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RESEARCH AND REVIEWS IN ANIMAL SCIENCE

VOLUME I

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Research and Reviews in Animal Science Volume I

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PREFACE

In the ever-evolving tapestry of scientific inquiry, the field of Animal Science stands as a cornerstone, where the intricacies of the animal kingdom are explored, understood, and celebrated. As we embark on this journey through the pages of "Research and Reviews in Animal Science," we are poised to delve into a realm where curiosity meets discovery, where questions find their answers, and where the pulse of innovation beats vibrantly.

This compendium represents not just a collection of scholarly works, but a testament to the collective efforts of passionate researchers, scholars, and practitioners who dedicate their intellect and expertise to unraveling the mysteries that surround the diverse species with which we share our planet. Within these pages, readers will encounter a rich tapestry of studies, analyses, and insights that span the breadth and depth of Animal Science.

From the intricacies of animal behavior to the dynamics of livestock production systems, from the exploration of nutritional needs to the pursuit of sustainable practices, the topics encapsulated within this volume are as diverse as the creatures they seek to understand. Each chapter represents a thread in the fabric of knowledge, woven together to form a comprehensive mosaic of understanding.

As we navigate the terrain of contemporary research and review in Animal Science, it is imperative to acknowledge the tireless efforts of the contributors whose dedication fuels the advancement of our understanding. Their commitment to excellence, coupled with their relentless pursuit of truth, serves as a guiding light illuminating the path toward scientific enlightenment.

Moreover, in an era marked by unprecedented global challenges, the insights contained within these pages hold profound implications for the well-being of both animals and humans alike. Whether addressing issues of animal welfare, public health, or environmental sustainability, the research delineated herein serves as a catalyst for positive change, inspiring action and advocacy in pursuit of a more harmonious coexistence with the natural world.

As we embark on this intellectual odyssey, I extend my deepest gratitude to the authors, editors, and reviewers whose contributions have shaped this volume into a beacon of knowledge and discovery. May the insights contained within these pages spark curiosity, provoke contemplation, and inspire a renewed commitment to the pursuit of truth.

With anticipation and reverence for the boundless wonders of the animal kingdom, let us embark upon this voyage of exploration, guided by the spirit of inquiry and the pursuit of enlightenment.

Editors

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A NOTE ON FIRST SIGHTING RECORD OF ASIAN PALM CIVET AT SALEKASA OF GONDIA DISTRICT, MAHARASHTRA

S. D. Puri

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Abstract:

Total five individuals including one adult female and four puppies of Asian Palm Civet in live condition were observed evidently on 14th August 2023 in the laboratory of Shankarlal Agrawal Science College, Salekasa of Gondia district, Maharashtra State, India. This animal was resting at the corner of the laboratory of college in the darkness. The Asian Palm Civet Paradoxurus hermaphroditus also known as Common Palm Civet and locally called as Ud Manjar or Masnya Ud in Maharashtra. As the Civet was observed unexpectedly in the laboratory of Shankarlal Agrawal Science College, Salekasa that was in aggressive mood; rescue operation done by the expert team of forest department of Salekasa. At that time, the author taken the photographs, videos and notes of the Civets. That was the first sighting record for this area of Salekasa tehsil in Gondia district of Maharashtra State, India.

Keywords: First sighting record, Asian Palm Civet, Salekasa, Gondia district, Maharashtra.

Introduction:

The Asian Palm Civet *Paradoxurus hermaphroditus* also known as Common Palm Civet and locally called as *Ud Manjar or Masnya Ud* in Maharashtra. This animal feeds generally at night and it is believed that its fear for predators during daytime is the reason for its nocturnal habitat. Hence, it is generally seen during night time and at the places where having darkness. This animal is omnivorous as eats small insects, frogs, small snakes, house lizards and some small creatures. Also, they eat the fruits and flowers of palms, mango, chiku and coffee in their natural habitats.

It has a wide spread distribution from central to south-eastern Asia, in India this species has been recorded as far North as the Narmada river along with Himalayan hills, lower Bengal Sikkim, Assam and North east India. In some locations of Bali Island which was very suitable for coffee plants, so civets reside there because they can easily find a source of food from these coffee plantations (Winaya *et al.,* 2020). Total 11 civets with six subspecies were recorded live or dead in videos or photographs during field visits at Bharatpur, Chitwan in Nepal (Chaudhary,

2021). But in Maharashtra it is occurred rarely in some districts having some records of the occurrence of this species in Maharashtra State. Chunekar *et al*. (2018) were reported coat colour variations in Common Palm Civet based on the observations from Karnataka and Maharashtra States, India. Very few individuals were also observed in the forest of Nawegaon National Park in Gondia district of Maharashtra State. Evidently one individual of female Asian Palm Civet with her four puppies were cited in the laboratory of Shankarlal Agrawal Science College, Salekasa in the district Gondia of Maharashtra State. This was the first record of sighting of Asian Palm Civet for this area.

This note describes the first sighting of the Asian Palm Civet from Salekasa of Gondia district, Maharashtra State, India.

Materials and Methods:

The study was accomplished by direct observation method in the laboratory at 1st floor of building of Shankarlal Agrawal Science College, Salekasa (21.305627, 80. 482753) in Gondia district of Maharashtra State as the Civets seen unexpectedly and evidently.

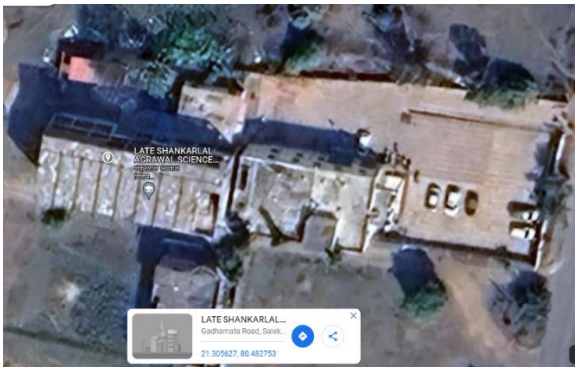

Figure 1: Satellite view of study area

Figure 2: A view of study area (1st floor)

The normal camera of mobile of Vivo Y50 which was available at that time of model number Vivo 1935 of 13 Megapixel was used to take photographs and videos of the Civets. The observation data sheets, pen, pencil, eraser, etc. was used for taking the notes of the Civet

(Chaudhary, 2021). As the Civet was observed unexpectedly in the laboratory of Shankarlal Agrawal Science College, Salekasa that was in aggressive mood. Hence, we called the expert team of the Forest Department, Salekasa to capture the Civets. The expert team caught the Civets from the laboratory by using their techniques and skills. They caught one female adult individual of Asian Palm Civet in the Laboratory and one puppy Civet outside the laboratory. At that time, the author taken the photographs, videos and notes of the Civets. That was the first sighting record for this area of Salekasa tehsil in Gondia district of Maharashtra State. Other three puppies ran away from this area. Later on the female adult and one puppy of Asian Palm Civet were abandoned in the forest by the expert team.

Details of the sighting

One individual of adult female Asian Palm Civet was sighted on 14th August 2023 near about 02:00 p.m. unusually and evidently with her four puppies while walking in the laboratory of Shankarlal Agrawal Science College, Salekasa. This animal was resting at the corner of the laboratory of college in the darkness. As this species generally not seen during the daytime, hence it was at the dark place of the laboratory. This animal may be living or resting in the dark corner place of the laboratory where waste materials kept since many days. It seems that a few months or days ago this animal entered in the laboratory through window or through a hole of the wall as there was a tree close to the wall of the laboratory. It was concluded that as she was with four puppies, she was pregnant and delivered these four puppies in the darkness of the laboratory. Hence, this species of Asian Palm Civet was occurred and sighted first time in the laboratory of the college of Salekasa tehsil of Gondia district, Maharashtra State, India.

Field characters and behaviour

Asian Palm Civet has stocky body covered with coarse shaggy hairs usually greyish in colour. It has a white mask across the forehead, a small white patch under each eye, a white spot on each side of the nostrils, and a narrow dark line between the eyes. The morphological study of dead adult Civet was carried out which was accidentally died. The neck girth, abdominal girth, heart girth were 26cm, 33cm and 34.5cm, respectively and the body length was 67cm (Choudhury *et al*., 2015). The Asian Palm Civet is thought to lead a solitary lifestyle, except for brief periods during mating. It is both terrestrial and arboreal, showing a nocturnal activity pattern with peaks between late evening until after midnight. The behavioral sequences of seven palm civets were observed using a contingency table representing the six behavioural states viz., resting, feeding, comfort behaviour, social behaviour, sniffing behaviour and locomotion (Krishnakumar *et al*., 2002). Scent marking behaviour and olfactory response to various excretions such as urine, faeces, and secretion of the perineal gland differs in males and females.

Results:

Total five individuals including one adult female and four puppies of Asian palm civet in live condition were observed in the laboratory of Shankarlal Agrawal Science College, Salekasa of Gondia district, Maharashtra State, India (Table-1).

Table 1: Number of individuals of Asian Palm Civet observed at study area

Sr. No.	Scientific Name	Common Name	Local Name	Recorded stage	Sex	Recorded numbers
1	*Paradoxurus hermaphroditus*	Asian Palm Civet or Common Palm Civet	*Ud Manjar* or *Masnya Ud*	Adult	Female	1
2	*Paradoxurus hermaphroditus*	Asian Palm Civet or Common Palm Civet	*Ud Manjar* or *Masnya Ud*	Puppy	Not known	1
3	*Paradoxurus hermaphroditus*	Asian Palm Civet or Common Palm Civet	*Ud Manjar* or *Masnya Ud*	Puppy	Not known	1
4	*Paradoxurus hermaphroditus*	Asian Palm Civet or Common Palm Civet	*Ud Manjar* or *Masnya Ud*	Puppy	Not known	1
5	*Paradoxurus hermaphroditus*	Asian Palm Civet or Common Palm Civet	*Ud Manjar* or *Masnya Ud*	Puppy	Not known	1

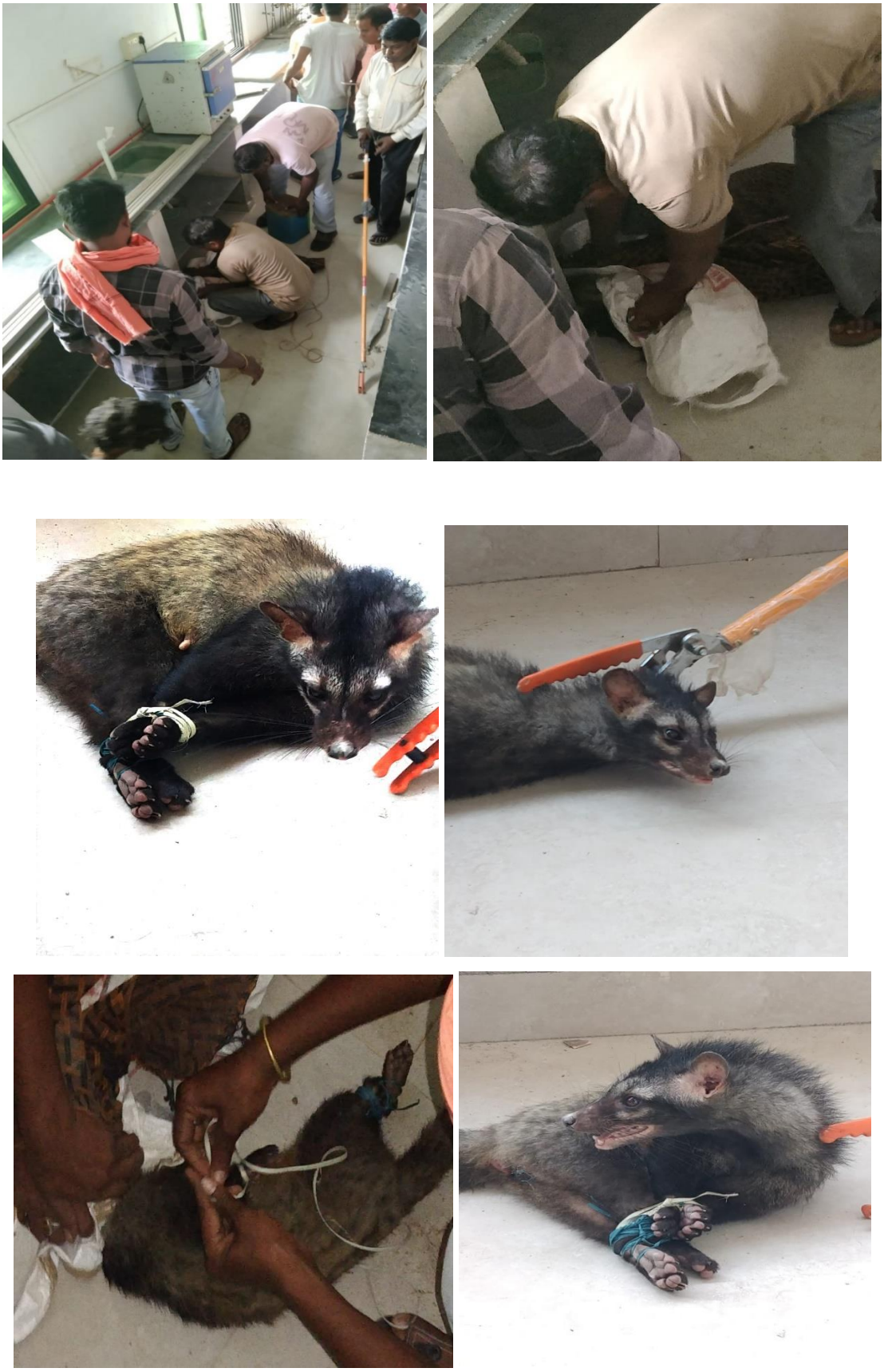

Figure 3: Photographs of Asian Palm Civet (At the time of Rescue)

Recommendations:

As this omnivore animal feeds on small prey and fruits and defecates viable seeds, making it a potential seed disperser. Every living organism is the part of the ecosystem, hence, there is need to conserve the Asian Palm Civet. As this animal is very rare in this area, therefore, the plantation of fruit bearing plants near the study area and other urban areas is highly recommended in addition to developing awareness in conservation of the Asian Palm Civet at the community level. Some people doing excessive hunting of this Civet, hence it is also protected under Wild Life Act 1972. Some Civets may die in the rescued cases, hence proper medical care should be provided immediately at the time of rescue.

Acknowledgement:

Author is special thankful to the expert team of the forest department of Salekasa and the Principal of Shankarlal Agrawal Science College, Salekasa for their cooperation and help. Also, the author is very much thankful to the researchers whose research articles were cited for this chapter.

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REVIEW ON ANTIMICROBIAL ACTIVITY OF FISH PROBIOTICS SUPPLEMENTS

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Abstract:

Probiotics are live bacteria that give the host physiological advantages when administered in appropriate doses. Different probiotic bacteria groups exist. Prebiotics are characterized as nondigestible food components that selectively promote the growth or activity of specific bacteria in the colon to produce beneficial impacts on the host. Lactic acid bacteria (LAB) create compounds known as Bacteriocin-like inhibitory substances (BLIS). Peptides or active peptide complexes known as bacteriocins and bacteriocin-like inhibitory substances (BLIS) typically show antibacterial (bactericidal or bacteriostatic) actions on closely related species. future studies using multiomic approaches will concentrate on the structural and functional characterization of molecules involved in the recorded research work. In the end, this should be a better alternative to the use of antibiotics.

Keywords: Probiotics, Lactic acid bacteria, Bacteriocins, Antimicrobial activity.

Introduction:

Probiotics are live bacteria that give the host physiological advantages when administered in appropriate doses. Different probiotic bacteria groups exist. Prebiotics are characterized as nondigestible food components that selectively promote the growth or activity of specific bacteria in the colon to produce beneficial impacts on the host. This definition was initially laid out in the Journal of Nutrition in 1995 by Gibson, Roberfroid. While it is a basic definition, many people have modified it. Prebiotics usually include fructooligosaccharides; additional carbohydrates, such as nonstarch polysaccharides, plant wall polysaccharides, and pectins, can also be prebiotic agents but generally classified as dietary fiber. Therefore, not all fiber is prebiotic, and not all prebiotics are made up of from fibers, nevertheless, both prebiotics and fiber share the characteristic of not being able to be broken down by human enzymes. Instead, the intestine's microbiota metabolizes and breaks them down (Gibson *et al.*, 1995; Dhingra *et al.*, 2011).

Serious biological and ecological issues have been raised by the prevalent and systematic use of antibiotics, particularly with relation to the emergence of antibiotic resistance. Beneficial microbes, or probiotics, are being recommended as an effective and environmentally conscious antibiotic substitute. Although they were initially used in aquaculture species over three decades ago, major focus was not given to them until the early year 2000. Probiotics can be potentially living or dead, or they can even be a part of the microorganisms that function in many ways to help the host or its surroundings. Numerous probiotics, most of which are of host origin, have been identified and employed in fish (Silvaa *et al.,* 2020).

In contrast to certain disease control measures which are being developed and utilized in the aquaculture sector, whereby evaluates are unilateral, Probiotics have tremendous potential due to the various ways in which they benefit both the host fish and the environment in which they are grown. Probiotics have been the subject of a staggering amount of items in aquaculture, which highlights their many benefits and places them prominent in the never-ending search for healthier substitutes for farmed fish. An update on probiotic utilization in finfish aquaculture is given in this publication, with special attention to their mechanisms of action. The current understanding of their nutritional and geographical competitiveness, inhibitory metabolites, ability to affect the environment, immunomodulatory potential, and stress-alleviating mechanism is studied. This timely give an opportunity to future researchers will wish to work upon probiotic research and development while emphasizing the role they play to promote sustainable aquaculture strategies.

Proper nutrition is inherently linked to proper development and effective immune responses. Thus, research has demonstrated that the microbiota plays an important role for healthy growth and pathogen defense in both humans and animals (Dawood *et al.,* 2019).

The microbiota of many species is greatly influenced by the conditions of aquatic environments, and many of these creatures are potential sources of microbes of biotechnological interest. In order to determine the probiotic profile and antimicrobial properties. efficacy of bacterial strains obtained from aquatic habitats against fish and food diseases, bioprospected the strains.

Lactic acid bacteria (LAB) create compounds known as Bacteriocin-like inhibitory substances (BLIS). Peptides or active peptide complexes known as bacteriocins and bacteriocin-like inhibitory substances (BLIS) typically show antibacterial (bactericidal or bacteriostatic) actions on closely related species.

Role of BLIS (Bacteriocin-like inhibitory substances)

Amin, Muhamad, *et al.* have clearly indicates that the foodborne pathogen was shown to be antagonistically active against three of the fifty-two lactic acid bacillus. Of these three, strain MA115 (>400 AU) produced the isolate with the highest inhibitory activity. Strain MA115 exhibited 99% similarity to *Enterococcus faecium* based on its 16S rDNA sequence, Additional in vitro tests demonstrated the antilisterial compound's high Proteinase K sensitivity, indicating that it may be a Bacteriocin-like inhibitory substance. Furthermore, the Bacteriocin-like inhibitory substances demonstrated resistance variable pH range viz. 2–10, as well as extreme temperatures ranging from 30 to -121°C and 4 to -20°C. Furthermore, the Bacteriocin-like inhibitory substances demonstrated bactericidal activity against *Listeria monocytogenes* in salmon fillets that were intentionally contaminated and kept at 0°C. Finaly he shows that while more research is needed to precisely identify the bacteriocin-like inhibitory substances for biopreservation reasons, the bacteriocin-like inhibitory substances produced by Enterococcus faecium strain MA115 has the potential to be applied to improve food safety (Amin *et al.,* 2023).

Isolation of various probiotics strains showing antimicrobial activity

Wanka *et al*. have been shows that *Tenacibaculum maritimum* exhibited antagonistic activity against thirteen isolates (7.4%). Psychrobacter genus isolates from wild turbot comprised four of these isolates, from farmed turbot, seven isolates exhibiting antagonistic action were identified as Acinetobacter heamolyticus. Lastly, two strains of wild European flounder were identified that had antagonistic action and were closely linked to Enterovibrio calviensis. None of these isolates showed any antagonistic action toward *Edwardsiella tarda* or *Listonella anguillarum* (Wanka *et al*., 2018).

Gutiérrez Falcón *et al*. have been shows that out of the meagre and seabass intestinal gut and gill samples, 122 bacterial strains were identified. However, only three of these strains exhibited inhibitory action against at least one pathogenic strain tested. Table 3 displays the strains' MALDI-TOF mass spectrometry system identification as well as their antagonistic effect profiles on growth against various Ph. damselae subsp. piscicida strains. All of the studied strains of Ph. damselae subsp. piscicida is inhibited by the bacterium Alcaligenes faecalis subsp. faecalis -1. On the other hand, only one pathogenic strain, Ph. damselae subsp. piscicida EP04, was shown to be antagonistically affected by the bacteria *Alc. faecalis subsp.* faecalis -2 and *Pseudomonas viridiflava* (Gutiérrez Falcón *et al*., 2021).

Tatsuro Hagi *et al*. have focused upon probiotic LAB which was been isolated from *Cyprinus carpio* gut region. further the results obtained from present investigation shows seasonally, prospective probiotic LAB from the adult common carp gut was screened for

aquaculture. From the most common LAB in the summer and winter, respectively, lactococcus lactis h2 and lactococcus raffinolactis h47 were chosen because they exhibit strong antibacterial activity against fish pathogens and cholic acid tolerance. The strain of Enterococcus pseudoavium h50 that exhibited the highest level of antibacterial activity after a year of isolation was also chosen.

Strongly antibacterial Streptococcus iniae I1 was chosen from the dominating LAB in the intestine of juvenile common carp. Seasonal direct screening for LAB resistant to cholic acid was also conducted. After testing the isolates' antibacterial activity, Lactobacillus fuchuensis K11 was chosen from the summer isolates. Five potential strains were also chosen from the winter samples. The degrees of the candidates' levels of antibacterial activity and cholic acid resistance matched or exceeded those of the matching type strains. Every contender expanded throughout a broad range of temperatures.

Probiotics, particularly lactic acid bacteria, have been utilized as dietary supplements in recent years to shield fish against a variety of illnesses. Here, we looked at Lactobacillus rhamnosus' ability to shield tilapia *Oreochromis niloticus* from an experimental *Edwardsiella tarda* infection. Fish fed with probiotics had a considerably reduced cumulative mortality rate than fish in the control group. Probioticsupplemented fish showed earlier and more pronounced pyogranulomatous responses in a histological assessment than did the control fish. When pyogranuloma-participating cells were subjected to immunohistochemistry with an anti-*E. tarda* antibody, more positive signals were seen, suggesting an improved capacity for phagocytolysis. In comparison to the control group, the probiotic groups exhibited considerably greater alternative complement activity. These findings imply that *L. rhamnosus* improved the fish's alternative complement system (Hagi *et al.*, 2004).

Methodology for antimicrobial study

The susceptibility of isolates to a few high-consumption and clinically significant antibiotics was assessed using the disc diffusion method. Isolates were streaked across the solidified MRS medium for this purpose, and antibiotic disks were then placed on top of the medium and incubated for the entire night at 37°C. Using a digital caliper, the inhibition zone diameters surrounding the disks can be measured at the conclusion.

Conclusion:

There was a pattern in the ability of the possibly probiotic bacteria to provide antimicrobial effects that were particular to each strain and pathogen. In order to draw the conclusion that the presence of bactericidal proteins in probiotics can lead to the development of antimicrobial drugs and food supplements for fish to reduce pathogenic load from it, future

studies using multiomic approaches will concentrate on the structural and functional characterization of molecules involved in the recorded research work. In the end, this should be a better alternative to the use of antibiotics.

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ASSESMENT OF PHYSICO-CHEMICAL PARAMETERS OF WATER OF THE ULHAS ESTUARY

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Introduction:

Despite the fact that water is a renewable resource, careless use and poor management of water systems can lead to major issues with water availability and quality. Water is essential to both natural ecosystems and human development. It is required for a variety of activities including drinking, cooking, industrial, agricultural, and recreational use. Water can become contaminated by a variety of chemical or biological mechanisms, rendering it unsafe for drinking and other uses. Water is responsible for around 80% of human ailments. As a result, the availability of potable water is an essential characteristic for increasing longevity by protecting against waterborne infections. The quantity and concentration of contaminants in natural water vary by location. The sorts of impurities/contaminants mostly depend on their sources, such as sewage and industrial waste, plant and animal decomposition, bacterial, algae, and viral proliferation, soil leaching, and the atmosphere in the form of dissolved gasses.

The Ulhas River is a river in the Indian state of Maharashtra. It is located in Thane, Raigad, and Pune districts in that state. Ulhas river estuary (72°55′E,19°N-73°E, 19°15′N). Untreated garbage pollutes water, affecting and altering its physicochemical qualities. The Ulhas River is a significant body of water in Thane District, near Mumbai, Maharashtra, India. It starts in the Sahyadri highlands at Khandala and runs through industrial sectors of cities like Badlapur Ambernath, Ambivli, Ulhasnagar, Kalyan, and Dombivli before reaching the Arabian Sea. Dombivli, located near Mumbai in the Indian state of Maharashtra, has also experienced considerable development (Jadhav *et al.,* 2015). The river recessive form of industrial wastewater (effluents) from the different types of industries e.g. chemical industry textile industry etc. and residential complexes. The harmful and wastewater effluents highly affected the river water physicochemical parameter which includes pH, temperature, salinity, and hardness (Menon *et al.,* 2010)

The water sources in this area are being poisoned at an alarming rate due to the unmanaged and uncontrolled disposal of industrial effluents, hospital discharge, sewage, and

drainage. Furthermore, the demand for freshwater has skyrocketed due to unplanned urbanization, rapid population expansion, agricultural pesticide and fertilizer use, and so on. Water quality gives up-to-date information on the concentrations of various solutes at any given location and time. Its quality standards serve as the foundation for determining whether water is suitable for its intended purposes and for improving existing circumstances (Ftsum Gebreyohannes *et al.*, 2015). The deterioration of water quality has led to the destruction of ecosystem balance, contamination and pollution of ground and surface water resources. Water quality degradation worldwide is due to many anthropogenic activities which release pollutants into the environment thereby having an adverse effect on aquatic ecosystems.

Materials and Methods:

The Ulhas River is a westbound running river that originates on the western slopes of the Sahyadri range in Maharashtra, India, almost 400 meters above mean sea level. Following the westerly winds It enters the Arabian Sea at Vasai (19° 16′ N and 72° 45′ E) after a 100-kilometer journey. In this region, the pre monsoon season lasts from March to May, followed by the southwest monsoon from June to September. The post-monsoon season lasts from October through January.

The water samples for analysis were taken from Three distinct sites along the Ulhas estuary stretch, sites following

Site 1: Durgadi Ganesh Ghat, Durgadi Chowk, Annabhau Sathe Nagar, Kalyan WestA kind of riverfront and (immersion place embarked for Ganesh Idols and Durga Mata Idols hence the name Ganesh Ghat) located near the historical Durgadi Fort. 7429+6C7, Annabhau Sathe Nagar, Khadakpada, Kalyan, Maharashtra 421301, India Lat 19.249861°, Long 73.117158°.

Site 2: Gandhari the location for sampling is situated beside the Gandhari Bridge. 749R+XXP Bapgaon Gaonthan Fence, Kalyan, Maharashtra 421305, India Lat 19.268776°, Long 73.142702°

Site 3: Shahad 7546+X5, Ulhasnagar, Maharashtra 421103, India Long 73.160385°, Lat 19.257539°.

Sample planning, collection and preservation

The pollution research along the Ulhas River lasted six months. Sampling was done on three sides. Water samples were collected in plastic bottles. The bottles were carefully cleaned and washed with distilled water. The bottles were cleaned once again with the water sample that was to be taken. The water sample had entirely filled the bottles. To maintain a constant temperature collected sample was kept in thermocol box along with ice bags and then the sample was transported from the sampling side to the laboratory.

The parameters for analysis of water includes pH, salinity, temperature, TDS, TSS, chlorinity, salinity, electrical conductivity and hardness. The analyss was carried out as per the procedure of standard analytical procedures for water analysis by hydrology project.

pH (Potentiometric method)

Take a 50 mL Water sample in a beaker and measure the pH using a calibrated pH meter.

Salinity and chlorinity

Take 10 ml of the Water sample in the burette and add 4 drops of 10% potassium chromate indicator. This is then titrated against silver nitrate ($AgNO_3$). The endpoint is a colour change from pale yellow to pale pinkish red.

Formula:

1 ml of 1N Ag NO_3 =35.46/1000 g Cl

Chlorinity $$\frac{\text{CBR x 0.144 x 35.46 x 1000 g/kg}}{\text{Volume of sample}}$$

0.144 = normality of $AgNO_3$

35. 46 = eq. wt of

1000 = for converting g to kg

Salinity = 1.80655 x Chloriity

Temperature (Mercury thermometer method)

The immerse thermometer in the sample up to the mark specified by the manufacturer and read the temperature after equilibration

TDS (Total Dissolve Solid)

Take 50 ml of sample water and add it to a porcelain dish And wight it. The water of the sample is heated and evaporated. After the sample is evaporated porcelain dish is transferred from the electrical oven for 60 min at 104 ± 1°C. Then transfer Desiccater and cool down and weigh the porcelain dish and compare the reading.

Formula,

Mg dissolve solid /L=[(A-B) * 1000]/mL sample

A= Weight of porcelain dish + water sample

B=Wight of dry porcelain dish

TSS (Total Suspended Solid)

Dry filter paper for 15 min. in an electric oven at 70°C. weight of Filter paper. Filter the water sample. Transfer the Filter paper to the Electrical oven At 104 ± 1°C for 60 min. Then take out the Filter paper and cool the weight of the filter paper

Formula,

mg dissolve solid /L=[(A-B) * 1000]/mL sample

A= weight of filter paper

B=Weight of dry filter paper

Electrical Conducting Meter (Conductivity Cell Potentiometric Method)

Take a 50 mL Water sample in a beaker and measure the Electrical conductivity using calibrated Electrical conducting Meter

Hardness (EDTA Method)

Take 50 mL of water sample in a conical flask. Add 5-6 drops of Ericrom black T indicator solution, and turn into wine red colour. Titrate against with stander EDTA solution Endpoint is a Colour change from wine red to blue colour

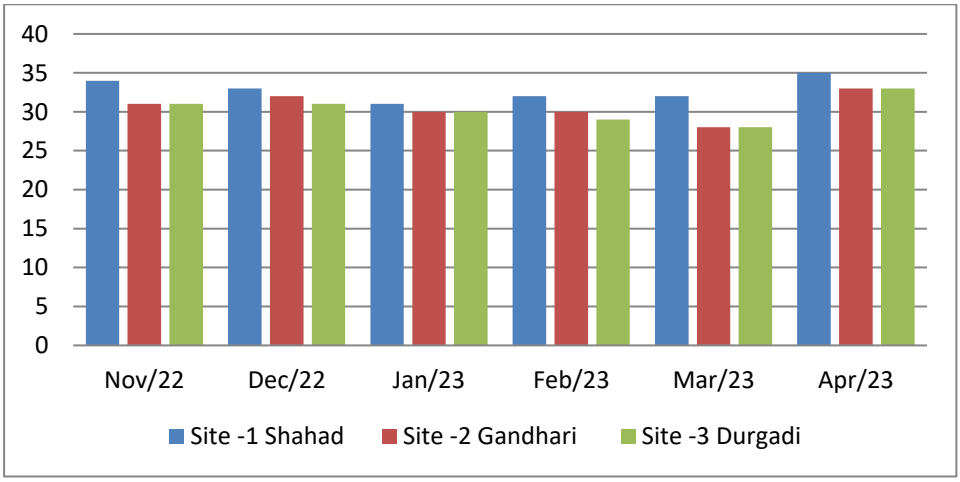
Formula –

Total hardness of water mg/L (CaCO3 Scale) = ml of EDTA used (unboiled) *100/ml of sample.

Results and Discussion:

Temperature

Sr.No.	Sample No	Location	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23
1	Site -1	Shahad	34	33	31	32	32	35
2	Site -2	Gandhari	31	32	30	30	28	33
3	Site -3	Durgadi	31	31	30	29	28	33



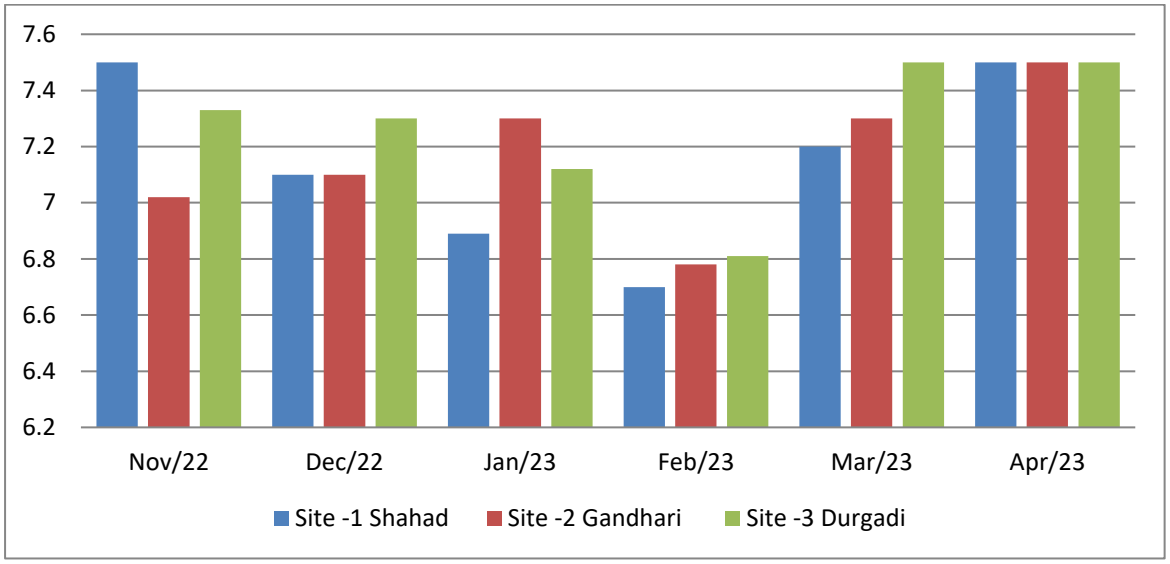
River water temperature plays a fundamental role in shaping the dynamics and health of aquatic ecosystems. It affects physical and chemical processes, influences the distribution and behavior of aquatic organisms, and interacts with climate change to impact the delicate balance of river ecosystems. Understanding the importance of river water temperature is crucial for

effective conservation and management strategies to protect and preserve these vital ecosystems for future generations. This experiment observed heist temperature in all month from site 1 Shahad as compared other two site. Lowest was observed in the month of March from all sites.

pH

River pH is a fundamental parameter that significantly influences the health and diversity of aquatic ecosystems. It serves as an indicator of water quality and provides insights into the well-being of aquatic organisms. By recognizing the importance of river pH and implementing effective monitoring and management practices, we can ensure the preservation and sustainable use of these vital freshwater resources for present and future generations. the study observed pH range neutral to slightly acidic in the all month from all sites. Various natural and human-induced factors can influence the pH of a river. The primary natural factors include geology, soil composition, and the presence of dissolved minerals. Human activities such as industrial pollution, agricultural runoff, and urban development can significantly alter river pH levels.

Sr.No.	Sample No	Location	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23
1	Site -1	Shahad	7.5	7.1	6.89	6.7	7.2	7.5
2	Site -2	Gandhari	7.02	7.1	7.3	6.78	7.3	7.5
3	Site -3	Durgadi	7.33	7.3	7.12	6.81	7.5	7.5

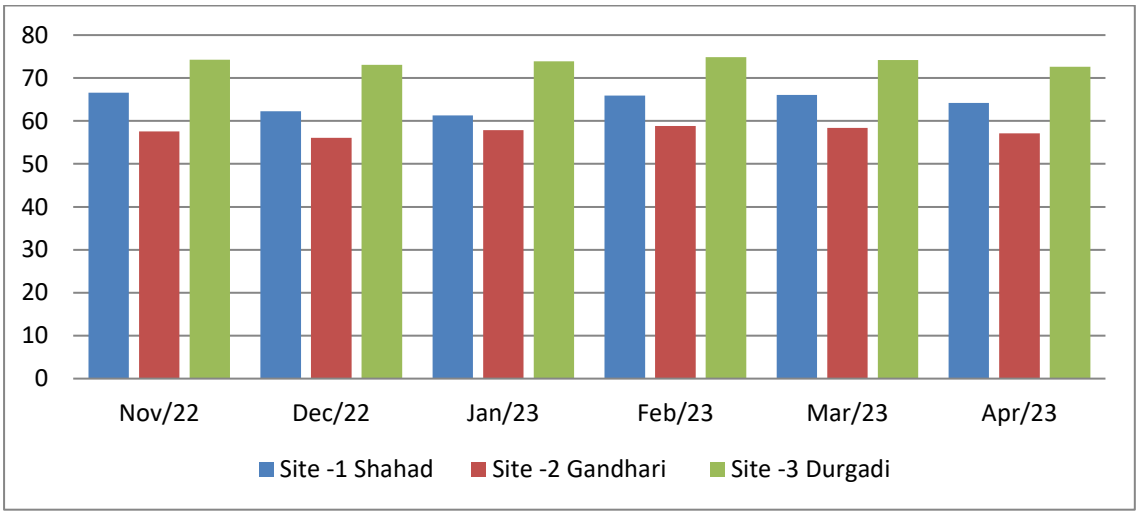


Conductivity

River water conductivity serves as a vital parameter in understanding the quality, health, and sustainability of river ecosystems. Its measurement and monitoring play a crucial role in water quality assessment, environmental management, agriculture, and scientific research. By recognizing the significance of river water conductivity and implementing appropriate measures

to maintain optimal levels, we can ensure the preservation and conservation of our precious freshwater resources for generations to come. This experiment observed highest conductivity from side 3 durgadi in all month as compared to other two sites .and site 1 shahad observed maximum and gandhari is minimum value of water conductivity in all months.

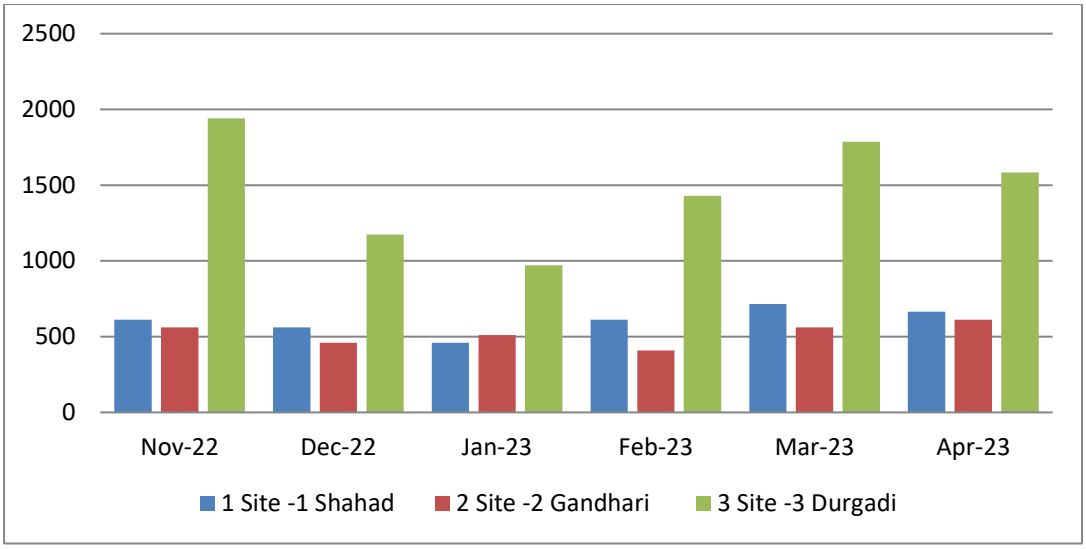
Sr.No.	Sample No	Location	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23
1	Site -1	Shahad	66.6	62.3	61.3	65.9	66.1	64.2
2	Site -2	Gandhari	57.6	56.1	57.9	58.8	58.4	57.1
3	Site -3	Durgadi	74.3	73.1	73.9	74.9	74.2	72.6



Chlorinity

River water chlorinity is a crucial parameter for assessing water quality, understanding ecosystem health, and managing human impacts on aquatic environments. It serves as an indicator of pollution, affects aquatic life and biodiversity, influences drinking water quality, and has implications for agriculture. Regular monitoring and management of chlorinity levels are essential to protect the health of rivers, ecosystems, and human populations. By recognizing the importance of river water chlorinity, we can work towards preserving and restoring the integrity of our freshwater resources for current and future generations. This experiment observed highest Chlorinity from side 3 durgadi in all month as compared to other two sites .and site 1 shahad observed maximum and gandhari is minimum value of water chlorinity in all months.

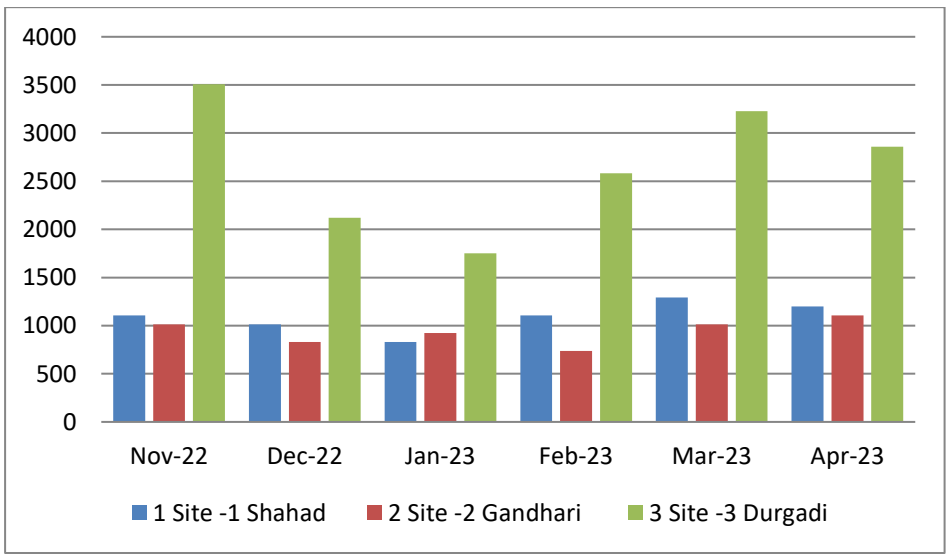
Sr.No.	Sample No	Location	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23
1	Site -1	Shahad	612.74	561.6864	459.5616	612.7488	714.8736	663.8112
2	Site -2	Gandhari	561.68	459.5616	510.624	408.4992	561.6864	612.7488
3	Site -3	Durgadi	1940.37	1174.4352	970.1856	1429.7472	1787.184	1582.9344



Salinity

River water salinity is a significant parameter that impacts both the environment and society. Understanding its role in aquatic ecosystems, water supply, agriculture, infrastructure, and coastal areas is crucial for sustainable water management. By maintaining an appropriate balance of salt content in rivers, we can ensure the health and resilience of ecosystems and support the well-being of communities relying on these vital water resources. This experiment observed highest Salinityt from side 3 durgadi in all month as compared to other two sites .and site 1 shahad observed maximum and gandhari is minimum value of water Salinity in all months

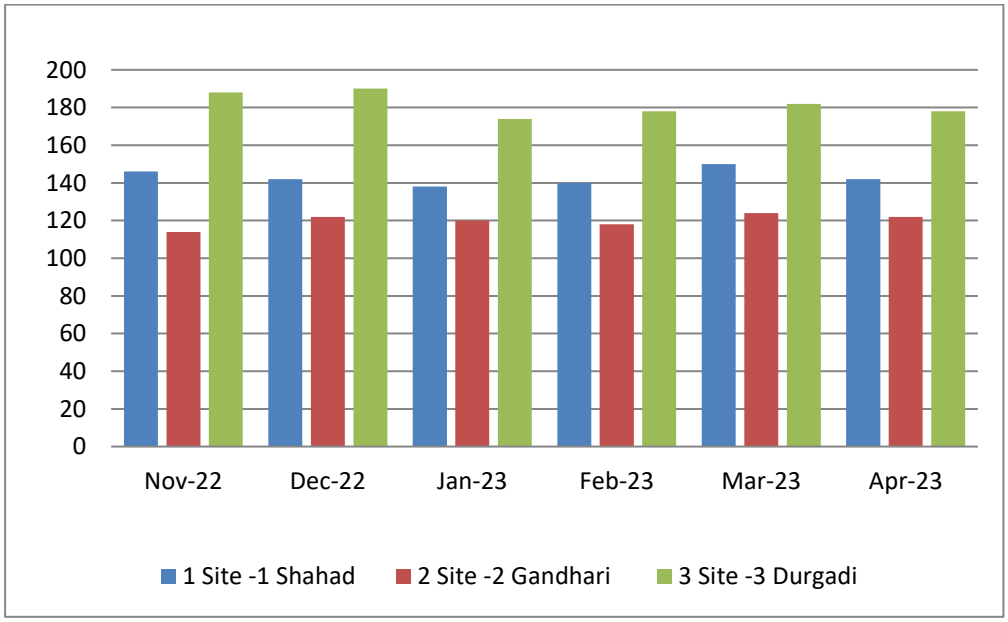
Sr.No.	Sample No	Location	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23
1	Site -1	Shahad	1106.96	1014.71	830.22	1106.96	1291.45	1199.2
2	Site -2	Gandhari	1014.71	830.22	922.46	737.97	1014.71	1106.96
3	Site -3	Durgadi	3505.37	2121.67	1752.68	2582.9	3228.63	2859.65



Hardness

The hardness of river water plays a vital role in the overall health and functioning of ecosystems and influences various human activities. While high hardness levels can present challenges for industrial processes and domestic use, moderate levels are generally beneficial for aquatic life and nutrient availability. Understanding and managing river water hardness are essential for maintaining sustainable water resources and balancing the needs of both ecosystems and human societies. In this experiment side 2 gandhari found lowest value of water hardness in all month and highst value was observed from side 3 durgadi in all month.

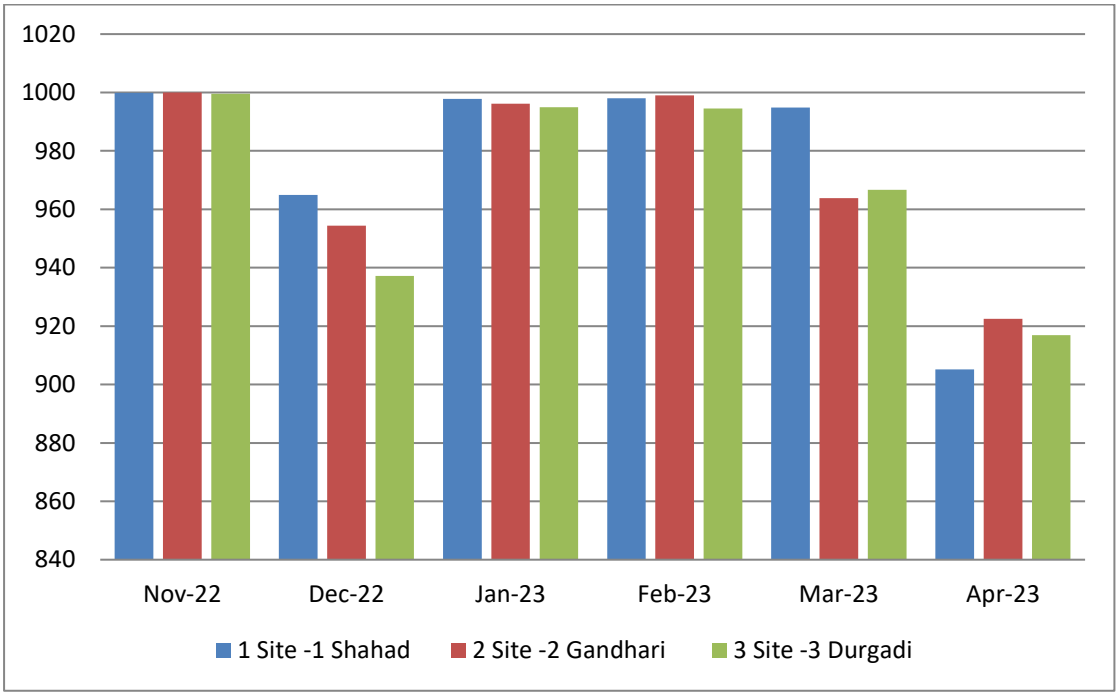
Sr.No.	Sample No	Location	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23
1	Site -1	Shahad	146	142	138	140	150	142
2	Site -2	Gandhari	114	122	120	118	124	122
3	Site -3	Durgadi	188	190	174	178	182	178



Total Dissolved Solid (TDS)

Total Dissolved Solids (TDS) in river water have a significant impact on the health of aquatic ecosystems, water quality, agricultural productivity, and human well-being. Maintaining an optimal TDS range is crucial for preserving the delicate balance of aquatic life, sustaining agricultural practices, and safeguarding public health. Monitoring and managing TDS levels in river water are key steps towards ensuring the availability of clean and sustainable water resources for both present and future generations. This Experiment are observated maximum 999.96 in month of November from side 2 gandhari. And minimum value found from side 1 in month of April as compaird to other month.

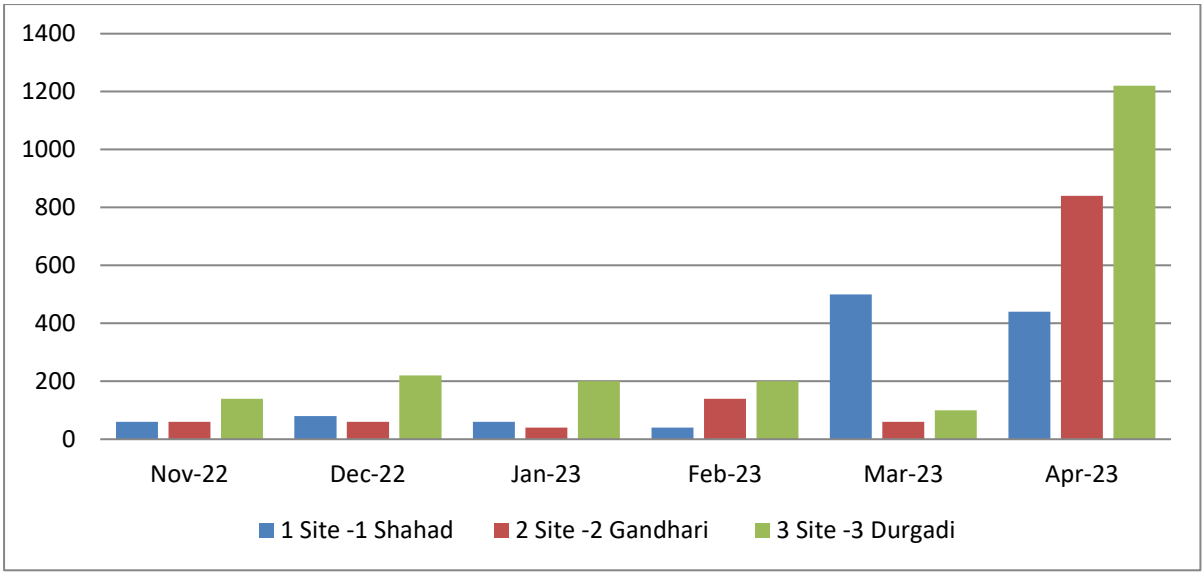
Sr.No.	Sample No	Location	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23
1	Site -1	Shahad	999.84	964.94	997.78	998	994.88	905.16
2	Site -2	Gandhari	999.96	954.44	996.14	998.98	963.86	922.54
3	Site -3	Durgadi	999.58	937.142	995	994.56	966.62	916.92



Total Suspended Solid (TSS)

Total Suspended Solids (TSS) plays a crucial role in river water quality assessment, environmental management, and human well-being. Monitoring TSS levels helps identify pollution sources, assess the health of aquatic ecosystems, and ensure compliance with regulatory standards. By understanding the role and importance of TSS, we can work towards preserving and safeguarding our precious river water resources for future generations.The experiment showed variation in all months. Highst value was observed in all months from site 3 durgadi and side 1 sahad found the highst value in the month of march and April.side 2 was observed lowest value in all months.

Sr.No.	Sample No	Location	Nov-22	Dec-22	Jan-23	Feb-23	Mar-23	Apr-23
1	Site -1	Shahad	60	80	60	40	500	440
2	Site -2	Gandhari	60	60	40	140	60	840
3	Site -3	Durgadi	140	220	200	200	100	1220



Conclusion:

River water temperature plays a fundamental role in shaping the dynamics and health of aquatic ecosystems This experiment observed highest temperature in all months from site 1 Shahad as compared other two sites. The lowest was observed in the month of March from all sites. Because of Site 1 Shahad water depth is low as compared to the Durgadi and Gandhari the study observed a pH range of neutral to slightly acidic for all months from all sites. River pH It serves as an indicator of water quality and provides insights into the well-being of aquatic organisms. River water conductivity serves as a vital parameter in understanding the quality, health, and sustainability of river ecosystems. This experiment observed the highest conductivity from side 3 durgadi in all months as compared to the other two sites. Durgadi is the nearest to human Activities. The study observes Maximum Chlorinity in side 3 Durgadi in all months. And lowest chlorinity was observed on side 2 in Gandhari. It serves as an indicator of pollution, affects aquatic life and biodiversity, influences drinking water quality, and has implications for agriculture. This experiment observed the highest Salinity from side 3 durgadi in all months as compared to the other two sites. evaporation exceeds the influx of fresh river water, there is an increase in salinity River water salinity is a significant parameter that impacts both the environment and society. In this experiment, the highest value was observed from side 3 durgadi in all months as compared to two sides While high hardness levels can present challenges for industrial processes and domestic use, moderate levels are generally beneficial for aquatic life and nutrient availability. This Experiment observed a maximum in the month of November from side 2 Gandhari. And minimum value was found from side 1 in the month of April as compared to other months. Higher TDS intrusion of seawater into their groundwater supply. The highest value Of TSS was observed in all months from site 3 Durgadi. side 2 was

observed lowest value in all months. The suspended particles released from dirt and soil can settle out across the water. Total Suspended Solids (TSS) play a crucial role in river water quality

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METALLOTHIONEIN

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Abstract:

Low molecular weight (6000-7000 Da) protein- metallothioneins (MTs) having metal affinity are reported from almost all the groups of animals, some plants, microorganisms and humans. They have been localized in almost all the tissues viz. liver, kidney, intestine, pancreas, thymus, bone marrow, brain, testes etc of mammals. Commonly found in cell cytoplasm yet nuclear localization has also been observed in cells. Moreover, their concentration in tissues seems to depend upon the tissue metal concentration and developmental stages and is under the control of house keeping genes found on 16th chromosome of human beings and 8th chromosome of mice. Structurally MT is made up of two domains A and B linked by a linker region with capability of binding seven bivalent metal atoms or equivalence of this. Commonly cadmium, copper /zinc are bound though occasionally mercury, platinum, gold and silver may also bind reflecting its ability of forming contrasting thermodynamic stable and kinetic labile metal clusters. Biosynthesis depends upon several nutritional and physiological factors such as metal ions (Cu, Zn, Cd) and hormones (cytokines). Zinc deficiency has been shown to decrease MT concentration whereas induce MT biosynthesis was reported during bacterial infection, physical and inflammatory stress. Scavenging free radicals and excess of reactive heavy metal ions along with supply of copper and zinc for synthesis of metalloprotein and metalloenzyme are one of the several functions performed by MTs. They have been referred to as "metal transfer agents" due to their role in removing or depositing metal from zinc dependent proteins. Metallothioneins too have binding sites on the spermatozoal membrane both specific as well as non-specific, thus playing a biological role.

Introduction:

Metallothionein is a sulphydryl rich, low molecular weight metal binding protein that exists in almost all the tissues and is easily induced by various internal and external stimuli, including glucocorticoids, interferon, interleukin-1, progesterone, vitamin D3, endotoxins, serum factors, heavy metals, and the regulation of cellular zinc storage. MTs were initially detected in

the late 1950s as cadmium binding proteins in equine renal cortex (Margoshes and Vallee, 1957) and thereafter have proven to be of great interest in a variety of scientific fields including biological and physical chemistry, molecular biology, toxicology and oncology. Metallothioneins (MTs) demonstrate a dual role in shaping the destiny of tumor cells. They exert influence over cellular growth and survival through their established metallo-regulatory involvement in repair processes, growth, and differentiation. Additionally, MTs act as guardians against oxidative stress, safeguarding cells from apoptosis induced by free radical (Tan *et al.,* 1999). Occurrence of MT not only, in the animal kingdom, in higher plants, eukaryotic microorganisms and in some prokaryotes (Waalkes, 1996) but also in humans (Miles *et al.*, 2000).

Structure of MT

The characteristic features of metallothionein are its low molecular weight (6000-7000 Da) and its unusual amino acid composition i.e. cysteine which accounts for 30% of the residues although aromatic amino acids are absent (Waalkes, 1996). The cysteine residues along the polypeptide chain are fixed, for example mammalian MT have twenty cysteines, and the 61-residue chain is interspersed with a series of cys-x-cys, cys-cys or cys-x-x-cys sequences where x stands for an amino acid residue other than cysteine. Most amino acid substitutions are conservative, both with respect to the chemical and the space filling properties of the residues (Wlostowski, 1993). All cysteines occur in the reduced form and are coordinated to the metal ions through mercaptide bonds, giving rise to spectroscopic features characteristics of metal - thiolate clusters (Nordberg and Kojima, 1991). The metal content of purified MT is highly variable and depends on organism, tissue and heavy metal exposure.

The twenty cysteines of mammalian MT bind a total seven equivalents of bivalent ions such as Zn, Cd, Hg, Bi, Sn, Co, Ni, Rb or Tc. Higher stoichiometries twelve equivalents were observed with univalent d(10) ions such as Cu(I), Ag(I) and Au(I). MTs have two metal binding domains {-α} (31-61) and {-β} (1-29) with diameters of about 15-20A^0 which contain two metallothiolate clusters (A) and (B) of different structures and with different affinities to individual metals (A - three metal cluster, preference Cu, Zn, Cd; B-four metal cluster preference Cd, Zn, Cu) (Kabzinski 1997) where each metal in coordinated tetrahedrally to cysteines (Waalkes 1996). The sulfhydryl groups, normally involved in metal binding are highly reactive and render the protein particularly sensitive towards oxidation. Oxidation of sulfhydryl groups in MT may occur both *in vivo* and *in vitro*, either intra- or inter-molecularly, the later leading to dimeric or polymeric forms of MT (Hongfang *et al.,* 2020).

2-Dimensional - NMR and crystallographic studies revealed that MTs are flexible molecules undergoing dynamic fluctuations within the clusters and the polypeptide chain. This lack of rigidity is disclosed by the quality of the 113Cd NMR resonances (Otvas *et al.*, 1989) and

other spectroscopic features (Vasak, 1986), as well as by the free accessibility of most peptide hydrogens to D_2O (Messerle *et al.,* 1990) and by the ready reactivity of the buried cysteine side chains with alkylating agents (Bernhard *et al.,* 1986). These features also reveal substantial differences between the two domains. Thus, in the mammalian forms, the amino terminal domain is more loosely structured than the carboxyl terminal domain (Messerle *et al.,* 1990, Bernhard *et al.,* 1986). The high structural flexibility of the MTs has its likely physical basis in the kinetic lability of the many coordination bonds to group IIB metal ions (Me(II)) in the clusters (Carson and Dean 1982). Thus, while thermodynamically stable (Vasak and Kagi, 1983), the Cys-Me(II)-Cys cross - links are to be visualized as undergoing a continuous breaking and reforming of their non-covalent bonds. A patent manifestation of this "fluxional" state is the facial exchange of the metal ions within the cluster (Otvos *et al.,* 1987) , with metal ions in solution and surprisingly, also with metal ions in clusters of other MT molecules (Nettesheim *et al.,* 1985) . This capacity of the metal- thiolate clusters to facilitate inter-molecular metal ion transfer is fundamental to the biological roles of MT. It may represent the mechanistic basis for the chaperoning function that MT was suggested to have in channeling and regulating the flow of essential metals, in particular of zinc, to and from their many sites of action (Otvas *et al.,* 1989). Thus, MT is primarily an elongated, single-chain molecule with a high degree of random structure. MT is also a very heat - stable protein and is stable at low pH characteristics often used in purification or quantitation.

Classification of MT

MTs have been classified on two different basis:

(I) one basis for classification of MTs was Phenotypically related metal thiolate polypeptides (Ebadi and Iversen, 1994). It was further categorized into: (a) Class I single chain polypeptides with location of cysteine residues closely related to those in equine renal MTs eg. mammalian MTs. (b) Class II single chain polypeptides with location of cysteine residues distantly related to those in equine renal MTs such as yeast MT. (c) Class III MTs are often oligomeric structure made up of two or more polypeptide chains of variable length which are atypical non-translational and synthesized metal thiolate polypetides such as cadystin and phytometallothionein or phytochelatin (Robinson, 1989). However Torres et al. (1997) reported that Class III MTs are composed of the repeating dipeptide unit γ -glutamyl cysteine with a single carboxy terminal glycine residue and general structure being $(\gamma\text{-Glu-Cys})_n$ -Gly, where n=2-11 .

Class I and II proteins are encoded by structural genes whereas class III proteins are secondary metabolites not directly encoded by structural genes.

However, in mammals, there are multiple MT genes giving rise to a family of isoforms with highly conserved cysteine rich region (Mahon *et al.,* 1995).The number of isoforms varies

between species and tissues. MT-I and MT-II isoforms are the most widely distributed MT isoforms, being expressed in virtually all the cells (Hidalgo and Carrasco, 1998). Searle et al. (1984) found MT-I and MT-II isoforms in all the organs examined in adult mice but MT-I is about twice as abundant as MT-II. MT-III isoform, also known as a growth inhibitory factor (GIF) and later on characterized as the central nervous system (CNS) specific isoform, possessing an additional seven amino acids, is expressed mostly in brain and to a very minute extent in the intestine and pancreas (Ebadi *et al.,* 1999) whereas MT-IV is expressed only in certain stratified squamous epithelial tissues (Quaife *et al.,* 1994). MT- III has additional glutamate residues which bestow acidic properties to it. On the contrary MT-I and MT-II have alkaline properties. Recently Nothern blot - analysis have demonstrated that MT-II mRNA is present in several organs other than the brain whereas, MT-III mRNA have been detected in the testies, prostate, epididymis, seminal vesicles, ovary, uterus, stomach, heart and tongue of rat (Moffatt and Seguin 1998). Further, the MT-III mRNA levels in the testis, epididymis, prostate and tongue were 22% of those in brain while in ovary, uterus and stomach it was 4% of the brain level being still lower in the other organs. Although, MT-III gene could not be induced by CdCl2 or lipopolysaccharide in the rat testis and prostate but RT-PCR analysis in combination with MT-III specific primer and an anti-MT-III antibody, expression of MT-III has been demonstrated in various organs including testis of rat and mice (Suzuki *et al.,* 1998).

Thus MT-III tissue specific gene expression is broader than previously reported. However, in pregnant mice, the four MT isoforms reported to be expressed in the maternal deciduum (Liang *et al.,* 1996) with genes encoding each isoform being clustered on mouse chromosome 8 and human chromosome 16 and share a similar intron / exon structure (Ebadi *et al.,* 1996).

(II) The basis of another classification of MT was taxonomic parameters and pattern of distribution of cysteine residues along with MT sequence (Binz and Kägi, 1999). Fifteen families of proteinaceous MT was reported under this category. Human MTs were placed in family 1 which expresses four main isoforms- MT1 (with subtypes A,B,E,F,G,H,L,M,X), MT2, MT3 and MT4. Two of them (MT1 and MT2) while MT3 reported in Brain and MT4 in stratified epithelium. Plant MTs were placed in Family 15 , which in 2002 have been further classified by Cobbet and oklsbrough into four types : 1,2,3 and 4 depending on the distribution of their cystein residues and a cystein devoid region referred as spacers , a characteristic feature of plant MTs. . Family 2-7 belongs to different invertebrate MTs and fungal MTs were placed in family 8-13. Family 14 include prokaryotic MT (Binz and Kägi, 1999).

Localization and concentration of MT

In animals, MTs were originally identified in equine kidney cortex but subsequently were reported in parenchymatous tissues such as liver, pancreas and intestine and has a significant role in nutrient homeostasis as well as in the reproductive tract during pregnancy (Dalton *et al.,* 1996). Using immunostaining technique, Nishimura et al. (1991) observed that MT is related mainly to the turn over rate of a tissue eg. the relatively short turnover of the cornea epithelium (3-4.5 days) accounts for stronger MT immunostaining in this tissue than in the epidermis in which keratinocytes are replaced with cells migrating from the basal layer with a turnover of 15-30 days. The intense MT immunostaining has been also demonstrated in spermatogonia and basal cells of seminal vesicles and the ejaculatory duct in rats (Nishimura *et al.,* 1991) as well as in human uroepithelium displaying dysplastic changes or carcinoma (Bahnson *et al.,* 1991).

During cell cycle, MT protein has been localized in the cytoplasm in G1 phase while in the early S phase it has been mainly localized in the cell nuclei. The nuclear localization of MT may be related to the interaction of metal ions like zinc with various nuclear constituents during cell cycle regulation and cell differentiation. This could also modulate gene expression by exchange of Zn binding between MT, histone transcription factors and nuclear acidic proteins (Cherian *et al.,* 1999). Concentration of MT in the nucleus has been reported not only to be energy dependent but also serve as a mean for supplying zinc to this organelle (Woo *et al.,* 1996). Thus, intracellular changes of MT are closely related to cell proliferation (Wlostowski, 1993). Northern analysis demonstrated that metallothionein expression was proportional to dietary zinc intake in some tissues. It was greatest in kidney, followed in descending order by liver, intestine, spleen and heart (Cousins and Lee-Ambrose, 1992). MT although is mainly found intracellularly in many organs but lacks an identifiable signal sequence and is thus not a classical secretory protein. There have been several reports of low levels of MT (ng/ml) in extracellular fluids (DeLisle *et al.,* 1996). Nishimura et al. (1990) reported that MT was localized in the nucleus of rat sperm in the testis by an immunohistochemical study. Under physiological conditions MT is localized mainly in the spermatocytes, spermatids, spermatozoa and Sertoli cells of the adult rats, but not in the interstitial cells (Nishimura *et al.,* 1990). This observation was further substantiated by *in situ* hybridization studies which showed that the level of MT mRNA is maintained at a high level in the mouse spermatid as compared to the mature sperm cells where its level was reported to be low (De *et al.,* 1991). Recently, Sugihara et al. (1999) cloned cDNA by RT-PCR on RNA from testes (2.2 and 1.8 kb in mouse and 2.1 kb in human) that encodes a cysteine -rich 32 kDa, protein with metallothionein- like motif, called as tesmin. It is specifically being expressed as early as on day 8 of postnatal life and this coincides with entry of germ cells into meiosis i.e. spermatocytes stage. The gene has been assigned to mouse

chromosome 19B. Suzuki *et al.* (1994) suggested that epididymal spermatozoa binds MT secreted from the prostate gland at the cell membrane and that MT plays a biological role for spermatozoa. Electron microscopic autoradiographic studies revealed grains of labelled MT mainly in the cell membrane of the head and proximal portion of the tail and partly in nucleus which suggests that MT has both specific and nonspecific binding sites on the spermatozoal membrane. In prostate, MT was found in the cytoplasm of the epithelia especially the supranuclear area, nucleus and secretory products in the lumen. Electron microscopic localization of MT has been reported in the nucleus, nucleolus, rough endoplasmic reticulum, Golgi apparatus, secretory vesicle of the epithelium and secretory products in the lumen. The concentration of MT is highest in the lateral lobe, followed by that in the dorsal lobe and lowest in the ventral lobe (Umeyama *et al.,* 1987). MT in prostatic fluid is secreted by prostatic cells and its concentration in prostatic fluid was lower in acute prostatitis, highest in chronic prostatitis without pyuria and was still higher in benign prostatic hyperplasia than that in normal patients. Acute prostatitis may possibly damage functions of prostatic cells and chronic prostatitis may induce MT synthesis in the prostate. MT content in prostatic fluid may possibly be used as a marker of secretory function in the prostate (Suzuki *et al.,* 1992).

MT is found at strikingly elevated levels in fetal liver and neonatal thymus (Olafson, 1985) -organs which make critical contributions to the development of the immune system. New born rat liver, for example, contains as much as 1.4 mg MT/g liver (Borghesi and Lynes 1996). The presence of MT is detected in rat liver as early as 18^{th} day of gestation and the level of MT is increased to a maximum at birth (Templeton *et al.,* 1985). The high levels of zinc and MT are maintained for about 2-weeks in neonates and then decrease to adult levels at weaning (Asokan and Cherian, 1985). In adult liver, metallothionein is mainly localized in the cytoplasm. However, it has also been localized in the hepatocyte nuclei in human fetal liver and fetal and neonatal rat liver as determined by immunohistochemical staining with a specific MT-antibody. The translocation of MT from the nucleus to the cytoplasm of the hepatocytes has also been demonstrated in rats during postnatal development (Panemangalore *et al.,* 1983). In most human tissues, higher levels of MT were observed compared to the rats. Moreover, human MT levels, particularly in liver and kidney showed greater inter-individual variations eg. the concentration of human hepatic MT varied between 11 and 1000 µg/g. The extraordinarily high levels of MT in human kidney and liver may reflect a different transcriptional control of the protein, the occurrence of multiple subtypes of human MT isoforms or the high exposure of man to environmental metals (Heilmaier *et al.,* 1987).

Placental MT has been localized predominately in the various layer of junctional zone and yolk sac with very little MT being detected in the labyrinthine trophoblasts in both C57BL/6

and MT-I mice placenta (Lau *et al.,* 1998). It is feasible that MT localization in placenta is dependent on the gestational age. Lau *et al.* (1998) also indicated that at late pregnancy the junctional zone is the principal source of MT.

In the central nervous system (CNS), MT-I and MT-II are conspicuously absent from neuronal populations yet abundant in fibrous and protoplasmic astrocytes. MT-III is expressed predominantly in glutamatergic neurons that sequester zinc in synaptic vesicles and that it protects against excessive neuronal stimulation possibly by protecting glutamatergic neurons against damage induced by oxidative stress (Aschner 1998). MT-III is absent from glial elements whereas MT-I and II mRNA and protein levels are particularly abundant within these area. The relative abundance of CNS MT mRNA is as follows: MT-II << MT - III << MT-I (Choudhuri *et al.,* 1995). In 1-3 weeks old Wistar rats intense immunostaining was observed in ependymal cells, choroid piexus, epithelium, arachnoid and pia matter (Nishimura *et al.,* 1992).

Induction of MT synthesis

The most remarkable feature of MT is its inducibility. The vertebrate protein is induced by a wide range of metals (cadmium, zinc, copper, mercury, bismuth) and hormones, as well as by various non-metallic chemicals, physical stress, generation of superoxide and hydroxyl radical on exposure to radiation etc (Ryvolova, 2011) and can serve as biomarker/ indicator in toxicological studies (Sakulsak, 2012). Some of these components are considered to be direct inducers, eg. cadmium, zinc, copper, glucocorticoids, interleukin-1 and interleukin-6, which can elicit MT synthesis response both *in vivo* and in cell culture. By contrast, indirect inducers (eg. endotoxin, radiation, oxidative stress agents, ethanol, iron, chromium and lead) stimulate MT synthesis *in vivo* only. Therefore, in general, three groups of inducers involved in MT synthesis may be distinguished. The first group consists of heavy metals (cadmium, zinc, copper) and glucocorticoids, which after being bound to nuclear factors (trans-acting elements), interact with the specific DNA regulatory sequences (Cis-acting element) in the promoter region, thereby initiating transcription. The second group belongs to hormones and cytokines which act on MT gene expression through membrane receptors and second messengers such as calcium. The third group comprises of different non-metallic compounds, some metals (iron, cadmium, lead) and physical stress that induce MT synthesis probably through cytokines released from inflamed sites (Wlostowski, 1994). MT can not be induced in male genital organs by various stimuli including heavy metals. The low inducibility of MT may possibly be due to the hyper methylation status of the gene in the rat testis (Bhave *et al.,* 1988) since DNA methylation controls MT-I gene expression in murine lymphoid cells (Compere and Palmiter 1981). Contrary to this, large amounts of MT protein (Nishimura *et al.,* 1990) and MT mRNA (Compere and Palmiter, 1981) have been detected in the testis under physiological conditions. Consistent with these

observations transgenic mice carrying the interferon gene under the control of the MT promoter expressed high levels of interferon mRNA in the testes in the absence of an exogenous inducer such as Cd (Iwakura *et al.,* 1988). Although oestrogen, progesterone and testosterone have been reported to be involved in the regulation of MT gene expression (Tohyama *et al.,* 1996), yet the mechanisms by which MT gene expression is regulated in the male genital organs still remains to be studied.

Regulation of gene expression

More than 20 MT genes have been cloned from a wide range of species (38,79) and the sequences of 62 MTs have been published (Kagi, 1993). The expressions of these 20 MT genes are regulated mainly at the transcriptional level (Butt *et al.,* 1984). Transcriptional activation is mediated by cis-acting DNA regulatory sequences in the promoter region of MT genes that have been designated as UASc for yeast cells (85) and metal responsive element (MRE) for vertebrates (Furst *et al.,* 1988) and trans-acting DNA binding transcription factors (Thiele 1992) in response to metals.

The simplest mode of eukaryotic MT gene expression occurs in yeast by the action of a Cu or Ag activated sequence specific DNA-binding proteins (ACE-1) and (AMT1) respectively. These metallated monomeric trans factors rapidly bind to distinct cis-acting regulatory sites within the promoter region, (termed as upstream activation sequence) thereby initiating transcription (Thiele, 1992). However, the regulation of MT gene expression is more complex in higher eukaryotes. The vertebrate MTs can be induced by a variety of metal species such as Cd, Zn, Hg and Cu (Otsuka *et al.,* 1994). Their promoter region contains not only a TATA box preceded by several proximal metal regulatory elements (MREs) with the consensus core sequence TGCA(G)CNC (Stuart *et al.,* 1985), but also a glucocorticoid regulatory element and regulatory DNA sites bind other transcription factors such as AP-1, AP-2, SP-1 (Koizumi *et al.,* 1991). The presence of so many cis- and trans-acting elements may well account for an additive MT production and degree of MT expression is directly related to the number of cis- and trans-acting factors simultaneously being engaged. Some of the DNA-binding metal regulatory protein(s) (referred to as MRP, MRF, MTF-1, MBF-1, MEP-1, ZRF, p39, MafY) which recognizes the MRE(s) acts as a positive transcription factor in the presence of metal (Labbe *et al.,* 1993).

In addition to the well defined transcription factors SPI, USF/MLTF, which interact with the specific sequences on the MT gene, other protein factors that modulate MT expression also have been characterized. MTF 1 is a 70 to 80 kDa polypeptide with 6 zinc fingers (Radtke *et al.,* 1993) that is required for the basal as well as induced transcription of MT gene by heavy metals (Heuchel *et al.,* 1994) and in response to oxidative stress (Dalton *et al.,* 1996). Further, two

nuclear protein factors one from rat liver and other from a rat hepatoma (Aniskovitch and Jacob 1998) that can trans-activate the mouse MT-I promoter were identified. The liver protein, a dimer of a 33 kDa polypeptide, and the tumor protein, a monomer with a molecular size of 28 kDa, interact with the MRE-c' sequence located between - 108 and - 135 bp positions with respect to the +1 site of mouse MT-1 gene, an element that is involved in the basal transcription of the gene (Datta and Jacob, 1994). However, Koizumi *et al.* (1992) indicated that there may also be negative transcription factors eg. MREBP, which lose the affinity to MREs in the presence of higher concentrations of Zn/Cd (Wlostowski, 1994). Another is Ku protein. Ghoshal *et al*. (1998) demonstrated that the cells over expressing the large submit (p80) of Ku do not induce MT-1 whereas MT-1 induction in response to the heavy metals proceeds unabated in the parental rat fibroblast cells or the cells over expressing either the small subunit (p70) of Ku or Ku heterodimer. The specificity of this inhibitory effect of the Ku subunit was demonstrated by the continued induction of another stress-responsive protein, namely, hsp 70. The nuclear run-on experiment has shown that this repression of MT-1 expression in Ku-80 cells is at the level of transcription, and it is specific for MT, as expression of ribosomal RNA and GAPDH remained unaffected. It is possible that the repressor in Ku-80 cells directly interacts with the basal transcription machinery (Ghoshal *et al.,* 1998).

In some circumstances, there is evidence for post transcriptional control of MT gene expression. Inhibition of protein synthesis stabilizes MT mRNA (McCormick *et al.,* 1991) and the MT mRNA half life depends specifically on the inducing metals, thereby suggesting that there is a regulation of MT mRNA turnover. However, there are several observations which suggest that the regulation of MT protein synthesis may also occur at the level of translation. For instance, it has been shown that *in vitro* translation of polyribosomal mRNA from Cd-treated rats did not consistently give greater synthesis of MT protein than that from control rats. In addition, studies on development of rat have shown that after birth, total MT-1 and MT-2 mRNA levels remained high although MT protein levels (Lehman-McKeeman *et al.,* 1988) and MT synthesis rates decreased (Piletz *et al.,* 1983). Furthermore, during the induction of MT by Zn, mRNA levels remained higher than control values for upto 36 hrs, whereas the rate of synthesis of MT remained same as that of control for 24 hrs. (Lehman-McKeeman *et al.,* 1988). Studies on synergistic induction of MT also suggested a possibility for post transcriptional regulation, with mRNA levels again not correlating with protein levels (Lijima *et al.,* 1990).

Metallothionein and heavy metals

Because MT gene expression is metal responsive and MT protein binds metals with high affinity, exhibiting a significant role in metal homeostasis. MT I and II function in metal

metabolism by both regulating the availability of essential metals and sequestering toxic metals (Bremner 1993) by its ability to neutralize the toxic effects of several elements (Sakulsak, 2012).

The metal content of purified MT is highly variable and depends on organism, tissue and history of heavy metal exposure, for example, MT isolated from human liver autopsied samples contained almost exclusively zinc, whereas MT from kidney contained substantial levels of cadmium and copper. These differences probably reflect both the natural heavy metal exposure of the organs and the expression of different isoforms (Ebadi and Iversen, 1994).

Normally MT binds seven atoms of cadmium or zinc to sulphur donors of four cysteine which serves as bridging ligands per molecule or 12 atoms of Copper. A tetrahedral arrangement of four sulfur atoms surrounds each Cd/Zn atom, while a trigonal arrangement surrounds the copper atom. The metals are localized in two polynuclear clusters in two distinct domains, each containing half of the polypeptide chain. The c-terminal or α-domain contains four cadmium or zinc atoms whereas the N-terminal or β-domain contains three atoms of the metals. Each domain also has the potential to bind six copper atoms. Interestingly, copper binding occurs preferentially in the β-domain and cadmium or zinc in the α-domain. Moreover, copper in its cuprous form binds more firmly, than cadmium, which in turn binds more firmly than zinc (Wlostowski, 1993). Krezel and Maret (2007) reported interaction of MT with zinc in Zn_3S_9 and Zn_4S_{11} cluster although affinity for binding varied as observed by log K=11.8 (for four zinc ions), log K=7.7 (for one zinc ion) and log k=10 (for remaining two zinc ions).

Metallothionein is capable of both chelating and donating metals *in vitro*. Apo MT (thionein) can remove zinc atoms from zinc-finger transcription factors resulting in their inactivation or increase in free zinc induces thionein which then is responsible for attaining its function (Maret, 2008), while zinc bound to MT can be donated to apometalloenzymes leading to restoration of functions (Maret, 2008). The binding of zinc to MT is thermodynamically stable which makes MT an ideal zinc reservior *in vivo* (Kang, 2006).

Zinc ingestion induces intestinal MT which represents a redundant mechanism for protection against excess zinc. Cousins (1985) reported that the induction of MT by zinc leads to copper deficiency because MT binds copper with higher affinity than zinc supported by the fact that the dietary copper deficiency induced pancreatic lesions similar to those seen in zinc toxicosis (Rao *et al.,* 1993). This led to the fact that MT may also protect against zinc toxicosis (Kelly *et al.,* 1996). However, in contrast to this, study of Reeves (1998) on MT-null mice suggest that MT induction is not required for the development of low copper status in mice fed a high zinc diet and that the actual mechanism may involve the modulation or inhibition of a Cu transporter protein by Zn. Thus, MT may not be involved in reducing Cu absorption when induced by zinc.

The unique properties of high thermodynamic stability and high kinetic lability of metal binding sites together with the order of affinity of different metals for MT (eg. Hg (II) >> Cd (II) >> Zn (II)) confer on MT a unique ability to "rescue" or "repair" structures that have been compromised by inappropriately binding toxic metals with a greater affinity for MT than zinc (Roesijadi *et al.,* 1998).

In adult rats, zinc deficiency decreases MT concentrations dramatically in the pancreas and modest in the kidney with little effect on the liver or brain (Onosaka and Cherian, 1982). However Giralt *et al.,* (2000) reported significant effect on brain neocortex, CA1-CA3 neuronal layer and dentate gyrus of hippocampus and purkinjee neuronal layer of cerebellum due to zinc deficiency which reflects impairment of brain MT response. In brain because of the presence of blood brain barrier MT-I and -II are induced only when zinc is injected i.c.v. (intra cerebro ventricular injection) (Gasull *et al.,* 1994). Astrocytes, neurons and microglia (Kramer *et al.,* 1996a, 1996b) have been reported to produce more MT-I and II in the presence of zinc, copper or both. The human and mouse MT-III genes contain MREs (Naruse *et al.,* 1994) but it seems that MT-III expression is not affected by zinc in astrocytes (Kramer *et al.,* 1996a) whereas they are slightly decreased in neurons (Kramer *et al.,* 1996b).

Further, Dalton et al. (1996) illustrated that during zinc deficiency, zinc bound to MT represents a bioavailable pool of zinc and that the concentrations of MT decline as the need for zinc increases and pancreatic MT concentrations are remarkably sensitive to dietary zinc and zinc demands during pregnancy. Thus, the pancreatic zinc MT pool is labile and may contribute to zinc efflux pathway. Under conditions of zinc deficiency, pre-existing MT may provide zinc for biological processes, resulting in the gradual depletion of the zinc-MT pool. Zinc lost from the pancreas during zinc deficiency may be released directly into the bloodstream via zinc efflux (Palmiter and Findly, 1995) from the basal surface of acinar cells or more likely, it may be lost via pancreatic secretion (McClain 1990). Therefore, the larger pool of zinc MT (pancreatic MT) provides a biologically important labile pool of zinc during periods of zinc deficiency (Dalton *et al.,* 1996). Maternal zinc supply is a major determinant of MT concentrations in the neonatal rats (Andrews *et al.,* 1987). Zinc deficiency also increases free radical production and reduces MT as a scavenger of superoxide and hydroxyl radicals (Ebadi and Iversen 1994). In animals where zinc is the major and often the sole metallic constitutent, it has been postulated that by controlling the flow of zinc, MT may serve a metalloregulatory role in zinc-dependent processes such as replication, transcription and translation (Karin, 1985). Thus, MT may serve as a zinc dispensing and collecting system that both protects the cellular constituents against fluctuations in zinc supply and modulates the action of zinc-dependent processes fundamental to cell activation in proliferation and differentiation.

Hormones, cytokines and MT

Induction of hepatic MT by glucocorticoids is more modest and nearly fivefold than induction by metals. Dexamethasone appears to be more potent than natural glucocorticoids (such as cortisol and corticosterone) and longer lasting for hepatic MT induction (Brady, 1992). Glucocorticoids are also major regulator of the brain MT-I and -II isoforms (Hamer, 1986). These hormones control the rate of transcription of the MT-I +II genes after binding to their specific receptors which then gain affinity for specific sequences of the MTs promoter region known as glucocorticoid response elements (GREs). Rat brain MT-I+II protein levels are increased in most of the brain areas by glucocorticoids and also involved in the maintenance of basal MT-I+II (Hidalgo *et al.*, 1997a). Despite the absence of GREs in the MT-III gene promoter region (Naruse *et al.,* 1994) glucocorticoids have some role in MT-III regulation (Hidalgo and Carrasco 1998, Hidalgo *et al.*, 1997a).

Glucagon and angiotensin II (Helvig and Brady, 1984) are also modest inducer of rat hepatic MT. Glucagon communicates via the adenylate cyclase cascade and angiotensin II via the phospholipase C cascade. Another peptide hormone such as Arg-vasopressin, do not induce hepatic MT *in vivo* (Brady, 1992). However, a variety of organic chemicals also induce hepatic MT. This is probably due to the cytokines that are liberated during the acute phase response. Of current interest, is the role of hepatocyte-stimulating factor / interleukin-6. This cytokine has multiple functions on many cell types, but it appears to be responsible for the stimulation of synthesis in liver of the acute phase proteins. Metallothionein is inducible by agents that causes the acute phase response (Brady, 1992). Interleukin-1 induces Zn-MT and also enhances the activity of protein kinase C (Ebadi and Iversen, 1994). Since the synthesis of MT is induced by interleukin -1 (IL-1) and tumor necrosis factor, MT is now considered as acute phase protein. Since the growth of astrocytes (in CNS) by IL-1 is associated with the production of IL-6, IL-8, tumor necrosis factor, nitric oxide synthase and prostaglandin E2, it is presumed that the production of IL-1 by astrocytes takes place when injury or inflammation occurs in the central nervous system (Ebadi *et al.,* 1999). IL-1 and heavy metal induce the synthesis of MT and its mRNA for a prolonged period of time. Sawada *et al.,* (1994) believed that in inflammation, the level of IL-1 increases which in turn induces the synthesis of long acting MT and hence protects against free radicals and oxidative stress caused by inflammation and tissue injury in the CNS.

Functions of MT

The function of metallothionein has been the subject of much speculation and experimentation. Metallothionein has been proposed to be involved in metal storage and detoxification, development, differentiation, control of cellular metabolism, protection from free radical toxicity and in UV response (Karin, 1985). The evidence that metallothionein is involved

in these activities tends to be correlative i.e. enhanced synthesis of mRNA or protein is observed during some phase of development and/or differentiation or in response to a certain chemical or physical assault on the cells (Ecker *et al.,* 1986). Because of their high affinity for metals, metallothionein may play a physiologic role in the absorption, storage and metabolism of important trace metals such as Zn and Cu as well as in detoxification of certain toxic metals such as cadmium and mercury. It is believed that after the initial pretreatment with Cd or Hg, the MT synthesis is induced and newly synthesized protein provides the sequestration capacity necessary to substantially reduce the toxic action of Cd^{+} (Waalkes, 1996).

MT also have antioxidant function in the cell (Sato and Bremner, 1993). Studies with MT+/+ and MT -/- cells revealed that MT functions as an antioxidant to maintain the normal cell cycle against excessive generation of reactive oxygen species (ROS) (Takahashi *et al.,* 2004). It is proposed that MT constitutes a cellular defense system actively responsive to electrophilic agents or reactive metabolites. This is likely to be related to the high sulfhydryl content of MT, which could provide a high degree of "neutralizing" nucleophilic equivalents (Otvos *et al.,* 1987). MT is an effective scavenger of hydroxyl radicals *in vitro* (Thornally and Vasak, 1985). Preinduction of MT reduces the toxic effects of carbon tetrachloride or x-ray irradiation which are mediated by reactive metabolites and free radicals respectively (Cagen and Klaassen, 1989). Miura *et al.,* (1997) suggest that MT scavenged hydroxyl radical ($OH^{\bullet}$) to protect DNA from the oxidative attack by microsomes. Thornalley and Vasak (1985) suggested that the SH groups in MT are the primary target for $OH^{\bullet}$ attack. MT scavenges only free $OH^{\bullet}$ but not site specific $OH^{\bullet}$. Further, MT effectively scavenged free peroxyl radicals of AAPH (2,2′-azobis-(2-amidinopropane)- dihydrochloride. Lipid peroxyl radical predominantely continues the peroxidation reaction at the propagation stage (Maiorino *et al.,* 1989). Therefore, the inhibitory activity of MT against lipid peroxidation is due to its ability to scavenge lipid peroxyl radicals on the membrane surface.

Thus, antioxidant activity of MT seems to be due to MT scavenging free $OH^{\bullet}$ and peroxyl radicals (Miura *et al.,* 1997). MTs may also scavenge nitrosonium (NO^{+}) equivalents, attenuating their oxidative injury. Reaction of NO (a free radical gas) with nonheme iron - containing proteins, and the inhibition of enzymes containing FeS clusters are also important cellular target for the damaging actions of NO (Aschner, 1998). Schwarz et al. (1995) suggested that the antioxidant properties of MT may be due to the formation of iron - thiol nitrosyl complexes, secondary to the sequestration of transition metal iron by MT. The rate constant of MT for its reaction with hydroxyl radical is very high (Sato and Bremner, 1993) since thiols are known targets for both $ONOO^{-}$ and ONOOH (Radi *et al.,* 1991) it is plausible that MT isoforms may intercept both these oxidizers (Aschner, 1998). The presence of MT in nucleus may be important

to protect DNA from Fe-chelate induced oxidative damage because nucleus does not contain antioxidant enzymes such as superoxide dismutase, catalase and peroxidase (Meheghini, 1997). Thus, the nuclear localization of MT may be an important factor to determine the role of MT in protection from genotoxic effect of chemicals (Cherian *et al.,* 1998). MT also provides a storage depot for cysteine, particularly during development (Zlotkin and Cherian, 1988). MT also has several significant intrinsic immunomodulatory properties. MT is a modest lymphoproliferative agent on its own and acts synergistically with the polyclonal activators concanavalin A (ConA) and lipopolysaccharides (LPS) *in vitro* to induce a significant increase in lymphoproliferation (Lynes *et al.,* 1990). This stimulation appears to depend upon the thiols in MT, since proliferation is significantly reduced by the presence of 2-mercaptoethanol and thiol alkylations abrogates this property of MT (Borghesi and Lynes 1996). It was also found that MT interacts with the macrophage at the plasma membrane and triggers activation of the macrophages, which was indicated by increased superoxide production and consequently reduced proliferation of TH lymphocytes in the context of antigen presentation (Youn *et al.,* 1995). Therefore, MT may represent a novel class of immune mediators and manipulations of extracellular MT may offer new approaches to the control of damage caused by these toxins. Simpkins *et al.,* (1996) showed that in isolated rat liver mitochondria, MT causes mitochondrial swelling, depolarization of inner membrane and enhances succinate oxidation with depression of ADP- induced oxygen consumption during stress conditions. Therefore, MT can scavenge oxygen free radicals and nitric oxide (Kennedy *et al.,* 1993). Spermine could act as counterbalance to metallothionein, preventing it from disrupting mitochondrial functions while allowing it to scavenge gaseous mediators.

MT-III is particularly abundant in zinc containing neurons of the hippocampus, zinc rich neurons and astrocytes in the cortex as well as amygdale and it is likely to play an important role in neuromodulation by zinc containing neurons and to act as a sink for free zinc in addition to all housekeeping functions attributed to all MT isoforms (Palmiter *et al.,* 1992). It may also play an etiologic role in various pathophysiological conditions associated with increased extracellular zinc. Studies have demonstrated • that MT-III prevents neuronal sprouting *in vitro*, appears to be down regulated in Alzheimer's disease and that MT- III `Knockout' mice appear highly sensitive to kainate induced seizures. This has focused growing attention on the etiologic role of MT-III in neurodegeneration (Aschner, 1998). Yang et al. (1998) by differential pulse polarographic study suggested that MT may serve as an early warning signal prior to the occurrence of cell death. Similarly Tsangaris and Tzortzutou-Stathopoulous (1998) suggested that MTs induction is rapid and transient in response to stress and / or environmental stimuli. Thus, these results indicate that MT constitute a cellular protective mechanism, neutralizing external apoptotic signals. Hanada et

al. (1998) observed that the skin of MT-null mouse is readily injured by UV-B irradiation and provides direct evidence of the photoprotective effect of cellular MT in the skin.

MT may also have an important role in chemotherapy of certain cancers, both in the development of tolerance to chemotherapeutics and as a potential therapeutic adjunct to reduce toxic side effects (Waalkes, 1993). Of some anticancer agents such as cis-diamine dischloroplatinum (cisplatin) and adriamycin (without affecting their antitumor activities) (Imura *et al.,* 1988) MTs may modify cisplatin toxicity by binding platinum and in the case of adriamycin - which is known to generate toxic free radicals - MT may act as a free radical scavenger (Imura *et al.,* 1990). The concentration of MT is elevated in neoplasm and represents one mechanism of resistance to important groups of anticancer drugs (Ebadi and Iversen 1994).

Degradation of MT

Lysosomes probably represent an important subcellular compartment that might be involved in the degradation of MT *in vivo*. Proteases (cathepsins) that function at acidic pH are probably involved in MT degradation. Studies of Klaassen et al. (1994) suggested that Apo-MT was degraded about 400 times faster by lysosomal fraction than by cytosolic fraction. Leupeptin, which inhibits cathepsins B and L, inhibits the degradation of apo-MT by 80% implying that cathepsins B and/or L might be very important in the intracellular turnover of MT. Cathepsin D appears to be least significant, because apo-MT degradation was reduced by about 20% by inhibiting cathepsin D i.e. the ability of different cathepsins to degrade apo-MT was in the following order: Cathepsin B >>>> Cathepsin C >> Cathepsin D. In addition to this, apo • MT is more susceptible to degradation than Zn MT and Cd MT. This indicates that metals protect MT from rapid proteolysis. The half-lives for constitutive MT-1 and II in adult rats are about 4 hrs., whereas in neonates they are 49 and 73hrs respectively (Klaassen, 1997). Induction of MT by ethanol, $ZnCl_2$ or $CdCl_2$, increases MT half-lives to approximately 9, 26 and 60 hrs. respectively (Kershaw and Klaassen, 1992), indicating that the degradation of MT is dependent on age of the animals and the induction status of MT.

In *in vitro* systems, the amount of metal bound to MT is very important in determining the rate of MT degradation. MT is degraded more rapidly when there are less than 5 atoms of metal associated with each molecule of MT (McKim *et al.,* 1992). The major physiological factor known to influence metal dissociation from MT is hydrogen ion concentration. Metals begin to dissociate from MT when the pH drops from 7 to 5 and at pH 3, almost all the metals are released resulting in higher degradation rates of MT (McKim *et al.,* 1992). Degradation of MT is yet poorly defined but may well be an important aspect of its physiological or toxicological functions.

Methods for detection of MT

Some of the methods used to study the structure and function of MT are:

(a) Fluropore – FluZin-3 has been used to analyze the zinc binding properties of human metallothionein-2 (Krężel and Maret 2007)

(b) Modification of thiols with eosin-5-iodoacetamide(E-5-I) for analysis by SDS-PAGE (Haase and Maret 2008)

(c) Use of MT-FRET (fluorescence resonance energy transfer) sensors (Pearce *et al.,* 2000, Maret 2009)

(d) Chromatography-gel filtration on Sephadex G-50 followed by ion chromatography on DEAE-Cellulose, HPLC-ICPMS and capillary zone electrophoresis (Połeć *et al.,* 2002, Nischwitz *et al.,* 2003).

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NAVIGATING DRUG BEHAVIOR: INSIGHTS FROM PHARMACOKINETICS AND PHARMACODYNAMICS IN ANIMAL MODELS

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Abstract:

This abstract provides a concise overview of a study exploring the intricacies of drug behavior through a comprehensive examination of pharmacokinetics and pharmacodynamics in animal models. The research aims to unravel the dynamic interplay between drug absorption, distribution, metabolism, and excretion, along with their corresponding effects on physiological responses. By utilizing animal models, the study seeks to elucidate the complex relationships between drug administration, dosage, and the resulting behavioral outcomes. Through a systematic analysis of these pharmacological processes, this research aims to contribute valuable insights into predicting and understanding drug behavior, ultimately paving the way for improved drug development and therapeutic interventions.

Keywords: Pharmacokinetics, Pharmacodynamics, Drug Behavior, Animal Models, Absorption, Distribution, Metabolism, Excretion, Dosage, Physiological Responses, Drug Development, Therapeutic Interventions.

Introduction:

In the dynamic landscape of pharmaceutical research and development, the principles of pharmacokinetics and pharmacodynamics stand as pillars essential for unraveling the intricate behaviors of drugs within living organisms. This exploration becomes even more nuanced when applied to animal models, where understanding the intricate interplay between drug absorption, distribution, metabolism, and excretion (pharmacokinetics) and the ensuing pharmacological responses (pharmacodynamics) is paramount. This introduction delves into the fundamental concepts of pharmacokinetics and pharmacodynamics in the context of animal models, highlighting their significance in shaping therapeutic outcomes, optimizing drug regimens, and ensuring the safety of pharmaceutical interventions.

The diverse array of animal models serves as invaluable tools in unraveling the complexities of drug behavior, offering insights that contribute not only to preclinical drug development but also to the broader understanding of human pharmacology. As we embark on

this exploration, we navigate the absorption kinetics of drugs in the gastrointestinal tract, their distribution across various tissues and organs, the intricate pathways of metabolism, and the routes of elimination, all of which collectively shape the pharmacokinetic profile within diverse animal species.

Complementing this is the examination of pharmacodynamics, where the focus shifts to understanding how drugs exert their effects on the body. Through the lens of receptor pharmacology, dose-response relationships, and the temporal dynamics of drug effects, we unravel the intricate tapestry of drug action within the living systems of animals. The application of mathematical models to predict drug concentrations and pharmacological responses further enhances our ability to tailor drug interventions with precision.

This journey through the realms of pharmacokinetics and pharmacodynamics in animal models not only provides a foundation for advancing drug development but also prompts reflection on the translational relevance of findings from bench to bedside. It underscores the importance of recognizing and addressing species-specific considerations, laying the groundwork for a comprehensive understanding that facilitates the successful transition from preclinical studies to clinical application. In essence, this exploration serves as a gateway to a deeper comprehension of drug behavior in the intricate milieu of animal physiology, offering insights that propel the pharmaceutical landscape towards safer, more effective, and tailored therapeutic interventions (Zhao *et al.*, 2021).

Pharmacokinetics in animal models:

Pharmacokinetics, the study of drug absorption, distribution, metabolism, and excretion within a biological system, is a fundamental discipline that shapes our understanding of how drugs behave in living organisms. In the context of animal models, investigating pharmacokinetics is essential for translating preclinical findings into meaningful insights that contribute to drug development, dosage optimization, and ultimately, the safe and effective use of pharmaceuticals. This exploration focuses on the key aspects of pharmacokinetics in animal models, unraveling the intricate processes that dictate the fate of drugs within diverse biological systems.

Absorption:

The journey of a drug begins with its absorption, a process heavily influenced by the anatomical and physiological characteristics of the gastrointestinal tract. In animal models, variations in gut structure and function necessitate a nuanced understanding of how drugs traverse biological barriers. Factors such as pH, solubility, and active transport mechanisms contribute to the diversity in drug absorption profiles observed across different species.

Distribution:

Following absorption, drugs embark on a voyage through the bloodstream, disseminating into various tissues and organs. The distribution phase of pharmacokinetics is shaped by factors like blood flow, tissue composition, and the affinity of drugs for specific binding sites. Variations in these factors among animal species contribute to the unique distribution patterns observed in different models.

Metabolism:

Metabolism plays a pivotal role in drug transformation, often occurring in the liver through enzymatic processes. Animal models exhibit distinct metabolic pathways and enzyme activities, influencing the rate and extent of drug metabolism. Recognition of species-specific variations in drug-metabolizing enzymes is crucial for predicting metabolic outcomes in preclinical studies.

Excretion:

The elimination of drugs from the body, primarily through renal and hepatic routes, marks the culmination of the pharmacokinetic journey. Variability in renal function, glomerular filtration rates, and biliary excretion mechanisms among animal species contributes to differences in drug elimination kinetics. Understanding these variations is pivotal for predicting drug clearance and optimizing dosing regimens.

Pharmacokinetic Modeling:

The complexities of pharmacokinetics in animal models are often distilled into mathematical models that offer predictive insights. Pharmacokinetic modeling involves the integration of data to estimate parameters such as drug half-life, bioavailability, and clearance. These models aid in optimizing drug doses and predicting plasma concentrations, contributing to the rational design of preclinical experiments (Czerniak, 2020).

Pharmacodynamics in animal models:

Pharmacodynamics, the study of how drugs exert their effects on living organisms, is a pivotal discipline that complements pharmacokinetics in unraveling the complexities of drug behavior. In the context of animal models, understanding pharmacodynamics provides crucial insights into the relationships between drug concentration and the observed physiological responses. This exploration delves into the key aspects of pharmacodynamics in animal models, shedding light on the intricate mechanisms that define drug actions within diverse biological systems (Griffith and Dudley, 2021).

Receptor pharmacology:

At the heart of pharmacodynamics lies the interaction between drugs and their molecular targets, often receptors. Animal models serve as invaluable tools for elucidating the dynamics of these interactions. Receptor binding studies, coupled with functional assays, unveil the specificity and potency of drugs in modulating cellular responses. Differences in receptor expression and sensitivity among animal species contribute to variations in drug responses and therapeutic outcomes.

Dose-response relationships:

The relationship between drug dose and the magnitude of the pharmacological response is a central tenet of pharmacodynamics. Animal models enable the construction of dose-response curves, offering a quantitative representation of drug efficacy and potency. Understanding the shape and characteristics of these curves provides critical information for determining therapeutic dosages and potential toxicities.

Time course of drug effects:

Pharmacodynamics extends beyond instantaneous responses, encompassing the temporal aspects of drug effects. The onset, peak, and duration of pharmacological actions are influenced by factors such as drug absorption, distribution, and elimination. Animal models facilitate the elucidation of these temporal dynamics, offering insights into the kinetics of drug action and guiding the development of dosing regimens.

Pharmacodynamic modeling:

The complexities of drug effects are often distilled into mathematical models that encapsulate the relationship between drug concentration and observed responses. Pharmacodynamic modeling aids in quantifying parameters such as efficacy, potency, and the duration of drug action. These models contribute to the refinement of dosing strategies and the prediction of pharmacological outcomes in diverse animal species.

Applications and implications:

1. **Translational relevance:**
   - Bridging the gap between preclinical findings in animal models and the extrapolation to human pharmacodynamics.
   - Recognizing and addressing species-specific considerations to enhance the translational impact of pharmacodynamic data.
2. **Drug development and safety assessment:**
   - Evaluating the efficacy and safety of pharmaceutical interventions through comprehensive pharmacodynamic studies.

- Assessing potential adverse effects and therapeutic margins in preclinical models.

3. Therapeutic drug monitoring:

- Implementing pharmacodynamic principles in therapeutic drug monitoring protocols.
- Tailoring dosages based on observed pharmacological responses for optimized therapeutic outcomes.

Application of pharmacodynamic principles to optimize drug efficacy and safety in animals

Pharmacodynamics, the study of how drugs exert their effects on living organisms, is a critical discipline that holds the key to optimizing therapeutic outcomes while ensuring the safety of pharmaceutical interventions. This exploration focuses on the application of pharmacodynamic principles to enhance drug efficacy and safety in animals, acknowledging the pivotal role of these principles in tailoring drug regimens for diverse species within the realm of veterinary medicine and preclinical research (Amore *et al.*, 2020).

Precision in dose-response relationships:

Understanding the dose-response relationships is fundamental to achieving optimal drug efficacy and safety. By precisely characterizing the relationship between drug dosage and the magnitude of the pharmacological response, researchers can determine the therapeutic window—the range of doses that elicit a beneficial effect without causing unacceptable toxicity. This precision allows for the identification of optimal dosages that maximize therapeutic benefits while minimizing the risk of adverse effects.

Individualized treatment strategies:

Pharmacodynamics facilitates the development of individualized treatment strategies by recognizing variations in drug responses among different animals. Factors such as species-specific differences in receptor expression and sensitivity contribute to the need for tailored approaches. By considering these variations, veterinarians and researchers can optimize drug regimens, accounting for the diversity in physiological responses and ensuring that each animal receives the most effective and safe treatment.

Temporal dynamics and therapeutic timing:

Pharmacodynamics extends beyond instantaneous responses to encompass the temporal dynamics of drug effects. Understanding the onset, peak, and duration of pharmacological actions is crucial for optimizing therapeutic timing. This knowledge allows for the development of dosing regimens that align with the natural rhythms of the animal's physiology, enhancing the overall efficacy of the treatment while minimizing the risk of adverse events.

Monitoring and adjusting dosages:

Continuous monitoring of pharmacodynamic responses provides real-time insights into the effectiveness and safety of drug interventions. By employing pharmacodynamic principles, practitioners can assess the therapeutic outcomes and adjust dosages as needed. This iterative process allows for dynamic optimization, ensuring that the drug regimen remains effective while mitigating the risk of toxicity or suboptimal responses over time.

Balancing efficacy and safety:

The ultimate goal in the application of pharmacodynamic principles is to strike a delicate balance between drug efficacy and safety. This involves a comprehensive understanding of the drug's mechanisms of action, its impact on the target receptors, and the potential for off-target effects. Pharmacodynamic insights guide decision-making to achieve the desired therapeutic effects while minimizing the occurrence of adverse reactions, providing a foundation for evidence-based and patient-centric veterinary care.

Conclusion:

In the dynamic realm of pharmacodynamics and its application to optimize drug efficacy and safety in animals, a nuanced and individualized approach emerges as the cornerstone of responsible veterinary care and preclinical research. As we navigate the intricacies of dose-response relationships, therapeutic timing, and the delicate balance between efficacy and safety, several key insights come to the forefront.

The precision afforded by understanding dose-response relationships enables veterinarians and researchers to tailor drug regimens with meticulous accuracy. Recognizing the diversity in drug responses among different animal species underscores the importance of individualized treatment strategies, acknowledging the unique physiological nuances that influence pharmacodynamic outcomes.

Temporal dynamics play a pivotal role, guiding the optimal timing of drug interventions. This temporal awareness aligns dosing regimens with the natural rhythms of the animal's physiology, enhancing the therapeutic impact while minimizing the risk of adverse events. Continuous monitoring and the ability to adjust dosages in response to real-time pharmacodynamic feedback provide a dynamic framework for refining treatment plans throughout the course of therapy.

The art of balancing efficacy and safety emerges as a central theme. Pharmacodynamic principles empower practitioners to make informed decisions, considering the mechanisms of action, receptor interactions, and potential off-target effects. This comprehensive understanding

guides the quest for the optimal therapeutic window, where the benefits of a drug are maximized, and the risks are minimized.

In conclusion, the application of pharmacodynamic principles in the realm of animal healthcare and preclinical studies embodies a commitment to excellence, precision, and the welfare of animal patients. It propels veterinary medicine towards a future where treatments are not only effective but also tailored to the individual needs of each species. As our understanding of pharmacodynamics evolves, so too does our ability to harness its principles for the betterment of animal health, offering a testament to the ongoing pursuit of excellence in veterinary pharmacology and therapeutics.

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SOMATIC CELL HYBRIDIZATION

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Introduction:

Somatic cell hybridization is the fusion of diploid somatic cells. Cultured cells of two different cell types or from two different species can be fused to produce somatic cell hybrids. The fused cell has common cytoplasm and a single continuous plasma membrane resulting in formation of new somatic hybrids with diploid genome of both cell lines (or of both species). The rate of fusion can be greatly increased by using certain viruses and chemicals.

Discovery: Frye and Edidin (1976) carried out the first cell fusion experiments in which mouse and human cells were fused.

Types of somatic cell hybrids:

The somatic cell hybrids may be of the following types:

1. Heterokaryon or Heterokaryocyte
2. Synkaryon or Synkaryocyte
3. Cybrid or Cytoplasmic hybrid
4. Protoplast fusion

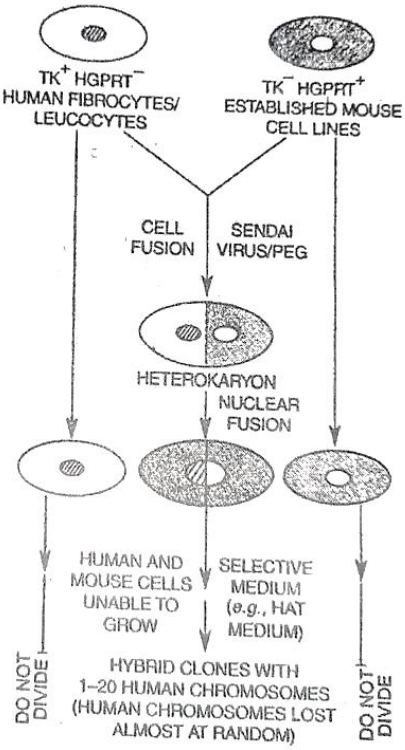
Mechanism of somatic cell hybridization: In any case, the hybrid nature of cells selected by any strategy must be confirmed by cytogenetic or molecular techniques. somatic cells of different types can be fused to obtain hybrid cells. hybrid cells are useful in variety of ways In hybrid cell, chromosome of human is lost at cell division so hybrid retains all mouse chromosomes and after several round of replication hybrid stabilizes retaining only one or few human chromosome under ideal condition. Hybrid cells are not produced in large number. Same cell don't fuse and same fusion involves same cell. To eliminate all these things, cell mixture is placed on selective medium that allow the growth of only hybrid cell. the most commonly used selective medium is HAT as it contains more chemical additives.

Somatic cells of different types can be fused to obtain hybrid cells a generalized scheme ofsomatic cell hybridization may be described as follows:

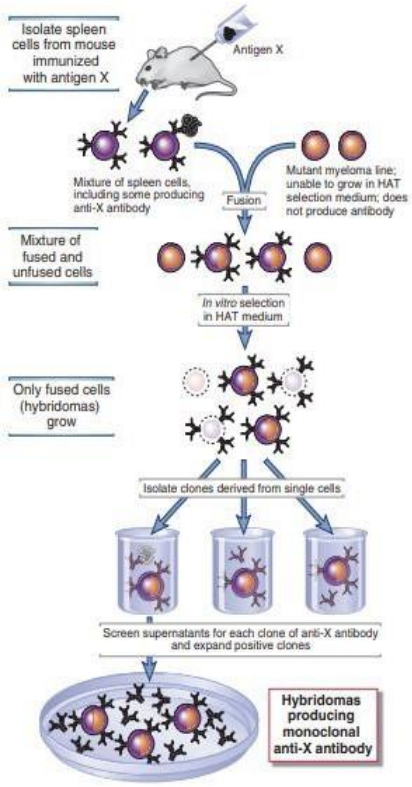
1. Appropriate human and mouse cells are selected and mix together in presence of

inactivated Sendai virus or polyethylene glycol to promote self fusion. This treatment is brief that is about 1 minute to avoid cell death

2. After the fusion treatment, the cells (human mouse and hybrid cells) are watched, suspended in complete medium, incubator overnight, and then are plated on a selective medium. E.g., HAT medium which allows multiplication of hybrid cells only. HAT medium is supplemented with hypoxanthine, aminopterin and thymidine, hence the name HAT.
3. On HAT medium, only those cells that have active HGPRT and TK enzymes can proliferate, while those deficient in these enzymes (HGPRT and/or TK^-) cannot divide (since they cannot produce purines and pyrimidines due to aminopterin present in the HAT medium).
4. For using HAT medium as a selective agent, human cells for fusion must be deficient for either the HGPRT or TK while mouse cells must be deficient for other enzyme of this pair
5. Thus one May fuse HGPRT deficient human cells with TK deficient mouse cells. Their fusion products will be TK and HGPRT and will multiply on HAT medium, while the man and mouse cells will fail to do so.
6. Experiments with other selection media can be planned in a similar fashion



A schematic representation of somatic cell hybridization



Detection of monoclonal antibodies

Applications

1. To study gene or chromosome mapping
2. For production of monoclonal antibodies by producing hybridoma and antibody producing hybridoma lymphocyte
3. To improve crop by protoplast fusion in plants
4. To study the control of cell division and gene expression
5. To investigate malignant transformations

Hybridoma technology:

Most antigens offer multiple epitopes and therefore induce proliferation and differentiation ofa variety of B-cell clones, each derived from a B cell that recognizes a particular epitope. The resulting serum antibodies are heterogeneous, comprising a mixture of antibodies, each specific for one epitope Such a polyclonal antibody response facilitates the localization, phagocytosis, and complement-mediated lysis of antigen; it thus has clear ad vantages for the organism in vivo. Unfortunately, the antibody heterogeneity that increases immune protection in vivo often reduces the efficacy of an antiserum for various in vitro uses. For most research, diagnostic, and therapeutic purposes, monoclonal antibodies, derived from a single clone and thus specific for a single epitope, are preferable. Direct biochemical purification of a monoclonal antibody from a polyclonal antibody preparation is not feasible. In 1975, Georges Kohler and Cesar Milstein devised a method for preparing monoclonal antibody, which quickly became one of immunology's key technologies. By fusing a normal activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a hybrid cell, called a hybridoma, that possessed the immortal growth properties of the myeloma cell and secreted the antibody produced by the B cell. The resulting clones of hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely. The development of techniques for producing monoclonal antibodies, gave immunologists a powerful and versatile research tool. The significance of the work by Kohler and Milstein was acknowledged when each was awarded a Nobel Prize.

In this procedure, spleen cells from a mouse that has been immunized with a known antigen or mixture of antigens are fused with an enzyme-deficient partner myeloma cell line, with use of chemicals such as polyethylene glycol that can facilitate the fusion of plasma membranes and the formation of hybrid cells that retain many chromosomes from both fusion partners. The myeloma partner used is one that does not secrete its own Igs. These hybrid cells are then placed in a selection medium that permits the survival of only immortalized hybrids; these hybrid cells are then grown as single cell clones and tested for the secretion of the

antibody of interest. The selection medium includes hypoxanthine, aminopterin, and thymidine and is therefore called HAT medium. There are two pathways of purine synthesis in most cells, a denovo pathway that needs tetrahydrofolate & a salvage pathway that uses the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Myeloma cells that lack HGPRT are used as fusion partners, and they normally survive usingdenovo purine synthesis. In the presence of aminopterin, tetrahydrofolate is not made, resulting in a defect in denovo purine synthesis and also a specific defect in pyrimidine biosynthesis, namely, in generating TMP from dUMP. Hybrid cells receive HGPRT from the spleenocytes and have the capacity for uncontrolled proliferation from the myeloma partner; if they are given hypoxanthine and thymidine, these cells can make DNA in the absence of tetrahydrofolate. As a result, only hybrid cells survive in HAT medium

The antibodies secreted by many hybridoma clones are screened for binding to the antigen of interest, and this single clone with the desired specificity is selected and expanded. The products of these individual clones are monoclonal antibodies that are each specific for a single epitope on the antigen or antigen mixture used to identify antibody secreting clones. Desired hybridomas are selected for screening. This medium is diluted into multi-well plates such that each well will contain only one cell. Since antibodies in a well are produced by same spleen cell (B-cell of spleen) they will be migrate towards the epitope of injected antigen & thus a monoclonal antibody.

Applications

Monoclonal antibodies have many practical applications in research and in medical diagnosis and therapy. Some of their common applications include the following:

Identification of phenotypic markers unique to particular cell types: The basis for the modern classification of lymphocytes and other leukocytes is the recognition of individual cell populations by specific monoclonal antibodies. These antibodies have been used to define clusters of differentiation (CD) markers for various cell types

Immunodiagnosis: The diagnosis of many infectious and systemic diseases relies on the detection of particular antigens or antibodies in the circulation or in tissues by use of monoclonal antibodies in immunoassays.

Tumor detection: Tumor-specific monoclonal antibodies are used for detection of tumors by imaging techniques and by staining tissues with labeled antibodies. Radiolabeled monoclonal antibodies can be used in vivo for detecting or locating tumor antigens, permitting earlier diagnosis of some primary or metastatic tumors in patients. For example, monoclonal antibody to breast-cancer cells is labeled with iodine-131 and introduced into the blood to detect the spread

of a tumor to regional lymph nodes. This monoclonal imaging technique can reveal breast-cancer metastases that would be undetected by other, less sensitive scanning techniques.

Therapy: Advances in medical research have led to the identification of cells and molecules that are involved in the pathogenesis of many diseases. Monoclonal antibodies, because of their exquisite specificity, provide a means of targeting these cells and molecules. A number of monoclonal antibodies are used therapeutically today. Some examples include antibodies against the cytokine tumor necrosis factor (TNF) used to treat rheumatoid arthritis and other inflammatory diseases, antibodies against CD20 for the treatment of B cell leukemias and for depleting B cells in certain autoimmune disorders, antibodies against the type 2 epidermal growth factor receptor to target breast cancer cells, antibodies against vascular endothelial growth factor (a cytokine that promotes angiogenesis) in patients with colon cancer, and so on.

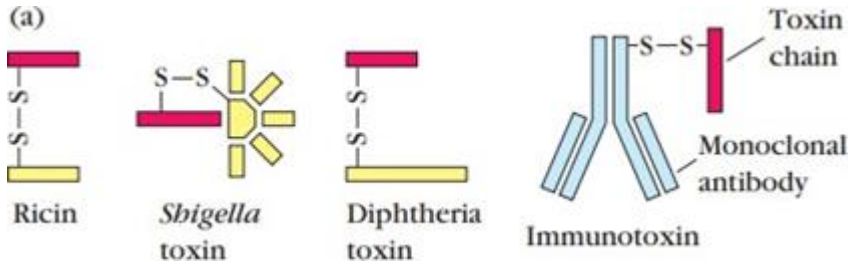
Table 1: Monoclonal antibodies of therapeutic significance

Target	Effect	Diseases
CD20	B cell depletion	Rheumatoid arthritis, multiple sclerosis, other autoimmune diseases
VEGF	Blocking of tumor angiogenesis	Breast cancer, colon cancer
HER2/Neu	Depletion of tumor cells with HER2 amplification	Breast cancer
TNF	Inhibition of T-cell mediated inflammation	Rheumatoid arthritis, Crohn's disease

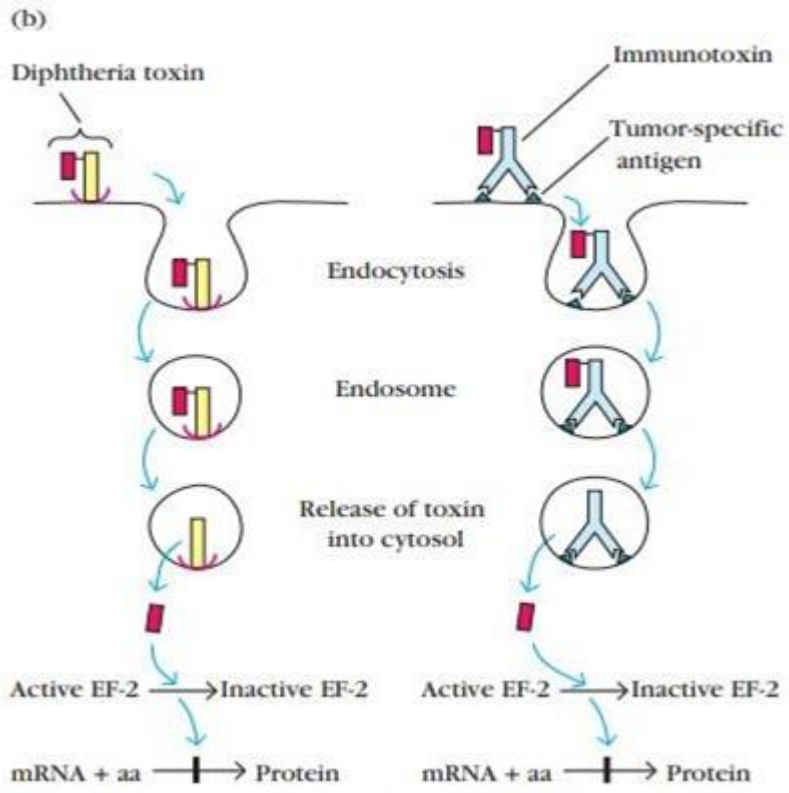
Functional analysis of cell surface and secreted molecules: In biologic research, monoclonal antibodies that bind to cell surface molecules and either stimulate or inhibit particular cellular functions are invaluable tools for defining the functions of surface molecules, including receptors for antigens. Monoclonal antibodies are also widely used to purify selected cell populations from complex mixtures to facilitate the study of the properties and functions of these cells.

Immunotoxins: Composed of tumor-specific monoclonal antibodies coupled to lethal toxins are potentially valuable therapeutic reagents. The toxins used in preparing immunotoxins include ricin, Shigella toxin, and diphtheria toxin, all of which inhibit protein synthesis. These toxins are so potent that a single molecule has been shown to kill a cell. Each of these toxins consists of two types of functionally distinct polypeptide components, an inhibitory (toxin) chain and one or more binding chains, which interact with receptors on cell surfaces; without the binding polypeptide(s) the toxin cannot get into cells and therefore is harmless. An immunotoxin is prepared by replacing the binding polypeptide(s) with a monoclonal antibody that is specific for

a particular tumor cell. In theory, the attached monoclonal antibody will deliver the toxin chain specifically to tumor cells, where it will cause death by inhibiting protein synthesis. The initial clinical responses to such immunotoxins in patients with leukemia, lymphoma, and some other types of cancer have shown promise, and research to develop and demonstrate their safety and effectiveness is underway.



(a) Toxins used to prepare immunotoxins include ricin, Shigella toxin, and diphtheria toxin. Each toxin contains an inhibitory toxin chain (red) and a binding component (yellow). To make an immunotoxin, the binding component of the toxin is replaced with a monoclonal antibody (blue).



(b) Diphtheria toxin binds to a cell-membrane receptor (left) and a diphtheria-immunotoxin binds to a tumor-associated antigen (right). In either case, the toxin is internalized in an endosome. The toxin chain is then released into the cytoplasm, where it inhibits protein synthesis by catalyzing the inactivation of elongation factor 2 (EF-2)

Detection of pregnancy: Pregnancy test kits use monoclonal antibodies. These have been designed to bind with a hormone called HCG which is found only in the urine of pregnant woman. Monoclonal antibodies are attached to the end of a pregnancy test stick onto which a woman urinates. If she is pregnant, HCG will be present in her urine and will bind to monoclonal antibodies on the test stick. This will cause a change in color or pattern which will indicate pregnancy. These specific monoclonal antibodies in a pregnancy test kit will only bind with HCG.

Limitations of monoclonal antibodies:

One of the limitations of monoclonal antibodies for therapy is that these antibodies are most easily produced by immunizing mice, but patients treated with mouse monoclonal antibodies may make antibodies against the mouse Ig, called a human anti-mouse antibody (HAMA) response. These anti-Ig antibodies eliminate the injected monoclonal antibody and can also cause serum sickness. Genetic engineering techniques have been used to expand the usefulness of monoclonal antibodies. The complementary DNAs (cDNAs) that encode the polypeptide chains of a monoclonal antibody can be isolated from a hybridoma, and these genes can be manipulated in vitro. Only small portions of the antibody molecule are responsible for binding to antigen; the remainder of the antibody molecule can be thought of as a framework. This structural organization allows the DNA segments encoding the antigen-binding sites from a mouse monoclonal antibody to be "stitched" into a cDNA encoding a human myeloma protein, creating a hybrid gene. When it is expressed, the resultant hybrid protein, which retains the antigen specificity of the original mouse monoclonal but has the core structure of a human Ig, is referred to as a humanized antibody. Humanized antibodies are far less likely than mouse monoclonals to appear "foreign" in humans and to induce anti-antibody responses.

Monoclonal antibodies are also very expensive to produce.

Ethical issues: An ethical issue is one in which people disagree for religious or other moral reasons. The first step in making a monoclonal antibody is to inject a mouse with an antigen. After it has produced antibodies, a small operation removes spleen cells, which then continue make this use of animals to produce monoclonal antibodies in 2006, a drug trial involving humans using monoclonal antibodies to treat conditions such as arthritis and leukemia went wrong. Despite the individuals being given very low doses, it resulted in organ failure but was not fatal. The monoclonal antibodies had been safely used in other animal trials before being used in human trials.

ELISA (Enzyme-Linked Immunosorbent Assay)

It was first described by Engvall and Perlmann in 1971. Enzyme-linked Immunosorbent Assay, commonly known as ELISA (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called a chromogenic substrate. A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and galactosidase. These assays approach the sensitivity of RIAs and have the advantage of being safer and less costly.

Principle of ELISA:

The test is based on ability of antibodies to couple with enzymes to produce conjugates. It can detect antigen and antibodies in the serum of the patient.

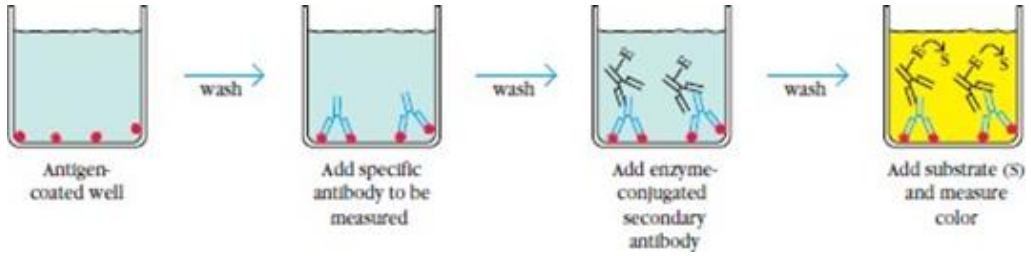
Types of ELISA:

A number of variations of ELISA have been developed, allowing qualitative detection or quantitative measurement of either antigen or antibody. Each type of ELISA can be used qualitatively to detect the presence of antibody or antigen.

There are following types of ELISA:

1. Indirect ELISA
2. Sandwich ELISA
3. Competitive ELISA

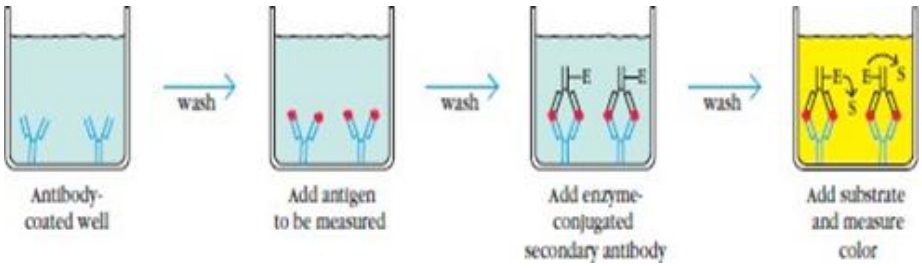
1. Indirect ELISA



Antibody can be detected or quantitatively determined with an indirect ELISA. Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody. Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds. Indirect ELISA is the method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV),

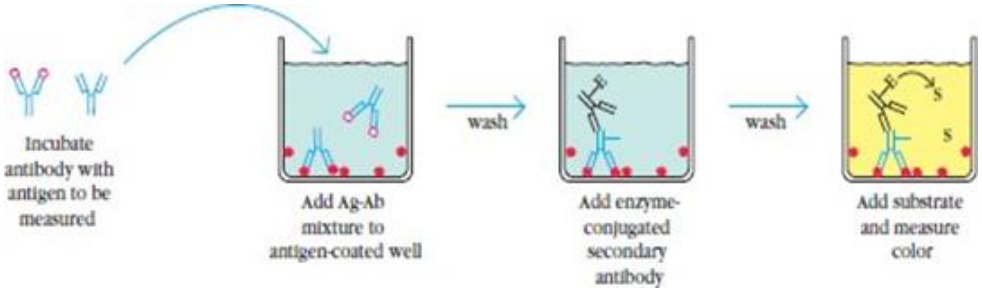
the causative agent of AIDS. In this assay, recombinant envelope and core proteins of HIV are adsorbed as solid-phase antigens to microtiter wells. Individuals infected with HIV will produce serum antibodies to epitopes on these viral proteins. Generally, serum antibodies to HIV can be detected by indirect ELISA within 6 weeks of infection.

2. Sandwich ELISA:



Antigen can be detected or measured by a sandwich ELISA. In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the antibody. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

3. Competitive ELISA:



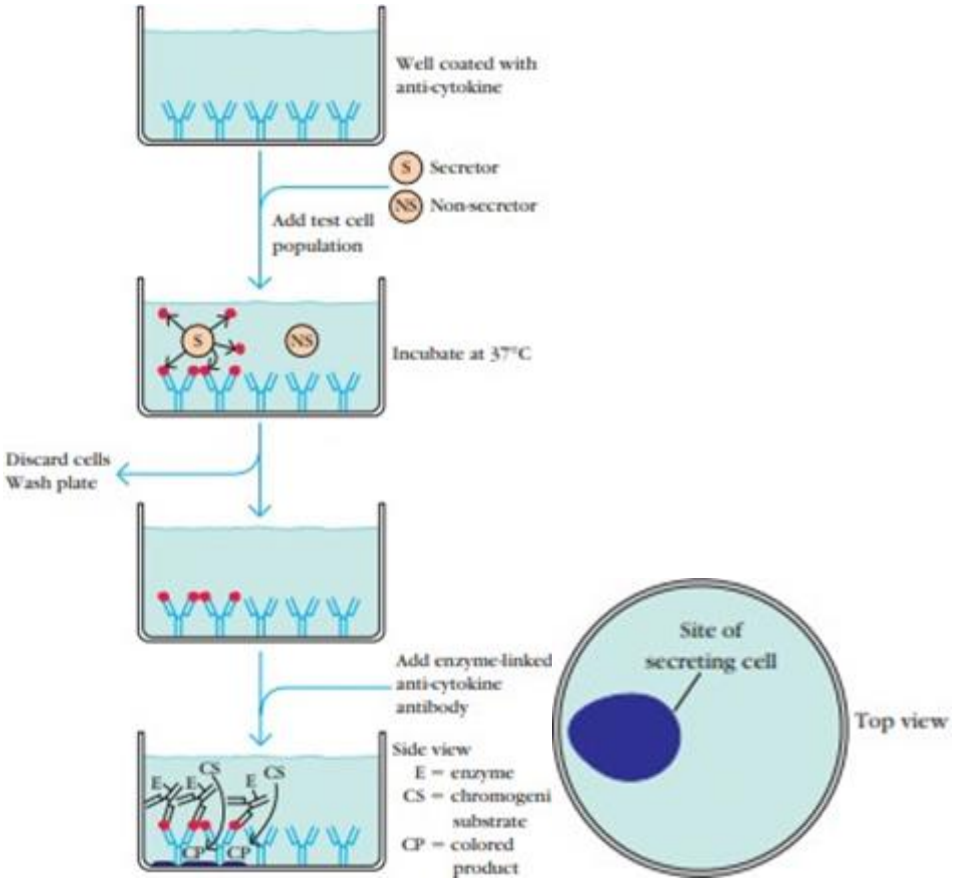
Another variation for measuring amounts of antigen is competitive ELISA. In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigen coated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In competitive assay, the higher the concentration of antigen in the original sample, the lower the absorbance.

Applications:

1. It has applications in bacteriology, virology and parasitology.
2. It is used to detect the infectious diseases like viral Hepatitis B, AIDS, etc.

3. It also detect TB, cysticercosis, leprosy, Herpes, influenza, measles and some fungal diseases.
4. It is useful for hormonal assay like oestrogen, progesterone, HCG, etc.

ELISPOT assay



ELISPOT assay

A modification of the ELISA assay called the ELISPOT assay allows the quantitative determination of the number of cells in a population that are producing antibodies specific for a given antigen or an antigen for which one has a specific antibody. In this approach, the plates are coated with the antigen (capture antigen) recognized by the antibody of interest or with the antibody (capture antibody) specific for the antigen whose production is being assayed. A suspension of the cell population under investigation is then added to the coated plates and incubated. The cells settle onto the surface of the plate, and secreted molecules reactive with the capture molecules are bound by the capture molecules in the vicinity of the secreting cells, producing a ring of antigen-antibody complexes around each cell that is producing the molecule of interest. The plate is then washed and an enzyme-linked antibody specific for the secreted antigen or specific for the species (e.g., goat anti-rabbit) of the secreted antibody is added and allowed to bind. Subsequent development of the assay by addition of a suitable chromogenic or

chemiluminescence-producing substrate reveals the position of each antibody- or antigen-producing cell as a point of color or light.

In the ELISPOT assay, a well is coated with antibody against the antigen of interest, a cytokine in this example, and then a suspension of a cell population thought to contain some members synthesizing and secreting the cytokine are layered onto the bottom of the well and incubated. Most of the cytokine molecules secreted by a particular cell react with nearby well-bound antibodies. After the incubation period, the well is washed and an enzyme-labeled anti-cytokine antibody is added. After washing away unbound antibody, a chromogenic substrate that forms an insoluble colored product is added. The colored product (purple) precipitates and forms a spot only on the areas of the well where cytokine-secreting cells had been deposited. By counting the number of colored spots, it is possible to determine how many cytokine-secreting cells were present in the added cell suspension.

Bone marrow transplantation

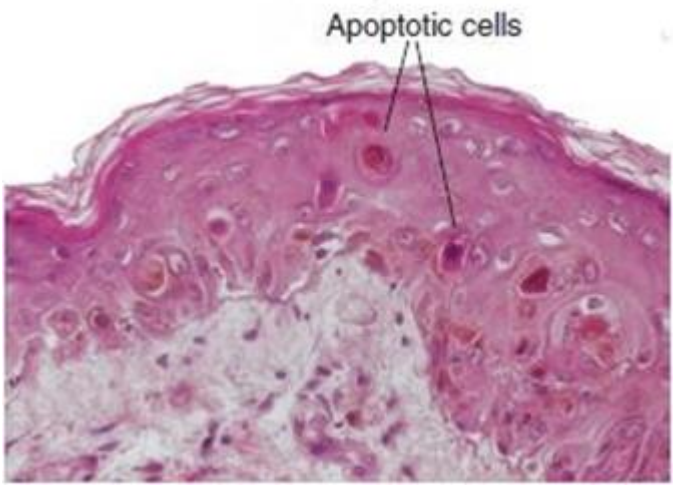
A bone marrow transplant is a procedure that replaces a person's faulty bone marrow stem cells. Doctors use these transplants to treat people with certain diseases, such as Leukemia. Severe blood diseases such as thalassemias, aplastic anemia, and sickle cell anemia, multiple myeloma and certain immune deficiency diseases. Before you have a transplant, you need to get high doses of chemotherapy and possibly radiation. This destroys the faulty stem cells in your bone marrow. It also suppresses your body's immune system so that it won't attack the new stem cells after the transplant. In some cases, you can donate your own bone marrow stem cells in advance. The cells are saved and then used later on. Or you can get cells from a donor. The donor might be a family member or unrelated person. Bone marrow transplantation has serious risks. Some complications can be life-threatening. But for some people, it is the best hope for a cure or a longer life.

Graft-versus-host disease

Graft-versus-host disease (GVHD) is caused by the reaction of grafted mature T cells in the marrow inoculums with alloantigens of the host. It occurs when the host is immunocompromised and therefore unable to reject the allogeneic cells in the graft. In most cases, the reaction is directed against minor histocompatibility antigens of the host because bone marrow transplantation is not performed when the donor and recipient have differences in MHC molecules. GVHD may also develop when solid organs that contain significant numbers of T cells are transplanted, such as the small bowel, lung, or liver.

GVHD is the principal limitation to the success of bone marrow transplantation. As in solid organ transplantation, GVHD may be classified on the basis of histologic patterns into acute and chronic forms.

Acute GVHD is characterized by epithelial cell death in the skin, liver (mainly the biliary epithelium), and gastrointestinal tract. It is manifested clinically by rash, jaundice, diarrhea, and gastrointestinal hemorrhage. When the epithelial cell death is extensive, the skin or lining of the gut may slough off. In this circumstance, acute GVHD may be fatal.



Chronic GVHD is characterized by fibrosis and atrophy of one or more of the same organs, without evidence of acute cell death. Chronic GVHD may also involve the lungs and produce obliteration of small airways. When it is severe, chronic GVHD leads to complete dysfunction of the affected organ. In animal models, acute GVHD is initiated by mature T cells present in the bone marrow inoculum, and elimination of mature donor T cells from the graft can prevent the development of GVHD. In clinical hematopoietic stem cell transplantation, efforts to eliminate T cells from the marrow inoculum have reduced the incidence of GVHD but also decrease the graft-versus-leukemia effect that is often critical in treating leukemias by this type of transplantation. T cell–depleted marrow also tends to engraft poorly, perhaps because mature T cells produce colony-stimulating factors that aid in stem cell repopulation. One approach that has been tried is to combine removal of T cells with supplemental colony-stimulating factor treatment to promote engraftment.

Although GVHD is initiated by grafted T cells recognizing host alloantigens, the effector cells that cause epithelial cell injury are less well defined. On histologic examination, NK cells are often attached to the dying epithelial cells, suggesting that NK cells are important effector cells of acute GVHD. CD8+ CTLs and cytokines also appear to be involved in tissue injury in acute GVHD.

The relationship of chronic GVHD to acute GVHD is not known and raises issues similar to those of relating chronic allograft rejection to acute allograft rejection. For example, chronic GVHD may represent the fibrosis of wound healing secondary to loss of epithelial cells.

However, chronic GVHD can arise without evidence of prior acute GVHD. An alternative explanation is that chronic GVHD represents a response to ischemia caused by vascular injury. Both acute and chronic GVHD are commonly treated with intense immunosuppression. It is not clear that either condition responds very well. A possible explanation for this therapeutic failure is that conventional immunosuppression is targeted against T lymphocytes, which may be only one of several mediators of GVHD. Cyclosporine and the metabolic toxin methotrexate are also used for prophylaxis against GVHD. Various new therapies are being studied in clinical trials, including rapamycin, anti-TNF antibodies, and regulatory T cell transfer.

Immunodeficiency after bone marrow:

Transplantation

Bone marrow transplantation is often accompanied by clinical immunodeficiency. Several factors may contribute to defective immune responses in recipients. Bone marrow transplant recipients may be unable to regenerate a completely new lymphocyte repertoire. Radiation therapy and chemotherapy used to prepare recipients for transplantation are likely to deplete the patient's memory cells and long-lived plasma cells, and it can take a long time to regenerate these populations.

The consequence of immunodeficiency is that bone marrow transplant recipients are susceptible to viral infections, especially cytomegalovirus infection, and to many bacterial and fungal infections. They are also susceptible to Epstein-Barr virus–provoked B cell lymphomas. The immune deficiencies of bone marrow transplant recipients can be more severe than those of conventionally immunosuppressed patients. Therefore, bone marrow transplant recipients commonly receive prophylactic antibiotics and anti-cytomegalovirus therapy and are often actively immunized against capsular bacteria such as pneumococcus before transplantation.

There is great interest in the use of pluripotent stem cells to repair tissues with little natural regenerative capacity, such as cardiac muscle, brain, or spinal cord. One approach is to use embryonic stem cells, which are Pluripotent stem cells derived from the blastocyst stage of human embryos. Although embryonic stem cells have not yet been used clinically, it is highly likely that a major barrier to their usefulness will be their alloantigenicity and rejection by the recipient's immune system. A possible solution to this may be to use induced Pluripotent stem

(iPS) cells, which can be derived from adult somatic tissues by transduction of certain genes. The immunologic advantage of the iPS cell approach is that these cells can be derived from somatic cells harvested from the patient, and therefore they will be syngenic to the patient.

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ISOLATION AND BIOCHEMICAL ANALYSIS OF *E. COLI* FROM THE SAMPLES OF PANI PURI

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Abstract:

Street food is mostly liked by the citizens of every part of the world. It is ready to eat which is prepared so easily. Pani poori is one of the major street foods which is liked by children and youth but it is the main reason for so many health problems such as digestive tract infections. It is estimated that it contains various types of microorganisms which cause infections. *E. coli* is one of the major microorganisms present in paani puri. *E. coli* is one of the microorganisms present in the human body as normal flora. If the count of *E. coli* increases in the body, it leads to infection. It affects our digestive system as well as urinary system also, which causes urinary tract infections. The juice which is used in pani puri contains microorganisms, especially *E. coli*. To test this, the samples of juice in the paani puri is taken from various sources. The juice is tested in the microbiology laboratory. The samples are grown on NA medium, McConkey agar and tested by gram staining and biochemical methods. The presence of *E. coli* in them is a warning signal for human health.

Keywords: Street Foods, Pathogenic. Microorganisms, Panipuri, *E. coli*, Digestive System Problems

Introduction:

Microorganisms are present everywhere in the world. They are called omnipresent. There are various types of microorganisms, such as bacteria, viruses, algae, fungi etc. Bacteria is identified by Antony won leavenhook, when he introduced the microscope. He named them as animalcules. Bacteria are a type of microorganisms. They are classified based on various methods. Sir Christian Gram introduced a method called, gram staining, in which classification is done based on gram's nature of the bacteria. They are of two types, gram positive as well as gram negative bacteria. *E. coli* is the most common bacteria, which is found everywhere in the world. It was discovered by Theodore Escherich in the year 1885. He was a pediatrician, battling against neonatal dysentery, he isolated these coli forms from the stool samples of the infants. Later it was named as Escherichia coli. *E. coli* is present in the digestive system of human beings

as normal flora as well as they present in the soil, water, lakes and ponds and even it was found in the food items which were consumed by humans. Pani poori is one of the famous street foods, which is liked by children and youth. Due to unhygienic maintenance of these places, *E. coli* has entered into this. The juice which is used in pani puri, contains *E. coli*, which damages the digestive system of human beings as well as it causes urinary tract infections.

Morphology:

E. coli is a gram negative, rod-shaped facultative anaerobe. Extraintestinal *E. coli* is encapsulated.

Availability:

E. coli is available in soil, water, ponds, lakes and in the food. It is present in the soil and it can be found in the soil of subtropical and tropical regions. *E. coli* is present in the water such as lakes, ponds and sewage water.

***E. coli* in panipuri:**

Panipuri is a famous street food. To save money, people started consuming pani puri as it is available almost everywhere in the world (Tulandhar and Singh, 2012). It is found by so many studies that pani puri contains a variety of microorganisms (Frelund *et al.,* 1987).

Studies revealed that it has 70% of bacterial pathogens (Nagendra Yadav, 2019). Such as staphylococcus aureus, *E. coli* etc. Analysis of food samples from various places, showed that it had microorganisms. It causes problems in human health (Garode *et al.,* 2012). Panipuri juice mainly contains pathogenic microorganisms as it is prepared under unhygienic conditions. It contains water, cumin powder, coriander powder, red chili powder, jaggery etc. *E. coli* may cause urinary tract infections, meningitis, abdominal and pelvic infections, diarrhea (Das, 2012). According to research, panipuri contains various types of bacteria which are pathogenic in nature.

Microorganisms.

Total number (Ghimire *et al.,* 2021)

- *Staphylococcus aureus* : 37
- *Klebsiella spp.* : 14
- *Salmonella typhi* : 7
- *Salmonella paratyphi* : 12
- *Shigella spp.* : 11
- *Vibrio cholerae* : 2
- *E. coli* : 34

Various street foods contain pathogens as they cause food borne infectious diseases as well, especially in India (Das *et al.,* 2012).

Because panipuri is put openly on the streets or roads which makes the quality of the food a big question (Barro *et al.,* 2006). Because the makers of panipuri are not aware of food safety and food safety rules and regulations etc (Mensah *et al.,* 2002). It is revealed that 77.5% of stuffing, 67.5% of puri and 52.5% of pani samples contained pathogenic microorganisms (Ghirime *et al.,* 2021).

Importance of maintaining food safety:

It is very important for the application of food safety standards as it affects human health. Making the street vendors aware of food safety and health is one of the best way to avoid food borne as well as water borne infection.

Hypothesis:

Microbial analysis of juice used in panipuri, specifically to isolate escherichia coli and biochemical analysis of isolated colonies.

Methodology:

Research includes methods and methodologies of how the research was conducted.

Sample collection: The samples were collected from the panipuri shops near the new nallakunta area, Hyderabad and sent to the laboratory.

Microbial analysis of samples:

The juice of the panipuri is taken and it is sent to the lab, department of microbiology, Sreevani Women's college Hyderabad for microbial analysis. To grow microorganisms in the laboratory, nutrient agar media is prepared.

Preparation of nutrient agar media:

It is a common microbiological media that contains nutrients to grow the microorganisms.

Beef extract - 3.0g
Agar. - 20.0g
Peptone - 5.0g
Distilled water - 1000ml
pH. -7.0 - 7.2

To isolate microorganisms from any samples, first, serial dilution is performed, to dilute the sample. The 1ml sample is diluted in various dilutions. To isolate bacteria, 6th and 7th dilutions were selected for pure culture methods.

Pure culture methods:

To isolate a single colony from the mixed populations of bacteria, pure culture methods are used. First spread plate method is used, the loop full of culture is spreaded on a nutrient agar plate. The plates were kept in the incubator for 24-48 hours. After 24-48 hours, the plates were taken out and observed colonies.

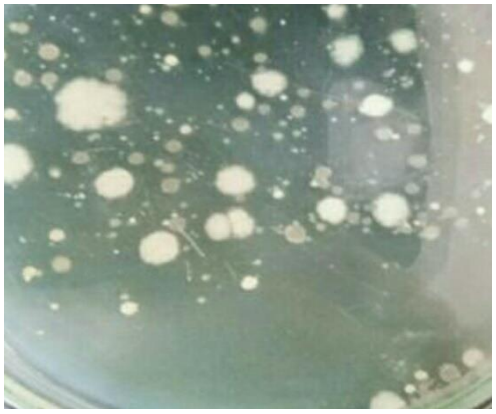

Figure 1: Spread plate method

Figure 2: Streak plate method

The selected five colonies on the plate were taken for a streak plate method to isolate a single colony. The selected colonies are streaked on nutrient agar media.

Among five plates, to get to know the presence of *E. coli*, the specific media, which is used to grow *E. coli* is used.

MacConkey agar: It is a specific media, used to grow *E. coli*.

Composition:

Proteose peptone or polypeptone	- 3 g
Peptone or gelysate.	- 17g
Lactose	- 10g
Bile Salts mixture.	- 1.3 g
Nacl.	- 1.5 g
Neutral red.	- 0.03 g
Crystal violet.	- 0.001g
Agar.	- 13.5 g
Distilled water.	- 1000ml

The media was prepared and the five colonies were streaked on five different plates of macconkey agar.

Among five plates, only one plate contained *E. coli* colonies, which showed green metallic sheen on the plate.

Gram staining:

Process of gram staining procedure is done to the colonies which are grown on macconkey agar. The loop full of culture is taken on a slide and staining is done.

First the culture on the slide is spreaded with a loop, and heat fixed. Then crystal violet, gram's iodine, alcohol and saffranine is added, air dried and observed under microscope.

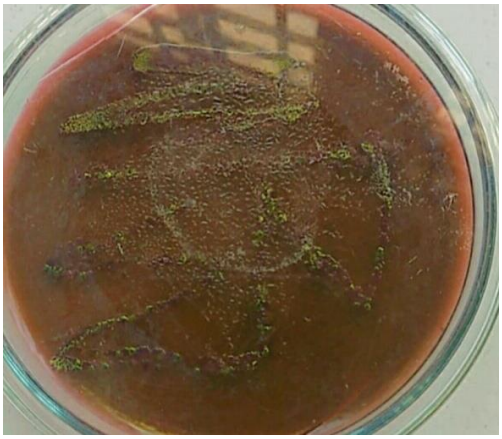

Figure 3: *E. coli* colonies on macconkey agar

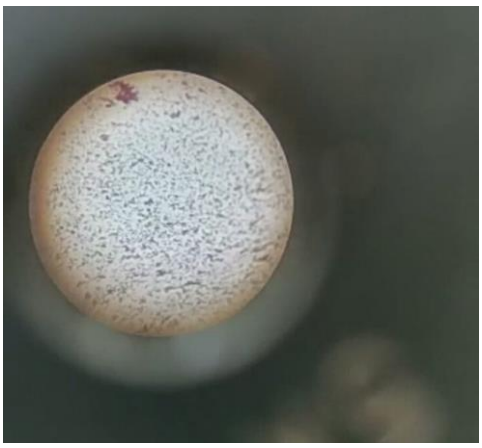

Figure 4: The colonies observed under microscope

The colonies were gram negative and rod shaped.

Biochemical tests:

Biochemical tests are the laboratory procedures to study the biochemistry of microorganisms. There are used to identify particular bacteria based on the reactions of microorganisms with different chemicals.

They are common tests used in the laboratories.

1. Indole test:

The uninoculated bacteria is introduced into the tryptone broth. The 48-hour old *E. coli* culture is grown at 37 degrees Celsius. It shows positive for *E. coli* by indicating the presence of indole. It formed a red-coloured layer by the addition of kovac's reagent.

2. Methyl red -voges proskauer test:

This test basically used to identify which fermentation pathway is used to utilize glucose by bacteria. It uses MR broth and VP broth. The *E. coli* culture is added to the MR broth, when it is added with the reagent, methyl red, it remains in red color. It showed that the *E. coli* is positive for methyl red test. When the culture is inoculated into the broth and added alpha naphthol and potassium hydroxide, if it turns red it is positive for the VP test. *E. coli* is negative for the voges proskauer test.

3. Citrate utilization test:

This is the test where the ability of a particular bacteria is tested whether it utilizes sodium citrate as a carbon source and inorganic ammonium hydrogen phosphate as a nitrogen source. Simmons citrate agar is used in the test. The inoculum is added to the medium and if it turns into blue color then the test is positive. If it remains green, then the test is negative. Citrate utilization test is negative for *E. coli*.

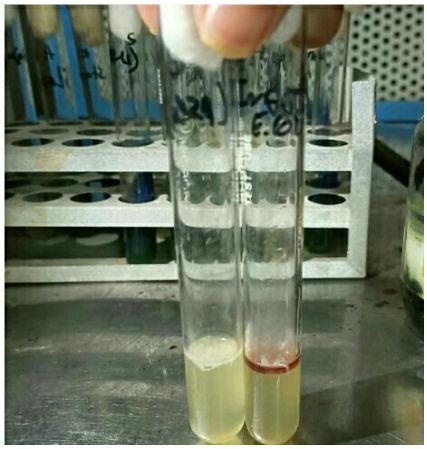

Figure 5: Indole test for *E. coli*

Figure 6: MR-VP and citrate utilization test for *E. coli*

Results and Discussion:

By following various microbiological laboratory methods to identify the bacteria. The samples are identified with *E. coli* and to confirm the presence, staining and biochemical methods were performed and confirmed the presence of *E. coli* in the juice of panipuri samples.

Presence of *E. coli* causes problems to human health if the consumption is more. It affects the digestive system by causing diarrhea and indigestion. It may also cause urinary tract infections and abdominal, pelvic infections. Avoiding consumption of these street foods reduces infections (Mensah *et al.,* 2006). Morphological configuration and biochemical nature of the samples are analyzed from the colonies grown on macconkey agar plates. It questions the microbiological quality of other popular street foods in India.

Conclusion:

The study revealed the nature of microorganism i.e *E. coli* is isolated from the samples and analysed morphology, biochemical nature and importance of maintaining food safety as far as human health is concerned.

Acknowledgements:

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SUMMER SEASON MANAGEMENT IN SERICULTURE

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Abstract:

The intensity of summer is increasing and the farmers engaged in sericulture must take some measures during this period. Sericulture mainly depends on two aspects which include mulberry fields and well-constructed rearing houses. Summer has a great impact on mulberry plants and silkworms, so farmers need to cultivate mulberry scientifically. Especially, the success of the silk industry depends on temperature and humidity, sustaining these two things in summer is a tough job. This article focuses on summer season management for the protection of silkworm rearing and mulberry cultivation.

Keywords: Summer, Sericulture, Management.

Introduction:

Impact of temperature on silkworm growth and cocoon development:

Ueda and Suzuki (1967) found that a silkworm body temperature (20-30°C) affects physiological activity, food intake, and economic parameters. Higher temperatures resulted in a lower rate of leaf-silk conversion. Shen (1986) reported remarkable conversion efficiency in silkworm larvae raised at low temperatures. Muniraju *et al.* (1999) found that maintaining a low temperature (26°C) during the raising stage improved silk conversion and better survival rates in bivoltine silkworms. This study examined how environmental conditions affect *B. mori* food intake, assimilation and silk production. Over the past two decades, the introduction of productive bivoltine hybrids and rearing management in India has led to a significant rise in yield and income for sericulture farmers, as well as increased output of high-quality raw silk. Bivoltine hybrids offer significant benefits for farmers with high input and administrative skills. These hybrids cannot adapt to India's extreme temperatures and humidity levels. To improve silk output, it is crucial to produce silkworm breeds that are resistant to diseases and unfavourable climatic circumstances. Additionally, appropriate climate management measures must be implemented during silkworm rearing (Rahmathulla and Suresh, 2012).

Temperature is one of the most important abiotic factors influencing silkworm growth and productivity (Ueda *et al.,* 1975; Benchamin and Jolly, 1986). There is substantial research suggesting that good-quality cocoons are generated within a temperature range of 22-27°C and that cocoon quality declines above these ranges (Krishanswami *et al.,* 1973). However, polyvoltine breeds raised in tropical regions are known to endure somewhat higher temperatures and adapt to tropical climatic circumstances (Datta, 1992). High temperatures have an unfavourable effect on practically all biological processes, including the rates of biochemical and physiological reactions (Hsieh *et al.,* 1995). They can eventually impact the quality or number of cocoon crops in the silkworm and, as a result, the amount of silk produced. Willmer et al., 2004 found that silkworms were more sensitive to high temperatures in the fourth and fifth stages.

Management of temperature control:

During the winter and rainy seasons, control batches with optimal temperature and humidity showed much higher nutritional efficiency. The amount of ingesta and digesta required for producing one gram of cocoon/shell was likewise lower in control batches for all seasons except summer. This could be related to silkworms' physiological adaption to cope with stress throughout the summer season. Cocoon production should be of good quality for this, it is necessary to use high-quality mulberry leaves. Mulberry leaves fed to silkworms are of low moisture content due to rising temperatures in summer. This causes the leaves to dry quickly and this affects the feeding of the larvae. Decreasing the amount of leaf feeding in larvae harms larval growth.

Temperature plays an important role in the flooding of mulberry trees because of the growth of mulberry and the ability to store water in the leaf depending on the temperature. About 13^0C to 40^0C. The ability of mulberry roots to survive in temperature increases the ability of the root to find water as the temperature increases. The result of this can be seen in the growth of mulberry and the reduction of art in the leaf. Considering all this, the main purpose is to maintain the moisture content of the leaves of the mulberry tree in the morning for the larvae to feed on the leaves. Applying early and in the evening can reduce the chance of leaf wilting. Also, if the mulberry leaves are soaked in water for a while before feeding them to the larvae, they can stay fresh longer. This allows the larvae to eat those leaves easily. Doing all these things can prevent wastage of leaves and keep larvae healthy.

Management of construction of rearing house:

The silkworm rearing house should be built in a cool place or with tree shade in the North-South direction. This will prevent sunlight from entering during the afternoon and protect the larvae and leaves from the sun. It also helps keep the air in the rearing house fresh. Brick,

cement or mud should be used in the construction of rearing houses. Electrical temperature and humidity control devices should be used if possible, and insulating roofs help keep temperatures under control. If cement sheets are used, then the screed should be placed on this sheet (Forage) Straws should be placed around the rearing house. A Mulberry nursery is useful if thick measures of soil, leaves, colas or asbestos sheets are used. Pesticides Household use of coconut branches, rice husks, grass etc. helps to reduce the temperature. The temperature can be reduced by applying certain types of dyes available in the market. Planting trees or a mulberry garden around the perimeter of the nursery has a double benefit, planting a variety of crops around the nursery can help retain moisture.

Thermometer Measures Temperature

Well-constructed Rearing House

Management of silkworm rearing:

Scientific rearing of silkworms is essential for a successful silk industry. If the ambient temperature increases, the temperature of the silkworm automatically increases. This affects the body of the silkworm and in turn the life cycle. This stunts the growth of the worm and reduces its ability to produce thread. If the temperature rises, the digestion of the silkworm slows down, which can lead to the decomposition of water and infection of various diseases. Almost 32^0C temperature affects the production of eggs by producing an impotent male pattern in the oviposition stage. This results not only in the loss of the thread but also in the cocoon becoming weak, and the cocoon becoming double and brittle. All these factors affect the quality and yield of silk production, so the temperature in rearing should be between 20^0C and 30^0C. Hang wet gunny cloths about the rearing shed to allow cold, humid air to flow inside. Summer stimulates sucking pests such as thrips, pink mealybugs, papaya mealybugs, and others in the mulberry garden, reducing leaf quality. Spray forceful jets of water to remove pests from the plants.

Table 1: Temperature (^{0}C) required for stages of growth in pure and hybrid silkworms.

Stages of Silkworm	Pure Variety	Hybrid Variety
First instar	26-27 ^{0}C	26-28 ^{0}C
Second instar	25-26 ^{0}C	26-28 ^{0}C
Third instar	24-25 ^{0}C	24-26 ^{0}C
Fourth instar	23-24 ^{0}C	24-25 ^{0}C
Fifth instar	22-23 ^{0}C	23-24 ^{0}C

Management of after-hatching stage silkworm:

In hatching, the temperature is high and the humidity is low during the egg-hatching period. At this time, put waxy paper or blue plastic paper in the tray and put a piece of paper on top. Wet burlap on top of the trays can be helpful to keep the larval beds open at the time of hatching should be kept After the air in the tray is freed for some time, the bed should be covered again by adding a sheet. If the pieces of leaves are grown in summer, the leaves will not dry up, the rest of the stand should be watered at harvest by cutting the lateral leaves on the lower side of the stand.

Silkworm Laid Eggs on Paper

Management of rearing of adult stage silkworm:

Due to low temperatures and low humidity in summer, wet cloth or gauze should be applied to the doors or windows to prevent air from reaching adulthood. Temperature and humidity can be controlled by sprinkling water on it from time to time. Windows should be kept open at night and in the morning. In summer, shoot feeding (branch method) should be used as food.

Adult Silkworm Feeding on Mulberry Leaves

Silkworm Larva Spinning Cocoon

Management of cocoon:

Keeping the Mountages in cool and fresh air for hatching will reduce larval mortality at high temperatures, the hatching temperature should be 24^0C to 25^0C and keeping the humidity between 60 and 70 % gives good cocoon formation.

Cocoon on Mountages

Cocoon Kolar Gold

Mulberry Management:

Mulberry cultivation is important all over the summer season. It has a significant impact on the growth and development of mulberries. Summer cutting, thinning, fertiliser, tillage, weeding, drainage, and insect control are the most common activities in a mulberry garden. During cutting the old mulberry garden requires stem cutting, whereas the new mulberry garden requires cutting around 35cm from the ground; if it is a young mulberry that was cut once last summer in the mulberry garden, the summer cutting should be enlarged by 5-10cm; at the same time, the leaves and small side branches of the mulberry tree base must be removed to reduce nutrient consumption and promote rapid germination of new shoots. After the summer cut, when the summer silkworm reaches a length of 20-30 cm, the buds must be thinned down, leaving 8-10 roots per plant. The method is separate weak and strong, dense and thin, internal and external, with a consistent distribution.

Summer tillage, when combined with summer manure, can improve soil structure while also weeding, holding moisture, and regulating soil temperature. Summer cultivation should be shallow, with an average depth of 12-15cm. Clay should be deep, sand should be shallow; old mulberry should be deep, fresh mulberry should be shallow; rows should be deep, plants shallow. Weeding should be combined during the summer, usually once or twice. Weeding must be done "early removal, small removal, and removal". Chemical weeding can be utilised when the weeds are dense and thick. To spray the stems and leaves, mix 1 kilogramme of glyphosate (15% active chemicals per mu) with 10 kg of water and 0.2 kg of washing powder as a spreading agent.

Mulberry trees soil moisture content ranges between 70 and 80 % of the field's maximum water capacity. If it reaches this limit, it will impact mulberry tree growth. If the mulberry garden floods, the ditch should be dredged in time to drain the water from the ground. Irrigation is essential throughout the summer, for up to 7-10 days of sustained drought. In the summer, plough the soil early to remove diseases and insects, and keep the mulberry garden well-ventilated, transparent, and dry. Alternatively, pests can be controlled using medication control and artificial culling.

Conclusion:

Overall, this article provides immense information to farmers and researchers about protecting silkworm rearing and mulberry cultivation during the summer season.

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A GENERAL ACCOUNT OF FISH *Catla catla* (HAMILTON 1822)

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Abstract:

Varieties of fishes are cultivated in India and Indian subcontinent. Inland fish resources of India are the richest source of fishes in the world. The river Ganga and its tributaries are the natural and vast habitat of Indian major carps like *Catla catla, Labeo rohita and Cirrhinus mrigala.* These are the most important major carp culture in Asia and commercially culture in India and Indian subcontinent. *Catla catla* (Hamilton 1822) is the fresh water Indian carp native to India, Bangladesh, Myanmar, Nepal, and Pakistan. It is also introduced in many other countries as exotic species. *Catla catla* is a very rich source of proteins and the important lipids especially ω-3 fatty acids Because of its high nutritive value, it is a highly priced food fish having a great demand in the market. It is an economically important fish, having a great commercial value. *Catla catla* is the very rich source of protein.

Keywords: Indian Major Carp, *Catla catla*

Introduction:

Carps are the fishes belonging to the family Cyprinidae of class Osteichthyes. These are the toothless fishes and cycloid scales form the external covering of the body. The carps having more than 11 undivided fin rays are called as major carps while the carps having less than 11 undivided fin rays are called as minor carps (Murthy, 2002). An important cultivable species of carps are the Indian major carps *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, Exotic carp, Grass carps and common carps. These are the most common food fishes having great economic importance (Sarder *et.al.*, 2011). They are generally produced for local consumption rather than for export**.** Carp culture is the traditional practice which recently received the broad scientific base and hence responsible to increase the pond productivity (Alikunhi, 1957).

Varieties of fishes are cultivated in India and Indian subcontinent. Inland fish resources of India are the richest source of fishes in the world. There are nearly 21000 fish species cultivated in Indian subcontinent out of which 40% are fresh water grows in tropical regions like lakes, rivers, canals, brackish water estuaries (Azim *et.al.*, 2022). The river Ganga and its tributaries are the natural and vast habitat of Indian major carps like *Catla catla, Labeo rohita and Cirrhinus mrigala*. The Indian major carp have great market demand due to their taste and

flesh and contribute about 67% of total freshwater fish production (ICLARM, 2001)**.** These Indian major carps are cultivated together in composite fish farming as they have a different feeding habitat and can utilize the resources efficiently to give a better yield (Hansen *et.al.*, 2006).

***Catla catla* (Hamilton 1822):**

Catla catla (Hamilton) is the fresh water Indian carp native to India, Bangladesh, Myanmar, Nepal, and Pakistan. It is also introduced in many other countries as exotic species. *Catla catla* is a very rich source of proteins. It is an economically important fish, having a great commercial value (Chalchisa, 2023). It occurs mainly in fresh water ecosystem in abundance. *Catla catla* is one of the fastest growing Indian major carp which can grow upto 182 cm in length and about 50 kilograms in weight, and it can attain a maximum size within short period of time, hence consider as the fastest growing Indian major carp (Karima *et al.*, 2023).

Catla catla has a characteristically large, upturned mouth with a prominent protruding jaw. Its upturned mouth and large gill rackers are well adapted to feed on numerous free floating organisms present in water. It is a surface and mid-water feeder, mainly omnivorous which can feed on zooplankton as well as phytoplankton. Its juveniles of *Catla catla* feed on aquatic and terrestrial insects, water fleas, detritus matter and phytoplankton until they reach a length of about 15 to 20 millimeters. *Catla catla* is the very rich source of protein and the important lipids specially ω-3 fatty acids. Because of its high nutritive value, it is a highly priced food fish having a great demand in the market.

Geographical distribution:

Catla catla is found in both the fresh water as well as brackish waters and can be seen in the tidal areas. The original natural distribution of *Catla catla* was fresh water rivers thought the India, Pakistan and Burma, because of its successful transportation from the beginning of 20th century the fish has now spread over the whole of peninsular India. Nowadays the fish is distributed all over Pakistan, India, Nepal, Bangladesh, Burma. *Catla catla* has been introduced into almost all riverine systems, reservoirs and tanks all over India. It is mostly observed in the broad deep pulls of the fresh water rivers during the winter and summer months. For breeding purpose, the fish shows migration, during the period of the monsoon, June- September. *Catla catla* can polyculture along with the rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). The composite culture of the *Catla catla* is also carried out successfully with the two Indian major carp rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) and the three species of exotic carp that is common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*).

Taxonomic position:

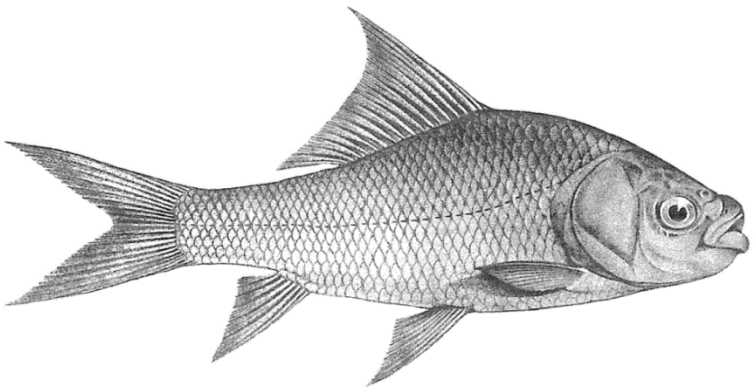
Phylum	-	Vertebrata
Sub phylum	-	Craniata
Super Class	-	Ganthostamata
Class	-	Teleostomi
Sub Class	-	Actinopterygii
Sub Order	-	Cyprinoidei
Family	-	Cyprinidae
Genus	-	Catla
Species	-	*Catla-catla* (Hamilton 1882)

Related taxa:

Today *Catla catla* is found in most of the places in India and hence it has different local names in the different regions some of which are mentioned below.

State	**Name**
Maharashtra	Catla
West Bengal	Catla
Assam	Baudhekra
Gujarat	Tambra
Hindi	Chepti
Orissa	Barkur
Punjab	Theil
Tamil Nadu	Tayoppa
Andhra Pradesh	Bacha
Kerala	Kazkatla

Morphology:



Morphology of fish *Catla catla*

The body of fish *Catla catla* is dull silver white but it is also darkish in color in weedy water. Body is deep and the dorsal side is more convex than the ventral side. Head is large with large rounded eyes, mouth is wide with prominent lips, dorsal fin is quite large, caudal fin is bilobed. The fry of *Catla catla* are distinguished by the relatively large head with reddish gills.

- Body deep its depth 2.5 to 3 times in standard length
- Head enormously large.
- Mouth wide and upturned, with prominent, protruding lower jaw.
- Snout is surrounded by thin integument.
- Eyes in front of head, absence of barbells.
- Dorsal fin commences in advance of ventral pectoral fin long extend to pelvic fin, anal and caudal fin short.
- Scales conspicuously large.
- Lateral line central and continuous till the base of caudal fin and having 40-43 scales.
- Upper lip absent, lower moderately thick having Continuous and free posterior margin. In large specimens, some pores on the Snout are visible.
- No barbells, gill rakers are long, fine and closely set.

Fin formula:

D 3-4/14-10,p21;v9 A3/5;c19;11;40-43;L.tr.71/2/9

Color:

Grayish on the back side and flanks silvery white from below. Fishes inhabiting turbid or weedy ponds possesses darker colouration.

Maturity:

Catla catla attains the maturity in the second year of life. The body length of mature *catla* varies in different species; the estimated length of matured *Catla catla* is approximately 441.559 mm to 558.8 mm Natarajan and Jhingran (1961).

Reproduction:

Catla catla can grow upto 15- 18 inches in length in the first year but it cannot attain sexual maturity at this age. In the second year *Catla catla* attains maturity in the second year that is they are ready to breed in the third season after hatching. In monsoon season *Catla catla* shows spawning migration towards the upper river stretches, where male and female breed in shallow peripheral regions. The spawning season lasts between May and August and in north India and Pakistan from June to September.

In India *Catla catla* breed in rivers during the south-west monsoon that is from June to August, after sudden heavy rain. Fecundity of *Catla catla* varies from100,000-200,000/kg BW,

depending on fish length and weight. The seeds are brought by water flow to the downstream areas where they are caught by seed collectors. Early fish are available in large numbers during June-July in Assam, Bengal and Bihar, and during July-August in Orissa, Punjab and Andhra Pradesh are collected in millions and stocked in nursery ponds.

Fecundity:

"Fecundity can be defined as the number of ova laid by a fish during the spawning season." It is the measure of the reproductive capacity of the female fish. Ntarajan and Jhingran (1961) found the fecundity of *Catla catla* to vary from 2309831 to 492022509 depending upon the length and weight of the fish and the weight of the ovary.

Feeding habit:

Catla catla is surface or column feeder. It has large upturned mouth and large gill rakers hence *Catla catla* is well adapted to feed on numerous organisms floating in water. The young ones of *Catla catla* start feeding from the third day after hatching. At fifth day the mouth parts, alimentary canal and its associated structures are fully formed and the fish starts to feed on natural diet. The young ones of *Catla catla* feed almost exclusively on water fleas and other animalcules in the water. The adult *Catla catla* feed on the diversity of the feed present in water that is on aquatic and terrestrial insects, water fleas, detritus matter, phytoplankton and zooplanktons which occurs naturally in the water. Adult *Catla catla* can also accept the artificial diet in the form of powder or pellets.

Food and feeding of fish *Catla catla*:

Feeding management plays an important role in success of fish culture**.** Feeding of fish with a balanced diet is very important as low feeding leads to poor growth while the excess feeding may lead to the fish death and makes financial losses, it also deteriorates the water quality Nandeesha *et.al.,* (2013). Fish *Catla catla* have become adapted to a wide variety of food. It can feed on the phytoplanktons as well as zooplanktons which are present naturally in the water bodies and hence shows the omnivores feeding habitat. It can also accept the artificial food.

Nutrient requirement of fish *Catla catla*:

Proper growth and survival of the fish is influenced by the availability of the right type of food in the right proportion. Generally, fish food constitutes the nutrients and energy sources essential for the growth, reproduction, and health of fish. For profitable growth of fishes, the feed must be adequate in their nutritional composition. For any organism including fish also carbohydrates, proteins and fats are of primary importance and deficiencies of these substances can reduce growth rates or lead to diseases, but in excess amounts it also leads to a reduction in

growth rate Rangasamy *et.al.,* (2021). Carbohydrates, proteins and fats are of essential significance for the normal growth and development of the fish. Micronutrients like vitamins and minerals also play an important role in fish growth, so it is important to study the role of these basic nutrients. Protein, lipid and carbohydrate functions as the metabolic reserve in the fish body, as they are necessary for reproduction, growth and development of the cellular membrane structure.

Economic importance of *Catla catla:*

Catla catla (Hamilton) is an important Indian major carp that got importance both in culture and capture fisheries. It is one of the most valuable food fish, mostly cultivated in freshwater ponds and lakes in the absence of carnivorous fish. It is the fastest-growing fish species among the three Indian major carps, (Chalchisa, 2023). *Catla catla* is largely employed for stocking in tanks. The fish is fleshy and noted for its delicacy and valued very high in the market. It is best for consumption. Catla is also game fish. It is used as a sport fish. It is used as the surface feeder in Indian major carp polyculture systems. Being a surface feeder that is highly preferred by the consumer (Kunguma *et.al.*, 2019), Catla forms an integral component in carp polyculture systems (Rahman *et.al.,* 2017).

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ANIMAL BREEDING AND GENETICS

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Introduction:

This chapter serves as a comprehensive introduction to the foundational concepts of animal science, providing essential insights for the course. It delves into the intricacies of animal breeding and genetics, encompassing various types and systematic classifications, along with a thorough examination of the positive and negative implications associated with these practices in the realm of animal breeding and genetics. Animals, integral to our existence, assume multifaceted roles in our lives, serving as subjects of reverence, sustenance, admiration, and companionship. Our dependence on them extends to nutrition, sociology, and daily life. Given their paramount importance, a dedicated branch of science, namely animal science, explores the intricacies of domestic animals.

Much of our reliance on animals is rooted in their contributions to the global food supply. To optimize agricultural output, humanity has devised an intricate resource management system known as agriculture—a fusion of scientific principles and artistic cultivation techniques applied to crops and livestock. Within this framework, domestic flora and fauna are carefully maintained to meet human requirements. Agriculture, a practice cultivated for millennia, forms the cornerstone of daily sustenance for every individual globally. Consequently, all human occupations are inherently interconnected with agriculture, particularly evident in developed nations where the urban industrial complex relies on agricultural surpluses for sustenance. Human utilization of domestic animals extends beyond food production, encompassing diverse areas such as sports, recreation, manufacturing, religion, scientific research, and companionship. Animals, therefore, form an integral component of virtually all facets of our lives, often operating at the core of our culture, influencing the landscape, and impacting our day-to-day activities. The timeline of individual animal species' domestication remains uncertain, with the transition from hunter-gatherer practices to agriculture representing a significant cultural revolution in human history. Initially, the dependence on tamed and domesticated animals by early societies was marked by the continuation of traditional uses such as meat, bones, and skins. Additional functionalities, including milk production, clothing, labour, military applications,

sports, and status symbols, evolved over time as human settlements adopted a more sedentary lifestyle.

The transformation from hunters and gatherers to farmers marked a crucial step towards civilization. Concurrently, the acquisition of domestic animals prompted the need for their management, care, and strategic utilization. This necessity led to the emergence of the academic discipline known as animal science. Animal science encompasses a comprehensive study of domestic animals, spanning from conception to death. This includes examinations of behaviour, management, physiology, nutrition, reproduction, and product distribution. Rooted in the observations of early societies engaged in domestication, animal science has evolved into a vast body of knowledge, necessitating its division into specialized disciplines for better comprehension. One such discipline is genetics, focusing on heredity and the variation of inherited characteristics. Another key area is animal breeding, utilizing biometry and genetics to enhance farm animal production.

Animal breeding:

Animal breeding falls under the realm of animal science, focusing on assessing the genetic worth of livestock through methods like best linear unbiased prediction. The process involves choosing animals with exceptional estimated breeding values (ebv) in areas such as growth rate, egg, meat, milk, or wool production, and other desirable traits. This approach has significantly transformed global livestock production. The scientific foundation of animal breeding integrates population genetics, quantitative genetics, statistics, and, more recently, molecular genetics. This field builds upon the groundbreaking contributions of sewall wright, jay lush, and charles henderson. Animal science encompasses various disciplines related to the study and management of animals, and animal breeding is a significant component within this field. In the context of animal breeding, animal science involves applying scientific principles to understand and manipulate the genetic traits of livestock. This includes evaluating and selecting animals based on desired characteristics such as growth rate, reproduction, and product quality.

Within animal breeding, scientists utilize population genetics, quantitative genetics, and statistical methods to assess genetic values and predict the potential performance of offspring. Advances in molecular genetics have also become integral, allowing for a more precise understanding of the underlying genetic mechanisms. Animal scientists work towards enhancing the efficiency and productivity of livestock by selectively breeding animals with favourable traits. This interdisciplinary approach combines biology, genetics, and practical husbandry techniques to achieve the desired genetic improvements in various agricultural settings. Overall,

animal science in the context of animal breeding aims to optimize the genetic potential of livestock for improved production and sustainability.

Animal genetics:

Genetics involves the exploration of genes and their impact on living beings. The details within an organism's genes serve as a biological plan, dictating its appearance, functionality, and ability to thrive. This genetic information plays a pivotal role in shaping the similarities and distinctions between different organisms. In the realm of livestock, genetics holds paramount importance, significantly shaping animal production and health outcomes.

The animal production and genetics unit spearhead the fao's initiatives aimed at assisting nations in effectively managing the genetic aspects of their livestock populations. Their efforts focus on optimizing genetic traits to enhance livestock production and overall well-being, acknowledging the fundamental influence of genetics on these crucial aspects. Some of the classifications of animal genetics,

1. Basic genetics
2. Molecular genetics
3. Genetic engineering

1. Basic genetics

In animal genetics involves studying the principles governing the inheritance of traits from one generation to another. Traits are determined by genes, which are segments of dna located on chromosomes. In animal breeding, understanding basic genetic concepts is essential for making informed decisions in selecting and mating animals to improve desired characteristics. Some of the characteristics are,

- **Genes and alleles:** genes are units of heredity that carry information for specific traits. Animals inherit two alleles (gene variants), one from each parent.
- **Dominance and recessive ness:** some alleles may be dominant, expressing their traits over recessive alleles. Understanding dominance relationships is crucial in predicting trait expression.
- **Genotype and phenotype:** genotype refers to an animal's genetic makeup, while phenotype is the observable expression of traits. The interplay between genotype and environmental factors influences the phenotype.

- **Mendelian inheritance:** mendel's laws describe the inheritance patterns of traits, such as segregation and independent assortment, providing a foundation for understanding genetic inheritance.
- **Heterozygosity and homozygosity**: animals can be heterozygous (two different alleles) or homozygous (two identical alleles) for a specific gene. This impacts the variability and predictability of trait expression.

2. Molecular genetics

Molecular genetics in animal science involves studying the genetic material at the molecular level to understand traits, diseases, and breeding strategies in animals. Researchers use techniques like dna sequencing to analyse genes, identify mutations, and explore genetic variations influencing animal characteristics. This field plays a crucial role in improving livestock health, productivity, and developing breeds with desired traits through selective breeding and genetic engineering. Molecular genetics explores the intricate structure of dna at the molecular level, investigating its cellular processes, including replication, and its role in shaping an organism's overall characteristics. Genes, the fundamental units of heredity, are composed of dna and serve as both the physical and functional blueprint for traits. While some genes provide instructions for creating proteins, others do not encode proteins. In the human genome, genes exhibit size variation from a few hundred to over 2 million dna bases. The field of molecular genetics involves manipulating an organism's genetic material. Beyond agricultural contexts, it has been applied to craft personalized bacterial cultures. In the domain of dairy animals, molecular genetics facilitates the development of transgenic individuals with unique traits not attainable through conventional breeding methods.

3. Genetic engineering

In animal science, genetic engineering involves manipulating the dna of animals to achieve various objectives. This encompasses modifying genetic traits, bolstering disease resistance, improving productivity, and addressing genetic disorders. Techniques may include altering individual base pairs, adding or deleting dna segments to in still desired features. The utilization of genetic engineering in animal science is pivotal for advancing studies, refining livestock breeding, and crafting animals with specific attributes for both agricultural and medical purposes. The process of genetic engineering, also known as genetic modification, employs laboratory technologies to modify an organism's dna. This can encompass changing a single base pair (a-t or c-g), removing or adding dna regions. Applied in animal production, genetic engineering yields practical benefits, such as generating disease-resistant transgenic animals,

enhancing animal productivity, treating genetic disorders, and manufacturing vaccines. A genetically modified animal is one whose genetic makeup has been adjusted by adding, altering, or removing specific dna sequences in a manner not occurring naturally. This alteration aims to introduce new traits, like disease resistance. Transgenic animals are purposefully designed to explore the role of genes in disease development. Additionally, these animals serve as model organisms in research to devise cures for diseases, contributing to the development of medicines.

Genomic technology:

Genomic technologies, encompassing methods to manipulate and analyze genomic information, originated notably with dna cloning in the 1970s, marking a transformative era in the late 20th century. The historical significance of these technologies is profound, especially with the widespread availability of genome sequences for various organisms. This evolution offers a novel perspective on biology. Key advancements include dna cloning, macromolecular structure analysis (x-ray crystallography and nmr), dna sequencing, synthesis, polymerase chain reaction (pcr), and the development of transgenic animals.

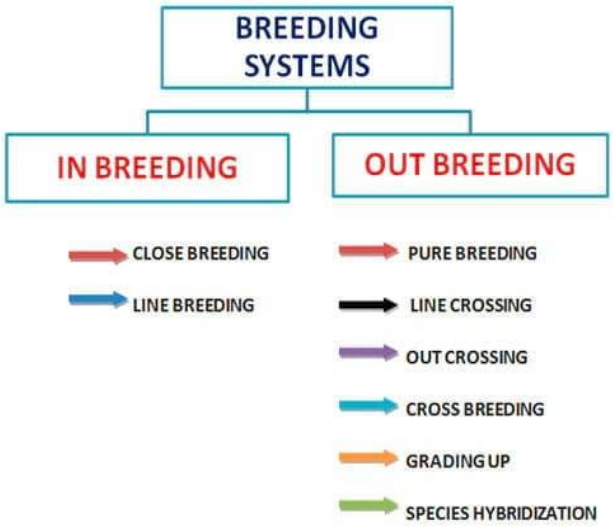
In animal science, genomic technology utilizes sophisticated tools like dna sequencing, genotyping, and bioinformatics analysis to explore an animal's complete set of genes (genome). This approach provides insights into genetic traits, identification of markers for desirable characteristics, and the improvement of selective breeding programs. The application of genomic technology is pivotal for understanding the genetic basis of complex traits, enhancing disease resistance, and optimizing livestock production for improved animal health and performance. Notably, genomic selection empowers breeders to address the rising demand for animal protein sustainably and ethically. Traditionally, breeders focused on studying observable traits like productivity and disease resistance, relying on phenotypic data and pedigrees to gauge genetic variation. The unravelling of genomes in various species now equips breeders with a transformative tool, reshaping perspectives on traits and the value of animals in breeding. This newfound genetic knowledge presents unique opportunities to contribute to future challenges, fostering production systems with healthy animals that deliver more with fewer resources. Additionally, genetic insights enable the selection of more social animals, positively impacting welfare, and the identification of cattle with reduced environmental footprints, such as limiting methane emissions.

Breeding of species

Breeding is the intentional propagation of domestic animals to enhance desirable qualities. This practice, ongoing for centuries, involves modifying animals to better serve human needs. Selective breeding integrates knowledge from various sciences like genetics, statistics,

reproductive physiology, computer science, and molecular genetics. This overview delves into the fundamental principles governing the transformation of animal populations through these sciences. It briefly touches on molecular genetics, immunogenetics, and modern reproductive technologies. Populations in animal breeding refer to interbreeding groups, often designated as purebred or nonpure bred. Purebred animals have meticulously recorded genealogy maintained in herd or studbooks by official associations, offering valuable services to their members.

Types of breeding:



In breeding:

In-breeding occurs when animals mate with relatives more closely related than the average relationship within their breed or population. On the other hand, outbreeding refers to matings between animals that are less closely related than the average within the given breed or population. In animal science, inbreeding refers to the practice of mating animals that are more closely related than the average relationship within a particular breed or population. This can lead to an increased likelihood of expressing recessive genetic traits and a reduction in overall genetic diversity.

Close breeding:

In the realm of intensive breeding, animals closely linked and tracing back to multiple common ancestors are involved. On the other hand, crossbreeding entails the mating of animals from different breeds. The enhanced characteristics observed in the offspring of crossbreeding are termed hybrid vigor or heterosis. In animal science, close breeding refers to a breeding practice where animals with close familial ties are paired for reproduction. This approach often involves mating individuals that share common ancestors, aiming to concentrate desirable traits within a specific lineage.

Line breeding:

In animal science, line breeding refers to a breeding strategy where animals with a common ancestor are selectively paired, but the degree of relatedness is less close than in strict inbreeding. This method aims to maintain desirable traits within a lineage while minimizing the potential negative effects associated with close relatives mating. Breeding animals with a shared ancestry, but not closely related, is known as linebreeding. This approach involves selecting parents that trace back to a common ancestor but are more distantly connected compared to close inbreeding.

Out breeding:

In animal science, outbreeding involves mating individuals from different genetic backgrounds or distinct lineages. This strategy is employed to introduce genetic diversity and reduce the risk of undesirable traits associated with close relatives mating. Outbreeding can enhance the overall health and adaptability of the population by promoting a broader genetic mix. Outbreeding, the opposite of inbreeding, involves producing offspring by mating individuals with no or distant ancestral connections. The extent of outbreeding is gauged by the coefficient of inbreeding, with f approaching 0, depending on the genetic separation between parents. Outbreeding, also known as outcrossing, allogamy, or xenogamy, entails the exchange of gametes between genetically dissimilar individuals. The overall benefit of outbreeding is fostering a rise in phenotypic diversity within a population.

Pure breeding:

Pure breeding in animal science involves the intentional mating of individuals with similar or identical genotypes to consistently produce offspring with uniform traits. This breeding strategy aims to establish and perpetuate specific desirable characteristics within a population, resulting in a genetically homogenous lineage. By selecting and pairing animals that exhibit the desired traits, breeders seek to create a stable and predictable genetic makeup. Pure breeding is commonly employed in the development and preservation of distinct animal breeds, ensuring that specific traits are consistently passed down through successive generations. This method plays a crucial role in maintaining the integrity and standardization of various breeds in the field of animal science.

Line crossing:

Line crossing in animal science refers to the breeding strategy where individuals from different lines or breeds are crossed to create hybrid offspring. This method aims to exploit heterosis or hybrid vigor, resulting in improved performance traits such as growth rate, disease resistance, and reproductive success. The process involves mating genetically distinct individuals

to capitalize on the complementary strengths of the parent lines. Line crossing is widely used in livestock production to enhance overall productivity and resilience in animals. This approach helps address specific breeding goals and can lead to more robust and economically valuable animal populations. However, careful planning and selection of parent lines are essential to achieve the desired genetic improvements in the offspring.

Out crossing:

Outcrossing in animal breeding involves mating individuals that are unrelated or distantly related within the same breed or species. This breeding strategy aims to introduce new genetic material into a population, increasing genetic diversity. Outcrossing can be beneficial for avoiding inbreeding depression, which occurs when closely related individuals mate, leading to a decrease in fitness and performance traits. By outcrossing, breeders seek to maintain or enhance desirable characteristics while minimizing the risk of inheriting recessive genetic disorders. However, careful selection of outcrossing partners is crucial to ensure compatibility and the preservation of specific traits within the breeding program. Overall, outcrossing contributes to the long-term health and adaptability of animal populations.

Cross breeding:

Crossbreeding, also known as outbreeding, is a breeding strategy that involves mating individuals from different breeds or genetic lines within the same species. This approach aims to capitalize on the benefits of heterosis or hybrid vigor, resulting in offspring with improved traits compared to the parent breeds. Crossbreeding can enhance characteristics such as growth rate, disease resistance, and reproductive performance. The genetic diversity introduced through crossbreeding helps mitigate inbreeding depression and promotes overall population resilience. However, effective crossbreeding requires careful selection of parent animals to complement each other's strengths and align with breeding goals. Balancing genetic diversity and maintaining specific traits are essential considerations in successful crossbreeding programs within animal science.

Grading up:

Grading up in animal breeding refers to the practice of incorporating superior genetics into a population by continuously mating purebred or higher-grade individuals with animals of lower genetic merit. This is often applied in outbreeding programs to improve the overall quality of a herd or population. The process typically involves using a purebred or higher-grade male to mate with females of lesser genetic quality. The resulting offspring have a higher percentage of the superior genetics, gradually improving the overall genetic makeup of the population over successive generations. Grading up is a strategic approach to enhance specific traits, such as

productivity, adaptability, or disease resistance, within a herd or breed, while maintaining the foundation of the original breeding stock. Successful grading up requires careful selection and rigorous breeding practices to achieve the desired genetic improvements.

Species hybridization:

It seems there might be a slight confusion in your question, as hybridization in the context of spices typically refers to plant breeding rather than animals. However, if you're referring to hybridization in the context of animal breeding, it generally involves crossing individuals from different breeds or lines to achieve desirable traits. In animal science, outbreeding through hybridization can be employed to enhance specific characteristics such as disease resistance, growth rates, or reproductive performance. Careful selection of parent animals is crucial to ensure compatibility and the expression of desired traits in the offspring. Hybridization in animal breeding aims to capitalize on heterosis, also known as hybrid vigor, which can result in improved overall fitness and productivity in the hybrid offspring. While spices generally pertain to plants, the principles of hybridization in animal breeding remain centered around introducing genetic diversity to achieve specific goals within a breeding program.

Breeding system with its classification:

Animal breeding systems encompass a diverse array of methodologies that are strategically employed to enhance specific traits in livestock populations. These systems are broadly classified into inbreeding, outbreeding, crossbreeding, selective breeding, and line breeding, each serving distinct purposes and facing unique challenges. Inbreeding involves the mating of closely related individuals within the same breed. This strategy aims to fixate desired traits and achieve uniformity within the population. However, it comes with drawbacks such as an increased expression of recessive defects and a potential loss of vigor due to the accumulation of deleterious alleles.

On the other hand, outbreeding involves mating unrelated individuals, often from different breeds. This approach promotes hybrid vigor, where the offspring exhibit superior qualities compared to their parents. The advantages include improved health and performance, but challenges may arise in maintaining uniformity and achieving trait fixation. Crossbreeding is a method where individuals from different breeds are mated to combine desirable traits. This technique leverages complementary characteristics and harnesses hybrid vigor for enhanced productivity and adaptability. However, it requires careful consideration of the initial cost and challenges in maintaining specific traits over generations.

Selective breeding entails choosing specific individuals with desired traits as parents to improve those traits over successive generations. This method offers precision in trait selection but is a slow process that may lead to a potential loss of genetic diversity within the population. Line breeding involves breeding within a particular family line to reinforce specific traits. This strategy aims at the concentration of desirable characteristics within a population. While it allows for the retention of specific genetic traits, there is a risk of inbreeding depression, highlighting the importance of balancing the benefits and drawbacks. In conclusion, the selection of an appropriate breeding system depends on the goals of the breeding program, the targeted species, and the specific traits to be enhanced. Successful animal breeding requires a thoughtful combination of these systems, considering their respective advantages and disadvantages to ensure sustainable and genetically diverse livestock populations.

Applications:

1. **Improved productivity:** animal breeding and genetics contribute to enhanced productivity by selecting individuals with higher growth rates, better feed conversion, and increased reproductive efficiency.
2. **Disease resistance:** breeding programs can focus on developing animals with increased resistance to diseases, reducing the need for antibiotics and improving overall herd health.
3. **Efficient resource utilization**: through genetic selection, animals can be bred for optimal resource utilization, minimizing waste and environmental impact.
4. **Adaptability:** breeding for traits related to environmental adaptability ensures that animals thrive in various climates and conditions.
5. **Quality of meat and milk:** genetics play a crucial role in determining the quality of meat and milk, including traits such as tenderness, marbling, and milk composition.
6. **Reduced environmental footprint:** selective breeding can lead to livestock with lower methane emissions and decreased environmental impact, contributing to sustainable agriculture.
7. **Enhanced reproductive performance:** genetic improvements in reproductive traits lead to increased fertility, shorter calving intervals, and overall better reproductive performance.
8. **Economic viability:** breeding for economically important traits, such as faster growth or improved feed efficiency, directly impacts the profitability of livestock operations.
9. **Conservation of rare breeds:** breeding programs aid in the conservation of rare and endangered animal breeds, preserving genetic diversity within the species.
10. **Optimized feed efficiency:** genetic selection for efficient nutrient utilization helps in reducing the amount of feed required for optimal growth.

11. **Resistance to environmental stress:** animals can be bred to withstand environmental stressors such as heat, drought, or limited forage availability.
12. **Enhanced wool and fiber quality:** in the case of fiber-producing animals, genetics influences the quality and quantity of wool or other fibers.
13. **Improved disease resilience:** selective breeding for disease-resistant traits helps in reducing the impact of various pathogens on livestock populations.
14. **Consistent milk production:** breeding for consistent milk production throughout lactation ensures a stable and predictable milk supply.
15. **Optimal body composition:** genetic selection can influence body composition, helping to achieve desired fat and muscle ratios for meat production.
16. **Behavioral traits:** selecting for favorable behavioral traits, such as docility, can improve handling and reduce stress in animals.
17. **Reduced input costs:** breeding for traits like feed efficiency and disease resistance can lead to lower input costs for farmers.
18. **Optimal egg production:** in poultry breeding, genetics plays a key role in achieving optimal egg production rates and egg quality.
19. **Improved eggshell strength:** genetic selection can enhance the strength of eggshells, reducing the risk of breakage during transportation and handling.
20. **Biodiversity conservation:** animal breeding and genetics contribute to the conservation of biodiversity by maintaining unique genetic traits within different livestock populations.

Positive effects of animal genetics:

Animal genetics allows for selective breeding, enhancing desirable traits such as increased milk production in cows or faster growth in poultry. Disease resistance, genetic engineering can create animals with enhanced resistance to diseases, reducing the need for antibiotics and promoting overall animal health. Higher productivity, selective breeding can lead to animals with higher reproductive rates, contributing to increased food production. Efficient resource utilization genetic modifications can result in animals that convert feed into meat or other products more efficiently, reducing resource wastage. Enhanced nutritional content, genetic modifications can be used to enrich animal products with essential nutrients, providing healthier options for consumption.

Negative effects of animal genetics:

Unintended consequences genetic modifications may lead to unintended side effects, affecting the overall well-being of the animals or causing unforeseen ecological impacts.ethical concerns, manipulating animal genetics raises ethical questions about the welfare of the animals,

especially if the modifications cause suffering or compromise their natural behaviors. Loss of biodiversity, focusing on specific genetic traits may lead to a reduction in genetic diversity within animal populations, making them more vulnerable to diseases or environmental changes. Unknown long-term effects, the long-term consequences of genetic modifications are not always fully understood, posing potential risks to ecosystems and human health. Economic disparities, access to advanced genetic technologies may create economic disparities among farmers, favoring large-scale operations over smaller ones. Public perception and acceptance negative public perception and resistance to genetically modified animals can hinder the adoption of beneficial technologies, even if they have potential advantages. Balancing the positive and negative aspects of animal genetics is crucial to ensure responsible and sustainable practices in agriculture and animal husbandry.

Conclusion:

In this comprehensive overview highlights the pivotal role of animal science, particularly in the domains of animal breeding and genetics. The intricate interplay between human reliance on animals, the historical evolution from hunter-gatherer societies to agriculture, and the emergence of animal science as a discipline underscores the profound impact of animals on various facets of human life. The exploration of animal breeding elucidates its significance in enhancing livestock production through the meticulous selection of desirable traits. From inbreeding to outbreeding, each breeding system comes with its advantages and challenges, emphasizing the need for a thoughtful combination to ensure sustainable and genetically diverse livestock populations. The applications of animal breeding and genetics extend beyond food production, influencing disease resistance, environmental adaptability, and the overall well-being of animal populations. Furthermore, the discussion on animal genetics delves into the molecular intricacies of genes and their manipulation through genetic engineering. The positive effects, such as increased productivity and disease resistance, are juxtaposed with potential negatives, including unintended consequences and ethical concerns. The emerging role of genomic technology adds a layer of sophistication to breeding programs, enabling a more precise understanding of genetic traits and contributing to the long-term health and adaptability of animal populations. In navigating the complexities of animal breeding and genetics, a delicate balance between positive advancements and potential drawbacks is essential. Responsible and sustainable practices, coupled with public awareness and acceptance, will play a crucial role in shaping the future of agriculture and animal husbandry. Overall, the integration of scientific principles, ethical considerations, and practical applications is fundamental for optimizing the genetic potential of livestock and ensuring a harmonious coexistence with the animal kingdom.

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ANIMAL BEHAVIOUR

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Introduction:

Behavior means "all the activities and reactions of the whole or part of organisms in relation to the environment". Most of the animal behavior is a sequence of responses (Movements of muscle contractions) related to some need of the species, such as getting food, acquiring and maintaining living space, protection, reproduction etc. In a broad sense "behaviour includes all those processes by which an animal's senses the external world and internal state of its body and responds to stimulations it perceives". Thus behaviour may be viewed as stimulus response relationship. Behavioural traits of organisms are controlled by many factors. Often behaviour is controlled by complex interactions of endogenous and environmental factors. Some of the endogenous factors are genotype, nervous system, endocrine system, pheromone system etc. and exogenous factors are innumerable.

Genetic aspect of behaviour

Genotype of an individual plays basic role in composing behavioural traits of an organism. Any behaviour pattern is constrained by the way in which genetic information is processed by the animal. The internal information-processing systems are established during the course of development from the fertilized egg, to the embryo, to the adult animal. Through the study of development of one individual viz, ontogeny, we can discover the ways in which genetic and environmental factors interact to give rise to the behaviour of organisms. Many workers studied the influence of genes and chromosomes upon behaviour using different organisms. McClearn and De Fries (1973); Ehrman and Parsons (1976) have studied chromosome anomalies in humans beings a number of genetically induced behavioural disorders including epilepsy, depression, mental retardation, Schizophrenia etc. Homozygous twins are very similar in their temperament because they have almost similar genotype. Homosexuality and Lesbianism behaviours are due to genetic reasons.

Evolutionary aspect of behaviour

Most of the behaviours of animals (Coelenterates to mammals) are expression of ability of organized form of nervous system. Learnt behaviours such as habituation, imprinting,

conditioned reflex, trial and error, latent learning etc. are coordinated and conducted by nervous system of the organism concerned. It took. more than a billion years to evolve from irritability of protoplasm to its cognition stage viz., the formation of nervous system, reflex action; conditioned reflex, trial and error etc. are under nervous control.

Hormonal aspect of behaviour

The endocrine system acts as a behaviour regulating factor in organisms through the production of hormones. Hormones may influence behaviour through their effect upon (i) The whole organism (ii) Morphologic structures employed in specific response pattern (iii) Peripheral receptor mechanisms and (iv) Integrative function of the nervous system.

Hormones are extremely potent chemicals which produce rapid changes in their various target organs. Such changes influence behavioural traits of organism indirectly. Example: Female pigeons do not normally lay eggs if kept alone or with only females, but she begins to lay eggs soon after a male pigeon is introduced to her. The pituitary secretion is influenced by the presence of male. Simple tests show that only the sight of a male is enough to cause ovulation in female pigeon.

Pheromones in behavior

Chemical substance contained in the secretion of an animal capable of producing communication between different individuals is known as pheromone, In other words "pheromones are scents secreted by exocrine glands and are transmitted as liquid or gases producing any kind of communication between different individuals". Pheromones are both intraspecific- Communicating between individuals of same species and interspecific - Communicating between individuals of different species. Pheromones may cause immediate behavioural response or it may alter the physiology of the receiving organisms, usually through the endocrine systems.

Types of behaviour

Animal behaviour can be divided into that which is shown by all members of a species (Species characteristic behaviour) and that which varies from one individual to another (individual characteristic behaviour). The species- characteristic behaviour includes the stereotyped behaviour patterns distinctive of particular species, for example courtship and copulation of many animals. The individual characteristic behaviour includes the behaviour learned by animal during its life time, for example, 'tricks' performed by individual dogs.

- **Stereotyped animal behaviour**

In the case of stereotyped behaviour, organism is to a large extent stimulus bound where a pattern of stimuli trigger a sequence of responses. Since this kind of behaviour is essentially the

outcome of inherited properties of the nervous system of the organism, it is also known as innate or inborn or inherent behaviour.

Stereotyped behaviour is of following three types- i) Taxes, ii) Reflexes, iii) Instincts.

i) Taxes

It is one of three types of stereotyped behaviours. Taxis means the orientation of the body with respect to the source of stimulation. It is a simple movement influenced by the direction of the stimulus. In taxis, the animal's body takes up a particular direction which may be combined with locomotion so that the animal moves towards, away from or at a fixed angle to the source. The simplest form of adaptive behaviour is the orientation of the organism in respect to some aspect of its environment. It may also be seen that in taxis, the orientation of body may also involve movement in respect to the stimulus. But all the orientations are not taxes. In a very simple case, the orientation may be nothing more than a series of random movement coupled with occasional avoidance or approach movements in response to a stimulus. Such orientation continuously and specifically guided by external stimuli is called taxis.

Types of taxes

Phototaxis:

Oriented locomotory movement caused by the light either towards source or away from it is called Phototaxis. e.g. Euglena, Ranatra etc. move towards the source of light (positive Phototaxis) while Planarians, Earthworms, Slugs, Copepodes, Siphonophores etc. move away from the source of light (Negative Phototaxis). Amoeba and Paramecium avoid both direct sun light and total darkness. They respond positively to normal or weak light. The housefly Musca domestica show positive response to the light while mosquito exhibits the negative response to it. Bed bugs display negative response to light whereas Hydra shows positive phototropism.

Thermotaxis:

Response to temperature is known as Thermotaxis. Animals strive to remain in a different temperature range e.g. Paramecium lies between 24°C and 28°C, which is temperature optimum for them. They avoid temperatures higher and lower than this. Animals thrive best at the optimum temperature e.g. the optimum temperature for Amoeba lies between 20°C and 25°C. An avoiding reaction is given to the temperatures higher and lower than this.

Chemotaxis:

Response of animals to the chemical substances in called Chemotaxis. It is negative is most of the cases e.g. Amoeba is negatively chemotactic to strong solution of alkalis

and to sugars. It also avoids substances unsuitable for it. Paramecium shows avoiding reaction to the salt solution, however, a positive responses occurs with a drop of weak acid solution.

Geotaxis:

Animal's response to gravity is known as geotaxis. Some of the animals show negative response to the gravity while others show positive response, e.g. Amoeba exhibit mostly positive response as it drops to the bottom of the container filled with water. Paramecium is generally geotactic in a culture containing in a test tube they gather close to the surface film with their anterior end pointed upwards: House fly *Musca domestica* also show negative response to the gravity. The fruit fly Drosophila when placed in a vertical glass tube moves upwards in the tube showing negative response to gravity.

Rheotaxis:

Response to current of air or water is called Rheotaxis. Most of the animals while Paramecia show positive rheotaxis orienting themselves with their anterior prefer to be drifted along the flowing water or air. Amoeba show negative response, ends upstream and swimming against the current. Some of the fishes are also positively Rheotactic. In winds, birds and insects mostly fly upwind although they may go in any direction.

Thigmotaxis:

Response to contact is called Thigmotaxis. Paramecium when slowly moves comes in contact with any object like an alga or a plant stem, becomes quiet but an avoiding reaction is seen when its anterior end is strongly touched with a solid object.

Galvanotaxis:

The response to electric current is called Galvanotaxis. A positive or negative response is seen in animals towards electric current. In positive galvanotaxis animals move towards the negative pole or cathode when a weak electric current is given. Such a behavior is seen in Paramecium when they are exposed to weak electric current. Paramecium move backwards towards anode when a strong electric current is given.

ii) Reflexes

Reflexes are very similar to taxes because they are relatively stereo typed and they may fit into the definition of innate behaviour in the sense they are outcome of inherited neural mechanism. Occasionally it is difficult to differentiate between taxes and reflexes. Taxes involved orientation of the whole body which may involve a number of specific reflex responses whereas reflexes involve all or most of the body like the flexion of leg in response to painful stimuli or the pupil constriction to intense light. Such reflexes are adaptive and are irreversible. There are two classes of reflexes which are as follow:

(a) Tonic reflexes: These are slow, long lasting adjustments which maintain muscular tone, posture and equilibrium.

(b) Phasic reflexes: These are rapid, short lived adjustments as seen in the flexure response. Reflexes may be organized at various levels of the nervous system and occur in varying degrees of complexities. Ex. Withdrawal of hand from a hot object, bathing of eyelids when anu object comes close.

iii) Instinctive

Among animals, there are many examples of elaborate behaviour that show adherence to a 'plan' that is relatively constant throughout a species and is not acquired through previous experience or learning. These types of behavioural patterns are considered to be 'instinctive'. We talk about actions as "instinctive" when they are done without conscious planning. The early ethologists considered innate behaviour to be behaviour that is determined by heredity and that is part of animal's original makeup and therefore that is independent of experience of the animal. In 1951, Timbergen postulated that various instinct centres in the body were arranged in a hierarchy. This helps the animal in saving the energy required for the stimulation of a particular behaviour. For example, initiation of reproduction in animals leads to a number of associated behaviours, such as nest-building, courtship and parental care.

Instinctive behaviour is of two types: Closed and Open

Closed instincts:

These are preprogrammed fixed motor patterns that are functional from the moment the neural circuitry is in place and are not modified by the environment. For example, males of the grasshopper Gomphocerippus rufus possess a complex courtship repertoire with a variety of components including head rocking, stridulation, antennal flicking and loud song. These elements are organized in an invariant sequence; the entire courtship pattern is performed for the female over and over again until either she accepts the male and copulates or the male wearies and goes elsewhere. There is no indication that the courtship sequence is ever altered in any way; instead, they are the product of a closed "motor tape" that can be played back time and again.

Open instincts:

In many species, behaviour that is functional when first performed, is capable of modification as a result of interaction with the environment. For example, by presenting cardboard models to chicks of the laughing gull of different ages, Hailman (1967,1969) found the following responses: (i) Older birds were better able to place most of their pecks on the bill of a model and not off to one side or the others. (ii) Pecking efficiency improved as well, with

the chicks growing better at judging the distance between themselves and the beak. Initially they might be right on target, but would strike the bill so powerfully that they would be knocked head over heels or be so far away from the bill that they failed to reach it. (iii) The chicks became progressively more selective in what they would peck at as time passed. During the study of food begging behaviour of chicks of laughing gulls they were offered a series of different models, some close in appearance to a laughing gull adult, others not remotely similar to a laughing gull. Chicks that initially pecked at almost anything would later refuse to bet from models other than those that were fairly accurate representations of the head of a laughing gull.

Adaptive significance of instincts:

Instincts are helpful for animal. An instinct renders the animals to acquire a pre hand knowledge of aversive situations in the environment and thus the animal is always ready to meet the challenges.

- **Motivation**

Motivation we mean fluctuation in the physiological state of an animal that result in animal responding in different ways to the same stimulus at different time (Barlow, 1977). One may substitute the term motivation with certain more familier terms such as drive, mood or tendency. For example, an animal eats or drinks in response to food and water to satiate its hunger or thirst. A bird builds a nest to complete its life's most vital activity- the reproduction.

- **Acquired animal behavior**

The acquired behaviour becomes more variable and modifiable through experience. The adaptation of the individual organism may develop uniquely in its life history through the process of learning. Such behaviour patterns are known as acquired behaviour.

Learning

"Learning" it can be defined as process which brings about certain adaptive changes in the behaviour of an individual as result of experience of individual. The ability to learn is a striking feature seen in most of the living organism. Thus, a process through which life experiences leave their mark on the individual through which it develops a new adaptation. Every organism can learn almost any response provided it is rewarded for that. Pavlov studied conditioned learning in dogs. His studies were concerned with the salivation of dog when they were provided with food. He found that a dog showed drooling response at a light flash or tickling of a metronome are present with enough food. He has also observed conditioned responses in several animals like chickens, rats and even in human beings. From current studies it is now known that it is not possible to link any response to any stimulus. Such stimuli in

behavioural limitations are seen in an animal which depends broadness or narrowness of the niche.

Types of learning

Learning is often classified into the following five major categories:

1. Non – associative learning: i) Habituation; ii) Sensitization
2. Associative learning: i) Pavlovian learning (Classical conditioning); ii)Operant conditioning (Trial and error learning)
3. Latent learning
4. Insight learning: i) Reasoning; ii) Intelligence; iii) Cognitive thinking
5. Phase-specific learning: i) Imprinting; ii) Avian song learning; iii) Language learning

1) Non – associative learning:

The mode of learning which develops in the absence of its association with any reinforcement (reward or punishment) is called non-associative learning. It is of following two types:

i) Habituation:

It is a simple learning not to respond to repeated stimuli which tend to be without significance in the life of animal. Unlike the other forms of learning, habituation involves not only the acquisition of new responces but the loss of old ones. If an animal is repeatedly given a stimulus which is not associated with any reward or punishment, it ceases to respond. Thus, the phenomenon in which repeated application of stimulus result in decreased responsiveness is called habituation. Habituation was first reported in 1887 by investigators testing the reaction of spiders to vibrating tuning forks. Initially, when the fork was vibrated, a spider would drop from its web by a thread to a distance of half metre. It would remain there for a time before returning to the web. With repeated tests the spider gradually reduced the distance to which it dropped and shortened the time of its return. After one month's training one spider remained on the web in spite of vibrations.

ii) Sensitization:

It is the opposite kind of change. While habituation means to become less sensitive to a stimulus, sensitization means to become more sensitive to a stimulus. For example, if an Aplysia receive an alarming stimulus such as an electric shock on the tail, it then respond more readily to other stimuli (such as prods to the siphon) to which it would otherwise have less responsive. It has become more sensitive, for if an Aplysia receives a dangerous stimulus naturally, it probably means some hazardous entity nearby, and it will pay to be careful.

2) Associative Learning:

Associative learning is a process whereby we acquire new responses and new capacities. In associative learning, a previous neutral stimulus or action has sufficiently important consequences to be singled out from other such events. Associative learning is of following two types.

i) Classical Conditioning:

Ivan Pavlov (1849-1936) discovered that it is possible to train a dog to salivate at the sound of a bell. This type of learning is called classical conditioning. Pavlov's classical experiment with dogs often involved the salivary reflex. Dogs salivate when food is put into their mouths and Pavlov could measure the strength of their response by arranging a fistula through the cheek from the salivary duct, so that the drops of saliva fell from a funnel and could be counted. A hungry dog was placed on a stand, restrained by a harness and every precaution was taken to exclude disturbances. In this position, it could be given various controlled stimuli such as lights, sounds or touch and meat powder could be puffed into the mouth through a tube. A standard quantity of meat powder caused the secretion of a certain amount of saliva. Now Pavlov preceded each ration of powder by the sound of a metronome (an instrument which sounds a bell) ticking. At first, the stimulus caused no response, viz. the dog pricked up its ears momentarily. However, after five or six pairings of metronome followed by food, saliva began to drip from dogs fistula soon after the metronome started and before the meat powder arrived. Eventually the amount of saliva produced to the metronome alone was the same as that which was given by the meat powder. Thus, the dog had learnt to respond to a new stimulus, previously neural, which Pavlov called conditioned stimulus. The salivation response to the conditioned stimulus (CS) is the conditioned response (CR). Prior to learning, only the meat powder called unconditional stimulus produced salivation as an unconditioned response.

ii) Instrumental (Operant) Conditioning

Instrumental (Operant) Conditioning is a type of learning where the animal has some control over the stimuli it receives and often over the responses it produces; its behavior will influence its situation. In 1930s, B. F. Skinner developed an apparatus that made it possible to demonstrate operant conditioning. This device, now called a skinner box, typically is a sound dampened and constantly illuminated box an animal had to press a small bar in order to receive a pellet of food from an automatic dispenser. Trial and error learning is a type of operant conditioning involving maze-running.

Trial and Error learning:

This can be done by giving the animal a choice of stimuli to respond to one with one response and other with another response. Here the animal is forced with a large number of alternative responses to a given stimulus where animal has to choose that response which result into better survival of it. Animal tries all the responses one by one and ultimately it learns to exhibit the right response. In the beginning of trial and error learning, animal makes large number of errors choosing wrong responses but with the time it learns to avoid these errors till when the animal makes the right response without making any error. This type of learning, forms a very major proportion of whole learning processes of animals because the animals are always surrounded by many alternative situations and responses from which they have to learn, to select the right situation and responses for their survival, e.g. the animal may be confronted with two doors one light and one dark, where food is behind light door, the dark door is locked and the animal must learn to approach or jump to the light door whether it appears to the right or the left. The animal here is required to make a discrimination of brightness but the same test could be applied by using two different colours triangle and circle. A loud and soft tone etc. in this type of situation, the animal is more obviously involved in a process of trial and error learning in which correct responses are encouraged by reward and incorrect responses discouraged by withholding reward or in some cases by giving punishment.

3) Latent learning:

When acquisition of a response is not displayed during learning but remains hidden (latent) and is expressed later, it is called latent learning. For instance, most animals will have knowledge of their surrounding because they explore them out of their curiosity. Insects and birds once fix a home territory, always have flight oriented toward their home. Latent learning is a peculiar form of learning which remains unrewarded at the time of learning. Wild animals judiciously utilize it in avoiding their predators and in searching their food.

4) Insight learning:

Insight learning is the highest form of learning in which the animals solve their problems too rapidly without a normal trial-error approach. For example, a male chimpanzee in sight of a bunch of banana in his cage, which was out of his reach, uses unsuccessfully a short stick. But failing to get fruits, he rakes a long stick he uses it to get the fruit. This demonstration of Kohler (1927) shows that chimpanzees used first latent learning of playing with sticks, but they applied it in some unusual context which speaks for insight learning.

i) Reasoning:

Reasoning is the ability to solve complex problems by behaving according to general principles rather than simply responding to the situation with simple trial-and-error behaviour or modification of stimulus-response behaviour. The animal should be able to put together elements from its past experience into new arrangements to meet different situations. Several types of tests have been devised to test an animal's reasoning ability. For instance, a detour problem consists of placing the animal in an environment where it must follow a circuitous route to a food source (or an escape path). A direct pathway to the food is blocked and the animal must go away from the food to succeed. The question is can the animal successfully solve the problem on the first try, or must it go through a series of trial-and-error procedures? Only the higher primates are good at this type of problem, although other animals may display the ability in rudimentary form. Dogs, rats and raccoons use trial-and-error learning to solve the detour problem. Another example of a reasoning problem is the discrimination learning test in which animal must choose between two or more responses depending upon the conditions. We can set up such a test using two doors, one black and the other white. The animal must learn to choose the white door if the light is on, but it must choose the black-door if light is off. This is an if-then reaction: if the light is on, then choose the white door; if the light is out, then choose the black. Higher primates are good at this type of reasoning problem. Other types of reasoning tests include the oddity principle where an animal is presented with several objects and it must choose the one that is different from the others; the delayed reaction test, in which the animal must find an object that has been hidden from view for a specified time; the triple-plate problem, in which the animal must learn a prescribed series of steps (such as pressing a number of levers in a correct order) in order to receive a reward or escape punishment.

ii) Intelligence:

Intelligence means the ability to learn or understand or to deal with new or trying situation. There are two main ways of assessing the intelligence of animals: one is to make a behavioural assessment and the other is to study the brain. Albert Binet in 1905 to intelligence behaviourally, considerable progress has been made in improving and refining them. Following tests have been designed to known the speed of learning which is an impotant measure of animal’s intelligence:

1. Intelligence test- a test designed to determine the relative mental capacity of a person to learn, e. g. achievement test, aptitude test etc.

2. Intelligence quotient (IQ)- It is a number used to express the apparent relative intelligence of a person that is the ratio multiplied by 100 of the mental age as reported on a standardized test to the chronological age.

5) Phase specific learning:

Imprinting

A more complex process than instinctive interaction between parent and young, occurs in species where the young are able to move around from the moment they are born or hatched. When such animals live in groups, both parents and young are faced with the problem of recognizing each other. There is a selective pressure on parents to care only for their own young and this creates a selective pressure on the offspring to approach only their own young and this creates a selective pressure on the offspring to approach only their own parents. Even, when such animals are solitary, the young need to keep in touch with their parents. In such species, there is a rapid kind of learning that is predisposed to happen at the time of birth or hatching. Imprinting is a specialized form of learning that is seen clearly in many kinds of birds during their early period of life. What seems to happen, at least in ducks, geese and chickens, the young bird is predisposed to learn the characteristic of any object that it sees and hears during a fairly short period soon after hatching, called critical period but now-a-days referred to as the sensitive period. The exact timing of the sensitive period differs between species. Domestic chick, for example, only follow objects they have seen during the first three days after hatching, whereas for mallard ducklings, the phase lasts for 10-15 days after hatching. The chick will not imprint on objects seen after that time. Normally what the young bird will see and hear during the sensitive period is its mother. But if we arrange things so that what it sees is either a human being or a red watering can or a blue flashing light borrowed from a police car, then it will learn the characteristics of that object instead. Bright, coloured, noisy, moving objects are most effective stimuli for imprinting experiments. But if no such stimuli are available, then chick will even imprint on their own feet. Although imprinting is most often studied in ducks and chickens, it occurs in many other species. For example, an imprinting process has been held to underlie mutual recognization by many goats and their kids. Unless a goat is able to see and smell her kid very shortly after birth, she will subsequently refuse to suckle it. Although later on it was suggested that the mother may take an active role by marking the kid in some way, there is obviously a sensitive period here, whatever the mechanism. It has also been suggested that imprinting process may be involved in the formation of bonds between human mother and their babies. Imprinting, thus, is a method of rapid learning of the mother's appearance by

newly hatched chick. This type of learning is preprogrammed to take place as part of the normal process of development and in whatever circumstance pertaining at the time.

Type of imprinting:

a. Filial imprinting:

It takes place in many species of birds and mammals. These are the kinds of animals with most extensive parental care. Imprinting is adaptive because it enables the young to recognize and follow their parents. They will grow up in a world of many hostile enemies and one or two protective parents. It is important that the young should choose the right animals to follow. The kind of imprinting that we have been considering so far is called filial imprinting. It is the imprinting of the following response which young animals make to their parents.

b. Sexual imprinting:

Foron, it concerns the species to which the animal will direct its sexual behaviour. In geese, for example, the sexual imprinting and filial imprinting are two different processes. A goose which follows a human being as if it were its parent does not have its sexual behaviour anything like so disturbed: when it grows up it will court other geese. Sexual imprinting also occurs early in life. Most experiments on sexual imprinting have been conducted on birds. It has been found that birds are most easily sexually imprinted on their own species, fairly easily on closely related species, and only with difficulty on very different species. Herring gulls and lesser black-backed gulls are similar species, and black-backed gull reared by herring gull parents (because some ethologist moves eggs between nests) become sexually imprinted on the herring gull i.e., the adult lesser black-backed gulls so produced will try to mate with herring gulls. They are sometimes successful. Most of the gulls which are hybrids between the two species around the British Islands are the result of these experiments, Sexual imprinting normally functions in the wild to ensure that the animal will, when it grows up, choose a mate of the correct species.

Memory

Learning is nothing without memory. We must be able to store the results of experience and recall them to our benefit later. Indeed one of the most remarkable properties of the nervous system is that it can retain some representation of past events for almost a life-time. - for few years in some cases.

Memory has the following parts:

1. Learning. The process of acquiring some activity or knowledge.

2. Remembering. The process where by the effects of past learning manifest themselves in the present.

3. Forgetting. Not directly observable, but manifested by a failure to remember.

4. Retention. The storage of learnt material in the brain.

Nature of memory:

The nervous system operates by transmitting electrical impulses along defined pathways. The process of learning involves heightened activity in those channels that record sensory impressions and their outcomes. It seems unlikely however, that heightened activity by itself could constitute memory, i.e., a memory could be stored in the form of a continuous train of nerve impulses running for years around the same pathways. Such ideas of "self-reverberating circuiting circuits" (for memory).

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