body colour.

Fig 1: A pair of red deer stags (*Cervus elaphus*) competing for possession of a female in the rutting season.

- ## Intersexual Selection and Intrasexual Selection

Before we delve into the types of sexual selection, it's worth taking a moment to differentiate between inter and intra. *Inter* means between groups. For example, an *inter*state highway goes between the states. Or the *Inter*net is a network that goes between computers. Contrast this with *intra*, which means within groups. An intrastate highway is a highway that travels inside a state's boundaries. Or intramural sports are sports that are played within your college, but not against other colleges.

Okay, so let's start with **intersexual selection**, which is sexual selection that is *between* two sexes. For example, consider a male peacock (Fig 2). Their giant, brightly coloured tails make avoiding predators difficult, so what's the advantage of having such tails? Well, females choose male peacocks with brightly coloured tails, so even though it's risky to be parading around predators with a bright tail, your chances of mating (and reproducing) are increased and thus this trait gets passed down. The peacock is an example of sexual selection *between* the two sexes, or intersexual selection. Just like a muscular, bearded human, it's assumed that female peacocks choose brightly coloured male peacocks because in order to produce such a large, colourful tail the male peacock must have good genes. These good genes, in turn, will contribute to the success of the offspring.

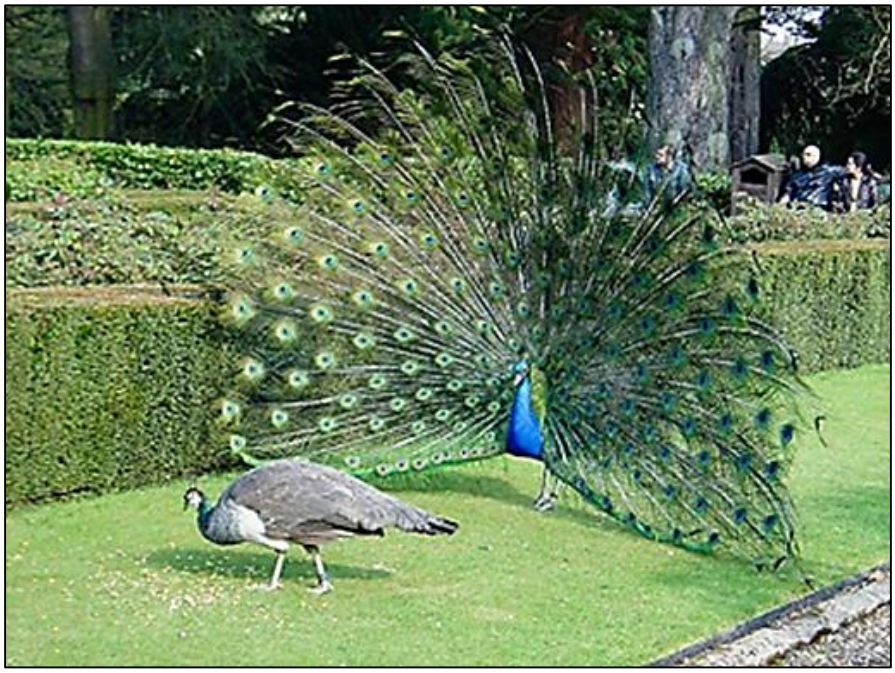

Fig 2: Female peacocks choosing males with brightly coloured, large tails is an example of Intersexual Selection

Contrast this with **intrasexual selection**, which is sexual selection *within* a sex. An example here is the competition seen in male primates. In many primates, a male will attempt to keep other males away from females so he can be their primary mate. Large, aggressive male primates with large canine teeth are usually the ones that are able to mate and pass along their genetic material. So here it is not selection from a different sex, its selection within the same sex. Again, this ensures the female gets the best genetic material from the male because in order for a male to grow large canines and defeat other males, he must be healthy and carry good genetic material.

How Does Sexual Selection Operate?

Sexual selection can operate both intra- and inter-sexually, either sequentially or simultaneously. During intrasexual selection, members of the same sex attempt to outcompete rivals, often during direct encounters. Intrasexual selection is typically responsible for the evolution of male armaments such as deer antlers, beetle horns, and large body size, which provide individuals with an advantage when fighting off potential competitors. Individuals who are better able to exclude competitors, have a greater chance to acquire mates and father offspring. For example, dominant male red deer monopolize a group of females (also known as harem) by constantly fighting off competitors, and they father most of the offspring produced by the females. By contrast, intersexual selection results from interactions between the sexes,

typically involving mate choice. The evolution of elaborate behavioural displays and morphological traits can often be explained as the result of intersexual selection. Usually, females tend to be choosier, evaluating morphological and behavioural traits from potential mates to determine which will maximize their fitness. Males tend to compete with one another to gain the female's attention. An extreme example of intersexual selection can be found in species where males form leks where multiple males gather to display to females.

- **Bateman's principle**

Bateman's principle, in evolutionary biology, is that in most species, variability in reproductive success (or reproductive variance) is greater in males than in females. It was first proposed by Angus John Bateman (1919–1996), an English geneticist. Bateman suggested that,

i. Since males are capable of producing millions of sperm cells with little effort, while females invest much higher levels of energy in order to nurture a relatively small number of eggs, the female plays a significantly larger role in their offspring's reproductive success.

ii. Females should be the choosier sex because eggs are expensive to produce and because a female's potential reproductive success is limited compared with that of a male.

iii. Female's greater choosiness in mate selection should translate into greater variance in the reproductive success of males (Bateman, 1948).

Bateman's paradigm thus views females as the limiting factor of parental investment, over which males will compete in order to copulate successfully.

Darwin argued that, all else being equal, any male trait that confers mating and fertilization advantages and is passed down across generations will, over time, increase in frequency in a population, because males with such traits will produce more offspring than their competitors. Darwin's idea about the struggle among males for mating opportunities forms one basic foundation of our current understanding of sexual selection. Of course, from a proximate perspective, the ways in which hormones, neurobiology, development, environment, and many other factors operate on any particular behaviour play a very important role in the sexual selection process as well.

Competition for mates can take many forms, depending on ecology, demography, and cognitive ability. For example, males may fight among themselves, occasionally in dramatic "battles to the death," but often in less dangerous bouts, to gain mating opportunities with females. This latter form of male-male sexual competition is illustrated by male stag beetles and red deer (*Cervus elaphus*), which use their "horns" (enlarged jaws) and antlers, respectively, in physical fights over females; the winners of such contests mate more often than the losers.

❖ Aggression

Introduction

- In psychology, the term aggression refers to a range of behaviours that can result in both physical and psychological harm to yourself, others, or objects in the environment. This type of behaviour centres on harming another person either physically or mentally. It can be a sign of an underlying mental health disorder, a substance use disorder, or a medical disorder. Predatory behaviour between members of one species towards another species is also described as aggression.
- To exhibit aggression towards members of another species is common.

  Examples: Lions are aggressive hunters of antelopes; Eagles are aggressive hunters of small mammals.

- Aggression is defined as an agonistic behaviour which is a system of behaviour pattern that has the common function of adjustment in situations of conflict among conspecifics.
- The term agonistic includes all aspects of conflicts such as threat, submission, chases and physical combat.
- Most agonistic behaviour involves competition for some resources. Like most behaviours, aggression can be examined in terms of its ability to help an animal reproduce and survive.
- Animals may use aggression to gain and secure territories, as well as other resources including food, water, and mating opportunities. Researchers have theorized that aggression and the capacity for murder are products of our evolutionary past.

The Nature of Animal Aggression

Aggression sometimes occurs when parents defend their young from attack by members of their own species. Female mice, for example, defend their pups against hostile neighbours, while male stickleback fish defend eggs and fry against cannibalistic attack. More frequently, however, animals fight over resources such as food and shelter—e.g., vultures fight over access to carcasses, and hermit crabs fight over empty shells. Another important resource over which fighting commonly occurs is potential mates. In this case the biology of gamete production has an influence on aggressive behaviour because a female's eggs are larger, are fewer in number, and require more energy to produce than a male's sperm. Competition among males over females is usually more frequent and intense than competition among females over males. As a result, the most spectacular fights among animals, whether they are crickets, salmon, tree frogs, chaffinches, or stags, occur between males over fertile females.

Aggression may be focused on a specific area, such as a defended territory from which rivals are vigorously excluded. A notable example is shown by mudskippers. Intertidal fish that

defend small territories where they browse on microscopic plants. The fish build mud walls around the borders of their territories, and at low tide water is retained within the walls (incidentally permitting the human observer to visualize the mosaic of territories in a colony of these fish). Territorial behaviour is also shown by rag worms and fiddler crabs when they defend their burrows, by male dragonflies and sticklebacks defending breeding grounds, by male tree frogs, sage grouse, and Uganda kob defending high-quality sites for courting and mating, and by spiders, reef fish, and hyenas when they defend feeding areas.

A common feature of aggression in most species is that fights tend to start with relatively harmless displays or postures. For example, aggressive interactions between two red deer stags begin with an exchange of deep roars followed by a display of "parallel walking," in which the stags strut side by side assessing their relative size. The aggression may then escalate to direct attacks during which the stags charge at each other, stabbing and wrestling with their antlers. Most confrontations are resolved early while displaying, but many others continue to the point of intense and dangerous fighting.

Contrary to previous assumptions, injury and death during animal fights are not uncommon. In species where animals live in established groups, however, overt fighting is often replaced by a set of relationships in which a subordinate individual consistently defers to a dominant one. Wolf packs, for example, are known for their clear hierarchical relationships. When two group members meet, the dominant animal adopts an upright stance, with raised ears and tail, while the subordinate flattens its body to the ground with the ears against the head and the tail lowered, a submissive posture that serves to protect it from attack. In a number of bird species, variations in plumage act as "badges of status," especially in large winter flocks. The black throat patch or bib of the house sparrow and the dark chest stripe of the great tit are signals of status; dominant individuals have more-conspicuous bibs or stripes than do subordinates and thus have preferential access to food.

Types of Aggression

Psychologists distinguish between two different types of aggression:

- **Impulsive Aggression:** Also known as affective aggression, impulsive aggression is characterized by strong emotions, usually anger. This form of aggression is not planned and often takes place in the heat of the moment. When another car cuts you off in traffic and you begin yelling and berating the other driver, you're experiencing impulsive aggression. Research suggests that impulsive aggression, especially when it's caused by anger, triggers the acute threat response system in the brain, involving the amygdala, hypothalamus, and periaqueductal grey (PAG).

- **Instrumental Aggression:** Also known as predatory aggression, instrumental aggression is marked by behaviours that are intended to achieve a larger goal. Instrumental aggression is often carefully planned and usually exists as a means to an end. Hurting another person in a robbery or car-jacking is an example of this type of aggression. The aggressor's goal is to obtain money or a vehicle, and harming another individual is the means to achieve that aim.

Other Forms of Aggression

- *Territorial*– Exclusion of others from a physical space to maintain its individual distance. E.g. New Zealand gannets.
- *Dominance*– Animals remember each other by previous encounters and maintain their status as dominant or submissive.E.g. dogs, monkeys, wolves and birds. Dominance may be linear, triangular and Coalition.
- *Sexual* – Use of threat and physical punishment, usually by males to obtain and retain females called as combat.
- *Parent-offspring* – Disciplinary action against off-springs.
- *Weaning* – Restriction of access of off-springs to milk.
- *Predatory* – Act of predation, possibly includes cannibalism. This is called as aberrant behaviour.
- *Behavioural*-Behaviour between members of the same species shows different aggression such as- violent, resolve conflicts, establish social order, warnings, defence of resources, instil fear in others, communication and defend offspring

Purposes of Aggression

Aggression can serve a number of different purposes, including:

- To express anger or hostility
- To assert dominance
- To intimidate or threaten
- To achieve a goal
- To express possession
- A response to fear
- A reaction to pain
- To compete with others

Factors affecting aggression

Physiological Causes of Aggression

The immediate cause or motivation of an attack by one animal on another lies in the attacker's response to certain cues or stimuli. Such cues can be visual (robins will vigorously attack a bunch of red feathers placed in their territory), auditory (robins will also attack a tape recorder playing the song of another robin), tactile (spiders respond to vibrations set up by rivals entering their web), olfactory (the scent of urine from another male mouse elicits vigorous attack from a territorial male), and even electrical (to deter territorial intruders, gymnotid and mormyrid fish use electrical signals generated by modified muscles). Often full attack is elicited by a combination of such cues. And yet aggression is not an inflexible response inevitably triggered by a particular stimulus or by collections of stimuli. Depending on the internal state of the potential attacker, the same opponent may be attacked on one occasion but ignored on another. In particular, an individual's tendency to attack a rival is influenced by the activity of key structures and pathways in the nervous system and by the levels of particular hormones circulating in the blood.

Neuroendocrine influences

The neuroendocrine mechanisms that generate aggressive responses and modulate the levels of aggression are complex and far from fully understood. They have been best-documented in invertebrates, particularly in lobsters and crayfish, where the neural circuits responsible for the performance of displays during fights have been partially identified. These crustaceans fight readily, and, after a series of interactions between the same individuals, a hierarchical relationship is established whereby the victor consistently takes a dominant posture, with raised legs and forward-directed antennae, while the loser adopts a submissive posture and avoids future fights. The neurohormone serotonin is clearly involved in the control of aggression and dominance, as is octopamine (an invertebrate analog of norepinephrine, or noradrenaline, which in vertebrates acts in response to stressful situations). Serotonin injections cause lobsters to take up the dominant posture, while octopamine injections induce submissive postures. In addition, when the levels of serotonin in subordinate animals are experimentally increased, the willingness of the animals to fight also increases. At least two pairs of serotonin-containing nerve cells have been identified in the central nervous system (CNS). These have connections with the motor neurons responsible for generating dominant and subordinate postures and with the motor neurons promoting more intense attack and escape. How the system is activated varies depending on the social status of the animal concerned; activation of the serotonergic neurons, and the consequent release of serotonin, is facilitated in dominant animals and suppressed in subordinates, probably as a result of input from higher centers in the CNS.

The vertebrate nervous system is significantly more complicated than the invertebrate nervous system, and it is much more difficult in vertebrates to associate specific behavioral functions with particular neural networks. However, research suggests that in mammals, too, the performance of aggressive behavioral patterns, and the modulation of an animal's tendency to fight, are controlled by a hierarchical system of neural structures. Many of these structures are found in the limbic system, that part of the forebrain involved predominantly with emotional behaviour and motivation. The aforementioned neural structures interact with biochemical produced both within and outside the nervous system. For example, in several vertebrate species, electrical stimulation of the midbrain and hindbrain elicits stereotyped and undirected patterns of aggressive behaviour, whereas stimulation of the hypothalamus and the nearby pre-optic region (both found in the forebrain) elicits well-coordinated attacks on other members of the same species. Lesions in these areas reduce aggression. These and other observations imply that the hypothalamus and the pre-optic area of the forebrain are involved in the generation of coordinated aggressive behaviour that are, in turn, produced in lower brain regions. The activity of this system is modulated by higher centers, including areas of the limbic system—specifically the septum, which lies above the hypothalamus and has an inhibitory effect on aggression, and the amygdala, found deep in the temporal lobes and having the opposite effect.

The limbic system is rich in neurons containing serotonin and norepinephrine. Observations suggest that high levels of serotonin are associated with reduced aggressiveness and that high levels of norepinephrine are associated with increased aggressiveness. In a range of vertebrate species, fighting experience has a marked effect on brain biochemistry, especially on the limbic system. For example, in rainbow trout and in lizards, dominant animals show transient activation of the brain serotonin systems, whereas subordinates show longer-term elevation of these systems.

Factors affecting aggression

A) Internal factors:

a. Limbic system – Hypothalamus is involved in defence and escape behaviour e.g. pigeons, cats, monkeys.

b. Hormones – Neurosecretion and hormones like epinephrine are related to physiological arousal. Dopamine or serotonin also may affect aggressiveness. Testosterone also makes males more aggressive during breeding. E.g. elephants 'Mast' stage.

The influence of testosterone

Many vertebrate brain structures involved in the control of aggression are richly supplied with receptors that bind with hormones produced in the endocrine system, in particular with steroid hormones produced by the gonads. In a wide range of vertebrate species, there is a clear relationship between a male's aggressiveness and his circulating levels of androgens such as testosterone, a hormone produced in the testes. From fish to mammals, aggression levels rise and fall with natural fluctuations in testosterone levels. Castration has been found to reduce aggression dramatically, while experimental reinstatement of testosterone—for instance, through injection into the blood—restores aggression. Circulating testosterone can even influence the structures and signals used during fights. In stags the neck muscles needed for effective roaring enlarge under the influence of rising testosterone levels. In male mice the scent of another male's urine, which contains the breakdown products of testosterone, elicits intense aggressive responses.

The close link between aggression and testosterone is not surprising, given that males of many species fight over access to fertile females, but the connection is complex. For instance, the more elaborate the social structure of a species, the less drastic are the effects of castration on aggression. In addition, testosterone of nongonadal origin (i.e., produced by the adrenal gland) may be important in aggression outside the breeding season, as in the case of birds such as the song sparrow that maintain nonbreeding territories in the winter. Furthermore, hormones other than testosterone and its derivatives also may be involved in the modulation of aggression. For example, in several species of mammals and birds, the distribution of the neuropeptide hormones arginine vasotocin (AVT) and arginine vasopressin (AVP) in the pre-optic and septal regions of the brain differs between the sexes. Aggression in males is facilitated by implants of AVT in the limbic system and inhibited by implants of AVP. Finally, while a causal link between circulating testosterone levels and aggression has been well established, it is also clear that the link can work in the opposite direction, with participation in a fight having rapid effects on hormone secretion. In particular, many vertebrates that win fights show increased testosterone levels, while losers exhibit not only reduced levels of testosterone but also elevated levels of the stress hormone cortisol. Changes in hormonal levels in turn modulate future aggressiveness. Such multiple and multidirectional links between brain biochemistry, circulating hormone levels, and aggression are a key part of the mechanisms whereby behaviour in conflict situations is adapted to both past experience and current circumstances.

c. Physical Factors: Epilepsy, dementia, psychosis, alcohol abuse, drug use, and brain injuries or abnormalities can also influence aggression.

B) External factors:

a. Learning and experience – Researchers generally accept that some external factors trigger the aggression in animals. Previous experience can produce semi-permanent changes in the individual and agonistic behaviour is exhibited accordingly as dominant or submissive.

b. Pain and frustration – Noxious stimuli such as noise; injuries etc. cause individuals to attack each other or any objects. Frustration due to limited resources and the high crowding may trigger disruption of the limbic and hormonal control and the individuals become aggressive. Such instances are common in aggregated animals.

c. Xenophobia – crowding (strangers, group size), breeding (cats) and feeding activity (fishes cannibalism) exhibit aggression normally during limited resources are available.

Restraint of aggression

1. Displays – aggressive displays like large antlers of deers, explicit coloration in fish and birds, postures and gestures in fish, mammals, are used to convey aggressive and to avoid conflicts. This behaviour has importance to avoid unnecessary killing or wounding.

2. Evolutionary – aggression and threats take less energy and physical combat are advantageous to individuals and the phenomenon has taken ages in the evolutionary course.

3. Social control and disorganization – Cichlid fish has shown that when strange fish of the same species are continually introduced in the group, fighting remains at a high level. So introduction of strangers disrupts the social control. In other instance when the male or female dominant *Rhesus* monkey dies or leaves the group, aggression within group increases called as social disorganization.

Cannibalism:

- **Cannibalism** is the act of one individual of a consuming all or part of another individual of the same family as food. To consume the same species or show cannibalistic behaviour is a common ecological interaction in the animal kingdom and has been recorded for more than 1,500 species. Human cannibalism is well-documented, both in ancient and recent times.
- Cannibalism seems to be especially prevalent in aquatic ecosystems, in which up to approximately 90% of the organisms engage in cannibalistic activity at some point in their life cycle. Cannibalism is also not restricted to carnivorous species, but can also be found in herbivores and detritivores.

Types of Cannibalism

1. Survival Cannibalism:

In the wild, the animals begin their struggle for survival. They start by eliminating their siblings, thus ensuring themselves of an extra helping of food. Cannibalism is also used to display dominance over other species at times.

e.g. - The golden eagle usually lays two eggs that hatch a few days apart. The one that hatches first turns out to be the stronger one, grabbing on to most of the feed that comes their way. In cases of extreme food shortage, the stronger one does not hesitate in making a meal out of its weaker sibling.

2. Sexual Cannibalism:

Sexual cannibalism as the consumption of a mate before, during or after copulation.

e.g. -Praying mantis. When the female mates on an empty stomach, she happily bites off her mate's head to kill those hunger pangs. However, this doesn't happen as often as it is portrayed to be. Praying mantises are also known to indulge in elaborate courtship rituals, with the males taking considerable efforts to woo the lady. In other instances, the males have been observed to be prudent enough to avoid courting a hungry lady.

3. Filial Cannibalism:

Filial cannibalism is when the parents consume their young ones, or when the adults eat up members of their own species.

e.g. - It is commonly observed in teleost fish families, where the eggs are eaten for their nutritive value, and are seen as an immediate source of energy. It is sometimes done to declutter an unmanageable brood.

Functions and evolution of aggression

Group versus individual selection

The nature of animal aggression, in most cases animals fight over food, shelter, and mates or over territories where these can be found. Therefore, in functional terms, it is easy to explain why animals fight: they do so to gain access to valuable resources. A more difficult question to answer is why conflicts are often resolved conventionally, by displays and threats, rather than by out-and-out fighting. For example, why does a stag, instead of using its antlers in an all-out bid for victory, withdraw from a fight after an exchange of roars, thus leaving its rival in possession of a group of fertile females?

For a long time the generally accepted answer was that animals refrain from engaging in overt fighting because the high level of injury that this can cause is disadvantageous for the species as a whole. According to this view, conventional fighting evolved because groups whose members behaved in this self-sacrificing way did better than, and gradually replaced, groups

in which individuals fought fiercely in their own interest. This "for the good of the species," or group selection, explanation has been rejected by most biologists for two main reasons. The first is that in a group consisting of altruists who fought conventionally, an individual who broke the rules by fighting as fiercely as possible would inevitably win fights, gain resources, and leave many offspring—some of whom would inherit the non-altruist's disposition toward fighting, thus passing on non-altruistic traits to more individuals of future generations. In this way natural selection at the level of the individual would be stronger than selective processes at the group level. Except in highly unusual circumstances, therefore, group selection simply does not explain why the majority of aggressive encounters are settled without recourse to overt fighting. The second reason why the theory has been rejected is that conventional fighting can be explained easily once it is recognized that, in addition to bringing benefits to the winner, aggression imposes costs on both opponents.

Cost-benefit analysis

Current understanding of the functions and evolution of behaviour has been greatly influenced by the economic approach that is central to the discipline of behavioral ecology. In this framework, both the costs and the benefits of particular actions are determined, ultimately in terms of their Darwinian fitness, which is an individual's genetic contribution to the next generation (through production and rearing of offspring) compared with that of other individuals. The cost-benefit analysis is then used to predict how animals should behave during fights in order to maximize their net fitness gains. Thus, the actual behaviour of animals can be compared with the predicted behaviour to see if the positive and negative effects of fighting on fitness have been correctly identified. This is not to suggest that animals make rational calculations about the consequences of their behaviour. Rather, it is assumed that natural selection, acting over thousands of generations, has resulted in the evolution of animals that are able to adjust their behaviour to the circumstances in which fights occur, by mechanisms that may well be unconscious (like the neuroendocrine effects).

The positive consequences for fitness, gaining preferential access to food and shelter and acquiring mates, are easy to specify if not always easy to measure. The negative consequences (or costs) of fighting are not so evident, but they include expenditure of energy and loss of time that might be devoted to other activities. For example, male sparrows that continue to fight over territories after they have acquired a mate neglect the care of their young, which do poorly as a consequence and in a diverse array of species, from crabs to crickets to sage grouse, aggressive displays and intense fighting have been shown to increase rates of aerobic and anaerobic respiration and to deplete energy reserves. Additionally, an important cost of fighting is the risk of injury; the fiercer the fighting, the greater the risk. Putting these adverse effects into the cost-benefit equation has helped to explain many puzzling aspects of animal aggression. These include the fact that subordinate animals accept their low status that animals

sometimes reduce the size of their territory or even abandon it altogether, and that, once a fight does get under way, animals do not always compete to the limit of their capability.

That subordinate animals accept their low status, even though by fighting they may ascend the hierarchy and gain advantages, can be explained in terms of the costs of fighting for the challenger. Subordinate animals are often small or young and are less likely to be able to challenge a dominant animal successfully. Since the fight is likely to be fierce and the risk of injury high, the costs of challenging outweigh the potential benefits of winning. Therefore, the individual fitness of a subordinate animal may be greater if it submits to a rival rather than launching a challenge. If the animals concerned must live in a group in order to survive, as is the case with wolves, then subordinate individuals may be "making the best of a bad job" by accepting long-term subordinate status. On the other hand, dominant individuals pay a high price for their status. Often challenged by rivals that are closer to themselves in size and strength, they must frequently engage in energetic and potentially dangerous fights, which may shorten their tenure as the dominant group member. For example, dominant red deer stags defending large groups of hinds end the breeding season in very poor condition, and they rarely retain their high status for more than a few years. Younger subordinate males, by keeping out of trouble until they become stronger and the dominant animal weaker, may actually increase their chances of ultimately achieving high status, with its accompanying benefits. Subordinate animals may even use tactics other than fighting to gain resources. For example, subordinate red deer stags sneak mating opportunities with fertile females while dominant males are busy fighting each other. In salmon, subordinate juveniles acquire food by foraging at times when their dominant neighbours are satiated. Badges of status, such as the Harris sparrow's black throat and crown feathers, facilitate the process of establishing and maintaining stable hierarchical relationships because only dominant animals can afford to pay the costs of getting involved in fights. In the case of the sparrows, subordinate males whose stripes have been enlarged experimentally are attacked by larger or stronger birds against whom they cannot adequately defend themselves.

Territorial behaviour

Territorial animals sometimes reduce the size of their defended area or even abandon it altogether. e.g., during the winter, pied wagtails are often seen to switch between defending and sharing their feeding territories along riverbanks. Such flexible behaviour can be explained in terms of the shifting balance between the costs and benefits of fighting over space. In brief, animals will defend territories when the distribution of resources and the density of competitors make it economically advantageous for them to do so, but they will abandon territorial defense when this ceases to be the case. This can be seen most clearly in the context of feeding territories, where the benefits gained from ownership (energy taken in) are in broadly the same currency as the costs of defense (energy expended).

The simple graphs shown in the figure 3 illustrate the costs and benefits of defending territories of different sizes. The model assumes that the energetic costs of fighting increase exponentially with the size of the territory because the defended area of a circular territory increases as the square of its radius. It also assumes that the benefits gained level off at larger territory sizes because there is a maximum rate of feeding beyond which animals cannot utilize more food. (Other models assume different shapes for these two curves, thus altering the predictions.) The net gain (or cost) for each territorial size is measured by the distance between the cost and benefit curves, as shown in the figure. The optimum territory size is the one corresponding to the maximum distance between the cost and benefit curves, indicating maximum net gain.

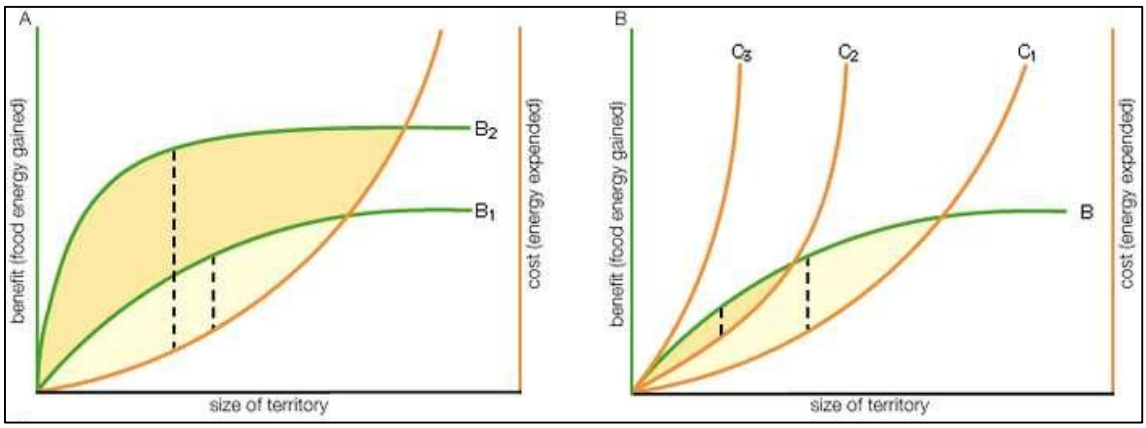

Fig 3: A cost-benefit analysis of territorial behaviour

Graph A shows that an increase in the density of available food (from B1 to B2) shifts the optimal territory size (i.e., the size that maximizes net gain) to the left, which means that owners should reduce the size of their territory. Thus, the model predicts that there should be a reduction in territory size in response to increased availability of food within it—a prediction shown to be true for species ranging from limpets to trout, hummingbirds, and squirrels. On the other hand, the cost of defending a territory of a given size can change; for example, it may increase as the number of individuals competing for a given patch increases.

Graph B in the figure shows that the size of a territory for which the benefits of ownership outweigh the costs of defense (i.e., there is net gain) becomes smaller as the cost of territorial defense increases from C1 to C2. Eventually a territory of any size ceases to be economically defensible (i.e., when C2 increases to C3). Therefore, the model predicts that territorial defense should be abandoned when a certain level of cost has been exceeded. Such an effect has been described for a variety of animal species, including migrating sunbirds defending patches of nectar-rich flowers and salmonid fishes defending feeding sites in streams.

Competition

Competition is a relationship between organisms in which one is harmed when both are trying to use the same resource related to growth, reproduction, or survivability. Competition stems from the fact that resources are limited. There are simply not enough of some resources for all individuals to have equal access and supply. Competition can occur between organisms of the same species, or between members of different species.

Competition between species can either lead to the extinction of one of the species, or a decline in both of the species. However, this process can often be interrupted by environmental disturbances or evolution, which can change the rules of the game. Competition is often involved when species are limited in their range, often by direct competition from other organisms.

Intraspecific Competition

Intraspecific competition is a *density-dependent* form of competition. "Intra" refers to *within* a species, as opposed to "inter" which means *between*. Intraspecific competition can be summed up in the image below (Fig 4).

Fig 4: Intraspecific competition

In this image, two wild dogs known as *Dholes* fight over a carcass. The carcass is a *resource*, something both organisms need to survive. Intraspecific competition is density dependent for one reason. The more *Dholes* you have, the less food each one gets. To the individual *Dhole*, food is everything. With very few predators of their own, the most successful *Dholes* (the ones who survive and reproduce the most) often are simply the ones who eat the most.

Thus, while these *Dholes* may have coordinated to take down this deer, they are now competing to see which one will get to eat first. The one that eats first will get more, and be more likely to survive and reproduce. The other one (or the last one if there are many) will not get as much. This will lower its survivability and the chances it will get to reproduce. Since evolution relies mainly on which organisms reproduce, this form of competition can quickly lead to changes in a population if only a few of the individuals are surviving and reproducing.

Interspecific Competition

Interspecific competition is between individuals which are different species. This could be between any two species, as long as they are competing over a resource. An interesting example of interspecific competition is found in coastal marine environments, like the coral reef in the picture below (Fig 5).

Fig 5: Interspecific competition

In this picture, there are dozens of species. There are several species of fish. Behind them, as a backdrop many people would ignore, is a canvas of dozens of species of coral. Coral, while it may look like some sort of rock or plant, is actually a colony of tiny animals. These tiny animals filter organic material from the water, and use stored bacteria to photosynthesize sunlight for additional energy. Thus, each coral species is competing with not only the other corals, but also with the fish for available nutrients and sunlight.

While corals might not seem like a competitive bunch, they are actually directly competitive with other corals. When an enemy coral is encroaching on their space, they can deploy chemical warfare to counter their rival. Often, coral fights end in one of the corals being killed by the other. While the corals are not *predators* of each other, the competition still ends in the death of one of the corals. The victorious coral was simply fighting for the resources it needs.

Direct and Indirect Competition

There is also another aspect of competition that can be applied to scenarios of limited resources, and that is the idea of direct vs indirect competition. Direct competition is like both of the scenarios above, and there are many more examples of it. Any time two or more animals fight or have a symbolized confrontation, this is probably some sort of competition for a resource.

However, *indirect competition* is when the two animals do not interact, but the presence of both animals in the same territory causes the competition. Think of the fish in the example above. If those fish feed on the same resources used by the corals, then the fish are in competition for the limited resources. Coral, being more or less anchored to the ocean floor, have little chance of directly attacking the fish. Instead, this would be referred to as an *asymmetrical indirect competition*. The fish eat as much of the food as they want, and the coral are limited to scraps. The coral has no way of competing. Luckily for most coral reef systems around the world, the ocean has plenty of food for most.

Outcomes of Competition

Competition is not a static process. Once set in motion, it can go a number of different ways. While the models may show that it will eventually drive one species to extinction, in reality a number of things can happen. First, an environmental disturbance, such as a fire or large wave, can upset the ecosystem and destroy the advantage the best competitor had. Typically, a pinewood forest is made mostly of pine trees because they are the best competitors in the environment. However, after a forest fire the most populous plants are small, opportunistic plants that grow quickly. The fire causes a change in the environment, which completely changes the dynamics of competition.

Further, most competition is also an evolutionary pressure on both parties. Animals from both sides that compete the best are able to survive and reproduce. Thus, over time the competition tends to resolve itself. More often than not, the competition can devolve as the species adapt to use different resources or change the way it uses a resource. This is known as *character displacement*. It is most well-documented in finches. When two different species of finch live on separate islands, their beaks are the same size because they prefer similar seeds. When they

occupy the same island, one of their beaks gets smaller while the other gets larger. This separates the resources they consume and alleviates the competition.

The niche concept

A species' **niche** is its ecological role or "way of life," which is defined by the full set of conditions, resources, and interactions it needs (or can make use of). Each species fits into an ecological community in its own special way and has its own tolerable ranges for many environmental factors. For example, a fish species' niche might be defined partly by ranges of salinity (saltiness), pH (acidity), and temperature it can tolerate, as well as the types of food it can eat.
[Is that the only way to define a niche?]

As we'll see, two organisms with exactly the same niche can't survive in the same habitat (because they compete for exactly the same resources, so one will drive the other to extinction). However, species whose niches only partly overlap may be able to coexist. Also, over long periods of time, they may evolve to make use of more different, or less overlapping, sets of resources.

The Competitive Exclusion Principle (Gause's Law of competitive exclusion)

To explain how species coexist, in 1934 G. F. Gause proposed the competitive exclusion principle: species cannot coexist if they have the same niche. The word "niche" refers to a species' requirements for survival and reproduction. These requirements include both resources (like food) and proper habitat conditions (like temperature, pH). Gause reasoned that if two species had identical niches (required identical resources and habitats) they would attempt to live in the exact same area and would compete for the exact same resources. If this happened, the species that was the best competitor would always exclude its competitors from that area. Therefore, species must at least have slightly different niches in order to coexist.
Peter Grant and colleagues tested Gause's principle by studying seed-eating finches (birds) that live on the Galapagos Islands in the Pacific Ocean. They found that different finch species can coexist if they have traits that allow them to specialize on particular resources. For example, two finch species, *Geospiza fuliginosa* and *Geospiza fortis,* vary in a key trait: beak size. Beak size is a critical trait because it determines the size of a seed that a finch can eat: Individuals with small beaks eat small seeds, individuals with intermediate sized beaks can eat intermediate size seeds and individuals with large beaks can eat large seeds. *G. fuliginosa* and *G. fortis* do compete for intermediate sized seeds because each species has some individuals with intermediate sized beaks. However, *G. fuliginosa* specializes upon smaller seeds because it has more individuals with small beaks. Conversely, *G. fortis* specializes upon larger seeds because

it has more individuals with large beaks. Thus, these species niches differ slightly because a specific trait, beak size, allows them to specialize upon a particular seed size.

Joe Connell also tested Gause's principle by studying barnacles (shelled marine organisms) that live on rocks along European coastlines. In 1961, Connell found that two barnacle species, *Balanus* and *Chthamalus,* can coexist because they differ in two traits: growth rate and vulnerability to desiccation. *Balanu* 's growth is rapid, which allows it to smother and crush the slower-growing *Chthamalus. Balanus,* however, dies close to shore because it gets too dry during low tide. In contrast, *Chthamalus* tolerates these dry conditions. Consequently, even though *Balanus* is a better competitor for space, these barnacles coexist because *Chthamalus* can survive in areas that *Balanus* cannot survive. These and many other examples support the competitive exclusion principle: Species can only coexist if they have different niches.

Dominance

Dominance in the context of biology and anthropology is the state of having high social status relative to one or more other individuals, who react submissively to dominant individuals. This enables the dominant individual to obtain access to resources such as food or access to potential mates, at the expense of the submissive individual, without active aggression. The opposite of dominance is submissiveness. Dominance may be established by fighting, or merely by threatening displays or interchanges; once established, however, dominance relationships may reduce the level of aggression between the individuals concerned.

Dominance may be a purely dyadic relationship, in which case the fact that individual A is dominant over B has no implications for whether or not either of them is dominant over a third individual C. Alternatively, dominance may be hierarchical, with a transitive relationship, so that if A dominates B and B dominates C, A always dominates C. This is called a linear dominance hierarchy or pecking order.

In hierarchical societies, the dominant individual in a group may exert control over others; more commonly, however, decision-taking about the actions of the group is dissociated from social dominance.

In animal societies, dominance is typically variable across time (as individuals age, gain or lose social status, or change their reproductive condition), across space (in territorial animals, territory owners are dominant over all others on their own territory but submissive elsewhere) or across resources. Even with these factors held constant, perfect Dominance hierarchies are rarely found in groups of any size (at least in the wild; dominance hierarchies may be more frequently found in captivity, since they tend to be induced by focused resources such as limited supplies of food supplied in a fixed place). Nonetheless, there are some species

in which clear dominance hierarchies are seen, and in any case establishing the dominance relationships between individuals is the usual first step in describing the social relationships within any animal group.

Dominance is a fact. Nonhuman (and human) animals dominate one another in a number of ways. Individuals may dominate or control

(1) access to various resources including food, potential and actual mates, territory, resting and sleeping areas, and the location in a group that's most protected from predators;

(2) The movements of others; or

(3) The attention of others.

Even if dominance interactions are rare, they do occur, and that is why it's important to log many hours observing known individuals. As one gets to know individuals in a group, he or she also learns more and more about the subtle ways in which a wide variety of social messages are communicated, including those used in interactions in which one individual controls another.

❖ Infanticide

Infanticide is any behaviour that makes a direct and significant contribution to the immediate death of an embryo or newly hatched or born member of the pardoner's own species. Two principal types of infanticides can be distinguished; viz., non-kin infanticide and kin infanticide. The latter type results, in sacrificing shared genes for some presumed compensating benefits to the perpetrator's inclusive fitness. Kin infanticide can be further divided into two categories, viz., parental infanticide (done by the parent) and siblicide (done by a sibling, and sometimes called fatricide or cainism).

S.B. Hardy (1979) has classified infanticide in animals into five categories, viz., exploitation (i.e., cannibalism), resource competition, sexual selection and social pathology. Direct contribution to infanticide indicates that either plain aggression or abusive neglect, or both, are involved. But the death need not be caused by any particular blows. Thus, aggressive intimidation of a nest-mate as an infanticidal act (i.e., siblicide) is included if, as a consequence, the victim starves to death, but the starvation would not be regarded as infanticide if it resulted from aggressive sibling competition.

Infanticide and Cannibalism

Infanticide mayor may not lead to cannibalism. Several cases are known in the animal world where cannibalism is the end and apparently the only purpose of infanticide, and it is not

clearly distinguishable from predation; here we also include 'ovicide', the killing (and eating) of eggs by conspecifics. Many fishes and insects eat their eggs and young ones.

A recent example is that given by RoonwaJ. and Rathore (1975) who observed deacylated brooding termites, microtermes microphagous of the Indian Desert, eating young larvae in the colony: "alates often attack and eat up young larvae, a process which is finished in about 5 minutes. Sometimes the larvae are merely bitten and then left over in dead or dying condition"; it was not possible to say whether the cannibals included the parents of the larvae. Similarly, I have seen the Old-World Desert Locust (*Schistocerca gregaria*) attack and eat the older (fourth and fifth stage) hoppers while they were undergoing moulting; it is possible that the hoppers were eaten not merely for the flesh but also for water. Steiner (1972) reported ground squirrels eating conspecific infants. I have seen a mother rat, *Rattus rattus*, eating young infants front her own litter, and a bitch eating a member of her own litter a few hours after giving birth, in both can apparently due to hunger.

Infanticide in Fishes:

In fishes, intraspecific predation of young stages (eggs, embryos, larvae and juveniles) is of common occurrence. Here, infanticide always results in cannibalism which is far more common than in the terrestrial vertebrates. Several factors have contributed to this situation. Among them, the more important ones are the following:

(i) High fecundity, and the resulting smaller' offspring;

(ii) The predominance of external fertilization, resulting in the deposition of helpless embryo in a potentially hostile environment; and

(iii) Filial cannibalism, which is favoured by the frequent occurrence of parental care of offspring by males in contrast to the general prevalence of care by the mother in most other animal groups (Dominey and Blumer 1984).

Infanticide in the Amphibia:

In the Amphibia to the highest mortality occurs in- the younger stages (eggs and larvae), and the problem has been reviewed by Simon (1984). This is due to various causes, e.g., predation by the vertebrates and the invertebrates, fungal and other infections, competition, disease, starvation, hostile environment (extremes of temperature), etc. In some salamanders and frogs the father guarding the eggs is said to eat them occasionally (filial cannibalism).

Infanticide in Human:

Human infanticide can take many forms ranging from outright physical killing of the infant to its neglect, abuse, etc. It includes destroying the foetus by abortion, or by killing it outright by

piercing the abdomen with a sharp instrument, etc. The infant may be drowned in water, placed in a basket and floated down the river; left alone in a jungle or the middle of a desert to die of starvation or be killed by predators. The subject has been reviewed by several authorities, notably Chandrasekhar (1959, India), Dickeman (1975, 1984), S.B. Hardy. (1979), Harris (1977), Williamson (1978), Scrimshaw (1984), Johansson (1984), Daly and Wilson (1984) and Bugos Jr. and McCarthy (1984).

Infanticide is found in all societies, from the most primitive to the most civilised, throughout the world and over the centuries. Earliest man, in the hunter-gatherer, horticulturist and early agrarian societies, practised it for supposed purposes as diverse as population control and maintenance of the social structure (Dickeman 1975). In London in the 19th century: infants were commonly left abandoned in parks and ditches to die, and similar practices to kill infants were found in many parts of Europe (Scrimshaw 1984). Although infanticide seems to have disappeared from most parts of the world, it is said to be still practised in China. As stated in a large number of cases, human infanticide can be caused in a variety of ways, viz., deliberate killing (sometimes in rituals to propitiate a god, goddess, or to obtain kingship), placing the child in a dangerous situation, "accidental" death, excessive physical punishment, and lowered biological support. Whatever the immediate method or methods used for infanticide, the practice has certain limited biological 'consequences. Among the most obvious of these is population control, particularly where the infant killed is a female. On the whole, however, it may be pointed out that infanticide in man was on so small a scale, compared to the total population in an area, that it probably did not have any large-scale biological consequences, and the various theories advanced to account for infanticide (particularly, comparisons with the non-human primates and other animals) are mostly idle speculations. Infanticide in man is simply a case of social pathology.

Theories of infanticide

a) Establishing social bonds:

This theory was advanced by Sugiyama (1964, 1965a, b, 1966, 1967) on the basis of his observations on the langurs (*Presbytis entellus*) in Dharwar, Southern India. He noted that the loss of the infant stimulated the onset of oestrus in the infants' mother. This situation helped in establishing social bonds between the group of females and the new male.

b) Sexual excitement of males:

Mohnot (1971), who was the first author to eye-witness infant killing, by males In the Hanuman langur, *Presbytis entellus*, in Jodhpur (India), stated that infanticide was induced by sexual excitement. He wrote "The aggressive attitude of the attackers, their sexual excitement (with

the erection of the penis), their probable influence on the oestrus of the females, presentation by the oestrus females towards the invader, subsequent copulations by them and their overstay with the sexually excited females indicates that the probable motive behind the attacks has some relationship with the sexual urge of the 'males. " All the infant deprived females came into oestrus quickly (within 6 days of the death of the infant), and mostly copulated with the killer males.

c) Xenophobia:

The killing of conspecific strangers is widespread among animals and has been reported to occur in several groups of animals, e.g., insects, fowl, geese, wolves, hyenas, primates and man. For example, in the Indian termite, *Anacanthotermes macrocephalus*, when two workers from different colonies are brought together, they often attack each other viciously, and within minutes one of them is seen lying fatally wounded or even cleanly decapitated (Roonwal, personal observations).

d) Dominance assertion:

From inferred cases in the Hanuman langur, *Presbytis entellus*, Parthasarathy and Rahaman (1974), postulated that infanticide was committed by the male to assert. His dominant status. In the red howler monkey, *Alouttaseniculus*, Crockett and Sekulic (1984), who analysed several inferred cases of infanticide, concluded that the infant killers were new or newly dominant males.

e) Sexual selection:

The sexual selection theory was proposed by S.B. Hardy (1974, 1977) to account for infanticide in the Hanuman langur, *Presbytis entellus*, and postulated that it evolve as a resultof male-male competition for gaining reproductive advantage. Following infanticide, the mother often comes into oestrus quickly and copulate with the killer male, a situation which provides support to the theory. On the other hand, the expectation that the attacking male attacks and kills only those infants who were not sired by him, is seldom proved for the simple reason that he cannot possibly recognise his own offspring (even man cannot do so).

g) Resource competition:

On the basis of observations in the New World howler monkey, *Alloutaseniculus*, Rudran (1979 a,b) concluded that increasing male fitness through the reproductive strategy of infanticide (Hardy's Sexual Selection Theory, supra) is not a primary reason of infanticide but merely a secondary consequence. Infanticide is primarily an extension of the process of eliminating unrelated food competitors who do not benefit the invading male in any way. Regulation of population growth is another consequence of infanticide. According to Rudran, the differences in the ratios of benefits to cost of the process may explain the observed differences in this

pattern of social change between the red howler monkey (*Alouatta seniculus*) and other primates (e.g., the langur, *Presbytis entellus*).

h) Population control (density-dependant infanticide) and prevention of inbreeding:

On the basis of data from the Hanuman langur, *Presbytis entellus*, in Dharwar, Sugiyama (1967) suggested that infanticide is the product of high population densities and is a possible mechanism for population control; Eisenberg et al (1972) came to the same conclusion. In man, infanticide probably serves as a means of population control and prevents inbreeding (Dickman 1975, Ripley 1979, 1980). The device serves as a means of ensuring genetic polymorphism in the, tangurs also where the social structure and breeding pattern favour inbreeding (Seger 1977). Boggess (1984) has brought forward several factors which go against this theory.

I)'Following response' induction:

Pirta (1981) emphasised that a wide range of behaviour patterns are employed by males to induce a "following response" in females. A male's glance and movement are sometimes enough for this purpose during consort relations. At other times, a male may clasp, pull and bite a female, as in, the hamadryas baboon, *Papio hamadryas* (Kummert and Kurt 1965). In the Olive baboon, *Papioanubis*, the male stole an infant from a female several times to induce a following response. In extreme cases, a male wound (usually by biting) or even kills an infant, as in the-Hanuman langur (*Presbytis entellus*) or in the Rhesus macaque (*Macaca mulatta*).

j) Parental manipulation:

Infanticide in traditional human societies, in contrast to other primates, appears to be primarily a sort of parental manipulation of their progeny to achieve various ends (Alexander 1974, Dickman 1975, Roonwa11977). The death of an infant and the termination of parental investment will sometimes improve the chances of survival of either the mother or ber older offspring, or will increase the reproductive fitness of both the parents (Hardy and Hausfater 1984).

Probable questions:

1. Write short notes on sexual selection. Give examples.
2. What is intersexual selection and intrasexual selection?
3. Differentiate between intersexual selection and intrasexual selection.
4. Elaborate Bateman’s principle.
5. What is aggression?

6. Discuss different types of aggression.
7. What is impulsive aggression?
8. Discuss various forms of aggression with suitable example.
9. Mention the purposes of aggression.
10. Discuss the role of neuroendocrine influences in aggression.
11. Discuss on internal factors affecting aggressive behaviour.
12. What is xenophobia?
13. What is cannibalism?
14. Discuss different types of cannibalism.
15. Write a detailed discussion on cost-benefit analysis of territorial behaviour.
16. What do you mean by interspecific competition and interspecific competition? Give example.
17. Elaborate the Gause's Law of competitive exclusion.
18. What is the relationship between infanticide and cannibalism?
19. Discuss different theories of infanticide.

Suggested Readings:

1. Alcock, J. (2001). Animal Behaviour: An Evolutionary Approach. , Sinauer Associate Inc., USA.

2. Chattopadhyay, S. (2012). Life: Evolution, Adaptation, Ethology. 3rd Edn. Books and Allied, Kolkata.

3. Dujatkin, L.A. (2014). Principles of Animal Behaviour. 3rd Edn. W.W.Norton and Co.

4. Mandal, F. (2010). A Text Book of Animal Behaviour. Pentice Hall India.

5. Mathur, R. (2005). Animal Behaviour. Rastogi Pub. Meerut.

6. Refinetti, R. (2000). Circadian Physiology. CRC Press, Boca Raton.

7. Ruhela, A. and Sinha, M. (2010). Recent Trends in Animal Behaviour. Oxford Book Co. Jaipur.

Unit VII

Game theory - Models and strategies

Objectives: In this unit you will know about Game theory - Models and strategies.

Introduction

what is game theory? In optimality theory it is assumed that we can predict the best behaviour for a particular (focal) animal irrespective of what others are doing. However, the frequency with which others are performing a particular behaviour is often highly relevant to the fitness consequences of acting a certain way. Thus, the crucial aspect of game theory is that selection among alternative behaviours depends to a large degree on what others are doing. A behaviour that works very well when rare in a population (for instance, some type of deception) may not be nearly as advantageous to its actors when it becomes common. Thus, explorations of game theory involve studies of a form of frequency-dependent selection.

Here's a brief example. Let's return to calling behaviour. Let's assume that females are attracted to calling males and travel to them to mate. But let's also assume that arriving females are not infallible in spotting the male that actually was making the call to which they were attracted. Or, females may be intercepted by males as they approach and sometimes the intercepting males were not the ones that attracted the female in the first place.

What's the best thing for a male to do? If no one is calling, it is probably best to call l and become conspicuous to females interested in mating. However, if there are many callers, it might be best to keep quiet (avoiding energy and predation risk costs) and try to intercept females as they approach the calling male. Such a male is termed a satellite.

As a strategy satellite could well be as or more fit than calling, depending on its frequency relative to callers. Thus, even though a satellite might (per day) have fewer opportunities for mating (lower benefit), the fact that his costs are less means that he might have as many if not more lifetime opportunities for mating. However, as should be obvious, his relative success does not simply reduce to costs and benefits as in optimality theory {instead, his fitness depends very much on the frequency of callers (versus satellites) in the population. If few others call, satellite is probably not a good strategy and relatively speaking, calling is an excellent strategy (or taking it to an extreme, if no one is calling, satellite (simple silence) makes very little sense since females are attracted to calling males). On the other hand, as more and more callers are present, satellite works better and at some frequencies might, over a lifetime payoff better than calling. Thus, frequency dependence distinguishes this example from simple optimality.

Careful observations show that contests occur commonly in numerous and diverse animal taxa, both under water and on land. Because natural resources are limited, individuals cannot avoid either intra-specific or interspecific competition over territory, food items, mating opportunities and other essential resources, regardless of whether they are living solitarily or in groups. Contests in some species are mild, involving only threatening, intimidatory displays, and conventional resolution, whereas contests in other species are more aggressive and may involve many fierce physical interactions, which usually lead to severe injuries or even death. Typically, winning a contest means successfully obtaining or defending a valuable resource item, while losing a contest means being forced to leave and paying a cost. The outcomes of contests have a significant effect on each individual's fitness, which, in a Darwinian sense, refers to an individual's survival and reproductive success. Considering the costs and benefits in a contest, over millions of years of evolution, the most successful characteristics that bring individuals maximal fitness given their living environment will be selected, and spread out in the population. These characteristics include certain kinds of physical features and behavior patterns that individuals routinely exhibit in their competitions with other individuals.

Mathematical models have long been used in the analysis of ecological and biological issues, and have proved to be powerful and useful. The real-world system is abstracted and simplified into a model, and mathematical tools or computer simulations are applied to obtain conclusions. These conclusions then are interpreted in the real-world context, and people compare the predictions with the observed behaviors; sometimes, empirical experiments are done to validate the predictions. Mathematical models can modify our intuition by revealing that apparently sensible assumptions are problematic, or even that seemingly implausible assumptions are quite reasonable. The most striking results often relate to behavior or biological features that are at first sight paradoxical from the conventional evolutionary standpoint. Examples are the classical results of how altruism can evolve, through kin selection or through reciprocal altruism, and the explanation of biological ornaments, such as the peacock's tail, as signals of mate quality. Mathematical models can also reveal different phenomena in different regions of ecological parameter space.

4. Animals Playing Games

As cooler temperatures descend on the Rocky Mountains during the fall, male elk (*Cervus elpahus*) enter a state of heightened sexual activity referred to as "rut." A rutting male spends considerable time calling to attract females into a harem that the male defends from other males. When another male attempts to mate with a female from the harem, the harem holder races in to drive off the interloper. In some cases, these disputes lead to fighting between the

two rival males, and the loser may be wounded during the contest. Even the winner may fare poorly in the long run, as the constant battles to protect his harem often leave him weak and in poor condition at the onset of winter [McCullough 1969]. Animal conflicts also occur over access to food resources. A group of vultures feeding on a dead wildebeest, for example, squabble with one another over access to the carcass. In this case, the resource being contested is food and not access to females, but the rules of the contest are essentially the same. At first, it might seem that we could apply optimality models to animal conflicts by determining the costs and benefits and solving for the optimal strategy. Unfortunately, this is not possible, because the optimal strategy for one individual depends on the behavior of its competitors in the population. Game theory is similar to optimization theory (e.g., the study of optimal foraging) in that it attempts to identify the best strategy based on the costs and benefits of some required resource. There are, however, important differences. Unlike optimization theory, where an individual's reproductive success depends only on its own behavior, game theory involves more than one contestant and a contestant's success depends on the behavior of all the other players.

5. Rules of the Game

In the simplest terms, economic game theory deals with two or more players each attempting to select the best response to the anticipated strategy of their opponents. Evolutionary game theory, however, tends not to focus on individual players, but on strategies available to different categories of individuals (i.e., male vs. females, dominants vs. subordinates, experienced vs. inexperienced, etc.). Each type of player has a set of strategies that it can adopt in response to its opponent's anticipated strategy. Players that adopt the best strategies contribute more offspring to future generations and therefore by definition have higher reproductive fitness. If the best strategy is also heritable, it will become the dominant strategy for this category of player over evolutionary time. In theory, the game stabilizes when all categories of players have adopted their best response to each of their opponents. As Mesterton-Gibbons and Adams [1998] point out, in evolutionary game theory the "best" solution to the contest is the strategy that can be expected to evolve by natural selection. In other words, any possible alternative behavior would yield lower reproductive fitness or else it would have already spread throughout the population. This "best" solution is termed an evolutionary stable strategy (ESS). Informally, an ESS is a strategy that cannot be replaced by any (rare) alternative strategy that appears in the population (i.e., a mutant strategy). An ESS may be pure (consist of a single strategy) or mixed (consist of several strategies in a stable equilibrium). The fitness of a genotype (sometimes) depends on the genetic composition of the population (it is "frequency-dependent"). In the language of game theory, this means that the payoff from a particular strategy depends on the strategies of the other players, so multiplayer game models are needed. In most cases, evolutionary games are played within a single species

by a local population in a specific environment. In addition, game theory models allow an individual to play more than one strategy over time. Multiple strategies allow animals to modify their behavior in different conditions. A harem-holding male elk, for example, would behave differently if it were displaced and had to take on the role of intruder.

6. Types of Evolutionary Games

A game is a model describing the behavioural interactions of two or more individuals whose interests may conflict. In order to model the potentially conflicting interactions between players, we must be precise about

• who is involved in the game,

• what "moves" are possible, and

• how one player's success depends on the behavior (moves) of the other players in the game [Hammerstein 1998].

In other words, a game has

• a set of players or categories of players;

• a strategy set—a list of alternative behaviors or morphologies that each category of player could use; and

• a set of payoffs (in terms of evolutionary fitness) for each possible combination of strategies. Virtually all evolutionary games can be classified based upon four criteria. Different combinations of these four criteria yield different types of games each with its own set of assumptions, methods, and ESSs.

The four criteria are:

• type of strategy set,

• type of player symmetry,

• number of opponents at one time,

• the number of sequential decisions in the game.

A strategy set can include discrete strategies (e.g., fight or flee), continuous strategies (e.g., vary the frequency of a call over a range of possible frequencies), or some combination of behaviors (e.g., produce pheromone A 30% of the time and pheromone B 70% of the time). If the strategy contains only one behavior, it is called a pure strategy. When a player (or category of players)

performs a combination of alternative strategies, the combination is called a mixed strategy. For example, male frogs often call to attract mates. Suppose there are two genotypes for calls and 75% of male frogs always give call A (one genotype) while 25% percent of males always give call B (second genotype). In a second species of frogs, each male can perform each call type and each male uses call A 75% of the time and call B the remaining 25% of the time. Both are examples of a mixed strategy (in each case, females encounter a 3:1 ratio of calls).

Games can also be classified by the type of player symmetry. If the game is symmetrical, then all players use identical strategy sets and players are essentially interchangeable. Two male warblers of identical age and size displaying over access to a suitable nest site would be playing a symmetrical game if each could use the same set of display behaviors. Asymmetrical games, however, are probably more common in animal populations. Asymmetrical games have at least two categories of players, with each category having "access to different alternative strategies, different probabilities of winning with a given strategy, different payoffs when they win with a given strategy, or some combination of these conditions" [Bradbury and Vehrencamp 1998]. Interactions between dominant and subordinate individuals within a social group, or between males and females, are asymmetrical, because the players are not likely to use the same strategy set and there are different fitness payoffs to each type of contestant.

A simple contest involves a player and a single opponent at a time, such as two male yellow warblers disputing the boundaries of a territory. In contrast, n-person games or scrambles involve more than two players at once. Imagine a harem-holding bull elk defending his harem against a series of bachelor males. Here the possible payoffs to the bull depend on the frequency with which alternative strategies are used by the bachelor males. If all bachelors elect to fight, the payoffs are different than if only 40% elect to fight and the other 60% elect a strategy of sneaking copulations when the harem master is otherwise occupied. In other words, payoffs for n-person games are frequency-dependent. A discrete symmetric contest requires a different model from an asymmetric n-person game. For each type of game, there are unique assumptions, different sets of ESSs possible, and different ways of finding those ESSs. In this article. we discuss contests involving two players.

7. Game Theory

Four key elements are necessary for a game. First, a game must have a number of players, who make strategic decisions and obtain rewards or take a penalty accordingly. Players can be specific individuals or randomly drawn from a large population. If these players are specific individuals, then the game is a community game; if they are randomly drawn from a large population, then the game is a population game. In biology we deal with population games, which for our purposes can be regarded as two-player games, in which Player 1 is an arbitrary

focal individual and Player 2 represents every other individual in the population. Second, players may encounter a number of points in a game where they have to take actions from possible choices. The chosen actions in every position are defined as a strategy. A pure strategy specifies what actions to take in every situation. For example, in the so-called Rock-Scissors-Paper or RSP game, the possible pure strategies are “play Rock,”” play Scissors” and” play Paper.” If players take each pure strategy with a certain probability, then such strategies are called mixed strategies. For example, in the RSP game, a player may choose to play Rock half of the time, play Paper a quarter of the time, and play Scissors a quarter of the time. When modelling a real biological scenario, strategies are constrained by the information structure of an interaction. Both mathematical and biological factors must be taken into consideration, which requires strategy sets to be of reasonable size as well as realistic.

Third, a payoff is the measure of how successful a strategy is, and it involves rewards and costs or punishments. In biology, the payoff of a game is some kind of contribution to an individual’s fitness, and thus natural selection leads to the spread of strategies that are associated with the highest payoff. Mathematically, payoffs are given by functions defined on the set of all feasible strategy combinations. When a player is playing a mixed strategy p = (p1, p2, . . .) against another player playing a mixed strategy q = (q1, q2, . . .), where pi or qj is the first or second player’s probability of playing its i-th or j-th pure strategy, we evaluate the expected payoffs to individual players. If aij denotes the payoff to the i-th pure strategy against the j-th pure strategy, then the expected payoff to Player 1 in the game is

E1[p, q] = pAqT

and the payoff to Player 2 is

E2[q, p] = qApT

where A is the payoff matrix, which has aij in row i and column j. In particular, in the RSP game, where Rock beats Scissors, Scissors beats Paper and Paper beats Rock, the payoff matrix for Player 1 is

$$A = \begin{pmatrix} 0 & 1 & -1 \\ -1 & 0 & 1 \\ 1 & -1 & 0 \end{pmatrix}$$

and that of Player 2 is −A. For example, if Player 1 plays strategy p = (1 2 , 1 2 , 0) and player 2 plays strategy q = (1 2 , 0, 1 2), then E1[p, q] = − 1 4 and E2[q, p] = 1 4 = −E1[p, q]. We see that the payoff to a player’s strategy depends on the strategies employed by its opponents.

The last element of a game is the solution concept to the game. The classic concept for games is Nash equilibrium, a strategy that every rational individual in the community should adopt, since no individual will gain more by unilaterally changing to an alternative strategy. In biology, a feature is evolved through thousands of years of natural selection, rather than "chosen" by the will of animals, and there may be several different behaviors that result in the same payoffs.

1. Hawk-Dove game

In the 1970s, Maynard Smith established a framework for modelling dyadic animal contests, in which two individuals are competing over a resource item with value v, and pay the cost c if injured from the conflict. Maynard Smith assumed that there are two kinds of behaviors in contests; one is performing aggressively, referred as" Hawk", the other is referred as" Dove", who simply displays at first and will retreat immediately if its opponent is aggressive. In the original Hawk-Dove game, if both contestants act as Dove, then they share the resource equally or each of them has 50% chance to obtain the whole resource. If one is Dove and one is Hawk, then the Hawk individual obtains the resource and the Dove gets nothing. If both are playing Hawk, then each individual has 50% chance of injury, and 50% chance of winning the contest. The corresponding payoffs to each situation are summarized in Table 2.1. Let p $*$ describe the population strategy,

Table 2.1: Payoffs in the Hawk-Dove game.

Player A \ Player B	Hawk	Dove
Hawk	$(\frac{v-c}{2}, \frac{v-c}{2})$	$(v, 0)$
Dove	$(0, v)$	$(\frac{v}{2}, \frac{v}{2})$

where p * is the probability of a contestant playing Hawk. Consider a mutant that adopts strategy p. The payoff to the mutant is

$$w(p, p^*) = \frac{(1-p^*)(1-p)v}{2} + p(1-p^*)v + \frac{pp^*(v-c)}{2}. \qquad (2.4)$$

From Table 2.1 we can see that the expected payoff to Hawk is always larger than that to Dove if v > c: playing Hawk is the pure ESS if v > c. When v < c, however, w(p, p$*$) obtains its maximum when p = p $*$ = v c . To prove that p $*$ = v c is the ESS, we verify that w(p, p$*$) = w(p

∗ , p∗) and w(p ∗ , p) > w(p, p) for any p 6= v c (by Equation (2.1), (2.2), (2.3) and w(p ∗ , p) − w(p, p) = c(p ∗−p) 2 2 > 0).

2. War-of-Attrition game

Now imagine that two Doves encounter, both displays, and the one that is prepared to display a bit longer gets the reward v. The interactions involved in this contest cause no apparent injuries, but each individual pays a cost proportional to the length of the contest. Costs in this game can be interpreted as opportunity costs for being in the contest, and thus not being able to participate in some other activities. This is the War-of-Attrition game. For simplicity, it assumes that the time an individual prepares to display is determined before the contest and cannot be adjusted by the performance of its opponent during the contest. Some biological examples may fit into the framework of a war of attrition if the accumulation of injuries does typically not affect the basic ability to continue fighting

Let the pure strategy that an individual prepares to display for time $t > 0$ be S_t. Then there can be infinitely many pure strategies. A mixed strategy for an individual is a measure p on $[0, \infty)$given by a density function $p(x)$, that is, the probability that an individual leaves between x and $x+dx$ is $p(x)dx$. For given strategies p and q, the expected payoff to a strategy-p adopter against a strategy-q adopter is

$$E[p,q] = \iint_{(x,y)\in[0,\infty)^2} E[S_x, S_y]dp(x)dq(y) \qquad (2.5)$$

where $E[S_x, S_y]$ is the payoff to an individual playing S_x against an individual playing S_y.

Hammerstein and Parker [45] considered a war of attrition where costs and rewards are differentfor the two players, that is, players are either in state A or in state B. This involves different rewardsfrom competing for the same resource or different costs associated with display. Let w_{IJ} be the probability that Player 1 identifies with state I while Player 2 identifies with state J, for $I, J = A, B$. Players may be in the same state with probability w_{AA} or w_{BB}. Let v_{IJ} be the resource value toPlayer in state I (A or B) when its opponent is in state J (A or B). Let c_{IJ} be the unit cost, where Player 1 is in state I and Player 2 is in state J. A player knows its own state but not that of its opponent. It is assumed that the rewards to an individual depend on its own properties but not that of its opponent, i.e. $v_{AB} = v_{AA} = v_A$, $v_{BA} = v_{BB} = v_B$. A strategy played by the playersis the amount of time that they are willing to spend. The length of a contest is decided by the smaller amount

of time spent by the loser. A payoff is the reward minus the cost. The reward forthe winner of the contest is the resource value; the one that displays longer gets the reward. If both individuals leave at exactly the same time, then the reward will be won by one of them at random. Thus, the payoff function to an individual in state A playing x against an individual in state B playing y is

$$E_{AB}(x,y) = \begin{cases} v_{AB} - c_{AB}y & \text{if } x > y \\ v_{AB}/2 - c_{AB}x & \text{if } x = y \\ -c_{AB}x & \text{if } x < y. \end{cases} \tag{2.6}$$

In this framework, Suppose A is the favored state, that is $\frac{v_A}{c_{AB}} > \frac{v_B}{c_{BA}}$.

Then Hammerstein and Parker [45] proved that the evolutionarily stable strategy for Player A and Player B in thisgame are not pure strategies, but mixed strategies with exponential distribution functions that have separated time domains:

$$p_A(x) = \begin{cases} 0 & \text{if } 0 \le x < s, \\ \frac{c_{AA}}{v_A} \exp(\frac{c_{AA}}{v_A}(s-x)) & \text{if } x \ge s \end{cases} \tag{2.7}$$

and

$$p_B(x) = \begin{cases} \frac{1}{v_B}\frac{w_{BA}c_{BA}+w_{BB}c_{BB}}{w_{BB}} \exp(-\frac{c_{BB}}{v_B}x) & \text{if } 0 \le x < s, \\ 0 & \text{if } x \ge s \end{cases} \tag{2.8}$$

where

$$s = \frac{v_B}{c_{BB}} \ln(\frac{w_{BA}c_{BA} + w_{BB}c_{BB}}{w_{BA}c_{BA}}) \tag{2.9}$$

The existence of exactly one ESS as in (2.7) and (2.8) depends essentially on the assumptions that neither w_{AA} nor w_{BB} equals 0, the asymmetry is payoff-relevant and the cost of attrition rises linearly. From (2.7) and (2.8), we can see that at the ESS, individuals in state A always display longer than individuals in state B. Therefore,

individuals in state *A* always receive the reward. When the contestants are in different states, and so the variability in chosen display times influencesonly conflicts between individuals in the same state: specifically, each of two state-*A* or state-*B* individuals wins with probability 1/2.

Probable questions:

1. What is game theory?
2. Discuss about Hawk and Dove model of game theory.
3. Discuss about war and attrition game.

Suggested Readings:

1. Alcock, J. (2001). Animal Behaviour: An Evolutionary Approach. , Sinauer Associate Inc., USA.

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Unit VIII

Communications: Channels, functions

Objectives: In this unit you will know about Communications; more preciously channels of communications and functions.

Introduction

Communication

Communications is fundamental to the existence and survival of humans as well as to an organization. It is a process of creating and sharing ideas, information, views, facts, feelings, etc. among the people to reach a common understanding. Communication is the key to the directing function of management. A manager may be highly qualified and skilled but if he does not possess good communication skills, all his ability becomes irrelevant. A manager must communicate his directions effectively to the subordinates to get the work done from them properly.

The widest includes any method by which one animal or cell demonstrably influence the behaviour of another (Wilson, 1975), is known as communication.

It is not difficult to select examples of behaviours which obviously qualify as some sort of communication - such as warning cries, or the release of chemical attractants. There is little agreement, however, on a formal definition to cover less obvious cases. The widest includes any method by which one animal (or cell) demonstrably influences the behaviour of another (Wilson, 1975). This is straightforward enough, but it is usual to qualify it in ways related to intuitive notions of functional specialization. The natural world is sustained by one animal eating another: an act of this kind undoubtedly alters the behaviour of both, but is certainly not primarily communicative. Predation can easily be excluded, and for most purposes it is convenient only to consider interactions between members of the same species, although symbiotic relations between different species, and deception of predators by prey, may involve interesting specializations. The behaviour is reasonable to suppose have been selected because of the benefit too both the sender and receiver of the transient messages thus conveyed.

An animal responds to stimuli send out by another. The one who sends out stimuli is the 'Signaller' and one who receives it and respond accordingly is the 'receiver'. There it is a cooperative transform of information from signaller to receiver which is called 'Communication'.

Thus, communication always involves the passage of information. Communication is adaptive for both the sender and receiver of the signal who change information perhaps concerning the location of food sources, presence of threat or their readiness to mate.

Communications Process

Communications is a continuous process which mainly involves three elements viz. sender, message, and receiver. The elements involved in the communication process are explained below in detail:

1. Sender

The sender or the communicator generates the message and conveys it to the receiver. He is the source and the one who starts the communication

2. Message

It is the idea, information, view, fact, feeling, etc. that is generated by the sender and is then intended to be communicated further.

3. Encoding

The message generated by the sender is encoded symbolically such as in the form of words, pictures, gestures, etc. before it is being conveyed.

4. Media

It is the manner in which the encoded message is transmitted. The message may be transmitted orally or in writing. The medium of communication includes telephone, internet, post, fax, e-mail, etc. The choice of medium is decided by the sender.

5. Decoding

It is the process of converting the symbols encoded by the sender. After decoding the message is received by the receiver.

6. Receiver

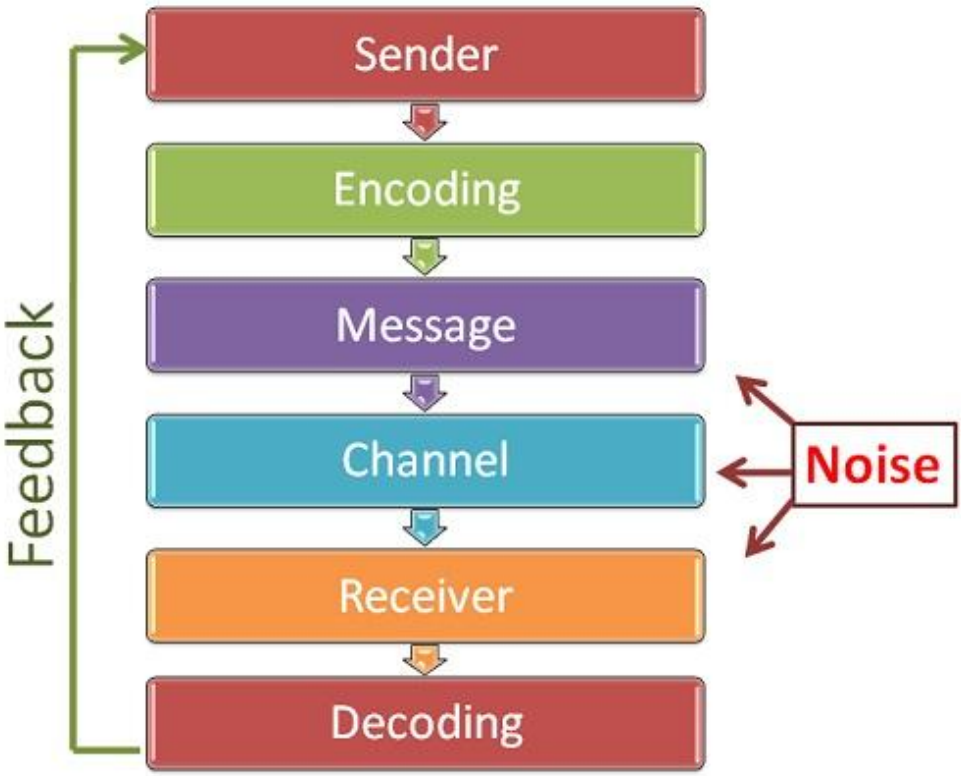
He is the person who is last in the chain and for whom the message was sent by the sender. Once the receiver receives the message and understands it in proper perspective and acts according to the message, only then the purpose of communication is successful.

7. Feedback

Once the receiver confirms to the sender that he has received the message and understood it, the process of communication is complete.

8. Noise

It refers to any obstruction that is caused by the sender, message or receiver during the process of communication. For example, bad telephone connection, faulty encoding, faulty decoding, inattentive receiver, poor understanding of message due to prejudice or inappropriate gestures, etc.



Components of communication

In the simple communication system, a sender encodes and transmit a signal which is detected by receiver who decodes the signal into meaningful terms. The components are –

i. *Sender* - This is sending a message.

ii. *Message* - It is designed communication as information.

iii. *Signal* – It is the physical import of the message.

iv. *Code* – Set of specific language in the signal.

v. *Receiver* – target of the signal.

vi. *Meaning* – is what the receiver makes out of the signal.

Effective of communication is may be influenced by many factors.

1. Error in receptor that may occur as result in
2. Error in detection may also occur in 2 basic ways –
   a. By missing some aspect of signal (missed detection).
   b. By reacting to some other stimuli which are not true stimuli (false alarms)

Influence of physical environment is great in effectiveness of communication. Fundamental problems of transmission of signal, particularly of sound which depends upon physical nature of habitat – attenuation or distortion.

Function of communication (or, why animal communicate)

The over-riding explanatory principle for animal behaviour is that of the theory of evolution, most often expressed not just in terms of the advantage of the individual, but by reference to the individual's genes. Behaviours under genetic control persist only if they optimise transmission of the individual's genes to the next generation. The initial presumption is that of selfishness, and this does not suggest that communicative mechanisms which provide mutual benefit should be at a premium. Communicative activities, if specialized, must entail costs of time, energy and computational effort to both sender and receiver: if both are selfish why do they not confine themselves to securing individual comfort and well-being?

General theories of social behaviour supply two kinds of qualification. The first is **kin-selection**. In genetic terms selfishness can encompass parental care, and indeed more extreme forms of self-sacrifice in return for the survival of sufficient numbers of close relatives. Observations of interactions between parents and young form a substantial part of the discipline of ethology - the approach to the study of animal behaviour which has supplied some of the conceptual framework for analyses of communication. The second qualification is "**reciprocal altruism**", which can be mathematically justified, but is suggested partly because of observations of elaborate social interactions not otherwise readily explicable. The most selfish individual should co-operate with others if its own benefits are increased as a result, which can happen if favours are returned, or if collective activities benefit all co-operators more than cheaters or individualists.

The direct relevance of these corollaries of evolutionary theory to the analysis of animal communication is that they both require some kind of individual and group *recognition*. If parental care is to be justified by kin-selection it ought to be directed at the correct offspring: in relatively solitary species a minor constraint, but a major one in those breeding in colonies or flocks. Where group behaviour is co-operative, recognition of particular individual conspecifics is implied by the rules of reciprocal altruism, while where groups are formed as fewer complex aggregations, presumptively on "safety in numbers" grounds, there needs to be at least a sensitivity to whom else to aggregate with.

1. *Recognition*

A first function for communication is therefore recognition of species: more detailed social interactions for a variety of purposes need to be with conspecifics. Species recognition may be in many cases built in to sensory systems, but in some birds and mammals infants may acquire it from interactions with their parents, this process being known as *imprinting.* Acquired recognition is more obvious when local group

membership is a factor in the details of communication, as for example with local dialects of vocalization (some song-birds) or local variations in group odour (some rodents). Within communities of social insects caste distinctions are typical, and within small groups of birds and mammals there is usually recognition of group members in terms of size, age and gender, and often also, as in the peck-orders of chickens and in other dominance hierarchies, recognition of each individual by any of the others. Generally, it is assumed that animal communicative behaviours are genetically determined, and common to all members of the same species. Paradoxically however, it can be demonstrated in higher vertebrates, if not in insects, that one of the functions of the innate mechanisms is to enable individuals to acquire information which is peculiar to their own circumstances of life, which can include recognition of the identity of unique individuals, and membership of local groups. Even recognition of species membership may be learned, as with imprinting.

2. Courtship and mate selection

Even the most solitary species must engage in the social interactions required for sexual reproduction (parthenogenesis is extremely rare). Evolutionary theorists from Darwin himself onwards have deduced that mate choice is likely to produce via sexual selection effects not predictable from the more humdrum necessities of day-to-day survival. Hence the antlers of the stag and the tail-feathers of the male peacock, and the behaviours that accompany these. Dispersed species must communicate at a distance to bring males and females into proximity. Especially where several similar species co-exist in the same geographical area (not unusual for insects) fine distinctions between species-specific signals need to be made. Some species differences may be very straightforward, as with the different frequencies of calls made by species of cricket which differ in size. In other cases, there appears to be more specialization in the evolution of particular signals, as in the patterning of acoustic messages in some other species of crickets, and more notably in bird song. The flashing of different species of firefly is also distinguished by temporal patterning. A further source of differentiation of species (and individual) recognition in mate selection lies in sequential "handshaking" between members of a pair. Male fireflies typically discriminate females of their own species by the precise time-delay between their own signal and the female's flash in response. Detailed sequential ordering of male-female interactions is common in courtship involving visual recognition, with an example being found in the ethologist Tinbergen's classic studies of the "zig-zag dance" during which a male stickleback leads a female back to his nest for spawning.

The degree of social interaction involved in mate selection varies from a single act of spawning to much more protracted shared parental activities, which are common in birds, where a male and female often pair permanently, or for several seasons, in some species remaining together throughout the year, but in others separating

outside the breeding period. Among mammals similar "pair-bonding" occurs in for instance beavers, when dam construction and/or maintenance is shared, in wolves and other canids, in which food is brought to pregnant or nursing females and young, and gibbons, where the functional reasons for the pairing are less obvious. Clearly methods of individual recognition, and signals for shared activities (e.g. turn-taking in incubation) are necessary in these cases. Sometimes the pair engage in elaborate gestural "ceremonies" during courtship, as in Great Crested Grebes, and these may contribute to both species and individual recognition via the visual modality. Individual recognition by smell is fairly standard in terrestrial mammals, but the use of all the sensory modalities available to any species is to be expected. Striking examples of vocal communication occur in species which use "dueting" or antiphonal singing between mated pairs. In East African shrikes, which forage together in dense foliage, each pair develops some idiosyncratic patterns among the several heard in antiphonal singing, while other patterns represent geographically localized dialects. Of seven species of pair-bonding lesser apes (gibbons and the Siamang) all but one have antiphonal singing between pairs (the other has lengthy all male and all female chorusing between adjacent groups). In two of these six species the male and female contributions never overlap. In the other four they do: in one species the male produces similar sounds to the female but at a lower pitch; but with the rest the male makes limited additions to a more complex female song.

3. *Aggression and Threat*

In deer and peacocks and many other species there is competition among males for access to females, along with male self-advertisement. Much but not all bird song is in this category. Aggression is of course also found in relation to disputes over territory and food. It is communicative in the specialized sense in so far as the interaction in the form of assertive calls and snarls, or gestural displays and postures, as alternatives or additions to direct physical struggle, which is usually the case. In highly social species including wolves and many monkeys' aggressive displays may be finely tuned according to the status of the interacting individuals in the group structure, and gestures of submission and appeasement from subordinates may be as important as those of threat from the more dominant. In these instances, communication is usually over short distances, and indeed may include tactile sensation. Threat between neighbouring groups or between dispersed individuals usually involves greater distances and is well-served by loud vocalization, or territorial scent marking. It has been shown that bird song (even from a loudspeaker) deters other males from entering the vicinity of its location. The roars of the howler monkey can be heard a kilometre away, and may reasonably be assumed to have a territorial function. The elaborate chest-beating display of the male gorilla, which includes hooting, the shaking of branches and the slapping of the ground, is often directed at a neighbouring troop but is also probably related to the maintenance of dominance relations with closer observers.

4. *Alarm and Distress*

A more benign form of social signalling is anything which serves as a warning to others of danger from predators. There is an explanation for pure and simple social aggregation from the benefits to any single individual in terms of predator avoidance. But a selfish individual, on detecting danger, might seem best advised in the short term to make a disguised retreat, and indeed to maximize rather than reduce the disadvantage to its conspecific competitors. In groups with a high degree of consanguinity warning to others, or indeed self-sacrificial defence of them, would be explicable in terms of kin-selection, and this is obviously the case for the social insects (which have single breeding queens for each community, and sterile workers; and, except for termites, have complicated increases in consanguinity because all males come from unfertilized eggs). Lesser degrees of consanguinity may contribute to the prevalence of alarm signals in small groups, particularly in monkeys and apes, where longevity, long-term group coherence and mutual dependence may foster reciprocal altruism. It has been suggested that in other cases giving an alarm call may in fact directly benefit the transmitting individual. That acoustic warnings (as opposed to alarms which elicit defensive aggression by the group) have evolved so as to be difficult to localize indicates that they are not without risk. However, it may to the advantage of an alerted individual to elicit collective evasive action - for instance, an individual bird in a ground feeding flock would probably be unwise to fly off on its own if it sights a hawk. Alternatively, signals given in reaction to predator proximity which are ostensibly altruistic may not be what they seem: it is now believed that the leaping ("stotting") of gazelles when fleeing advertises the vigour and alertness of the fleeing individual to the predator, rather than the presence of the predator to alternative prey. For whatever reason, apparently communicative reactions to predator presence are common, and are sometimes utilized by species other than that of the transmitter. They may differ in the same transmitting species, according to circumstances, including predator type. Mobbing calls in birds differ from other alerting or warning calls, in particular by being easy to localize, and the different cries made by vervet monkeys in response sightings of leopards, hawks, or pythons cause others to run to trees, to look up, or to look down, respectively. It is believed that infants learn the individual meanings of these calls, and experimental evidence suggests that fear of both natural and artificial objects is induced in both primates and birds if the young observe adults making alarm reactions to their presence.

Vocal or chemical signals elicited by fear or pain may function as warning messages, but as a special case, infant distress signals clearly function to elicit parental assistance. In experiments mother rats, cats, and hens react to the vocalization of a misplaced infant by taking steps to retrieve it (the specificity of the response in hens having been demonstrated by the fact that they ignore offspring visible distressed but acoustically isolated under a glass bell). Much less common than signs of distress in infants are "satisfaction messages" but the familiar purring of domestic cats, and

similar sounds produced by only the young of wild carnivores, are sometimes given this description. As these sounds are accompanied by vibrations it is thought they may function to confirm bodily contact.

5. *Signals related to feeding*

The most celebrated example of animal communication is the system by which foraging honey bees convey to their fellow's information about the location of recently used food sources (related bee species, and many species of ant, individuals lay olfactory trails to known food sources which are subsequently used by other colony members). But this kind of specific communication of the location of distant food sources is known only in the social insects. The most general form of communication in this category is simply the visual observation by one animal of another feeding, which has behavioural effects which are readily obvious in seagulls and other flocking birds. There is rarely any suggestion that the feeding animal emits signals with a specialized communicative role. However, among social primates "food finding calls" have been identified, such as the low grunting of the chimpanzee when it discovers highly preferred food such as bananas or palm fruit. Food sharing occurs within chimpanzee groups and the rarity of food-finding signals is undoubtedly related to the fact that feeding is usually competitive. In chimpanzees and baboons there is occasional hunting of the young of other species by males, with its products being shared with females and infants, among whom there is the intuitively understandable begging gesture of holding out the hand, palm upwards. In wolves and other canids meat sharing is routine in family groups, the reactions of the young, such as licking the muzzle of the returning parent, often being required to elicit regurgitation.

A similar pattern is very common in birds, and the interactions between parental feeding and the begging responses of nestlings was an early focus of attention for ethologists. A gaping response, with vocalization, occurs in the nestlings of many species, and the probability of being fed is related to the vigour of the begging. In gulls, nestlings peck at the parental beak to elicit regurgitation, and the precise visual features of the parent's head and beak which act best as stimuli for these pecks have been studied by the use of models. In fowl, where the young feed themselves from birth, there are usually special calls used by both parents to attract the young to food, and these are sometimes also included in courtship feeding of females by males.

6. *Co-ordination of group behaviours*

Flocking, herding and schooling require that individual animals influence one another, but these kinds of co-ordination may occur without the emission of specialized signals, as long as individuals are sensitive to the movements of others. Thus, social facilitation is sometimes distinguished from co-ordination via known communicative mechanisms. However, in most social species, especially of birds and mammals, many more communicative behaviours are observed than can be accounted for in terms of the specific functions of mate selection, alarm calls and so on listed above. There are of course other functional categories, such as group

migration and co-operative hunting, which have not been listed. Another area is juvenile play, in carnivores and primates, which is recognizable from specialized gestures, cries and facial expressions, and which is assumed to have some functions which are related to socialization. But there are also more general categories of behaviour which may serve vaguer functions such as social cohesion and affiliation. Thus, species are said to use, for instance, "greeting displays" and "bonding messages" (vocal chorusing being an example of the latter). As the last point in this section on functions of communication therefore, one should note that although functionality is an underlying assumption it is by no means the rule that the functions of any agreed instance of animal communication can be readily identified.

Types of channels of communication

a. Tactile communication– is important in many animals. Touch is a very basic and faithful channel of communication but its score of transmitting information is limited. Social interaction of many invertebrates depends on tactile communication such as blind workers of termite colony which never leave their underground tunnel. Earthworm also rely on touch when they emerge from their burrow at night. Generally tactile communication operates only at closed range. Some animal has long antennae like cockroach and prawns who explore beyond their body length but those also has a limit. Some animals rely their effective length of touch by sensing mechanical disturbances of the medium by special chemoreceptors.

b. Visual communication– It is found in animal during many events and usual signal operate both at short and long range. Exhibition of immense and magnificent tail with numerous eye spot is a visual signal sent by peacock to peahen.Such a courtship signal operate at a closed range. Remarkable flashing of flies is also visual communication but operate over long distance.

When white tail deer (*Odoceolius sp*.) leap away sensing a potential danger, their tail is elevated, revealing their brilliant and also underside and white hairs of rump. This tail elevation is an alarm signal of white tail deer, an example of visual communication to fellow members of this group.

c. Acoustic Communication– It has been adopted by many insects, amphibians, birds that produce species specific acoustic signal trilling falls of male cricket are actually this signal to female announcing their readiness to mate. Bull frog males call to females by inplating and discharging air from their vocal sac. The females have no problem to distinguish a conspecific males call from a chorus of call in the same habitat. Songs of male birds are complex sounds with notes and phrase which are very species specific.

d. Chemical Communication– It is particularly important in insects, mammals. The chemical messengers known as pheromones are specific for species, used for communication individual of same sp. for various reasons including sex attraction. Female silk worm (*Bombyx mori*) produce pheromone form their gland associated with

their reproductive system called Bombycol. Male silk moth contains numerous sensory receptors for Bombycol on the antennae. This receptor is so extraordinary sensitive to the stimuli that male can respond to a concentration of Bombycol as low as 1 molecule or 10^7 molecules on air. Workers are communicated with other workers are communicated with other workers of their nest by a trail of sent-which is chemical signal. Many larger mammals like tiger, deer, use chemical signal to announce the ownership to territory.

Importance of Communication

1. The Basis of Co-ordination

The manager explains to the employees the organizational goals, modes of their achievement and also the interpersonal relationships amongst them. This provides coordination between various employees and also departments. Thus, communications act as a basis for coordination in the organization.

2. Fluent Working

A manager coordinates the human and physical elements of an organization to run it smoothly and efficiently. This coordination is not possible without proper communication.

3. The Basis of Decision Making

Proper communication provides information to the manager that is useful for decision making. No decisions could be taken in the absence of information. Thus, communication is the basis for taking the right decisions.

4. Increases Managerial Efficiency

The manager conveys the targets and issues instructions and allocates jobs to the subordinates. All of these aspects involve communication. Thus, communication is essential for the quick and effective performance of the managers and the entire organization.

5. Increases Cooperation and Organizational Peace

The two-way communication process promotes co-operation and mutual understanding amongst the workers and also between them and the management. This leads to less friction and thus leads to industrial peace in the factory and efficient operations.

6. Boosts Morale of the Employees

Good communication helps the workers to adjust to the physical and social aspect of work. It also improves good human relations in the industry. An efficient system of communication enables the management to motivate, influence and satisfy the subordinates which in turn boosts their morale and keeps them motivated.

Probable questions:

1. What do you mean by communication?
2. Discuss in details about different types of communication process.
3. Discuss the functions of communication.
4. Discuss different types of channels of communication.
5. Briefly discuss about visual communication.
6. Briefly discuss about acoustic communication.
7. What is the importance of communication?

Suggested Readings:

1. Darwin, C (1872/1965) *The Expression of the Emotions in Man and Animals*. Chicago University Press.

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5. Pinker, S and Bloom, P (1990) Natural language and natural selection. *Behavioural and Brain Sciences, 13*, 707-784.

6. Walker, S.F. (1985) *Animal Thought*. Routledge & Kegan Paul, London.

7. Wilson, E.O. (1975) *Sociobiology*. Harvard University Press: Cambridge, MA

Unit IX

Communications: origin and modification of signal, signal receiving mechanism

Objectives: In this unit you will know about origin and modification of signal, signal receiving mechanism.

Introduction

Origin of Signal:

A communication signal requires two activities (1) a producer capable of generating a receiver capable of detecting it and (2) integrate corporation that is require in communication arose.

One views that most animals are preadapted to generate signal because their normal everyday activity provides potential information. As for e.g. when a water strider walks on water-on-water surface, it creates small waves. It is now known that these waves carrying information about the presence of strider who generates waves to communicate with other.

Raw materials for communication signal may be provided by action of animals conflict behaviour may occur in animals, though it reduce frequency. When two rival's herring gulls meet at the border between their territory, they may attempt to attack and to escape from the opponent. The conflict may cause a male to show pecking behaviour to its opponent (without attack action) or may redirect attack to a set subject (Clump of grass) rather than rival male. Such conflict behaviour has been involved in the origin of certain stereotype behaviour.

Cost and Benefit of signal:

Adaptive value of communication, signal can be analysed by cost and benefit approach. This approach based on assumption that a specific trait with evolve and with spread through a population only when a benefit to its processors exceeds its cost by grater margin that another alternative forms of the trait. Usually such an assumption leads to the expectation that individual with transfer information to other only when they have reproductive interest.

Signal giving is a threat if it is an evolve adaptation, we can expect its benefits to be relatively high and its cost to be relatively low.

Male song birds sing in the spring, one bird may generate several thousand full throated song in a day. The clear substantial cost of singing are time and energy. Moreover, a singer

may provide information through a predator about its location which can be great disadvantage to the singer.

There is straight forward alternative hypothesis on the benefit of signalling by singing. Because the singers are males, the songs are design to defend a breeding site against rival males if then at a distance- the male repulsion theory. Alternatively, song may be designed to attract female through singers- Female attraction hypothesis. The experiment shows some male birds sing to defend territory, yet some sing to attract female, there is also evidence that males of some species do sing at a time when males are most fertile- Male guarding hypothesis. In all cases, singing in males increases their reproductive success.

Signal receiving mechanism

8. *Chemical Signals*

All forms of life must selectively detect and take in chemicals, and so chemical signaling occurs at many levels in all cells. Hormones operate within an organism, pheromones are signals between conspecifics, and allomones are intended for interspecies communication. In this section we will focus on pheromones.

Two different systems of reception are employed for chemical communication. Airborne and waterborne chemicals received at a distance from their source are detected by olfactory reception, or smell. Other chemicals require contact reception, direct contact of the receiver with the source of the pheromone. Pheromones are usually produced by glands located on the skin, or by ducts within the body that have ducts leading to the body's exterior; the second variety of glands are known as exocrine glands. Mammals produce pheromones in the sudiferous (fluid-producing) gland and the sebaceous (waxy-substance producing) gland. Some mammals have other specialized glands. Insects produce a large variety of pheromones, most notably from their mandibular glands, thoracic glands, and stingers.

Some important examples of chemical signalling

A good example of a multipurpose pheromone is the Queen substance employed by some eusocial bees. This pheromone motivates and attracts workers, releases swarming, and is a sex pheromone. Absence of queen substance indicates the colony has grown too large (the queen is too far away to smell her) and so workers will build queen cells to rear new queens. An abrupt absence of Queen substance results in emergency queen rearing, since that absence is probably an indication of the queen's death.

Other examples of chemical signaling include alarm pheromones, such as bee stings. Isopentyl acetate, the chemical injected into a sting wound not only serves to wound an enemy, but to alert other bees to danger, and in some case cause swarming. The reason "killer bees" are so deadly is not because they have a more venomous sting, but rather

because these bees have a lower threshold for alarm pheromone and so an entire swarm will react and sting the enemy to death. Ants and snakes employ trail pheromones to mark the path to a food source. These chemicals are laid out along a trail, and the next ant will follow the trail by means of contact reception. Many animals, from moths to cats, use pheromones to attract mates.

9. Visual Signals

Visual signals are limited because they require a direct line of sight and lighted conditions, and they only last as long as the sender is signaling. However, studies of communication have overemphasized visual communication, most likely because humans and primates are much more dependent on this type of communication than non-primate animals. We will not spend much time on visual signals because we are already familiar with them from our daily lives. The sender can send a signal by performing a display or by assuming a specific body posture. The receiver views the signal by means of eyes, which the brain translates into a visual image. Visual images are received in real-time, and so are generally dynamic signals.

Visual signals allow for a certain amount of cheating; that is, deceptive signals can lure receivers into responding to the benefit of the sender and the detriment of the receiver. Photuris fireflies are the only predatory species of firefly. By mimicking the female response of the prey species, the "femme fatale" Photuris female lures in males, and then preys upon them. Wary males are careful in responding to female displays of their own species for fear of being preyed upon by the Photuris females. In this way, the prey males experience conflicting pressures from natural selection, which demands both individual survival and mating for species survival. This example reveals another problem with visual signals--they are not receiver specific. Any animal can potentially react to the visual signal of any species.

10. Representational Information

Most displays reveal information about the signaler, whether it be fitness, disposition, or location. Representational information imparts information about the environment external to the sender. This is a more complicated form of communication, as it requires first assimilating information about the environment, and then divulging that information to others. The honeybee dance language is an example of representational information, imparting both the distance and the direction from the hive to food. A forager will return from a food source and, by performing a directed series of movements, can inform a second wave of foragers as to the location of the same food source.

11. Acoustic Signals

Acoustic forms of animal communication have greater superficial similarity to human speech than chemical or visual signals. Apart from this, this acoustic channel shares with

olfaction the virtues of broadcast transmission over considerable distances, and availability in the dark or when lines of sight are interrupted. It is arguable that in many species (songbirds, crickets) hearing as a sense must be specialized for the purpose of social communication, as opposed to food seeking and predator avoidance. In others, by contrast, (bats and dolphins) the acoustic sense is utilized as the major avenue for prey detection - by echo-location. This is sometimes considered to be "solipsistic communication", but falls outside the present social perspective. Many other predators (cats, owls) have highly developed hearing which is used for detecting sounds produced by prey.

Acoustic signals are energetically costly, but can travel great distances, degrading with increasing distance. Many animals produce sounds to impart information, however only humans have a well-developed language. There is some evidence that Vervet monkeys have a language consisting of three distinct words: snake, eagle, and leopard. As it turns out, these alarm calls actually represent the type of threat, rather than the specific type of predator. The snake call warns conspecifics of the presence of a slow predator on the ground. Vervets respond to this call by standing up and looking around. The eagle call indicates a fast-flying predator. Vervets will run for cover and look up. The leopard call alerts the monkeys to a fast-running predator, and they respond by running up a tree.

12. Tactile Signals

Physical contact is limited in its ability to communicate because it is extremely short-range. Many invertebrates use antennae as the first line of contact with objects and organisms. The honeybee waggle dance used to explain the location of a food source is often performed in a dark hive, and so the foragers receive their information by interpreting the dance with their antennae. The most common use of tactile communication occurs during copulation. Tactile stimulation by males will often let a female know when to adopt a sexually receptive posture, as in rodents. In primates, grooming is an extremely important social activity. It functions to remove parasites, but also to secure social bonds. This is also true of humans, for whom touch is an intimate form of communication.

13. Electrical Signals

Sharks and some fish have electroreceptors that are used to detect objects and to socially communicate. Electro location is a form of auto communication; signalers send and receive their own signals. The difference between the emitted and received signals yield information about the environment through which the signal has passed. Species that use electrical signals for social communication are nocturnal or inhabit murky waters where visual communication is limited. Electrical signals are useful because they are extremely precise; they are limited to use in aquatic environments, though, because air is ineffective as an electrical insulator or conductor.

Comparison of signal types

As we have seen, a wide variety of signals are used in animal communication. Of course, each has its advantages and disadvantages, and are more useful in certain situations than in others. Otherwise, evolution would have only produced one type. In, we can see a comparison of visual, acoustic, chemical, and tactile signals. Acoustic and chemical signals are useful when obstacles stand between the signaler and the receiver, whole tactile and visual signals are not useful unless there is a clear path. Chemical signals can persist for long periods of time, while other signal types occur in real time, and so are only fleeting messages.

	Visual	Acoustic	Chemical	Tactile
Range	Good	Very Good	Very Good	Poor
Ease of locating source	Very Good	Good	Varies	Very Good
Going around obstacles	Poor	Very Good	Very Good	Poor
Speed	Very Good	Very Good	Poor	Very Good
Persistence	Poor	Poor	Very Good	Poor

Figure: Comparison of Signal Types

Communication and ecology

The analysis of animal communication is directed at discovering its functions, broadly characterised earlier in this article. Variations in modalities used, and in mapping relationships between signals produced and responses elicited by them, should in theory be explicable in terms of the details of the environmental circumstances a given species is adapted to, even though this is only supported by case-by-case examination. The physical environment clearly limits choices of modality in the example of the absence of visual communication in nocturnal species, and more detailed inspection reveals correspondences between, for instance, the precise characteristics of vocalizations and those most appropriate for optimal dispersion in given geographical conditions. The social environment of a species and individuals within it is no less important. For instance, in the example of Bonelli's warbler, above, the fact that transposing its song upwards in pitch slightly severely reduced responses to it, while transformation downwards had little effect, is almost certainly because this species often co-exists with another which has a similar but higher-pitched song. More general comparisons suggest

that both song-birds and forest primates which need to communicate acoustically over relatively short distances, with comparatively little "noise" from other similar species, have a larger species repertoire of calls with greater amounts of individual variation, than those in which group or species members are more widely dispersed, with greater likelihood of between-species confusions.

Probable questions:

1. Write short notes on origin of signals.
2. Discuss about the cost and benefits of signal in communication.
3. How chemical signal helps in receiving the signal in communication?
4. Discuss on the mechanism of receiving Visual Signals.
5. Discuss the importance of acoustic signals in communication.
6. What is tactile signal? Mention the importance of tactile signal in communication.
7. What is electrical signal? Mention the importance of electrical signal in communication.
8. Elaborate the relationship between communication and ecology.

Suggested Readings:

1. Darwin, C (1872/1965) *The Expression of the Emotions in Man and Animals*. Chicago University Press.
2. Halliday, T R and Slater, P J B (1983) *Communication*. Blackwell Scientific Publications, Oxford.
3. Hart, S. (1996) *The Language of Animals*. Henry Holt, New York.
4. Hauser, M.D. (1997) *The Evolution of Communication.* MIT Press, Cambridge, MA
5. Pinker, S and Bloom, P (1990) Natural language and natural selection. *Behavioural and Brain Sciences, 13*, 707-784.
6. Walker, S.F. (1985) *Animal Thought*. Routledge & Kegan Paul, London.
7. Wilson, E.O. (1975) *Sociobiology*. Harvard University Press: Cambridge, MA

Unit X

Evolution of feeding behaviour: optimal foraging theory

Objective: In this unit you will know about evolution of feeding behaviour and optimal foraging theory.

Foraging is searching for wild food resources. It affects an animal's fitness because it plays an important role in an animal's ability to survive and reproduce.[1] Foraging theory is a branch of behavioral ecology that studies the foraging behavior of animals in response to the environment where the animal lives.

Introduction

Foraging Behaviour:

Expression of all behaviour in all animals requires energy. Animals are essentially heterotrophs and food are prime source of energy in animals. Food may be gained in various ways, all of which are together called "Foraging". This behaviour is collectively called Foraging Behaviour.

On the basis of range of food item animals consume, they can be grouped into 2. Those primarily feed exclusively on only one kind of food are called "Specialized", some ant ate only Spider's egg, in contrast those taking many kinds of food are called generalized.

Food finding ability of one species may depend on foraging strategy of another species. Foraging investment of one species may be parasitized by another species Lapwing *Vanelus* sp. feeds on earth worm which are also favourite food for Black Headed Gulls. Gulls often undertake Arial chasing of Lapwing to catch whole of Earthworm but is often expensive. Thus, a Gull often attacks a Lapwing just when it obtains a prey.

Selective Food:

After finding potential food, a forager has to decide which to attack and which to ignore for its own benefit. The first factor that influences a consumer decision in selecting food is Palatability of alternate food. Both herbivore and carnivore foragers select good and less edible food on the basis of potential toxicity. Plant eating animals like leaf cutting ant (*Atta cephalotes*) or Passel-eared squirrel select plenty as their food item, which contain low amount of terpenoids. Terpenoids are toxic substances, which plants incorporate in their leaves to repel consumers. Similarly, a bat chooses a frog rather than a poisonous toad through their calls though. Later is more abundant in locality.

➢ Factors influencing foraging behaviour

Several factors affect an animal's ability to forage and acquire profitable resources.

1. Learning

Learning is defined as an adaptive change or modification of a behavior based on a previous experience.[3] Since an animal's environment is constantly changing, the ability to adjust foraging behavior is essential for maximization of fitness. Studies in social insects have shown that there is a significant correlation between learning and foraging performance.

In nonhuman primates, young individuals learn foraging behavior from their peers and elders by watching other group members forage and by copying their behavior. Observing and learning from other members of the group ensure that the younger members of the group learn what is safe to eat and become proficient foragers **(Figure** 1**)**.

Figure 1**:** A troop of olive baboons (*Papio anubis*) foraging in Laikipia, Kenya. Young primates learn from elders in their group about proper foraging.

One measure of learning is 'foraging innovation'—an animal consuming new food, or using a new foraging technique in response to their dynamic living environment. Foraging innovation is considered learning because it involves behavioral plasticity on the animal's part. The animal recognizes the need to come up with a new foraging strategy and introduce something it has never used before to maximize his or her fitness (survival). Forebrain size has been associated with learning behavior. Animals with larger brain sizes are expected to learn better.[5] A higher ability to innovate has been linked to larger forebrain sizes in North American and British Isle birds according to Lefebvre et al. (1997). In this study, bird orders that contained individuals with larger forebrain sizes displayed a higher amount of foraging innovation. Examples of

innovations recorded in birds include following tractors and eating frogs or other insects killed by it and using swaying trees to catch their prey.

Another measure of learning is spatio-temporal learning (also called time-place learning), which refers to an individual's ability to associate the time of an event with the place of that event. This type of learning has been documented in the foraging behaviors of individuals of the stingless bee species *Trigona fulviventris.* Studies showed that *Trigona fulviventris* individuals learned the locations and times of feeding events, and arrived to those locations up to thirty minutes before the feeding event in anticipation of the food reward.

2. Genetics

Figure 2: A European honey bee extracts nectar. According to Hunt (2007), two genes have been associated with the sugar concentration of the nectar honey bees collect.

Foraging behavior can also be influenced by genetics. The genes associated with foraging behavior have been widely studied in honeybees with reference to the following; onset of foraging behavior, task division between foragers and workers, and bias in foraging for either pollen or nectar. Honey bee foraging activity occurs both inside and outside the hive for either pollen or nectar. Similar behavior is seen in many social wasps, such as the species *Apoica flavissima*. Studies using quantitative trait loci (QTL) mapping have associated the following loci with the matched functions; Pln-1 and Pln-4 with onset of foraging age, Pln-1 and 2 with the size of the pollen loads collected by workers, and Pln-2 and pln-3 were shown to influence the sugar concentration of the nectar collected (**Figure 2)**.

3. Predators and parasites

The presence of predators while a (prey) animal is foraging affects its behaviour. In general, foragers balance the risk of predation with their needs, thus deviating from the foraging behaviour that would be expected in the absence of predators.[9]

Similarly, parasitism can affect the way in which animals forage. Parasitism can affect foraging at several levels. Animals might simply avoid food items that increase their risk of being parasitized, as when the prey items are intermediate hosts of parasites. Animals might also avoid areas that would expose them to a high risk of parasitism. Finally, animals might effectively self-medicate, either prophylactically or therapeutically.[10]

Types of foraging

Foraging can be categorized into two main types. The first is solitary foraging, when animals forage by themselves. The second is group foraging.

1. Solitary foraging

Solitary foraging includes the variety of foraging in which animals find, capture and consume their prey alone. Individuals can manually exploit patches or they can use tools to exploit their prey. For example, Bolas spiders attack their prey by luring them with a scent identical to the female moth's sex pheromones. Animals may choose to forage on their own when the resources are abundant, which can occur when the habitat is rich or when the number of conspecifics foraging are few. In these cases, there may be no need for group foraging. In addition, foraging alone can result in less interaction with other foragers, which can decrease the amount of competition and dominance interactions an animal deals with. It will also ensure that a solitary forager is less conspicuous to predators. Solitary foraging strategies characterize many of the phocids (the true seals) such as the elephant and harbor seals. An example of an exclusive solitary forager is the South American species of the harvester ant, *Pogonomyrmex vermiculatus*. The theory scientists use to understand solitary foraging is called optimal foraging theory, which predicts that foragers alter their behavior (e.g., when to move to the next foraging area) to maximize energy intake. See the book section on "Optimal Foraging Theory" for more information.

2. Group foraging

Group foraging is when animals find, capture and consume prey in the presence of other individuals. In other words, it is foraging when success depends not only on your own foraging behaviors but the behaviors of others as well. An important note here is that group foraging can emerge in two types of situations. The first situation is frequently

thought of and occurs when foraging in a group is beneficial and brings greater rewards known as an aggregation economy. The second situation occurs when a group of animals forage together but it may not be in an animal's best interest to do so known as a dispersion economy. Think of a cardinal at a bird feeder for the dispersion economy. We might see a group of birds foraging at that bird feeder but it is not in the best interest of the cardinal for any of the other birds to be there too **(Figure 3)**. The amount of food the cardinal can get from that bird feeder depends on how much it can take from the bird feeder but also depends on how much the other birds take as well.

Figure 3: A male northern cardinal at a bird feeder. Birds feeding at a bird feeder is an example of a dispersion economy. This is when it may not be in an animal's best interest to forage in a group.

In red harvester ants, the foraging process is divided between three different types of workers: nest patrollers, trail patrollers, and foragers. These workers can utilize many different methods of communicating while foraging in a group, such as guiding flights, scent paths, and "jostling runs", as seen in the eusocial bee *Melipona scutellaris*.

Chimpanzees in the Taï Forest in Côte d'Ivoire also engage in foraging for meats when they can, which is achieved through group foraging. Positive correlation has been observed between the success of the hunt and the size of the foraging group. The chimps have also been observed implying rules with their foraging, where there is a benefit to becoming involved through allowing successful hunters first access to their kills.

- **Cost and benefits of group foraging**

Figure 4: Female lions make foraging decisions and more specifically decisions about hunting group size with protection of their cubs and territory defense in mind.[21]

As already mentioned, group foraging brings both costs and benefits to the members of that group. Some of the benefits of group foraging include being able to capture larger prey, being able to create aggregations of prey, being able to capture prey that are difficult or dangerous and most importantly reduction of predation threat. With regard to costs, however, group foraging results in competition for available resources by other group members. Competition for resources can be characterized by either scramble competition whereby each individual strives to get a portion of the shared resource, or by interference competition whereby the presence of competitors prevents a forager's accessibility to resources. Group foraging can thus reduce an animal's foraging payoff.

Group foraging may be influenced by the size of a group. In some species like lions and wild dogs, foraging success increases with an increase in group size then declines once the optimal size is exceeded. A myriad number of factors affect the group sizes in different species. For example, lionesses (female lions) do not make decisions about foraging in a vacuum (**Figure 4)**. They make decisions that reflect a balance between obtaining food, defending their territory and protecting their young. In fact, we see that lion foraging behavior does not maximize their energy gain. They are not behaving optimally with respect to foraging because they have to defend their territory and protect young so they hunt in small groups to reduce the risk of being caught alone. Another factor that may influence group size is the cost of hunting. To understand the behavior of wild dogs and the average group size we must incorporate the distance the dogs run.

Optimal Foraging Theory:

Introduction

Optimal foraging theory a theory, first formulated in 1966 by R. H. MacArthur and E. R. Pianka, stating that natural selection favours animals whose behavioural strategies maximize their net energy intake per unit time spent foraging. Such time includes both searching for prey and handling (i.e. killing and eating) it. The theory was originally devised in an attempt to explain why, out of the wide range of foods available, animals often restrict themselves to a few preferred types. The prediction is that an animal strikes a balance between two contrasting strategies: spending a long time (i.e. using more energy) searching for highly `profitable' food items, or devoting minimal time (i.e. using less energy) to more common but less profitable food items. Various factors can cause animals to deviate from optimal foraging. For example, the risk of predation may force the animal to select less profitable food items in a relatively safe location, rather than opting for the energetically most efficient feeding strategy.

The absolute limits of the range of food types eaten by a consumer in a given habitat are defined by morphological constraints, but very few animals actually eat all of the different food types they are capable of consuming. Optimal foraging theory helps biologists understand the factors determining a consumer's operational range of food types, or diet width. At the one extreme, animals employing a generalist strategy tend to have broad diets; they chase and eat many of the prey/food items with which they come into contact. At the other extreme, those with a specialist strategy have narrow diets and ignore many of the prey items they come across, searching preferentially for a few specific types of food. In general, animals exhibit strategies ranging across a continuum between these two extremes.

When food differs in size but not in toxicity selecting a larger prey is more profitable because it is likely to contain more calorie. Optimal Foraging is forwarded in the analysis of such foraging behaviour. Optimal theory predicts optimal solution to an ecological problem and optimal solution is that which raise the fitness of an individual more than other alternatives (Fig 5).

Many birds take mussels as their food, Oyster catcher (*Haematopus* sp.) is such a species the mussels larger in size (Greater than 50 mm in length) provide more calorie. Therefore, birds should prefer large mussels as their food. Observation supports that bird select larger than average sized mussel (> 35 mm and < 45 mm) not really the large one. To solve this mystery, it has been found that larger mussels are difficult to break by catchers so it is not beneficial to handle. A very large prey though it is profitable apparently. Again, most large sized mussels are covered by barnacles; each additionally makes them tougher to break open. Thus, considering the factor-

1. Calorie richness and
2. Time wasted to open the mussel, bird select > 30 mm to < 45 mm size in a realistic view.

Not only calorie richness, food selection may also be influenced by predation risk many animals which are easy prey to their predators, are extremely reluctant to travel far from their retreats though there are vast food resources nearby. Eg: *Marmota* sp. are small herbivore mammals. Juvenile marmots are involved and less experienced and are easy prey of eagles. They travel a short distance from the burrows than the adults do and spend more time to look around for approaching predators, than they spend for foraging.

Retozacs made several observations while watching foraging crows.

1. Crows picked up only large Whealks about 3.5 to 4.4 cm long.
2. They flewed up about 5 m to drop their chosen Whealks
3. They kept trying until the Whealks brone, even if more fling were required.

Zacs sound to explain the close behaviour by determining whether the birds' decision was optimal in terms of maintaining whelks available for consumption, per unit of time spend foraging. The optimal hypothesis yields following prediction.

1. Large Whelks should be more likely than small one to shutter after a drop of 5m.
2. Drops of less than 5m should yield a reduced breakage rate whereas drop of much more than 5m should not greatly improve the chance of opening the Whelks. The probability that a Whelks will break should be independent of the number of times, it has already been dropped.

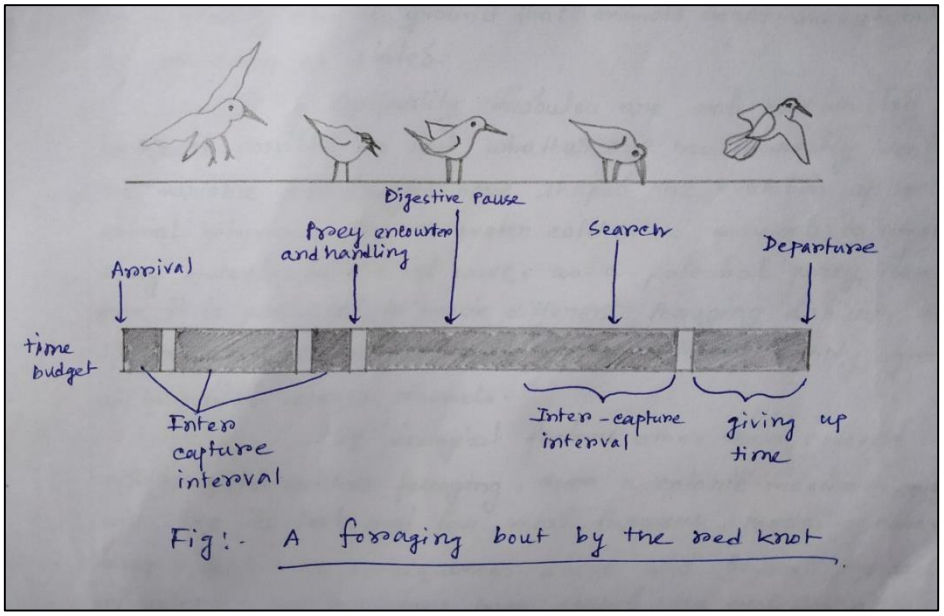


Fig 5

Importance

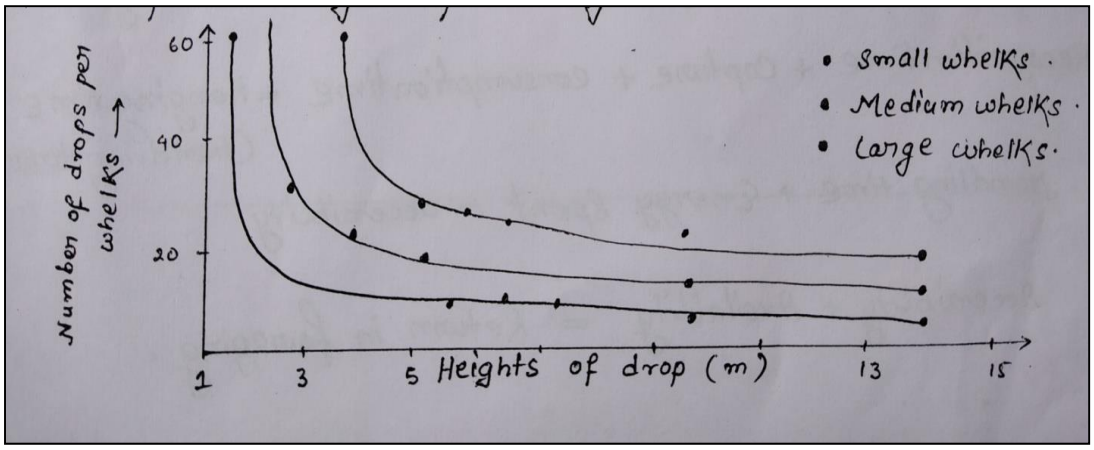
Foraging is critical to the survival of every animal. More successful foragers are assumed to increase their reproductive fitness, passing their genes on into the next generation.

- **Criticism of Optimal Foraging Theory:**

North-western coast and European oyster catchers choose prey that provides maximum calories in relation to time spent in foraging. But some birds have been under controversy, and some researchers have criticized the use of optimality on the ground that animals do not always behave as efficiently as stated.

Optimality modules are not constructed to make it possible to test whether one has correctly identified the variable that might have shaped the evolution of an animal behaviour. If an oyster catcher is assumed to consider every mussel in a tidal wave, as a potential prey item than it is predicted to made different foraging decision than if the modular assumes that oyster catchers simply ignore all barnacles covered mussels.

If ecological factors other than calorie intake effect oyster catcher foraging, then a calorie-maximum model will take its test and for most foragers, foraging behaviour does indeed have consequences above and beyond the acquisition of calorie. The predators have shaped the evolution of an animal's foraging behaviour, then the kind of optimality model you might choose to construct and test could not focus solely on calorie gain vs calorie expenditure. If foraging expresses an animal to risk of sudden death, then when the risk is high, we could expect foragers to sacrifice short term choice of calorie gain for long term survival.



Foraging efficiency is a matter of trade-off between different alternative

Some species spend much energy while foraging but relatively little time. On contrary other species have low foraging energy expenditure but lot of time in foraging. The rate at which energy is spent, depend upon availability and accessibility of food. In fact, foraging efficiency is a matter of trial between priorities. They may include energy gain vs energy expenditure or energy gain vs losing to rivals etc.

In foraging animals, it can be compared with shopping in a supermarket. First and foremost, limit of a shopper is the availability of money equivalent to amount energy spent in foraging. Second limitation is amount of time available to select on item. In

foraging animal this is equivalent to hanging time required to recognize, capture and process the food item. Finding a prey spend upon its availability. If available, an animal needs some time to recognize it. If prey captured successfully, animal take some time to consume it, therefore {Hanging time= (recognition+capture+consumption time)}

Hanging time plus energy of an animal spent to obtain its prey may have accessibility. The returns of an animal obtained per unit time spent in foraging depend on availability and accessibility of the resource.

- Density of prey in environmental= availability.
- Recognition time+capture+consumption time= hanging time or handling time.
- Hanging time + energy spent= accessibility
- Accessibility+ availability= return in foraging

When the prey is profitable for a predator, can be calculated. The net energy value of a prey item is determined by the substitution of energy spent in handling and digestion of prey from gross energy value of the prey.

Net energy/ handling time= profitability.

Animals having a nest or colony, may take capture to home. This type of foraging is called central based foraging and the (distance between central place and foraging area= travel distance)

Net energy value of prey equivalent to-

(Gross energy values- energy spent in handling and digesting).

• Case Studies on Sit and Wait Predator

a. Foraging of Kingfisher

Kingfisher is a sit-and-wait predator (Fig 6) whose optimal foraging strategy is to maximize the energy that it gains during each foraging course. It usually waits for prey to come within a striking distance. It waits in one place for long periods of time and makes decisions regarding when to hunt or when not to hunt the prey that it sees. A major part of this decision depends on how far the prey is from its predator. The Common Kingfisher hunts from a perch above the water, on a branch, beak pointing down as it seeks for prey. When food is detected, it dives steeply down to grab its prey. For example, consider a kingfisher waiting on a perch on a branch, looking down at a river and choosing which fish to go for. For the sake of understanding, let us assume that the pattern of the foraging area as a semicircle around it and the size and behaviour of all the fishes are same. When a kingfisher takes decision to grab a particular fish, it dives from its perch, seizes the fish, and comeback to its perch.

Fig 6: Kingfisher, a sit-and-wait predator

- **Optimal foraging in crows**

Crows (*Corvus caurinus*) in coastal area of Canada feed on shellfish (Fig 7). They hunt for whelks (*Thais lamellosa*) at low tide on the west coast. Having found a whelk, they fly with it in their beak to above a nearby rock.

Fig 7: Flying Crow; natural scavenger

They stall and drop the whelk from the air hence to smash its shell on the rock thereby exposing the flesh inside it. A crow has to drop each whelk several times to break the whelk open.

A crow has to expand its energy to fly upwards. The crows might drop whelks from a height at which the crows would minimize the total upward vertical flight required per whelk eaten. If whelks are dropped from near the ground, many drops are required to break open the shell.

Zach calculated the dropping height that minimizes the total upward vertical height is close to average of 5.2 m. Zach also suggested that greater the height from which a whelk is dropped, the more the chance that the whelk will fragment into small fragments, among those some are too small to retrieve. This may be the reason why the crows usually fly to a height of about 5 to 5.5 m rather than above 10m.

- **Factors affecting the foraging behaviour**

1. The energy spent in waiting and pursuit of the prey depends on the size of the foraging area. Increasing the size of the foraging area decreases the time and energy spent in waiting for a fish (prey) to come into sight as there is a chance of choosing more fishes.
2. Increasing the size of the foraging area increases the average time and energy spent in capturing the prey, because the kingfisher has to fly longer distance to catch its prey.
3. The area of the foraging system determines the abundance of the prey in that ecosystem (More prey in the maximum foraging area) In case of Kingfisher, this means that increasing the size of the foraging area decreases the waiting time of the kingfisher for fish to appear. Waiting time is then proportional to the reciprocal of the total abundance.

Probable questions:

1. What do you mean by foraging behaviour?
2. What do you mean by selective food?
3. Discuss briefly the optimal foraging theory.
4. Discuss optimal foraging theory and its relevance in ecology.
5. Discuss the factors which affects the foraging behaviour.
6. Write down the foraging behaviour of Kingfisher.

Suggested Readings:

1. Alcock, J. (2001). Animal Behaviour: An Evolutionary Approach., Sinauer Associate Inc., USA.

2. Dujatkin, L.A. (2014). Principles of Animal Behaviour. 3rd Edn. W.W.Norton and Co.

3. Mandal, F. (2010). A Text Book of Animal Behaviour. Pentice Hall India.

4. Mathur, R. (2005). Animal Behaviour. Rastogi Pub. Meerut.

5. Refinetti, R. (2000). Circadian Physiology. CRC Press, Boca Raton.

Module	Unit	Content	Credit	Page No.
ZCORT- 411 (Animal Behaviour and Microbiology)	**Group B (Microbiology)**			
	XI	History of microbiology.	**3**	
	XII	Bacteriology: Structure and function of capsule, pili, flagella, cell wall, cell membrane, outer-membrane, chromosome and plasmid		
	XIII	Virology: Structural organization of viruses, Prions and viroids, Lytic cycle of bacteriophages, Lysogeny and lysogeny control, lysogenic conversion, induction and significance.		
	XIV	Animal and Veterinary Microbiology: Microbial interactions with animals (marine and freshwater invertebrates, ruminants), symbiotic light production, sulfide-based mutualism.		
	XV	Disease causing microbes: *Escherichia coli* and *Streptococcus* spp.		
	XVI	Culture techniques: Microbial nutrition and growth; types of culture media, sterilization of culture media; culture techniques: pure cultures.		
	XVII	Industrial microbiology: Microbial fermentation; production and commercialization.		
	XVIII	Mode of transmission, pathogenicity and prevention of microbial diseases: Air-borne (Tuberculosis), Food and waterborne (Typhoid) and Arthropod borne (JE and Yellow fever), SARS-COV 2 (infection and concept of herd immunity).		

	XIX	Mode of transmission, pathogenicity and prevention of microbial diseases: Food and waterborne (Typhoid) and Arthropod borne (JE and Yellow fever), SARS-COV 2 (infection and concept of herd immunity).		
	XX	Mode of transmission, pathogenicity and prevention of microbial diseases: SARS-COV 2 (infection and concept of herd immunity).		
	Total counselling session 18hrs.			

Unit XI

History of microbiology

Objective: In this unit you will know about history of microbiology.

Introduction

Microbiology is a branch of science that deals with the study of diverse groups of microorganisms that include bacteria, fungi, algae and protozoa. It also includes viruses and acellular components that are sometimes considered non-living. The study of microbiology deals with the nutrition and functioning of microorganisms as well as their effect on plants and animals.

Microbiology has been derived from Greek words micros (small), bios (life) and logos (science). The name microbiology indicates that it includes microorganisms that cannot be seen with the naked eye. However, there are some microbes that can be seen with naked eyes such as filamentous algae, bread moulds, mushrooms, and some bacteria.

Microbiology is an exploration of beneficial microbes and it deals with the structure and function of the microorganism. The study of medical microbiology plays a significant role to analyse the microbes in human as well as animal diseases. On the other hand, agricultural microbiology plays an important role in the plant disease that illustrates soil fertilization and discusses the spoilage of agricultural products.

History of Microbiology

- Physics began in ancient times, mathematics even earlier, but the knowledge of tiny living things, their biology, and their impact on human lives have only been around since the late 19th century.
- Until about the 1880s, people still believed that life could form out of thin air and that sickness was caused by sins or bad odors.
- Opinions about why diseases afflicted people differed between cultures and parts of society and the treatments differed as well. Diseases were thought to be caused by
    i. Bad smells, treated by removing or masking the offending odor
    ii. An imbalance in the humor of the body, treated with bleeding, sweating, and vomiting
    iii. Sins of the soul, treated with prayer and rituals
    - Although the concept of contagion was known, it wasn't attributed to tiny living creatures but to bad odors or spirits, such as the devil.

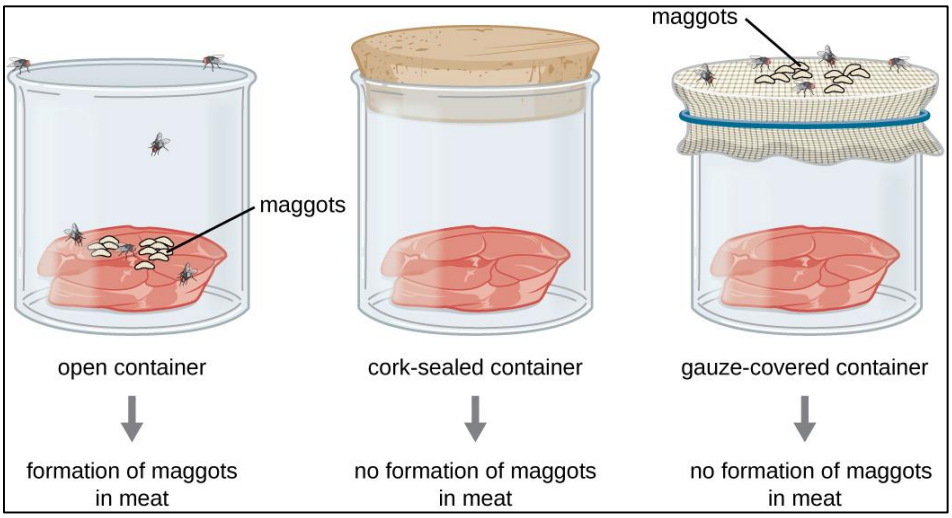
Early history of microbiology

Varo and Columella in the first century BC postulated that diseases were caused by invisible beings (*Animalia minuta*) inhaled or ingested.

Fracastorius of Verona (1546) proposed a *Contagium vivum* as a possible cause of infectious disease and **Von Plenciz (1762)** suggested that each disease was caused by a separate agent.

- **Robert Hooke,** a 17th-century English scientist, was the first to use a lens to observe the smallest unit of tissues he called "cells." Soon after, the Dutch amateur biologist **Anton van Leeuwenhoek** observed what he called "animalcules" with the use of his homemade microscopes.
- **Antonie van Leeuwenhoek (1632-1723)** of Delft, Holland (Netherland) was the first person to observe and accurately describe microorganisms (bacteria and protozoa) called 'animalcules' (little animals) in 1676.
- Actually, he was a Dutch linen merchant but spent much of his spare time constructing simple microscopes composed of double convex lenses held between two silver plates. He constructed over 250 small powerful microscopes that could magnify around 50-300 times.
- Leeuwenhoek was the first person to produce precise and correct descriptions of bacteria and protozoa using a microscope he made himself. Because of this extraordinary contribution to microbiology, **Antonie van Leeuwenhoek is considered as the "Father of microbiology".**
- **Antonie van Leeuwenhoek is also considered to be the father of bacteriology and protozoology (protistology).**

After van Leeuwenhoek died, the study of microbiology did not develop rapidly because microscopes were rare and the interest in microorganisms was not high. In those years, scientists debated the theory of **spontaneous generation**, which stated that microorganisms arise from lifeless matter such as beef broth. This theory was disputed by **Francesco Redi**, who showed that fly maggots do not arise from decaying meat (as others believed) if the meat is covered to prevent the entry of flies.



Redi's experiment

Redi experiment (1665)

To test the **spontaneous generation** hypothesis, **Francesco Redi** placed fresh meat in open containers. As expected, the rotting meat attracted flies, and the meat was soon swarming with maggots, which hatched into flies. When the jars were tightly covered so that flies could not get in [middle, above], no maggots were produced. To answer the objection that the cover cut off *fresh air* necessary for spontaneous generation, Redi covered the jars with several layers of porous gauze instead of an air-tight cover. Flies were attracted to the smell of the rotting meat, clustered on the gauze, which was soon swarming with maggots, *but the meat itself remained free of maggots*. Thus, ***flies are necessary to produce flie***s: they do not arise spontaneously from rotting meat.

Redi went on to demonstrate that dead maggots or flies would *not* generate new flies when placed on rotting meat in a sealed jar, whereas live maggots or flies would. This disproved both the existence of some essential component in once-living organisms, and the necessity of fresh air to generate life.

An English cleric named **John Needham** advanced spontaneous generation, but **Lazzaro Spallanzani** disputed the theory by showing that boiled broth would not give rise to microscopic forms of life.

The Golden age of microbiology

The Golden age of microbiology began with the work of Louis Pasteur and Robert Koch who had their own research institute. More important there was an acceptance of their work by the scientific community throughout the world and a willingness to continue and expand the work. During this period, we see the real beginning of microbiology as a discipline of biology.

Louis Pasteur and the germ theory

Louis Pasteur worked in the middle and late 1800s. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame. Pasteur called attention to the importance of microorganisms in everyday life and stirred scientists to think that if bacteria could make the wine "sick," then perhaps they could cause human illness.

Pasteur had to disprove spontaneous generation to sustain his theory, and he therefore devised a series of **swan-necked flasks** filled with meat broth.

Louis Pasteur is known as the "Father of Modern Microbiology / Father of Bacteriology.

Pasteur boiled a meat broth in a flask that had a long neck that curved downward, like a goose. The idea was that the bend in the neck prevented falling particles from reaching the broth, while still allowing the free flow of air. The flask remained free of growth for an extended period. When the flask was turned so that particles could fall down the bends, the broth quickly became clouded. In detail, Pasteur exposed boiled broths to air

in vessels that contained a filter to prevent all particles from passing through to the growth medium, and even in vessels with no filter at all, with air being admitted via a long tortuous tube that would not allow dust particles to pass. Nothing grew in the broths unless the flasks were broken open, showing that the living organisms that grew in such broths came from outside, as spores on dust, rather than spontaneously generated within the broth. This was one of the last and most important experiments disproving the theory of spontaneous generation.

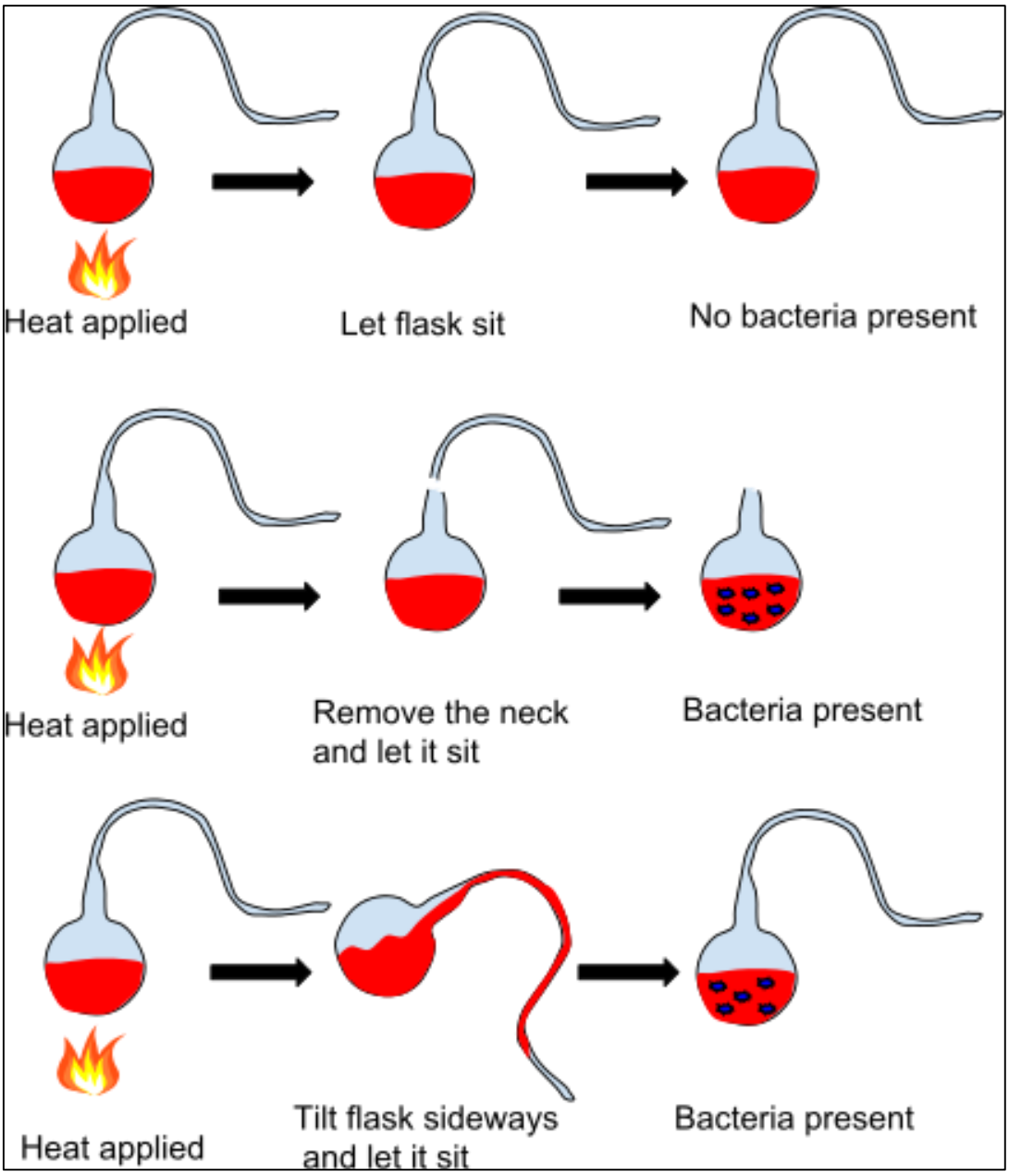


Figure: ***Pasteur's test of spontaneous generation****: By sterilizing a food source and keeping it isolated from the outside, Pasteur observed no putrefaction of the food source (top panel). Upon exposure to the outside environment, Pasteur observed the putrefaction of the food source (bottom panel). This strongly suggested that the components needed to*

create life do not spontaneously arise. Louis Pasteur's pasteurization experiment illustrates the fact that the spoilage of liquid was caused by particles in the air rather than the air itself. These experiments were important pieces of evidence supporting the idea of germ theory of disease. (CC BY-SA 4.0; Kgerow16).

His work also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulated the **germ theory of disease**, which states that microorganisms are the causes of infectious disease.

Pasteur's attempts to prove the germ theory were unsuccessful. However, the German scientist **Robert Koch** provided the proof by cultivating anthrax bacteria apart from any other type of organism. He then injected pure cultures of the bacilli into mice and showed that the bacilli invariably caused anthrax. The procedures used by Koch came to be known as **Koch's postulates**. They provided a set of principles whereby other microorganisms could be related to other diseases.

Koch's four postulates are:

- The organism causing the disease can be found in sick individuals but not in healthy ones.
- The organism can be isolated and grown in pure culture.
- The organism must cause the disease when it is introduced into a healthy animal.
- The organism must be recovered from the infected animal and shown to be the same as the organism that was introduced.

Development in Medicine and Surgery

- Once scientists knew that microbes caused disease, it was only a matter of time before medical practices improved dramatically. Surgery used to be as dangerous as not doing anything at all, but once **aseptic (sterile) technique** was introduced, recovery rates improved dramatically. Hand washing and quarantine of infected patients reduced the spread of disease and made hospitals into a place to get treatment instead of a place to die.
- **Lord Joseph Lister (1827-1912):** A famous English surgeon is known for his notable contribution to the antiseptic treatment for the prevention and cure of wound infections. Lister concluded that wound infections too were due to microorganisms. In 1867, he developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds by the application of phenol on surgical dressings and at times it was sprayed over the surgical areas. He also devised a method to destroy microorganisms in the operation theatre by spraying a fine mist of carbolic acid into the air, thus producing an antiseptic environment. Thus, Joseph Lister was the first to introduce aseptic techniques

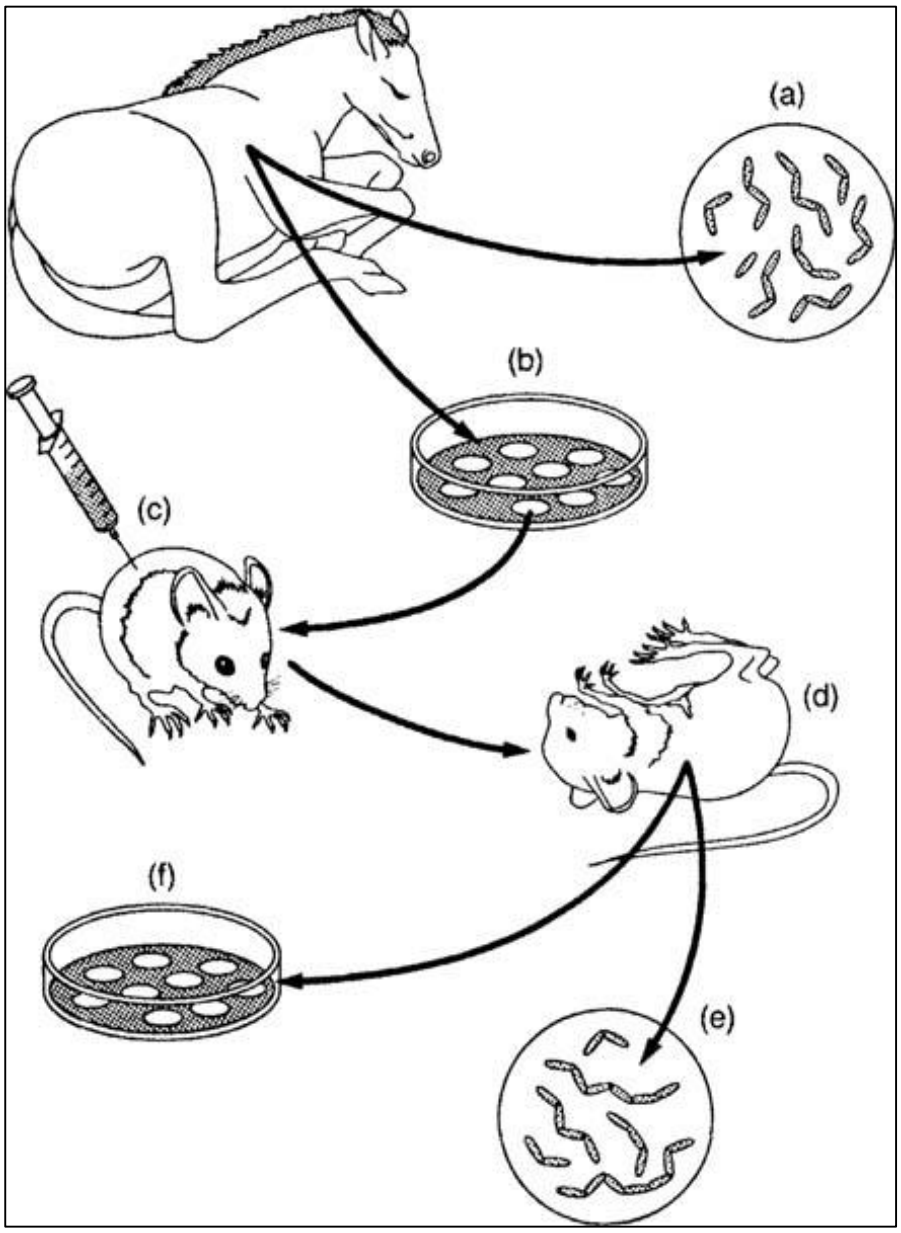
for control of microbes by the use of physical and chemical agents which are still in use today. Because of this notable contribution, **Joseph Lister is known as the Father of Antiseptic surgery.**

Development of Vaccine

- **Vaccination** was discovered before germ theory, but it wasn't fully understood until the time of Pasteur. In the late 18th century, milkmaids who contracted the nonlethal cowpox sickness from the cows they were milking were spared in deadly smallpox outbreaks that ravaged England periodically. The physician **Edward Jenner** used pus from cowpox scabs to vaccinate people against smallpox.
- **Edward Jenner (1749-1823)** an English physician was the first to prevent small pox. He was impressed by the observation that countryside milk maid who contacted cowpox (Cowpox is a milder disease caused by a virus closely related to small pox) while milking was subsequently immune to small pox. On May 14^{th}, 1796 he proved that inoculating people with pus from cowpox lesions provided protection against small pox. Jenner in 1798, published his results on 23 successful vaccinators. Eventually this process was known as vaccination, based on the latin word 'Vacca' meaning cow. Thus the use of cow pox virus to protect small pox disease in humans became popular replacing the risky technique of immunizing with actual small pox material.
- Jenner's experimental significance was realized by Pasteur who next applied this principle to the prevention of anthrax and it worked. He called the attenuated cultures vaccines (Vacca = cow) and the process as vaccination. Encouraged by the successful prevention of anthrax by vaccination, Pasteur marched ahead towards the service of humanity by making a vaccine for hydrophobia or rabies (a disease transmitted to people by bites of dogs and other animals). As with Jenner's vaccination for small pox, principle of the preventive treatment of rabies also worked fully which laid the foundation of modern immunization programme against many dreaded diseases like diphtheria, tetanus, pertussis, polio and measles etc.
- **Elie Metchnikoff (1845-1916)** proposed the phagocytic theory of immunity in 1883. He discovered that some blood leukocytes, white blood cells (WBC) protect against disease by engulfing disease-causing bacteria. These cells were called phagocytes and the process phagocytosis. Thus, human blood cells also confer immunity, referred to as cellular immunity.

Modern microbiology. Modern microbiology reaches into many fields of human endeavor, including the development of pharmaceutical products, the use of quality-control methods in food and dairy product production, the control of disease-causing

microorganisms in consumable waters, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acids, enzymes, and growth supplements. They manufacture many foods, including fermented dairy products (sour cream, yogurt, and buttermilk), as well as other fermented foods such as pickles, sauerkraut, breads, and alcoholic beverages.



One of the major areas of applied microbiology is **biotechnology.** In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood-clotting factors and clotdissolving enzymes, and a number of vaccines. Bacteria can be reengineered to increase plant resistance to insects and frost, and biotechnology will represent a major application of microorganisms in the next century.

Fig: The steps of Koch's postulates used to relate a specific microorganism to a specific disease. (a) Microorganisms are observed in a sick animal and (b) cultivated in the lab. (c) The organisms are injected into a healthy animal, and (d) the animal develops the disease. (e) The organisms are observed in the sick animal and (f) reisolated in the lab.

The development of microbiology. In the late 1800s and for the first decade of the 1900s, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a **Golden Age of Microbiology** during which many agents of different infectious diseases were identified. Many of the etiologic agents of microbial disease were discovered during that period, leading to the ability to halt epidemics by interrupting the spread of microorganisms.

Despite the advances in microbiology, it was rarely possible to render life-saving therapy to an infected patient. Then, after World War II, the **antibiotics** were introduced to medicine. The incidence of pneumonia, tuberculosis, meningitis, syphilis, and many other diseases declined with the use of antibiotics.

Work with viruses could not be effectively performed until instruments were developed to help scientists see these disease agents. In the 1940s, the **electron microscope**was developed and perfected. In that decade, cultivation methods for viruses were also introduced, and the knowledge of viruses developed rapidly. With the development of vaccines in the 1950s and 1960s, such viral diseases as polio, measles, mumps, and rubella came under control.

Scope of microbiology

The scope of microbiology is his duties deliverance and involvement in day-to-day science. The development of microbiology in various fields such as medicine, pharmacy, clinical research, dairy industry agriculture, water industry, and Chemical Technology has been evaluated throughout these years. Thereby in today's science, the microbiologist can make their careers into the research as well as non-research fields of microbes that can level the scope of microbiology and the forecasted future.

What is the importance of microbiology?

Microbiology is one of the most important and effective subsectors of biology. The microbiologist plays a crucial role in combating the disease and creating chemical products for the Welfare of human beings and Agriculture. It significantly also helps to keep the planet healthy in an effective way. Moreover, the microbiologist helps to identify microorganisms that exist in food-related products. The study of microbiology helps to understand the microorganism which is present in the food and that can help to prevent the food from spoilage and can hinder the infection of the food. Thereby it can be said that it makes the food safer, as most scientists use good bacteria against the pathogenic bacteria in order to prevent the contamination of the food.

On the other hand, microbiology in the nursing profession helps to understand the basic concepts of morphology, reproduction, genetics, and biochemical characteristics. It helps to make people aware of the new disease as well as modern molecular identification. That can significantly prevent several diseases of a human being from bacteria. It helps to prevent as well as control in order to respond to the epidemic threats of day-to-day life.

Probable questions

1. Describe the early history of microbiology.
2. State the history golden age of microbiology.
3. Who invented microscope and state the role of microscope in the history of microbiology.
4. What is spontaneous generation?
5. Describe the Pasteur's test of spontaneous generation.
6. Describe the Redi's experiment of spontaneous generation.
7. State Koch's postulate.
8. What is the importance of microbiology?
9. Recently how progression of microbiology is related with biotechnology.
10. Who is the Father of Antiseptic surgery. State the role of Joseph Lister in surgery related to development of microbiology.
11. State the role of Edward Jenner in development of vaccine.
12. State the history of development of vaccine.

Suggested Literature:

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4. Microbiology with diseases by taxonomy. Bauman Robert W. Pearson Education (latest edition).
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6. Engelkirk, P. G., Duben-Engelkirk, J. L., & Burton, G. R. W. (2011). Burton's microbiology for the health sciences. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.
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11. https://www.cliffsnotes.com/study-guides/biology/microbiology/introduction-to-microbiology/a-brief-history-of-microbiology
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Unit XII

Bacteriology: Structure and function of capsule, pili, flagella, cell wall, cell membrane, outer-membrane, chromosome and plasmid

Objective: In this unit we will learn about Structure and function of capsule, pili, flagella, cell wall, cell membrane, outer-membrane, chromosome and plasmid of bacteria.

Introduction

Bacteria are microorganisms of single cells, renowned for their tremendous capacity to adapt and multiply as well as for their ancient history. Some of the earliest recognized fossils are those of bacteria-like organisms— nearly 3.5 billion years old. While some bacteria cause disease and mortality, others are useful or even useful, breaking down or generating antibiotics. Classified by form, there are three types of bacteria:

(i) Spherical (coccus),

(ii) Rod shaped or cylindrical (bacillus), and

(iii) Spiral (spirillum) or spirochetes.

Types of bacteria

i. The Cocus

The coccus bacteria, like a berry, are spherical. The name is actually obtained from the Greek term "kokkos," meaning berry. These are some of the lowest and easiest bacteria with an average diameter of approximately 0.5 to 1.0 micrometers. (A micrometer is approximately 1/1,000,000 per meter.) This classification includes an amount of pathogenic (disease-causing) organisms.

Some instances of cocci are streptococcus, which may trigger strep throat and scarlet fever. Staphylococcus, specifically Staphylococcus aureus, which may trigger food poisoning and toxic shock syndrome; and meningococcus, which may trigger a number of meningococcal illnesses, including epidemic bacterial meningitis.

ii. The Bacillus

The form of the bacillus bacteria is rod-like. These bacteria are a little more complicated than the class of coccus and are 0.5 to 1.0 microns broad by 1.0 to 4.0 microns long on average.

Some of these bacteria are pathogenic, such as Yersinia pestis, which can trigger bubonic and pneumonic plague or the anthrax-causing Bacillus anthracis. But beneficial bacteria, like those used to create antibiotics, as well as those that colonize the human intestinal tract, also belong to this community, helping with digestion.

iii. The Spirochete

The bacteria of spirochete are in the form of a spiral. They appear almost worm-like when regarded under a microscope, wiggling wildly and shifting about. Two of the spirochete family's most well-known representatives are Treponema pallidum, the syphilis-causing bacteria, and Leptospira, which produces leptospirosis.

Beneficial spirochetes include symbiotic spirochetes that enter the stomachs of ruminants such as sheep, cattle, and goats where cellulose and other hard-to-digest plant polysaccharides are converted into nourishing meat and fiber for their host. Beneficial spirochetes also reside in termites' intestines and assist in wood and plant fiber digestion. This enables termites to help remove rotted and diseased wood and discharge organic matter into the soil to enrich its value.

The bacterial cells show a typical prokaryotic organization consisting of the following parts

1. Outer covering,

2. Cytoplasm, and

3. Nucleoid.

1. Outer Covering of the Cell:

The outer covering in most of the bacterial cells consists of the following layers:

(a) Capsule,

(b) Cell wall, and

(c) Plasma membrane.

A. Capsule

An amorphous viscid secretion of bacterial cell is present as a loose undermarketed region outside the cell, called slime layer (e.g., Leuconostoc). But, when it originates as a sharply defined structure outside the cell wall, it is called capsule (e.g., Pneumococcus). The capsule is about 0.2 μm in width.

The capsules those are much narrower than true capsule, called microcapsule (e.g., *Haemophilus influenzae*). Both the layers usually consist of polysaccharide and occasionally polypeptide. The capsule contains 2% solid and 98% water. The solid portion is comprised of complex polysaccharide (e.g., Pneumococcus, Enterobacter) or polype) ide (e.g., anthrax bacillus) or hyaluronic acid (e.g., Streptococcus).

Both the layers serve as a protective covering and protect the cells from antibacterial substances like bacteriophage, enzymes etc. They enhance the virulence of bacteria.

The glycocalyx is an extracellular, hygroscopic network of mostly polysaccharides and occasionally of polypeptide or of both. The chemical components of glycocalyx are synthesized by the cell and transported through the cell membrane and cell wall, and finally deposited outside the cell, to form extracellular covering.

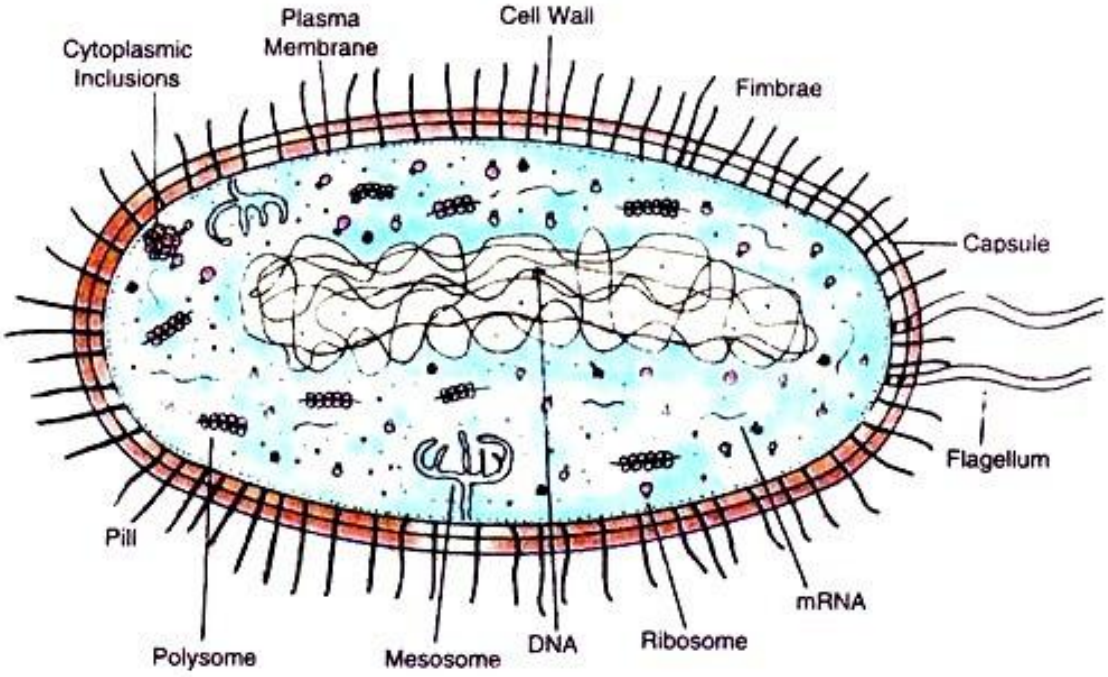


Fig. 4.2 : Structure of a typical bacterial cell (diagrammatic)

In addition to flagella, there occur some hair-like or peg-like outgrowths on the surface of some bacterial cells. These are called pili or fimbriae. They are composed of helically arranged units of special protein called pilin. Pilin helps in the attachment of bacterial cells to some other objects, and in some they act as conjugation channels through which DNA of donor cell moves into the female cell.

- ***Functions of Capsule:***

(i) The capsule may prevent the attachment of bacteriophages.

(ii) It protects the bacterial cells against desiccation as it is hygroscopic and contains water molecules.

(iii) It may survive in natural environment due to its sticky property. After attachment they can grow on diverse surfaces e.g. plant root surfaces, human teeth and tissues (dental carries, respiratory tract), rocks in fast flowing streams, etc.

(iv) They may inhibit the engulfment by WBCs (anti-phagocytic feature) and, therefore, contribute to virulence. Capsule protects from phagocytosis for example the capsulated strains of Streptococcus pneumoniae causes pneumonia and un-capsulated strain is phagocytized.

(v) S. mutans uses its capsules as a source of energy. It breaks down the sugars of capsule when stored energy is in low amount.

(iv) Capsule protects the cell from desiccation, maintains the viscosity and inhibits the movement of nutrients from the bacterial cell.

B. Flagella of Bacteria:

Most of motile bacteria (e.g., Spirochaetes) possess long (5-20 μm), thin (12-30 nm), helical appendages, called flagella. Electron microscopy shows that the flagellum consists of three distinct regions — filament, hook and basal body (Fig. 2.14). Filament is attached at one end through the cell wall to the cell membrane by the hook, which, in turn, is attached to the basal body. The rings of basal body remain attached to the cell membrane and cell wall. The filament lies external to the cell.

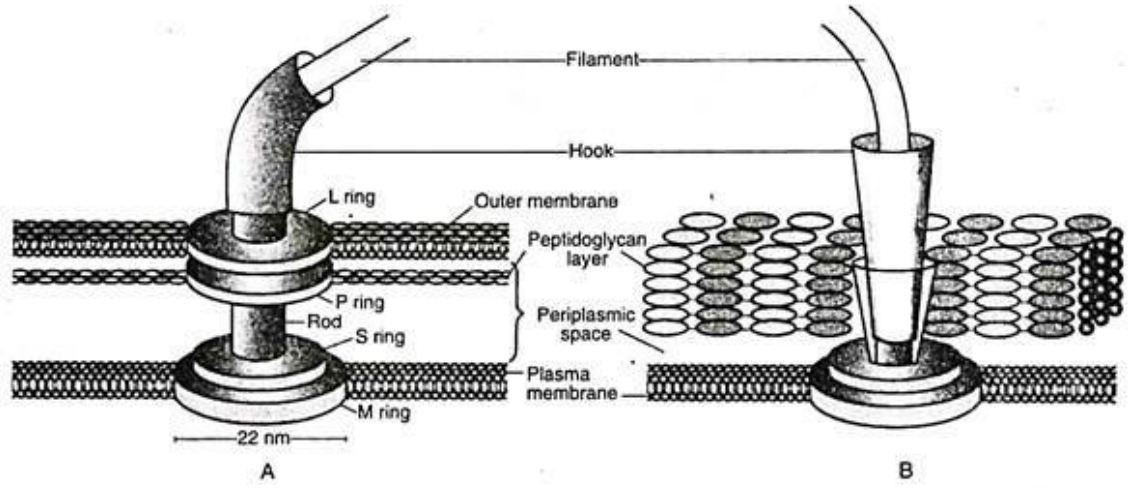


Fig. 2.14 : The ultrastructure of bacterial flagella showing flagellar basal bodies and hooks. A. Gram-negative bacteria and B. Gram-positive bacteria

Filament is made up of identical subunits (3 or more), arranged helically along the axis to give a hollow tube (Fig. 2.15). These subunits are made up of protein molecule, called flagellin. Each subunit is about 4.5 nm thick.

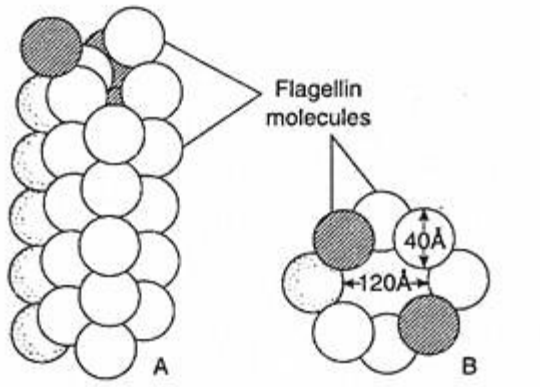


Fig. 2.15 : Structure of filament. A. Arrangement of flagellin molecules showing hollow cylinder and B. T.S. of a filament

The filament ends with a capping protein. Some bacteria have sheath surrounding the flagella (e.g., Vibrio cholerae has a sheath of lipopolysaccharide).

Structurally, the hook and basal body are quite different from the filament (Fig. 2.14). The hook is slightly wider than the filament and made up of protein subunits. The structure of basal body is quite different in gram (-ve) and gram (+ve) bacteria. Gram (-ve) bacteria like *E. coli* and others have 4 rings connected to a central rod. The outer L arid P- rings are associated with the lipopolysaccharide and peptidoglycan layers.

The inner two rings, i.e., S and M rings, are associated with plasma membrane (Fig. 2.14). On the other hand, in gram (+ve) bacteria like Clostridium sporogens, Bacillus subtilis and others, have only two rings attached with the plasma membrane. The structural differences of flagella between gram (-ve) and gram (+ve) bacteria are given in Fig. 2.14.

Some bacteria are devoid of flagella and are non-motile, called atrichous (Fig. 2.16A) e.g., Cocci, Lactobacillus, etc.

The number and arrangement of flagella on a cell are useful for identification and classification of bacteria. On the basis of arrangement of flagella, the bacteria are categorized into the following types (Fig. 2.16):

i. Polar flagellation:

(a) Monotrichous (Fig. 2.16B). Single fla- gellum at one pole of the cell, e.g., Vibrio cholerae.

(b) Amphitrichous (Fig. 2.16C). Each single flagellum is attached at both ends, e.g., Alkaligenes faecalis, Nitrosomonas.

(c) Cephalotrichous (Fig. 2.16D). Two or more flagella at one end only, e.g., Pseudomonas fluorescens.

(d) Lophotrichous (Fig. 2.16E). A tuft of flagella at both ends, e.g., Spirillum volutans.

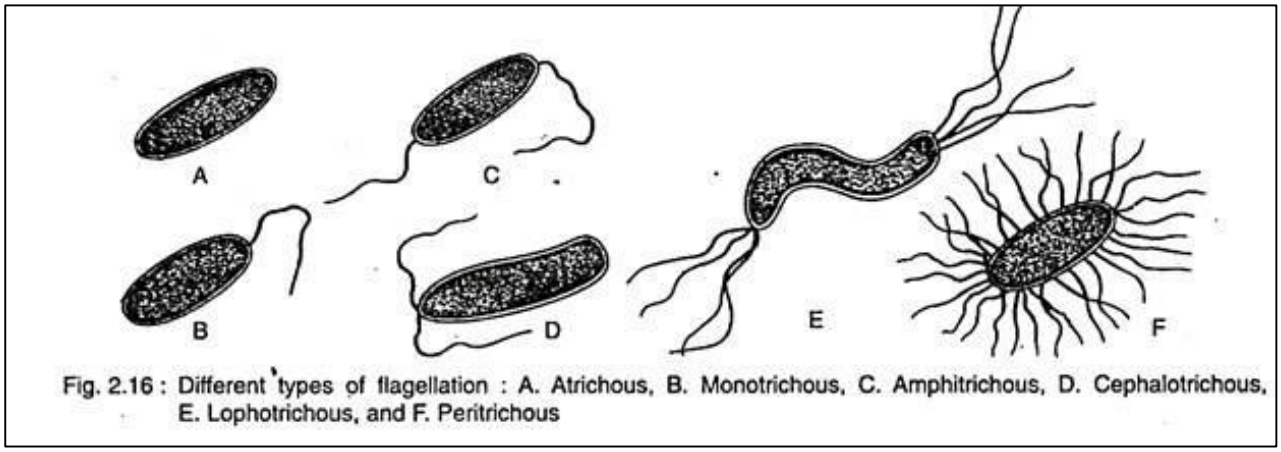


Fig. 2.16 : Different types of flagellation : A. Atrichous, B. Monotrichous, C. Amphitrichous, D. Cephalotrichous, E. Lophotrichous, and F. Peritrichous

ii. Non-polar flagellation:

Peritrichous (Fig. 2.16F). Numerous flagella are distributed all over the surface of the cell e.g., Bacillus typhosus, Clostridium.

C. Fimbriae or Pili:

The term fimbriae (sing, fimbria) were introduced by Duguid et al. (1955) and pili (sing. pilus) by Brinton (1959). Fimbriae are observed mostly in Gram-negative rods (Salmonella typhi, typhoid fever; Shigella dysen- teriae, bacillary dysentery) and also in cocci (Neisseria gonorrhoeae, gonorrhoea). Gram- positive bacilli like *Corynebacterium* sp. also have fimbriae or pili.

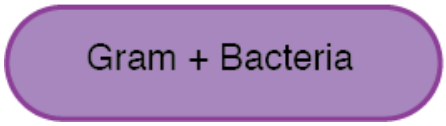
These are extremely thin and short, filamentous, non-flagellar appendages projecting peritrichously from cell surface. Their number is 100-500 per cell and measure 0.5-20 µm in length and 3-25 nm in diameter. They are made up of subunits of protein, the pilin, arranged helically and form hollow filament.

They are antigenic. Pili are of 3 types: Common pili, sex pili or F (fertility) pili and col I (colicin) pili. The common pili are responsible for adhesion; the sex pili (1-5 per cell) help in the transfer of genetic material during conjugation and the col I for colicin or hemolysin production.

D. Cell Walls

It is important to note that not all bacteria have a **cell wall**. Having said that though, it is also important to note that **most** bacteria (about 90%) have a cell wall and they typically have one of two types: a **gram-positive** cell wall or a **gram-negative** cell wall.

The two different cell wall types can be identified in the lab by a differential stain known as the **Gram stain**. Developed in 1884, it's been in use ever since. Originally, it was not known why the Gram stain allowed for such reliable separation of bacterial into two groups. Once the electron microscope was invented in the 1940s, it was found that the staining difference correlated with differences in the cell walls. Here is a website that shows the actual steps of the Gram stain. After this stain technique is applied the gram-positive bacteria will stain purple, while the gram-negative bacteria will stain pink.



- **Overview of Bacterial Cell Walls**

A cell wall, not just of bacteria but for all organisms, is found outside of the cell membrane. It's an additional layer that typically provides some strength that the cell membrane lacks, by having a semi-rigid structure.

Both gram positive and gram-negative cell walls contain an ingredient known as **peptidoglycan** (also known as **murein**). This particular substance hasn't been found anywhere else on Earth, other than the cell walls of bacteria. But both bacterial cell wall types contain additional ingredients as well, making the bacterial cell wall a complex structure overall, particularly when compared with the cell walls of eukaryotic microbes. The cell walls of eukaryotic microbes are typically composed of a single ingredient, like the cellulose found in algal cell walls or the chitin in fungal cell walls.

The bacterial cell wall performs several functions as well, in addition to providing overall strength to the cell. It also helps maintain the cell shape, which is important for how the cell will grow, reproduce, obtain nutrients, and move. It protects the cell from **osmotic lysis**, as the cell moves from one environment to another or transports in nutrients from its surroundings. Since water can freely move across both the cell membrane and the cell wall, the cell is at risk for an osmotic imbalance, which could put pressure on the relatively weak plasma membrane. Studies have actually shown that the internal pressure of a cell is similar to the pressure found inside a fully inflated car tire. That is a lot of pressure for the plasma membrane to withstand! The cell wall can keep out certain molecules, such as toxins, particularly for gram negative bacteria. And lastly, the bacterial cell wall can contribute to the pathogenicity or disease –causing ability of the cell for certain bacterial pathogens.

- ## Structure of Peptidoglycan

Peptidoglycan is a polysaccharide made of two glucose derivatives, **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**, alternated in long chains. The chains are cross-linked to one another by a **tetrapeptide** that extends off the NAM sugar unit, allowing a lattice-like structure to form. The four amino acids that compose the tetrapeptide are: **L-alanine, D-glutamine, L-lysine or *meso*-diaminopimelic acid (DPA),** and **D-alanine**. Typically, only the L-isomeric form of amino acids is utilized by cells but the use of the mirror image D-amino acids provides protection from proteases that might compromise the integrity of the cell wall by attacking the peptidoglycan. The tetrapeptides can be **directly cross-linked** to one another, with the D-alanine on one tetrapeptide binding to the L-lysine/ DPA on another tetrapeptide. In many gram-positive bacteria there is a cross-bridge of five amino acids such as glycine (**peptide interbridge**) that serves to connect one tetrapeptide to another. In either case the cross-linking serves to increase the strength of the overall structure, with more strength derived from **complete cross-linking**, where every tetrapeptide is bound in some way to a tetrapeptide on another NAG-NAM chain.

While much is still unknown about peptidoglycan, research in the past ten years suggests that peptidoglycan is synthesized as a cylinder with a coiled substructure, where each coil is cross-linked to the coil next to it, creating an even stronger structure overall.

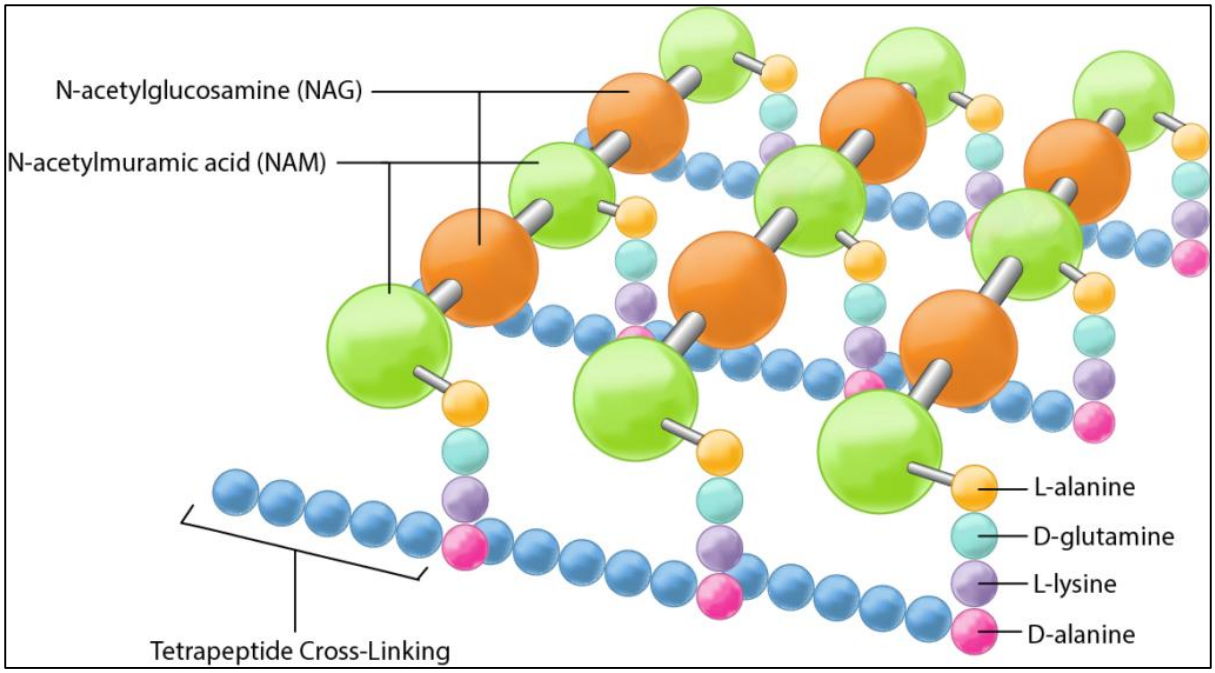
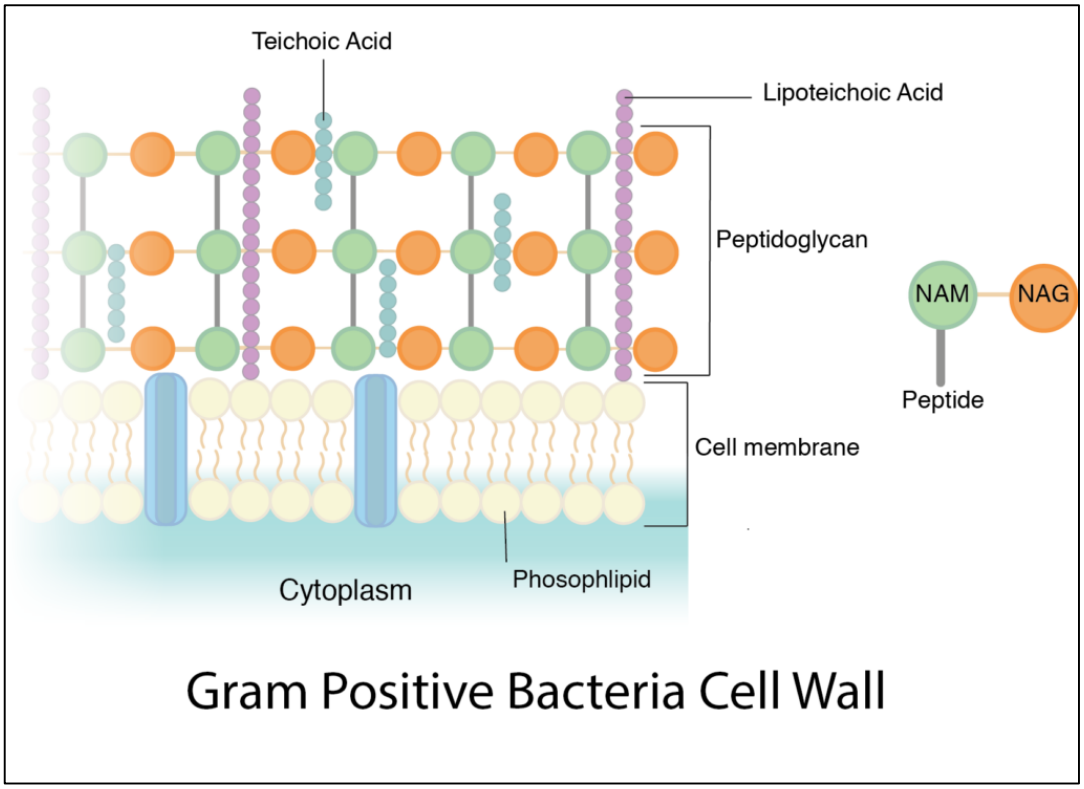


Fig: Peptidoglycan Structure

- **Gram Positive Cell walls**

The cell walls of gram-positive bacteria are composed predominantly of peptidoglycan. In fact, peptidoglycan can represent up to 90% of the cell wall, with layer after layer forming around the cell membrane. The NAM tetrapeptides are typically cross-linked with a peptide interbridge and complete cross-linking is common. All of this combines together to create an incredibly strong cell wall.

The additional component in a gram-positive cell wall is **teichoic acid**, a glycopolymer, which is embedded within the peptidoglycan layers. Teichoic acid is believed to play several important roles for the cell, such as generation of the net negative charge of the cell, which is essential for development of a proton motive force. Teichoic acid contributes to the overall rigidity of the cell wall, which is important for the maintenance of the cell shape, particularly in rod-shaped organisms. There is also evidence that teichoic acids participate in cell division, by interacting with the peptidoglycan biosynthesis machinery. Lastly, teichoic acids appear to play a role in resistance to adverse conditions such as high temperatures and high salt concentrations, as well as to β-lactam antibiotics. Teichoic acids can either be covalently linked to peptidoglycan (**wall teichoic acids or WTA**) or connected to the cell membrane via a lipid anchor, in which case it is referred to as **lipoteichoic acid**.



Since peptidoglycan is relatively porous, most substances can pass through the gram-positive cell wall with little difficulty. But some nutrients are too large, requiring the cell to rely on the use of **exoenzymes**. These extracellular enzymes are made within the cell's cytoplasm and then secreted past the cell membrane, through the cell wall, where they function outside of the cell to break down large macromolecules into smaller components.

- ## Gram Negative Cell Walls

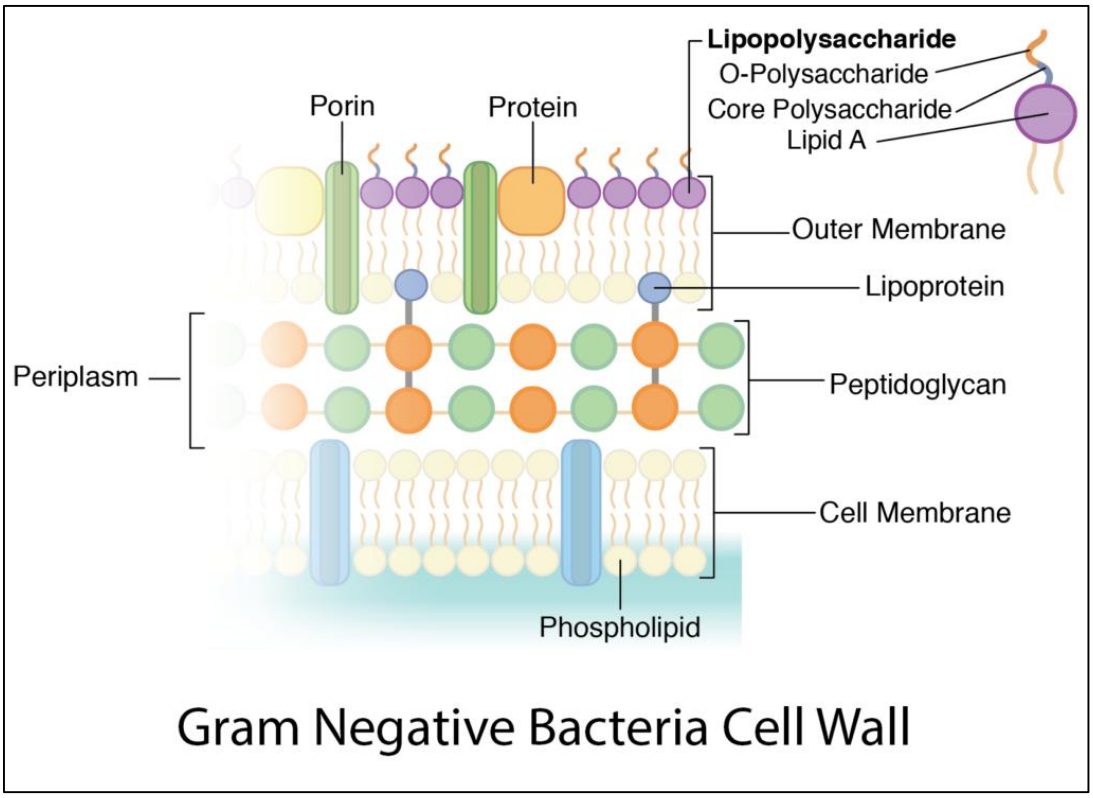
The cell walls of gram-negative bacteria are more complex than that of gram positive bacteria, with more ingredients overall. They do contain peptidoglycan as well, although only a couple of layers, representing 5-10% of the total cell wall. What is most notable about the gram-negative cell wall is the presence of a plasma membrane located outside of the peptidoglycan layers, known as the **outer membrane**. This makes up the bulk of the gram-negative cell wall. The outer membrane is composed of a lipid bilayer, very similar in composition to the cell membrane with polar heads, fatty acid tails, and integral proteins. It differs from the cell membrane by the presence of large molecules known as **lipopolysaccharide (LPS)**, which are anchored into the outer membrane and project from the cell into the environment. LPS is made up of three different components:

1) the **O-antigen or O-polysaccharide**, which represents the outermost part of the structure,

2) the **core polysaccharide**, and

3) **lipid A**,

which anchors the LPS into the outer membrane. LPS is known to serve many different functions for the cell, such as contributing to the net negative charge for the cell, helping to stabilize the outer membrane, and providing protection from certain chemical substances by physically blocking access to other parts of the cell wall. It is also known that LPS can play a role in the response that a host (think human or other animal) develops to an infection by a pathogenic gram-negative bacterium. Specifically, the O-antigen portion of LPS triggers an immune response in the host, causing the generation of protective antibodies that identify a particular O-antigen (think of E. coli O157: H7). It is also known that lipid A can act as a toxin in humans, specifically an **endotoxin**, causing general symptoms of illness such as fever and diarrhea. A large amount of lipid A released into the bloodstream can trigger endotoxic shock, a body-wide inflammatory response which can be life-threatening.



The outer membrane does present an obstacle for the cell. While there are certain molecules it would like to keep out, such as antibiotics and toxic chemicals, there are nutrients that it would like to let in and the additional lipid bilayer presents a formidable barrier. Large molecules are broken down by enzymes, in order to allow them to get past the LPS. Instead of exoenzymes (like the gram-positive bacteria), the gram-negative bacteria utilize **periplasmic enzymes** that are stored in the **periplasm**. Where is the periplasm, you ask? It is the space located between the outer surface of the cell membrane and the inner surface of the outer membrane, and it contains the gram-negative peptidoglycan. Once the periplasmic enzymes have broken nutrients down to smaller molecules that can get past the LPS, they still need to be transported across the outer membrane, specifically the lipid bilayer. Gram negative cells utilize **porins**, which are transmembrane proteins composed of a trimer of three subunits, which form a pore

across the membrane. Some porins are non-specific and transport any molecule that fits, while some porins are specific and only transport substances that they recognize by use of a binding site. Once across the outer membrane and in the periplasm, molecules work their way through the porous peptidoglycan layers before being transported by integral proteins across the cell membrane.

The peptidoglycan layers are linked to the outer membrane by the use of a lipoprotein known as **Braun's lipoprotein** (good ol' Dr. Braun). At one end the lipoprotein is covalently bound to the peptidoglycan while the other end is embedded into the outer membrane via its polar head. This linkage between the two layers provides additional structural integrity and strength.

- **Function of the Cell Wall**

The cell wall is an integral component of the plant cell and it performs many essential functions. Following are some of the major cell wall functions observed:

i. The plant cell wall provides definite shape, strength, and rigidity
ii. It also provides protection against mechanical stress and physical shocks
iii. It helps to control cell expansion due to the intake of water
iv. It helps in preventing water loss from the cell
v. It is responsible for transporting substances between and across the cell
vi. It acts as a barrier between the interior cellular components and the external environment

E. Cell membrane

Beneath the cell wall there lies a thin living membrane, the plasma membrane which forms the outer boundary of cytoplasm. The plasma membrane is 75 Å thick unit membrane and is composed chiefly of proteins and phospholipids.

Some amount of carbohydrate, DNA and RNA have also been reported from plasma membrane but it still needs substantial proof. The lipids in the prokaryotic plasma membrane are polar lipids which may be glycophosphates or glycolipids. Small amount of quinone, Co-enzyme-Q, vitamin K and carotenoids may also be found in the bacterial plasma membrane.

This membrane is a selective barrier to the surrounding medium. The plasma membranes of bacteria contain enzymes involved in oxidation of metabolites or respiratory chain and many multienzyme complexes. It performs the functions including oxidative phosphorylation which are usually done by mitochondria in eukaryotic cells.

Bacterial plasma membrane contains many specific transport systems for compounds of the sugars, amino acids, mineral ions etc. The plasma membrane is capable of not only transporting materials by simple diffusion but is also involved in active transport against concentration gradient.

At certain places membrane may be infolded to form whorls of convoluted membranes called mesosomes. The mesosomes perform several important metabolic activities such as respiration, secretion, etc. They are thought to increase the surface area for transporting systems of the cells. They are also sites of DNA replication enzymes and nucleoid separation.

Mesosomes (Chondroids):

These are convoluted multi-laminated localised infoldings of the cytoplasmic membrane into the cytoplasm (Fig. 2.12). Their number is usually 2-4, but often found to be more in cells with high respiratory activity, e.g., Nitrosomonas.

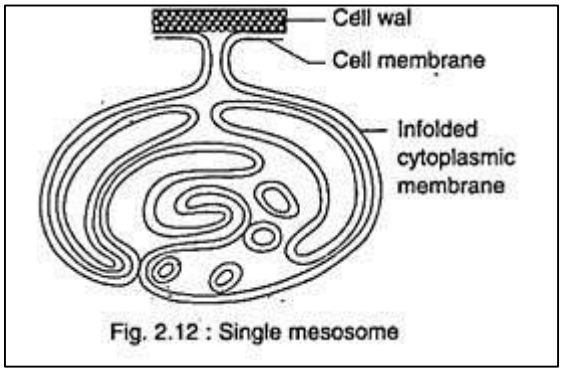


Fig. 2.12 : Single mesosome

Perhaps it serves to accommodate more spaces for respiration. In photosynthetic bacterial (Rhodopseudomonas), they are the site of photosynthetic pigments. The mesosomes are of two types — septal mesosome and lateral mesosome. The septal mesosomes are involved in DNA segregation and in the formation of transverse septum during cell division.

- ## Functions of Cell membrane

(i) Transport:

(a) Active:

Being the site of many enzymes like oxidase, polymerase etc., it is involved in the active transport of selective nutrients. It is impermeable to ionised substances and macromolecules.

(b) Passive:

The passive transport of fat-soluble micromolecular solutes takes place by diffusion.

(ii) Energy production:

It is the site of electron flow in both respiration and photosynthesis leading to phosphorylation and, therefore, the membrane is the site of carriers and enzymes in these reactions.

(iii) Polymer production:

Cell membrane is the site of polymerising enzymes necessary for synthesis of cell wall.

F. Bacterial Chromosomes:

Bacterial chromosome is a double-stranded circular DNA. In general, bacterial DNA ranges from 1100 pm to 1400 μm in length. An *E. coli* cell contains 4. 2 x 10^6kbp DNA

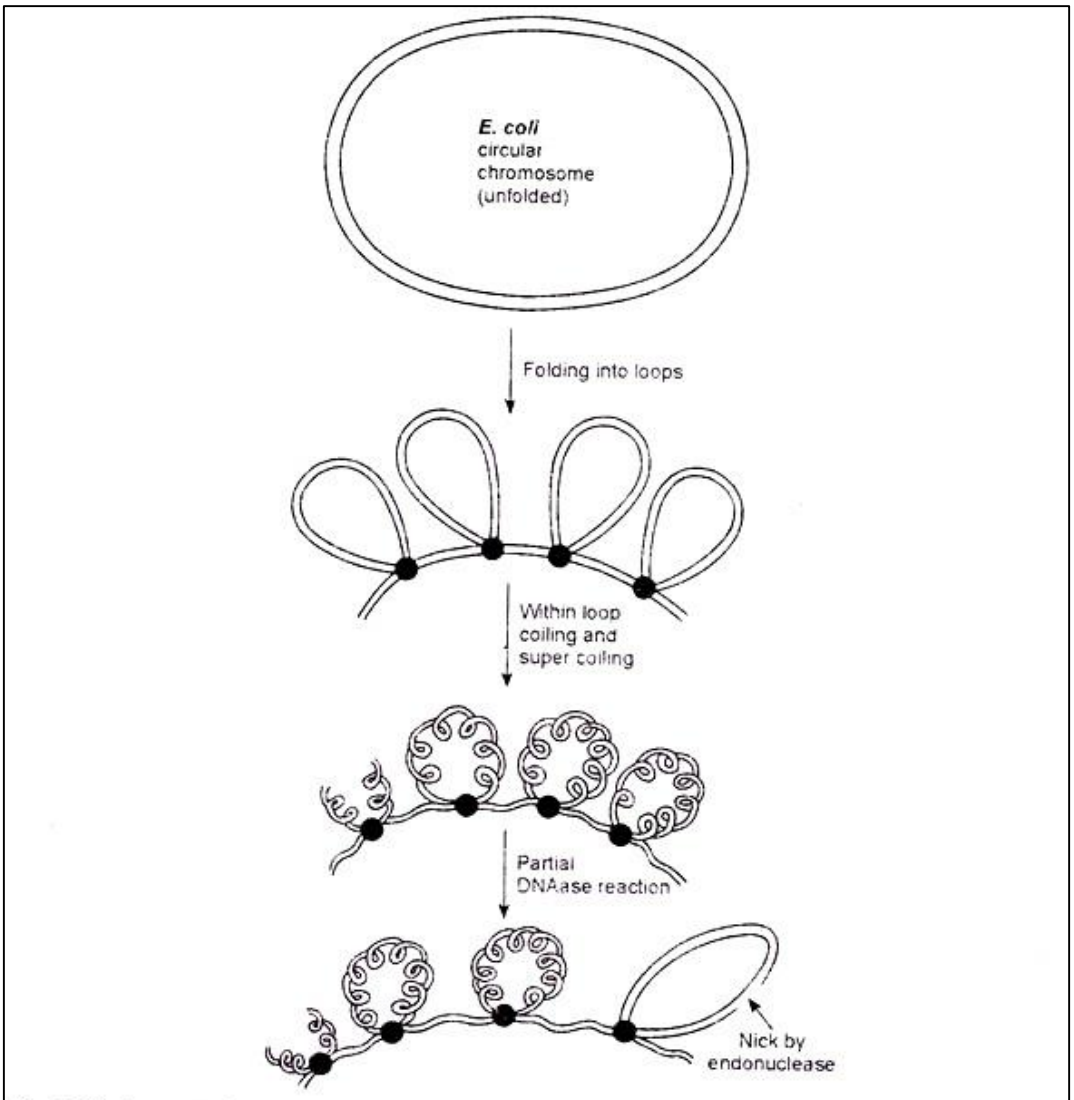


Fig. 5.26. A model of the genome of *E. coli*. The chromosome is folded into ~100 loops which undergo supercoiling. As a result, the chromosome becomes much shorter so that it is able to be packaged into the cell. When an endonuclease makes a cut in one strand of one domain only, that becomes unfolded and enlarged while the other domains remains unaffected.

which is about 1.3 mm (1300 μm) in length. Such a long DNA molecule must be greatly folded to be packaged in a small space of 1.7 x 0.65 μm. The bacterial chromosome is folded into loops or domains which are about 100 in number. A chromosomal domain

may be defined as a discrete structural entity within which supercoiling is independent of the other domains.

Thus, different domains can maintain different degrees of supercoiling. The DNA chain is coiled on itself to produce supercoiling (Fig. 5.26). The ends of the loops or domains are bound in some way which does not allow rotational events to propagate from one domain to another.

If an endonuclease puts a nick in DNA strand of one domain, this loop becomes larger due to the uncoiling, but the other domains are not affected. Each domain contains about 40 kbp (13 μm) of DNA. The loops are bound by some mechanism that may involve proteins and/or RNA but the mechanism is not clearly understood.

In *E. coli*, a number of proteins have been isolated which have some similarities with the eukaryotic chromosomal proteins. These proteins are HU, IHF (integration host factor). HI (H-NS) and R It is suspected that HU is involved in the nucleoid condensation.

The protein HI probably has effects on gene expression. The amino acid sequence of P has some similarity with the protamine's (DNA of certain sperms is bound with protamine's). However, the functions of the P protein are not known.

- ## Functions of the Bacterial Chromosome

The chromosome is the genetic material of the bacterium. Genes located along the DNA are transcribed into RNA molecules, primarily messenger RNA (mRNA), transfer RNA (tRNA, and ribosomal RNA (rRNA). Messenger RNA is then translated into protein at the ribosomes.

- **Transcription:** Ribonucleic acid (RNA) is synthesized by complementary base pairing of ribonucleotides with deoxyribonucleotides to match a portion of one strand of DNA called a gene. Although genes are present on both strands of DNA, only one strand is transcribed for any given gene. Following transcription of genes into mRNA, 30S and 50S ribosomal subunits attach to the mRNA and tRNA inserts the correct amino acids which are subsequently joined to form a polypeptide or a protein through a process called translation.
- **Translation**: During translation, specific tRNA molecules pick up specific amino acids, transfer those amino acids to the ribosomes, and insert them in their proper place according to the mRNA "message." This is done by the anticodon portion of the tRNA molecules complementary base pairing with the codons along the mRNA.

In general, then, DNA determines what proteins and enzymes an organism can synthesize and, therefore, what chemical reactions it is able to carry out.

G. Plasmid:

In addition to bacterial chromosome (nucleoid), bacterial cells normally contain genetic elements in their cytoplasm. These genetic elements exist and replicate separately from the chromosome and are called plasmids. The very existence of plasmids in bacterial cytoplasm was revealed by Lederberg in 1952 while working on conjugation process in bacteria.

Lederberg coined the term 'plasmid' to refer to the transmissible genetic elements that were transferred from one bacterial cell to another and determined the maleness in bacteria.

Plasmids are ring-like double stranded DNA molecules which may contain about 100 genes having the molecular weight range from 5×10^7 to 7×10^7 or less. The replication of plasmid seems self-controlled. They contain different non-essential characters.

- **Properties of Plasmids:**

(i) They are specific to one or a few particular bacteria.

(ii) They replicate independently of the bacterial chromosome.

(iii) They code for their own transfer.

(iv) They act as episomes and reversibly integrate into bacterial chromosome.

(v) They may pick-up and transfer certain genes of bacterial chromosome,

(vi) They may affect certain characteristics of the bacterial cell,

(vii) Plasmids differ from viruses in following two ways.

(viii) They do not cause damage to cells and generally are beneficial.

(ix) They do not have extracellular forms and exist inside cells simply as free and typically circular DNA.

- **Types of Plasmids:**

(i) F-Plasmid:

The F-plasmid, also known as the fertility factor or sex-factor, determines the sex of E. coli bacteria. The cells containing this plasmid are designated as F^+ and those without it as F^-. F^+ bacteria are considered as male, because they can act as donor of not only the plasmid, but also chromosomal genes to the F^- cells which act as recipient and are, therefore, considered as female.

The process of transfer takes place by conjugation of the F^+ cell with the F^- cell. The F-plasmid is a conjugative plasmid.

(ii) R-Plasmids:

R-plasmids conferring resistance to various drugs individually or multiple resistance to several antibacterial agents were first discovered in Japan in the 1950s in the gastroenteritis-causing Shigella dysenteriae. Since then, these plasmids have been found in E. coli and other enteric bacteria. Such plasmids have proved a great threat to the medical science.

(iii) Col-Plasmids:

The Col-plasmids are present in different strains of *E. coli* and they contain genes controlling synthesis of a class of proteins called colicines. Colicines are able to inhibit the growth of related bacteria which lack a Col-plasmid (Cor).

Several different types of Col-plasmids have been discovered, each of which produces colicines having a different mode of inhibition of susceptible bacteria. For example, Col B induces a damage of the cytoplasmic membrane of the target bacteria and Col E2 and Col E3 cause degradation of nucleic acids.

Like R-plasmids, Col-plasmids may be self-transmissible or non-self-transmissible. Large Col plasmids, like Col I and Col V-K94 having molecular weights of 60 x 10^6 Daltons or above are self- transmissible. They have a small copy number, usually 1 to 3 copies per cell. Small Col-plasmids, like Col-El, have molecular weight weighs of about 4 to 5 x 10^6 Daltons.

(iv) Ti-Plasmid of Agrobacterium:

Ti-plasmid is a tumour-inducing large extra-chromosomal double stranded circular DNA which is present in Agrobacterium tumefaciens, a plant-pathogenic bacterium causing the crown-gall disease in many dicotyledonous species. Crown-gall is a tumour produced at the collar region of plants by agrobacteria which possess the Ti-plasmid. Bacteria lacking the plasmid are non-virulent.

(v) Degradative Plasmids:

Degradation or dissimilation of organic compounds in course of mineralization is often controlled by plasmid-borne genes in many microorganisms. Such plasmids with genes coding for enzymes that catabolize complex organic molecules are known as degradative or dissimilation plasmids. For example, in species of Pseudomonas, both chromosomal and plasmid genes produce enzymes for breakdown of complex compounds.

Some of the plasmid genes code for enzymes which degrade such unusual compounds like camphor, toluene, naphthalene, salicylate and complex hydrocarbons of crude petroleum. With the help of these enzymes, the bacteria can utilize these compounds as source of carbon and energy.

As a result, bacteria possessing such degradative plasmids stand a much better chance of survival under conditions where only such unusual compounds are available. Normal bacteria without such plasmid-coded enzymes would perish under similar conditions.

- ***Use of Plasmids as Cloning Vectors:***

Significance of plasmids dramatically increased with the advent of recombinant DNA technology as they became the first cloning vectors, and even today they are the most widely used cloning vectors especially in gene cloning in bacteria.

They enjoy this status because they have very useful properties as cloning vectors that include:

(i) Small size, which makes the plasmid easy to isolate and manipulate;

(ii) Independent origin of replication, which allows plasmid replication in the cell to proceed independently from direct chromosomal control;

(iii) Multiple copy number, which makes them to be present in the cell in several copies so that amplification of the plasmid DNA becomes easy; and

(iv) Presence of selectable markers such as antibiotic resistance genes, which make detection and selection of plasmid-containing clones easier.

The plasmid vector is isolated from the bacterial cell and at one site by restriction enzyme. The cleavage converts the circular plasmid DNA into a linear DNA molecule.

Now the two open ends of linear plasmid are joined to the ends of the foreign DNA to be inserted with the help of enzyme DNA ligase. This regenerates a circular hybrid or chimeric plasmid, which is transferred to a bacterium wherein it replicates and perpetuates indefinitely.

One of the most widely used plasmids in gene cloning in bacteria is pBR322, which has both resistance genes for ampicillin and tetracycline and many restriction sites. When a foreign DNA is inserted into the ampicillin resistance gene of pBR322, the plasmid is no longer able to confer resistance to ampicillin.

Probable questions:

1. What are the basic characteristics and functions of the cell wall in *Bacteria*?
2. What is the Gram stain and how does it relate to the different cell wall types of *Bacteria*?
3. What is the basic unit structure of peptidoglycan? What components are present and how do they interact? Be able to diagram peptidoglycan and its' components.
4. What is cross linking and why does this play such an important role in the cell wall? What different types of cross-linking are there?
5. Why are D-amino acids unusual and how does having D-amino acids in the peptidoglycan keep this macromolecule stable?

6. What are the differences between gram positive and negative organisms in terms of thickness of peptidoglycan, different constituents of PG and variations in cross linkage and strength, and other molecules associated with cell wall?
7. What is teichoic acid and what are its' proposed roles and functions? What are lipteichoic acids?
8. What is the periplasm of gram-negative bacteria? What purpose can it serve? What alternatives are available for cells?
9. What is the general composition of the outer membrane of gram-negative microorganisms, its function and toxic properties? How is it linked to the cell? What is a porin and what are their functions?
10. What group of bacteria lack peptidoglycan in their cell wall? What advantage does this confer?
11. What group of bacteria normally does not have cell walls and how do they maintain themselves?

Suggested Literature:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)
2. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).
3. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press.
4. Microbiology with diseases by taxonomy. Bauman Robert W. Pearson Education (latest edition).
5. Medical microbiology and parasitology. Nagoba BS & Asha P. Elsevier India. (Latest Edition).

Unit XIII

Virology: Structural organization of viruses, Prions and viroids, Lytic cycle of bacteriophages, Lysogeny and lysogeny control, lysogenic conversion, induction and significance

Objective: In this unit we will discuss about Virology: Structural organization of viruses, Prions and viroids, Lytic cycle of bacteriophages, Lysogeny and lysogeny control, lysogenic conversion, induction and significance.

Introduction

Viruses (Latin Venum – poisonous fluid) are simplest forms of life. They are not cells, but their study has provided a great deal of information about cells. Study of viruses is a branch of biology called Virology. Viruses are cellular parasites. They are smaller than bacteria and have a much more simplified organization.

- **Nature of Viruses:**

Viruses are infective microorganisms. They show several differences from typical bacterial cells:

Bacteria	Virus
1.Bacteria are totally living in nature.	1. Viruses are present in both living and nonliving form.
2. Cell wall of Bacteria is made up of lipopolysaccharide or peptidoglycan.	2. Cell wall is absent but a capsid is present in them.
3. Size of Bacteria is between 900-1000nm.	3. Size of the Virus is between 30-50nm.
4.They can survive without a host.	4.They cannot survive without a host.
5. Bacteria reproduce mainly by binary fission.	5. They reproduce by lytic fission and produce their similar copies.
6. Ribosomes are present in Bacteria.	6. Ribosome is absent in Bacteria.
7. RNA and DNA float freely inside cytoplasm.	7. RNA and DNA are present inside the proteinous covering.
8. Antibiotics are effective for Bacterial infections.	8. Antivirals are effective for viral infection.
9. Bacteria causes disease like food poisoning, ulcers, pneumonia,etc.	9. Virus causes infection like AIDS, common cold, chickenpox, etc.

10.Most of the Bacteria can reproduce without a host.	10. Viruses need a host for their reproduction process.
11. Bacterias are both pathogenic and parasitic in nature.	11. They are only parasitic in nature.
12. Bacterial infections are generally localised.	12. Viral infections are systemic.
13.Incubation period of Bacterias is 1-2 weeks.	13. Incubation period of the Virus is 20 days-2 months.
14. Example of Bacterias: Vibrio cholerae, Staphylococcus, etc.	14. Example of Viruses: HIV, RhinoVirus,etc.

- **Difference between bacterial infection and viral infection**

Bacterial infections	Viral infections
• They stem from bacteria, which are single-celled microorganisms. • Bacteria may be within or on the human body. • Not all bacteria are harmful to humans. • Pathogenic bacteria refer to microorganisms that can make humans sick. • In some cases, bacterial infections may be treated with antibiotics.	• They stem from viruses, which are a piece of genetic material, such as DNA or RNA, coated with protein. • Viruses feed off of healthy cells in the body, sometimes killing their host cells as they multiply. • Certain types of viral infections are treated with antiviral medications. • Antibiotics can't cure a viral infection.

Classification of Virus

Viruses are classified on the basis of:

1. Structure 2. Shape 3. Type of host 4. Nucleic acid

1. On the Basis of Structure

a. **Cubical Virus:** they are also known to have icosahedral symmetry Virus. Eg: Reo Virus, Picorna Virus
b. **Spiral Virus:** they are known as helical symmetry Virus. Eg: Orthomyxo Virus
c. **Radial symmetry Virus:** Eg: bacteriophage
d. **Complex Virus: Eg**: pox Virus

2. **On the Basis of Shape**

a. **Rabies Virus:** Bullet shaped
b. **Ebola Virus:** Filamentous shaped
c. **PoxVirus:** Brick shaped

3. **On the Basis of Host Body**

a. **Animal Virus:** The Viruses which infect and live inside the animal body including man are called animal Viruses. Eg; influenza Virus, rabies Virus, mumps Virus, polioVirus etc. Their genetic material is RNA or DNA.

b. **Plant Virus:** Virus which lives inside the plant body is known as plant Virus. Eg: The tobacco mosaic Virus, potato Virus, beet yellow Virus and turnip yellow Virus etc.

c. **Bacteriophage:** It is a kind of Virus which infect Bacterial Cells known as bacteriophage.

4. On the basis of nucleic acids, viruses are:

a. **DNA viruses:**

These viruses possess DNA as the genetic material. On replication this DNA produces new DNA. DNA transmits information for protein synthesis through RNA. (DNA → RNA → PROTEIN).

b. **RNA viruses:**

These viruses possess RNA as the genetic material. The RNA replicates directly to produce new RNA. Information for protein synthesis passes from RNA to protein without involment of DNA. (RNA → RNA → PROTEIN).

c. **DNA – RNA viruses:**

In a group of RNA tumour viruses called leukoviruses or rousviruses the genetic material is alternately DNA and RNA. In addition to the normal mode of transfer found in DNA viruses (DNA → RNA → PROTEIN) the rousviruses also transfer information from RNA to DNA (RNA-DNA-RNA -PROTEIN).

- ## Structure

1. Viruses are much smaller than bacteria. A virus particle is called virion. The virions vary widely in size. The smallest virus measures about 10 mm in diameter (e.g., foot-and-mouth disease virus). The largest virus, (e.g., poxvirus) measures about 250 nm, i.e., as large as the smallest bacteria or mycoplasma.

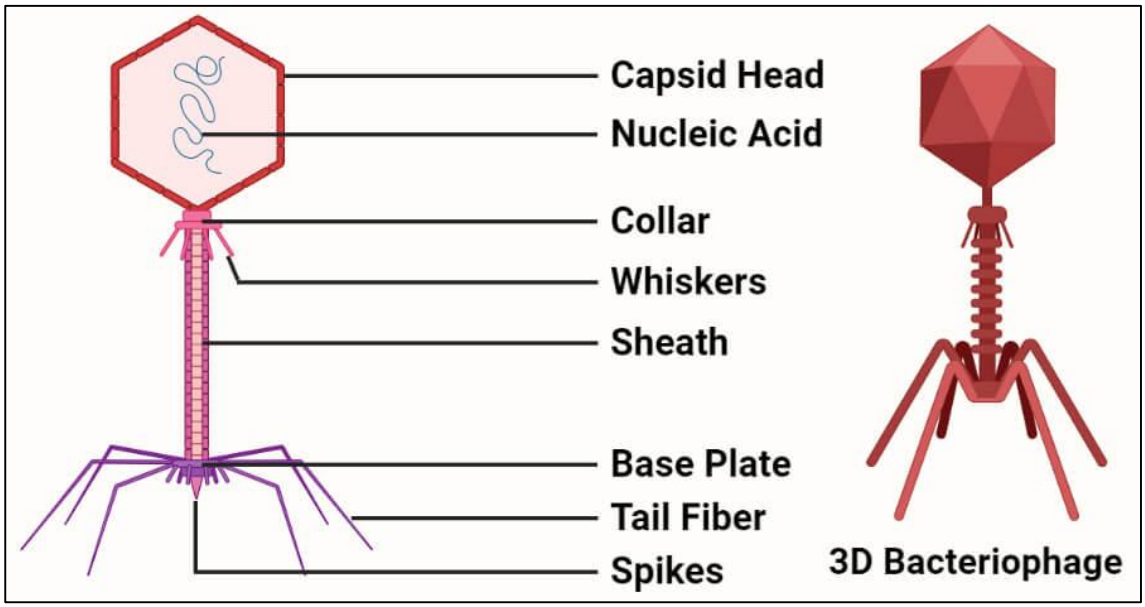
2. Being ultramicroscopic, the viruses can be seen only by an electron microscope.

3. Generally, plant viruses are smaller than animal of bacterial viruses.

4. The shape of the virions is highly variable in different groups of viruses. They may be rod shaped, bullet shaped, brick-shaped, oval, irregular and pleomorphic, or even like a piece of coir rope.

5. In 'tailed' or T-bacteriophages the virion is made up of complex head and an attached tail.

6. A virus particle or virion consists of nucleic acid core surrounded by a protein coat or capsid. The capsid with the enclosed nucleic acid is called nucleocapsid. The capsid is made up of many morphological units, called capsomeres. Chemically, the units of capsid are polypeptide molecules, which form an impenetrable shell around the nucleic acid core.



7. The envelope of the viruses is derived from the host-cell membrane, and is lipoproteinaceous in nature. Recent researches have shown that the lipid of the lipoprotein is of host-cell origin, whereas its protein is of viral origin.

8. The tail consists of a hollow core (Figs. 300, 301) surrounded by a contractile sheath.

9. At the terminal end of the tail is present an end- plate which has attached tail fibres.

10. At the head end the tail connects the head through a thin disc or collar.

11. The nucleic acid in the head of the T-even bacteriophages is double stranded DNA.

12. The end-plate is hexagonal. It has a pin at every corner and remains connected to six very long tail fibres. Bacteriophages remain attached to the host cell through these tail fibres.

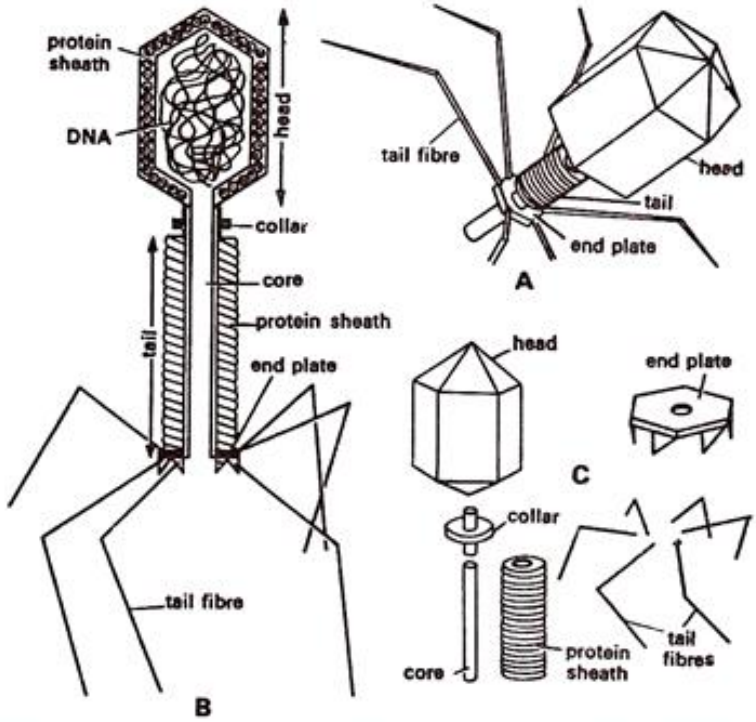


Fig. 301. Morphology of bacteriophage virus. A, External morphology; B, diagrammatic representation of L.S. of a bacteriophage; C, Various components of a bacteriophage.

Viroids and Prions

Prions and viroids are pathogens (agents with the ability to cause disease) that have simpler structures than viruses but, in the case of prions, still can produce deadly diseases.

Infectious agents include bacteria, fungus, protozoans, viruses, viral vectors, and prions. Viroids and prions are tiny infectious particles with virus-like characteristics. These forms, nevertheless, diverge fundamentally from a conventional viral particle. Viruses have two primary elements: genetic material and a protein capsid. Viruses and prions both include genetic information as well as protein capsids. Viroids are contagious RNA molecules that make people sick in the plant kingdom and are tiny and transparent. Prions are microscopic proteinaceous molecules that cause disease in humans and other animals. The main distinction between prions and viroids is that prions lack nucleic acids while viroids lack protein.

- **<u>Viroids</u>**

The Viroids were initially found in 1971 by a plant pathologist named Theodor Otto Diener. When working for the Agricultural Research station Service, he discovered an acellular particle that he termed viroid, which means “virus-like.” There are now 33 viroid varieties recognised. Viroids are the tiniest infectious pathogens, consisting only

of a single strand of circular, single-stranded self-replicating RNA with no protein coating.

On the other hand, Viroids are composed of nucleic acid, which will not code for almost any protein. They lack a protein covering that would safeguard the genetic data. Viroids are known to cause a variety of plant diseases. They use the phloem vascular conduits and plasmodesmata to attach to the owner's protein and then migrate inside the cell (Plant). Chlorosis, stunting, veinal discolouration, epinasty, vein clearing, isolated necrotic patches, mottling of leaflets, and demise of the host are all indications of viroid illness. The first viroid was discovered in tuber spindles syndrome, which caused plantlets to sprout slowly and develop numerous abnormalities. The viroid's replication method is based on the RNA polymerase II enzyme. This enzyme aids in the manufacture of mRNA molecules from DNA in the host cell. Instead, it catalyses "rolling circle" RNA synthesis utilising the viroid's RNA as a template in the viroid. They may have catalytic activity, allowing them to self-cleave and ligate unit-size genomes from bigger replication intermediate. Avsunviroids (such as the Avocado Sun-blotch viroid) and Pospiviroids are two types of viroids (e.g., Potato spindle tuber viroid).

- **Prions**

The name 'prion' was coined to characterise unknown infectious organisms that cause numerous neurodegenerative disorders in invertebrates, particularly Creutzfeldt-Jakob disease (CJD) in humans. The term comes from the phrase "protein infect particles," which alludes to the once-controversial idea that now the infectious agent causing such diseases is made entirely of protein and lacks a nucleic acid genome. (Nucleic acids, which allow viruses and bacteria to proliferate, are found in all prior knowledge diseases.) The prion hypothesis explains why the unknown infected individual is immune to ultraviolet light, that dissolves nucleic acids, yet vulnerable to proteins disrupting chemicals." Researchers made a huge breakthrough when they identified that now the infectious agent is mostly made up of a particular protein in a regular cellular membrane, but with a different shape, or orientation. Some researchers speculated that now the deformed protein may attach to those other molecules of the same type and cause those to change their shape as well, resulting in a chain of events that spreads the sickness and generates fresh infectious material. The genome for this protein has now been mapped, and investigations with transgenic mice have supported the prion hypothesis. The proof in favour of the idea is becoming very robust, if not infallible.

- **Difference between Viroids and Prions**

VIROIDS	PRIONS
1. Viroids are the tiniest infectious pathogens, consisting only of a single strand of circular, single-stranded self-replicating RNA with no protein coating.	1. Prion is an aberrant or misfolded protein that causes fatal sickness in animals and humans by infecting normal variations of the same protein with its misfolded structure.

2. It is an infectious RNA particle	2. It is an infectious protein particle.
3. It's made up entirely of single-stranded circular RNA.	3. It is only composed of Protein.
4. Nucleic acid is present.	4. Nucleic acid is absent.
5. Protein coat is absent.	5. Protein coat is not known.
6. Viroids are inactivated by ribonuclease digestion.	6. Proteinase K and trypsin digestion inactivate prions.
7. Resistant to proteinase K and trypsin digestion	7. Resistant to ribonuclease treatment.
8. Viroids are smaller than virus	8. Smaller than viroids.
9. Viroid infects only higher plants (Exception: hepatitis D virus in humans is similar to viroid)	9. Prions infect animals causing neurological degenerative diseases
10. Example: Potato Spindle Tuber Disease and Chrysanthemum Stunt Disease are two common plant diseases.	10. Examples: Mad cow disease in cows and Scrapie disease in sheep and goat, Creutzfeldt-Jakob Disease (CJD), Kuru.

Lytic cycle of bacteriophages (T_2)

Bacteriophages exhibit two types of replication cycle – virulent or lytic cycle and temperate or lysogenic cycle.

- The **lytic cycle**: The phage infects a bacterium, hijacks the bacterium to make lots of phages, and then kills the cell by making it explode (*lyse*).
- The **lysogenic cycle**: The phage infects a bacterium and inserts its DNA into the bacterial chromosome, allowing the phage DNA (now called a **prophage**) to be copied and passed on along with the cell's own DNA.

The lytic cycle consists of five steps

(a) Adsorption,

(b) Infection (Fig. 2.44),

(c) Synthesis of phage components in host cell,

(d) Formation of new phage particle, and

(e) Liberation of phages from the host cell.

(a) Adsorption:

The interaction between the phage specific organelle — the tail and the receptor site of the host cell is called the adsorption. The adsorption is facilitated by the negatively charged carboxyl groups on the host surface and the positively charged amino-group of protein present at the tip of the phage tail.

In T-even phages, the tip of the tail fibre first attaches to the cell surface. The tail fibre then bends and allows the tail pins to attach on the host surface that makes an irreversible attachment.

(b) Infection:

After adsorption, the phage particle secretes an enzyme which hydrolyses the murin complex of the host cell wall and forms a pore. The sheath of the tail then contracts and pushes the central tubular part, i.e., core of the tail, into the host wall, like an injection needle. The nucleic acid of the phage then passes through the core and enters the host bacterium.

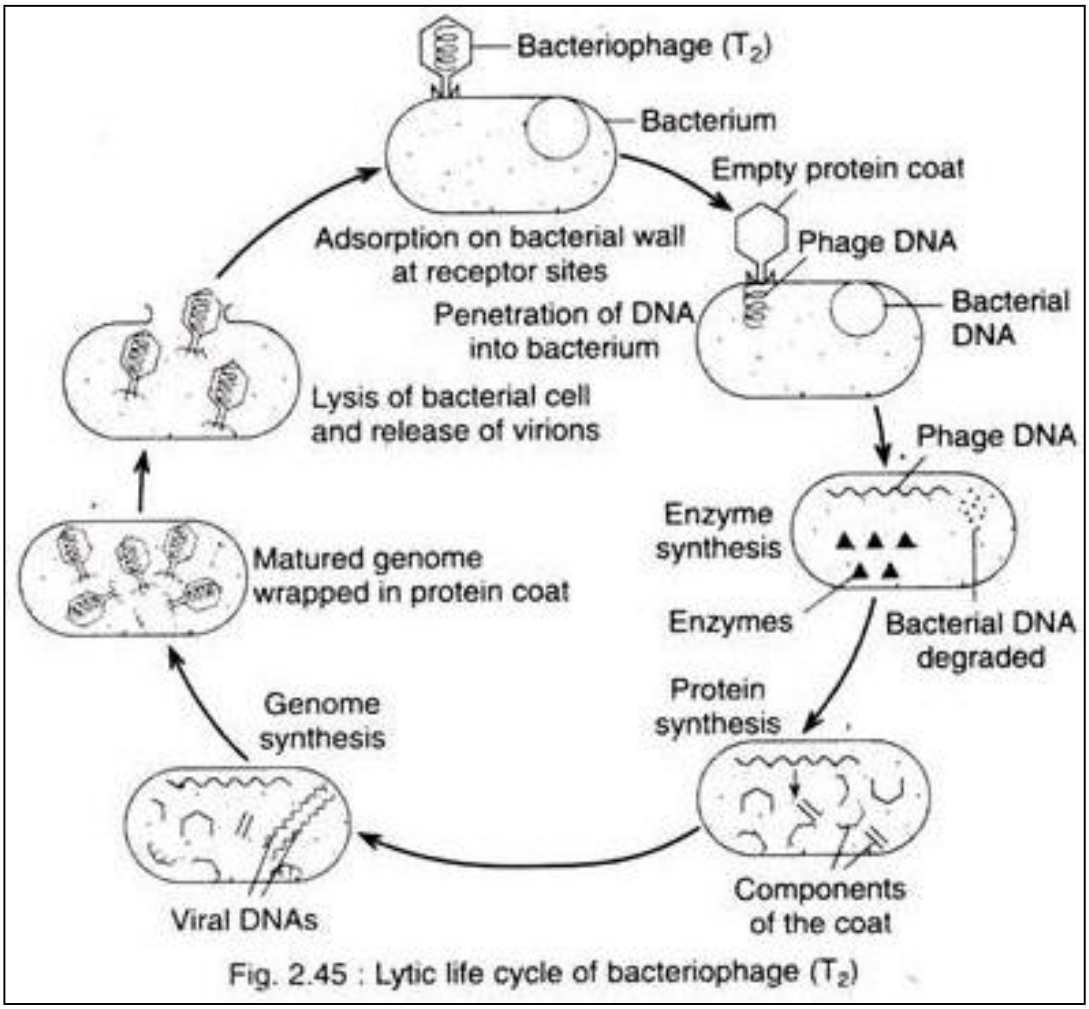


Fig. 2.45 : Lytic life cycle of bacteriophage (T_2)

The empty protein shell of the phage is called ghost, which may remain attached even after release of nucleic acid. Once the bacterial cell receives the nucleic acid of a phage, it becomes resistant to the other phages.

(c) Synthesis of Phage Components in Host Cell:

Once the phage nucleic acid takes the entry inside the bacterial cell, it suppresses the synthesis of bacterial protein and directs to synthesise the proteins of the phage particle (Fig. 2.45).

The DNA of phage replicates following the semi-conservative process. Majority of the DNA acts as a template for its own synthesis and the rest is used as template for the synthesis of viral specific m-RNA by utilizing the RNA-polymerase of the host.

The newly formed m-RNA directs the host cell to synthesize the proteins which are used to build up the protein coat of the phage particle (Fig. 2.45). Almost at the end of replication of phage nucleic acid, a protein, the phage lysozyme, is synthesized.

(d) Formation of New Phage Particle:

The new phage particles are formed by the assemblage of nucleic acid and protein. This process is called maturation, which is controlled by viral genome (Fig. 2.45). In this process, initially the condensation of nucleic acid molecule takes place.

The protein sub-units then aggregate around the nucleic acid molecule and form the head of the phage. By this time the tail formation starts. Initially the core tube is attached with the basal plate and then sheath becomes assembled around the core tube. In this stage, the tail becomes attached to the base of the head taking a collar in between. At last, the tail fibres are attached to the basal plate.

(e) Liberation of Phages from the Host Cell:

In a cycle of phage development, about 200 phages are formed which take about 30-90 minutes. In the host cell, the phage DNA secretes lysozyme (an enzyme) which causes the lysis of host cell wall. As a result of lysis, the phage particles are liberated (Fig. 2.45).

Lysogeny and lysogeny control

The lysogenic cycle is described as one of two cycles of viral reproduction, the other being the lytic cycle. Contrasting the lytic cycle, the lysogenic cycle doesn't result in immediate lysis (destruction) of the host cell. In this cycle, the viral DNA gets incorporated into the host cell's DNA, replicates as the cell divides, causing no harm to the host under normal conditions. Thus, it's often referred to as the dormant cycle.

Lysogenic cycle

In this way, the DNA of λ-phage is integrated with the bacterial DNA at a specific site to become a prophage and thus the infected host bacterium becomes lysogenic. Thus the phage DNA (prophage) replicates along with bacterial DNA. During this process the genes of phage DNA controlling lytic cycle becomes inhibited by a repressor — the λ-repressor.

Replication of Virus by Lysogenic Cycle:

This cycle can be compartmentalised into five crucial steps: attachment, penetration, integration, latency or prophage state, and induction.

- **Attachment:** In this initial phase, the bacteriophage adheres to the bacterial host cell, recognising it through specific receptor sites.
- **Penetration:** Following the successful attachment, the bacteriophage injects its DNA or RNA into the host cell.
- **Integration:** The assimilation stage involves the integration or combination of the phage DNA with the host's DNA, forming a prophage. This is typically performed by a DNA-cutting enzyme called integrase.
- **Latency (Prophage State):** Once integrated, the prophage remains idle within the host cell, not producing any viral proteins. The host cell lives and multiplies as usual, and the prophage DNA is replicated and distributed to new bacterial cells alongside the host DNA.
- **Induction:** During this final stage, environmental stress or some other trigger may cause the prophage to detach or excise from the host DNA, ready to enter a lytic cycle. The viral genes get transcribed and translated, producing mature phages which then cause the host cell to burst or lyse, releasing many copies of new phages into the environment.

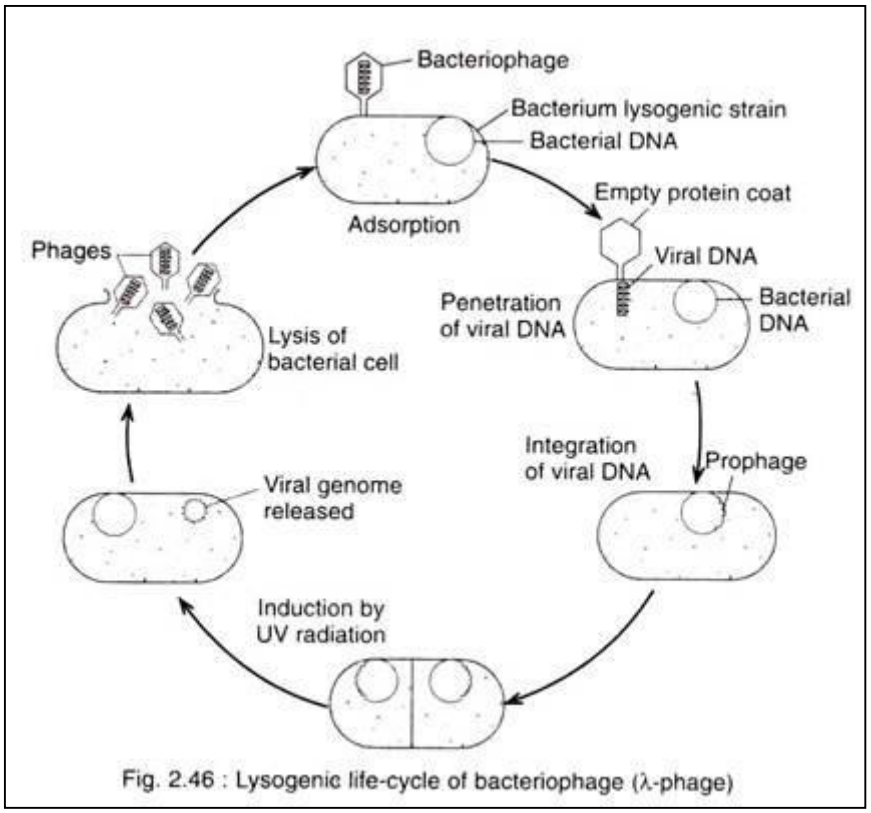


Fig. 2.46 : Lysogenic life-cycle of bacteriophage (λ-phage)

Detailed molecular mechanism of lysogeny and induction by lambda (λ) phage:

Being non-cellular the viral particles have no capacity of independent metabolism and reproduction like other organisms. Thus, they need others' help for their multiplication

almost like the activity of a terrorist of present day. Multiplication of viruses commonly takes place by infection, multiplication and lysis of the host bacterium, called lytic cycle.

But in many others, the mechanism is different, those show different modes of parasitism including infection, integration with genome, multiplication along with host genome; and, later, it gets separated from the host genome, causes multiplication and comes out through lysis of host cell, called as lysogenic cycle.

The phage involved in this cycle is called temperate phage, the bacterium as lysogenic strain, the injected phage DNA integrated with the host genome as prophage and the entire process is called lysogeny.

The temperate phage, after infection, may undergo any one option of multiplication i.e., either lytic cycle like a virulent phage, or lysogenic cycle. Artificial disruption of lysogenic host cell does not show the presence of infectious phages. In other way, it indicates that phages must be present in non-infectious state.

Before going through the cycle one has to know the structure and circularisation of genome of λ-phage.

Activity of the circular λ-DNA:

After circularisaion of λ-DNA inside the bacterial cell (E. coli K12), it functions in either of two alternative ways:

Induction of Lysogeny:

Lysogeny is an indefinite process is not true. The phage genome (pro-phage) can dissociate from the bacterial DNA and start progeny production to result in the lysis of the bacterial cell (i.e., lytic cycle). Dissociation of pro-phage from bacterial DNA, its conversion into virulent phage, and initiation of lytic cycle is called induction (Fig. 13.13).

Induction may occur spontaneously at a low frequency in about one out of 10^2 to 10^5 cells, or may be due to external agents including ultraviolet irradiation, X-rays, and DNA-damaging chemicals such as the nitrogen mustards.

The process of induction is triggered by a drop in the level of lambda repressor protein. The recA gene of E. coli synthesizes recA protein, which normally plays role in genetic recombination. The recA protein acts as a protease (proteolytic enzyme) and cleaves the lambda repressor protein chain between its two domains.

The separated domains fail to assemble to form the normal active repressor protein and, as a result, the inactive form of lambda repressor protein can no longer bind to the promoter inhibiting transcription of the lytic genes and the lytic genes become active again.

There is some recent evidence that recA protein may not directly clear lambda repressor protein; it may instead bind to the lambda repressor protein and stimulate it to proteolytically clear itself.

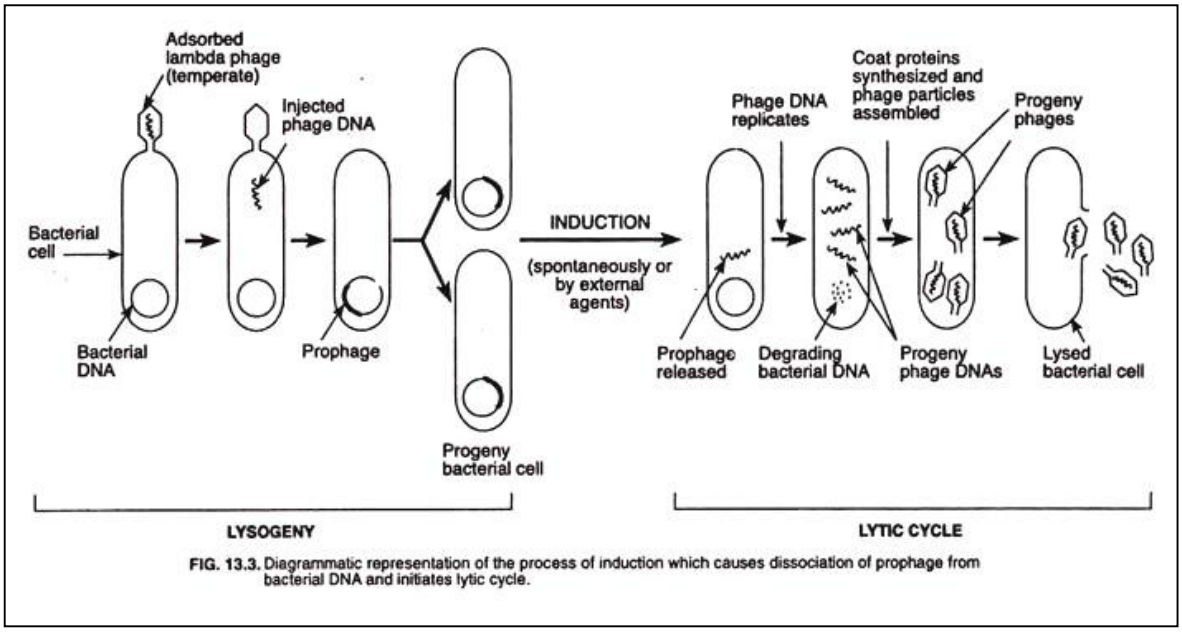


FIG. 13.3. Diagrammatic representation of the process of induction which causes dissociation of prophage from bacterial DNA and initiates lytic cycle.

However, axis gene located next to the int gene codes for the synthesis of an excisionase protein that binds to the integrase enzyme and enables it to reserve the integration process and free the pro-phage. In this condition, the synthesis of another protein, called the cro-protein, by cro genes is initiated. The cro-protein switches the process of synthesis of viral components and the lytic cycle then proceeds normally.

- **Choice between Lysogeny and Lytic Cycle: The Genetic Switch:**

The lambda phages (also some other temperate phages) possess ability to follow the route of either lysogeny or lytic cycle. What controls whether the phage will take the lysogenic or lytic route?

It is considered that the lambda and such other temperate phages have a genetic switch that controls which route will be followed. This genetic switch mainly consists of two proteins, namely, cro-protein (by cro gene) and lambda repressor protein (by cI gene). Both these proteins are repressor proteins and are simultaneously produced.

Since both proteins can block the transcription by each other, there is a race between the production of cro-protein and lambda repressor protein. If the lambda repressor protein wins, the race the linear lambda genome becomes circular, integrates into the bacterial genome, and the lysogeny is followed. But, if the concentration of cro- protein increases so that it wins the race, the lytic cycle is initiated.

The cro-protein binds to O_R (operator right) and O_L (operator left), turns off the transcription of repressor gene (as well as inhibiting the expression of ether early genes), and represses P_M (promoter maintenance) function to switch off the lysogenic cycle.

There are three similar but non-identical sites at O_R where the cro-protein can bind. It does so first at site 3, and then site 2 and only when these two sites are filled, at site 1.

This 3-site binding of cro-protein blocks P_R. Once P_R and P_L are blocked, no more cII or cIII proteins are produced. These proteins are required to enter the lysogenic cycle, and so when cro-protein concentration goes high the lambda phage initiates the lytic cycle.

- ## Significance of Lysogeny:

Lysogeny is probably of major significance to temperate bacteriophages because most bacteria isolated from natural habitats are lysogenic for one or more bacteriophages.

1. Lysogeny confers immunity to lysogens to infection by the same type of phage:

The temperate bacteriophage does not exist free in the lysogen's cytoplasm. Instead, it remains integrated into the bacterial DNA and replicates along with it as long as its lytic cycle genes are not expressed. Typically, it is the phage repressor protein that maintains this control.

The phage repressor protein, which is encoded by phage gene, not only controls the lytic cycle genes situated in pro-phage but also prevents the expression of any incoming genes of the same type of phage. This results in the lysogens having immunity to infection by the same type of phage.

2. Lysogeny is advantageous to phage in nutrient deficiency:

Bacteria enter dormancy in a phage- infected culture that becomes nutrient-deficient, and they degrade their own mRNA and protein. In this condition, the phage reproduction is confined only in actively metabolizing bacterial cells and is usually permanently interrupted in mRNA and protein degrading bacterial cells.

This difficult situation can be avoided if the phage turns to be dormant (lysogenic) simultaneously with the host, i.e., the nutrient deficiency docs favour lysogeny.

3. A high multiplicity of infection (MOI) stimulates lysogeny:

Temperate bacteriophages also ate advantageous in situations when each bacterial cell is subjected to infection by many viruses, i.e., there is a high multiplicity of infection (MOI). In this situation when every cell becomes infected, the last round of replication will destroy all host cells.

Thus, there is a risk that the phages may be left without a host and directly exposed to environmental hazards. Lysogeny therefore comes forward to avoid this prospect; some bacteria that become lysogenized manage to survive, carry the phage genome, and increase their population by reproduction.

When bacterial population increases, the phage genome may dissociate from the bacterial DNA and may enter into lytic cycle to reproduce its own progeny. Therefore, not surprisingly, a high multiplicity of infection (MOI) does stimulate lysogeny.

4. Lysogeny can also confer new properties on the bacterial cell:

Lysogeny may induce a change in the phenotypic property of the host cell employing lysogenic conversion and thus confers a new property on it.

There are several examples of pathogenic bacteria such as Corynebacterium diphtheriae (the cause of diphtheria), Clostridium botulinum (the cause of botulism), Vibrio cholerae (the cause of cholera), and streptococci (the cause of scarlet fever), whose virulence is due, at least in part, to the lysogenic bacteriophage they harbor.

For convenience, when non-toxin producing strains of C. diphtheriae are lysogenized by bacteriophage β, they are converted to toxin producing strains. These strains produce the powerful exotoxin called diphtheria toxin, which inhibits eukaryotic protein synthesis and thus kills infected cells of the host.

Probable questions:

1. Describe the structure of a bacteriophage
2. Difference between bacterial infection and viral infection.
3. What is viroid and mention its function.
4. What do you mean by prion?
5. Describe lytic cycle?
6. What is prophage?
7. What is lysogenic cycle?
8. Discuss the significance of lysogenic cycle.
9. Discuss about the choice between Lysogeny and Lytic Cycle of bacteria.

Suggested Literature:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)
2. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).
3. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press.
4. Microbiology with diseases by taxonomy. Bauman Robert W. Pearson Education (latest edition).
5. Medical microbiology and parasitology. Nagoba BS & Asha P. Elsevier India. (Latest Edition).
6. https://www.biologydiscussion.com/viruses/structure-of-viruses-with-diagram-botany/54588

Unit-XIV

Animal and Veterinary Microbiology: Microbial interactions with animals (marine and freshwater invertebrates, ruminants), symbiotic light production, sulfide-based mutualism

Objectives:

In this section we will discuss on animal and veterinary microbiology: microbial interactions with animals (marine and freshwater invertebrates, ruminants), symbiotic light production, sulfide-based mutualism.

Microbial interactions with freshwater animals:

Microbial interactions play an essential role in aquatic ecosystems and are of the great interest for both marine and freshwater ecologists. Recent development of new technologies and methods allowed to reveal many functional mechanisms and create new concepts. Yet, many fundamental aspects of microbial interactions have been almost exclusively studied for marine pelagic and benthic ecosystems. These studies resulted in a formulation of the Black Queen hypothesis, a development of the phycosphere concept for pelagic communities, and a realization of microbial communication as a key mechanism for microbial interactions. In freshwater ecosystems, especially for periphyton communities, studies focus mainly on physiology, biodiversity, biological indication and assessment, but the many aspects of microbial interactions are neglected to a large extent. Periphyton plays a great role for aquatic nutrient cycling, provides the basis for water purification, and can be regarded as a hotspot of microbial biodiversity, we highlight that more in-depth studies on microbial interactions in periphyton are needed to improve our understanding on functioning of freshwater ecosystems.

Principles and mechanisms of microbial interactions have been studied intensively for planktonic communities and cultures. Based on recent studies on microbial interactions in plankton, Grossart (2010) proposed the concept of an intertwined microbial network between free-living and surface-attached (particle-associated) bacteria, where surface associated bacteria may play a considerable role for nutrient cycling and biomass production. Later studies of free-living and particle-associated bacteria revealed that the connectivity between these communities increased along nutrient gradients. Also, it was shown that interactions in particle associated community are stronger, than in free-living. Microbial interactions mediated by chemical substances, e.g. allelopathy, seem to be a widespread strategy in both planktonic and benthic communities. Unlike phytoplankton, biofilms represent specific organismic communities embedded in an extracellular polysaccharide matrix providing a favorable environment for short-distance microbial interactions. Later, in stream ecosystems, biofilms have been called “jungles” providing numerous ecological niches for a high number of organisms such as algae, protozoa,

nematodes and even some insects, which are “among the top consumers in the stream biofilms”. At the same time, both local environment and biotic interactions act as drivers for local community composition. We suppose that aside of various close interactions between microbial organisms in biofilms, periphyton communities form an intertwined network with free-living bacteria and dead organic matter in the surrounding environment. Initially, in aquatic ecology, the “phycosphere” concept includes interactions between auto- and heterotrophic microorganisms – analogous to the rhizosphere concept in soils. Thereby, the phycosphere depicts an immediate region surrounding an algal cell, chain, or colony and represents a zone, “in which bacterial growth is stimulated by the extracellular products of the alga”. Later studies on plankton confirmed that phytoplankton is surrounded by a diffusive boundary layer, in which excreted phytoplankton molecules accumulate in concentrations, high enough to be detected by bacteria allowing for intense communication and interaction with the algae. According to the concept proposed by Grossart (2010) this microbial community is interconnected by chemical means with its own members and with the surrounding periphytic and planktonic communities. Moreover, since in periphyton all organisms are in the most intimate contact, it leads to tight interactions among them. There are two main types of interactions: competitive and cooperative. The cooperative concept lately was developed via the Black Queen Hypothesis, which proposes that cooperation can be a result of coevolution of coexisting bacteria and adaptive gene loss. It is well known that bacteria are able to stimulate growth, morphogenesis and zoosporogenesis of algae as well as the synthesis of algal extracellular compounds. Bacteria serve as an ultimate source of cobalamin for vitamin B dependent algae and promote algal growth via excretion of substances with hormonal nature. In turn, dissolved organic carbon (DOC), excreted by algae serve as nutrients for alga-associated heterotrophic microbial community. Besides of synergistic interactions, a multispecies community inevitably faces competitive antagonistic interactions among species. Last, studies have shown that in the absence of carbon limitation, heterotrophs can out-compete algae for nutrients and thus suppress algal growth. The inhibiting substances include antibiotics and enzymes, which can be transported to competitors with extracellular vesicles and affect cellular differentiation, protein synthesis and inhibit cell-to-cell signaling. Studies on various microbial biofilms reveal that cell-to-cell chemical signaling plays a crucial role in a shaping of the biofilm community, e.g. by controlling processes such as invasion and colonization. Since many signaling molecules are small, they can diffuse freely through the highly hydrated biofilm matrix, depending on their hydrophobicity. However, the extracellular polymeric substances have a high chemical heterogeneity creating environmental gradients which may serve as signal filters or fluid channels. At the same time, flow of water may dilute the pool of signaling molecules or transfer them to the more distant biofilm areas resulting in chemical interactions with the surrounding environment.

- **“Black Queen Hypothesis”** Recent applications of new molecular methods for studies on bacterial interactions of free-living lineages of oceanic plankton have resulted in the proposition of the so-called “Black Queen Hypothesis” (BQH). It suggests that some

species of free-living bacteria are dependent on functions of co-occurring bacteria due to loss of a number of genes during reductive genome evolution. The hypothesis of cross-feeding assigns co-existing species as “helpers” and “beneficiaries”. The scenarios of co-existence in microbial communities are diverse and include a number of strategies (Elias and Banin, 2012). Unlike many theories of coevolution, e.g., the Red Queen Hypothesis, proposes that relationships between helpers and beneficiaries doesn’t necessarily arise from direct interactions, but beneficiaries can simply stop a costly function that is provided by their helpers (loss-of-function mutation). This new paradigm caused an intense discussion in the scientific community and suggests that bacteria often form interdependent cooperative interactions in communities and develop a clear genomic signature via complementary in loss of shared diffusible functions. Experiments with mixed algal-bacterial co-cultures have shown that in periphyton heterotrophic bacteria play a crucial ecological role and that algae-only assemblages develop slower and are less stable than in the presence of bacteria. Therefore, it is difficult to obtain representative axenic cultures of periphytic algae. This functional interconnectedness between photo- and heterotrophic consortia in algal-bacterial associations and, particularly, in periphyton is consistent with the above portrayed BQH. Yet, such interconnectedness raises the following questions: is this functional interrelationship obligatory and a result of: i) gene loss described by the BQH (when organism with lost-of-function mutation relies on self-sufficient organisms)? ii) the proposed cross-feeding scenarios? or iii) phenotypic plasticity caused by the present environmental conditions? Loss-of-function mutations are predicted to be the most widespread among intimately interacting bacteria, e.g. biofilm and rhizosphere consortia. Yuan and Meng (2020) found a difference in interactions in mobile (flocks) vs. those in surface-attached biofilms. In flocks, the prevailing interactions were more competitive corresponding more with the Red Queen Hypothesis. In surface-attached biofilms, however, the relationships were less competitive and more cooperative.

Microbial symbiosis in marine animals:

Symbiotic relationships between marine invertebrates and chemoautotrophic bacteria have been found in a variety of ecosystems, ranging from shallow coastal waters to deep-sea hydrothermal vents. Symbiosis is a way for marine organisms to find creative ways to survive in a very dynamic environment. They are different in relation to how dependent the organisms are on each other or how they are associated. It is also considered a selective force behind evolution in some scientific aspects. The symbiotic relationships of organisms have the ability to change behavior, morphology and metabolic pathways.

- **Coral reef symbiosis** The most notable display of marine symbiotic relationship would be coral. Coral reefs are home to a variety of dinoflagellate symbiont, these symbionts give coral its bright coloring and are vital for the survival of the reef. The symbionts provide the coral with food in exchange for protection. If the waters warm or

become too acidic, the symbionts are expelled, the coral bleaches and if conditions persist the coral will die. This in turn leads to the collapse of the entire reef ecosystem.

- **Bone eating worm symbiosis** *Osedax*, also called the bone eating worm is a siboglinid worm from polychaete genus. It was discovered in a whalefall community on the surface of bones, in the axis of Monterey Canyon, California, in 2002. *Osedax* lacks a mouth, a functional gut and a trophosome. But female *osedax* have a vascularized root system originating from their ovisac which contains heterotrophic endosymbiotic bacterial community dominated by γ-proteobacteria clade. They use the vascularized root system to access the whale bones. The endosymbionts help the host utilize nutrients from the whale bones.

- **Hawaiian squid and *Vibrio fischeri* symbiosis** Hawaiian sepiolid squid *Euprymna scolopes* and bacterium *Vibrio fischeri* also show symbiosis. In this symbiosis, symbiont not only serve the host for defence, but also shapes the host morphology. Bioluminescent *V. fischeri* can be found in epithelial lined crypts of the light organ of the host. Symbiosis begins as soon as a newly hatched squid finds and houses *V. fischeri* bacteria. They emit light during night time to camouflage themselves against the moon and star light coming down the ocean. It helps them to avoid predators.

The symbiosis process begins when Peptidoglycan shed by the sea water bacteria comes in contact to the ciliated epithelial cells of the light organ. It induces mucus production in the cells. Mucus entraps bacterial cells. Antimicrobial peptides, nitric oxide and sialyted mucins in the mucus then selectively allow only *V. fischeri* which encode gene rscS to adhere and win over gram positive and other gram-negative bacteria. The symbiotic bacteria are then guided up to the light organ via chemotaxis. After successful colonization, symbionts induce loss of mucus and ciliated sites to prevent further attachment of bacterial cells via MAMP (microbe associated molecular pattern) signalling. Also, they induce changes in protein expression in the host symbiotic tissues and modify both physiology and morphology of light organs. After bacterial cells divide and increase in population, they begin expressing enzyme luciferase as a result of quorum sensing. Luciferase enzymes produce bioluminescence. Squids can then emit the luminescence from the light organ. Because *Euprymna scolopes* emerges only during night time, it helps them avoid predation. Bioluminescence allows them to camouflage with the light coming from moon and stars to ocean and avoid predators.

- **Pompeii worm** *Alvinella pompejana,* the Pompeii worm is a polychaete, found in the far depths of the sea, typically found near hydrothermal vents. They were originally discovered by French researchers in the early 1980s. They can grow as large as 5 inches long and are normally described as having pale gray coloring with red "tentacle-like" gills protruding from their heads. Their tails are most likely found in temperatures as high as 176 degrees Fahrenheit, while their heads, which stick out from the tubes they live in are only exposed to temperatures as high as 72 degrees Fahrenheit. Its ability to survive the

temperatures of hydrothermal vents lies in its symbiotic relationship with the bacteria that resides on its back. It forms a "fleece-like" protective covering. Mucous is secreted from glands on the back of the Pompeii worm in order to provide nutrients for the bacteria. Further study of the bacteria led to the discovery that they are chemolithotrophic.

- **Hawaiian sea slug** *Elysia rufescens* grazes on *Bryopsis* sp., an alga that defends itself from predators by using peptide toxins with fatty acids, called kahalalides. A bacterial obligate symbiont produces many defensive molecules, including kahalalides, in order to protect the alga. This bacterium is able to use substrates derived from the host in order to synthesize the toxins. The Hawaiian Sea Slug grazes on the alga in order to accumulate kahalalide. This uptake of the toxin, which the slug is immune to, allows it to also become toxic to predators. This shared ability, both originating from the bacteria, provide protection within the marine ecosystems.

- **Marine sponges** The sponge holobiont is an example of the concept of nested ecosystems. Key functions carried out by the microbiome (coloured arrows) influence holobiont functioning and, through cascading effects, subsequently influence community structure and ecosystem functioning. Environmental factors act at multiple scales to alter microbiome, holobiont, community, and ecosystem scale processes. Thus, factors that alter microbiome functioning can lead to changes at the holobiont, community, or even ecosystem level and vice versa, illustrating the necessity of considering multiple scales when evaluating functioning in nested ecosystems.
Besides a one-to-one symbiotic relationship, it is possible for a host to become symbiotic with a microbial consortium. In the case of the sponge (phylum Porifera), they are able to host a lot of wide range of microbial communities that can also be very specific. The microbial communities that form a symbiotic relationship with the sponge can actually comprise up to 35% of the biomass of its host. The term for this specific symbiotic relationship, where a microbial consortia pairs with a host is called a holobiotic relationship. The sponge as well as the microbial community associated with it will produce a large range of secondary metabolites that help protect it against predators through mechanisms such as chemical defense. Some of these relationships include endosymbionts within bacteriocyte cells, and cyanobacteria or microalgae found below the pinacoderm cell layer where they are able to receive the highest amount of light, used for phototrophy. They can host approximately 52 different microbial phyla and candidate phyla, including *Alphaproteobacteria, Actinobacteria, Chloroflexi, Nitrospirae, Cyanobacteria,* and the candidate phylum *Poribacteria,* and *Thaumarchaea*.

- ***Endozoicomonas*** This type of bacteria was first described in 2007. It is able to form symbiotic relationships with a wide range of hosts in the marine environment such as cnidarians, poriferans, molluscs, annelids, tunicates, and fish. They are distributed through various marine zones from extreme depths to warm photic

zones. *Endozoicomonas* is thought to acquisition nutrients from nitrogen/carbon recycling, methane/sulfur recycling, and synthesize amino acids and various other molecules necessary for life. It was also found that it has a correlation to photosymbionts which provide carbon and sulfur to the bacteria from dimethylsulfopropionate (DMSP). They are also suspected to help regulate bacterial colonization of the host by using bioactive secondary metabolites or even probiotic mechanisms like limiting pathogenic bacteria by means of competitive exclusion. When *Endozoicomonas* is removed from the host, there are often signs of lesions on corals and disease.

Rumen and microbiota

The evolution of the gastrointestinal tract is a history that has been pockmarked by many innovations and compromises; however, the defining element of this history is the tacit acknowledgment of defeat: colonizing microbes are here to stay. Some mammals—particularly carnivorous species—increase their absorption of nutrients from food by using a shorter gastrointestinal tract and decreasing food transit time to reduce feed energy losses to microbes in their large intestines. Other, primarily herbivorous, species form a symbiosis with microbial colonizers, for which they provide a suitable environment for microbial growth; in return, the microbes provide their host with nutrients from otherwise indigestible food sources. It is in this context that the symbiosis between cattle and microbes becomes apparent, as the rumen is a highly specialized organ designed to contain and promote a collection of mutualistic microbial species while simultaneously harvesting nutrients derived from their digestion of plant fibre and cellular material.

The rumen interior is lined by a stratified squamous epithelium (epimural layer) that changes texture and color in response to mechanical and chemical stimuli. A critical adaptation in the evolution of the rumen was an expansion in its volume, relative to the rest of the gastrointestinal tract. In mature Holstein cows, for example, the rumen volume generally ranges from 70 to 100 L, and the rumen and its contents account for about one-seventh of the animal's total BW. This expansion established a solids retention time long enough to permit microbial degradation of fibrous plant materials to acetate and other VFA. However, this retention time is too short to permit the establishment of slow-growing, thermodynamically constrained microbes capable of VFA degradation such as proton-reducing acetogens (which convert C_3–C_6 VFA to H_2 and acetate) or aceticlastic methanogens (which cleave acetate to methane and CO_2). By preventing catabolism of VFA by other members of the ruminal community, up to 75% of the energy in the original digestible organic matter in the feed is retained within the VFA for absorption from the rumen and intestines, although a large fraction of the VFA is consumed by oxidation in the ruminal epithelial cells. Each VFA is used for a different function, with acetic acid used for ATP and lipid synthesis, propionic acid for gluconeogenesis in the liver, and butyric acid converted immediately into a ketone body by enzymes in the rumen epimural layer for use as an energy source for many tissues of the body. Apart from the conversion of butyric acid in the rumen epimural layer, the majority of enzymatic activity aiding cattle digestion is provided by a diverse group

of commensal microbes inhabiting the lumen of the rumen; henceforth called the "rumen microbiome."

The productivity of ruminants relies heavily on rumen function, which can be regulated by interactions between host genes and the environment (e.g., diet, management), leading to the alteration of rumen microbial ecology. In particular, the type of diet can strongly influence rumen function by altering the microbial populations and fermentation activities. Roughage has a significant impact on rumen development and expression of the genes involved in VFA absorption in the rumen epithelial cells. Therefore, diet, which is regarded as one of the most significant factors, has been modified in large feeding operations of ruminants to improve feed efficiency.

The tight and constant interaction between the host and rumen microbiota is a vital prerequisite for ensuring host health and optimal productivity. The rumen provides a permissive residence for the microbes, and the rumen microbes can secrete special enzymes that aid feed digestion in the host. If any 1 of these 2 factors becomes abnormally, the regular metabolic mechanism will be interrupted in ruminants. Therefore, regulating the host–microbiota interaction may bring benefits to the host.

Diet is the key factor determining and maintaining the host–microbiota interaction. It is a universal means to reach the genetic potential of the animals by providing a properly matched diet. Researchers have proved that the ruminal microbiome-host crosstalk stimulates the development of the ruminal epithelium. It was shown that the abundances of genes involved in sugar degradation decreased in the rumen of lambs fed starter, but the abundance of glycoside hydrolase family 13 encoding α-amylase increased; simultaneously, the expression of the genes involved in cell growth modules, such as *MAPK1*, *PIK3CB*, *TNFSF10*, *ITGA6*, *SNAI2*, *SAV1,* and *DLG*, were upregulated, whereas the genes encoding the proapoptotic protein BAD's promotion of cell death was down-regulated in the rumen epithelial cells. If the same results are present in goats, sheep, and cows, it will emphasize the importance of diet in the regulation of host growth. Firmicutes and Bacteroidetes are the dominant bacterial phyla of the rumen microbiota irrespective of host species, and both participate in fibre degradation and produce VFA. The various species in these 2 phyla have their special niches, and they can be influenced by dietary types. Specific fibre sources can alter the rumen microbiota, modulate innate immune systems, and affect intestinal mucosa barrier integrity and colon pH, ultimately improving the absorption of nutrients. It may be an effective way to maintain high efficiency between cellulolytic bacteria and host gut health by changing the type and amount of fibre in the diet. Formulating appropriate composite diets can stimulate the development of the immune system during not only the early life but also the adulthood, which may have an impact on the economic return in animal farming.

The micro-ecological environment of GIT is another important factor affecting the metabolism and health of the host by regulating the host–microbiota interaction. Species of bacteria with specific functions may be identified, cultured/enriched, manufactured, and used as feed additives. Using direct-fed microbes is an efficient method to provide some functional microbes to the host. Yet, it remains a challenge to identify the specific function of microbes and to acquire the microbes of interest. Currently, researchers focus

on microbial transplantation to enhance feed efficiency, productivity, and host health. For example, Hu et al. (2018) reported that fecal microbiota transplantation from diarrhea-resistant pigs to susceptible piglets significantly prevented early weaning stress-induced diarrhea in Landrace × Yorkshire crossbred piglets. *Lactobacillus gasseri* LA39 and *Lactobacillus frumenti* were confirmed as 2 bacterial species mediating diarrhea resistance in pigs. Gassericin A, a bacterial secretory circular peptide, was found to play a vital role in diarrhea resistance via a mechanism involving keratin 19 (KRT19) on the plasma membrane of intestinal epithelial cells, which reduces the levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), contributing to diarrhea resistance by promoting intestinal epithelial fluid absorption and reducing fluid secretion. These observations suggest that *L. gasseri* LA39 and *L. frumenti* could have a practical value for preventing diarrhea in piglets and may be used to support early weaning and pig raising. To improve feed efficiency in beef cattle, the rumen contents from high and low feed efficiency cattle were exchanged, and the results showed that the brief substitution of the content was not an effective strategy to improve feed efficiency. Thus, it is necessary to identify the key microbes that are associated with feed efficiency.

The rumen microbiome consists of microbial groups that represent all 3 of Woese's domains of life: bacteria, archaea, and eukarya. The bacteria comprise thousands of different species and approximately 60% of the microbial mass, and are primarily responsible for most of the feed biopolymer degradation and fermentations of the resulting monomers and oligomers. This community is dominated by 2 phyla: *Firmicutes* (particularly represented by the genera *Butyrivibrio*, *Lachnospira*, *Succiniclasticum* and *Ruminococcus*) and *Bacteroidetes* (particularly represented by the genus *Prevotella*), that together comprise ~90% of the 16S rRNA gene abundance (a proxy for community abundance) in bulk ruminal contents. The archaea, which comprise only ~1% of the microbial mass, are primarily hydrogenotrophic methanogens, mostly of the genus *Methanobrevibacter*, with additional contribution from methylotrophic methanogens, particularly *Methanothermoplasmata* . The eukaryotes are primarily represented by various genera of protists ("protozoa") that can comprise one-third or more of the microbial mass, plus anaerobic chytrid fungi that comprise only ~1% of microbial mass in ruminants from temperate climates but are more prevalent in tropical ruminants. It is important to note that rumen microbial content differs between the 2 different phases of rumen digesta material: the liquid phase and solid phase contain different community compositions. Of these 2 phases, the solid phase can contain 80% or more of the bacterial community and is likely to contain the most important microbes related to digestion of feed particles.

Symbiotic light production:

Bioluminescence is a form of chemiluminescence, which is the production of visible light by a chemical reaction. Scientists call the process "bioluminescence" when the reaction

occurs in living organisms. Bioluminescence is usually blue or blue-green. But it can be nearly violet (bright purple), green-yellow, and less often, red.

- **What is Bioluminescence?**

Bioluminescence definition can be described as the natural phenomenon wherein certain living organisms light up because of certain chemical reactions. As a result, the chemical energy readily changes into light energy.

It is noteworthy that the occurrence of bioluminescence in land and freshwater is quite rare when compared to its appearance in oceans. As much as 90% of animals harbouring in the deep ocean are luminescent.

Furthermore, bioluminescent animals on land tend to emit light in blue-green hues or shades belonging to the yellow spectrum. On the other hand, the marine organisms emit light which has a blue-green glow.

- **Chemistry**

The chemical reaction that results in bioluminescence requires two unique chemicals: luciferin and either luciferase or photoprotein. Luciferin is the compound that actually, produces light. In a chemical reaction, luciferin is called the substrate. The bioluminescent color (yellow in fireflies, greenish in lanternfish) is a result of the arrangement of luciferin molecules. Some bioluminescent organisms produce (synthesize) luciferin on their own. Dinoflagellates, for instance, bioluminesce in a bluish-green color. Bioluminescent dinoflagellates are a type of plankton—tiny marine organisms that can sometimes cause the surface of the ocean to sparkle at night. Luciferase is an enzyme. An enzyme is a chemical (called a catalyst) that interacts with a substrate to affect the rate of a chemical reaction. The interaction of the luciferase with oxidized (oxygen-added) luciferin creates a byproduct, called oxyluciferin. More importantly, the chemical reaction creates light. Bioluminescent dinoflagellates produce light using a luciferin-luciferase reaction. The luciferase found in dinoflagellates is related to the green chemical chlorophyll found in plants. Bioluminescent dinoflagellate ecosystems are rare, mostly forming in warm-water lagoons with narrow openings to the open sea. Bioluminescent dinoflagellates gather in these lagoons or bays, and the narrow opening prevents them from escaping. The whole lagoon can be illuminated at night.

- **Luminous Bacteria - Habitation and Taxonomy**

Bioluminescence or emission of light by biological organisms is an interesting aspect of energy metabolism. Many different kinds of organisms, both prokaryotic and eukaryotic, show this property. Among the bacteria, bioluminescent species occur in the genera, Photo-bacterium, Vibrio and Beneckea. Many marine dinoflagellates also show this property. At least two fungi — *Armillaria mellea* and *Panus stipiticus* — are known to be

bioluminescent. Luminous bacteria are the most widely distributed light-emitting organisms with the majority existing in seawater and the remainder living in the terrestrial or freshwater environment. While most species of luminescent bacteria are capable of living free, the majority are found in nature associated in symbiosis with host organisms (Figures 1 to 4) (i.e., fishes, squids, crabs, nematodes, etc...).

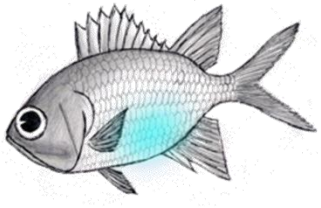


Figure 1. Pinecone fish utilize luminous bacteria, colonized in the ventral cavity, to illuminate the surroundings as well as for intra-species communication.

In symbiosis, the bacteria are nourished with readily available food sources for growth, and at the same time the host utilizes the adopted illumination to communicate, to attract prey, and to masquerade itself from predators.

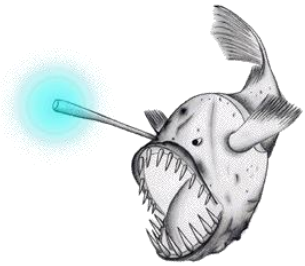


Figure 2. The deep-sea Angler fish carries luminous bacteria in a light emitting rod, which attracts prey to the front of its mouth.

However, there are certain species of luminescent bacteria, which are obligatory symbionts, requiring unique nutritional supplements, which are exclusively available from the host. Although the presence of these obligatory symbionts has been detected, they are not separable from their host, and therefore are unable to be cultured in the laboratory for further study.

There are three major genera, into which most luminous bacteria are classified; *Photobacterium*, *Vibrio*, and *Photorhabdus*. Species existing in the marine environment are mainly categorized into the *Photobacterium* and *Vibrio* genera, and the terrestrial species are classified into the *Photorhabdus* (previously designated as *Xenorhabdus*) genus. Species within the Photobacterium genus are generally light organ symbionts of marine animals, whereas the Vibrio species exist as free-living forms as well as symbionts in the sea.

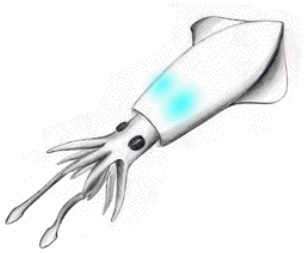


Figure 3. Luminous bacteria reside in symbiosis on a pair of light organs in the mantle body of the squid. Utilization of the illumination function is believed to frighten nearby predators, allowing the squid to escape.

Many luminous bacteria are parasitic, with *Photobacterium* and *Vibrio* families infecting marine crustacea, and *Photorhabdus luminescens* infecting terrestrial insects, such as caterpillars, with nematodes as the intermediate host for the bacteria (Figure 4). In addition, free-living luminous bacteria that are dispersed in the seawater can often be found in both the gut tract and on the skin surface of almost all marine animals as non-specific parasites.

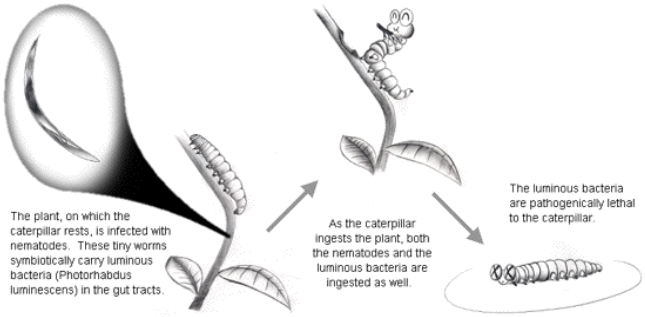


Figure 4. Infection with the terrestrial luminous bacteria (Photohabdus luminescens) is pathogenically lethal to the caterpillar.

For luminous bacteria residing within the gut tracts of marine animals, the extra-cellular chitinase produced on the cell wall of all luminous bacteria facilitate the decomposition of the ingested chitin (Figure 5) (e.g., from the exoskeletons of crustacea).

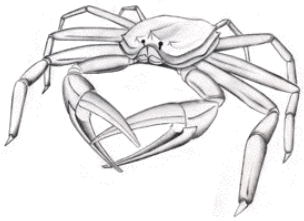


Figure 5. Luminous bacteria are non-specific parasites of crustacea. The exoskeletons of Tanner crabs are the sites of colonization of luminous bacteria, whose infection causes lesions on the surface of its appendages.

Each species of luminous bacteria differs in a number of properties, including the specific growing conditions (nutritional requirements and growth temperature), and the reaction kinetics of the luciferase involved in light generation; however, all luminous bacteria are

rod-shaped, gram-negative microorganisms with flagella facilitating motion. Luminous bacteria are also facultative anaerobes capable of growth when the supply of molecular oxygen is limited. Despite the physiological diversity among different species of luminous bacteria, all luminescent microorganisms utilize highly homologous biochemical machineries to produce light. The onset and the energy output of this light-producing molecular machinery are tightly regulated under a central signaling pathway. The text as well as the Figures below will describe the essential biochemical components of bacterial bioluminescence, and the sequential molecular interactions/reactions along the reaction pathway. The final sections will walk briefly through the signaling pathways, by which the induction and the repression of the illumination function in luminescent bacteria are carefully controlled by cellular factors and intercellular communication.

Sulphide based mutualism:

The phototrophic bacteria are a large and diverse category of bacteria that do not represent a taxon but, rather, a group of bacteria that use sunlight as their primary source of energy. This group contains both Proteobacteria and nonproteobacteria. They use solar energy to synthesize ATP through photosynthesis. When they produce oxygen, they perform oxygenic photosynthesis. When they do not produce oxygen, they perform anoxygenic photosynthesis. With the exception of some cyanobacteria, the majority of phototrophic bacteria perform anoxygenic photosynthesis. One large group of phototrophic bacteria includes the purple or green bacteria that perform photosynthesis with the help of bacteriochlorophylls, which are green, purple, or blue pigments similar to chlorophyll in plants. Some of these bacteria have a varying amount of red or orange pigments called carotenoids. Their color varies from orange to red to purple to green and they are able to absorb light of various wavelengths. Some green sulfur bacteria are able to photosynthesize at the bottom of the ocean using the light wavelengths emitted from geothermally heated rocks around hydrothermal vents! Traditionally, photosynthetic bacteria are classified into sulfur and nonsulfur bacteria; they are further differentiated by color (e.g. purple sulfur bacteria). The sulfur bacteria perform anoxygenic photosynthesis, using sulfites as electron donors and releasing free elemental sulfur. Nonsulfur bacteria use organic substrates, such as succinate and malate, as donors of electrons. The purple sulfur bacteria oxidize hydrogen sulfide into elemental sulfur and sulfuric acid and get their purple color from the pigment's bacteriochlorophylls and carotenoids. Bacteria of the genus *Chromatium* are purple sulfur Gammaproteobacteria. These microorganisms are strict anaerobes and live in water. They use carbon dioxide as their only source of carbon, but their survival and growth are possible only in the presence of sulfites, which they use as electron donors. Chromatium has been used as a model for studies of bacterial photosynthesis since the 1950s. The green sulfur bacteria use sulfide for oxidation and produce large amounts of green bacteriochlorophyll. The genus Chlorobium is a green sulfur bacterium that is implicated in climate change because it produces methane, a greenhouse gas. These bacteria use at least four types of chlorophyll for photosynthesis. The most prevalent of these, bacteriochlorophyll, is stored in special vesicle-like organelles called chlorosomes. Purple nonsulfur bacteria are

similar to purple sulfur bacteria, except that they use hydrogen rather than hydrogen sulfide for oxidation. Among the purple nonsulfur bacteria is the genus *Rhodospirillum*. These microorganisms are facultative anaerobes, which are actually pink rather than purple, and can metabolize ("fix") nitrogen. They may be valuable in the field of biotechnology because of their potential ability to produce biological plastic and hydrogen fuel.

The green nonsulfur bacteria are similar to green sulfur bacteria but they use substrates other than sulfides for oxidation. *Chloroflexus* is an example of a green nonsulfur bacterium. It often has an orange color when it grows in the dark, but it becomes green when it grows in sunlight. It stores bacteriochlorophyll in chlorosomes, similar to Chlorobium, and performs anoxygenic photosynthesis, using organic sulfites (low concentrations) or molecular hydrogen as electron donors, so it can survive in the dark if oxygen is available. *Chloroflexus* does not have flagella but can glide, like Cytophaga. It grows at a wide range of temperatures, from 35 °C to 70 °C, thus can be thermophilic.

Probable questions:

1. What are the roles played by periphyton in aquatic environment?
2. What do you mean by phycosphere?
3. Explain Black Queen Hypothesis.
4. Give examples of microbial symbiosis in marine animals.
5. State the role of bacteria in digestion of ruminants.
6. What do you mean by bioluminescence? Describe the chemistry behind bioluminescence
7. Write a short note on sulfur bacteria.

Suggested reading:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)

2. Essentials of microbiology. Amita J & Parul J. Elsevier (Latest edition).

3. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).

4. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press.

Unit XV

Disease causing microbes: *Escherichia coli* and *Streptococcus* spp.

Objective: In this unit we will learn about two disease causing microbes namely *Escherichia coli* and *Streptococcus* spp.

Escherichia Coli

Introduction

Since 1885, when it was first isolated from children's faeces and described by the German bacteriologist Theodor Escherich, scientific attention has been lavished on Escherichia coli to such an extent that it is today probably the best understood free-living organism.

Escherichia Coli is an almost universal inhabitant of the gut of humans and other warm--blooded animals where it is the predominant facultative anaerobe though only a minor component of the total microflora.

Generally, a harmless commensal, it can be an opportunistic pathogen causing a number of infections such as Gram-negative sepsis, urinary tract infections, pneumonia in immunosuppressed patients, and meningitis in neonates.

Its common occurrence in faeces, ready culturability, generally non-pathogenic character, and survival characteristics in water led to the adoption of Escherichia Coli as an indicator of faecal contamination and the possible presence of enteric pathogens such as S. typhi in water. This usage has been transferred to foods where greater circumspection is required in interpreting the significance of positive results.

They are not very common causes of foodborne illness in developed countries, but an important cause of childhood diarrhoea in less developed countries. ETEC is also frequently associated with so-called traveller's diarrhoea.

1) Morphology of Escherichia Coli:

E. coli is Gram-negative straight rod, 1-3 μ x 0.4-0.7 μ, arranged singly or in pairs (Fig. 28.1). It is motile by peritrichous flagellae, though some strains are non-motile. Spores are not formed. Capsules and fimbriae are found in some strains.

2) Cultural Characteristics of Escherichia Coli:

It is an aerobe and a facultative anaerobe. The optimum growth temperature is 37°C. On Nutrient agar, colonies are large, thick, greyish white, moist, smooth, opaque or translucent discs. The smooth (s) form seen in fresh isolation is easily emulsified in saline, whereas the rough (R) form often auto agglutinates in saline.

Some strains may form "**mucoid**" colonies. On MacConkey agar medium, colonies are bright pink due to lactose fermentation.

On selective media (Desoxycholate citrate agar-DCA; salmonella shigella-SS medium) used for the isolation of salmonella, their growth is inhibited, however their colonies are pink on DCA as it contains lactose and neutral red. In broth, there is generalized turbidity and deposit which disperses on shaking.

3) Biochemical Reaction of Escherichia coli*:*

Glucose, lactose, mannitol, maltose are fermented with acid and gas production, but sucrose is not fermented by typical strain of E. coli. In Triple sugar iron (TSI), acid and gas are produced.

The four biochemical tests widely used for entero-bacteriaceae classification are Indole (I), Methyl Red (MR), Voges Proskauer (VP) and Citrate (C) utilisation which are referred to by the mnemonic $IMV_{(1)}$ C.E. coli is Indole and MR positive VP and citrate negative ($IMV_{(1)}$ C++ —), H_2 S is not formed and urea is not hydrolysed.

4) Antigenic Structure of Escherichia Coli:

E. coli has Three Antigens:

Antigens are:

flagella and K (Kapsular) antigens. K antigen is an envelope antigen, which encloses the O antigen, renders the strain inagglutinable by the O antiserum and contributes to virulence by inhibiting phagocytosis.

It may be of three types —L, A and B. Though L type is common, the B antigen is medically important as it is found on enteropathogenic E. coli.

F (Fimbrial) Antigen:

The F antigen has no significance in antigenic classification of E. coli. Type I fimbriae mediates adhesion of bacterium to human and animal cells. Such adhesion enhances bacterial pathogenicity e.g. urinary tract infection in which type I fimbriae has some possible role to play.

Several fibrin structures resembling fimbriae have been demonstrated. They, most probably, play a very important role in pathogenesis of diarrhoeal diseases and urinary tract infection.

5) Toxin:

Besides the endotoxin associated with O antigen, some strains produce two types of exotoxin—enterotoxin and haemolysin. Enterotoxins responsible for diarrhoea are of two types—heat labile (LT); heat stable (ST).

LT is similar to cholera enterotoxin antigenically and in its mechanism of action—by stimulating the adenyl cyclase—cyclic adenosine monophosphate (cAMP) system to produce fluid accumulation in the intestinal lumen. ST appears to stimulate fluid secretion into the gut through the mediation of cyclic guanosine monophosphate (cGMP) resulting into dehydration.

6) Haemolysin by Escherichia Coli:

Three types of haemolysins produced by E. coli are not related to pathogenesis. E. coli forms a part of normal intestinal flora of man and animal and the commensal strains belong to several O groups. There are many strains of E. coli which include commensal strains as well as strains with virulence determinants that cause a wide variety of infections of all age groups of men and animals.

The virulent strains of E. coli are specific pathogens in the gut (enteritis) and of extra-intestinal sites (urinary tract infection, wound infection).

7) **Clinical infections caused by *Esch. coli* are:**

1. Urinary tract infection (UTI)
2. Septic infections of wound
3. Diarrhoea
4. Dysentery
5. Septicaemia
6. Pneumonia
7. Neonatal meningitis
8. Abscess in various organs.

Other pathogens of the family Entero-bacteriaceae causing UTI are Klebsiella, Proteus (P. mirabilis), Providence and Citrobacter. Gram-positive organisms (Staph, aureus, Enterococcus, Str. pyogenes) are also frequent pathogens of UTI.

The hospital acquired infections following instrumentation and catheterisation are mostly due to Pseudomonas and Proteus. Pregnant women (6-8%) do suffer from asymptomatic bacteriuria, if undetected and untreated, which may terminate into symptomatic infection later in pregnancy resulting to pyelonephritis and hypertension.

Calculi, enlarged prostate, pregnancy are predisposing factors in UTI. The reservoir of infection is the bacterial flora of colon. Urinary tract infection is usually from the perineum in ascending order via urethra.

A prerequisite for UTI is the colonisation of peri-urethral area by the pathogens. Due to shortness of urethra, the females are more prone to this infection. The haematogenous spread of this infection may be possible in newborn.

Diarrhoea

Four groups of *E. coli* responsible for diarrhoea in infants, children and adults are:

Enteropathogenic E. coli (EPEC); Enterotoxigenic E. coli (ETEC); Enteroinvasive E. coli (EIEC); Enterohaemorrhagic E. coli (EHEC).

I. EPEC:

EPEC is responsible for infantile diarrhoea. The pathogenic mechanism of EPEC has only recently been developed. They adhere to the intestinal mucosa, cause the loss of microvilli and prevent the entry of bacteria into the mucosa. They also produce a shigella-like toxin.

II. ETEC:

The enterotoxins are now known to produce diarrhoea in children with dehydration, traveller's diarrhoea in adults, and sometimes cholera infantum similar to cholera. ETEC also possess colonisation factors (pili, K antigen) to enhance their virulence.

III. EIEC:

EIEC do not produce enterotoxin but invade the intestinal mucosa like dysentery bacilli. They cause kerato-conjunctivitis on instillation into the eyes of guinea pig (Sereny test) which is a diagnostic method for EIEC.

Another diagnostic method is their invasion of HeLa cells in tissue culture. EIEC is late lactose fermenter and may be anaerogenic. They have antigenic relationship with shigella.

IV. EHEC:

EHEC has been very recently identified and is found to cause colitis with marked haemorrhage and absence of fever, produces Verotoxin (Cytotoxin) which affects the Vero cells in tissue culture.

8) Treatment of Escherichia Coli:

Infantile diarrhoea can be treated by Oral Rehydration Solution (ORS) as per World Health Organisation (WHO recommendation).

Medical Importance of Escherichia Coli:

E. coli is medically important because it

(a) Is used to produce insulin by adopting the genetic engineering technique,

(b) Produces certain vitamins in the intestine,

(c) Is used as parameter to determine the faecal contamination of drinking water,

(d) Is used for the plasmid study in the bacterial genetics.

Streptococcus spp.

Sore throat; Scarlet fever, Impetigo, Bacterial endocarditis, Rheumatic fever, Acute glomerulonephritis. Several species of Gram-positive cocci (i.e., pneumococci and streptococci found mostly in the throat and staphylococci predominantly found in the anterior nares) have their main habitat in the upper respiratory tract of man. Amongst these groups, there are commensals, pathogens and potential pathogens, but only the last ones affect tissues when the resistance is lowered causing bronchitis, after primary virus infection.

9) Morphology of Streptococci:

Streptococci are spherical in shape 0.6 to 1 micron in diameter, are Gram-positive and form chains Fig. 26.1. They are non-motile and do not form spores. Some strains are capsulated.

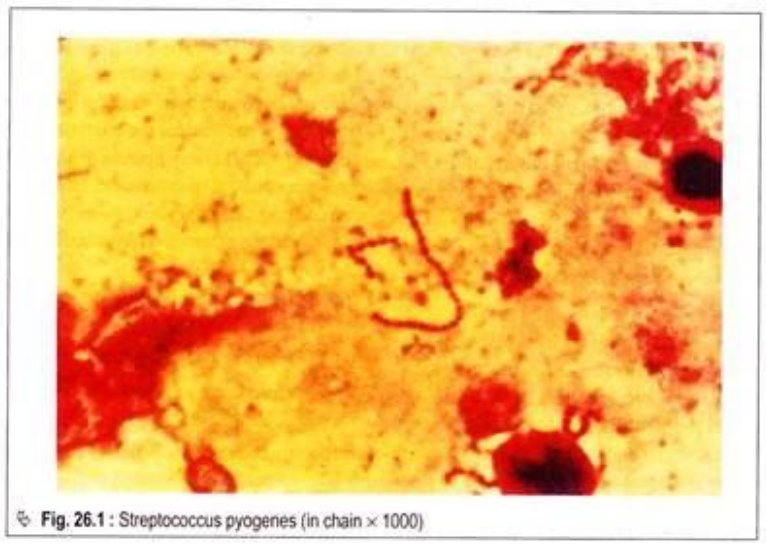


Fig. 26.1 : Streptococcus pyogenes (in chain × 1000)

10) Cultivation of Streptococci:

Streptococci are aerobic and facultatively aerobic; some are also anaerobic species and grow well on sugar, blood, serum or ascitic agar or broth. On solid media they produce small (0.5 -1 mm in diameter) transparent, greyish white colonies. On blood agar media, Strept.

Viridans produce (partial or greenish) α-haemolysis as a result of conversion of haemoglobulin into methaemoglobulin; Strept. faecalis cause no haemolysis (i.e., y-haemoly- sis); Strept. Pyogenes produce complete β-haemolysis. On sugar broth, growth is granular on the walls and at the bottom of the tube without turbidity.

1) *Strept. Viridans Infection:*

Tooth abscess. Streptococci of alpha type (Strept. viridans) are found in abscess in the roots of the teeth. The symptoms of these abscesses which are not definite may be found out by dentist X-rays plates; however, the organisms and their toxins may cause severe damage to the heart valve after setting up a focus of infection.

Heart Valve Injuries:

Injured heart valves may be infected by Strept. viridans and ultimately these organisms may cause sub acute bacterial endocarditis which is fatal. The exact mode of transmission of the organism is not yet well established, but it may, most probably, come via blood from infected teeth and tonsils, chronic sinus infection or from the intestine. The infection via blood is known as haematogenous or endogenous. In endocarditis caused by streptococci, there is inflammation of heart valve which is followed by thickening and shrinkage of heart valves causing them to leak. Arthritis may ensue if the joints are infected.

2) *Strept.pyogenes Infection:*

Strept. pyogenes produces several exotoxins. Two distinct haemolysins can be recognised; O-streptolysin which is oxygen labile and S-streptolysin which is not oxygen sensitive. This two streptomycin's are antigenically distinct and both are toxic to animals. O-streptolysin is found in rheumatic fever.

Antibody to O-streptolysin is generally determined; in the patient blood by anti-streptolysin – O titire (ASO titre); whereas S-streptolysin is responsible for β-haemolysis in blood agar medium. Other toxins are leucocidin, fibrinolysin (streptokinase), hyaluronidase, erythrogenic toxin which produces erythema when injected intradermally and plays a role in scarlet fever Streptococci gain entrance through injured skin and mucous membrane or enter the intestine with the food. They may be spread by the air droplet route. When the natural body resistance is lowered down, conditionally pathogenic streptococci normally present in the human body, may become pathogenic. Penetrating deep into the tissue they produce local pyogenic inflammation — streptoderma, abscess, lymphangitis, cystitis, pyelitis, cholecystitis and peritonitis.

Erysipelas, inflammation of the superficial lymphatic vessels and tonsillitis (inflammation of the pharyngeal and tonsillar mucosa) are among the diseases caused by streptococci. Invading the blood, streptococci produce a serious septicaemia. They are more commonly the cause of puerperal septicaemia than other bacteria.

Streptococci may cause secondary infection in patients with diphtheria, small pox, whooping cough, measles and other diseases. Chronic tonsillitis is attributed to Strept. viridans and adenovirus. Contamination of wounds with streptococci result in wound suppurations, abscess formation and traumatic sepsis.

3) Cellulitis:

Cellulitis is a most dangerous condition in which skin and underlying tissues are infected and it is caused by β-haemolytic streptococci: Group A streptococci (Strepto pyogenes) causes a very severe, rapidly spreading infection with much swelling followed by general symptoms due to potent exotoxin. The infection spreads in subcutaneous lymphatic tissue. Erysipelas is another form of acute infection of skin and underlying tissues due to P type streptococci Group A. It is rapidly progressive and frequently fatal.

Like staphylococci, beta type streptococci Group A sometimes invade the blood stream causing septicaemia and are often found in infections of lungs (pneumonia and empyema), meningitis, sinusitis and mastoiditis. Beta type streptococcal infections are controlled by broad spectrum antibiotics or penicillin or erythromycin.

4) Rheumatic Heart Disease:

Rheumatic fever is very serious and widespread disease with resulting heart disease which often cripples, is frequently fatal and is caused by beta type streptococci Group A. The allergy to Strept.pyogenes is, most probably, the underlying cause. "Relapses in rheumatic fever can be treated with sulphonamides or antibiotics".

5) Puerperal Infection:

Haemolytic streptococci gain access into the uterus:

(a) From the skin or external parts of vulva that have not been properly cleansed;

(b) From the unsterile dressings or instruments;

(c) From the hands of the doctor or nurse that have not been sufficiently disinfected, especially if the doctor or nurse is a carrier of haemolytic streptococci, a common situation.

The spread of puerperal fever or erysipelas infection can be prevented by the isolation of the infected patients. The discharges, packing's, dressings are highly infectious, so they should be discarded into the strong disinfectants or completely burnt. Clean obstetric techniques and antibiotics could prevent postpartum mortality due to streptococcal puerperal fever (child bed fever). A pregnant woman should prefer to go to hospital for delivery because of the aseptic technique available than at home. Thus, she can be protected from possible puerperal fever.

Since the open wound in the uterus, vagina and perineum of the recent post-partum patient is a very good portal of entry for streptococci, staphylococci and other microorganisms, the doctor, nurse and other personnel's should not be allowed, until and unless they follow all aseptic techniques and observe strict sterility. If these staffs have sore noses or throats, they should be forbidden from entry into the delivery room. Besides, the staffs having infectious lesions on their body are very dangerous to other patients.

6) *Bronchopneumonia:*

It may be caused by one or variety of organisms (staphylococci, alpha, beta type haemolytic streptococci). The causative agents of bronchopneumonia are usually found in the patient's own mouth or often gain access to the lungs by accidental inhalation of saliva and mucus or by inhalation of micro-organisms found in the droplets of sputum of saliva sprayed out while talking, sneezing or coughing. It is evident that the patients likely to develop bronchopneumonia must be protected from both the bacteria in their own mouth and from vomitus.

The greatest care to prevent carrying bacteria from patient to patient is to keep the mouth clean. The nursing care adopted for lobar pneumonia should be applied for bronchopneumonia. All persons (including medical and hospital personnel) with respiratory infections should be excluded.

Scarlet fever and Septic sore throat were formerly traceable to the consumption of unpasteurized milk from cows having mastitis due to Group A streptococci introduced by infected milkers. Pasteurization destroys the streptococci. Septic sore throat and Scarlet fever are different manifestations of the same infection. Strept pyogenes forms an erythrogenic toxin that produces rash on the skin which is a common manifestation in scarlet fever.

This toxin can be detected by Dick test (Skin test). Dick test is a biological test which determines the immune status of an individual to erythrogenic toxin. A diagnostic skin test, Schultz-Charlton reaction is sometimes made by injecting into the rash of the skin-a small dose of scarlet fever antitoxin. If the rash is due to scarlet fever, it promptly fades or is blanched; the blanching reaction.

Persons immunized to the toxin will not have the rash but may still contract the infection with Group A streptococci of other type. The infection may develop without rash and, therefore, it is called by another name—Septic sore throat. Acute glomerulonephritis is associated with specific serotype or nephritogenic type of Group A streptococci.

- *Transmission:*

Scarlet fever transmission from person to person is an excellent model of most diseases of upper respiratory tract. In scarlet fever, saliva or mucus from mouth or nose may contain streptococci; as a result, patient's hands and face may be contaminated with these streptococci; so kissing and contaminated handkerchiefs may transmit them and are very dangerous.

Convalescents released from isolation may become carriers for a longer period, spread the disease through any discharge from the nose, ears or any other parts of the body and disseminate streptococci. During epidemics in school children, cases of scarlet fever and septic sore throat in amongst school children can easily be detected by the nurses and well-informed intelligent school teachers and its further spread can be prevented.

- **Application to Nursing:**

It is well established that the discharges from the lesions with streptococci are very dangerous, as these discharges transmit streptococci to clean wounds, scratches, cuts, the post-partum mother, and possibly, to normal mucous membrane of the nose, throat and eyes.

Since pathogens fresh from the body are particularly virulent, the professional nurse should take care so that dressings from all septic wounds including those infected with staphylococci should be properly wrapped, securely fastened and burnt. Instruments and objects contaminated by the infected wounds should be well disinfected by boiling or with chemicals.

The intelligent nurse must always take into consideration the portal of entry and portal of exit, particularly in streptococcal infections (erysipelas, septic sore throat, scarlet fever and puerperal fever), e.g., the exudate from the erysipelas lesions is very dangerous; the nose and throat secretions of a septic sore throat or scarlet fever patient are highly infectious through only portal of entry.

Paper "wipes" or pieces of old linen or gauze can be used to collect all these secretions and ultimately discarded into a paper bag at the bedside. This paper bag should be discarded daily or frequently, according to necessity, after properly securing in a newspaper or it can be incinerated.

Dishes and other objects that were in contact with mouth or nose should be adequately disinfected by boiling or with chemicals. Above all, the nurse is responsible for prevention of transfer of the infection to non-professional workers in hospitals (food handlers, dish washers, servants who dispose off trash and garbage).

Probable questions:

1. What are the Cultural Characteristics of *Escherichia Coli*.
2. State the Application of *Streptococcus* spp. to nursing.
3. Discuss the role of *E. coli* in UTI.
4. Discuss the role of *Streptococcus* spp. in bronchopneumonia

Suggested reading:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)
2. Essentials of microbiology. Amita J & Parul J. Elsevier (Latest edition).
3. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).
4. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press.

Unit XVI

Culture techniques: Microbial nutrition and growth; types of culture media, sterilization of culture media; culture techniques: pure cultures

Objective: In this unit we will discuss about microbial culture techniques: Microbial nutrition and growth; types of culture media, sterilization of culture media; culture techniques: pure cultures.

Microbial nutrition and growth

Introduction

One of the most critical factors for microbial growth is the availability of nutrients and energy. Microbes need carbohydrates, fats, proteins, metals, and vitamins to survive, just like animals. The process of using nutrients and converting them into cellular material requires energy.

Micro and Macro Nutrients

The microbial cell is made up of several elements such as carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. These are also known as macro elements or macronutrients because these elements are required in high amounts by the microbes. Among these, C, H, O, N, S, and P are the major elements required for carbohydrates, lipids, proteins, and nucleic acids. Apart from these, the other macronutrients are found to have several biological functions. For example, Potassium ions (K+) involved in the activity of several enzymes, Calcium (Ca2+) is an important element of bacterial endospores, Magnesium (Mg2+) involved as cofactors of different enzymes, etc.

On the other hand, several other elements are also required by the microbes on a small level which are known as microelements or micronutrients or traces elements. These nutrients include manganese, zinc, cobalt, molybdenum, nickel, and copper. These are not essential elements for the growth of the microbes but these are involved in biological functions in several ways. For example, zinc (Zn2+) is present at the active site of several enzymes, manganese (Mn2+) involved in catalysis of the transfer of phosphate group, Mo (Mo2+) is essential for nitrogen fixation, etc.

The requirement for Carbon, Hydrogen, Oxygen, and Electrons

Each and every organism requires carbon, hydrogen, oxygen, and electrons for their growth and development.

Organic molecules are essential for microorganisms and a major constituent of these organic molecules is carbon. Hydrogen and oxygen are also found in the organic molecules.

Electrons have two main functions, which are the movement of electrons through electron transport chains and during other oxidation-reduction reactions can provide energy for use in cellular work and electrons also are needed to reduce molecules during biosynthesis.

The requirement of C, H, and O can be satisfied together, as most of the carbon sources often attached to hydrogen and oxygen. For instance, many heterotrophs—organisms that use reduced, preformed organic molecules as their carbon source—can also obtain hydrogen, oxygen, and electrons from the same molecules. Because the electrons provided by these organic carbon sources can be used in electron transport as well as in other oxidation-reduction reactions, many heterotrophs also use their carbon source as an energy source.

- **The requirement for Nitrogen Phosphorus and Sulphur**

i. Nitrogen is an essential element that plays an important role in the synthesis of amino acids, purines, pyrimidines, some carbohydrates, and lipids. Many microorganisms can use nitrogen in amino acids. Others can incorporate ammonia directly through the action of enzymes such as glutamate dehydrogenase or glutamine synthetase and glutamate synthase.

ii. Phosphorus is present in nucleic acids, phospholipids, nucleotides like ATP, several cofactors, some proteins, and other cell components. Almost all microorganisms use inorganic phosphate as their phosphorus source and incorporate it directly. Low phosphate levels actually limit microbial growth in many aquatic environments. Some microbes, such as *Escherichia coli,* can use both organic and inorganic phosphate. Some organophosphates such as hexose 6-phosphates can be taken up directly by the cell. Other organophosphates are hydrolyzed in the periplasm by the enzyme alkaline phosphatase to produce inorganic phosphate, which then is transported across the plasma membrane.

iii. Sulfur is needed for the synthesis of substances like the amino acids cysteine and methionine, some carbohydrates, biotin, and thiamine. Most microorganisms use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction; a few microorganisms require a reduced form of sulfur such as cysteine.

- **Growth Factors**

i. Most of the organisms are capable of producing enzymes required for biochemical pathways by the presence of nutrients; however, there are several organisms that lack specific enzymes required by the microbes. Therefore, they must obtain these

constituents or their precursors from the environment. Organic compounds that are essential cell components or precursors of such components but cannot be synthesized by the organism are called growth factors. There are three major types of growth factors such as Amino acids, purines, and pyrimidines and Vitamins. Some microorganisms require many vitamins; for example, *Enterococcus faecalis* needs eight different vitamins for growth. Other growth factors are also seen; heme (from hemoglobin or cytochromes) is required by *Haemophilus influenzae,* and some mycoplasmas need cholesterol.

ii. Understanding the growth factor requirements of microbes has important practical applications. Both microbes with known, specific requirements and those that produce large quantities of a substance (e.g., vitamins) are useful. Microbes with a specific growth factor requirement can be used in bioassays for the factor they need. The atypical assay is a growth-response assay, which allows the amount of growth factor in a solution to be determined.

The Growth Curve

Binary fission is the procedure by which microbial cell division occurs. The population of growth is understood by studying the growth curve. When microorganisms are cultivated in the liquid medium, they usually are grown in batch culture or closed system—that is, they are incubated in a closed culture vessel with a single batch of medium. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases

1. Lag Phase

At first, the microorganisms are introduced in a fresh medium, in that medium, the organisms will not multiply immediately. Hence, the cell division does not occur at the very first moment. There can be several reasons behind this, such as old cells that lack adequate ATP molecules, ribosomes, essential co-factors to start the cell division, the characteristic of the medium can be different from the previous one, the microbes can be injured and required time for recovery, etc.

The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in a longer lag phase. On the other hand, when a young, vigorously growing exponential phase culture is transferred to a fresh medium of the same composition, the lag phase will be short or absent.

2. Exponential or Log Phase

It is the second stage of the microbial growth curve. In this case, the microorganisms start dividing rapidly at its highest possible rate. The rate of division depends on the characteristics of the medium, genetic organization of the organism and other environmental factors. Their rate of growth is constant during the exponential phase; that is, the microorganisms are dividing and doubling in number at regular intervals. Because each individual divides at a slightly different moment, the growth curve rises smoothly rather than in discrete jumps. Uniform growth is observed throughout the exponential phase which helps the researcher to carry forward any physical or chemical activity studies in this phase of growth of microbes. Exponential growth is balanced growth. That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, unbalanced growth results. This stage is also known for the production of primary metabolites, which are elements produced by organisms essentially required for their growth. Primary metabolites are important to carry out physiological functions. Examples of primary metabolites are amino acids, nucleic acids, vitamins, etc.

3. Stationary Phase

After the log phase, the bacterial growth ceases almost completely due to the exhaustion of nutrients and the accumulation of toxic products. The number of progeny cells formed is just enough to replace the number of cells that die. The number of the viable cell remains stationary as there is almost a balance between dying cells and newly formed cells. Microbial populations enter the stationary phase for several reasons. One obvious factor is nutrient limitation; if an essential nutrient is severely depleted, population growth will slow. Aerobic organisms often are limited by O2 availability. Oxygen is not very soluble and may be depleted so quickly that only the surface of culture will have an O2 concentration adequate for growth. The cells beneath the surface will not be able to grow unless the culture is shaken or aerated in another way. Population growth also may cease due to the accumulation of toxic waste products. This factor seems to limit the growth of many anaerobic cultures (cultures growing in the absence of O2). For example, streptococci can produce so much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and growth is inhibited. Streptococcal cultures also can enter the stationary phase due to the depletion of their sugar supply. Finally, there is some evidence that growth may cease when a critical population level is reached. In phase secondary metabolites are produced by the organisms which are compounds with no direct connection with the growth of the organism. For example antibiotics, naphthalenes, alkaloids, etc.

4. Death Phase

After the period of the stationary phase, the bacterial population decreases due to the death of cells. The death phase starts due to the death of cells. The phase starts due to exhaustion of nutrients, accumulation of toxic products and autolytic enzymes. There is a

decline in the variable count and not in the total count. With autolytic bacteria, even the total count shows the phase of decline.

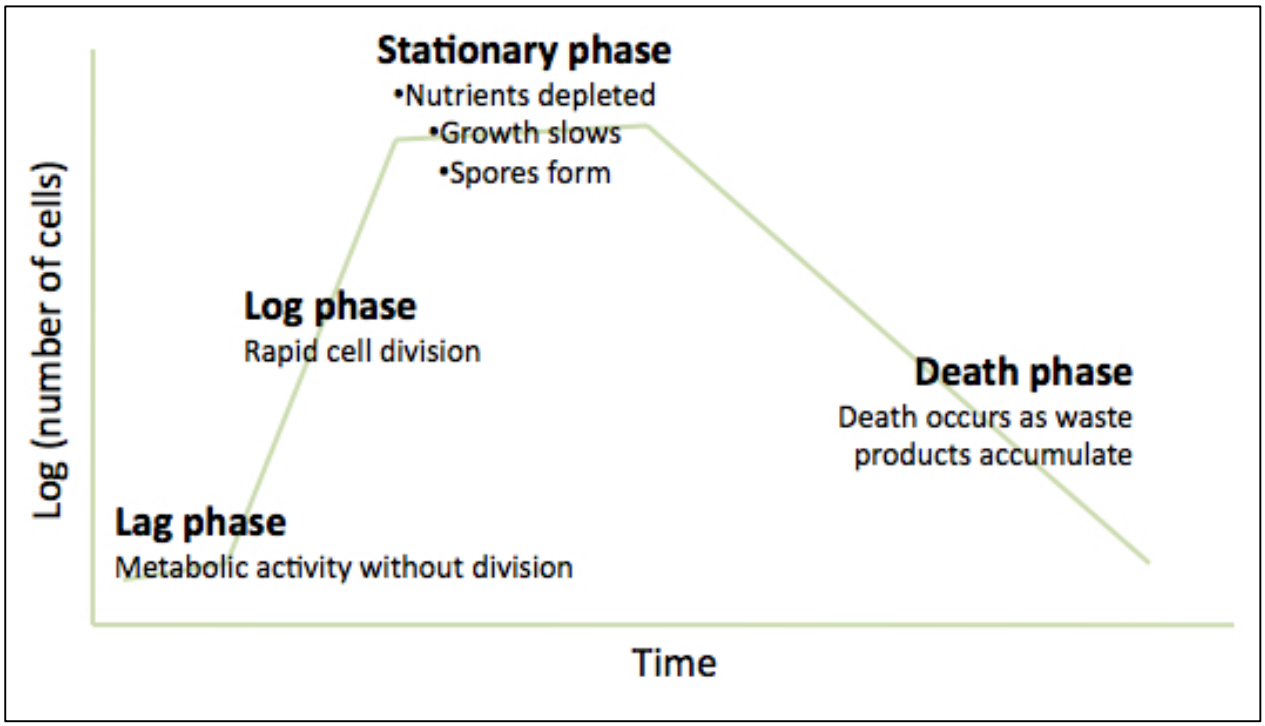


Fig: Growth curve of bacteria

Types of culture media

Bacterial cultural media can be classified in various ways.

Classification Bacterial culture media can be classified in at least three ways;

- Based on consistency
- Based on nutritional component
- Based on its functional use.

Based on consistency the media are of three types as 1) Liquid 2) Semisolid 3) Solid medium

1. Liquid medium: Agar is not added or used while preparing the medium. After inoculation and later incubation, the growth of cells becomes visible in the form of small mass on the top of the broth. eg. Nutrient broth

2. Semi-solid medium: Half quantity of agar is added This type of medium may be selective which promote the growth of one organism and retards the growth of the other organism.

3. Solid medium: If agar is added to a nutrient broth, it becomes solid medium. It is used for isolating microbes and to determine characteristics of colonies. It remains solid on incubation and not destroyed by proteolytic bacteria.

Based on nutritional component media can be classified further as Natural, complex, synthetic/defined and semi-synthetic based on the nutrients required for the growth of microorganisms. The bacteria which are able to grow with minimal requirements are said to be non-fastidious and those that require supplements or extra nutrients are said to be fastidious.

1.Natural medium: Culture media of which, the exact chemical composition is not known is called natural or empirical culture media. Examples- Milk, urine, diluted blood, vegetable juices, meat extracts, beef and tomato juice, blood etc

2. Complex medium: Media that contain some ingredients whose exact chemical composition is not known are complex media. These kinds of media are very useful as a single complex medium can be sufficiently rich and can be able to meet all the nutritional requirements of a diverse group of bacteria. Sometimes the exact nutritional requirement of a particular group of microorganisms is not known; hence they can be grown in complex media. This is especially applicable for the group of fastidious bacteria; some of them may even require a medium containing serum or blood (Table 1).

Some Common Complex Media

Nutrient Broth	Amount (g/liter)
Peptone (gelatin hydrolysate)	5
Beef extract	3
Tryptic Soy Broth	
Tryptone (pancreatic digest of casein)	17
Peptone (soybean digest)	3
Glucose	2.5
Sodium chloride	5
Dipotassium phosphate	2.5
MacConkey Agar	
Pancreatic digest of gelatin	17.0
Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Table 1: List of some complex media used in routine microbiological based works (Taken from Microbiology-Prescott, 2002).

Complex media generally contains undefined ingredients as peptones, meat extract and yeast extract. Peptones are protein hydro lysates which is prepared by incomplete proteolytic digestion of meat, casein, soya, meal, gelatin and other protein sources. They are sources of carbon, energy and nitrogen. Beef extract commonly used in complex media are sources of amino acids, peptides, nucleotides, organic acids, vitamins and minerals. Whereas yeast extract derived from brewer's yeast is a rich source of vitamin B as well as nitrogen and carbon sources. Three commonly used complex media are nutrient broth, tryptic soy broth and MacConkey agar as shown in Table 2.

3. Semi-synthetic: Culture media, the chemical components of which are partially known and partially obscure are termed as semisynthetic culture media. Examples- Potato dextrose agar (PDA), Czapek-Dox agar, oat meal agar (OMA), corn meal agar (CMA), beef peptone agar and nutrient agar.

4. Synthetic medium: Such media are composed of the substances that are chemically known. These media are very useful in studying the physiology, metabolic nature and nutritional requirements of microbes. Both autotrophs and heterotrophs can be grown in these media. Examples- Mineral glucose medium, Richard's solution, Raulins medium etc

Based on application or function, media can be classified as follows.

1. Basal Medium: are basically simple media that supports most non-fastidious bacteria. Ex- peptone water, nutrient broth and nutrient agar. They are generally considered for growing broad spectrum of bacteria
2. Minimal medium: Minimal media are those kinds of media that contain the minimum nutrients required for colony growth, generally without the presence of amino acids, and are often used in laboratories to isolate wild type microorganisms. Minimal media can also be used to select for or against recombinants bacteria.

Minimal medium typically contains:

- a carbon source for bacterial growth, which may be a sugar such as glucose
- various salts, which may vary among bacteria species and growing conditions; these generally provide essential elements such as magnesium, nitrogen, phosphorus, and sulfur to allow the bacteria to synthesize protein and nucleic acid
- water

3. Selective media: Used for a selected species. Provide nutrients that enhance the growth and predominance of particular microbe and don't enhance or may inhibit other types of organisms that may be present.

Examples of selective media:

- eosin methylene blue (EMB) that contains methylene blue – toxic to Gram-positive bacteria, allowing only the growth of Gram-negative bacteria

- YM (yeast and mold) which has a low pH, deterring bacterial growth
- MacConkey agar for Gram-negative bacteria
- Hektoen enteric agar (HE) which is selective for Gram-negative bacteria
- mannitol salt agar (MSA) which is selective for Gram-positive bacteria and differential for mannitol
- Terrific Broth (TB) is used with glycerol in cultivating recombinant strains of Escherichia coli.
- xylose lysine desoxyscholate (XLD), which is selective for Gram-negative bacteria
- buffered charcoal yeast extract agar, which is selective for certain gram-negative bacteria, especially Legionella pneumophila

4. **Differential media**: It allows differentiation among morphologically and biochemically related group of organisms. It employs the addition of one or more substances that allow differentiating between very closely related species based on specific biochemical or physiological properties. The differential contains in the culture medium contains specific chemicals to indicate which species possess and which lack a particular biochemical process.

   Examples of differential media include:

   - blood agar (used in strep tests), which contains bovine heart blood that becomes transparent in the presence of hemolytic Streptococcus
   - eosin methylene blue (EMB), which is differential for lactose and sucrose fermentation
   - MacConkey (MCK), which is differential for lactose fermentation
   - mannitol salt agar (MSA), which is differential for mannitol fermentation
   - X-gal plates, which are differential for lac operon mutants

5. Enriched medium: Natural environments usually contain numerous kinds of bacteria or other microorganisms. Some microorganisms are very particular in their growth requirements and are cultured only when they are provided with rich in special nutrients. These kinds of media are generally used to grow fastidious organisms. It is generally done by addition of extra nutrients in the form of blood, serum, egg yolk, etc. to basal medium to make them enriched media. Blood agar, chocolate agar are examples of enriched media.

Sterilization of culture media

Sterilization is the removal of all forms of microorganisms from the surface of an object. It includes both spore and vegetative forms. Here, let's glance at the definition and classification of sterilization notes.

In microbiology, sterilization can be defined as the complete removal of all forms of microorganisms, both vegetative and spore forms, from a surface or an object. Sterilization is carried out by various physical and chemical methods such that it eliminates around 10^6 log colony-forming units.

Sterilization is done to avoid the growth of microorganisms which may grow on the surface of an object if left without killing the germs. It is, however, different from disinfection or sanitisation where only reduction of the microorganisms takes place, rather than total elimination. After sterilization, an object becomes sterile or aseptic.

The Sterilization is carried out by the methods according to requirement. The methods are: 1. Moist Heat Sterilization 2. Dry Heat Sterilization 3. Gas Sterilization and Others.

Method # 1. Moist Heat Sterilization:

Moderate pressure is used in steam sterilization. Steam is used under pressure as a means of achieving an elevated temperature. It is important to ensure the correct quality of steam is used in order to avoid the problems which follow, superheating of the steam, failure of steam penetration into porous loads, incorrect removal of air, etc.

For aqueous preparations and for surgical dressing, heating in saturated steam under pressure is carried out. A number of time-temperature combinations have been proposed.

The British and European Pharmacopoeia (2001) recommended 121°C temperature and 15 $lb/inch^2$ maintained throughout the load for 15 minutes as the preferred combination for this method of terminal sterilization. It is used to sterilize aqueous parenteral solutions and suspensions, surgical dressing and fabrics, plastic and rubbers closures, metal instruments, glass apparatus etc.

Structure of Autoclave:

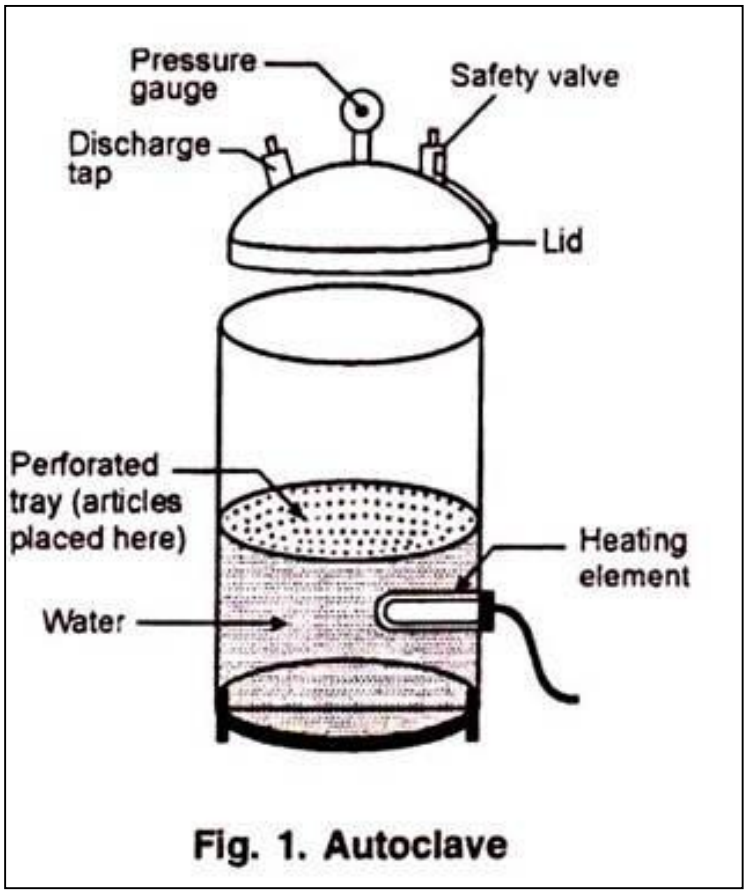


Fig. 1. Autoclave

A simple autoclave has vertical or horizontal cylindrical body with a heating element, a perforted tray to keep the articles, a lid that can be fastened by screw clamps, a pressure gauge, a safety valve and a discharge tap (Fig. 1). The lid is closed but the discharge tap is kept open and the water is heated.

As the water starts boiling the steam drives air out of the discharge tap, when all the air is displaced and steam starts appearing through the discharge tap, the tap is closed. The pressure inside is allowed to rise up to 15 lbs. per square inch. At this pressure the articles are heated for 15 minutes, after which the heating is stopped and the autoclave is allowed to cool.

Once the pressure gauge shows the pressure equal to atmospheric pressure, the discharged tap is opened to let the air in. The lid is opened and articles are removed. Culture media, dressing, certain equipment's can be sterilised by autoclave.

Method # 2. Dry Heat Sterilization:

Dry heat sterilization is used for heat-stable non-aqueous preparations, powders and certain impregnated dressings. It may also be used for sterilization of some types of container. Sterilization by dry heat is usually carried out in a hot-air oven. Heat is transferred from its source to load by radiation, convention and to a small extent by conduction.

Temperature-time exposures necessary to kill pathogen by dry heat indicates that a period of 90 minutes at 100°C destroys all vegetative bacteria but a period of 3 h at 140″C kills the spores.

Mould spores are of intermediate resistance and are killed in 90 minutes at 115°C. Most viruses have resistance similar to vegetative bacteria but some viruses are as resistant as bacterial spores e.g. virus that causes homologous serum jaundice.

The British Pharmacopoeia (2001) states a minimum temperature of 160°C for at least 2 h for dry heat sterilization. Other combinations of temperature and time are permissible

subject to first demonstrating a reproducible level of lethality in routine operation. Dry heat treatment, greater than 220°C, provides a useful method for sterilization and dehydrogenation of glassware in a particular container intended for a large volume of parenteral dosage.

This process can remove heat-resistant endotoxin. In each cycle it is important to ensure that the whole content of each container is maintained for an effective combination of time and temperature especially to allow temperature variations in hot-air ovens, which may be considerable. Dry heat is used to sterilize glassware, porcelain and metal equipment, oils and fats and powders i.e. talc, etc.

Method # 3. Gas Sterilization:

Gaseous sterilizing agents are of two main types, oxidizing and alkylating agents. Vapour phase hydrogen peroxide is an example of the former. Ethylene oxide and formaldehyde are examples of the alkylating agents. However, the BP states that gaseous sterilization is used when there is no suitable alternative. The main advantage of ethylene oxide is that many types of materials, including thermolabile materials, can be sterilized without damage.

The gas can diffuse through packaging materials and rubber, and diffuse out after sterilization. It has the disadvantages of being toxic and combustible and also requires the correct humidity. In practice, the relative humidity in the chamber atmosphere is usually between 40 and 50% with temperatures up to 6°C.

Low temperature steam with formaldehyde has been used as an option for sterilizing thermolabile substances. Both ethylene oxide and formaldehyde have health risks and strict monitoring of personnel exposed to the gases required to ensure protection from harmful effects.

Method # 4. Sterilization by Radiation:

Radiations can be divided into two groups: electromagnetic waves and streams of particulate matter. The former group includes infrared radiation, ultraviolet light, X-rays and gamma rays. The latter group includes alpha and beta radiations. Most commonly infrared radiation, ultraviolet light, gamma radiation and high-velocity electrons are used for sterilization.

(i) Ultraviolet Light:

A narrow range of UV wavelength (220-280 nm) is effective in killing the microorganism. The wavelength close to 265 nm and adjacent wavelengths are strongly absorbed by the nucleoproteins. The most serious disadvantage of UV radiation as a sterilizing agent is its poor penetrating power. This is the result of strong absorption by many substances. The application of UV radiation is limited.

(ii) Ionizing Radiations:

Ionizing radiations are suitable for commercial sterilization processes. It must have good penetrating power, high sterilizing efficiency, little or no damage effect on irradiated materials and are capable of being produced efficiently. The radiations that fulfill these four criteria are best high-speed electrons from machines and gamma rays from radioactive isotopes.

Sterilization by gamma rays is carried out using the radioactive isotope of [CO-60]. Articles for sterilization by radiations are packed in boxes of standard size which are sterilized by a series of slow passages around the gamma ray source. The absorbed dose for sterilization is 25 kGy.

The plastic syringes and catheters, hypodermic needles and scalped blades, adhesive dressings, single-application capsules of eye ointment, containers made of polythene and packaging materials using aluminium foil and plastic films are sterilized by gamma radiation.

Method # 5. Sterilization by Filtration:

Membrane filters are made from cellulose derivates or other polymers. There are no loose fibres or particles in membrane filters. They retain particles larger than the pore size on the filter surface such filters particularly useful in detection of small numbers of bacteria.

Sterilization by filtration is a method permitted by the British pharmacopoeia (2001) for heat sensitive solutions or liquids that are not sufficiently stable to withstand the process of heating in an autoclave.

Passage through a filter of appropriate pore size (e.g. 0.22 pm) can remove bacteria and moulds. Viruses and mycoplasma may not be retained. After filtration the liquid is aseptically distributed into previously sterilized containers which are later sealed. This method has disadvantages that specialized facilities and skilled operations are required.

The final preparations cannot be released until the manufacturing batch has passed the appropriate test for sterility.

Culture techniques: pure cultures

What is a Pure Culture?

The environment around us consists of a mixed population of microbes. Each of these groups of organisms contributes to the ecology. However, ever wondered how are the studies carried on such microbes? Is it feasible to study and experiment with all of these together? Well, no. It isn't. To carry out the studies on these microbes, it is essential to dissect the mixed culture into a pure culture.

Now, what is a pure culture? What purpose does it serve? What is pure culture microbiology? What are the pure culture methods or pure culture techniques? Let us help you understand all these terms in detail.

Pure Culture Definition

Pure culture is defined as a laboratory culture that contains just one species of organisms in microbiology. Microbes are usually in mixed cultures. But, the pure culture can be derived from it through the transfer of a bit of its sample in the new and sterile growth medium.

The process of pure culture isolation usually takes place by the dispersion of cells across the surface medium. This mainly involves thinning of the sample before it is inoculated into a new medium. There are several pure culture techniques utilized by scientists to carry out the process of the creation of discrete colonies of pure microbes.

The inculcation of such techniques of pure culture led to bacterias characterization that was responsible for causing anthrax, tuberculosis, and such major diseases. Following this, scientists developed similar procedures for protozoa, fungi, and algae.

Importance of Pure Culture in Microbiology?

- The organisms of pure culture can be grown, tested, characterized, and identified.
- It is feasible to study the clinical aspects and physiology of pure culture organisms.
- Irrespective of the number of times the test is done, the same results will be obtained for a particular culture of organisms during any sort of test.
- Spontaneous mutation occurs slowly in the case of pure culture, and the clone so formed is identical in all forms and aspects.

There are several ways to prepare pure cultures:

A. The Spread Plate method:

1. Mixture of cells is spread out on an agar surface at a relatively low density so that every cell grows into a completely separate colony, a macroscopically visible growth or cluster of microorganisms on a solid medium.

2. Each colony arises from a single cell and represents a pure culture.

3. The spread plate is an easy, direct way of achieving pure culture of bacteria. Steps involved in spread plate method:

a) A small volume of dilute microbial mixture containing around 30 to 300 cells is transferred to the centre of an agar plate.

b) The dilute microbial mixture is spread evenly over the surface with a sterile bent-glass rod.

c) The dispersed cells develop into isolated colonies.

d) As the number of colonies should be equal to the number of viable organisms in the sample, spread plates can be used to count the microbial population.

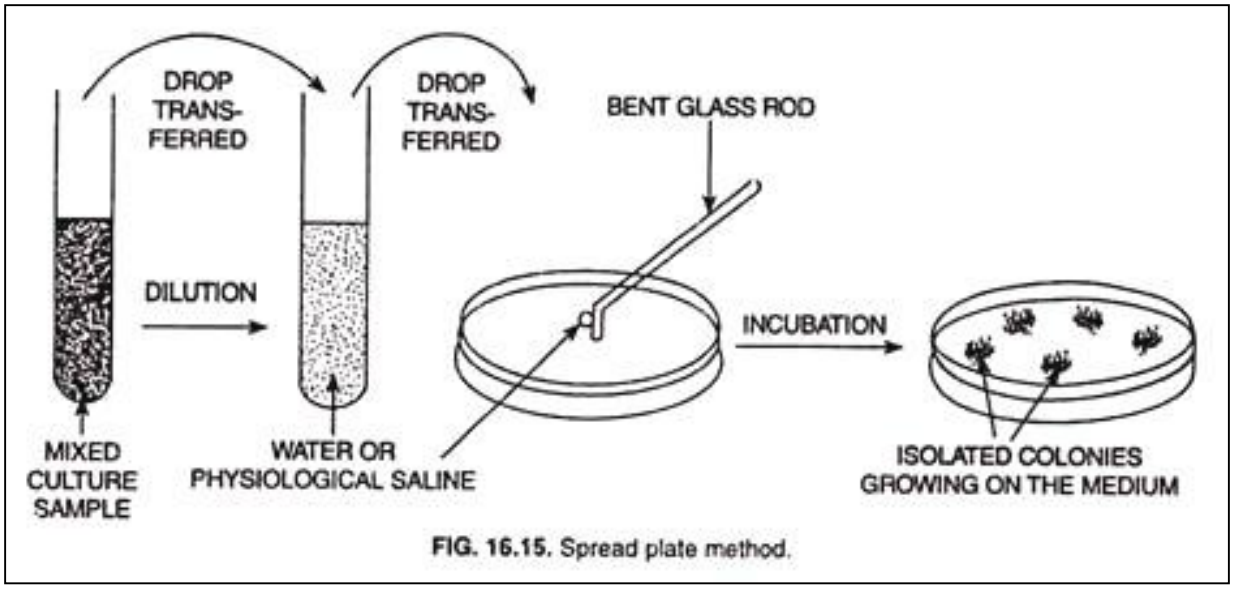


FIG. 16.15. Spread plate method.

B. The Streak Plate method: Pure colonies also can be obtained from streak plate method. Streak Plate Method is one of the most exercised and convenient methods for the

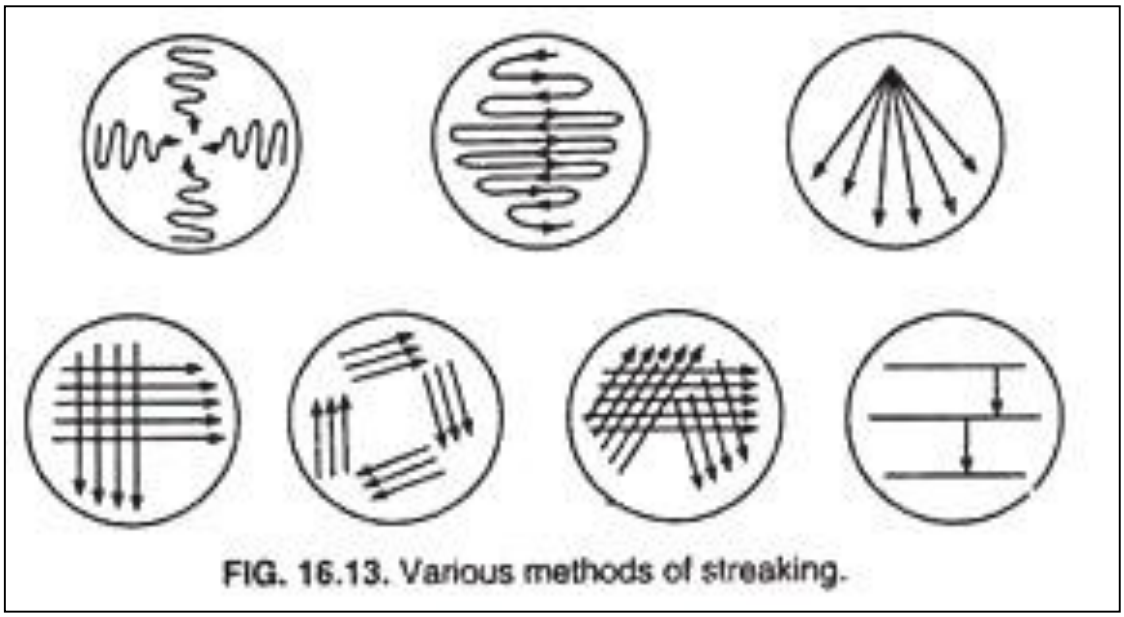


FIG. 16.13. Various methods of streaking.

isolation of bacteria from various samples. This method depends on first sterilizing and

charging the nichrome wire loop, which is then streaked/ rubbed on the solid surfaces of the agar medium in a manner that thins out and progressively dilutes the population of microorganisms in a way that helps get very well isolated colonies at the end.

C. The pour plate method: It is based on the principle of counting viable colonies of microorganisms using serial dilution. A serially diluted sample (usually 1 ml) is poured into the petri dish, and molten agar at 45-50℃ is added to the dish and swirled. After solidification, the plate is incubated at an optimal temperature. Viable microbial colonies can be observed on the plate after incubation that can be counted. The CFU/ml can be obtained by the following formula:

$$CFU/ml = \frac{Total number of colonies * dilution factor}{volume of specimen used (aliquot)}$$

Pour Plate Method Procedure

- Sterilise all the instruments, flasks, and media that are required for the streaking procedure.
- Clean your work area using a disinfectant to minimise any contamination.
- Set up the bunsen burner in your work area carefully.
- Wash your hands with an antiseptic solution before handling any microbial solution.
- Label the petri dish with all important information, such as your name, date, media used, and the culture being inoculated.
- **Sample Preparation:** If the sample is in semisolid or solid form, suspend it in sterile water or broth to prepare a liquid solution. If the sample is already in liquid form, prepare serial dilutions of the sample to lessen the load of microbial colonies in the range of 20-300 CFU/ml. You can prepare dilutions up to 10^{-10}.
- For inoculation, open the lids of the Petri dishes and pour 1 ml of the diluted sample. Take the molten agar, heat it a little and pour around 15-18 ml of it onto the sample. Keep in mind that the agar should not be either too hot or too cold. Close the lid of the dish and swirl it slowly.
- Another method for inoculation is to mix the diluted sample in the agar medium, mix it gently, and then pour it into the petri dish. However, this method is less commonly used.
- Let the plate solidify.
- Invert the plate and incubate it at an optimal temperature (usually 37℃) for 24-48 hours.

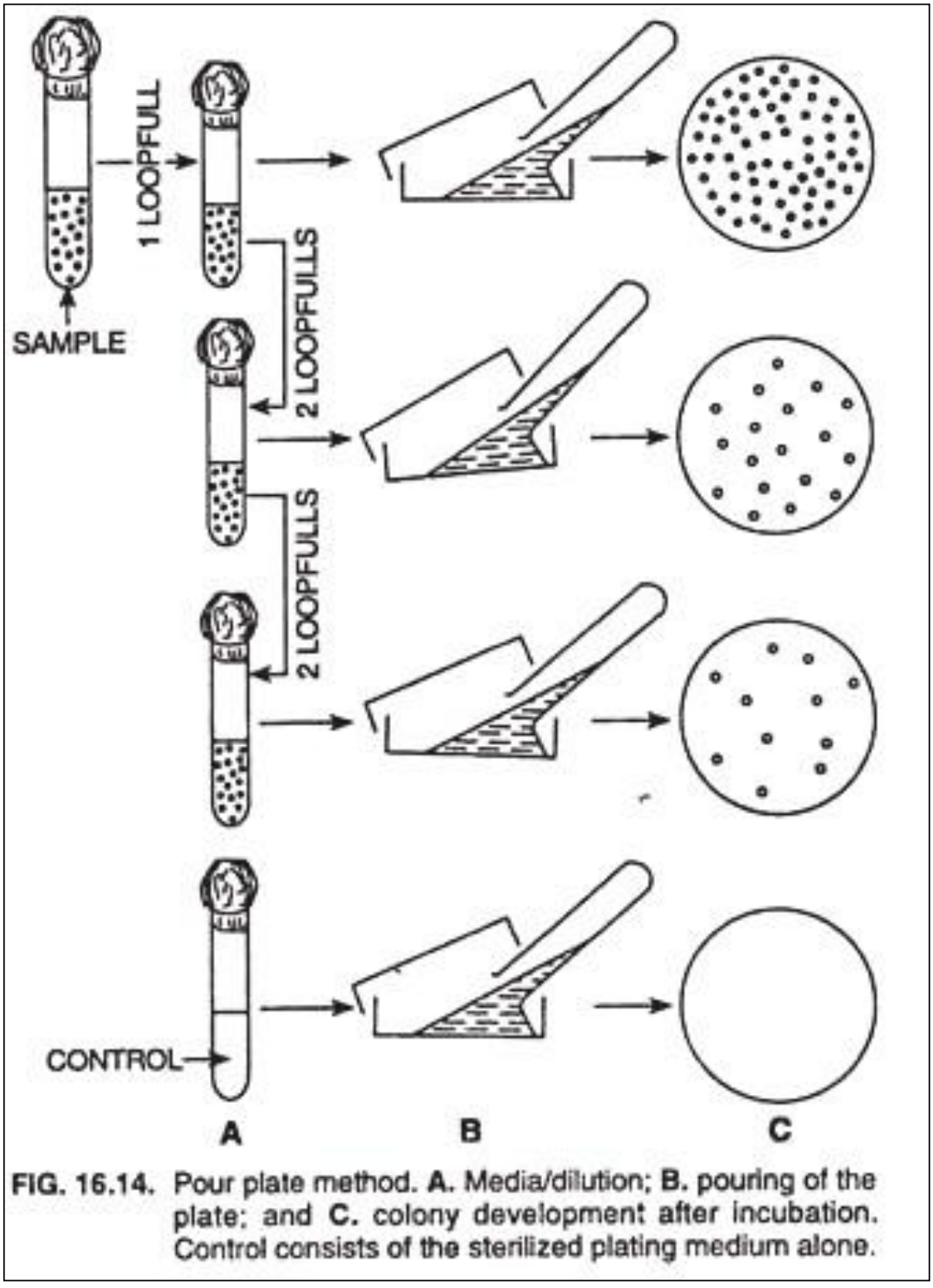


FIG. 16.14. Pour plate method. **A.** Media/dilution; **B.** pouring of the plate; and **C.** colony development after incubation. Control consists of the sterilized plating medium alone.

Advantages of the Pour Plate Method

- It is useful for counting viable colonies.
- It can detect very low loads of bacterial counts as well.
- It does not require previously solidified agar plates.
- It can also be used for clinical and environmental samples.

Pour plate method has certain disadvantages as follows:

- The picking up of subsurface colonies needs digging them out of the agar medium thus interfering with other colonies, and
- The microbes being isolated must be able to withstand temporary exposure to the 42-45° temperature of the liquid agar medium; therefore, this technique proves unsuitable for the isolation of psychrophilic microorganisms.

However, the pour plate method, in addition to its use in isolating pure cultures, is also used for determining the number of viable bacterial cells present in a culture.

4. Serial Dilution Method:

As stated earlier, this method is commonly used to obtain pure cultures of those microorganisms that have not yet been successfully cultivated on solid media and grow only in liquid media.

A microorganism that predominates in a mixed culture can be isolated in pure form by a series of dilutions. The inoculum is subjected to serial dilution in a sterile liquid medium, and a large number of tubes of sterile liquid medium are inoculated with aliquots of each successive dilution.

The aim of this dilution is to inoculate a series of tubes with a microbial suspension so dilute that there are some tubes showing growth of only one individual microbe. For convenience, suppose we have a culture containing 10 ml of liquid medium, containing 1,000 microorganisms (Fig. 16.16.), i.e., 100 microorganisms/ml of the liquid medium.

If we take out 1 ml of this medium and mix it with 9 ml of fresh sterile liquid medium, we would then have 100 microorganisms in 10 ml or 10 microorganisms/ml. If we add 1 ml of this suspension to another 9 ml. of fresh sterile liquid medium, each ml would now contain a single microorganism.

If this tube shows any microbial growth, there is a very high probability that this growth has resulted from the introduction of a single microorganism in the medium and represents the pure culture of that microorganism.

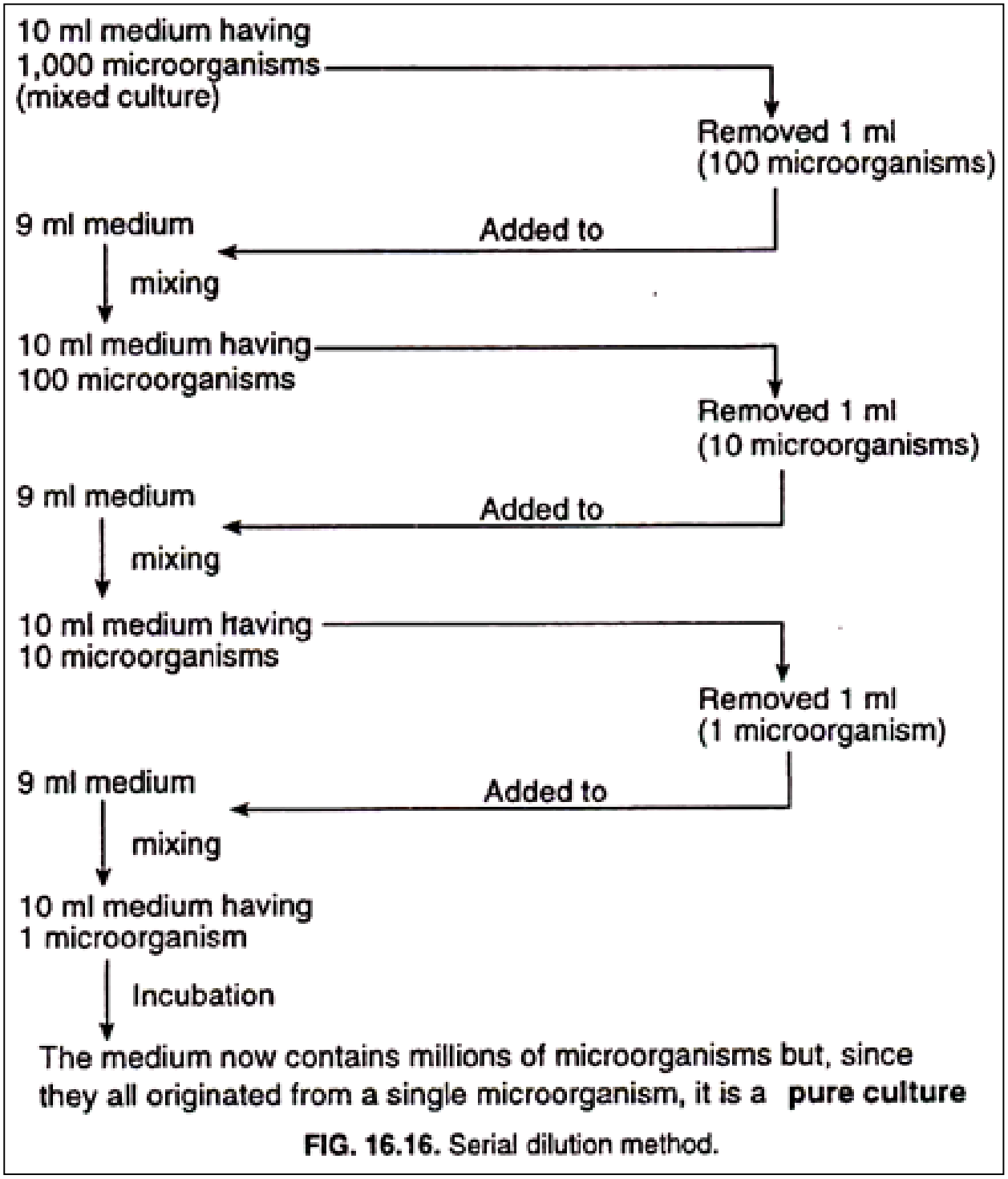


FIG. 16.16. Serial dilution method.

5. Single Cell Isolation Methods:

An individual cell of the required kind is picked out by this method from the mixed culture and is permitted to grow.

The following two methods are in use:

i. Capillary pipette method:

Several small drops of a suitably diluted culture medium are put on a sterile glass-coverslip by a sterile pipette drawn to a capillary. One then examines each drop under

the microscope until one finds such a drop, which contains only one microorganism. This drop is removed with a sterile capillars pipette to fresh medium. The individual microorganism present in the drop starts multiplying to yield a pure culture (Fig. 16.17).

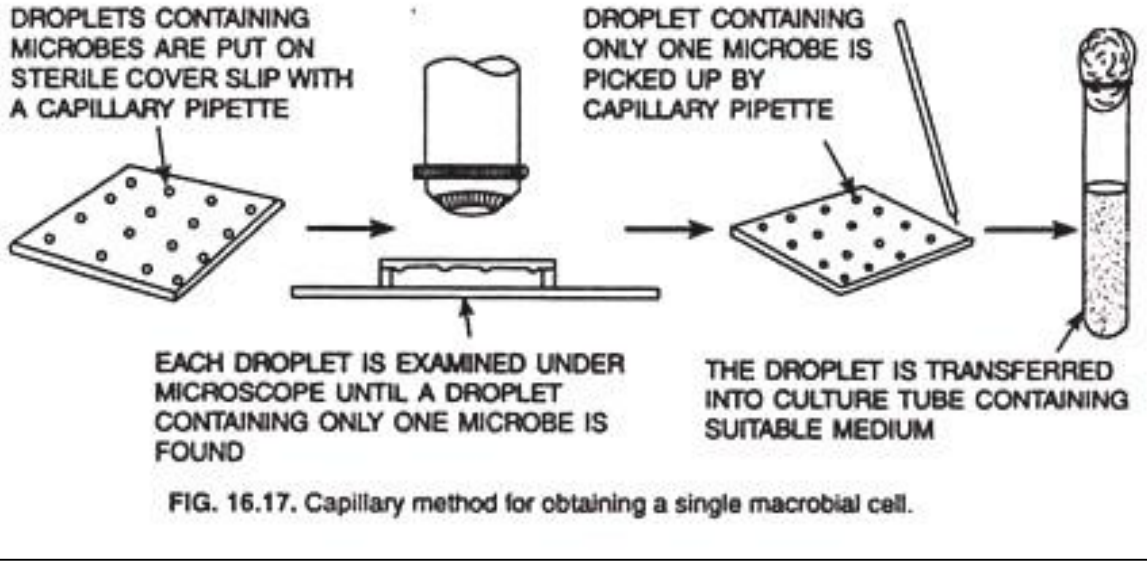


FIG. 16.17. Capillary method for obtaining a single macrobial cell.

ii. Micromanipulator method:

Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture. This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation. The micro-manipulator has micrometer adjustments by means of which its micropipette can be moved right and left, forward, and backward, and up and down.

A series of hanging drops of a diluted culture are placed on a special sterile coverslip by a micropipette. Now a hanging drop is searched, which contains only a single microorganism cell.

This cell is drawn into the micropipette by gentle suction and then transferred to a large drop of sterile medium on another sterile coverslip. When the number of cells increases in that drop as a result of multiplication, the drop is transferred to a culture tube having suitable medium. This yields a pure culture of the required microorganism.

The advantages of this method are that one can be reasonably sure that the cultures come from a single cell and one can obtain strains with in the species. The disadvantages are that the equipment is expensive, its manipulation is very tedious, and it requires a skilled operator. This is the reason why this method is reserved for use in highly specialised studies.

6. Enrichment Culture Method:

Generally, it is used to isolate those microorganisms, which are present in relatively small numbers or that have slow growth rates compared to the other species present in the mixed culture.

The enrichment culture strategy provides a specially designed cultural environment by incorporating a specific nutrient in the medium and by modifying the physical conditions of the incubation. The medium of known composition and, specific condition of incubation favours the growth of desired microorganisms but, is unsuitable for the growth of other types of microorganisms.

Probable questions:

1. What is lag phase?
2. What is bacterial growth curve?
3. What is complex media? Give example.
4. What is selective media? Give example.
5. What is spread plate culture technique of bacterial culture?

Suggested reading:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)
2. Essentials of microbiology. Amita J & Parul J. Elsevier (Latest edition).
3. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).
4. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press
5. Prescott, Harley, and Klein's Microbiology, 7th Edition.

Unit-XVII
Industrial microbiology: Microbial fermentation; production and commercialization

Objectives:

In this section we will discuss on industrial microbiology: microbial fermentation; production and commercialization.

Introduction:

Fermentation word is derived from Latin verb "fervere" which means to boil. This technology has been perceived in different sense by the world of microbiologist and biochemist. For biochemists, "fermentation is a catabolic process leading to generation of energy". Whereas, according to industrial microbiologist, "fermentation is the process of mass cultivation of micro-organisms that convert substrates into valuable products through aerobic or anaerobic route".

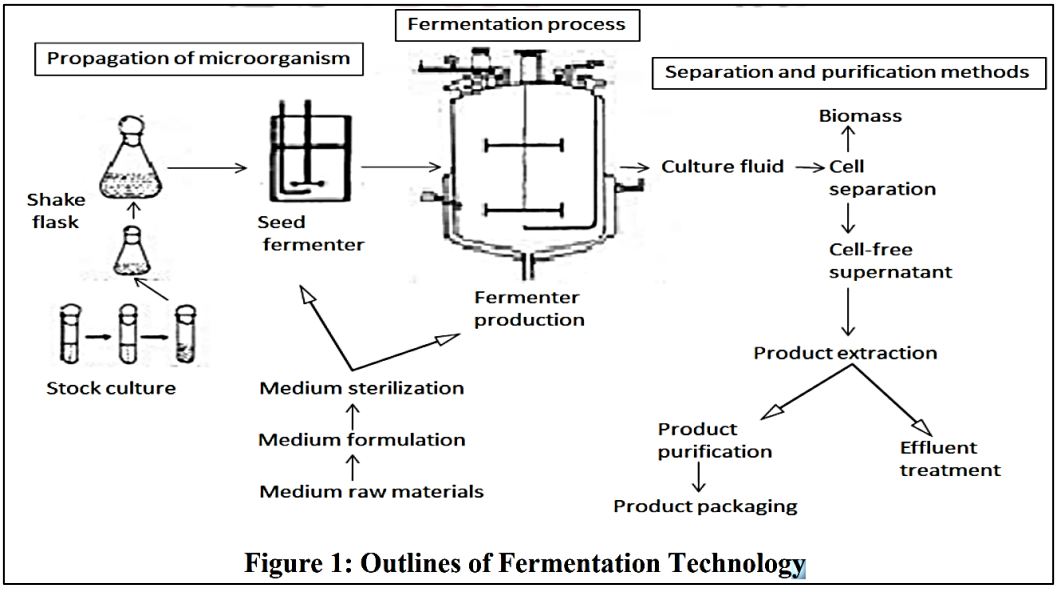


Figure 1: Outlines of Fermentation Technology

A general outline of the process is outlined in figure 1. Fermentation technology has wide application for the production of products such as organic solvents (acetone, alcohols), fermented beverages (wine, beer, whisky), and other products like enzymes, amino acids, vitamins, pharmaceuticals etc. The fermentation processes is dependent on microbial growth, which in turn is governed by many biochemical and physical parameters. The motive of all parameters is to provide the most suitable environment for the growth of micro-organisms.

Microbial Growth:

The most important criterion of a fermentation process is to achieve good yield of product which in turn is dependent on the proper microbial growth. The micro-organism requires optimum pH, temperature, oxygen, minerals, energy source and other raw material conditions to complete their life cycle under six phases as illustrated in figure 2 and discussed below:

1) **Lag Phase** While there is no measurable increase in the bacterial population, the bacteria are expected to be adapting to the new media and resources, with increased metabolic activity, a potential increase in mass and volume and changes in gene regulation. This is the most poorly understood step in the bacterial growth cycle. The duration of this lag phase varies according to the species along with many different factors, such as the composition of the media, temperature, size of the inoculation sample, etc.

2) **Exponential Phase** The exponential phase, or log phase, is the actual rapid rise of the bacterial population described above. This is also where the bacteria are the wealthiest, and the ideal moment to extract samples for inoculating other fresh cultures. In the exponential phase, you can calculate the experimental generation time by measuring the number of viable cells at regular intervals, for example using turbidity.

3) **Stationary Phase** In a closed system, such as a laboratory flask or petri dish, growth cannot be sustained indefinitely. The bacterial population will run out of resources, space or produce an overwhelming amount of toxic end products from high metabolic activity. Growth slows down to a negligible rate, but this phase is inadequate for a population count as it is difficult to differentiate between viable and dying cells. Bacteria will also start producing antibiotics and spores, which could affect the estimation of viable cells.

4) **Death/Decline Phase** Due to a shortage of resources or overpopulation, bacteria are badly harmed and start dying at an exponential rate, mirroring the lag phase. Importantly, bacterial samples from the death phase are even unable to start a new culture when transferred to fresh growth media.

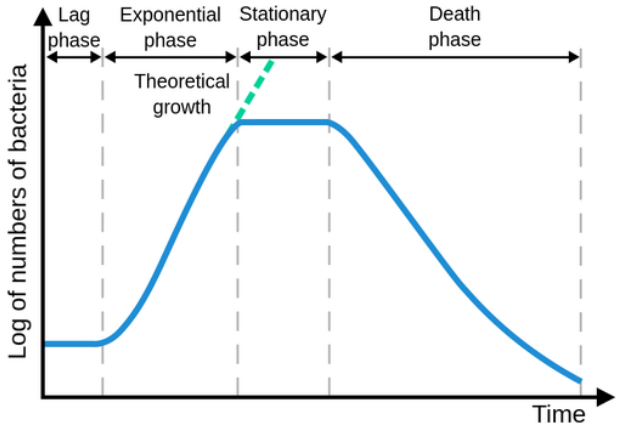


Figure 2: Microbial growth phases

Figure 2 depicts the microbial growth in a batch fermentation system where one time addition of microbial culture and sterilized media components is done. It is a closed

system, where no further addition or removal of materials are followed until all the stages of microbial life cycle are completed and product is formed. After completion, the product is removed and processed under various downstream processes. Another type of fermentation process is fed-batch, where nutrients are fed more than one times during microbial cultivation, but products are harvested only at the end of the process. This method provides opportunity to control the yield and productivity of the process by adding limiting nutrients at defined phase of cell growth. For example, the substrates such as ethanol, methanol or acetic acid may be added at the later stages of cell growth to avoid their inhibitory effect at initial growth phase. The most valuable method for high turnover of industrial products is the continuous fermentation that allows continuous supply of nutrients and raw materials. To maintain a static environment inside the vessel, products are also harvested continuously from the overflow of fermenter. The exponential growth of microbes is maintained for a prolonged period which is suitable for production of primary metabolites such as organic acids, amino acids, single cell protein etc.

Fermentation Process:

Fermentation takes place in the lack of oxygen (when the electron transport chain is unusable) and becomes the cell's primary means of ATP (energy) production. It turns NADH and pyruvate produced in the glycolysis step into NAD+ and various small molecules depending on the type of fermentation. In the presence of O_2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation, and it generates much more ATP than glycolysis alone. For that reason, cells generally benefit from avoiding fermentation when oxygen is available, the exception being obligate anaerobes which cannot tolerate oxygen. The first step, glycolysis, is common to all fermentation pathways:

$$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ Pi \rightarrow 2\ CH_3COCOO^- + 2\ NADH + 2\ ATP + 2\ H_2O + 2H^+$$

Pyruvate is CH_3COCOO^-. Pi is inorganic phosphate. Two ADP molecules and two Pi are converted to two ATP and two water molecules via substrate-level phosphorylation. Two molecules of NAD^+ are also reduced to NADH. In oxidative phosphorylation the energy for ATP formation is derived from an electrochemical proton gradient generated across the inner mitochondrial membrane (or, in the case of bacteria, the plasma membrane) via the electron transport chain. Glycolysis has substrate-level phosphorylation (ATP generated directly at the point of reaction).

Initially, Louis Pasteur described fermentation as "an anaerobic process carried out by yeast like organism to break complex sugars in simpler ones". But now fermentation includes anaerobic as well as aerobic processes that need maintenance of oxygen deficient or supplemented aseptic conditions. In case of anaerobic fermentation, no aeration device is needed as the gases generated during the process enable satisfactory mixing. The environment inside the vessel needs to be maintained oxygen scarce during

the whole process which is maintained by flushing a mixture of CO_2, H_2 and N_2 in the head space of the fermenter. On the other hand, large volumes of oxygen approximately 60 times the medium volume are required for aerobic fermentation processes. Such fermenters need provision of efficient aeration and uniform mixing of fermenter contents. To accomplish sterilized conditions along with uniform distribution of media, air, pressure and timely escape of products, a special vessel known as Fermenter is employed. In addition to above mentioned conditions, a fermenter should facilitate the following functions:

1. A fermenter should meet the requirement of containment that means to prevent the escape of viable cells during fermenter process or downstream processing.

2. It should provide continuous online monitoring of undergoing process through pH, temperature and pressure sensors.

3. A fermenter should provide stable environment required for the process with minimum consumption of operating power, maintenance labour, construction cost etc.

4. A pilot scale fermenter should provide the opportunity of scaling up the process. A typical bioreactor has the following parts with specified functions as illustrated in figure 3. Principles of chemical engineering are applied to design a fermenter.

a. Agitator is used for uniform mixing of media constituents and to keep the cells suspension homogenous. Agitation also approves air dispersion, and random transfer of oxygen and heat. The most important function of an agitator is to reduce shear forces and forming produced during the biochemical conversion. To achieve homogenous environment through the vessel, different types of stirrer or impellers such as disc turbine, vaned disc, open turbine and propeller are used. All these agitators are designed to achieve objectives of gas-phase and bulk fluid mixing.

b. Aeration System plays the crucial role along with agitators to supply sufficient oxygen to growing cells without causing any damage to them. The device used for this purpose is known as sparger. Sparger is needed for efficient aeration by bubbling of air through the medium, as surface aeration is inefficient for submerged cultures. The kind of aeration system required in a fermenter is characterized by the type of process and attributes of media constituents and microbial culture. Sometimes only aeration could achieve the functions of proper mixing and oxygen supply which eliminated the need of extra agitator. Such conditions are dictated by the less viscous broth, and bacterial cultures. For high viscosity broths, it is desired that Fermenters with height/diameter ration of 5:1 provide uniform mixing by aeration system only and save equipment and power cost. But, for fungal cultures proper mixing by agitators is required. The spargers have been employed with variable designs such as orifice sparger, nozzle sparger or combined sparger- agitator.

c. Baffles are the metal strips attached radially to the vessel wall to prevent whirl formation and to perform efficient aeration. Their length may be one tenth of the vessel diameter with diameter less than 3 meters and their number varies from 4 to 8. Wider

baffles are advised to have positive effect on agitation efficiency as compared to narrow baffles. A gap between the baffles and fermenter wall prevents microbial growth at the vessel wall.

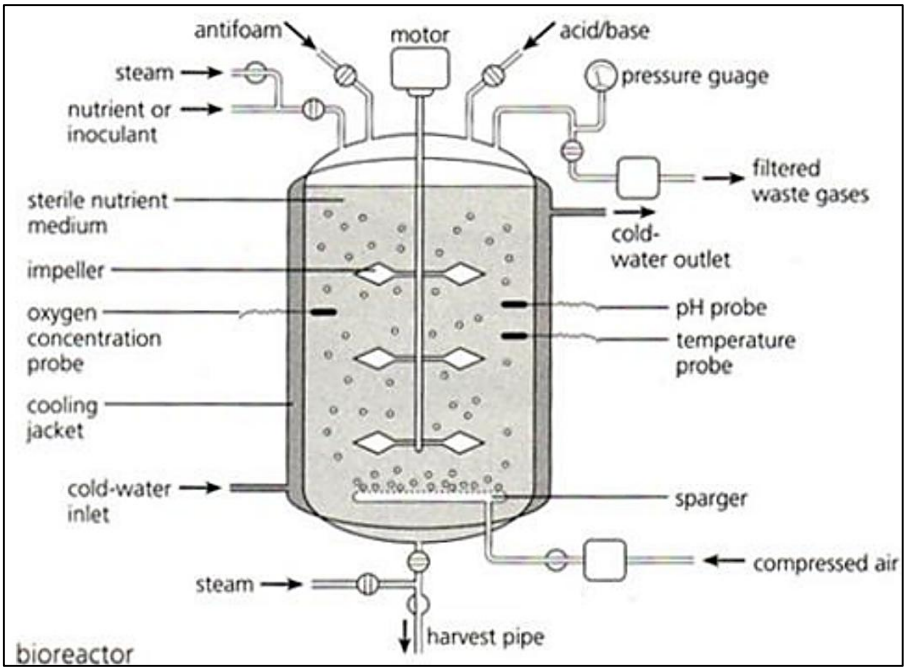


Figure 3: A typical design of a fermenter

Fermentation Techniques

i. Solid State Fermentation

Solid state fermentation is a type of fermentation used in the enzyme production. As the name suggests, fermentation occurs by microorganisms grown on a solid surface or solid substrate that has very low moisture content. A single insoluble substrate provides nutrients such as carbon, nitrogen, etc., for growing microbes. Microorganisms grow adhered to the solid substrate. Solid-state fermentation often uses composite and heterogeneous products of agriculture or by-products of agro-industries such as rice husk, wheat bran, sugar beet pulp, wheat and corn flour, etc. Hence, the substrates are cheaper and readily available.

Furthermore, filamentous fungi are the ideal microorganisms for solid-state fermentation. Also, bacteria, yeast and other fungi can also grow on solid substrates and can be used in solid-state fermentation.

- **Advantages of Solid-State Fermentation**

Similar to other fermentation processes, solid-state fermentation also has many advantages as listed below.

- The medium is simple, easily available, and inexpensive
- Substrates need less pretreatment compared to liquid media

- ❖ Contaminations are restricted since the moisture content is low
- ❖ Forced aeration is often easier
- ❖ Simplified and minimized downstream process and waste disposal
- ❖ Simple fermentation equipment
- ❖ High volumetric productivity

Although solid-state fermentation provides many advantages, it also has several disadvantages as well as listed below.

- **Disadvantages of Solid-State Fermentation**

- ❖ Low moisture level may restrict the growth of microorganisms
- ❖ Removing of metabolic heat is a problem in large scale solid-state fermentations
- ❖ Difficulties in monitoring the process parameters

ii. Submerged Fermentation

Submerged fermentation is another fermentation method we use in industrial enzyme production. Moreover, it needs a large-scale aseptic fermentation vessel which can provide a controlled environment that consists of optimum temperature, pH, degree of agitation, oxygen concentration, etc., for the growing microorganisms. Submerged fermentation occurs in a liquid medium where microorganisms are present. Thus, the water content is high, and all the nutrients are present in the liquid medium for the growth of microorganisms. Most importantly, the nutrients are evenly available throughout the medium for microorganisms in submerged fermentation. Agitation facilitates the even distribution of nutrients and the microbial cells.

Similar to solid-state fermentation, submerged fermentation also has advantages and disadvantages as mentioned below.

- **Advantages of Submerged Fermentation**

- ❖ Easiness of measuring process parameters
- ❖ Even distribution of nutrients and microorganisms
- ❖ Ability to control growth conditions
- ❖ Availability of high-water content for the growth of microbes

- **Disadvantages of Submerged Fermentation**

- ❖ Use of expensive media and expensive equipment
- ❖ Complex and expensive downstream process and difficulty in the waste disposal
- ❖ High power consumption

Solid State Fermentation	**Submerged Fermentation**
Fermentation may be carried out as batch or continuous	Fermentation may be carried out as batch
Medium is added in large vessel	Medium is added in flat vessel or trays

Surface area to volume height ratio is very less	Surface area to volume height ratio is very high
5-10% of inoculum is added	Less inoculum is added
Product yield are usually high as compared to input cost	Product yield is comparatively less
Lesser space is required	More space is required
Less contamination	More contamination
If a batch gets contaminated there is a loss of entire batch	If a tray gets contaminated there is a loss of only tray but not the batch
Entire fermentation media is utilized by microorganism for growth and product fermentation	There is wastage of fermentation media
Aeration and agitation of system is possible by use of sparger and impeller	Aeration is usually carried out by passing sterile air and no agitation

- **Application of Fermentation Technology:**

Advances in fermenter designing and fermentation technology have led to the commercialization of a number of fermented products. Various kinds of food and food additives are manufactured using industrial fermentation technology in developing countries. Wild type and recombinant microorganisms are used in the production of following products.

1. Alcoholic beverages- Whisky, rum, brandy, Beer and wine
2. Milk and Milk Products- Cultured milks, yoghurt, cheese
3. Microbial flavors- Vanillin, benzaldehyde and lactones
4. Biofuels- Ethanol, acetone-butanol
5. Microbial polysaccharides- Dextran, xanthan gum and pullulan
6. Food additives and ingredients- L-glutamic acid and L-aspartic acid
7. Vitamins- Vitamin A, C, B_{12}, and riboflavin
8. Enzyme- Amylase, invertase, Protease
9. Organic acid- Lactic acid, citric acid, acetic acid

The end product of a fermentation process depends upon the kind of microbe used for the process. Many bacteria, protists, fungi and animal cells produce lactic acid, water and carbon dioxide, whereas yeast and plant cells yield alcohol, water and carbon dioxide. Thus, most of the alcoholic beverages are produced by the action of yeast on carbon sources such as malted barley, millet, sorghum, cassava etc. Among all the fermented alcoholic beverages, beer making (brewing) is the oldest and the most researched process. So, the next section would be dedicated to discuss brewing process.

Ethanol Production process

- **Preparation of the medium**

Mashing (Saccharification), this means the grain starch is hydrolyzed to sugars with microbial enzymes or with the enzymes of barley malt. In all the others no hydrolysis is necessary as sugars are present in the fermenting substrate as in grape sugar and cane sugar. First, starch should be exposed to contact with water. Grinding makes small pieces, which can increase its surface area. Then, the increase in its surface area can enhance the contact between starch and water. At the grinding step, the outer shell of grain is almost completely removed, this step increases the surface area of each particle. These results make water penetrate through grain easily. However, there is still some undesirable area, which is hard to absorb water. To achieve effective enzymatic action, this micro-crystalline area should be removed. This is typically accomplished with two hot water processes. First, grain is treated with hot water, typically 85°C for between 20 to 60 minutes. Then, super-heated water, typically 110°C, is introduced with high pressure. With the first mixing with hot water, the starch absorbs water. Then, the structure of the micro-crystalline area becomes weak. Then, after introduction of super-heated water with high pressure, this area is completely broken. Without this water treatment, this area cannot be broken, which means the efficiency of the enzymatic action is lost. In an ethanol industry, two enzymes are usually employed, endoenzyme alpha-amylase and exoenzyme glucoamylase. Alpha-amylase attacks the alpha-1,4 linkages of starch. Then, starch is converted into dextrin. After the first hydrolysis with alpha-amylase, glucoamylase works. Glucoamylase removes one glucose from dextrin. Thus, glucoamylase cuts linkage of dextrin from its end.

- **Propagation of yeast inoculum**

In general, the inoculum is made of selected alcohol-tolerant yeast strains usually *Saccharomyces cerevisiae* grown aerobically with agitation and in a molasses base. Yeast inoculum with up to 5% (v/v). Progressively larger volumes of culture may be developed before the desired volume is attained.

- **Fermentation**

Yeast is a facultative anaerobe. In the aerobic environment, it converts sugars into carbon dioxide and water, while in the anaerobic environment, it converts sugars into carbon dioxide and ethanol. Thus, for an ethanol industry, it is important to exclude significant oxygen from its system. Alcohol-resistant yeasts, strains of *Saccharomyces cerevisiae* are used, and nutrients such as nitrogen and phosphate lacking in the broth are added. When the nitrogen content of the medium is insufficient nitrogen is added usually in the form of an ammonium salt. In all alcohol fermentations the heat released must be reduced by cooling and temperatures are generally not permitted to exceed 35- 37°C. The pH is usually in the range 4.5-5.0, when the buffering capacity of the medium is high, higher pH values tend to lead to higher glycerol formation. When the buffering capacity is lower, the pH is falls to about 3.5., the contaminations can have serious effects on the process (sugars are used up leading to reduced yields).

- **Distillation**

Distillation is one of the steps of the purification. Distillation is the method to separate two liquid utilizing their different boiling points. After fermentation the fermented liquor contains alcohol as well as low boiling point volatile compounds such as acetaldehydes, esters and the higher boiling, fuel oils. The alcohol is obtained by several operations. First, steam is passed through the liquor. The result is a dilute alcohol solution which still contains part of the undesirable volatile compounds. Secondly, the dilute alcohol solution is passed into the centre of a multi-plate aldehyde column in which the following fractions are separated: esters and aldehydes, fuel oil, water, and an ethanol solution containing about 25% ethanol. Thirdly, the dilute alcohol solution is passed into a rectifying column distils off at 95.6% alcohol concentration. The maximum alcohol concentration of 96.5% is obtained by azeotropic distillation. The principle of this method is to add an organic solvent which will form a ternary (three-membered) azeotrope with most of the water, but with only a small proportion of the alcohol. Benzene, carbon tetrachloride, chloroform and cyclohezane may be used, but in practice, benzene is used. In practice, four columns are usually used. The first and second columns remove aldehydes and fuel oils, respectively, while the last two towers are for the concentration of the alcohol.

- **Some Developments in Alcohol Production**

(1) Developments of new strains of yeast of *Saccharomyces uvarum* able to ferment sugar rapidly, to tolerate high alcohol concentrations, flocculate rapidly.

(2) The use of continuous fermentation with recycle using the rapidly flocculating yeasts.

(3) Continuous vacuum fermentation in which alcohol is continuously evaporated under low pressure from the fermentation broth.

(4) The use of *Zymomonas mobilis*, a Gram-negative bacterium which is found in some tropical alcoholic beverages. The advantages claimed for the use of *Zymomonas* are the following:

(a) Higher specific rates of glucose uptake and ethanol production than reported for yeasts.

(b) Higher ethanol yields and lower biomass than with yeasts.

(c) Ethanol tolerance is at least as high or even higher [up to 16% (v/v)] in some strains of the bacterium than with yeast.

(d) *Zymomonas* also tolerates high glucose concentration and many cultures grow in sugar solutions of up to 40% (w/v) glucose which should lead to high ethanol production.

(e) *Zymomonas* grows anaerobically and, unlike yeasts, does not require the controlled addition of oxygen for viability at the high cell concentrations used in cell recycle.

(f) The many techniques for genetic engineering already worked out in bacteria can be easily applied to *Zymomonas* for greater productivity.

- **Advantages of Fermentation Technology**

1. Preservation and enriches food, improves digestibility, and enhances the taste and flavors of foods.

2. Potential of enhancing food safety by controlling the growth and multiplication of number of pathogens in foods.

3. Important contribution to human nutrition, particularly in developing countries, where economic problems pose a major barrier to ensuring food safety.

4. Low energy consumption due to the mild operating conditions relatively low capital and operating costs relatively simple technologies.

5. They cause specific and controlled changes to foods by using enzymes.

6. Preservation and detoxification of the food.

7. Waste treatment.

8. Health related product.

- **Disadvantages of Fermentation Technology**

1. Hazardous contamination always exists in fermented food.

2. The uneven distribution of salt in lactic acid fermentation fish products and contamination of *Aspergillus flavus* in traditional starter culture for rice wine and soyabean sauce result in severe food poisoning incidences.

3. Health (obesity).

Commercialization of fermented food products:

Fermented foods have long been used as source of nutrition. The demand for fermented foods and the newly conceptualized functional foods are in the rise owing to its potential health benefits. These foods have been explored for their various medicinal properties in the recent past such as antihypertensive, antidiarrheal, blood glucose-lowering benefits, and antithrombotic properties, to name a few. The health benefits of the fermented foods can be accounted to the presence of bioactive compounds in the form of phytochemicals such as phenolics, flavones, fatty acids, and saccharides along with vitamins, minerals, and amino acids in substantial quantities as compared to their nonfermented forms. Fermented foods are known to accumulate bioactive compounds during the processing of the food which are naturally absent or present in very low amounts in the unprocessed counterparts. For instance, red ginseng roots contain bioactive compounds such as saponins (ginsenosides) and nonsaponins, while the fermented ginseng roots have

shown increased levels of saponins. These saponins are known to regulate the blood glucose and insulin levels. Fermented soybeans, a major staple food consumed in Korea, China, Japan, Indonesia, and Vietnam, has been reported for exhibiting antidiabetic effects. Such properties in soybean are partly due to both quantitative and qualitative changes in the small molecules during the processing of the fermented product. Dickerson and his colleagues (2015) have demonstrated defined antioxidant and immune-modulating potentials of fermented papaya. Certain foods and beverages fermented with specific LAB strains are known to represent immune regulation and antiallergenic properties. Recently, metabolomics in conjunction with microbial ecology and genomics are scientific tools intended toward finding out the disease-fighting properties of the bioactive compounds and discovering novel ones from fermented foods.

Global functional food market was worth USD 129.39 billion in 2015, and it is projected to be USD 255.10 by 2024 (www.grandviewresearch.com). The demand of milk and milk products is in an increasing trend for the last three decades. Global milk production has increased by more than 50%, from 500 million tons in 1983 to 769 million tons during 2013. In South Asia, consumption of milk and milk products is projected to increase by 125% during 2030. Similarly, beer possesses the largest market share among the alcoholic beverage industries with a global production of 1.96 billion hectolitres. China is the largest producer of beer (46.54 million kilolitres) in 2013 followed by the US (22.43 million kilolitres) and Brazil (13.46 million kilolitres) in the same year (http://www.worldatlas.com). The global wine production was 259 million hectolitres in 2016, and the consumption was 240 million hectolitres. The top wine producers are Italy (48.8 million hectolitres), France (41.9 million hectolitres), and Spain (37.8 million hectolitres) (www.bkwine.com). Commercially important fermented foods and the major producing regions are elucidated in Table 1.1.

Table 1.1 Commercial fermented foods and the major producing regions

Products	Microorganisms used	Type of fermentation	Major producers
Wine	*Saccharomyces cerevisiae*	Submerged	Italy, France, Spain
Beer	*Saccharomyces cerevisiae*, *Saccharomyces pastorianus*	Submerged	China, USA
Whiskey	*Saccharomyces cerevisiae*	Submerged	France, Scotland, USA, Canada
Yogurt	*Streptococcus thermophilus*, *Lactobacillus delbrueckii*	Submerged	France, Ireland, Canada, USA
Cheese	*Lactococcus*, *Lactobacillus*, *Streptococcus* sp., *Penicillium roqueforti*	Solid-state fermentation	Germany, the Netherlands, France, USA
Acidophilus milk	*Lactobacillus acidophilus*	Submerged	North America, Europe, Asia
Sauerkraut	*Leuconostoc* sp., *Lactobacillus brevis*, *Lactobacillus plantarum*	Solid-state fermentation	Europe
Fish sauce	Lactic acid bacteria (halophilic), *Halobacterium salinarum*, *Halobacterium cutirubrum*, *Bacillus* sp.	Submerged fermentation	Thailand, Korea, Indonesia
Fermented meat	*Lactobacillus sp,. Micrococcus sp., Staphylococcus sp.*	Solid/ submerged fermentation	Europe

Probable questions:

1. Define fermentation.
2. Describe the phases of microbial graph with a labelled diagram.
3. Explain the chemical basis of fermentation. What are the functions of a fermenter?
4. Describe the parts of a typical fermenter with a labelled diagram.
5. Differentiate solid state and submerged fermentation. State the advantages and disadvantages of each process.
6. Give a brief description of ethanol production process. What are the recent developments in alcohol production process?
7. Write a short note on commercialization aspect of fermented products.

Suggested reading:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)
2. Essentials of microbiology. Amita J & Parul J. Elsevier (Latest edition).
3. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).
4. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press.

Unit-XVIII
Mode of transmission, pathogenicity and prevention of microbial diseases: Air-borne (Tuberculosis)

Objectives:

In this section we will discuss on mode of transmission, pathogenicity and prevention of microbial diseases: Air-borne (Tuberculosis).

Introduction:

Tuberculosis (TB) is an infectious disease that most often affects the lungs and is caused by a type of bacteria. It spreads through the air when infected people cough, sneeze or spit.

Tuberculosis is preventable and curable.

About a quarter of the global population is estimated to have been infected with TB bacteria. About 5–10% of people infected with TB will eventually get symptoms and develop TB disease.

Those who are infected but not (yet) ill with the disease cannot transmit it. TB disease is usually treated with antibiotics and can be fatal without treatment.

In certain countries, the Bacille Calmette-Guérin (BCG) vaccine is given to babies or small children to prevent TB. The vaccine prevents TB outside of the lungs but not in the lungs.

Transmission:

The transmission of tuberculosis (caused by *Mycobacterium tuberculosis*) is predominantly airborne. An "open case" or a sputum positive case is the source of infection and the cause of transmission of tuberculous infection to other persons in the community. Tuberculosis is transmitted as airborne particles, or droplet nuclei which patients with pulmonary or laryngeal TB generate when they sneeze, cough, speak or sing. Normal air currents can keep infectious particles airborne for prolonged periods and spread them throughout a room or a building.

Recent studies have shown that those people who are in close vicinity to the infective TB cases have an increased chance of acquiring the disease.

These include:

1. Health care workers (HCW) - Doctors, Pathologists, Microbiologists, Nursing staff, Lab staff, etc.

2. Young children in the household

3. Other family members

In most instances, the infection is transmitted from pulmonary smear positive cases (open cases) to other people. Children are rarely sputum positive, hence are much less

likely to be source of infection for others. However, case reports of children who are sputum positive have been documented. These patients were later on traced as a source of infection for other persons including children in their vicinity.

Various risk factors facilitating the transmission of tuberculous infection from a source to contacts and its subsequent manifestations include:

1. Co-infection with HIV

2. Age of the patient

3. Co-morbid conditions like diabetes mellitus

4. Occupational exposure e.g., silicosis

5. Malnutrition and cachexia

6. History of smoking

7. Industrial worker

8. Delay in diagnosis of disease.

Various research studies indicate that health care setups are often at higher risk of transmission of tuberculous infection. The risk may be higher in places where health care providers directly encounter patients with tuberculosis especially before diagnosis and treatment or in setups where high risk procedures are carried out. Such procedures include:

1. Aerolised medication and treatment

2. Bronchoscopy

3. Endotracheal intubation

4. Suctioning procedures

5. Procedures that stimulate coughing

Environmental factors affecting the transmission include:

1. Exposure in relatively small and enclosed spaces

2. Lack of adequate ventilation to clean the environment through dilution or removal of infectious droplet nuclei

3. Recirculation of air containing infectious droplet nuclei.

Children as a special group are highly affected by these open cases. Although children are rarely sputum positive but they can transmit infection, as has been documented in a large school-based study and past experiences of community outbreaks. They are also more likely to develop disease after acquiring infection and are significantly more likely to develop extra pulmonary tuberculosis and disseminated tuberculosis i.e. tubercular meningitis and military tuberculosis. Paediatric tuberculosis is an indicator of recent transmission of tuberculosis and an indirect indicator of the success of the tuberculosis

control programme and paediatric health programme. Sub-Saharan Africa forms the depot store for tuberculosis with highest incidence of tuberculosis in all age groups.

Pathogenecity:

The pathogenesis and transmission of TB are inter-related. *M. tuberculosis* is almost exclusively a human pathogen and how it interacts with the human host determines its survival. From the perspective of the bacterium, a successful host-pathogen interaction is one that results in ongoing pathogen transmission.

- **Evolution of initial infection and host response**

At the time of initial infection, the distribution of inhaled droplet nuclei in the lung is determined by the pattern of regional ventilation. It thus tends to follow the most direct path to the periphery and to favor the middle and lower lung zones, which receive most of the ventilation. In immunocompetent hosts, it is theorized that alveolar macrophages ingest the *M. tuberculosis* organisms. Whether or not those macrophages destroy the bacteria depends on the degree to which they are non-specifically activated, on host genetic factors and on resistance mechanisms in the bacteria. If bacteria are successfully cleared, then immunological tests like the tuberculin skin test (TST) and IGRA will remain negative. When innate macrophage microbicidal activity is inadequate to destroy the initial few bacteria of the droplet nucleus, they replicate logarithmically, doubling every 24 hours until the macrophage bursts to release its bacterial progeny. New macrophages attracted to the site engulf these bacilli, and the cycle continues. The bacilli spread from the initial lesion via the lymphatic and/or circulatory systems to other parts of the body. This spread may, in fact, be critical to the induction of cellular immunity. It is also during this stage of the infection that seeding of the lung apices occurs, which is so critical to the later development of adult-type (infectious) pulmonary TB. After a period lasting from 3 to 8 weeks, the host develops specific immunity (cell-mediated immunity and delayed-type hypersensitivity) to the bacilli. This is when individuals first show positive results on the TST or IGRA. *M. tuberculosis*-specific lymphocytes then migrate to the site of infection, surrounding and activating macrophages localized to the site. As the cellular infiltration continues, the centre of the cell mass, or granuloma, becomes caseous and necrotic. Later, radiographically demonstrable fibrocalcific residua of the initial infection can be identified, including a calcified granuloma in the lung alone or in combination with a calcified granulomatous focus in a draining lymph node, called a Ranke complex. Infection and immune conversion are usually asymptomatic; any symptoms that do occur are self-limited. In a small proportion of those infected, erythema nodosum (a cutaneous immunologic response to an extracutaneous TB infection) or phlyctenular conjunctivitis (a hypersensitivity reaction) may develop.

- **Early disease progression (primary TB)**

A proportion of those who are recently infected are unable to contain the infection, despite the stimulation of cell-mediated immunity, and there is progression to disease in a matter of months. Such early disease progression is a function of age and immunologic

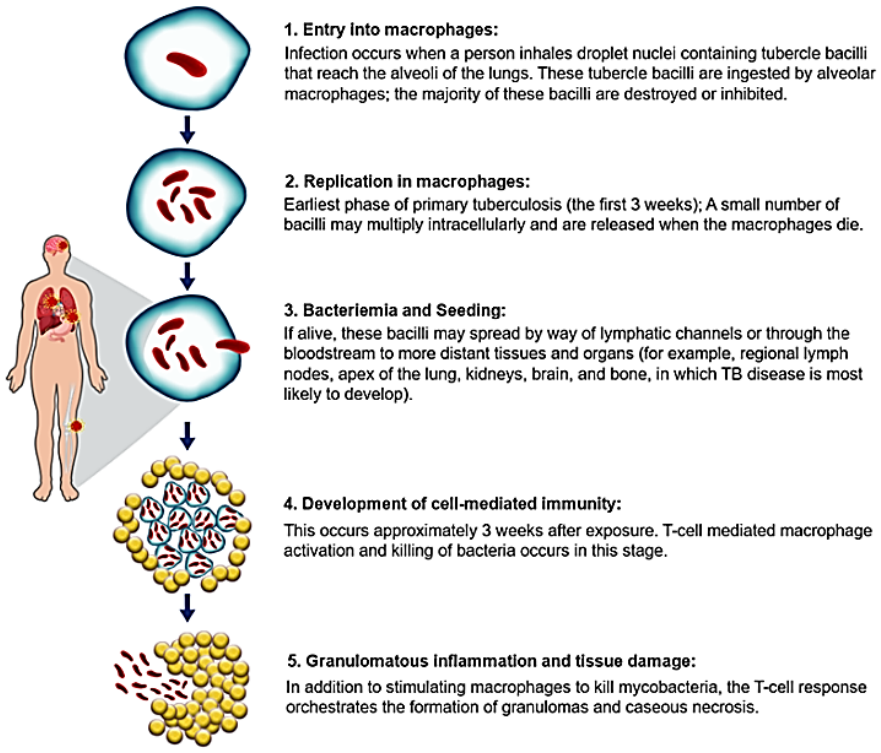
response; thus, disease is especially likely to occur in children 0-4 years of age and the immunocompromised. Local progression in the lung, or lymphohematogenous spread resulting in disseminated (miliary) disease and/or central nervous system disease, may occur as early as 2-to-6 months after infection in infants and severely immunocompromised hosts. Uncomplicated and asymptomatic lymph node disease (hilar or mediastinal lymphadenopathy without airway involvement) may also occur in the first 2-to-6 months of infection, although there is debate about whether this should be called active disease. At 4-12 months after infection, early disease manifestations include complicated lymph node disease (airway compression, expansile caseating pneumonia, infiltration of adjacent anatomic structures), pleural disease (most commonly a lymphocyte-predominant exudative effusion) and peripheral lymphadenitis (usually in the cervical lymph nodes). In immunocompetent children and adolescents, early disease is more likely to manifest as intrathoracic adenopathy and in adults as a unilateral pleural effusion. In severely immunocompromised people of any age (e.g., those with advanced HIV or AIDS), early disease may manifest as intrathoracic adenopathy. Newly infected children who are 10 years of age or older (pubertal) or adolescents, may develop adult-type pulmonary disease or other types of extrapulmonary TB (for example bone and joint TB) within the first 8-24 months of infection. For purposes of disease reporting, most but not all patients with a diagnosis of TB made within 18-24 months of infection should be considered to have "primary" disease. Those newly infected persons in whom TB does not develop in this time period have three possible outcomes: they may remain infected indefinitely and never develop disease, they may naturally clear their infection over time or they may progress to active TB disease at a later date, beyond the first 18-24 months. The concept of disease tolerance provides further insight into the aforementioned host-pathogen interactions.

- **Disease tolerance**

It is now increasingly understood that host defence strategies against infectious diseases comprise both host resistance and disease tolerance. Host resistance is the ability of the host to prevent invasion or to eliminate the pathogen, while disease tolerance is defined as limiting the tissue damage caused by the pathogen and/or the immune response. Since the discovery of *M. tuberculosis* more than a century ago, great progress has been made in defining mechanisms of host resistance to this respiratory pathogen. By contrast, our understanding of natural immunity in the 90 to 95% of infected individuals who remain disease-free is extremely limited. The inability of both the innate and adaptive immune system to eliminate the bacteria forces the host to develop a cellular barrier, referred to as a granuloma, around infected cells. Granuloma formation appears to be the point at which host immunity “switches” from resistance to tolerance. Indeed, studies have elegantly demonstrated that intercellular communication is organized in the granuloma such that pro-inflammatory signaling occurs at the core to control *M. tuberculosis* growth, while anti-inflammatory signaling at the periphery acts to limit tissue damage. Thus, the spatial compartmentalization of pro- and anti-inflammatory signaling is critical in granuloma function to prevent *M. tuberculosis* dissemination.

- **TB infection**

In the classical concept of tuberculosis infection (TBI), *M. tuberculosis* bacteria are believed to survive for years at the site of the original infection in the lung and draining lymph nodes and in the small granulomas or solid caseous material of lympho-hematogenously seeded foci. Presumably, local conditions, an intact cell-mediated immunity or the presence of inhibitors result in conditions unfavorable to replication. Recent mapping of the complete genome sequence of the bacterium demonstrates that the organism has the potential to synthesize enzymes involved in anaerobic metabolism. Although rapid death and autolysis occur after abrupt depletion of oxygen, the organism can shift into a state of dormancy if exposed to gradual reductions in oxygen tension. Therefore, although *M. tuberculosis* thrives in an aerobic environment, it possesses the genetic and biochemical capability to survive anaerobically in experimentally oxygen-depleted media. Granuloma formation, with its oxygen-depleted environment, is a defining characteristic of TB. It is this stage of infection that is termed TBI and is usually identified by a positive TST or IGRA in the absence of active disease.



- **Reinfection**

The elegant studies of R. G. Ferguson in the first half of the 20th century strongly suggest that it takes up to 18 months after the initial infection for cell-mediated immunity to fully mature. During this period, each successive exposure and infection appears to carry its own inherent risk of disease; the cumulative risk thus becomes a function of the number of infections. This may explain why disease is so much more common in newly infected close contacts of smear-positive cases than it is in newly infected close contacts of smear-negative cases; the former has a greater likelihood than the latter of repeated exposure

and reinfection. More recent studies have also reported a higher risk of disease with greater intensity of exposure. A meta-analysis of 23 cohorts from the pre-antibiotic era — largely health care workers — estimated that subsequent reinfection (after the first 18 months) of immunocompetent hosts carries a much lower risk of progression to TB disease, estimated to be 21% of the risk of an initial infection progressing to disease. It remains unknown whether prior infection without development of overt disease is simply a marker for people who are less susceptible to disease development, or whether it truly induces immunity that is better able to prevent progression after reinfection. In Canada, repeated exposure is rare in most settings, such that active TB generally reflects an initial infection — recent or remote — rather than reinfection. However, there is clear evidence for the important role of reinfection causing TB morbidity in high-incidence, high-transmission settings. This has been most consistently documented among persons living with HIV who are not receiving anti-retroviral therapy, among whom high rates of recurrent TB disease have been observed long after microbiologic cure of an initial disease episode. DNA fingerprinting has confirmed that many recurrent episodes relate to new infecting strains rather than to late relapse. Strong supporting evidence also comes from clinical trials among persons living with HIV in high-transmission settings. These demonstrated high rates of TB disease after completion of preventive therapy, attributable to reinfection after treatment. Reinfection can also lead to repeated illness in HIV-negative persons who were cured after an initial episode of TB disease, if they are in settings with extremely high TB incidence and transmission. This may be relevant in a few, very specific Canadian settings (eg, isolated communities with extensive outbreaks). Some persons in those settings with documented prior treatment for TB disease experienced recurrent disease that was shown by whole genome sequencing to reflect reinfection. These observations can lead to consideration, on a case-by-case basis, of retreatment of TB infection after new exposure to highly infectious source patients.

- **Reactivation TB**

In Canada, most TB is understood to be “reactivation” TB (ie, occurring in adolescents or adults). It usually presents as adult-type pulmonary disease (upper lung-zone fibrocavitary disease — previously referred to as postprimary TB — beginning in small foci that are the result of remote lympho-hematogenous spread), although it may also present as extrapulmonary TB. As mentioned earlier, adult-type pulmonary TB may on occasion be a manifestation of primary TB or a reinfection. In any population group, reactivation of TBI, leading to reactivation TB, is much more likely to occur in people who are immunocompromised. Patients with adult-type pulmonary TB are much more likely to show lung cavitation (created when caseous material liquefies) that erodes into the bronchi. Within the unique extracellular environment of cavities, host defenses are ineffectual, and bacteria multiply in large numbers. Because cavities are open to, and discharge their contents into, nearby bronchi, these same bacteria are directly communicable to the outside air when the patient coughs. From the perspective of public health and the organism’s ability to survive as a species, adult-type pulmonary TB is the most important form of TB disease. Persons with a history of untreated or inadequately

treated pulmonary TB or a "high-risk" lung scar (upper lung-zone fibronodular abnormality) on chest radiograph are thought to have a higher bacillary burden, even though "dormant," than those without such a history/radiograph and to be at increased risk of reactivation TB. This scenario is commonly seen among immigrants referred to public health authorities for medical surveillance.

- **Extrapulmonary TB**

The pathogenesis of extrapulmonary TB has been attributed to lympho-hematogenous spread at the time of initial primary lung infection, later dissemination from reactivated pulmonary TB or contiguous spread from adjacent organs. Abdominal disease may also result from direct infection through ingestion of infected sputum or contaminated milk (*M. bovis*). Extrapulmonary TB or combined pulmonary and extrapulmonary TB is more common in those who are severely immunocompromised. Among people coinfected with HIV and TB, the prevalence of extrapulmonary TB increases as the CD4 count decreases.

- **Evolution of initial infection and host response**

Recently, Behr et al. posit a more nuanced understanding of tuberculosis infection. In an analysis of studies spanning 5 decades, they concluded that the majority of TB-immunoreactive individuals have cleared their infection while retaining immunologic memory of it. As a result, such patients would not benefit from preventive therapy. Unfortunately, there is no currently available test to identify patients who still harbor viable *M. tuberculosis* and so would benefit from tuberculosis preventive treatment. Added to this conceptualization of infection is a more nuanced understanding of disease with 2 additional states of infection: incipient TB (an intermediate state that is likely to progress to active disease but does not cause detectable abnormalities) and a subclinical state of active TB due to viable *M. tuberculosis* that does not cause clinical TB-related symptoms, but causes other abnormalities that can be detected using radiologic and microbiologic assays. These newly described states are conceivably determined by the host's immunological response and the virulence of the pathogen, with the capacity of the host to shift between states. In the future, biomarkers may permit the early diagnosis and treatment of these intermediate states.

- **Risk factors for progression from infection to disease**

The risk of progression from TBI to active TB is largely dependent on the immune competency of the host. Age and sex appear to directly affect the immunologic response and the risk of disease: morbidity is greater among young children (<5 years of age), especially infants; young adults, especially females; and older adults, especially males. In high-burden countries, the population-attributable fraction of undernutrition for TB is 27% according to the WHO. In Canada, inadequate diet has been associated with acquiring *M. tuberculosis* infection in an Inuit community. In a study from Peru, biosocial household factors contributed to the risk of TB among contacts. The seasonality of TB (with the highest incidence in spring and early summer) has been attributed to reduced sunlight and vitamin D deficiency during the winter months in some studies but not in

others. Ethnic differences have been offered as factors determining host immune response, with some support. A growing body of evidence suggests that host genetic factors are important in determining susceptibility to TB as well. Most important from a clinical perspective are the many medical conditions that are well-known to affect host immunologic response and increase the risk of progression from TBI to active TB disease. Until recently, virtually all the efforts to develop a TB vaccine have been focused on conventional T cell-mediated immunity. However, there is no direct correlation between increased T-cell responses and protection against TB. Thus, it is not surprising that the results from clinical trials of T cell-based vaccine approaches have been disappointing. These studies collectively challenge the current dogma that conventional T cells are predominantly engaged in host resistance against TB, but rather indicate the critical role of T cells in disease tolerance and containment of infection. Contrary to focusing on the adaptive immune response, epidemiological data show that among close household contacts of highly infectious TB patients, up to 50% of exposed individuals do not convert their TST response from negative to positive, suggesting many of these individuals are intrinsically resistant to infection by *M. tuberculosis*. These studies support the idea that perhaps the best window of opportunity to eradicate *M. tuberculosis* is during the early phase of infection, when the bacteria are still in the airway and have not initiated adaptive immunity and granuloma formation. These studies indicate that developing a vaccine targeting innate immunity may prevent TB. However, designing such a vaccine will require a better understanding of innate immunity, especially the memory capacity of innate cells. Simple organisms such as plants and invertebrates, which only possess innate immune defences, have demonstrated immunological memory (ie, the primary exposure to a pathogen resulted in more efficient immunity to a subsequent challenge with the same pathogen). Similarly, innate immune cells in vertebrates can generate a memory-like response (termed trained immunity), which is more efficient in preventing subsequent infection by a broad spectrum of pathogens and that is largely driven by epigenetic modifications. Therefore, identifying the key determinants of trained immunity and their protective function will lead to new targets and vaccine strategies against *M. tuberculosis*.

Symptoms:

When tuberculosis (TB) germs survive and multiply in the lungs, it is called a TB infection. A TB infection may be in one of three stages. Symptoms are different in each stage.

- **Primary TB infection**

The first stage is called the primary infection. Immune system cells find and capture the germs. The immune system may completely destroy the germs. But some captured germs may still survive and multiply.

Most people don't have symptoms during a primary infection. Some people may get flu-like symptoms, such as:

- Low fever.
- Tiredness.
- Cough.

- **Latent TB infection**

Primary infection is usually followed by the stage called latent TB infection. Immune system cells build a wall around lung tissue with TB germs. The germs can't do any more harm if the immune system keeps them under control. But the germs survive. There are no symptoms during latent TB infection.

- **Active TB disease**

Active TB disease happens when the immune system can't control an infection. Germs cause disease throughout the lungs or other parts of the body. Active TB disease may happen right after primary infection. But it usually happens after months or years of latent TB infection.

Symptoms of active TB disease in the lungs usually begin gradually and worsen over a few weeks. They may include:

- Cough.
- Coughing up blood or mucus.
- Chest pain.
- Pain with breathing or coughing.
- Fever.
- Chills.
- Night sweats.
- Weight loss.
- Not wanting to eat.
- Tiredness.
- Not feeling well in general.

- **Active TB disease outside the lungs**

TB infection can spread from the lungs to other parts of the body. This is called extrapulmonary tuberculosis. Symptoms vary depending on what part of the body is infected. Common symptoms may include:

- Fever.
- Chills.

- Night sweats.
- Weight loss.
- Not wanting to eat.
- Tiredness.
- Not feeling well in general.
- Pain near the site of infection.

Active TB disease in the voice box is outside the lungs, but it has symptoms more like disease in the lungs.

Common sites of active TB disease outside the lungs include:

- Kidneys.
- Liver.
- Fluid surrounding the brain and spinal cord.
- Heart muscles.
- Genitals.
- Lymph nodes.
- Bones and joints.
- Skin.
- Walls of blood vessels.
- Voice box, also called larynx.

- **Active TB disease in children.**

Symptoms of active TB disease in children vary. Typically, symptoms by age may include the following:

- **Teenagers-** Symptoms are similar to adult symptoms.
- **1- to 12-year-olds-** Younger children may have a fever that won't go away and weight loss.
- **Infants-**The baby doesn't grow or gain weight as expected. Also, a baby may have symptoms from swelling in the fluid around the brain or spinal cord, including:
    - Being sluggish or not active.
    - Unusually fussy.
    - Vomiting.
    - Poor feeding.
    - Bulging soft spot on the head.

- Poor reflexes.

Prevention:

To help stop the spread of TB:

- If you're traveling to a place where TB is common, avoid spending a lot of time in crowded places with sick people.
- If you have a latent infection, take all your medication so it doesn't become active and contagious.

If you have active TB, you'll need to take care to protect others from infection during your first few weeks of treatment:

- Limit contact with other people. If you live with others, sleep in a separate room and isolate yourself as much as possible.
- Cover your mouth when you laugh, sneeze, or cough.
- Wear a surgical mask when you're around other people.
- Keep your space ventilated. Open windows, if possible, and use a fan. Germs spread more easily in poorly ventilated places.

- **Tuberculosis vaccine**

Bacillus Calmette-Guérin (BCG) is the live attenuated vaccine form of Mycobacterium bovis used to prevent tuberculosis and other mycobacterial infections. The vaccine was developed by Calmette and Guérin and was first administered to human beings in 1921. BCG is the only vaccine against tuberculosis. It is the most widely administered vaccine and usually a part of the routine newborn immunization schedule. BCG vaccine also offers protection against non-tuberculous mycobacterial infections like leprosy and Buruli ulcer. It is also used in the treatment of superficial carcinoma of the bladder.

BCG vaccine is a fairly safe vaccine and it is not associated with severe complications. Prior to the mycobacterial infection, vaccine-induced or acquired naturally can protect against subsequent infection due to mycobacteria including tuberculosis. Prior infection with nontuberculous mycobacteria and *Mycobacterium tuberculosis* can confer natural protection against tuberculosis infection. Protection against tuberculosis infection is usually due to the immune response to mycobacterial antigens. Prior contained latent infection with *Mycobacterium tuberculosis* can provide up to 80 percent protection against disease with subsequent exposure. In patients with previous active disease, there is an increased risk of recurrence of active tuberculosis due to distinct strains in both HIV-uninfected and HIV-infected patients. Bacille Calmette-Guérin (BCG) has been associated with a reduction in childhood mortality which is not attributable to tuberculosis. Reduction in childhood mortality may be due to epigenetic reprogramming of the nucleotide-binding oligomerization domain (NOD2) receptor.

BCG vaccine can be given either intracutaneously or intradermally. Research is currently being conducted on respiratory administration since natural infection, and sensitization to *Mycobacterium tuberculosis* in humans tend to occur in the respiratory system.

Probable questions:

1. Discuss the route of infection of *Mycobacterium tuberculosis*.
2. In which race of human highest incidence rate of tuberculosis is observed? Which indicator acts as marker for the success of tuberculosis control programme?
3. How tuberculosis infection is related to immunocompetency in humans?
4. What are the risk factors for the progress of tuberculosis infection?
5. Differentiate primary and latent tuberculosis infection.
6. How tuberculosis infection can be prevented?
7. Name the most wide-spread vaccine of tuberculosis.

Suggested reading:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)
2. Essentials of microbiology. Amita J & Parul J. Elsevier (Latest edition).
3. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).
4. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press.

Unit-XIX

Mode of transmission, pathogenicity and prevention of microbial diseases: Food and waterborne (Typhoid) and Arthropod borne (JE and Yellow fever)

Objectives:

In this section we will discuss on mode of transmission, pathogenicity and prevention of microbial diseases: Food and waterborne (Typhoid) and Arthropod borne (JE and Yellow fever).

Typhoid

Typhoid is an infection caused by the bacterium *Salmonella typhi*.

The bacterium lives in the intestines and bloodstream of humans. It spreads between individuals through direct contact with the feces of a person with an infection.

No animals carry this disease, so transmission is always from human to human. *S. typhi* enters through the mouth and spends 1–3 weeks in the intestine. Then, it makes its way through the intestinal wall and into the bloodstream.

From the bloodstream, it spreads into other tissues and organs. The immune system of the host can do little to fight back, because *S. typhi* can live within the host's cells, safe from the immune system.

Doctors diagnose typhoid by detecting the presence of *S. typhi* via blood, stool, urine, or bone marrow sample.

- **Transmission:**

By ingestion of:

- Food and water contaminated by faeces and urine of patients or carriers.
- Shellfish taken from sewage contaminated beds.
- Vegetables fertilized by night soil and consumed raw.
- Food contaminated by flies.
- Contaminated milk and milk products.

Transmission of typhoid fever depends primarily on direct contact with the stool of an infected individual, and risk is highest in densely populated areas that lack proper sanitation and access to safe drinking water. Household-level hygiene and food/water safety and handling practices, as well as close contact with an index case, are associated

with the direct transmission of typhoid in endemic areas. Because *Salmonella typhi* is exclusively human host-adapted, reservoirs of infection exist solely within groups of infected humans, a small number of which (1–6%) develop a chronic carrier state, which has allowed the disease to persist during inter-epidemic periods. Recent evidence suggests that environmental reservoirs of infection may also support disease transmission. The risk of typhoid fever is associated with environmental factors, including proximity to open sewers and highly contaminated water bodies, residence in low elevation areas, and rainy season. Major outbreaks of *S. typhi* have been linked to contaminated municipal water sources, and suggest waterborne transmission as an important environmental pathway. Whether environmental sources contribute to endemic transmission during non-outbreak periods is unclear.

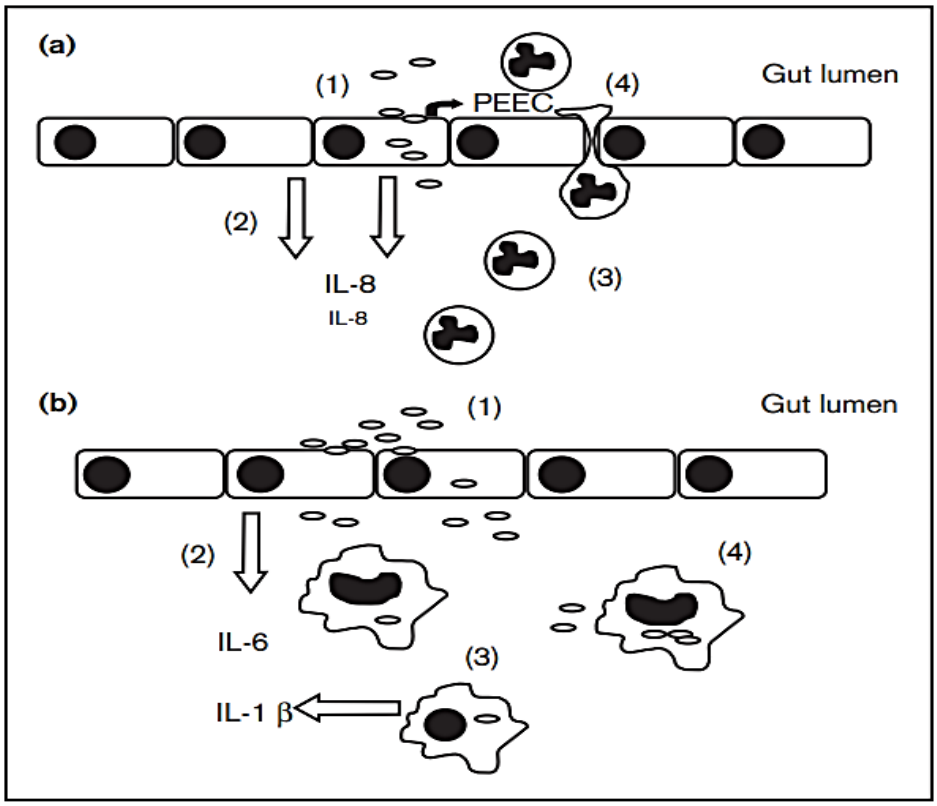
Pathogenicity:

Typhoid fever remains a major public health problem in many regions of the world with over 16 million cases being reported each year worldwide. Patients with typhoid fever are infected with *Salmonella enterica* subspecies I serovar *Typhi* (*S. typhi*) and usually present to the health care worker with a history of prolonged fever, headache, abdominal discomfort and general lethargy. Around 10% of these develop severe or complicated disease and without specific treatment 5±30% of all patients with typhoid fever may die. Serovars of *S. enterica* subspecies I (41300) cause infections in many warm-blooded animals and, despite a very close genetic relationship to each other, display differing ranges of host specificity. Some serovars are host restricted while others can infect a variety of mammals. *S. typhi*, for example, is adapted, and restricted, to the human host, whereas many isolates of *S. typhimurium* cause gastroenteritis or occasionally septicaemia in humans, lethal diarrhoea in calves or a systemic disease in genetically susceptible mice. Since *S. typhi* fails to establish infection in laboratory rodents most of the research reported on the pathogenesis of typhoid fever is based on in-vitro studies with human and murine cell lines and the infection of mice with *S. typhimurium*.

Adherence to and invasion of epithelial cells *S. typhi* is ingested in contaminated food or water. It passes through the stomach and invades the gut epithelium, possibly in the distal ileum. Active attachment promoted by the bacterium may be necessary before invasion can occur and this most likely involves unidentified adhesion molecules on the bacterium interacting with receptors on the host cell. The nature of this interaction for *S. typhi* is not clear but may involve fimbriae. *S. typhi* has 12 fimbrial operons of the chaperone usher assembly class, but none, including tcf (Typhi colonization factor) are unique to *S. typhi*. *S. typhi*, however, does possess a unique combination of fimbrial operons. The diverse family of fimbriae may be the result of selection by the host immune response. A type IVB pilus operon (pil) is also present in *S. typhi* 573 and wild type (pil^+) *S. typhi* are more able to adhere to and invade intestinal epithelial cells than are pilS::Km^r (pil^-) mutants. The cystic fibrosis transmembrane conductance regulator, previously reported to be used by *S. typhi* for entry into intestinal cells, has been suggested as a possible site for attachment mediated by type IVB pili. *Salmonella* spp. invade epithelial cells in vitro by a process of

bacterial-mediated endocytosis, involving cytoskeletal re-arrangement, disruption of the epithelial cell brush border and the subsequent formation of membrane ruffles. An adherent and invasive phenotype of *S. enterica* is activated under conditions similar to those found in the human small intestine (high osmolarity, low oxygen). The invasive phenotype is mediated, in part, by salmonella pathogenicity island (SPI)-1, a 40 kb region of the chromosome which encodes regulator proteins (e.g. HilA), a type III secretion system (TTSS) that delivers bacterial proteins from the salmonella cytosol into the host cell, and several effector proteins which induce changes within the host cell and promote bacterial uptake. The acquisition of SPI-1 probably enabled the ancestral *S. enterica* to adhere to and invade epithelial cells more efficiently, thus allowing the colonization of a new environmental niche. Although *S. typhimurium* can cause systemic disease in the human host, infection with this serovar usually results in a localized enteritis associated with a secretory response in the intestinal epithelium and recruitment and transmigration of neutrophils into the intestinal lumen. *S. typhimurium* induces human intestinal epithelial cells to secrete interleukin-8 and other pathogen-elicited epithelial chemo-attractants which direct the recruitment and transmigration of neutrophils into the gut lumen (Fig. 1).

Figure 2. Comparison of the events occurring within the gut lumen and gut-associated tissues which may determine whether an infection with *Salmonella* will be limited to the gut (a) or become systemic (b)



S. typhi is not typically associated with acute diarrhoea, suggesting that the initial interaction between this serovar and the human gut is less inflammatory than that seen with enteritis-causing Salmonella spp. The lack of acute inflammation and the absence of subsequent recruitment of neutrophils may allow *S. typhi* to invade into the deeper tissues of the gut, although there is little experimental evidence for this hypothesis. *S. typhi* does, however, stimulate IL-6 secretion from human epithelial cells. The magnitude of the response is dependent upon the isolate of *S. typhi* (clinical versus laboratory {Ty2}) and is higher than with *S. typhimurium*, possibly reflecting differences in adherence/invasion, which is greater for *S. typhi*. Shortly following invasion of the gut epithelium, invasive *Salmonella* spp. encounter macrophages within the gut-associated lymphoid tissue. The interaction between Salmonella and macrophage results in an alteration in the expression of a number of host genes including those encoding pro-inflammatory mediators (e.g. iNOS, chemokines, interleukin-1b), receptors or adhesion molecules (e.g. TNFaR, CD40, ICAM-1), and anti-inflammatory mediators (e.g. TGFb1 and 2). Other up-regulated genes include those involved in cell death or apoptosis (e.g. ICE protease, TNFR1, Fas) and transcription factors (e.g. Egr-1, IRF-1). Some non-typhi serovars can induce rapid macrophage death (within 30 min of infection) in vitro. This is mediated by the SPI-1 effector SipB, is dependent upon the host cell protein caspase-1 and has features common to both apoptosis and necrosis. Caspase-1 mediated cell death is a pro-inflammatory process that would seem to be counter-productive if an organism is to establish a systemic infection. The pro-inflammatory response and the subsequent recruitment of phagocytic cells to the site of the infection may facilitate systemic spread of the bacteria. In the absence of SPI-1 expression, Salmonella-infected macrophages can remain viable for several hours and thus serve as a cellular niche in which the bacteria can survive protected from the host immune system. Intracellular *S. typhimurium* are located within specialized Salmonella containing vacuoles that have diverged from the normal endocytic pathway. Within epithelial cells, lysosomal glycoprotein (lgb)-containing tubular structures, termed Salmonella-induced filaments (Sifs), can be seen radiating from Salmonella-containing vacuoles. Although Sifs are not seen in *S. typhimurium*-infected macrophages, sifA7 *S. typhimurium* are unable to survive well and replicate efficiently within these cells in vitro and are attenuated in vivo when administered intraperitoneally to mice. A high proportion of *S. typhi* in the bone marrow of the human host is intracellular and in the blood this percentage increases as the disease progresses. Shedding of *S. typhi* in the faeces of an infected individual is an essential step in the transmission of typhoid fever.

Symptoms:

Typhoid fever and paratyphoid fever have similar symptoms. People usually have a sustained fever that can be as high as 103–104°F (39–40°C). A sustained fever is a fever that does not come and go.

Other symptoms of typhoid fever and paratyphoid fever include

- Weakness
- Stomach pain
- Headache
- Diarrhea or constipation
- Cough
- Loss of appetite

Some people with typhoid fever or paratyphoid fever develop a rash of flat, rose-colored spots.

Prevention:

Typhoid fever is common in places with poor sanitation and a lack of safe drinking water. Access to safe water and adequate sanitation, hygiene among food handlers and typhoid vaccination are all effective in preventing typhoid fever.

Typhoid conjugate vaccine, consisting of the purified Vi antigen linked to a carrier protein, is given as a single injectable dose in children from 6 months of age and in adults up to 45 years or 65 years (depending on the vaccine).

Two additional vaccines have been used for many years in older children and adults at risk of typhoid, including travellers. These vaccines do not provide long-lasting immunity (requiring repeat or booster doses) and are not approved for children younger than 2 years old:

- an injectable vaccine based on the purified antigen for people aged 2 years and above; and
- a live attenuated oral vaccine in capsule formulation for people aged over 6 years.

Two typhoid conjugate vaccines have been prequalified by WHO since December 2017 and are being introduced into childhood immunization programmes in typhoid endemic countries.

All travellers to endemic areas are at potential risk of typhoid fever, although the risk is generally low in tourist and business centres where standards of accommodation, sanitation and food hygiene are high. Typhoid fever vaccination should be offered to travellers to destinations where the risk of typhoid fever is high.

The following recommendations will help ensure safety while travelling:

- Ensure food is properly cooked and still hot when served.
- Avoid raw milk and products made from raw milk. Drink only pasteurized or boiled milk.
- Avoid ice unless it is made from safe water.

- When the safety of drinking water is questionable, boil it, or if this is not possible, disinfect it with a reliable, slow-release disinfectant agent (usually available at pharmacies).
- Wash hands thoroughly and frequently using soap, in particular after contact with pets or farm animals, or after having been to the toilet.
- Wash fruits and vegetables carefully, particularly if they are eaten raw. If possible, vegetables and fruits should be peeled.

JE (Japanese encephalitis)

JE virus (causative agent of Japanese encephalitis) is maintained in nature between vector mosquitoes and vertebrate animals, especially pigs. The principal vector of JE virus is culex mosquitoes, mainly *Culex tritaeniorhynchus* in Asia. Wild birds probably play a role in the maintenance of JE virus in nature and in carrying the virus to new regions.

Humans are infected by the bite of infected mosquitoes. Pigs play a role as an amplifier for JE virus. Infectivity titres of JE virus reach high levels in pigs. Many mosquitoes are infected with the virus by biting infected pigs. The infected mosquitoes then transmit JE virus to humans. However, humans do not transmit virus to biting mosquitoes because of low titres of viremia; thus, humans are considered to be dead-end hosts.

- **Transmission:**

Japanese encephalitis virus is maintained in enzootic forms and appears as focal outbreaks under specific ecological conditions. They multiply in the tissues of arthropods without evidence of disease and damage. Man is an accidental, dead-end host for JE. The principal vector species is *Culex tritaeniorhynchus*, which is a rural mosquito, present in great density in rainy season in both tropical and temperate regions. The other minor hosts are cattle, buffaloes, goats, sheep, horses, rodents, monkeys, dogs and bats. It has an extrinsic incubation period of 10-12 days. The natural cycle of JEV consists of pig-mosquito-pig or bird mosquito- bird cycles. GIII was the only widely distributed genotype found in India until till when GI JEV strains were detected and isolated from 66 Acute Encephalitis Syndrome (AES) patients along with GIII strains. This detection indicates their co-circulation and association with humans. In the mid 1990's genetic shift had occurred in Japan, Korea and Vietnam that lead to disappearance of GIII and then progressively GI supplanted it. In India exact mode of introduction of GI is not clear, but it is possible that it may have been introduced through migratory birds. Pigs are the most important biological amplifiers and reservoirs. Generally direct person to person spread of JEV does not or rarely occurs until it is through intrauterine transmission. Blood and organ transplantation also serve as a mode of transmission. The risk for JE is more in rainy season both in temperate and tropical regions.

- **Maternal to Foetal Transmission**

JEV infection transmits from mother to fetus through vertical mode of transmission. This may be due to persistent maternal infection or due to pregnancy induced reactivation of virus.

Pathogenicity:

The pathogenesis of JE, including neuroinvasion, neuroinflammation, and neuronal cell damage, is complicated; therefore, it has not yet been understood completely. The currently accepted narrative is that JEV gains access to the central nervous system (CNS) by breaching the blood-brain barrier (BBB), followed by stimulation of profound neuroinflammatory response in glial cells (microglia and astrocytes) and subsequent neuronal cell damage. In the past several years, efforts have been made in order to deeply understand the biology and pathogenesis of JE, and thus, highlighted several cellular and viral factors that facilitate the JEV replication and enhance the JEV-associated neuropathology.

- **Neuroinflammation in JE**

An overview of JEV entry into the CNS is shown in Fig. 1. Once an individual is bitten by a JEV-infected mosquito, the virus infects the resident dermal cells (dendritic cells, fibroblasts, endothelial cells, and pericytes) and local lymph nodes, followed by the onset of primary asymptomatic viremia. Later, the virus disseminates via hematogenous route and efferent lymphatic system to multiple body organs (e.g., heart, liver, spleen, and muscle), causing secondary symptomatic viremia. In the periphery, JEV mainly replicates in the macrophage/dendritic cell precursor-derived $Ly6C^{hi}$ monocytes that express abundant surface CCR2, and subsequently, can migrate from the periphery to the CNS and contribute to the inflammatory response.

The ingress of the virus in the CNS is indispensable for inducing neuroinflammation and neuronal cell damage. Several lines of evidence demonstrate that the virus reaches the CNS via crossing the BBB, which is comprised of brain microvasculature endothelial cells together with neurovasculature units (pericytes, astrocytes, microglia, neurons, and extracellular matrix), and maintains the CNS homeostasis by regulating the transport of immune cells and soluble molecules from blood to brain. Endothelial cells in the brain microvasculature are considered as the key cells to provide structural and functional integrity to BBB. The interaction of neurovasculature cells with each other and their cooperation with endothelial cells is also vital for tight junctions and regulation of the BBB integrity.

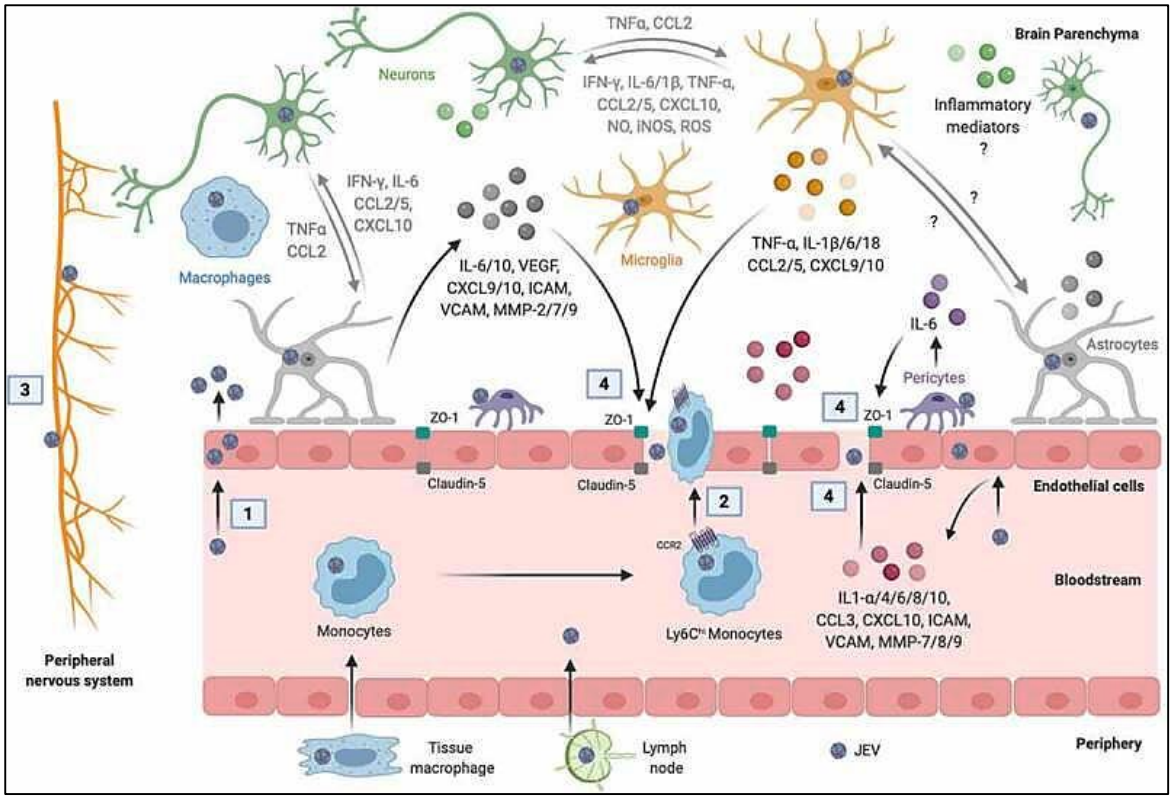


Figure 2.

Mechanisms of JEV neuroinvasion. The numbers in squares indicate the mechanisms of virus entry into the brain: 1, passive transport of virus particles across the endothelial cells; 2, diapedesis of infected leukocytes; 3, virus transport via the peripheral nervous system; and 4, virus transport through the BBB disrupted by inflammatory mediators released from cells of blood and brain sides of the BBB.

The mechanisms by which JEV crosses the BBB to enter into the CNS are supported by four possible modes: (1) proliferation of virus within endothelial cells without affecting the cell viability, followed by passive transport of viral particles across the endothelial cells, (2) diapedesis of virus-infected leukocytes between endothelial cell junctions, (3) transport via the peripheral nervous system, and (4) disruption of the BBB through the release of virus-induced inflammatory mediators from the cells of apical (blood) and basolateral (brain) sides of the BBB, which is considered to be the most common mode.

Infection of endothelial cells by JEV caused no cytotoxicity through the deactivation of cellular pro-apoptotic proteins, but increased permeability of endothelial cells, excluding the apoptosis of endothelial cells as a cell process associated with the BBB disruption. In an *in vitro* human BBB model, JEV has been shown to stimulate the release of inflammatory mediators (cytokines, chemokines, matrix metalloproteinases, and cellular adhesion molecules) from endothelial cells and astrocytes, which permit the virus entry into the brain by disrupting the BBB. In addition, activated microglia secrete inflammatory cytokines, which in turn promote the BBB leakage.

In contrast, JEV has also been observed to gain entry into the CNS prior to disrupting the BBB. Incubation of cultured monolayers of endothelial cells with brain extracts obtained from JEV-infected mice, but not with direct JEV particles, induced alterations in the permeability of the BBB through inflammatory mediators (CXCL10, CCL2/3/4, and IL-6) associated inhibition of tight junction proteins. CXCL10 also affects the distribution of ZO-1 and claudin-5 in endothelial cells and subsequently promotes the BBB damage by augmenting the activity of TNF-α through the JNK signaling pathway in infected mice.

- **Activation of glial and neuronal cells**

Once the JEV entered the brain, the substantial activation of glial (microglia and astrocytes) and neuronal cells is a hallmark feature of JE. Microglia, astrocytes, and neurons have employed several mechanisms to trigger the neuroinflammatory response. It is well established that these cells can detect structurally conserved pathogen-associated molecular patterns by expressing a wide array to pattern recognition receptors, resulting in the stimulation of immune signaling cascades that lead to the production of inflammatory mediators.

JEV infection of cultured mouse microglia resulted in the activation of TLR3- and RIG-I-mediated activation of ERK/MAPKp38/AP-1/NF-κB signaling cascades, leading to the production of inflammatory cytokines. In the neuron/glial co-culture system, JEV induced a ROS-dependent activation of Src/Ras/Raf/ERK/NF-κB signaling axis. The JEV-induced expression of CCR2 on the surface of microglia is positively correlated with the neurotoxic microglia activation phenotype and subsequent inflammatory response. The interaction between JEV subgenomic RNA (ssRNA) and TLR7, as assessed by co-immunoprecipitation, negatively regulated type-I IFN and inflammatory response in cultured neurons and mice brain tissues. Moreover, the deficiency of TLR7 caused the stimulation of TLR8-mediated compensatory immune response in JEV-infected mice brain, suggesting that TLR7 and TLR8 are interlinked to mediate immune response during JEV infection.

In addition to the direct modulation of immune response proteins in glia and neurons, the perturbation of the signatures of non-coding RNAs upon JEV infection in these cells has been associated with increased neuroinflammation. Of the non-coding RNAs, the functions of microRNAs (miRNAs) in regulating the neuroinflammatory response have been studied extensively in the last few years. Global RNA sequence profiling of JEV-infected mice brain tissues revealed differential expression of host-encoded miRNAs which were predicted to regulate antiviral immunity, apoptosis, neuronal differentiation, neurotrophin signaling, and transcription. The JEV-induced miR-155 positively regulated NF-κB activity by targeting SHIP1 in mouse microglial cells, whereas the same miRNA negatively regulated innate immune responses by attenuating IRF8 and NF-κB pathway mediators during JEV infection of human microglial cells, which indicates a species-specific mechanism. Apart from glial cells, JEV infection is found to modulate the expression of miRNAs in neuronal cells. The miR-301a blocked the type-I IFN response

and facilitated JE pathogenesis in mouse neuronal cells by suppressing the abundance of IRF1 and SOCS5.

RNA sequence analyses of JEV-infected mice brain tissues have also unveiled widespread alterations in the expression of other non-coding RNAs that include long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs). Functional enrichment analysis of differentially expressed lncRNAs and circRNAs exhibit a strong relationship with cellular processes related to innate immunity, inflammation, neurotransmission, and transcription dysregulation.

- **Neuronal cell damage in JE**

There have been several types of cell death modes described in the biomedical literature such as apoptosis, necroptosis, autophagy, pyroptosis, ferroptosis, phagocytosis, entosis, paraptosis, excitotoxicity, NETosis, and mitotic catastrophe. Each of such modes is triggered and propagated by cellular mechanisms that show a considerable level of linkage with each other.

- **Apoptosis**

The activation of glia or direct infection of neurons during JEV infection commences irreversible cellular responses, leading to neuronal apoptosis. During the direct infection of neuronal cells, JEV can induce unfolded protein response by stressing the endoplasmic reticulum (ER) as assessed by the stimulation of CHOP, MAPK p38, and the IRE-1α-dependent decay pathway, which results in the apoptosis of neuronal cells. Furthermore, the involvement of mitochondrion-dependent pathways in regulating JEV-induced neuronal apoptosis has also been elucidated. Infection of cultured neuronal cells caused the JEV NS2B-NS3 protease-induced or p21-Bax/p18-Bax-mediated release of mitochondrial cytochrome C in the cytoplasm, indispensable for the activation of caspase-3/7-mediated apoptosis pathways in these cells. Alternatively, the production of ROS and the stimulation of ASK1/ERK1/p38-MAPK pathway in infected cells is also linked to the NS4B-NS3-induced mitochondrion-dependent apoptosis. The activation/phosphorylation of NMDAR is also observed as a pathogenic mechanism during JEV of cultured neurons and mice brain tissues, resulting in increased calcium ion influx in infected neurons and enhanced neuronal toxicity.

- **Autophagy**

Apart from maintaining cellular homeostasis, autophagy is known to play a pivotal role during the replication of several viruses, including JEV. The propagation of some viruses is suppressed by autophagy, whereas other viruses can harness autophagy pathways to aid their replication. Autophagy positively regulates JEV replication by regulating the early step of virus replication, i.e., entry and virus-uncoating. The depletion of autophagy-related genes (Rab7, LAMP2, ATG5, and Beclin-1) in JEV-infected mouse or human neuronal cells displayed cellular apoptosis and suppressed virus replication, suggesting that JEV employs autophagy as an immune evasion mechanism.

In contrast to the above findings, a few reports suggested a negative correlation between autophagy and JEV replication, indicating autophagy as a host-defensive mechanism against JEV. The production of JEV infectious particles was observed to be significantly increased in mouse neuronal cell deficient in ATG7 protein, and these cells were highly susceptible to virus-induced cellular apoptosis. Furthermore, the JEV-induced expression of Nedd4 (E3 ubiquitin ligase) in human neuronal cells, but not in non-neuronal cells, promoted JEV replication by suppressing the autophagy as assessed by increased autophagosome accumulation in Nedd4-silenced cells, thus, implying it as a neuron-specific cellular mechanism.

Symptoms:

A person with Japanese encephalitis will probably have no symptoms at all, but if there are symptoms, they will appear 5 to 15 days after being infected.

A person with mild Japanese encephalitis might only develop a fever and a headache, but in more severe cases, more serious symptoms can develop quickly.

Possible symptoms include:

- a headache
- high fever
- tremors
- nausea
- vomiting
- stiff neck
- spastic paralysis

A person might also undergo changes to brain function, including:

- stupor
- disorientation
- coma
- convulsions in children

The testicles can also swell.

The brain symptoms of Japanese encephalitis can cause lifelong complications, such as deafness, uncontrollable emotions, and weakness on one side of the body.

The chance of surviving the disease varies, but children face the highest risk of fatal consequences.

Yellow fever

A certain species of mosquito can bite a person and transmit the virus that causes yellow fever. The tropics and subtropics of Africa and South America are home to these mosquitoes and yellow fever. When mosquitoes bite primates carrying the virus get infected.

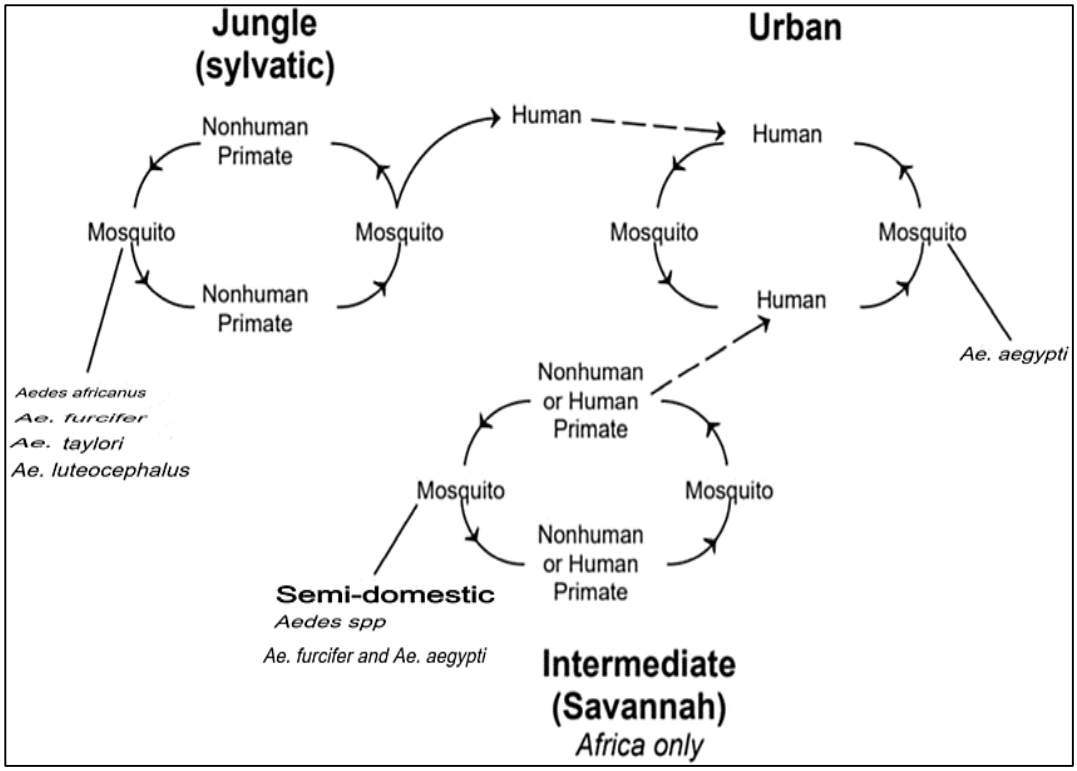
The symptoms of the yellow fever virus might vary. Some individuals could be symptom-free. When it's at its worst, it can be fatal but only exhibit minor flu-like symptoms. You can experience symptoms similar to the flu, including aches, pains, and fever, or you might start bleeding and get liver damage. About three to six days pass before symptoms appear.

Transmission:

Yellow fever virus is an RNA virus that belongs to the genus *Flavivirus*. It is related to West Nile, St. Louis encephalitis, and Japanese encephalitis viruses. Yellow fever virus is transmitted to people primarily through the bite of infected *Aedes* mosquitoes. Mosquitoes acquire the virus by feeding on infected primates (human or non-human) and then can transmit the virus to other primates (human or non-human). People infected with yellow fever virus are infectious to mosquitoes (referred to as being "viremic") shortly before the onset of fever and up to 5 days after onset.

Yellow fever virus has three transmission cycles: jungle (sylvatic), intermediate (savannah), and urban.

- The jungle (sylvatic) cycle involves transmission of the virus between non-human primates (e.g., monkeys) and mosquito species found in the forest canopy. The virus is transmitted by mosquitoes from monkeys to humans when humans are visiting or working in the jungle.
- In Africa, an intermediate (savannah) cycle exists that involves transmission of virus from mosquitoes to humans living or working in jungle border areas. In this cycle, the virus can be transmitted from monkey to human or from human to human via mosquitoes.
- The urban cycle involves transmission of the virus between humans and urban mosquitoes, primarily *Aedes aegypti.* The virus is usually brought to the urban setting by a viremic human who was infected in the jungle or savannah.



Pathogenicity:

Three clinical stages of illness have been described in humans in the classical picture of yellow fever, 3–6 days after the infecting bite. The disease begins with the acute onset of fever (approximately 39°C), headache, malaise, photophobia, backache, myalgia, irritability, restlessness, nausea, and vomiting. This symptomatology lasts for 3–5 days and is known as the period of infection (i.e. period of viremia). During this period, the blood is infectious to biting mosquitoes. The period of infection is followed by a period of remission, which lasts approximately 12 h–2 days. During this stage, the patient feels better, with a sensation of recovery, but suddenly, some patients become severely ill with typical signs of liver and renal failure, which characterizes the third stage, the period of intoxication. During this stage, patients develop severe hemorrhagic fever and multi-organ dysfunction accompanied by jaundice, oliguria or anuria, cardiovascular instability, and hemorrhagic diathesis during which episodes of hematemesis, melena, petechiae, ecchymosis, and other hemorrhagic manifestations are common. In this final stage, thrombocytopenia (a platelet count of less than 50 000 per millilitre), prolonged clotting and prothrombin times, and lower levels of liver clotting factors are observed. Laboratory clinical tests indicate diminished fibrinogen and factor VIII and elevated fibrin split products, which are characteristics of disseminated intravascular coagulation. Immediately after a YFV inoculation by an infected mosquito, the virus first replicates in the local lymph nodes and then disseminates to many organs, causing lesions, either as a consequence of the direct viral cytopathic effect or due to alterations secondary to the immune response of the host.

In yellow fever pathogenesis, the virus elicits two distinct and separate patterns of injury, viscerotropism and neurotropism. YFV is neurotropic in mice, and they succumb to an encephalitic infection. YFV is viscerotropic in primates, including humans, in whom it causes lesions in multiple organs, such as the liver, spleen, heart, and kidneys. The liver is the primary target organ for YFV. Indeed, YFV induces hepatocellular injury characterized by eosinophilic necrosis, apoptosis of hepatic cells, and macro steatosis and micro steatosis, mainly located in the midzone; a scarce portal inflammatory infiltrate is also found.

The pattern of liver involvement in yellow fever is intense, primarily in the midzone region (zone 2) when compared with the acini zones 1 and 3. This involvement has been frequently reported in both human cases and experimental monkey and hamster models. There is questionable evidence that explains the preferential lesion pattern observed for yellow fever and other arbovirus infections. This pattern shows a course of intense vascular involvement and more severe midzonal lesions. The pathophysiology of yellow fever and dengue clearly is of infectious vasculopathies, characterized by increased vascular damage which results in low-flow hypoxia. Therefore, the hemodynamic changes in connection with the liver tropism of both viruses explain the preferential observed midzonal focus in the course of dengue, yellow fever, and other hepatotropic flaviviruses.

In yellow fever, in all liver tissue, swelling of the hepatocytes is present, but it is more severe in zones 1 and 3. The common finding in other viral hepatitis infections such as ballooning degeneration is not observed during yellow fever. The presence of Councilman bodies is typical of yellow fever, and their morphology is impressively wide, with pleomorphic hepatocytes ranging from well-defined to bizarre cells distributed in all liver areas but clearly found more intensely in the midzone. These aspects may correspond to the different apoptotic stages observed in the yellow fever-infected liver. The characteristics of cells and their ultrastructural aspects confirm apoptosis as the main cell death mechanism during severe yellow fever. In parallel with the apoptosis, especially in the midzone, lytic necrosis of hepatocytes has been found in well-limited areas by minimal inflammation (predominantly lymphocytes and neutrophils). These two mechanisms of hepatic cell death, plus swelling and steatosis, are clearly associated with the liver failure classically described during the course of severe yellow fever and explain the high levels of both alanine and aspartate aminotransferase in the blood. Both steatosis and necrosis occur more intensely in the midzone area; the first is characterized by wide morphological variability, including microdroplets or a morula-like aspect ("morula cells") that apparently results from accumulation of large amounts of vesicles in the hepatocyte cytoplasm, called macrodroplet. Steatosis is one of the marked aspects of the yellow fever liver, and its intensity coincides with the intensity of apoptosis. During severe yellow fever, acinar mononuclear inflammatory infiltrate is a common finding but usually discrete and disproportionate to the severe degree of hepatic damage. Indeed, in the areas of lytic necrosis, the polymorphonuclear infiltrate is scarce. In the acini, the inflammatory infiltrate is more evident in zone 2, similar to the hepatocyte injury. The

edema is discrete in the portal spaces, and the presence of lymphomononuclear (lymphocytes and macrophages) cells is quite discrete and slightly more concentrated than that observed in the other acini areas and independent of the degree of parenchymal involvement. The portal and acinar inflammatory infiltrates in humans severely infected by yellow fever and also in experimental models of yellow fever consist of lymphomononuclear cells. Importantly, near the necrotic areas, a neutrophilic infiltrate is commonly observed. In contrast, the extensive apoptosis seems to be responsible for the disproportionate parenchymatous injury versus inflammation which results in a paucity cellular inflammatory response.

Apoptosis is a cardinal feature of liver injury in many infections. In the liver, two pathways of apoptosis have been observed: extrinsic and intrinsic pathways that are dependent and independent of death receptors, respectively. Death receptors are major mediators of the apoptosis pathway in the liver and have been implicated in the pathogenesis of viral hepatitis and other liver diseases. Mechanisms of liver injury are complex and involve the interaction of cytokines, reactive oxygen species, Kupffer cells, and immune cells. In the intrinsic pathway, oxidative stress and the mediators released in the endothelial lesions with microvascular alterations cause mitochondrial lesions with release of members of the Bcl-2 protein family that interact with the caspase cascade to induce cell death by apoptosis. Death receptors can also regulate apoptosis by external killers binding or ligating cell surface receptors. For example, Fas, TNF receptor 1, TNF-related apoptosis-inducing ligand receptor 1 (TRAIL), and TRAIL receptor 2 are common death cell receptors expressed in the human liver. During fatal yellow fever, ApopTag-labeled cells have been found in all regions of the hepatic lobule. Ligands for these receptors are expressed in human and rodent liver tissues by immunohistochemistry and molecular assays. It is a common observation that apoptotic hepatic cells correspond to the Councilman bodies in humans and to cells under apoptotic conditions in rodents. Several mechanisms are implicated in the apoptosis pathway following yellow fever and include the direct cytopathic effect of YFV, an important mitochondrial dysfunction due to low-flow hypoxia, and the influence of immune factors in the in situ immune response on the liver. This cell death process in the liver following yellow fever with minimal inflammatory infiltrate is explained because apoptosis does not induce an inflammatory response. The main consequence of this death phenomenon is the possibility of developing a therapeutic approach to protect the liver in severe cases of disease.

The pattern of cytokine expression in response to yellow fever has been described both in human and experimental infection models. A marked expression of TNF-α, IFN-γ, and intense immunolabelling for TGF-β has been demonstrated in fatal human cases. Other cytokines are not as highly expressed but are observed in all of the hepatic acini. Those cytokines show a greater expression in the midzone and portal tract and occur less frequently in other areas. Liver expression of IL1-α/β, IL-4, IL-8, and IL-10 is lower in the lobule areas and portal tract, and there is no preference for any area of the hepatic lobule. TNF-α and IFN-γ are of major importance because both cytokines can induce apoptosis in hepatocytes by binding to their respective receptors and activating the death domain,

which results in cell death. In the human host, TNF-α is incriminated with presentation of antigens to lymphomononuclear cells and particularly in binding of CD8+ T-cells to the surface of the hepatocytes, an important mechanism leading to cell death. It is clear that receptors such as Fas, which is especially expressed in fatal/severe yellow fever cases, may act as possible mediators for cell death by activating death receptors and inducing apoptosis. The intense immunolabelling for TGF-β in the yellow fever liver is an important characteristic of this infection. TGF-β is a cytokine with strong immunosuppressor and pro-apoptotic effects; it was demonstrated that TGF-β plays an important role in fatal human yellow fever possibly by inducing an intense tissue damage.

Following the infecting bite, yellow fever virus is initially recognized by antigen presenting cells (dendritic cells) and presented in the lymph nodes to the CD4+ lymphocytes, in which the virus replicates and spreads to other organs by viremia. In the liver (and in other organs), the immune response, coordinated by those lymphocytes and the liberation of several cytokines (especially TNF-α and INF-γ), will determine the midzonal apoptosis of hepatocytes and several pathologic changes (apoptosis in heart and acute tubular necrosis in the renal tissues), which culminates with multi-organ failure and hemorrhagic diathesis (vasculopathy). The humoral response produces specific antibodies against yellow fever virus by B lymphocytes, and the cellular response results in apoptosis and hepatocytic necrosis, which is accompanied by discrete inflammatory infiltrate due mainly to TGF-β action, which is an apoptotic inductor and inhibitor of the inflammatory response and the cytokine storm.

Symptoms:

Yellow fever has 3 stages:

Stage 1 (infection): Headache, muscle and joint aches, fever, flushing, loss of appetite, vomiting, and jaundice are common. Symptoms often go away briefly after about 3 to 4 days.

Stage 2 (remission): Fever and other symptoms go away. Most people will recover at this stage, but others may get worse within 24 hours.

Stage 3 (intoxication): Problems with many organs may occur, including the heart, liver, and kidney. Bleeding disorders, seizures, coma, and delirium may also occur.

Symptoms may include:

- Fever, headache, muscle aches
- Nausea and vomiting, possibly vomiting blood
- Red eyes, face, tongue
- Yellow skin and eyes (jaundice)
- Decreased urination

- Delirium
- Irregular heartbeats (arrhythmias)
- Bleeding (may progress to hemorrhage)
- Seizures
- Coma

Prevention:

Yellow fever can be best prevented by adopting following measures:

- Avoiding mosquito bites
- Vaccination
- Isolation

Avoiding mosquito bites is key to prevention. People who live in or visit areas where yellow fever is common can

- Apply DEET (diethyltoluamide) insect repellant to the skin.
- Use mosquito netting.
- Wear long-sleeved shirts and long pants.
- Stay in places that have air conditioning or that use window and door screens to keep mosquitoes out.
- Treat clothing and gear with permethrin insecticide (do not apply it directly to the skin).

For children, the following precautions are recommended:

- Do not use insect repellent on infants under 2 months old.
- Do not use products containing oil of lemon eucalyptus (para-menthane-diol) on children under 3 years old.
- For older children, adults should spray repellent on their own hands and then apply it to the children's skin.
- Dress children in clothing that covers their arms and legs, or cover the crib, stroller, or baby carrier with mosquito netting.
- Do not apply insect repellent to the hands, eyes, mouth, or cut or irritated skin of children.

A vaccine that is 95% effective at preventing yellow fever is available. A single dose of vaccine can provide life-long immunity against yellow fever.

Probable questions:

1. Discuss the route of infection of *Salmonella typhi*.
2. How *Salmonella typhi* gets adhered to gut epithelial cells leading to its damage?
3. State the symptoms of typhoid.
4. Discuss the route of transmission of JE virus.
5. Elucidate the mechanism of neuroinvasion of JE virus.
6. What are symptoms of Japanese encephalitis?
7. What are jungle cycle and urban cycle of yellow fever virus?
8. How the hepatocytes get damaged due to yellow fever virus invasion?
9. What are the three stages of symptoms of yellow fever?
10. Discuss the methods of mosquito prevention.

Suggested reading:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)
2. Essentials of microbiology. Amita J & Parul J. Elsevier (Latest edition).
3. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).
4. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press.

Unit-XX

Mode of transmission, pathogenicity and prevention of microbial diseases: SARS-COV 2 (infection and concept of herd immunity)

Objectives:

In this section we will discuss on mode of transmission, pathogenicity and prevention of microbial diseases: SARS-COV 2 (infection and concept of herd immunity).

Introduction:

The World Health Organization recognized SARS-CoV-2 as a public health concern and declared it as a pandemic on March 11, 2020. Over 12 million people have been affected across several countries since it was first recognized. SARS-CoV-2 is thought to commonly spread via respiratory droplets formed while talking, coughing, and sneezing of an infected patient. As several cases, with an absence of travel history to the majorly affected areas were identified, a strong possibility of community transmission could have been possible.

Modes of transmission:

This section briefly describes possible modes of transmission for SARS-CoV-2, including contact, droplet, airborne, fomite, fecal-oral, bloodborne, mother-to-child, and animal-to-human transmission. Infection with SARS-CoV-2 primarily causes respiratory illness ranging from mild disease to severe disease and death, and some people infected with the virus never develop symptoms.

- **Contact and droplet transmission**

Transmission of SARS-CoV-2 can occur through direct, indirect, or close contact with infected people through infected secretions such as saliva and respiratory secretions or their respiratory droplets, which are expelled when an infected person coughs, sneezes, talks or sings. Respiratory droplets are >5-10 μm in diameter whereas droplets ≤5 μm in diameter are referred to as droplet nuclei or aerosols. Respiratory droplet transmission can occur when a person is in close contact (within 1 metre) with an infected person who has respiratory symptoms (e.g. coughing or sneezing) or who is talking or singing; in these circumstances, respiratory droplets that include virus can reach the mouth, nose or eyes of a susceptible person and can result in infection. Indirect contact transmission involving contact of a susceptible host with a contaminated object or surface (fomite transmission) may also be possible.

- **Airborne transmission**

Airborne transmission is defined as the spread of an infectious agent caused by the dissemination of droplet nuclei (aerosols) that remain infectious when suspended in air over long distances and time. Airborne transmission of SARS-CoV-2 can occur during medical procedures that generate aerosols ("aerosol generating procedures"). WHO, together with the scientific community, has been actively discussing and evaluating whether SARS-CoV-2 may also spread through aerosols in the absence of aerosol generating procedures, particularly in indoor settings with poor ventilation.

The physics of exhaled air and flow physics have generated hypotheses about possible mechanisms of SARS-CoV-2 transmission through aerosols. These theories suggest that 1) a number of respiratory droplets generate microscopic aerosols (<5 μm) by evaporating, and 2) normal breathing and talking results in exhaled aerosols. Thus, a susceptible person could inhale aerosols, and could become infected if the aerosols contain the virus in sufficient quantity to cause infection within the recipient. However, the proportion of exhaled droplet nuclei or of respiratory droplets that evaporate to generate aerosols, and the infectious dose of viable SARS-CoV-2 required to cause infection in another person are not known, but it has been studied for other respiratory viruses.

A recent experimental model found that healthy individuals can produce aerosols through coughing and talking, and another model suggested high variability between individuals in terms of particle emission rates during speech, with increased rates correlated with increased amplitude of vocalization. To date, transmission of SARS-CoV-2 by this type of aerosol route has not been demonstrated; much more research is needed given the possible implications of such route of transmission.

Experimental studies have generated aerosols of infectious samples using high-powered jet nebulizers under controlled laboratory conditions. These studies found SARS-CoV-2 virus RNA in air samples within aerosols for up to 3 hours in one study and 16 hours in another, which also found viable replication-competent virus. These findings were from experimentally induced aerosols that do not reflect normal human cough conditions.

Some studies conducted in health care settings where symptomatic COVID-19 patients were cared for, but where aerosol generating procedures were not performed, reported the presence of SARS-CoV-2 RNA in air samples, while other similar investigations in both health care and non-health care settings found no presence of SARS-CoV-2 RNA; no studies have found viable virus in air samples. Within samples where SARS-CoV-2 RNA was found, the quantity of RNA detected was in extremely low numbers in large volumes of air and one study that found SARS-CoV-2 RNA in air samples reported inability to identify viable virus. The detection of RNA using reverse transcription polymerase chain reaction (RT-PCR)-based assays is not necessarily indicative of replication- and infection-competent (viable) virus that could be transmissible and capable of causing infection.

Recent clinical reports of health workers exposed to COVID-19 index cases, not in the presence of aerosol-generating procedures, found no nosocomial transmission when

contact and droplet precautions were appropriately used, including the wearing of medical masks as a component of the personal protective equipment (PPE). These observations suggest that aerosol transmission did not occur in this context. Further studies are needed to determine whether it is possible to detect viable SARS-CoV-2 in air samples from settings where no procedures that generate aerosols are performed and what role aerosols might play in transmission.

Outside of medical facilities, some outbreak reports related to indoor crowded spaces have suggested the possibility of aerosol transmission, combined with droplet transmission, for example, during choir practice, in restaurants or in fitness classes. In these events, short-range aerosol transmission, particularly in specific indoor locations, such as crowded and inadequately ventilated spaces over a prolonged period of time with infected persons cannot be ruled out. However, the detailed investigations of these clusters suggest that droplet and fomite transmission could also explain human-to-human transmission within these clusters. Further, the close contact environments of these clusters may have facilitated transmission from a small number of cases to many other people (e.g., superspreading event), especially if hand hygiene was not performed and masks were not used when physical distancing was not maintained.

- **Fomite transmission**

Respiratory secretions or droplets expelled by infected individuals can contaminate surfaces and objects, creating fomites (contaminated surfaces). Viable SARS-CoV-2 virus and/or RNA detected by RT-PCR can be found on those surfaces for periods ranging from hours to days, depending on the ambient environment (including temperature and humidity) and the type of surface, in particular at high concentration in health care facilities where COVID-19 patients were being treated. Therefore, transmission may also occur indirectly through touching surfaces in the immediate environment or objects contaminated with virus from an infected person (e.g. stethoscope or thermometer), followed by touching the mouth, nose, or eyes.

Despite consistent evidence as to SARS-CoV-2 contamination of surfaces and the survival of the virus on certain surfaces, there are no specific reports which have directly demonstrated fomite transmission. People who come into contact with potentially infectious surfaces often also have close contact with the infectious person, making the distinction between respiratory droplet and fomite transmission difficult to discern. However, fomite transmission is considered a likely mode of transmission for SARS-CoV-2, given consistent findings about environmental contamination in the vicinity of infected cases and the fact that other coronaviruses and respiratory viruses can transmit this way.

- **Other modes of transmission**

SARS-CoV-2 RNA has also been detected in other biological samples, including the urine and feces of some patients. One study found viable SARS-CoV-2 in the urine of one patient. Three studies have cultured SARS-CoV-2 from stool specimens. To date, however, there have been no published reports of transmission of SARS-CoV-2 through feces or urine.

Some studies have reported detection of SARS-CoV-2 RNA, in either plasma or serum, and the virus can replicate in blood cells. However, the role of bloodborne transmission remains uncertain; and low viral titres in plasma and serum suggest that the risk of transmission through this route may be low. Currently, there is no evidence for intrauterine transmission of SARS-CoV-2 from infected pregnant women to their fetuses, although data remain limited. WHO has recently published a scientific brief on breastfeeding and COVID-19. This brief explains that viral RNA fragments have been found by RT-PCR testing in a few breast milk samples of mothers infected with SARS-CoV-2, but studies investigating whether the virus could be isolated, have found no viable virus. Transmission of SARS-CoV-2 from mother to child would necessitate replicative and infectious virus in breast milk being able to reach target sites in the infant and also to overcome infant defence systems. WHO recommends that mothers with suspected or confirmed COVID-19 should be encouraged to initiate or continue to breastfeed.

Evidence to date shows that SARS-CoV-2 is most closely related to known beta coronaviruses in bats; the role of an intermediate host in facilitating transmission in the earliest known human cases remains unclear. In addition to investigations on the possible intermediate host(s) of SARS-CoV-2, there are also a number of studies underway to better understand susceptibility of SARS-CoV-2 in different animal species. Current evidence suggests that humans infected with SARS-CoV-2 can infect other mammals, including dogs, cats, and farmed mink. However, it remains unclear if these infected mammals pose a significant risk for transmission to humans.

Pathogenicity:

The genome of SARS-CoV-2 is comprised of approximately 29 kilobases of single-stranded positive-sense RNA. The envelope surrounding the genome consists of four structural proteins: (1) a membrane protein (M), (2) an envelope protein (E), (3) a nucleoprotein, and (4) a spike protein (S). Spike glycoprotein is a very important component because it is responsible for the entry of the virus into the host cell. The pathogenicity with its underlying clinical manifestations is discussed in detail.

- **Lung pathogenesis**

Upon exposure of a healthy person to a SARS-CoV-2 infected person, viral particles reach the superficial epithelium of the nasal cavity. The viral spike glycoprotein of SARS-CoV-2 binds to the target cells by ACE2, present on the epithelial cells of the nasal cavity. The entry of the virus into the epithelial cells is facilitated by transmembrane serine protease 2 (TMPRSS2), which helps in the fusion of the membrane. Children have a low-level expression of this ACE2 receptor, which explains the reason for the lower severity of the disease in children. From nasal epithelial cells, the pathway by which it enters into lower respiratory tract is not much clear. But there are two proposed theories. According to the first theory, microaspiration of virus particles leads to the spread of the virus to the lower respiratory tract. The other theory is the direct entry of virus particles into the lower

respiratory tract by bypassing the nasal cavity. Neuropilin-1 is abundantly expressed in the respiratory and olfactory epithelium and enhances the SARS-CoV-2 infectivity. Once the viral genome enters the host cell, its genes replicate, undergo transcription, and form new virions. After reaching the lower respiratory tract, the virus attaches to the alveolar epithelial cells through ACE2 receptors present on them. Type-2 alveolar epithelial cells are more involved than type-1. SARS-CoV-2 has a unique polybasic S1/S2 protease cleavage site and an insertion of tetra-peptide SPRR, which increases cleavage efficiency. Consequent to the engagement of spike with ACE2, TMPRSS2 present in the host cell membrane cleaves the above site and thus exposing S2 fusion protein, resulting in the fusion of the virus with the host cell membrane. After the membrane fusion, the internalization of viral RNA occurs, resulting in the replication and formation of viral proteins. SARS-CoV-2 nucleocapsid proteins bind with the viral RNA, covered by envelope and membrane proteins resulting in complete virion formation (Fig. 1).

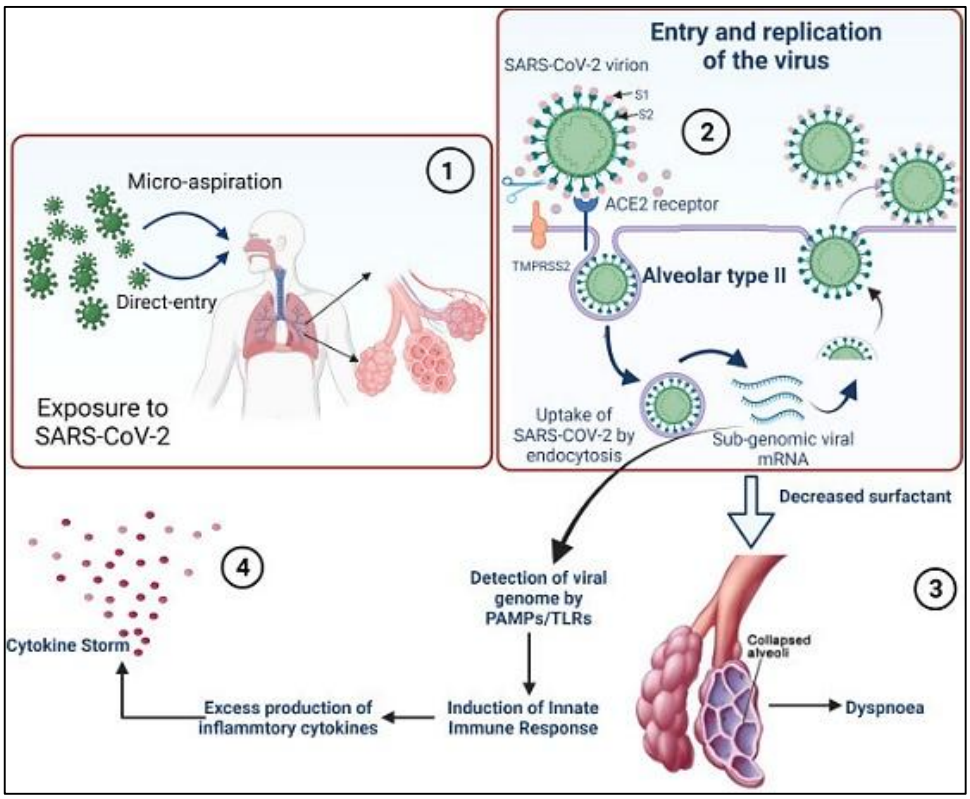


Figure 1: Pathophysiology of severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) induced lung injury. Following inhalation (1), SARS-CoV-2 infects type-2 alveolar cells, epithelial cells, and endothelial cells through binding of its spike protein with angiotensin-converting enzyme 2 (ACE2) receptor. The serine protease type-2 transmembrane serine protease (TMPRSS2) promotes viral uptake by cleaving spike. SARS-CoV-2 replicates in the host cells and generates new virions (2). SARS-CoV-2 damages type-2 alveolar cells and reduce surfactant synthesis, thereby increasing surface tension causing the collapse of alveoli (3). Damage-associated molecular pattern (DAMP) released by macrophages and alveolar cells recruit T lymphocytes, monocytes, and neutrophils. These cells secrete an excess number of inflammatory cytokines resulting in a cytokine storm (4).

SARS-CoV-2 damages type-2 alveolar cells, which are essential for surfactant synthesis and repair of damaged tissues. Thus, there will be an increase in the surface tension, causing dyspnoea. In addition, the viral genome and proteins act as a pathogen-associated molecular pattern (PAMP) and stimulate the innate immune system. In the cytoplasm, viral RNA is recognized by cytosolic receptor melanoma differentiation-associated gene 5 (MDA5), the viral RNA receptor retinoic-acid inducible gene I (RIG-I), and nucleotidyl transferase cyclic GMP-AMP synthase (cGAS). In the endosome, viral RNA is recognized by endosomal TLRs, leading to activation of downstream cascades, resulting in cytokine production, especially type-1I interferon (IFN-1). Other cytokines such as IL-1, IL-2, IL-4, IL-7, IL-10, IL-12, IL-13, IL-17, macrophage colony-stimulating factor (MCSF), granulocyte-colony stimulating factor (G-CSF), MCP-1, macrophage inflammatory protein-1 alpha (MIP-1α), interferon gamma-induced protein-10 (IP-10), IFN-γ, TNF-α, and hepatocyte growth factor (HGF) levels also increase during COVID-19 infection, causing massive a cytokine storm and thus worsening the patient's condition. Monocytes release proinflammatory cytokines responsible for pneumocyte apoptosis. Macrophages release chemokines and cytokines, which increase capillary permeability and also cause the recruitment of neutrophils. Excessive degranulation of neutrophils causes permanent damage to pneumocytes breaking the alveolar–capillary barrier. The end result of all these mechanisms is a transmigration of blood proteins resulting in alveolar and interstitial edema.

The common symptom in COVID-19 patients is hypoxia, if worsened, may lead to acute respiratory distress syndrome (ARDS). An unusual phenomenon seen in COVID patients is silent hypoxemia. The patients with silent hypoxemia experience no or mild respiratory discomfort and dyspnoea despite critically low partial pressure of oxygen (PaO_2). Lung fibrosis is a complication of ARDS which occurs in severe COVID-19 patients. It occurs due to profibrotic factors like transforming growth factor-beta (TGF-beta) released from the damaged lung tissues. Usually, it promotes tissue repair and causes resolution of infection-induced damage. But in severely affected patients, excessive secretion of TGF-beta and epithelial-to-mesenchymal transition and endothelial-to-mesenchymal transition results in fibrosis of the lung.

- **Hematological manifestations**

The hematological manifestations in COVID-19 patients include both thrombotic and bleeding disorders. Disseminated-intravascular coagulation (DIC) has been noted in COVID-19 patients. There are two types of DIC (1) enhanced fibrinolytic DIC and (2) suppressed fibrinolytic DIC. In enhanced fibrinolytic DIC, the thrombus is formed and is broken by fibrinolytic activation. D-Dimer is an important marker in the determination of the prognosis of COVID-19 patients. It is formed as a result of the degradation of fibrin polymer by plasmin. In suppressed fibrinolytic DIC, the thrombus is not broken down, resulting in only a mild increase in D-Dimer levels. One of the mechanisms responsible for thrombotic manifestations is endothelial cell disruption. Inflammatory cytokines

released due to SARS-CoV-2 infection causes endothelial activation, which also causes thrombosis. Damaged endothelial cells and/or activated endothelial cells release Weibel-Palade granules containing ultra-large molecular-weight multimers of von Willebrand factor (vWF). These ultra-large granules spontaneously bind to the platelets and cause microthrombosis. Usually, these large granules are broken down by ADAMTS13, a vWF-cleaving protease. But the activity of ADAMTS13 is reduced in COVID-19 patients. The elevated levels of vWF and increased clotting activity of factor-VIII are due to the acute phase reaction, resulting in thrombosis.

Complement activation is another mechanism responsible for hematological manifestations observed in COVID-19 patients. The complement pathway mediates during mild-to-moderate inflammation, leading to the removal of harmful agents and thus helping in tissue regeneration. Complement activation and cytokine production are interlinked to each other. Low complement levels inhibit cytokine production; however, uncontrolled action of complement pathway results in hyperinflammation and much collateral damage, which include excess production of cytokines by macrophages. The antimicrobial activity of neutrophils gets reduced, and endothelial cells produce tissue factor which increases thrombosis resulting in DIC. Adding to these, SARS-CoV-2 can increase the transcription of complement (C1r, C1s, C3) and coagulation genes (fibrinogen) in the lungs and in the liver.

COVID-19 patients present with various forms of thrombosis, including retiform purpura with extensive deposition of C3d, C4d, MAC, and viral spike protein (Lo et al., 2020). Moreover, SARS-CoV-2 nucleocapsid (N) protein has been shown to directly activate the complement pathway. It can increase mannose-binding lectin (MBL)-associated serine protease-2 (MASP-2)-dependent complement activation. Ficolin-2 expression gets upregulated in monocyte-derived macrophages, which further increases the MBL pathway of complement activation. This results in degranulation and increased production of cytokines, increased production of reactive oxygen species and eicosanoids. Owing to the membrane activation complex (MAC)-mediated cell death, damage-associated molecular patterns (DAMPs) are released, which acts as positive feedback and further increases complement production. The complement and coagulation pathways are interlinked in two ways. MAC and MASP2 can cleave prothrombin. Factor 10a, plasmin, thrombin can cleave C3. In this way, they together interact and cause an increase in thrombosis and thus explain inflammation around retiform purpura in COVID-19 patients.

- **Cardiovascular manifestations**

The frequent cardiovascular complications observed in COVID-19 patients are myocardial infarction, heart failure, venous-thromboembolic episodes, and palpitations. Acute myocardial infarction is due to atherosclerotic plaque rupture due to inflammation and hyperstability. Some of the COVID-19 patients experience palpitations which may be due to hypoxia, abnormal metabolism, and inflammatory stress. The pathology involved

in heart failure is not known, but it is most likely due to the exacerbation of previously undiagnosed heart failure. So, immune system dysregulation, hypercoagulability, increased metabolic demand are some causes of cardiovascular diseases in COVID-19 patients.

- **Kidney and excretory system**

COVID-19 patients with preexisting renal diseases have a poor prognosis and have high mortality. So, understanding the pathogenesis of kidney involvement and treating it is very much essential to reduce mortality in COVID-19 patients. In COVID-19 patients, acute kidney injury increases the severity of the disease, resulting in increased mortality and morbidity in the patients. Kidney damage involves tubular damage, impaired glomerular filtration, proteinuria and is associated with elevated levels of serum urea and serum creatinine. The virus can gain entry through ACE2 receptors present in proximal kidney tubules, making tubular damage more common. Inflammatory cytokines and hypoxia also aggravate sepsis-induced acute kidney injury (AKI). Rhabdomyolysis caused due to further hypoxia increases the damage to the kidney. Diarrhea caused due to GI injury leads to dehydration which may also play some role in damaging kidney function Fig. 2.

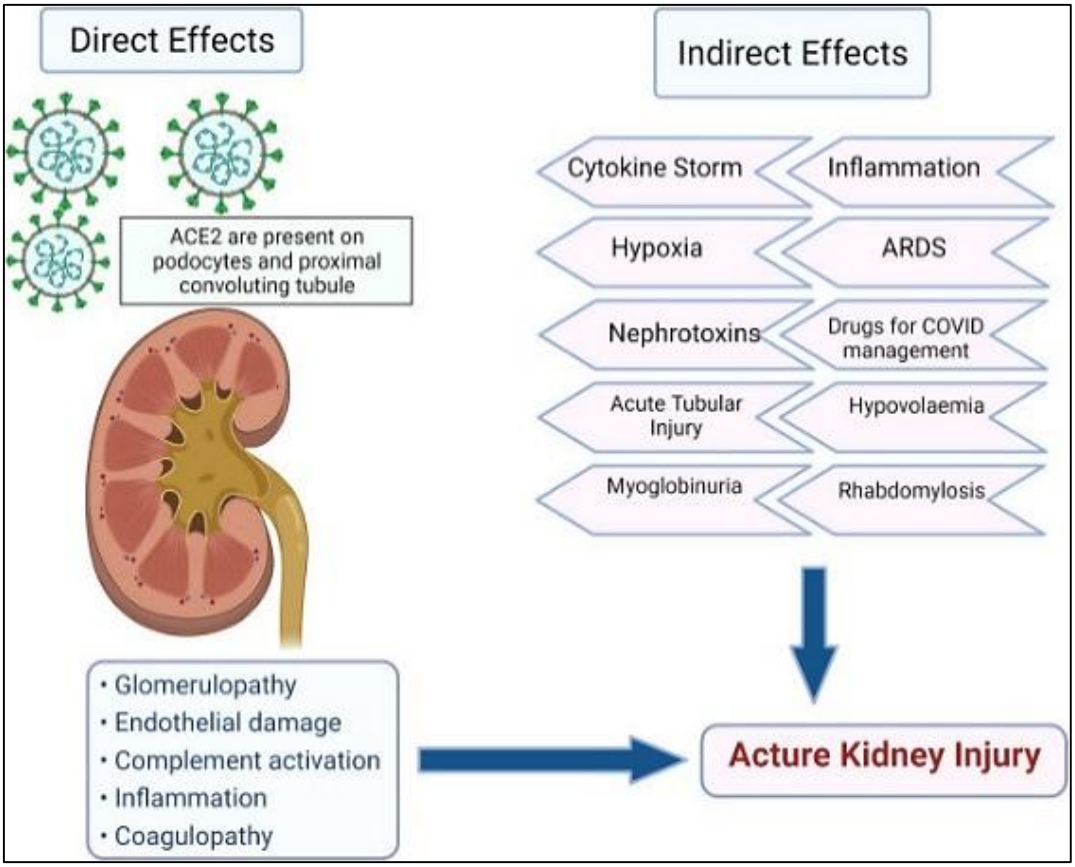


Figure 2: Pathophysiology of renal injury by severe acute respiratory syndrome-Coronavirus-2. Acute renal injury in Coronavirus disease-2019 patients involves tubular

damage, impaired glomerular filtration, and myoglobinuria. The virus can directly cause harm to the kidney by binding to angiotensin-converting enzyme 2 present in proximal renal tubules. Inflammatory cytokines, rhabdomyolysis, nephrotoxins, and hypoxia also aggravate acute kidney injury.

- **Neurological manifestations**

The neurological manifestations in COVID-19 can be divided into two groups: central nervous system (CNS) manifestations and peripheral nervous system (PNS) manifestations. The symptoms in CNS manifestations include headache and dizziness, and PNS include dysgeusia and hyposmia. One of the most common symptoms experienced by COVID-19 patients is anosmia. ACE2 and TMPRSS2 are expressed in vascular pericytes of the olfactory bulb as well as in the olfactory neuroepithelium. SARS-CoV-2 binds ACE2 present on the olfactory bulb; thus, via olfactory nerves, it spreads into the CNS through the cribriform plate, thus causing anosmia. Another way of transmission is the retrograde pathway through trigeminal and vagus nerves and also by entry of infected leucocytes through the blood–brain barrier (BBB) into the CNS, causing anosmia.

BBB is involved in many pathophysiological mechanisms resulting in neurological manifestations of COVID-19. ACE2 is expressed in varying levels on the vascular endothelium of BBB, which gets infected by SARS-CoV-2 triggers proinflammatory and procoagulable states and leads to vasculitis. SARS-CoV-2 binding to ACE2 receptors present in sympathoadrenal systems may disrupt the autoregulation of systemic and intracranial blood pressure, thus causing dysregulation of blood pressure. Systemic inflammatory responses also affect the integrity of BBB. Blood vessel-associated microglia maintain the integrity of BBB through tight junction proteins. Astrocytes help in the maintenance of BBB endothelium via posttranslational modifications of occludin, a component of tight junction proteins. Microglia gets activated by a systemic inflammatory response and thus disrupts the BBB, which further leads to increased neuro-inflammation and hyperexcitability, resulting in seizures, functional disturbance, fatigue, encephalopathy, and neuronal death. Severe hypoxia due to respiratory complications cause cerebral vasodilation and interstitial edema. Headache experienced by COVID-19 patients is due to the invasion of trigeminal nerve endings directly by the virus in the nasal cavity. Elevated levels of proinflammatory cytokines also affect the perivascular nerve endings, resulting in headaches.

- **Psychiatric manifestations**

The SARS-CoV-2 infection also results in psychiatric manifestations like psychosis, posttraumatic stress disorder, and even suicide in some patients. Sleep deprivation and feeling excessive fear are some of the reasons for depression and posttraumatic stress disorder in these patients. The psychiatric symptoms may be due to a direct neurotropic effect or may be due to a systemic immune-inflammatory response. The proinflammatory cytokines, including TNF-alpha, IL-1, IL-6 produced by cytokine storm, exert their effect

on indoleamine-2, 3-dioxygenase. This enzyme is responsible for the degradation of tryptophan which is important for the synthesis of serotonin, a mood elevator. The increase in the activity of this enzyme results in depletion of serotonin causing depressive symptoms and other psychiatric manifestations. Furthermore, prolonged stay of COVID-19 in the intensive care unit causes psychosis, delirium in some patients.

- **Dermatological manifestations**

Common dermatological manifestations observed in COVID-19 patients are maculopapular eruptions, acral areas of erythema with vesicles and pustules, urticarial lesions, vesicular eruptions, livedo or necrosis. The pathophysiological mechanism behind dermatological manifestations is the cytokine storm, which stimulates the dermal dendritic cells, macrophages, neutrophils, lymphocytes and promotes erythema, urticarial lesions, and eruptions. ACE2 receptors are also present in the basal layer of the epidermis and in the endothelial cells of dermal blood vessels, which form the target site for the action of the virus and result in acantholysis and dyskeratosis.

- **Reproductive system manifestations**

ACE2 receptors and Ang 2 are widely expressed on all the components of the female reproductive system, including the ovary, uterus, and vagina. ACE2 and Ang 1 regulate follicle development, ovulation, luteal angiogenesis, and degeneration. Ang 1 is an endogenous ligand for the G protein-coupled receptor Mas and specifically inhibits Ang 2 by the antagonism of AT1 receptors. Thus, SARS-CoV-2 affects ovarian tissue, granulosa cells and affects ovarian function, oocyte viability, resulting in infertility and miscarriage. It also damages endometrial cells and thus distressing the implantation of the embryo. In pregnant women, it causes vascular mal-perfusion of the placental bed, chorangio-hemangioma, and intervillous fibrin deposition. Male gender serves as a risk factor for COVID-19.

- **Gastrointestinal and hepatic manifestations**

The most common gastrointestinal (GI) symptom observed in COVID-19 patients is diarrhoea. Other symptoms include nausea, vomiting, abdominal discomfort, abdominal pain, and dysgeusia. The symptoms related to the involvement of the liver mostly include a change in the laboratory parameters. These include elevated levels of serum aspartate aminotransferase, alanine aminotransferase, bilirubin, prolonged prothrombin time, and elevated lactate dehydrogenase. Some of the symptoms are thought to be due to the interaction between CNS and the gut. The proinflammatory cytokines cause alteration in the gut-CNS axis of the vagal nerve or through the lymphatic or vascular system. The lateral hypothalamic nuclei control nausea and vomiting, and if it is affected due to neurological damage, it may also become the cause of vomiting and diarrhoea. ACE2

receptor is abundantly expressed in the GI tract, including the tongue, esophagus, stomach, ileum, rectum, and possess mucosa. The gastric acid secreted in the stomach kills the virus, but still, the virus finds its way into the duodenum and distal ileum, showing its effect. Once the virus gains entry into the cells, it divides rapidly and proliferates, resulting in cytopathic changes causing viral cytopathic effects. The cytokine storm seen in COVID-19 patients increases IL-2, IL-7, GM-CSF, TNF-alpha, which causes alteration in the GI motility and also plays a role in alteration of GI flora resulting in diarrhoea. The liver is much less affected in COVID-19 patients because it does not express many ACE2 receptors. But the cholangiocytes and biliary epithelium have more of these receptors, making them a potential site for the virus to act. The virus gains access into the biliary system through the portal vein. This way, it can directly cause hepatocytes' cytopathic effect, resulting in micro vesicular steatosis.

Prevention:

The WHO has stated that education, isolation, prevention, controlling the transmission, and treatment of infected persons are the critical steps in controlling contagious diseases like COVID-19. It is possible to minimize the spread of infection by making the following recommendations.

Staying at home (home quarantine) and avoiding any direct contact with any healthy (possible asymptomatic patients) or infected person, which has been called shielding; avoiding nonessential travel; observing social distancing rules like avoiding crowded public places and maintaining at least two meters of distance between each person, especially if they are coughing or sneezing; avoiding shaking hands when greeting others; frequently washing hands for at least 20 s with soap and water or hand sanitizer with at least 60% alcohol, especially after touching common surface areas, using the bathroom, or shaking hands, avoiding touching eyes, nose, and mouth with unwashed hands; and disinfecting surfaces using household sprays or wipes.

It should be mentioned that due to the long incubation period and presence of asymptomatic patients, using a medical mask (especially N95) or a respirator (especially FFP3) could be recommended. Also, sterilizing the used respirator, only reusing it for a limited time, and proper disposal of the used masks, have been recommended. Although respirators (the protective classes, including FFP1, FFP2, and FFP3) are produced as single-use items, they could be used again for a limited time unless there is a risk for contamination through the deposition of infectious particles on the surface. When the respirator becomes soiled or wet with bodily fluids or it can no longer be appropriately fitted, or if breathing via the respirator becomes difficult, it should be discarded. Also, masks should be discarded after being used during an aerosol-generating procedure (AGP). SARS-CoV-2 remains viable in the environment, including on the surface of different materials like cardboard, iron, or tissue for some time. This suggests that there is a risk for rapid contamination of the outer surface of respirators and surgical masks. Contamination of the respirator surface could be prevented through placing a medical

mask over it, or wearing a face shield that can be cleaned. Because of the severe contamination of respirators and surgical masks in the COVID-19 pandemic, several methods could be considered for the sterilization of used masks, including steam, hydrogen peroxide, or radiation.

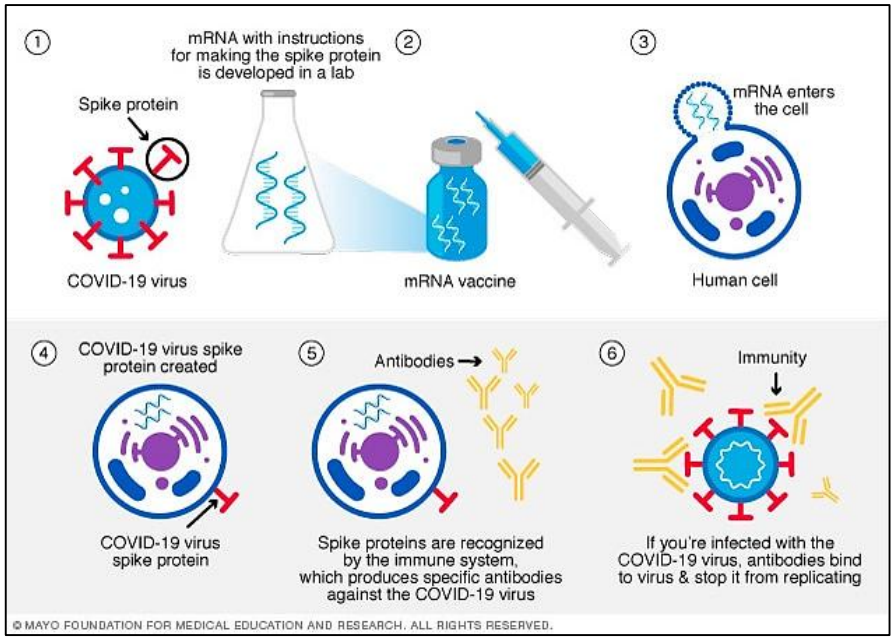
Besides, the use of medical shields or wearing protective suits is recommended, especially for health care workers. It should be mentioned that wearing gloves in public is not an adequate protection against COVID-19, because gloves can easily be contaminated. So, frequent washing of hands is the best way to protect against SARS-CoV-2 infection.

✓ Types of COVID-19 Vaccines:

Each COVID-19 vaccine causes the immune system to create antibodies to fight COVID-19. COVID-19 vaccines use a harmless version of a spikelike structure on the surface of the COVID-19 virus called an S protein.

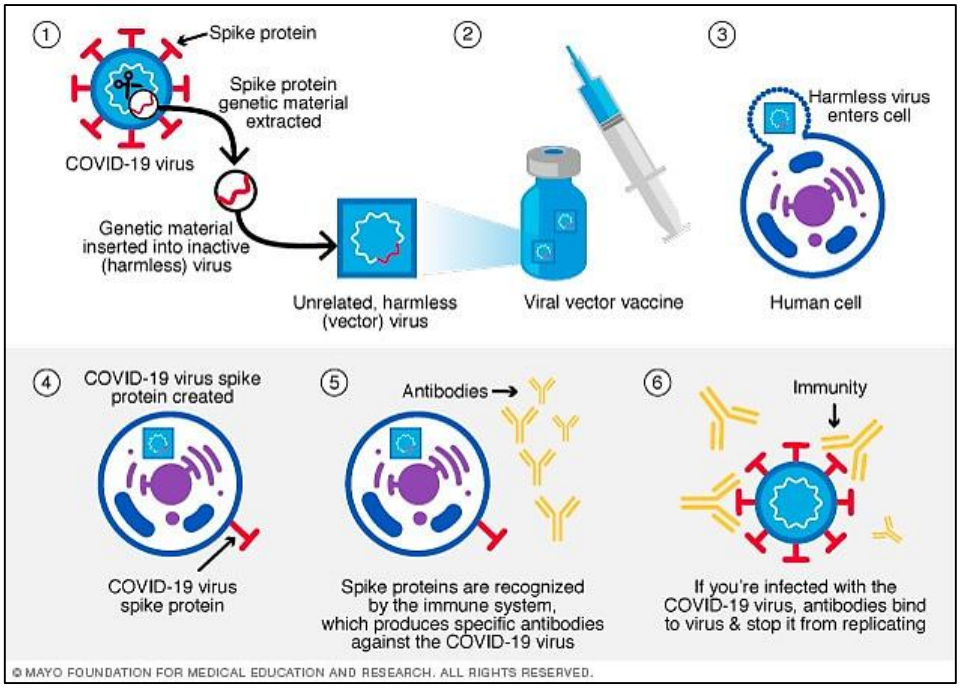
The main types of COVID-19 vaccines currently available in the U.S. or being studied include:

- **Messenger RNA (mRNA) vaccine.** This type of vaccine gives instructions to cells for how to make the S protein found on the surface of the COVID-19 virus. After vaccination, muscle cells begin making the S protein pieces and displaying them on cell surfaces. This causes the body to create antibodies which will protect the individual from future infection. The mRNA in the vaccine doesn't enter the nucleus of the cell, where DNA is kept. Both the Pfizer-BioNTech and the Moderna COVID-19 vaccines use mRNA.

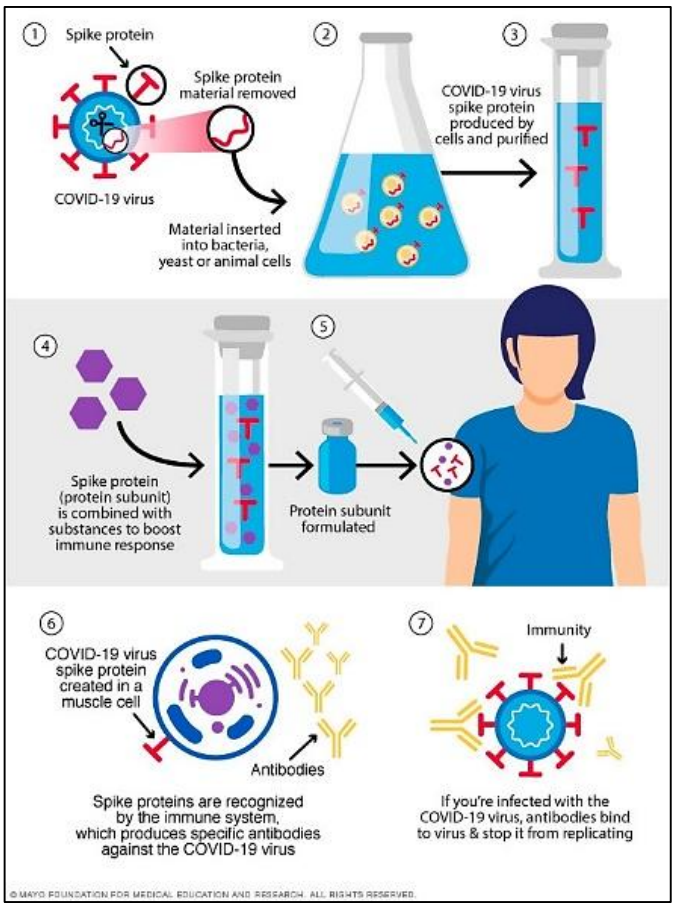


- **Vector vaccine.** In this type of vaccine, material from the COVID-19 virus is placed in a modified version of a different virus (viral vector). The viral vector gives

instructions to cells to make copies of the COVID-19 S protein. Once cells display the S proteins on their surfaces, immune system responds by creating antibodies and defensive white blood cells which will protect the individual from future infection. The Janssen/Johnson & Johnson COVID-19 vaccine is a vector vaccine. AstraZeneca and the University of Oxford also have a vector COVID-19 vaccine.



- **Protein subunit vaccine.** Subunit vaccines include only the parts of a virus that best stimulate the immune system. This type of COVID-19 vaccine contains harmless S proteins. Once immune system recognizes the S proteins, it creates antibodies and defensive white blood cells which will protect the individual from future infection. The Novavax COVID-19 vaccine is a protein subunit vaccine.



Concept of herd immunity:

The development of herd immunity is an important way of gaining protection from contagious diseases such as smallpox, measles, mumps, polio, and COVID-19. Once achieved, herd immunity can provide some level of protection even to non-vaccinated individuals primarily because of the significantly reduced chance of disease spreading within the community.

In other words, for a population where a considerable number of people are already immune, herd immunity can protect the vulnerable portion of the population by reducing the chance of physical contact between an infected person and a vulnerable person.

To develop herd immunity against a highly contagious disease, about 70% to 90% of a population needs to be immune. This is believed to be the threshold for herd immunity. However, depending on the severity of infection, the herd immunity threshold can be as low as 40%.

How is herd immunity developed?

There are two ways to develop herd immunity: vaccination and previous infection.

i. Vaccination

Vaccination is the best way to develop herd immunity, as it can break the chain of infection. The level of herd immunity-based indirect protection is directly proportional to the number of vaccinated people within a community. In this way, it is possible to protect individuals who cannot be vaccinated, such as new-borns, pregnant women, or immunocompromised patients (organ transplant patients; cancer patients receiving chemotherapy).

However, the indirect protection achieved through vaccine-based herd immunity can vary from one geographic location to another as it depends on geographically variable factors such as vaccine efficacy and coverage.

There are some disadvantages to developing vaccine-based herd immunity. For some vaccines, the efficacy reduces over time, and people who fail to receive the booster dose may lose the benefits. Moreover, people who do not complete the entire course of a vaccine may remain unprotected against vaccine-preventable diseases.

In a population with a lower vaccination rate, outbreaks of vaccine-preventable diseases may occur. One of the main reasons for such outbreaks is the lack of herd immunity. Moreover, people who do not believe in vaccination frequently live in the same community, leading to a drastic reduction in the percentage of vaccinated people in that community.

If the percentage drops below the threshold of herd immunity, exposure to infectious disease can lead to the rapid spreading of the disease within the community.

ii. Previous infection

Another way of developing herd immunity is having a previous infection. In this case, herd immunity can be achieved when a large portion of a population has been infected with and recovered from a contagious disease and subsequently has developed antibodies against the disease-causing pathogen.

For instance, people who recovered from the deadly influenza pandemic in 1918 eventually became resistant to the H1N1 influenza A virus.

However, the development of herd immunity through community infection can be risky if the antibodies developed against a pathogen fail to provide long-term protection. For example, studies have found that people previously infected with common coronaviruses can be re-infected with the same viruses after months or years. In the case of COVID-19, recent studies suggest previous infection with a novel coronavirus protects a person from future infection for only a limited time of around five months.

Probable questions:

1. What are the probable routes of transmission for SARS-COV 2?
2. What do you mean by aerosols? Differentiate aerosols and fomite.
3. Describe the molecular events that lead to alveolar damage due to SARS-COV 2 infection.
4. How SARS-COV 2 virus interacts with the immune cells of the human body?
5. Can home quarantine prevent SARS-COV 2 infection?
6. What are the types of vaccines available in the market to prevent SARS-COV 2 infection?
7. Elucidate herd immunity.

Suggested reading:

1. Microbiology an introduction. Gerard JT, Berdell RF and Christine LC. Pearson Education. (Latest edition)
2. Essentials of microbiology. Amita J & Parul J. Elsevier (Latest edition).
3. Text book of microbiology and immunology. Parija SC. Elsevier (Latest edition).
4. Text book of microbiology. Ananthanarayan R and Paniker CKJ. Universities press.

DISCLAIMER: This Self Learning Material (SLM) has been compiled from various authentic books, Journals articles, e-journals and other web sources.