Directorate of Distance Education
NALSAR University of Law, Hyderabad
&
Truth Labs, Hyderabad

Reading Material

Advanced Post Graduate Diploma
in
Criminal Law and Forensic Science

1.2.6 Forensic Medicine, Biology & DNA

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FOREWORD

Phenomenal advances in Science and Technology and application of some such knowledge put to use by the criminals for illegal purposes has recently led to proliferation of cyber crimes and technology based crimes, etc. At the same time many conventional crimes are now being committed using new technologies through use of smart phones, computer software and hardware usage based on SMS messages and telephone call data, etc and these evidentiary items are acting as an essential proof for investigating many sensational crimes like rape, murder, theft, assault, burglary, cheating, etc. In the light of all that, extensive use of forensic science to unravel truth became an indispensable tool for criminal investigation and trials.

Against this backdrop, the knowledge of forensic science and its deep understanding and appreciation by all the end users viz. Police, Prosecutors, Advocates as well as the Judiciary, is paramount for ensuring peace and tranquillity in the society.

It is therefore necessary to familiarize the legal fraternity, notably the lawyers and legal officers who are one of the first respondents to be contacted by the victims and perpetrators of crime to be afforded an opportunity to get acquainted with the sociological, criminological and psychological factors that affect a normal person to commit a crime, the laws that are enacted for tackling the crime followed by investigation and Prosecution of crime as well as punishment and the roles and responsibility of the agencies that are assigned the tasks.

Similarly, it is also necessary for them to understand the technological nuances and delicate scientific intricacies involved in handling various types of evidence analyzed in different branches of forensic science by the experts of a modern forensic laboratory.

The advanced PG diploma course in Criminal Law and Forensic Science now offered from NALSAR is primarily aimed at fulfilling the above objectives and accordingly the reading materials prepared for the purpose will serve as a basic guide for understanding the fundamental concepts, processes and procedures for all those involved in handling the issues.

Date: 1st October, 2019
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UNIT I

LEARNING OBJECTIVES

In this unit you will be introduced to:

1. The different roles of Medico-legal expert in crime investigations.
2. The definition and importance of Forensic Pathology.
3. The Human Anatomy and Physiology.
4. Different parts of the body.
5. Structure and systems of the body.
6. Parts of the skeleton.

UNIT I-A-Forensic Medicine

1.0 INTRODUCTION

The word 'Forensic is derived from Latin word 'forum' which means a public place. In ancient Rome people assembled at the forum for judicial transactions. Hence in modern parlance the word forensic is synonymous with the term legal.

Forensic medicine is a branch of medical science which aids in the administration of justice. It is broad in its perspective and embraces different areas of medical knowledge and practice. Every law-enforcing official dealing with crimes involving the human body must have a sound basic knowledge of forensic medicine for the effective discharge of his duties.

In the past people depended upon empirical and perfunctory methods of investigation to prove the guilt or innocence of the accused. Trial by fire and trial by ordeal perhaps have taken their toll of many innocent lives in the days of yore. In the later period (18^{th} – 19^{th} centuries) the accused were tried with the help of eye witnesses and Juries, definitely a progressive step in the administration of justice, but still not fool- proof, as the evidence of the eye witnesses is not infallible. The beginning of the twentieth century saw the introduction of scientific evidence in the courts of law. In the past few years trust and confidence in scientific evidence have undoubtedly improved even as the Forensic Sciences themselves have progressed in reliability and effectiveness.

Scientific observations often allow for more than one interpretation depending on the circumstances of the situation. It is necessary therefore that the expert is provided with adequate information on the background and circumstances surrounding the incident so that he may draw conclusions from his scientific observations with the correct perspective of the situation as a whole.

Proper collection and preservation of materials from the scene of crime will ensure reliability of results in scientific examination. Care should be exercised in the transportation of such material to laboratories for scientific examination by the specialists. To make sure that material is not lost during transit, misplaced or tampered with, it is necessary that an unbreakable chain of custody is maintained. Each person in the chain should take responsibility for proper transmission of material to the next person and should maintain a record of the time of recovery of the specimen, what was done with it, when it left his possession and to whom it was given. Needless to mention the chain should be as short as possible.
Scientific evidence, by itself, usually does not establish the guilt or the innocence of the defendant, nor does it establish independently, the degree of guilt. It only provides an expert opinion based upon an objective assessment of the indisputable facts which help evaluate the reliability and credibility of other witnesses. The scientific witness provides the connection between the facts of the crime and the testimony of persons whose version of the episode is at issue.

The system of justice aims to discover the facts and to ensure that truth has it's full sway on the judgments passed in courts of law, but it is not able to adhere to these aims due to the inherent shortcomings of the system. The aim of the court is to place responsibility upon the contending parties in a litigation to present the

relevant facts and hopefully determine the truth, but in reality the adversary system often undermines the pursuit of the truth, with opposing sides seeking to win at all costs, without obligation to reveal information which may be detrimental to them.

The lawyer aims at winning the matter to defend his client's version of the facts and not at aiding the court in the discovery of the facts. He does not want the trial court to reach a sound and educated guess, if it is likely to be contrary to his client's interest. This is a special privilege enjoyed by the lawyers. An expert witness on the other hand has no such privilege and has no axe to grind nor does he have a personal interest to protect a client. His evidence is therefore objective, unbiased and impartial.

Forensic medicine, contrary to general belief, is not a static science. It is vital and continually growing, taking into its fold new scientific discoveries and effectively turning them to its own use, in its relentless war against crime and criminals.

1.1 ROLE OF MEDICO-LEGAL EXPERT IN CRIME INVESTIGATION

Medico-legal Expert

A medico-legal expert is a graduate of modern medicine (vis. Possessing

M.B.B.S. degree) who is employed in the service of the Government or a local body and authorized to deal with Medico-legal cases.

1.1.1 MEDICAL CODE GUIDELINES

The Tamil Nadu Medical code has dealt in detail with the issues relating to medico-legal examinations including autopsies on dead bodies. (Chapter XVII section II).

Para. 606:

Government medical officers and medical officers appointed by local bodies such as Panchayath unions and municipalities are authorized to conduct postmortem examinations on corpses sent to them if requested to do so by competent government officers. (viz. Police or Executive Magistrates).

Para. 607:

When a Government Medical Officer is absent from the station either on casual leave or other duty, the medical officer in charge of the nearest Government or Panchayath union medical institution should attend to the postmortem examination on receipt of the communication from the police officer concerned.

Para. 608:

Routine Medical Practitioners in India medicines are not qualified to conduct the postmortem examination and should not be entrusted with postmortem work under and any circumstances by the police.

Para. 612:

No time should be lost in the dispatch of the dead body to the medical officer for autopsy as every hour's delay means further decomposition and therefore additional difficulty in detecting the cause of death.

Para. 614:

Postmortem examination should be done expeditiously on the same day the body is received. Even if the body is received late in the evening at least the external appearances should be noted immediately and subsequent examination done early next morning, except in places where cold storage facilities are available for the preservation of dead bodies.

Para. 36:

Practitioners of Indian Medicine are not prohibited from issuing ordinary wound certificates or of drunkenness and their services may be requisitioned by police officers where practitioners of modern medicine are not readily available. It will however be for the courts to decide whether the certificates produced before them are to be accepted or not with reference to the evidence adduced.

1.1.2 PRIVATE DOCTORS

Private medical practitioners are not empowered to deal with medico-legal cases such as postmortem examinations. However in any emergency where human life is in jeopardy as in cases of accidents or assaults it is the primary duty of every doctor to attend on the rescue him from the risk of death. Refusal to attend on 'medico-legal' cases by private medical practitioners has in turn taken its own toll. Hence the Supreme Court of India was constrained to pass a Judgment (of Justice Rangnath Misra quoted in Hindu dt. 30.08.90) which clearly amplified that doctors are duty bound to attend and protect the lives of injured victims (eg. of an accident) brought before them including those cases of a medico-legal nature and there after the provisions of criminal law should be allowed to operate.

1.1.3 MEDICO-LEGAL CONSULTANTS: COMPOSITE ANDHRA PRADESH STATE MODEL (1990)

The Professor of Forensic Medicine in each of the 9 medical colleges in the state of Andhra Pradesh has been designated as the medico-legal consultant for 2 or 3 districts which constitute his medico-legal Jurisdiction as specified in G.O.No.15 of Home Dept. (Police. E) dt. 06-01-89.

S. No.	Professor of Forensic Medicine of	Consultant for the districts of
1.	Osmania Medical College, Hyderabad	Mahaboobnagar, Nalgonda and Hyderabad City

2.	Gandhi Medical College, Hyderabad	Rangareddy, Nizamabad, Medak and Secunderabad.
3.	Kakatiya Medical College, Warangal	Warangal, Karimnagar, Adilabad
4.	Guntur Medical College, Guntur	Guntur, Prakasham.
5.	Siddhartha Medical College, Vijayawada	Khammam, Krishna
6.	Rangaraya Medical College, Kakinada	West and East Godavari
7.	Andhra Medical College, Visakhapatnam	Visakhapatnam, Vizianagaram, Srikakulam
8.	Kurnool Medical College, Kurnool	Kurnool, Kadapa, Ananthapur
9.	S.V. Medical College, Tirupati	Chittoor and Nellore

A part from dealing with the medico-legal work of the particular city/town the state medico-legal consultant also offers the following services:

Expert opinions: On the materials/cases referred by medical officers such as tissues, skeletal remains, Hyoid bone and age cases, etc.

Second opinions: On the medico-legal reports of doctors, by re-interpretation of their findings in examination when it is so required by the Investigating officer.

1.1.4 ROLE OF THE MEDICO-LEGAL EXPERT

The services rendered by a medico-legal expert extend to both the living and the dead individuals. 'Clinical Forensic medicine' deals with the medico-legal examination of the living and 'Forensic Pathology' with that of the dead. The cases commonly examined in these two fields are tabulated below:

Clinical Forensic Medicine	Forensic Pathology
Wounds	Autopsies
Drunkenness	Mutilated/Fragmentary remains
Sexual offences Age determination	Skeletal remains
Poisoning	Exhumation

1.2 SCOPE OF THE SERVICES

A. Clinical Forensic Medicine

Wounds cases are examined to decide the type (eg. abrasion), age and nature (Simple/Grievous) of the wound as well as the type of the weapon (Blunt/Sharp) responsible for the same. In sexual offences cases, both the accused and the victim are examined for signs of recent sexual intercourse. Further, the accused in particular is examined for potency and the victim for determination of her age, if required. Age determination is also conducted in cases such as kidnapping, Juvenile crime and disputed age claims.

B. Forensic Pathology

Dead bodies (whether fresh, decomposed or exhumed or and whether intact or fragmented and mutilated) as well as skeletal remains can be examined for establishing the cause and time since death and for possible clues to establish the identity of unidentified bodies.
Unnatural or violent deaths are commonly caused by injuries, poisoning or asphyxia while natural or sudden deaths are caused by disease processes.

A host of physical characteristics and traits help in identification of the body while the onset and progress of the postmortem changes are of use in estimating the time that has elapsed since death.

UNIT I-B-Human Anatomy & Physiology

1.3 INTRODUCTION TO HUMAN ANATOMY AND PHYSIOLOGY

Human anatomy is the study of the structure of the human body and physiology is the study of the functions of the body or its component parts.

An elementary knowledge of human anatomy and physiology is necessary for police officers to understand and appreciate the medico-legal reports issued by doctors. However when a medical term or concept is obscure a medical dictionary or an expert should always the consulted.

Anatomical position

For description of the human body and its parts, the body is assumed to be in the standing position with arms at the sides and palms facing forwards. This is known as the anatomical position and helps to standardize descriptions.

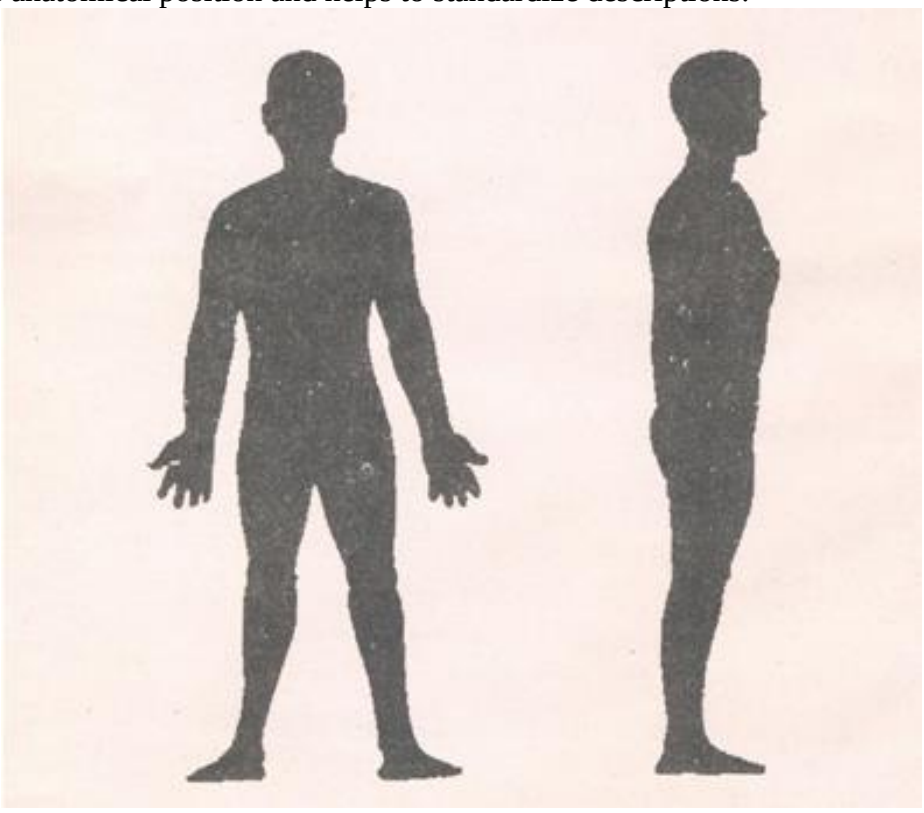

Fig. 1 Anatomical Position

1.4 TERMINOLOGY

Common descriptive terms used in medical reports are:

1. Sagittal plane : An imaginary line dividing the body into a right and a left portion.

2. Median plane : Mid-line of the body dividing it into right and left halves.
3. Coronal plane : Vertical plane at right angles to the median plane.
4. Anterior : Front of the body, limbs or organs
5. Posterior : Back of the body, part or organ
6. Superior : Towards the head end or upper part of the body
7. Inferior : Away from the head end or towards the lower part of the body.
8. Proximal : Nearer to the centre of the body (midline)
9. Distal : Farther from the centre of the body
10. Medial : Inwards, closer to the midline
11. Lateral : Outward; away from the midline

The terms 4 to 11 are used in a relative sense.

Parts of the body

The human body consists of the head and neck. The torso or the trunk and the limbs. The trunk consists of the thorax and abdomen which are separated internally by the diaphragm. The pelvis forms the lowermost part of the trunk.

Body cavities:

1. Cranial cavity : It is formed by the skull and contains the brain.
2. Thoracic cavity : It is formed by the rib cage and contains the lungs

and the heart enclosed in fibrous membranes or sacs, known as the pleura and pericardium respectively.

3. Abdominal Cavity : It contains the stomach, intestines, liver, spleen,

kidneys and pancreas.

4. Pelvic cavity : It contains the urinary bladder and the internal genital organs.

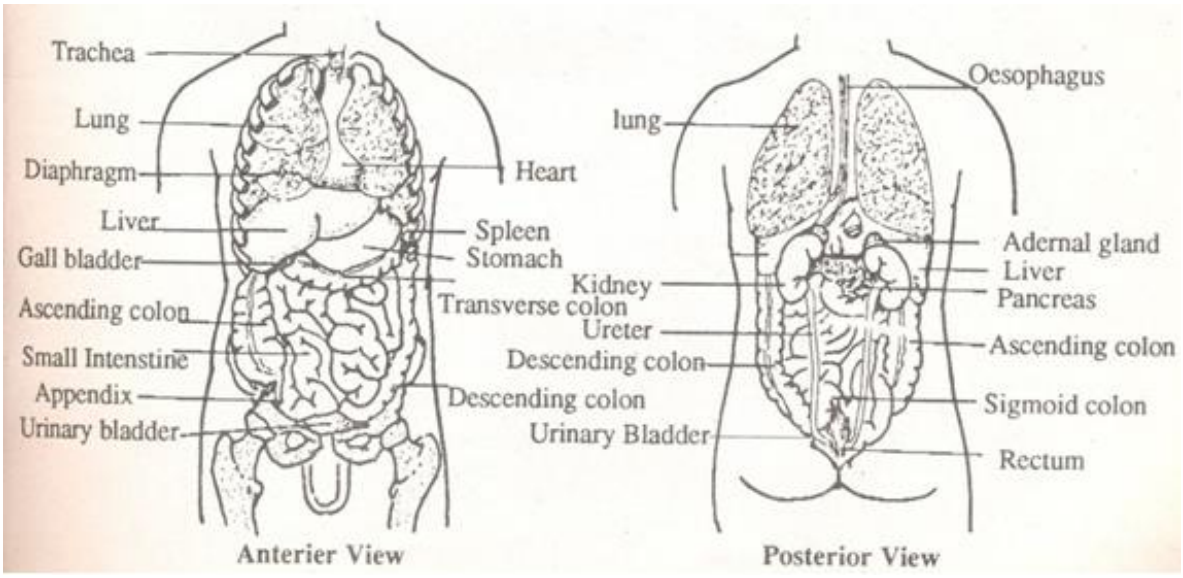

Fig. 2 Organs in the Thoracic, Abdominal and Pelvic Cavities

1.5 STRUCTURE OF THE BODY

Cells:

The smallest unit of every living organism is the cell, seen normally under a microscope and consisting of an external lining membrane celled the cell wall which encloses the living material (Protoplasm) around a highly organized structure, the nucleus, which controls all cell functions. The nucleus contains the genetic material called chromosomes which determine individual hereditary traits. There are 23 pairs of chromosomes in human cells of which one pair the sex chromosomes, are specialized in function.

Tissues:

A tissue is a collection of cells that form a basic functional unit. The 4 main types of human tissue are epithelial. Connective, muscular and nervous tissue. The epithelial tissue covers the body and lines the hollow viscera and protects other tissues. The connective tissues such as bone, cartilage, fat and fibrous tissue are mainly supportive to the other structures. Muscle tissue is contractile, while nervous tissue transmits impulses.

Organs:

Each organ in the body has a definite function, and is composed of different tissues, though predominantly it is made up of tissues associated with the specific functions of the organ e.g. Muscles. Organs with similar functions are united to form systems.

Systems of the Body:

1. Nervous system: Regulates and coordinates the activities of all the organs. It consists of the brain, spinal cord and the peripheral nerves.
2. Cardio-vascular system: It deals with blood circulation. The blood stream delivers nutrients and oxygen to all the organs and simultaneously carries away

the waste metabolites. It consists of the heart and blood vessels.

3. Respiratory system: It performs the function of gaseous interchange between the body and the environment receiving oxygen and expelling carbon dioxide. It consists of the lungs and the respiratory tract.
4. Musculo- skeletal system: This is concerned with locomotion. It consists of the bones, muscles and tendons.
5. Digestive system: The nourishment required by the body is received through it. Complex food substances are converted into simpler, water-soluble substances which are absorbed and assimilated. It consists of stomach and intestine and other digestive organs like the liver and pancreas.
6. Genito-urinary system: Genitals are concerned with reproduction. The urinary system, consisting of kidneys, ureters, bladder and urethra – deals with the excretion of waste products.
7. Endocrines: They secrete special substances called hormones which enter the blood and influence metabolic functions. The pituitary, thyroid and the adrenal are the chief endocrine glands.

1.6 HUMAN SKELETON

The bones of the skeleton provide support, protection and form to the body, and together with the muscles, aid bodily movement.

There are more than 200 bones in the human body (approximately 203 µ 3), varying in size and shape. They may be grouped as short, long, flat and mixed bones, based on shape. The bones of hands and feet are short bones. The bones of limbs, excluding hands and feet, are the long bones. Flat bones are present in the vault of the skull and mixed bones at its base.

1.6.1 Parts of the Skeleton

The skeleton comprises of the

A. Skull

B. Bones of the trunk

C. Shoulder girdle and upper limb bones

D. Pelvic girdle and the lower limb bones

A. Skull:

The skull is formed from a number of bones and is divided into a cranial and facial part.

The cranial part has a base and a dome or a vault and encloses the cranial cavity, which lodges the brain. It is formed by the frontal bone on the front, temporal bones at the sides, parietal bones at the top and occipital bone at the back. The less known sphenoid and ethmoid bones are present at the base.

The facial part is mainly composed of the maxilla or the upper jaw, the mandible or the lower jaw, the zygoma or the cheek bones and the nasal bones. In an adult there are 8 teeth in each half of the jaws.

The bones of the vault of the skull articulate with each other by joints known as sutures, which start fusing by 30-35 yrs. The two parietal bones articulate with each other forming the sagittal suture. They also articulate with the frontal, occipital and temporal bones, forming the coronal, lambdoid and temporal sutures respectively. The obliteration of the sutures helps in the assessment of age.

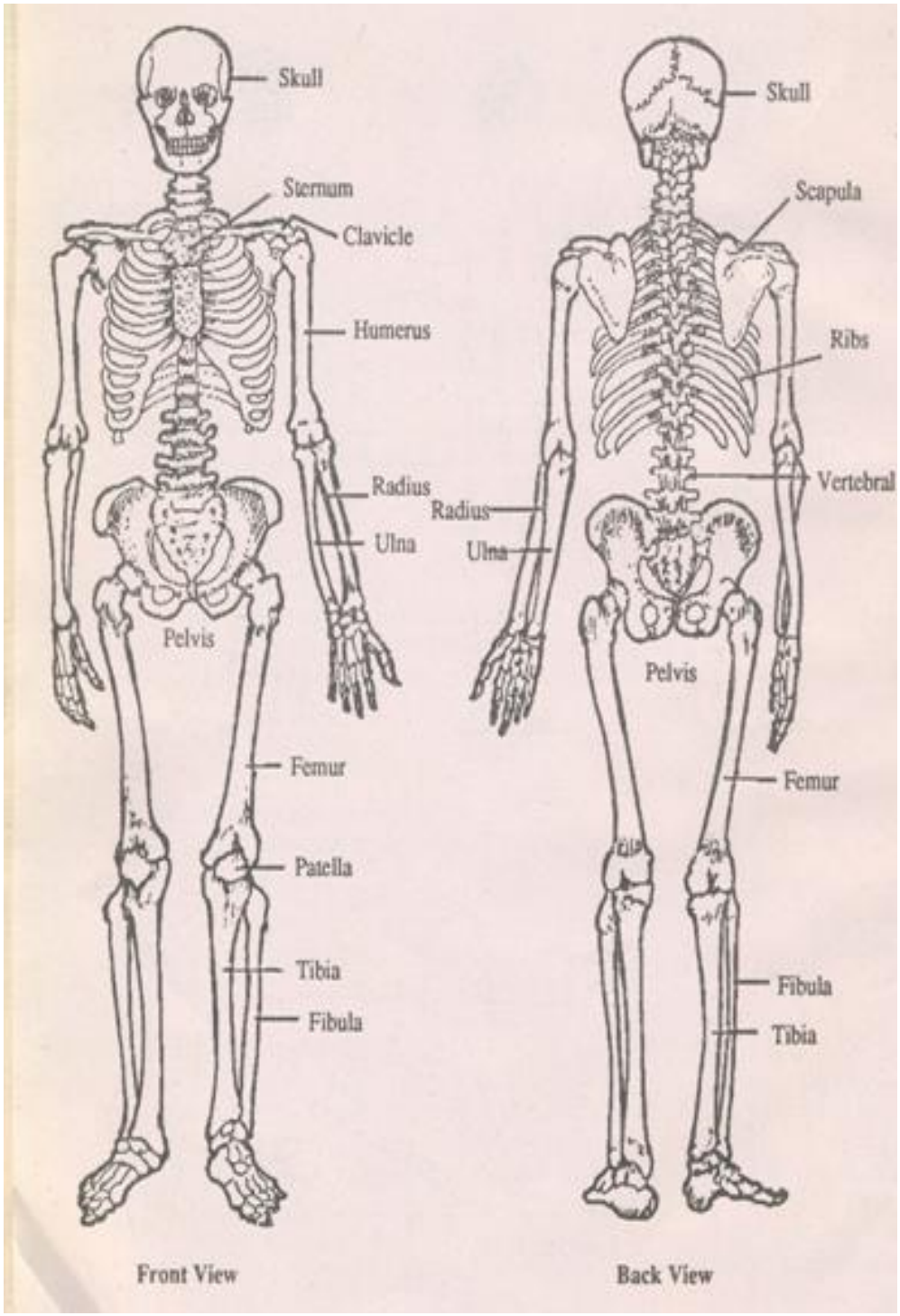

Fig 3: Bones of the Human Skeleton

B. Bones of the Trunk:

They consist of the spinal column and the rib cage or thorax.

The spinal column consists of 33 vertebrae. They are 7 cervical, 12 thoracic, 5 lumbar, 5 sacral and 4 coccygeal vertebrae. The first and second neck vertebrae are known as atlas and axis respectively and their fracture is immediately fatal. Injury to the lower cervical vertebrae may produce paralysis of all the 4 limbs (Quadriplegia).

The rib cage is formed by 12 pairs of ribs and the breast bone or sternum in the front. The first 7 pairs of ribs articulate with the sternum by rim (Costal) cartilages and are called the true ribs. The last two ribs are the shortest and their front ends are free and are hence called the floating ribs. The cartridges of the 8, 9 and 10^{th} ribs are attached to the overlying rib cartilage, forming the costal arch.

C. Shoulder girdle and upper limb bones:

The shoulder girdle is formed by the clavicle or collar bone in the front and the scapula or shoulder blade at the back.

The upper limb consists of the following bones: Humerus in the upper arm, Radius (on the outer side) and ulna (on the inner side) in the fore-arm, 8 carpal bones at the wrist, 5 metacarpal bones in the hands, and the phalanges in the fingers.

D. Pelvic girdle and the lower limb bones:

The pelvic girdle is formed by the two hip bones (each of which is formed by the fleum, ischium and pubis) which articulate with each other at the front (symphysis pubis) and with the sacrum at the back, together forming the pelvis.

The lower limb consists of the following bones: The femur in the thigh, tibia (on the inner side), fibula (on the outer side) in the leg, 7 tarsal bones at the ankle, 5 meta tarsal bones in the foot and the tarsal phalanges in the toes.

1.6.2 Joints and Muscles

The bones articulate with each other forming the joints. The main joints are at the shoulder, elbow and wrist in the upper limbs and the hip, knee and ankle in the lower limbs.
The muscles in the body are of 2 types namely the voluntary or striated muscles present in the head, trunk or extremities, which contract at the will of man, and the involuntary or smooth muscles which are present in the walls of the internal organs eg: Bladder and the blood vessels, whose contractions are not under conscious control. The heart muscle is unique in that it is striated in structure but yet involuntary in function.

Ossification: The long bones have a shaft in the middle called diaphysis and two ends at either side called epiphysis. To begin with, the epiphysis is made up of cartilage and subsequently converted into bone by the deposition of calcium salts. This process is known as ossification. Its onset, progress and completion in different bones is utilized for determination of the age. Ossification can be studied

even in the living with the help of x-rays.

Medico-Legal Aspects: Examination of the skeletal remains by medico-legal expert is helpful in establishing the victim's identity and the time and cause of death.

Skull, pelvis and femur are especially helpful in sex determination. The long bones are useful for estimation of the stature. The skull is also used for deciding the race from cephalic index and assessing specific identity by the Superimposition test. The cause of death is established from the injuries and evidence of poisoning if any.

1.7 VITAL SYSTEMS OF THE BODY

The nervous control, circulation and respiration are the vital functions in the body indispensable for life. The systems dealing with them are considered below:

1. Central nervous system:

The brain and the spinal cord constitute the central nervous system. They are formed of the grey matter consisting of nerve cells and white matter composed of the nerve fibres.
The brain weighs about 1200grams and is covered by membranes known as the Meninges. It consists of three main parts: Cerebrum, Cerebellum and Brain stem. The cerebrum consists of two halves known as the cerebral hemispheres, each of which has frontal, temporal, parietal and occipital lobes. Its surface is thrown into folds known as convolutions. A cavity known as the lateral ventricle is present in the substance of the cerebral hemisphere, containing cerebro-spinal fluid. This communicates with the other ventricles. The cerebellum is located below and at the back of the cerebrum. The brain stem is at the base of the brain and contains the mid brain, pons and medulla. The medulla continues downward and joins the spinal cord.

The cerebrum is the seat of higher nervous functions, like memory and intelligence. The cerebellum coordinates all the muscular movements of the body. The brain stem contains the vital centres which control circulation and respiration.

As the cranial cavity is a closed and limited space, injury to the head resulting in bleeding into it or swelling of the brain leads to coma and death unless duly attended to. In old age the diseased cerebral vessels may rupture under stress leading to cerebral haemorrhage and rapid death.

2. Cardio-vascular system:

It consists of the heart and blood vessels. The heart is a muscular pump which maintains blood circulation. The blood vessels basically are of 2 types the arteries, which carry pure (oxygenated) blood away from the heart, and the veins, which carry impure (de-oxygenated) blood towards the heart. The arteries and the veins are linked together by minute, microscopic blood vessels called the capillaries.

The heart is located in the chest between the two lungs in the mediastinum and is covered by a fibrous membrane known as the pericardium. It is roughly the size of

a person's fist and weighs about 300 grams.

The heart has 4 chambers. The upper ones are called the atria or auricles and the lower ones the ventricles. The right atrium communicates with the right ventricle by the Tricuspid valve and the left atrium communicates with the left ventricle by the Mitral valve.

Impure blood is brought to the right atrium by two major venous channels namely superior and inferior vena cava. From the right ventricle blood is pumped into the lungs through the pulmonary artery. The purified blood reaches the left atrium from the lungs by the 4 pulmonary veins. It is distributed from the left ventricle through the aorta and its various branches to all the organs and parts of the body. Thus the tissues of the body are constantly supplied with oxygen by the arterial blood. The heart muscle is known as the myocardium and is supplied by the right and left coronary arteries. Blockage of a diseased coronary artery by a thrombus causes sudden death from myocardial infarction.

3. Respiratory system:

The respiratory system consists of the lungs and the respiratory tract comprising the Larynx (Voice box) Trachea (windpipe), the two bronchii, and their terminal branches. The Nasopharynx connects the nose and the Larynx. The Larynx is located below the hyoid bone and is made up of the thyroid and cricoid cartilages. The opening in the larynx is closed during swallowing by the epiglottis.

The bronchii divide repeatedly in to minute branches in the lungs, which end in the air sacs or the alveoli, where the exchange of gases takes place. The oxygen from the inspired air enters the venous blood in the lung capillaries and the carbon dioxide from the same enters the air sacs and is expelled during expiration.
Any mechanical interference with respiration leads to rapid death from Asphyxia.

1.7.1 INTER-RELATIONSHIP

The functions of the vital organs are closely inter-related. The brain controls the circulation and respiration through its centres in the brain stem. The heart beats about 72 times per minute and keeps the blood (about 5 liters in total) in constant circulation. Inspiration and expiration succeed each other about 18-20 times per minute, breathing in and breathing out the air and purifying the blood in the lungs. These vital functions go on ceaselessly in the human body without the conscious knowledge of man, irrespective of work, rest or sleep, from cradle to grave.

GLOSSARY

1. Medico-legal
2. Forensic Pathology
3. Ossification
4. Humerus
5. Ethmoid
6. Zygoma
7. Bone
8. Bone
9. Cells
10. Tissues
11. Organs
12. Cavity

LEARNING OUTCOME

After studying this unit you should be able to:

1. Mention the different roles of Medico-Legal Expert during a crime investigation.
2. Understand the importance of Forensic Pathology.
3. Describe and understand the function of each part of the body.
4. Describe the different structures and systems of the body.
5. Importance and function of different parts of the skeleton.

SUGGESTED READINGS

1. Forensic Medicine and Toxicology – Dr. S. N. Tiwari.
2. Anatomy of Murder – Mr. P. K. Jain.
3. Criminalists Forensic Science and Crime – Mr. James.
4. Atlas of Forensic Pathology – Mr. Shetty B. S. K.
5. Forensic medicine for the Police – Mr. B. Umadethan.
6. Practical Manual on Human Physiology – Prof. Sunita Mishra.
7. Forensic Medicine and Toxicology – Dr. Anil Aggrawal.

UNIT II

LEARNING OBJECTIVES:

In this unit you will be introduced to:

1. The purpose of Identification of the Living and Dead.
2. The different acquired traits listed.
3. The types of occupation marks that the Investigator comes across at the scene of crime.
4. Investigation of Personal belongings.
5. Signs of Death (Immediate/early and late).

UNIT II-A-Identification of the Living and Dead

2.0 DEFINITION

Identification is the exact establishment of the individuality of a person, either living or dead.

2.0.1 CIRCUMSTANCES

Identification is commonly required in respect of: -

A. Living persons

a. Absconding criminals/soldiers

b. Missing persons

c. Mix-up of new-born babies in hospitals

d. Escaped prisoners/lunatics

e. Imposters

B. Dead bodies

a. Unknown dead bodies

b. Dead bodies which are decomposed, charred or disfigured

c. Human remains: fragmentary, mutilated or skeletal remains.

2.0.2 PURPOSE

Identification may be required both in Civil and Criminal Cases.

A. Civil: Identification is necessary in matters like insurance claims, matrimonial disputes, affiliation and inheritance cases, impersonation and issue of passports, etc.

B. Criminal: Identification a dead body is vital in the establishment of corpus delicti Identification may be required either of a single dead body or of multiple bodies involved in mass disasters like air crashes, railway accidents and conflagrations. The bodies to the identified may be entire and intact or available only in fragments, which may be in one place or scattered.

2.1 DATA USEFUL FOR IDENTIFICATION

1. Race
2. Religion
3. Sex
4. Age
5. Stature
6. Physical Features

7. Deformities and malformations

8. Impressions

a) Fingerprints

b) Footprints

c) Lip prints

d) Palato-prints

e) Nail-prints

9. Acquired traits

a) Tattoo-marks

b) Scars

c) Occupation marks

10. Personal belongings

11. Individual traits

a) Speech and voice

b) Bait

c) Handwriting

d) Mental caliber and knowledge

e) Mannerisms, habits

Identification of living persons is primarily a matter for police investigation while identification of the dead often requires the medico-legal expertise of doctors. While the last category of data mentioned above relates only to the living, the rest in general are helpful in identifying both the living and the dead.

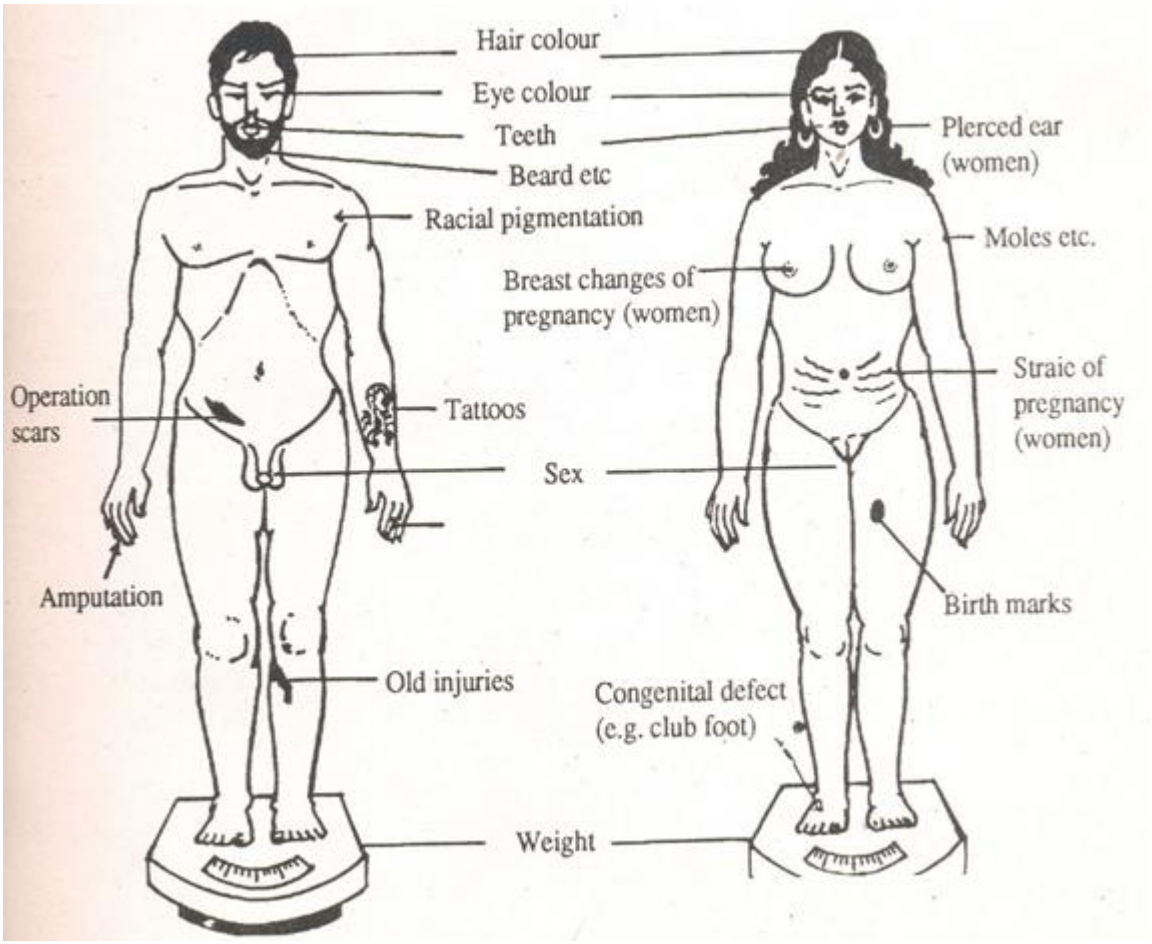

Fig. 1 Useful Data for Identification

2.1.1 RACE

Identification of the racial origin has assumed importance in recent times, as a result of the increase in international travel, foreign tourist and student influx, and consequent mix-up of the races.
The three main human races are:

1. Caucasians e.g. Europeans
2. Mongolians e.g. Japanese, Chinese
3. Negroid e.g. Negroes, West Indians

They can be distinguished on the basis of characteristic physical features.

However in doubtful cases expert advice should be sought.

Table: Identification of races

CHARACTER	Caucasian	Mongolian	Negroid
COMPLEXION	White	Yellow	Black
STATURE	Normal	Short	Tall

FEATURES	Normal	Small eyes	Thick, everted lips
SCALP HAIR	Blond or dark brown straight or curled	Coarse, black straight	Black, and kinky
SKELETON			
SKULL	Round	Square	Narrow and elongated
FACE	Small	Large and flat with Prominent cheek bones	Cheek bones Prominent Jaws projecting
LIMBS	Normal	Small	Large
CEPHALIC INDEX:	80 – 85	75 – 80	70 – 75
BREADTH ---------- OF SKULL X 100 LENGTH	Short-headed	Medium-headed	Long-headed

2.1.2 RELIGION

The Hindu and Muslim individuals may be distinguished as follows:

Table: Identification of Religion

HINDU MALES	**MUSLIM MALES**
Circumcision of penis absent	Boys are invariably circumcised by 11yrs
Tuft of hair (Pig-tail) may be present on the back of the head, especially in northerners	No pigtail is present
Sacred thread is worn is some communities	Not worn
Religious marks may be found on the forehead	Collosities are seen on the forehead, left knee and ankle due to the posture adopted at prayer (Namaj)
Necklace of wooden beads may be worn	Not worn
Tattooing may be present	Absent
HINDU FEMALES	**MUSLIM FEMALES**
Vermillion mark on the forehead or in the parting of hair	Vermillion mark absent
Ear lobules pierced	Rim (helix) of the ear may be pierced at multiple sites

Nose ring aperture to either nostril	Nose-ring aperture to the septum
Mangalsutra worn after marriage	Not worn
Tattoo marks may be present	Absent
Hindu widows (e.g. Brahmins) may shave off scalp hair	No shaving of the head

2.1.3 SEX

The male sex is characterized by the presence of testes and the female sex by the presence of ovary, which are the gonads or the sex glands. The function is indicated, after puberty, by seminal emission in the male and menstruation in the female.

Secondary sex characters: They are developed after puberty and strongly indicate the sex.

Table: Secondary sex differences

TRAIT	MALE	FEMALE
BUILD	Larger, muscular, shoulders broader then hips	Smaller with smooth contours. Hips broader than shoulders
CHEST HAIR	Generally present	Absent
PUBIC HAIR	Extends to the Navel	Confined to the Pubis
MOUSTACHE AND BEARD	Present	Absent
ADAM'S APPLE	Prominent	Not developed
VOICE	Low-pitched	High-pitched
BREAST	Rudimentary	Developed

Genitals

In general, the presence of external genitalia, such as the penis in the male and vagina in the female corroborate the sex, but in states of intersex or hermaphroditism, there may be various degree of commingling of the genital characters.

Nuclear sexing

Sex chromatin (Barr body) is a condensation of the nuclear material present in the nuclei of female cells. This can be studied in the mucosa of the mouth, tissue tags, fresh blood stains or the roots of hair.

Skeleton

Sex differences are distinctly seen in the bones of the skeleton after puberty. The male bones are as a rule heavier, longer and more rugged, with prominent muscle markings and larger particular surfaces.
The skull, pelvis and the long bones (especially femur) are very helpful in the determination of sex.
In decomposing bodies, the prostate in the male and (Non-pregnant) uterus in the female are dependable indicators of sex, as they resist putrefaction longer.

2.1.4 AGE

The age of an individual can be determined on the basis of the following:

A. Height and Weight

These are useful indicators mainly in early childhood (up to 5 yrs). The height and weight should be compared with the classified data in the I.C.M.R. bulletins on child growth.

B. Teeth

There are 2 sets of teeth namely deciduous or milk teeth and permanent teeth. The types of teeth encountered in each half of the jaw are incisors, canine, premolars and molars. They extend in that sequence from the front of the jaw to the back. Pre-molars are absent in the deciduous teeth.

Table: Eruption of the teeth

TYPE	TOTAL	DENTAL FORMULA I C PM M	ERUPTION
A. Milk teeth (Deciduous)	20	2 0 2 1	Starts in 6th month completed by 2 ½ yrs
B. Permanent teeth	32	2 3 1 2	Starts from 6th years completed by 17 - 25 yrs
I = Incisor;	*C = Canine*	*PM = Pre-molar*	*M – molar*

Thus a child below 6 yrs has only temporary teeth and one after 12 yrs of age generally has only permanent teeth. A person between 6 – 12 yrs has mixed dentition. Teeth are shed in old age. Eruption of teeth is not helpful for age determination after the individual crosses 21 years. However in dead bodies the examination of teeth by Gustafson's method may help in the estimation of age even in older individuals with a range of error of 3.5 years.

C. *Secondary Sexual Characters* Are seen after puberty

Table: Secondary sexual characters

TRAIT	MALE	FEMALE
PUBIC HAIR	Appearance: 14 yrs	Appearance: 13 yrs
AXILLARY HAIR	Appearance: 15 yrs	Appearance: 14 yrs
MOUSTACHE AND BEARD	Appearance: 16 – 18 yrs	Absent
VOICE	Breaking 12 – 14 yrs (low – pitched)	High - pitched
BREAST	Rudimentary	Development 12 – 14 yrs
MENSES	-------	Onset 13 – 14 yrs

D. Miscellaneous

Greying of the hair especially on the chest and pubis and the presence of Arcus Senilis in the eye are indicative of old age.

E. Ossification study

X-ray examination of the joints of the limbs for the appearance and fusion of the ossification centres is helpful to corroborate and confirm the clinical findings.

2.1.5 STATURE

The length of a dead body is usually 1.25-2 cm more than that in life due to loss of elasticity of the tissues and consequent flattening of the body curvatures. The body length should be taken by keeping two wooden blocks one touching the vertex and another touching the soles of the feet and measuring the distance between them.

Anthropometry (Bertillon's system)

It is an obsolete system wherein 11 physical measurements were taken for the identification of criminals. The measurements taken include standing and sitting height, length and breadth of the head and right ear, length of left cubit, foot, little and middle finger and span of outstretched arms. It has been replaced by Dactylography or the Fingerprint System.

2.1.6 PHYSICAL FEATURES

A. Complexion: May be dark, fair or medium. There is patchy loss of pigment in Leukoderma and total absence in Albinism.

B. Facial Features: An outline of the facial features helpful in identification is given below:

Eyes: Colour of the Iris - Black, brown, blue or grey

Nose: Length of nose, shape of the tip and bridge
Ears: Lobules: free or adherent
Mouth and lips: Outline: lip thickness; lip overlap; width

Teeth: Spaced or projecting front teeth; over-riding; teeth-gaps; dentures; discoloration; staining; wear and tear
Hair: Black, grey or coppery brown colour; bleached or dyed

C. Photograph: Front and profile views are routinely employed for identification, but the method is not infallible.

2.1.7 MALFORMATIONS AND DEFORMITIES

These are congenital or acquired; and when present are very useful for Identification.

A. Congenital: Moles

Birth Marks

Naevus Flammeus (port-wine stains) Hare-lip
Cleft palate

Supernumerary fingers/toes Webbed fingers/toes
Club-foot

B. Acquired: Mal-united/non-united fractures
Amputations

Rickety chest

Hump back due to TB spine

PLATE 3: FEATURES USEFUL IN IDENTIFICATION

Supernumerary fingers

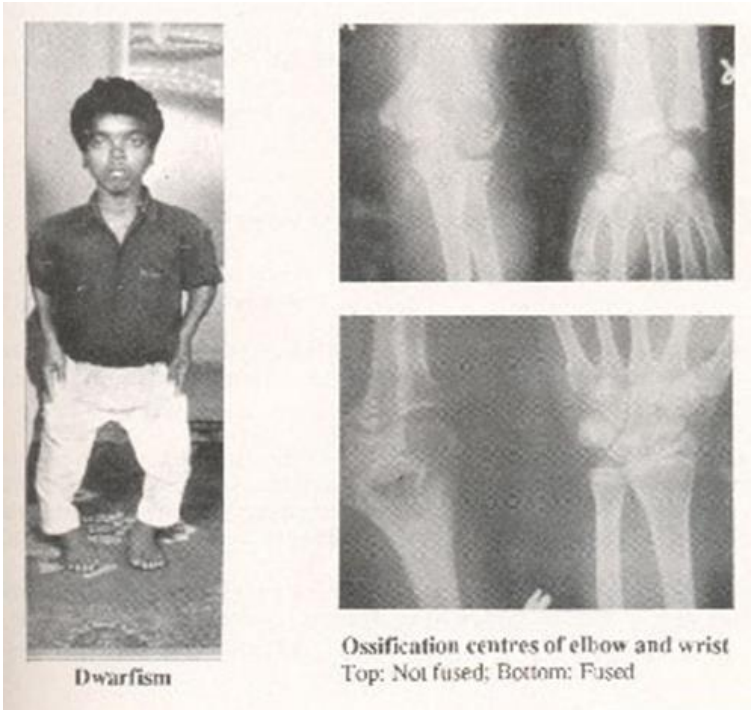

Fig 2: Features useful for identification

2.1.8 IMPRESSIONS

Fingerprints

Fingerprints are the impressions of the papillary ridges of the fingertips. Recording them is easy and inexpensive. Identification by them is absolutely specific; not even the twins from the same ovum have identical fingerprints. In partial prints, the microscopic study of the pores is used for identification (poroscopy).

Footprints

Footprints are specially helpful in identification of the newborn in hospitals and crew victims in air crashes. The imprint of feet or shoes left at the scene of crime can be preserved by casting and thereby used for identification.

Other Impressions

Apart from fingerprints and footprints, the following impressions are also useful in identification, as they are indelible, immutable and specific for each individual.

Lip prints: Divided into eight patterns.

Palato-Prints: The structural details on the front part of the palate, such as the rugae and furrows.

Nail-prints: The longitudinal striations on the nails of the fingers and toes.

2.1.9 ACQUIRED TRAITS

A. Tattoo Marks

These are produced by injection of insoluble pigments in the skin by multiple needle-punctures. Designs in red, green or blue are likely to fade or disappear in about 10 yrs, but those with the carbon pigment remain permanent. A faded tattoo-mark can be revealed by the use of a U.V. Lamp.

Type of designs: The designs may reflect personal details, religious or political symbols or emblems depicting sexual fantasies.
Removal: A tattoo-mark may be removed by

1. Surgical methods (a) Excision and skin grafting

(b) Production of a burn by means of red hot iron.

(c) Scarification

(d) Use of carbon dioxide snow

2. Electrolysis

3. Use of caustic substances. E.g. Zinc Chloride

4. Laser beam.

Significance: Tattoo-marks are more common in people of lower socio-economic status. The type of design may give a clue about the religion, political affiliation, linguistic origin and behavioral characteristics. Drug addicts may have tattoos in front of the elbows to mask needle puncture marks, while homosexuals may have tattoos of 'falcon' design at the base of the thumb on the back of the hand. Names when tattooed may clinch the 'identity' of a person.

B. Scars

A scar is the result of an injury to the true skin or dermis. In a clean aseptic incised wound a scar is formed in 1 – 2 weeks, while in a suppurating would it takes 2 – 8 weeks.

Examination: A faint scar can be rendered visible by rubbing or slapping or applying heat to the area. The scarred area appears pale, while the surrounding skin will be red.

Shape: Scars take after the shape of the wounds causing them. An incised wound results in a linear scar while a lacerated wound, an irregular scar. Scars of bullet wounds are disc-shaped and depressed while smallpox scars are pitted. The scars of burns are extensive and irregular with a tendency to keloid formation. Striae Albicantes: They are white streak-like scars seen in the skin resulting from stretching of the skin due to over-distention, seen in regions like abdominal wall, buttocks, and outer aspect of thighs.

C. *Occupation Marks:* They are of two types -
1. Recent and Temporary:

Trace evidence: Includes paint spots on painters, grease on engineers and mechanics, flour on bakers and millers, dyes on dye workers etc.
Microscopy: Examination of dust in clothing, pockets, trouser turn-ups and ear

wax may reveal coal dust in miners, grains of starch in bakers, pieces of hair in barbers and asbestos fibres in those employed in that industry.

2. Permanent or semi-permanent: Stigmata:

a. Callosity on the outer side of the distal phalanx of the right middle finger in a clerk.

b. Thickening of the palmar skin of the right hand is seen in butchers.

c. Horny and rough hands are seen in manual labourers.

d. Scars produced by burns are seen on the back of the hands of blacksmiths.

e. Tattooing by particles of coal is seen in the hands of coal miners.

f. Tailors have marks of needle punctures on their left index fingers.

g. Bricklayers have a flattening of the left thumb and index finger.

h. Carpenters have one shoulder higher than the other, with callosities on the thumb, index finger and the palms.

i. Opticians have small cuts on the tips of the thumb and index fingers.

j. Chemists and photographers have discoloured fingertips.

2.1.10 PERSONAL BELONGINGS

A. Clothing

The texture and value of clothing provides a clue about the social status. If it is a uniform, the occupation can be known. The marker's label and the dhobi mark are clues to identity.

B. Pocket contents

Wallet, diary, visiting cards, credit cards, letters, receipts, are useful in identification.

C. Jewellery / Articles

Rings, watches, keys, belt, buckles, spectacles, are helpful in identification. The clothing and personal belongings are specially helpful in identification of the victims of mass disasters. However the possibility of usage of second hand clothing or exchange / theft of personal belonging has to be borne in mind.

2.1.11 INDIVIDUAL TRAITS

A. Speech and Voice

Peculiarities of speech such as stammering, stuttering, lisping or nasal twang are helpful in identification. The voice can be recorded on a tape-recorder or graphically as a voice-print, which can be used for future identification. The voice however can be altered by diseases of the larynx and trachea or at one's own will as is done by ventriloquists and actors.

B. Gait

An individual can be recognized even from a distance by his gait but such evidence is far from conclusive in as much as the gait may be altered by an

accident or disease.

C. Handwriting

The formation of the written characters is accompanied by certain peculiarities which are guides to the identity of the writer. Though these peculiarities can be forged, their constant reproduction is difficult to achieve and such discrepancies can be detected by the expert in case of forged or questioned documents by photographic enlargement.
However evidence provided by handwriting cannot be regarded as conclusive as the character of writing may be altered by diseases of Central Nervous System, Muscles or Joints.

D. Mental Calibre and knowledge

A test of the educational standard and knowledge in relation to specific matters can help to readily detect an imposter.

E. Ticks, Manner and Habit

These may be hereditary e.g.: left handedness, or an individual characteristic e.g.: repetitive jerky movements of the shoulder or muscles of the face.

Amount of illumination required for identification:

(a) A flash of lightning produces sufficient illumination for the identification of an individual.
(b) In moonlight (full moon) a person, even if well known cannot be recognized beyond a distance of 17 yards.
(c) With regard to artificial light nothing definite can be said without conducting experiments with the said light.
(d) It is possible to identify a person with the flash produced by the discharge of a firearm if he is standing in close proximity on one side of the line of fire and the gunpowder used is a smokeless one.

UNIT II-B-Post-Mortem Changes

2.2 INTRODUCTION

The changes which occur after death can be divided into:

I.	Immediate changes		due to Somatic death.
II.	Early changes	-	due to molecular death.
III.	Late changes	-	due to decomposition and decay.

The immediate changes help to establish the occurrence of death, while the early and late changes help to determine the time since death.

2.3 IMMEDIATE SIGNS OF DEATH

The immediate changes or signs of death are loss of brain function and cessation of circulation and respiration. The cessation of the vital functions can be established by certain simple findings.

A. *Loss of brain function:*

a. No voluntary muscular movement.

b. No response to sensations, like touch or pain for e.g., in response to a pin prick.

c. No reflexes.

B. *Cessation of Circulation:*

a. No pulse felt at the wrist or neck.

b. No heart beat heard over the chest.

c. No blanching on pressing the finger nail.

d. No discoloration on tying a string at the base of a finger.

e. No sprouting of blood on cutting an artery.

C. *Cessation of Respiration:*

a. No movement of cotton wool or twig of a feather, held near nostrils.

b. No condensation of moisture over a mirror held in front of the nostrils.

c. No visible movements of the chest.

d. No visible movement on the surface of the water in a dish, kept over the chest.

e. No breathing sounds.

2.4 EARLY SIGNS OF DEATH

A. Eye changes:

a. Loss of Reflexes:

i. Absence of blinking on touching the cornea or conjunctiva with cotton wool.

ii. Absence of contraction of the pupil on exposure to light.

b. Haziness of cornea

c. Flaccidity of the eye ball due to loss of tension.

d. Distortion of the shape of the pupil by the application of pressure.

B. Skin changes:

a. Skin becomes pale and ash-white.

b. It loses its elasticity. Hence postmortem wounds do not 'gape' and 'contact flattening' occurs over areas of pressure.

C. Cooling:

The normal body temperature is 98.4^{o}F which is usually higher than that of the atmosphere. Hence dead bodies cool after death. Heat is lost from the body by conduction, convection and radiation, till equilibrium is established with the surroundings. The average rate of fall of temperature is 1.5^{o}F per hour. The temperature of the dead body can be measured by a special thermometer measuring from 0^{o}C to 50^{o}C which is introduced into the rectum. At least two readings should be taken at hourly intervals.

Time since death can be calculated from the formula:

$$\text{Time since death} = \frac{\text{Normal body Temperature} - \text{Rectal Temperature}}{\text{Rate of temperature fall per hour}}$$

The cooling of a dead body is not always uniform as it is influenced by several factors like clothing, body constitution and manner of death etc.

D. *Postmortem lividity:* (Postmortem staining; Hypostasis)

Postmortem staining or lividity is the discoloration of the skin and organs, after death, due to gravitational settling of the fluid blood in the most dependent parts of the body.

Timing: The staining begins to form in about a hour or two after death as small patches, which increase in size and merge subsequently. It is formed completely in about 6 hours and is 'fixed', i.e. not altered by position or pressure in about 8 to 10 hours.

Location: In a body lying on it's back, the postmortem lividity is seen on the back, excluding those areas on which the body exactly rests, which are flattened by pressure. In a case of hanging it is seen in the lower limbs and in drowning it is seen in the head, neck and shoulders.

Colour: Normal lividity is reddish-purple in colour. It is cherry-red in carbon monoxide poisoning, bright pink in cyanide poisoning and chocolate brown in poisoning by nitrites.

Importance: Apart from the time of death lividity indicates the position of the body after death, including shifting, if any.

Pitfalls: Postmortem staining should not be mistaken for bruises. The two are differentiated as follows:

Table: Differences between postmortem Lividity and Bruise

	LIVIDITY	**BRUISE**
LOCATION	Dependent parts	At the site of injury
ABRASION OF SKIN	Absent	May be present
CONTOUR	Not elevated;	May be elevated
MARGINS:	Well-defined	Margins: diffuse
COLOUR	Uniform	Changes with age
INCISION	Edges not stained	Edges infiltrated by blood

E. Rigor Mortis: (Cadaveric Rigidity)

Rigor mortis is a stiffening and shortening of the voluntary and involuntary muscles after death.

Cause: It occurs due to destruction of a chemical substance in the muscles known as ATP (Adenosine – Tri Phosphate) and accumulation of lactic acid.

Timing: Rigor mortis sets in about an hour or two after death. It is fully developed in the body in about 12 hours, persists for about 12 hours and passes off in another 12 hours. This is called the 'Rule of Dozens'.

Examination: The presence of rigor can be looked for by opening the eyes and mouth, by bending the head over the chest and by flexing the limbs. Resistance indicates the presence of rigor.

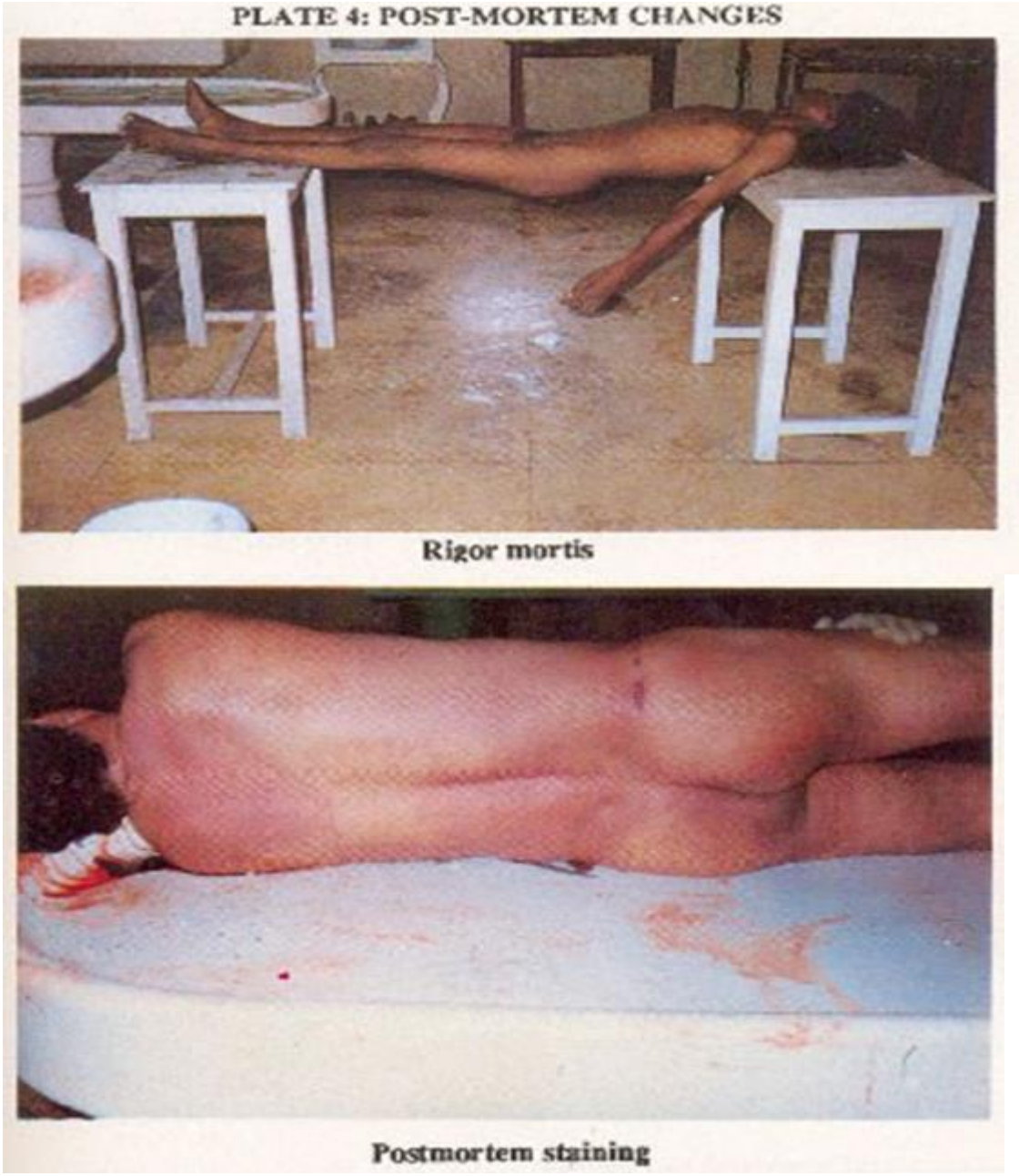

Fig 1: Post-mortem changes

Modifying Factors: Rigor mortis sets in and passes off earlier in deaths after prolonged illnesses like tuberculosis and cancer than in sudden deaths of healthy adults. It is delayed by cold weather and accelerated by hot weather.

Pit Falls: Stiffness in the muscles due to rigor mortis may be broken by excessive pressure as in careless handling, which might be mistaken at autopsy for absence of rigor mortis. Hence undue bending of the body should be avoided and the body should preferably be transported on a stretcher.

Simulating conditions: Rigor mortis is simulated by

1. Cadaveric spasm
2. Heat stiffening and
3. Cold stiffening.

They can be differentiated as follows:

Table: Features of cadaveric spasm and heat and cold stiffening

FEATURE	CADAVERIC SPASM	HEAT STIFFENIN	COLD STIFFENING
Occurrence	Violent deaths proceeded by severe mental tension	Burns	Exposure to cold
Mechanism	Unknown; a single group of voluntary muscles is usually involved	Coagulation of muscle protein	Solidification of bo fat
Rigor mortis	Develops subsequently	Does not develop	Can develop
Disappearance	Merges with Rigor	On decomposition	On thrawing
Medico-legal importance	Indicates the 'Last act of life'	Indicates exposure to temperatures above 50^{o}C	Indicates exposure temperatures below 3.5^{o}C

2.5 LATE SIGNS OF DEATH

A. Putrefaction:

Decomposition occurs due to

a. Action of enzymes on the tissues and

b. Proliferation of bacteria in the body

Manifestations:
Decomposition manifests itself externally by

(a) Colour changes and

(b) Accumulation of foul smelling gases

a. Colour changes:

(i) Greenish discoloration of the skin in the right flank (about 12 hours after death)

(ii) Demarcation of the venous network (marbling) (about 18-36 hours after death)

These changes occur due to the formation of a green pigment from the blood by the action of hydrogen sulphide gas on haemoglobin.

Putrefactive Gases:

Foul smelling gases such as hydrogen sulphide, nitrogen, methane, ammonia etc., are formed by the bacteria proliferating in the dead body, between 18-36 hours after death causing the following manifestations:

1. Bloating of features: e.g. bloating of the face, bulging of eyes, opening of mouth, protrusion of the tongue, distention of abdomen and scrotum in the male and breasts in the female, and gaping of the anus and female genitalia.
2. Blister formation and peeling of the cuticle.
3. Loosening of hair, nails and teeth.
4. Floatation of a dead body submerged in water.

Internally the organs and tissues undergo dissolution with gas formation and liquefaction.

Entomology:

Insects such as house-flies lay eggs on the body which hatch into larvae in 24 – 48 hours.

Pitfalls: In a decomposed dead body:

a. Interpretation of the wounds as to the type, time and nature is difficult.

b. The Pathognomonic autopsy findings of different deaths may be blurred, masked or lost.

c. Findings of putrefaction such as protrusion of the tongue, froth at the mouth and nose, oozing of blood from the wounds, expulsion of foetus from the womb or regurgitation of food into the respiratory tract may be mistaken and misinterpreted as ante-mortem leading to sinister conclusions.

d. The softening of the cartilaginous joints between the body and the horns of the hyoid bone, associated with abnormal mobility may be mistaken for a fracture.

B. Adipocere: (Saponification)

When a dead body is exposed to excessive moisture, the fatty areas of the body like the face, abdomen, thighs, buttocks etc., undergo a physico-chemical change and are transformed in to an adipocere. This is a white or faintly yellowish substance, lighter than water and having a rancid odour and a greasy texture.

Adipocere is formed in about 3 weeks to 3 months after death. It preserves the features of the body and also any wounds present, thereby helping to establish the identity and the cause of death.

C. Mummification:

Mummification occurs in a dead body which is exposed to excessive atmospheric heat, as on a hillock or on the sands of a beach or desert. It occurs due to rapid evaporation of water from the body. It is seen in about 3 months to 1 year after

death. The skin becomes dry, shriveled, rusty brown in colour and clings to the bones. The features and wounds, if any, are well preserved. It is helpful in establishing the cause and time of death and the place of burial or disposal of the body.

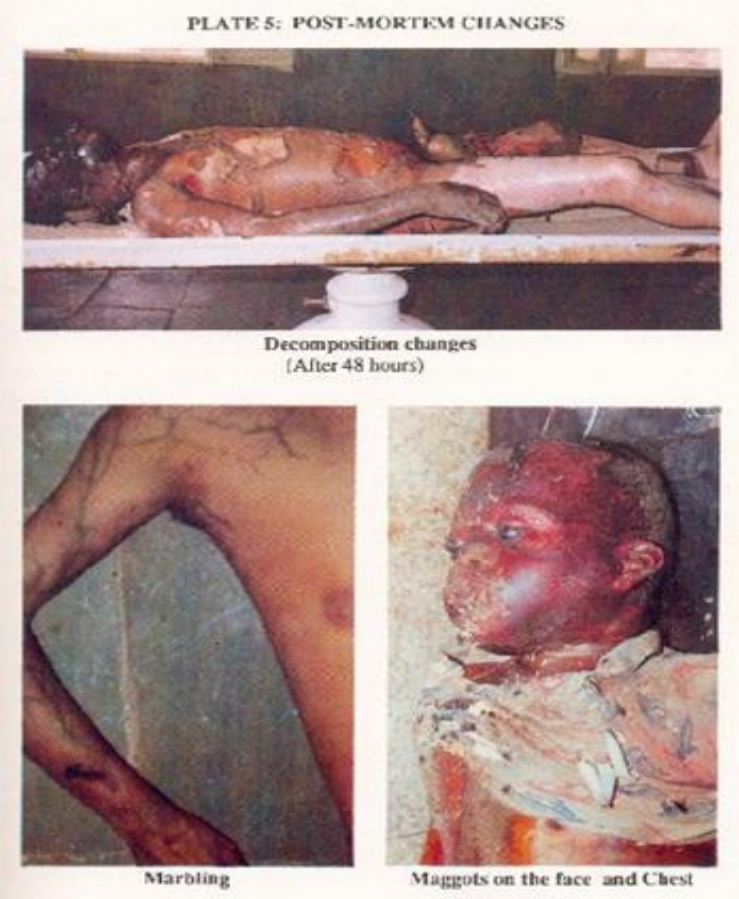

Fig 2: Post-mortem changes

2.6 POST-MORTEM INTERVAL

The time since death can be estimated from the various postmortem changes and the examination of the scene of death.

A. Postmortem Clock:

Postmortem changes	Timing
a. Cooling:	
Total time Rate of cooling	16 – 24 hrs
	1.5 ^{o}F / hr
b. Hypostasis	
Patchy mottling	3 Hrs
Confluent	6 Hrs

	Fixed	8 Hrs
c.	Rigor mortis:	
	Onset in the head	3 Hrs
	Onset in the limbs	6 Hrs
	Complete	12 Hrs
	Passes off	18 – 36 Hrs (summer)
		24 – 48 Hrs (winter)
d.	Decomposition:	
	(i) Greenish discoloration in the right flank	12 Hrs
	(ii) Hlister formation, peeling of the cuticle	18 – 36 Hrs (summer)
	marbling, bloating of features, gaseous distention	24 – 48 Hrs (winter)
	(iii) Presence of larvae of housefly	24 – 48 Hrs (variable)

(iv) Loosening of hair and nails 48 – 72 Hrs

Other Data

e. Stomach-emptying time (in relation to last meal) 3 – 5 Hrs

f. Hair growth (in relation to last shave) 0.4 mm/day

g. Gastro-intestinal and urinary tract:

(i) Empty stomach + full bladder and rectum
Death in the early hours of morning
(ii) Full stomach + empty bladder and rectum
Death in the early hours of the night.

Scene of death:

V

aluable information having a bearing on the time since death may be obtained from the examination of the scene of death.

Loosening and falling of the teeth,	3 – 5 days
liquifaction of the brain, autolysis of the spleen	
(v) skeletonization, adipocere and mummification	3 months or more

Examples

a. The state of the dress: e.g. officer uniform; night dress

b. Dates of mail and newspaper

c. State of food and utensils

d. Stopping of a watch: due to impact or immersion

GLOSSARY

1. Race
2. Religion
3. Sex
4. Age
5. Trait
6. Stature
7. Gait
8. Rigor Mortis
9. Post-Mortem
10. Saponification
11. Mummification

LEARNING OUTCOME

After studying this unit you should be able to:

1. Explain purpose of Identification of the Living and Dead.

2. The different types of acquired traits in a human being.
3. Understand the different types of occupation marks that the Investigator comes across at the scene of crime.
4. Describe the Investigation of Personal belongings.
5. Describe the different signs of Death (Immediate/early and late) based on the changes occurred on the dead body in different types of crime.

SUGGESTED READINGS

1. Forensic and Medico-legal aspects of sexual crimes and Unusual sexual practices – Dr. Anil Aggrawal.
2. Death Scene Investigation Procedural Guide – Mr. M. S. Maloney.
3. Forensic Gynecology – Mr. M. Dalton.
4. Forensic Biology – Dr. R. Krishnamurty.
5. Anthropometry – Mr. I. P. Singh.

UNIT III

LEARNING OBJECTIVES:

In this unit you will be introduced to:

1. The different types of injuries.

2. Nature of the injuries and the mode of death.

3. What is Asphyxial death and its various types.

4. Different guidelines for investigation.

5. What is Rape and other Unnatural sexual offences.

UNIT III-A-Injuries, Wounds

3.0 DEFINITIONS

Injury: The word 'Injury' denotes any harm whatever illegally caused to any person in body, mind, reputation or property. (Sec 44 IPC)
Wound: A wound is an injury to the body leading to a breach in the continuity of the tissues, usually skin or mucous membrane.
Fracture: It is a break in the integrity of a bone or cartilage.

Hurt: Causing of bodily pain, disease or infirmity is known as hurt (Sec. 319 IPC)

Grievous hurt:

The following kinds of hurt are designated as Grievous Hurt (Sec. 320 IPC)

(a) Emasculation

(b) Permanent privation of the sight of either eye.

(c) Permanent privation of the hearing of either ear.

(d) Privation of any member or joint.

(e) Destruction or permanent impairment of the powers of any member or joint.
(f) Permanent disfiguration of the head or face.

(g) Fracture or dislocation of a bone or tooth.

(h) Any hurt which endangers life or which causes the sufferer to be during the space of 20 days in severe bodily pain, unable to conduct his ordinary pursuits.

3.1 CLASSIFICATION

Depending on the method of causation, injuries are grouped into the following types:

Mechanical Injuries:

They are caused by mechanical force

I. Injuries caused by physical agents:
Thermal injuries:

1. Due to heat

a. Dry heat: Burns

b. Moist heat: Scalds

2. Due to cold

Frost bite: Trench foot

Electrical injuries:

a. Electrical burns

b. Lightning stroke

Chemical injuries

Burns, caused by corrosive acids and alkalies
Radiation injuries
Caused by

c. X-ray; UV rays; Sun light

d. Radioactive substances

B. Sharp force injuries:

Caused by a sharp cutting or penetrating weapon.

e. Incised wound

f. Stab wound (punctured wound)

g. Firearm injuries: Caused by either smooth-bored or rifled weapons.

(i) Shotgun wounds

(ii)

3.1.1.1 Abrasions

An abrasion is a destruction of the skin, affecting the superficial layers of skin (epithelium). On healing, the integrity of the skin is completely restored and no scar remains. Abrasions are caused by the impact of an object or a fall on a rough surface. They are also caused by the pressure of finger nails, teeth or a ligature.
There are 4 types of abrasions:

a. Scratch: Scratches are caused by nails or sharp objects like pins, thorns or needles. The object causing the scratch carries the torn epithelium in front of it, which is heaped up at one end. This indicates the direction of the application of the force.
b. Graze: Grazes are caused when there is movement and friction between the skin and a rough object or surface. They occur when the body is dragged on a rough surface and also in traffic accidents, when the body slides on the road surface and the skin is scraped.
Grazes show multiple, parallel, longitudinal lines with a serrated border at the beginning, and epithelial tags at the end which indicate the direction. They are often contaminated with dirt, the nature of which may give a clue about the scene of crime.
The impact or friction at the site of contact generates in some cases enough heat to cause a burn on the skin, known as a brush burn.

c. Pressure abrasions: They are caused by direct pressure on the skin, compressing and crushing the cuticle e.g. ligature mark; teeth bite marks; nail-marks. Often the object responsible leaves behind the pattern of its shape and form on the skin.

d. Impact abrasions: They are caused by impact with a hard object e.g. Tyre tread marks. Radiator grills impression. They often reproduce the pattern of the object causing them.

Pressure and impact abrasions are often grouped together and are known as ‘imprint’ or ‘patterned’ abrasions.

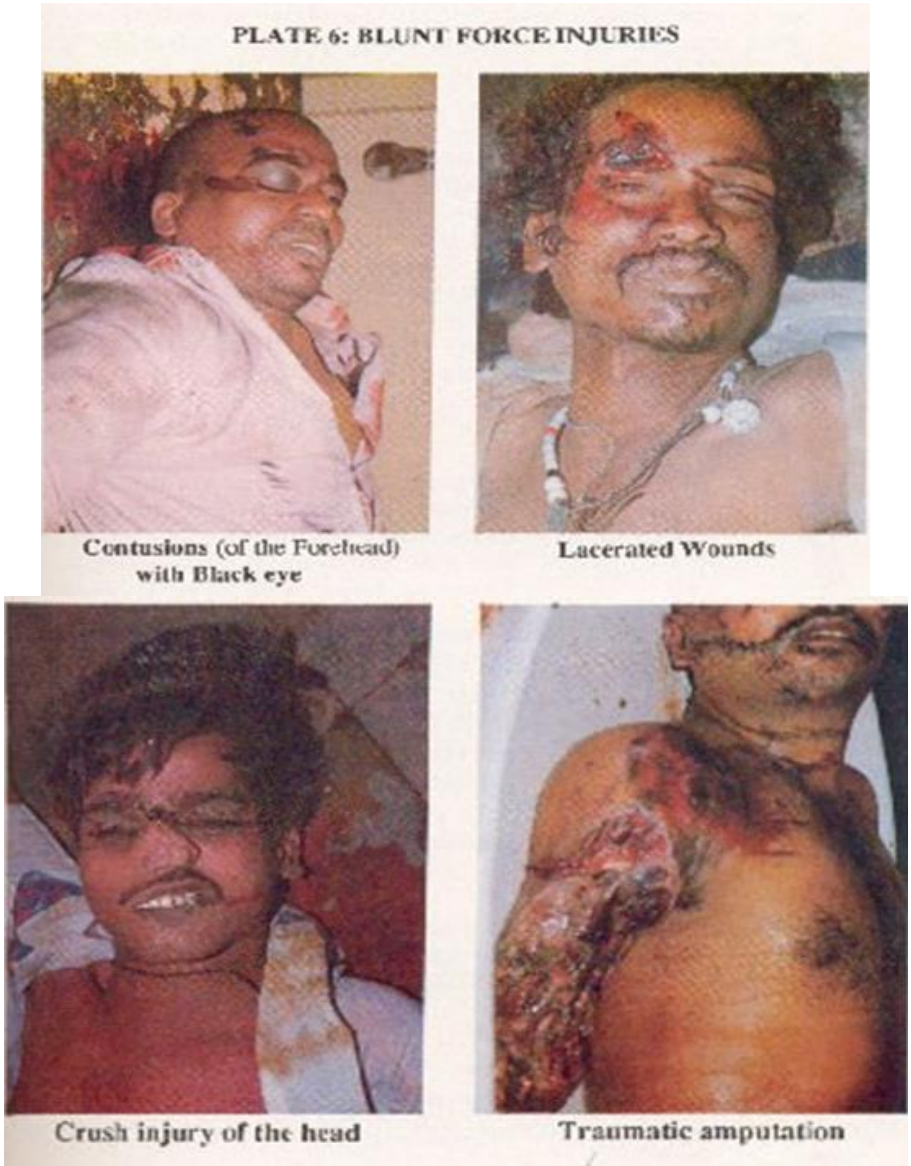

Fig. 1 Grazed abrasion

Medico-legal aspects:

Ante-mortem/Postmortem Abrasions:

Postmortem abrasions may be caused on a dead body by careless handling or during transportation. They can be differentiated as follows:

Table: Differences between Ante-mortem and Postmortem abrasions

TRIAL	ANTE-MORTEM	POSTMORTEM
Site	Anywhere on the body	Usually on a bony prominence
Colour	Red or reddish-brown	Yellowish and parchment like
Scab	May be present	Absent
Microscopy	Signs of healing present	absent

Ant Bites:

Ant bites on a dead body may simulate abrasions. They are present as superficial, brown erosions of the skin with irregular margins, seen mainly around the nose, mouth, eyes or genital orifices.

Age of an Abrasion:

Age of an abrasion can be assessed on the basis of the healing changes:

Fresh : Bright red

12 – 24 Hrs : Bright red scab formed due to drying of the blood/serum

2 – 3 days : Reddish-brown scab

4 – 7 days : Epithelium grows beneath the scab and closes the defects.

After 7 days : Scab falls off.

Forensic importance:

Abrasions indicate the site of impact and direction of the force. They may sometimes, be the only external evidence of a serious internal injury. The cause and mode of death and nature of crime can also be inferred from their presence.

3.1.1.2 Bruises (contusions):

A bruise is a blunt-force injury of the deeper layers of the skin (dermis) and underlying tissues, resulting in rupture of blood vessels and bleeding into the tissues. They are caused by the blunt force received from objects such as a fist, stick, stone, shoe, or whip.

Factors: The formation and extend of contusion depends on several factors. Women, children, old people and those suffering from bleeding diseases (e.g. scurvy) bruise more easily and profoundly than others. Bruises on the scrotum and eye tend to be extensive unlike those on palms and soles due to loose skin and greater blood.

Pit falls:

(a) Unlike an abrasion, the site of a bruise may not correspond with to the site of an injury. E.g. Injury to the fore-head or scalp may result in a bruise around the eye and injury to the hip may cause a bruise around the knee.
(b) Deep bruises appear late, usually about 1-2 days after an assault. Hence in living persons, a follow-up examination on second or third day and in dead bodies, incision of the suspected sites is necessary to elicit them.
Medico-legal aspects:

Age of bruise:

The age of a bruise is indicated by the colour changes which occur due to degradation of the blood pigment, haemoglobin.

At first:		Red
1 – 2 days	:	Blue
3 – 4 days	:	Bluish-black to Brown 5 – 7 days : Green
7 – 10 days	:	Yellow
Two weeks	:	Normal skin colour

Differences between bruise and Hypostasis:

While examining a dead body, the postmortem staining may be mistaken for a bruise, refer pg no: 42 for differentiating them.

Artificial bruise:

Bruises may be fabricated by applying irritant plant juices such as those of the Marking nut, Calatropis, etc. Such bruises are dark brown in colour with multiple vesicles in the margins and reddening and inflammation of the surrounding skin.

Patterned bruises:

The pattern of the shape of the object causing the bruises may be reproduced in them. E.g. bruises caused by cycle chain or whip.

3.1.1.3 Lacerations:

Lacerated wounds are caused by blunt force due to tearing and stretching of the tissues. They are caused by assaults with blunt objects, violent falls and vehicular and machinery accidents.
Characteristics:

a. The margins are ragged and irregular
b. The edges of the wound are contused and the hair blubs are crushed
c. Bleeding is minimal.
d. The wound is often contaminated with dirt, etc.
e. The underlying bone may be fractured.

There are 4 types of Lacerations:

a. Split-laceration: (Incised-like wound). It occurs when the skin is crushed between two hard objects (e.g. the weapon and the bone) as in hits or falls on the head or shin. The split skin wound resembles an in such cases are however irregular.

b. Cut laceration: It is caused by heavy sharp-cutting weapons like an axe or chopper. The edges of the wound are contused by the weight of the weapon.

c. Stretch laceration: Over-stretching of the skin may cause tears.

d. Avulsion: In grinding compression by weight as in run over accidents, the skin may be separated from the underlying tissues and muscles with the formation of a flap. This is called an avulsion.

3.1.1.4 Incised Wounds (cuts):

They are clean-cut wounds caused by sharp-cutting instruments such as a knife, razor, dagger, or by objects such as the edge of a metal sheet, broken glass or a blade of grass. The word 'slash wound' may be limited to incise wounds with great force e.g. a sword cut with evidence of random wielding of the weapon.

Characteristics:

a. The edges are clean-cut, regular and everted (turned-out)

b. The wound is spindle-shaped due to retraction of the edges

c. Length is more than the width or the depth.

d. Bleeding is profuse as the vessels are clean-cut.

e. The wound is deep at the origin and shallow towards the end. This 'tailing' is helpful to know the direction of the wound.

Chop wound: They are caused by heavy sharp-cutting weapons like an axe or a butcherÊs knife. The margins of the wound are bruised and the underlying structures such as the bones and muscles are usually cut.

3.1.1.5 Stab Wounds (punctured wounds):

The stab wounds are caused by piercing of the body by objects such as knife, dagger, spear, arrow, scissors, screw driver, bayonet, needles etc. They are called 'penetrating' wounds when they enter a body cavity and 'perforating' when they make an entry and an exit through the body.

Characteristics:

a. The length of a stab wound is slightly less than the width of the blade. This is because the skin is stretched at the time of penetration of the blade. Occasionally, the length may be more, when the weapon is rocked.

b. The depth of a stab wound is greater than its length or width. It is usually equal to or less than the length of the blade. Occasionally the depth may be more than the length of the blade, if the wound is sustained in the yielding parts of the body, such as the abdomen.

c. The margins are clean cut when caused by a sharp pointed cutting weapon. Punctured wounds caused by penetrating weapons such as crow bar, iron rod, or screw driver may have lacerated edges.

d. The shape of the stab wound is related to the cross-section of the weapon's

blade. Stab wounds caused by single-edged weapons are wedge-shaped and those caused by double-edged weapons are spindle-shaped. A pointed square shaped object and rounded object, when thrust cause wounds which are cruciate and circular respectively.

Concealed puncture wounds:

They are penetrating wounds usually caused by needles, in remote sites such as the angle of the eye, fontanellae, nape of the neck, roof of the nose, vagina and rectum, often escaping detection unless carefully looked for. They are generally encountered in cases of infanticide.

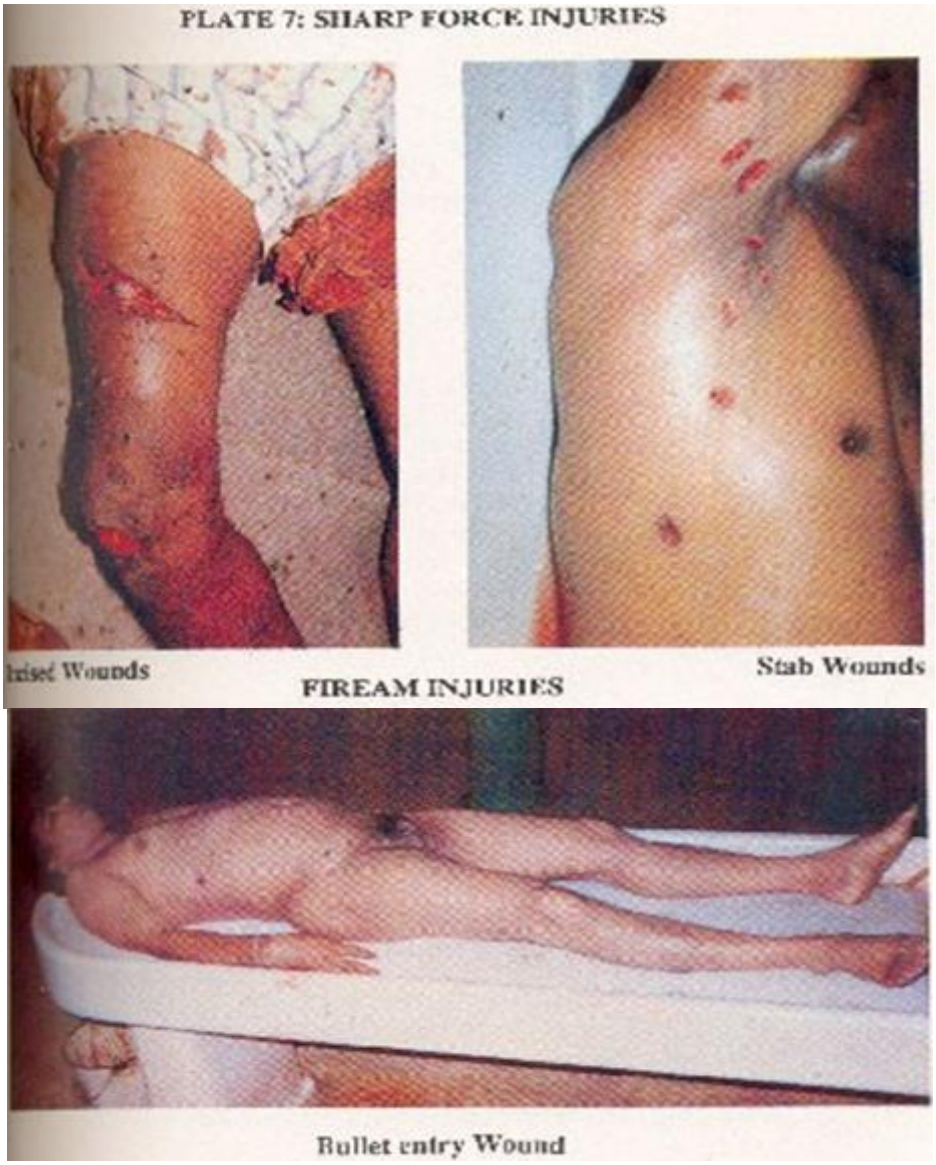

Fig 2: Stab wounds & Firearm Wounds

3.1.1.6 Firearm Injuries

There are two basic types of firearms: Smooth-bored and Rifled. The wounds produced by them vary widely, depending upon the type of the firearm and the

range of fire. When a firearm is used, the discharge from the muzzle end of the barrel consists of, apart from the projectile, flame, smoke and unburnt powder residue, which are responsible for singeing, scorching, blackening, and tattooing respectively. The injuries *per se* are caused by the pellets or lead balls in smooth bored weapons and by bullets in rifled weapons.

1) Shotgun Injuries:

The characteristics of the wound depend upon the range. Wounds of Entry:
Contact range:

The lead shot enter *en masse* causing a circular hole with ragged edges. Scorching, singeing and tattooing are seen around the wound. A circular burn caused by the hot muzzle end of the barrel is seen at the point of contact. When underlying bone is present, the tissues are torn by the pressure of gases.

a. *Near range*: up to 1 yard

The lead shot enter en masse and cause a single wound. Scorching, singing and tattooing are seen around the wound.

b. *Close range*: up to 3 yards

This is beyond the range of flame and smoke but not powder blast, so scorching and singing are absent but tattooing is present. Partial dispersal of the pellets starts beyond 1 yard. Hence, there is a central hole with satellite pellet holes at the periphery. The wad may be present in the wound and is found up to 2 yards.

c. *Distant range:* Beyond 3 yards.

There is complete dispersal of pellets resulting in independent pellet holes. The extent of spread in inches is approximately equal to the range of fire in yards.

Exit wounds:

At close range fire, exit wounds are seen with ragged everted margins caused by lead shot, individual pellets or splinters of bone, without any evidence of burning singeing, blackening or tattooing. In distant shots the pellets are mostly lodged in the body because of their lowered velocity.

2) Rifled Firearm Injuries:

They are characterized by a wound of entry and a wound of exit. Wound of Entry:
It shows the following:
Bullet Hole:

The spinning bullet, as it burrows into the body causes a circular or oval hole depending upon the angle of the entry. The dimensions of the hole are slightly less than the diameter of the bullet due to stretching and recoil of the skin. The margins of the bullet hole are inverted and fibers of the clothing may be driven in.
Abrasion collar:

It is caused by the spin of the bullet and is seen around the bullet hole.
Dirt or Grease collar:

It is formed by the deposition of grease and other foreign materials e.g. dust, carried from the barrel by the bullet.
Apart from the above, other features are seen depending upon the range of fire.
Contact range:

Circular muzzle imprint due to the branding by the hot barrel may be seen along

with pink discolouration of the edges due to carbon monoxide. Singeing, scorching and tattooing are present in the track but not around the wound. When there is underlying bone, the shape of the wound is stellate or cruciform due to rupture of the tissues caused by the expansible gases.

Close range (1‰ to 3‰)

Singeing, scorching, blackening and tattooing are seen around the bullet hole.

Near range (within 1-yard):

This is beyond the range of flame but within the range of powder blast. Hence singeing and scorching are absent but blackening and tattooing are present.

Distant range:

It is characterized by only the bullet hole with the abrasion and dirt collar.

Exit wound:

The exit wound is larger than the corresponding entrance wound and is characterized by eversion of edges, profuse bleeding, and beveling, if located in a bone. singeing, scorching, blackening and tattooing are absent.

Table: Differences between wound of entry and wound of exit:

TRAIT	**WOUND OF ENTRY**	**WOUND OF EXIT**
Size	Smaller than the diameter of the bullet	Larger than the bullet
Edge	Inverted	Everted, puckered or torn
Singeing, scorching, blackening and tattooing	May be present	Absent
Abrasion collar and grease collar	Present	Absent
Fibres of clothing	Turnedin and may be carried into the wound	Turned out
Bleeding	Less	more

The wound of entry may be differentiated from the wound of exit as follows:

Track of the bullet:

The direction of fire and the relative position of the assailant and victim can be determined from the track of the bullet which connects the wound of entry and the wound of exit or depending on whether the bullet is lodged in the body.

Peculiar appearances in firearm injuries:

a. The close range effects of flame and powder residue i.e. burning, blackening, and tattooing may be seen either on the clothing exclusively or the skin around the wound. Hence examination of the clothing is of vital importance in determining the range of fire.

b. The pattern of the wounds caused by country-made firearms at different ranges may not conform to the caused by standard firearms. Experimentation with individual weapons is necessary to determine the range of fire.

c. There may be a single entrance wound with multiple exit wounds when the bullet splinters a bone, or fragments itself, and in cases of 'Tandem' bullets entering the body at once but separating subsequently.

d. Exit wound may be absent when the bullet is impacted in the body or if it enters the trachea, stomach or rectum from where it may be coughed out, vomited or defaceated, respectively.

e. Multiple entrance and exit wounds may occur with a single bullet.

3.1.2 REGIONAL INJURIES

3.1.2.1 Head injuries

Injuries to the head may involve the scalp, skull or the brain.

a. Scalp

Injuries of the scalp are better felt than seen. The covering hair has to be clipped or shaved near the injuries in order to more clearly see them. Split lacerations of the scalp caused by blunt force look like incised wounds. Examination with a magnifying lens however shows that their margins are irregular. Cut lacerations are caused by exes in homicidal attacks. The heel end of the wound which is struck first in such cases is deeper and broader and is towards the assailant. The scalp may occasionally be intact even in serious head injuries with extensive damage to the skull and the brain.

b. Skull

Fractures of the skull may occur either to the vault or base, the latter being associated with bleeding from nose and ears. The different types of skull fractures are:

(a) *Fissured fracture*: Hair-line and crack like.

(b) *Depressed fracture*: Skull surface is depressed.

(c) *Comminuted fracture*: Bone is broken into more than two fragments.

(d) *Pond fracture*: Occurs in children. Owing to elasticity, the bone bends and is depressed but does not break.

(e) *Guttered fracture*: Gutter-like, caused by glancing bullets.

(f) *Ring fracture*: Seen in the base of the skull around formen magnum. It is caused by falls from a height.

c. Brain

Concussion is sudden loss of consciousness due to injury to the brain without any gross evidence. Contusion and laceration of the brain are caused by blunt force and occur either on the side of impact (coup) or on the side opposite to it (contre-coup). Injury of the brain may lead to Oedema (swelling) which may result in death from compression of the vital centres in the brain stem.

Intracranial Haemorrahages:

Head injury may lead to bleeding into the cranial cavity, which may collect between the meninges. Depending upon the location of this bleeding, such haemorrhage is termed extradural, subdural or subarachnoid.

Facial Injuries:

Disfiguration of the face by cutting of the nose and ears or by vitriolage i.e. throwing of corrosive acids on the face may generally be seen when sexual jealousy is the motive of the attack.

Throat:

Homicidal cut throat wounds are generally few, located on the sides of the neck, horizontal and deep. On the other hand suicidal cut throat wounds are associated with multiple, parallel superficial ÂhesitationÊ cutsÊ on the side of the neck (left side in right handed persons) running obliquely downwards and ÂtailingÊ towards mid-line on the front of the neck. Cadaveric spasm may be present in the body, with clasping of the weapon in the hand.

Spine:

Trauma to the vertebral column occurs due to blows or falls, leading to

dislocation of the vertebra and damage to the spinal cord. Fracture of the 1st and 2nd cervical (neck) vertebrae is instantly fatal. Fracture of the other cervical vertebrae causes paralysis of all four limbs (quadriplegia). Fracture of thoracic vertebrae causes paralysis of the lower limbs (paraplegia).

3.1.2.2 Chest Injuries

a. Blunt Force Injuries

Compression of the chest by blunt force results in fracture of the ribs, usually in the axillary or para-vertebral plane with contusion or laceration of the lungs. In massive compression of the chest, as in run over injuries, the heart may be torn off from the great blood vessels and the lungs are lacerated.

b. Sharp force Injuries:

Stab wounds penetrating the chest wall may injure the heart or the lungs. Blood may collect in the pericardial sac in stab wounds of the heart causing mechanical obstruction to the expansion of the heart, leading to death; this is known as ‘cardiac tamponade’. Penetrating injury to the lungs may lead to collection of air or blood in the pleural cavity or collapse of the lungs.

3.1.2.3 Abdominal Injuries

Blunt force injuries may produce rupture of hollow organs such as stomach or intestines, especially when they are full or of solid viscera such as the liver, spleen or kidney, whose friability is increased when enlarged due to any pathological condition.

3.1.2.4 Limb Injuries

Crush injuries of limbs with tyre imprints are seen in run over vehicular accidents. Traumatic amputation of the limbs is often encountered in railway or factory accidents. Cuts with sharp weapons are seen on the limbs, in the accessible parts in cases of suicide and generally involve blood vessels. Fabricated incised wounds are also seen in the accessible parts, but are multiple, parallel and superficial. In homicidal attacks incised wounds may be sustained by the victim on the palms and back of hands and forearm in attempts at self-defense.

3.1.2.5 Genital Injuries

Men: Mutilation of the penis is sometimes meted out as a punishment to a person accused of adultery or rape. Kicks on or squeezing of the testicles may cause sudden death from vagal inhibition.
Women: Women may be tortured by branding the genitals, by introducing chilli powder into the vagina or by lacerating with a blunt weapon. Often the motive is sexual jealousy.

UNIT III-A-Medico-legal aspects of Injuries.

3.2 INTRODUCTION

3.2.1 WOUND CERTIFICATE

Hospitals dealing with medico-legal cases attend upon the injured round the clock, the initial medical examination usually being conducted in the casualty by the medical officers on duty. When an injured person arrives or is brought directly to a hospital and the injuries are considered to have medico-legal implications, the doctor intimates the police about the case. Alternatively the victims of accidents, assaults or self- harm are referred and escorted by the police to the hospital for examination.

Whether such cases are admitted into the hospital or treated as outpatients, the details of the examination are entered in the 'Accident Register' maintained in all government hospitals.

The wound certificate is issued by the Medical Officer in the following proforma:

Nature of injury	Size	Site	Simple or grievous	Causative weapon	Remarks (Age)

3.2.2 POSTMORTEM CERTIFICATE

Autopsies are held on the bodies of victims of unnatural deaths and of fatal injuries. The cause and time of death are routinely certified by the doctor who also gives an opinion as to the mode of death whenever possible.

The following medico-legal aspects of wounds are relevant and significant and considered in the interpretation of the postmortem certificates.

3.3 CAUSES OF DEATH FROM WOUNDS

They may be immediate or remote.

3.3.1 IMMEDIATE CAUSES

i) Haemorrhage: Bleeding may be external or internal. Internal bleeding may occur in an organ, tissue or body cavity. Such bleeding, even in small quantities, may lead to death from compression effects e.g. Intra-cranial haemorrhage. External bleeding stains the clothing and the scene of crime. In general, the more rapid the loss of blood, the more sinister the outcome. Evidence of haemorrhage is indicated at autopsy by pallor of organs and shrinkage of spleen.

ii) Injury to a vital organ: Gross injury to a vital organ such as the brain, heart or lung results in death.

iii) Shock: Shock may be primary or secondary. Primary shock may result from extensive or severe injury causing intense pain, or even from a slight injury in vulnerable sites such as the neck, pit of the stomach, testicles, genital passages etc., which stimulates the vagus nerve. Secondary shock occurs due to excessive

bleeding. Symptoms of shock appear when 1 liter of blood is lost rapidly from the body; death occurs when the loss exceeds 2 liters.

3.3.2 REMOTE CAUSES

i) Infection: Infection wounds by bacteria causes sepsis (pus formation), and occasionally gas gangrene or tetanus.

ii) Embolism: An embolus is a foreign body such as a thrombus (blood clot), air bubble or fat in the blood vessels. A thrombus formed in the leg veins may dislodge and cause death from pulmonary embolism in bedridden accident victims. Death from fat embolism occurs after burns, soft tissue injury and long bone fractures. Death from air embolism occurs in cases of cut throat injuries.

iii) Aggravation of disease process: Pre-existing diseases in organs may be exacerbated by trauma. Organs like liver and spleen become more friable when enlarged and may rupture with minimal force.

iv) Complication of surgical procedures: A victim of injuries who is treated surgically may succumb to complications such as operative surgical shock.

3.4 NATURE OF THE INJURY

Postmortem injuries are caused on a dead body by mishandling, by the bites of insects and animals or by criminal means. They can be distinguished from ante-mortem injuries as follows.

Table: Differences between Ante-mortem and Postmortem wounds

	ANTE-MORTEM WOUNDS	POSTMORTEM WOUNDS
1.	Haemorrhage is copious	Haemorrhage is slight or none at all
2.	Marks of spouting of blood from arteries	No spouting of blood
3.	Blood is clotted	Not clotted
4.	Wound edges are deeply stained which is not removed by washing	Wound edges are feebly stained
5.	Wound edges show gaping	No gaping is seen
6.	Signs of healing may present	absent

3.5 MODE OF DEATH

Based on the character of injuries it is sometimes possible to infer the mode of death. The distinguishing characters of suicidal, homicidal and accidental injuries are enumerated below.

Table: Differences between suicidal, homicidal and accidental wounds

	Character	Suicidal	Homicidal	Accidental
1.	Position	In the accessible parts only, i.e. the front and sides of the body.	No fixed site, vital parts such as the head, chest and abdomen are involved	Anywhere; usually on exposed parts and bony prominences
2.	Nature	inised, gunshot	Usually gunshot, wounds lacerations	Usually lacerations, abrasions and contusions
3.	Direction	In right handed persons, from left to right and from above down wards	Any direction	Any direction
4.	Severity	Mostly superficial with 1 or 2 deep wounds	Mostly severe and extensive	Variable in severity
5.	Weapon	By the side of the body or may be grasped firmly in the hand	Usually absent	May be present
6.	Clothes	Not damaged as they are usually removed or avoided	May be damaged	May be damaged and stained with oil, grease mud, dirt etc.

3.6 VOLITIONAL ACTS AFTER INJURY

Voluntary acts such as walking, running, speaking, shouting, climbing stairs etc., may be performed by a person after receiving a fatal injury. However, individuals react differently to injuries and dogmatic opinions are untenable.

After receiving a head injury, a person may speak and act in a purposeful manner which he may not remember later (Post Traumatic Amnesia). A victim of a head injury after initial unconsciousness may be conscious for a period (Lucid interval)

before ultimately sinking into unconsciousness leading to death. During this Lucid interval voluntary acts may be performed by the individual.

Stab wounds or bullet injuries in the chest affecting the heart or the lungs are usually fatal and cause instantaneous or rapid death. On occasion, however, the victim may survive long enough to exercise powers of locomotion and volition. Injuries of auricles of the **heart are more rapidly fatal than those of ventricles.**

3.7 SEQUENCE OF INJURIES

Amongst multiple injuries those which are not likely to fell, disable or kill, are presumed to have been inflicted first.

3.8 PERIOD OF SURVIVAL

The period of survival of a victim of injuries can be inferred from the age of the injuries. The wound edges become swollen in 3-4 hours. White blood corpuscles collect in the edges of wound in about 6 hours and pus forms in about 36 hours. Granulation tissue is formed due to healing of the wound in about 7 – 5 days.

3.9 RELATIVE POSITION OF THE ASSAILANT AND VICTIM

The relative position of the assailant and victim may be inferred from the following details:

a. Tailing of an incised wound: An incised wound is deeper at the beginning and shallower towards the end.

b. Bevelling cuts: Overhanging of the edges of a wound indicates tangential blows.

c. Direction of stab wounds.

d. Track of a bullet.

3.10 GRAVITY OF INJURIES

a. An injury may itself be necessarily fatal when death is inescapable e.g. A crushing injury of the head.

b. Individual injuries may be non-fatal but their cumulative effect may be fatal.

c. An injury is considered sufficient in the ordinary course of nature to cause death when death is a very probable result of it. E.g. rupture of the heart.

d. An injury is considered likely to cause death when it endangers life and is likely to prove fatal in the absence of medical attention e.g. rupture of spleen.

3.11 NOVUS ACTUS INTERVENIENS

This is the breaking of the chain of causation of an injury by an intervening event which may be an act of God, the act of a third party, the plaintiff or the victim himself. This principle of criminal law is considered in deaths following injuries after an interval, with history or evidence of intervention e.g. Surgery.

3.12 BURNS

Burns are caused by flame, corrosive chemicals, electricity or by radiation. E.g.- ray. The commonest burns are those caused by flame. Scalds, on the other hand are lesions caused by boiling liquids or steam. Burns and scalds can be differentiated as follows:

Table: Differences between burns and scalds

TRAIT	BURNS	SCALDS
Cause	Dry heat e.g. flame or hot metallic object	Moist heat e.g. boiling liquid or steam
Blister	Found only in superficial burns	Found always. The skin is sodden and bleached
Location	At and above the site of application of heat	At and below the site of contact due to trickling down of the boiling liquids
Flame effects	Hair is signed and skin is scorched	No such effect
Shape	Shape of the object is seen in branding	Splashing may produce a pattern

Flame burns: Burns are commonly sustained during suicidal attempts of self-immolation; by dousing oneself with kerosene and then setting alight the clothing. They may be sustained accidentally when clothing catches fire from a hearth or stove. Homicidal burns are generally rare.

Classification: Burns are classified into the following degrees depending upon the depth of injury to the skin.

Table: Degree of Burns

	Type	Degree	Features
I.	Superficial	Epidermal	Redness; Blister formation
		Dermo-epidermal	Destruction of part or whole of the true skin
II.	Deep	Deep	Involvement of the muscles Destruction of the whole limb/bones

Extent: The body surface area involved in burns can be calculated by the Rule of Nines. The body for this purpose is divided into 11 areas each of which represents 9% of the total body surface, the genital area accounting for 1%. Burns are likely to be fatal when more than 40% of the body surface is involved.
Causes of Death: Death occurs in burns due to the following causes:

1. Shock:

(a) Primary shock: Due to severe pain caused by damage to the nerves.

(b) Secondary shock: Due to circulatory failure occurring from the reduced blood volume caused by oozing of serum.

2. Infection: Infection by bacteria occurs due to lowered resistance from loss of proteins.

3. Fat embolism

4. Kidney failure

Death may also occur at the scene from the following causes.

5. Suffocation: Due to inhalation of smoke and irrespirable gases.

6. Injuries: Caused by falling objects e.g. burning logs from the roof of a house.

Fig 1: Burns

3.12.1 EXAMINATION OF BURNT DEAD BODY

A. External Examination

1. Clothing: Burnt or partly burnt

2. Hair: Singed or completely burnt smell of fuel e.g. kerosene may be found.
3. Blackening: Occurs due to deposition of soot.

4. Position: Body may assume pugilistic (Boxer's) attitude with universal flexion of limbs.
5. Injuries: Heat ruptures of the skin may occur in the armpits or groin folds.

6. Burns: The degree and extent of the burns is assessed from the data given above.

Postmortem burns can be differentiated from ante-mortem burns as follows:

Table: Differences between Ante-mortem and postmortem burns.

	Trait	**Ante-mortem burns**	**Postmortem burns**
1.	Red line	Present at the junction of burnt and unburnt areas	Absent
2.	Blister	Contains serious fluid Base is red	Contains air Base is yellow
3.	Repair	Signs of healing may be present depending on the duration	Absent

B. Internal examination

An autopsy may yield the following findings:

1. Respiratory passages : Contain soot

2. Organs and blood : May show cherry red colour due to the presence of carbon- monoxide.

Occasionally the skull may show fractures due to expansion from heat (Heat fractures) and collection of blood in the form of a soft friable cloth between the meninges and skull (Heat haematoma), which together may be mistaken as evidence of assault on the head.

3.12.2 MEDICO-LEGAL ASPECTS

The ante-mortem nature of burns is confirmed on the basis of the external characteristics of the burns and by the presence of soot in respiratory passages and cherry red colouration of blood and internal organs.

3.12.3 OTHER TYPES OF BURNS

A. *Chemical burns:* The red line of reaction, singeing of hair and blister formation are absent. Distinctive staining may be seen.
B. X-ray burns: The skin is inflamed with a typical pigmentation and shedding of hair, with a tendency to the formation of ulcers which do not easily heal. Wart- like growths may also form at the finger-nails.

C. *Electrical Burns:* Electrical burns are of 3 types.

a. *Joule's burn:* This appears as a shallow crater bordered by a ridge of skin 1 – 3 mm in height. Its base is pale and lined by flattened skin. The surrounding area is red due to accumulation of blood.
b. *Flash burn:* Extensive areas are involved sometimes with burnt and unburnt areas alternating. The affected skin is pale due to closure of blood vessels.
c. *Exit marks:* The sites of exit of electric current are seen as splits, tears or ruptures of the skin.

D. *Burns due to lightning stroke:*

In lightning stroke a massive electric discharge passes from the clouds to the earth, causing the death of persons by the passage of electric current through the body and the effects of the blast wave in the air set up by it. Victims of a lightning stroke may have torn clothing and may receive limb injuries, due to the blast effect. There may be fusion of glass and metallic objects on the body as a result of the intense heat produced and the magnetization of any steel objects (like keys) due to the passage of the electric current.

The characteristic burns on the body that result from a lightning stroke are termed filigree burns or Arboresent marks. They are superficial, red in colour and have a branching tree-like pattern due to the passage of electric current through the blood vessels.

3.13 TRAFFIC ACCIDENTS

Injuries may be sustained by pedestrians, occupants of vehicles and the riders of motor cycles or bicycles.

A. Pedestrian Injuries: They are of 3 types:

1. *Primary impact injuries:* These are caused by the impact on parts of the body first struck by projecting parts of the vehicle such as bumper, mud guard, door handles, radiator grill, etc. The bumper causes contusion of the leg muscles with fracture of the bones of the leg, while the radiator grill or the rim of the head lamp may cause abrasions with the imprint of their shape.

2. *Secondary impact injuries:* They are caused by further impacts with the vehicle. When a person is hit by a vehicle while standing on the ground he may fall forward and may be run over by the vehicle. A run over injury may crush the limbs, avulse the skin, rupture the internal organs and leave tyre marks on the skin. On the other hand when a pedestrian is hit while walking, his body may be thrown into the air, and fall backwards and dash against the windscreen or may hit the roof of the vehicle.

3. *Secondary injuries:* They are caused when the victim is thrown off due to the impact of the vehicle, and hits some hard object on the road such as a stone, tree, metal pole or edge of a pavement.

B. Injuries to occupants of vehicles

The occupants of the vehicle are thrown against objects in front of them or they may occasionally be ejected when a door unlocks, thereby hitting the ground or they may get crushed by the vehicle itself. The front seat passengers may sustain contusions on the knees by hitting the dash board and may suffer cuts and lacerations on the face by hitting the rear-view mirror or the wind screen. When the vehicle comes to a sudden stop in an accident, the front seat passenger may hit the windscreen with his head; the resulting bending of spine may produce either a fatal contusion or laceration of the spinal cord without a fracture of spine, an injury known as whiplash injury. The rear seat passengers hit the front seat and the roof sustaining blunt injuries on the knees and the head.

C. Injuries to motorcyclists

Due to sudden deceleration of the vehicle in an accident, the rider of a two-wheeler is thrown off his vehicle, hitting the ground or other objects, thereby sustaining injuries to the head and the limbs. While speeding behind a lorry, he may run under the tail board sustaining injuries to the head, neck, and shoulders or may get impaled against any objects projecting from the vehicle, such as iron rods.

D. Injuries to pedal cyclists

Cyclists commonly sustain head injuries due to fall on the ground. Impact against projecting objects like handle bars, brake handles may cause lacerations of the limbs and rupture of internal organs like liver or spleen.

3.14 RAILWAY INJURIES

Railway injuries are characterized by relatively less bleeding and staining of the body and clothes with grease and oil. The injuries sustained fall into the following patterns:

a. *Decapitation:* Head is severed from the trunk.

b. *Transaction of the trunk:* May be complete or partial.

c. cTraumatic amputation: The limbs may be amputated by the traumatic force.

d. *Multiple contusions and lacerations: Caused by impact of a fall on the track* against the rails, sleepers and the granite chips or on the ground.

h. *Traumatic Asphyxia:* It occurs when a traveler on the roof of a train falls between the carriages and is crushed between the buffers.

3.14.1 MEDICO-LEGAL ASPECTS

While decapitation is generally indicative of suicide, trunk and limb injuries point towards accidental causation. However, it should be borne in mind that a person can be rendered unconscious by a head injury or drugged or intoxicated and in such a state left on the railway track for a train to pass over.

3.15 AIR CRAFT INJURIES

Injuries sustained in aircraft accidents are as follows:

a. *Head injury:* Due to impact against the back of the front seat.

b. *Seat belt injuries:* Compression by seat belt causes rupture of abdominal organs.

c. *Burns:* Often the body is charred beyond physical recognition.

d. *Carbon Monoxide Poisoning:* Occurs from the fumes.

e. *Anoxia:* Occurs due to low pressure in the cabin.

The victims of aircraft accidents are identified generally on the basis of the skeletal remains, personal belongings and the teeth. The autopsy of the pilot's body may offer clues to the causes of the accident, for instance, from the presence of natural disease such as coronary occlusion, myocarditis, and hypoglycemia or from the detection and use of drugs such as alcohol, antihistamines and tranquilizers.

3.16 BLAST INJURIES

Injuries due to an explosion e.g. of dynamite are caused in 3 main ways:

a. *Displacement of the body:* injuries are caused due to hitting the ground or other objects.

b. *Flying missiles of different kinds:* Boulders, stones, etc., may cause blunt force injuries and crush injuries.

c. *Physiological effects of the blast wave:* The blast wave produces patchy but often massive haemorrhages in the lungs due to stretching and recoil in the tissues.

3.17 BOMB INJURIES

Injuries caused in bomb explosions fall into the following types:

a. *Explosive Injuries:* Bodies of those close to the bomb, may be shattered with dismembering of limbs, and rupture of the trunk with extrusion of the organs.

b. *Missile Injuries:* The penetration of different missiles causes injuries in a Âpeppering patternÊ.

c. *Injuries by falling masonry:* May cause crush injuries of the head or chest or traumatic asphyxia.

d. *Blast Injuries:* Patchy massive haemorrhages are seen in the lungs

e. *Burns:* Burns of a minor degree occur in bomb explosions. Severe burning may result from conflagrations which follow a bombing.

3.18 BAROTRAUMAS

It is the damage and injury caused due to differences in the barometric pressures on the surfaces of vital organ. It occurs in deep sea diving. The pressure increases by 1 atmosphere with every 10 meter depth. Hence a greater amount of inspired gases are dissolved in the body fluids during diving, the oxygen causing convulsions and nitrogen Narcosis. During rapid ascent to the surface, the nitrogen may effervesce causing air embolism and disruption of the lung tissue from barotraumas.

UNIT III-B-Asphyxial Deaths

3.19 ASPHYXIA

Asphyxia (As = absent; sphyxis = pulse (Greek) is a condition in which there is interference with respiration, which in turn causes diminished supply (Hypoxia) or total deprivation (Anoxia) of oxygen to the tissues. Asphyxia is commonly brought about by:

A. Mechanical means: e.g. Compression of the Neck

B. Pathological conditions: e.g. Diphtheria, Tetanus

Stages: Mechanical interference with respiration causing asphyxia results in rapid death, usually within 3-5 minutes. The victim passes through 3 brief stages:

1. States of breathlessness
2. Stage of fits
3. Stage of respiratory paralysis.

Occasionally, death may be instantaneous from vaso-vagal shock.

3.20 GENERAL FEATURES

In general, asphyxial deaths are characterized by certain common features:

1. *Cyanosis:* It is the bluish colouration of the skin and mucus membranes of the body prominent in the nails of the fingers and toes and also in the lips and ears.
2. *Froth at the mouth and nose*: It occurs due to oedema of the lungs. It is either fine and white or blood-stained.

	Mechanism of respiratory obstruction	**Type of Asphyxial death**
A.	Constriction of the neck: 1. With suspension of the body 2. Without suspension a. By ligature b. By hands	Hanging Ligature strangulation Throttling
B.	Entry of fluid in to the respiratory passages	Drowning
C.	Obstruction by mechanisms other than the above	Suffocation
	1. Closure of mouth and nose	Smothering

	2. Stuffing mouth by a cloth piece	Gagging
	3. Blockage of respiratory tree from within	Choking
	4. Prevention of respiratory movements of chest	Traumatic asphyxia
	5. Vitiation of atmosphere	Suffocation
	6. Homicidal smothering in association with overlying	Burking

3. *Relaxation of Sphincters:* The anal and bladder sphincters relax at the time of death, resulting in voiding of faeces and urine and consequent soiling of the clothes.
4. *Capillaries and blood:* The permeability of capillaries is increased, with the result that fluid accumulates in the serous sacs such as the pleura and pericardium. The blood is dark and fluid in asphyxial deaths.
5. *Tardieu's spots:* They are petechial or pin-point haemorrhages first described by the French medico-legal expert Ambroise Tardieu in 1866. They occur due to the rupture of distended capillaries and are seen externally in the eyelids, face and scalp and internally on the surface of the heart and lungs.

3.21 TYPES OF ASPHYXIAL DEATHS:

Depending on the mode of respiratory obstruction, asphyxial deaths are differentiated as follows:

3.22 HANGING

Hanging is a form of death which is caused by suspension of the body by a ligature round the neck, the constricting force being the weight of the body.

3.22.1 TYPES

Hanging may be classified in 2 ways:

I. *Complete*: Suspension of the body is complete and the feet do not touch the ground. Death occurs rapidly.
Partial: Suspension of the body is partial. Hanging is committed in the sitting, kneeling, reclining or any other posture. Death occurs rather slowly.

II. *Typical:* Knot of the ligature is at the back of the head. Hence, compression of the neck is uniform.

Atypical: Knot is at the front or the sides of the neck. Hence, compression of the neck is not uniform.

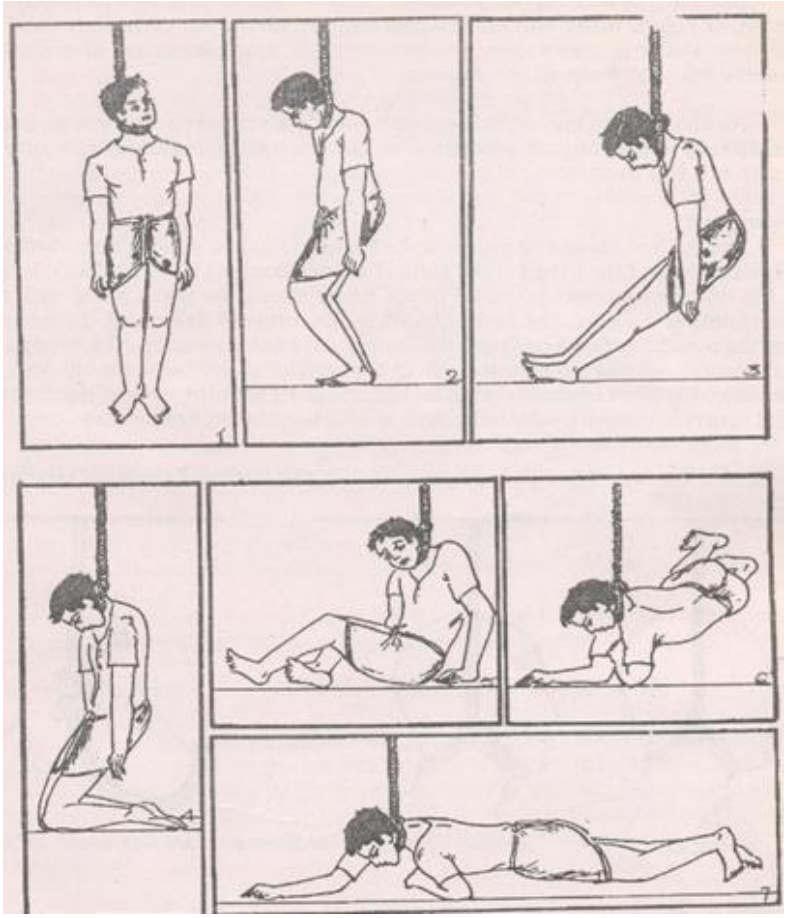

Fig. 1 Types of Hanging

3.22.2 LIGATURE

Any material which is easily available may be used for hanging. Coconut, jute, cotton, or plastic ropes, saris, dhotis and bed sheets are commonly used as ligatures. Cot tape, Iron strings, electric wire, belt, scarf, stockings, or any other material may also be used occasionally. Rarely, hanging may occur even without a ligature, when the neck is caught accidentally in the rungs of a ladder, edge of scaffolding, tail-board of a lorry or the branch of a tree.

3.22.3 PRECAUTIONS

The ligature should be photographed in situ, unless already disturbed and measurements of the height of the point of suspension and foot-rest if any, length of the ligature and circumference of the noose should be taken along with the description of the knot. The nose should be cut without disturbing the knot, so that the possibility or otherwise of self-application can be assessed. The strength of the ligature, whether adequate to hold the weight of the body should also be considered. It is preferable to send the ligature along with the body to the medico-legal expert to compare it with the ligature-mark at the time of autopsy.

3.22.4 CAUSES OF DEATH

The constriction of the neck impairs blood circulation to the brain by obstructing the (carotid) arteries and (jugular) veins and by interfering with air entry into the lungs, leading to rapid death in 5-8 minutes. Sometimes death may be instantaneous.

3.22.5 POSTMORTEM APPEARANCES

A. External:

a. The neck is elongated and the head is tilted to the side opposite to the knot.

b. Tongue is protruded and the tip is bitten between the teeth.

c. Dribbling of saliva is seen from the angle of the mouth.

d. Nails are livid.

e. Postmortem staining is seen in the lower limbs.
Occasionally, pin-head sized postmortem petechiae are seen in the place of hypostasis.

f. Sphincters relax with voiding of urine and faeces. Semen or blood may be seen at the urethral opening on the penis.

B. Internal:

a. The ligature mark is dry, hard and parchment-like and is located obliquely, above the level of thyroid cartilage with interruption towards the knot.
b. The imprint of the pattern of the ligature may be seen on the mark.

c. Smooth and broad ligatures produce a shallow and faint mark while firm rope or cord produces a narrow, deep and distinct ligature-mark.
d. The underlying tissues beneath the ligature mark are dry white and glistening due to passive compression.
e. The hyoid bone is fractured in about 20% of cases usually at the junction of the body with the greater horn.
f. The muscles may be bruised and the inner coats of vessels lacerated at the level of the ligature.
g. Fracture of the upper cervical spine with laceration of the spinal cord is seen in judicial hanging as well as in hanging with a long drop.

3.23 STRANGULATION

Fig. 2 Neck structures

Strangulation is an asphyxial death caused by constriction of the neck without suspension of the body.

3.23.1 TYPES OF STRANGULATION

	Constriction of the neck by	Type of strangulation
1.	Ligature round the neck	Ligature strangulation
2.	Compression by hands	Throttling
3.	Two bamboos/poles on the front and back of the neck	Bansdola
4.	Squeeze in the bend of the elbow	Mugging
5.	Ligature or metal collar	Garroting

Among the above, strangulation by ligature and throttling are the commonest. All the strangulation deaths must be considered homicidal, until proof to the contrary is available.

3.23.2 LIGATURE STRANGULATION

Compression of the neck is effected by a ligature round the neck, the constricting force being applied by the assailant. The ligature is wound round the neck often with multiple turns with or without a knot.

3.23.3 POSTMORTEM APPEARANCES

3.23.3.1 External

3.23.3.1.1 Face is swollen and cyanosed with prominent eye balls and dilated pupils.

3.23.3.1.2 Petechial haemorrhages are seen in the face, eyes and the scalp.

3.23.3.1.3 Bleeding may be present from the mouth, nose and ears.

3.23.3.1.4 Hands are clenched and nails are cyanosed.

3.23.3.1.5 Incontinence of urine and faeces is seen.

3.23.3.1.6 The ligature mark is dry, hard and parchment-like and is located horizontally at below the level of the Thyroid cartilage and completely encircling the neck. Evidence of multiple turns and imprint of the pattern of the ligature may be seen in the mark.

3.23.3.1.7 The underlying tissues beneath the mark are often ecchymosed.

3.23.3.2 Internal:

3.23.3.2.1 The neck muscles are bruised.

3.23.3.2.2 The innercoats of the carotid vessels are lacerated.

3.23.3.2.3 The thyroid and cricoid cartilages may be fractured but the hyoid bone often escapes injury.

3.23.3.2.4 Lungs are congested and haemorrhagic on cut section.

3.23.3.2.5 Frothy blood is present in the respiratory passages.

3.23.4 DIFFERENCES

Hanging and strangulation by ligature can be differentiated one from the other on the basis of the following features:

Table: Differences between Hanging and Strangulation

Feature	Hanging	Strangulation
External:		
1. Neck	Elongated	Not so
2. Face	Pale Petechial Haemorrhages are few	Congested, petechial Haemorrhages are numerous
3. Saliva	Dribbling from the lower angle of mouth	Not so
4. Bleeding	Rarely from urethra	Commonly seen from the mouth, Nose and ears
5. Ligature mark	Oblique, in-continuous and above the thyroid cartilage.	Horizontal, completely encircling the neck and at or below the level of thyroid cartilage.
6. Signs of struggle	Absent	Present
7. Hypostasis	In the lower limbs	In the dependent parts i.e. front or back

Internal:

1.	Tissues beneath the ligature mark	Dry white and glistening	Ecchymosed
2.	Neck muscles	Rarely injured	Bruised
3.	Fracture of larynx and cricoid cartilage	Rare	Common
4.	Inner walls of carotid vessels	Not lacerated except with long drop	Commonly lacerated
5.	Hyoid bone	Fractured about in 20% of cases	Not fractured

3.24 THROTTLING

In throttling compression of the neck is brought about by the hands of the assailant. The victims are often women and the crime may follow rape.

3.23.5 POSTMORTEM APPEARANCE

The evidence is often minimal and a painstaking search is necessary.

3.23.5.1 External:

3.23.5.1.1 Nail marks: Crescentric abrasions on the neck, caused by the nails

3.23.5.1.2 Finger marks: Bruises caused by the pressure of fingers, either discrete or confluent and seen on either side of the neck.

3.23.5.1.3 Sings of sexual interference in female

3.23.5.1.4 Signs of struggle like disheveled hair, torn clothes, broken bangles and injuries like scratches, bruises etc.

3.23.5.2 Internal:

3.23.5.2.1 Bruising of the tissues and muscles of the neck.

3.23.5.2.2 Haemorrhages beneath the capsules of the thyroid and salivary glands.

3.23.5.2.3 Fracture of the superior horn of the Hyoid bone at the junction of the outer 1/3 with inner 2/3 especially in individuals above the age of 40 years.

3.25 DROWNING

It is an asphyxial death occurring due to submersion of the body in water or in any fluid medium which prevents air entry into the lungs.

3.23.6 CIRCUMSTANCES

A. Drowning may occur in fresh water as in wells, canals, ponds, tanks, lakes or rivers, or in sea water. Death is more rapid in fresh water than in sea water.
B. The submersion of the body may be complete or only partial. Even a small quantity of water as is present in a ditch can drown a person when he falls face down in it and is unable to get up. This may occur in children or in adults due to intoxication or in an epileptic fit or heart attack.
C. Sudden inrush of water, especially when cold, may cause spasm of larynx causing asphyxia. It is known as dry drowning as no water enters the respiratory passages and lungs.

3.23.7 SEQUENCE OF EVENTS

When a person falls in water and does not swim, he sinks into the water column to a depth proportional to the momentum of the fall but rises to the surface due to the buoyancy and struggling movements. He shouts for help while at the water surface and instinctively grapples at any object such as the weeds in the water or the gravel or stones at the edge to save himself. Water enters the respiratory passages exciting a violent cough. He sinks and rises again repeatedly each time going deeper as the body becomes heavier due to further water intake. Ultimately the body sinks and rests at the water bed. The person dies usually in 5 – 8 mts due to biochemical changes in the blood following drowning. Occasionally there may be a state of suspended animation and survival up to ½ hr. Later decomposition sets in the dead body and gases of putrefaction accumulate bloating and lightening the body, which then floats to the surface in about 24 to 48 hours in winter and 18 – 36 hours in summer.

3.23.8 POSTMORTEM APPEARANCES

External:

a) Clothes may be wet

b) Mud, gravel, sand or small plants (e.g. Algae) may be found on the clothes and the body.

c) Copious, fine white, lathery, tenacious froth is seen at the mouth and nostrils.
d) Cadaveric spasm may be seen in the hands with weeds, sand or gravel in the grip of the hand.
e) Nodular or puckered appearance of the skin (Goose skin) due to contraction of the tiny muscles at the roots of the hair.
f) The skin of the palms and soles of the feet are pale, sodden and wrinkled (washer womanÊs hand appearance) due to imbibitions of water.
g) Postmortem injuries caused by aquatic animals like fish, crabs etc., may be found.

3.23.8.1 Internal:

a) Froth is seen in the respiratory passages.

b) Mud, sand or gravel or plant matter may be seen in the respiratory passages especially at the bifurcation of the trachea or the root of the lungs.

c) Coin-sized haemorrhages occur beneath the pleura on the surface of the lungs occurring due to rupture of the capillaries.

d) Water-logging is seen in the lungs. Lungs are massive and voluminous and pour out frothy blood stained fluid on cut section.

e) Water is present in the stomach, small intestine and also in the middle-ear.

f) All the organs may be congested.

3.23.9 LABORATORY TESTS

A. Diatoms Test:

Diatoms are microscopic plants with a sandy coat that makes them resistant to acid and heat. The bone marrow of sternum or Femur is examined for diatoms by the Acid digestion method. A litre sample of

water collected from the exact suspected site of drowning should be sent to the medical officer for comparison of the diatoms. Diatom test is of special value in detection of drowning in decomposed bodies and skeletal remains.

B. GettlerÊs Test:

The chloride concentration in the blood on the right and left sides of the heart estimated separately to decide the type of drowning. The chloride concentration on the left side of heart is 50% less in fresh water drowning and 30 – 40% more in salt water drowning as compared to the right side.

3.23.10 MODE OF DEATH

Drowning is mostly accidental or suicidal. Homicide should however be considered when there are signs of struggle on the body or associated poisoning, drugging or gross head injury. When boulders are found tied to the body by ropes the manner of tying and whether possible or not by the victim himself should be examined and assessed. Sudden submersion of the head of a person below water in a bath tub is possible with rapid unconsciousness and death following without any obvious injury.

3.26 SUFFOCATION

It is asphyxia due to mechanical obstruction other than constriction of the neck and drowning. Suffocation is a slow type of asphyxial death with well marked asphyxial signs. Specific signs indicative of the mechanism of obstruction are seen in individual cases.

3.23.11 TYPES OF SUFFOCATION

3.23.11.1 Smothering: Asphyxia is caused by closure of mouth and nose. It may be homicidal or accidental.

1. *Homicidal:* Infants or terminally ill or disabled old persons re killed by smothering by hands, pillows or bedclothes.

2. *Accidental:* Infants may roll over and get smothered in soft pillows or bed clothes. Incapacitated or intoxicated adults may be accidentally smothered when they fall face down in mud, sand, grain or loose soil.

Postmortem appearances: Evidence of smothering is minimal and must be looked for carefully. Often it is not more then contusion or splits on the inner aspect of the lips, flattening of the nose and occasional fracture of the nasal septum.

3.23.11.2 Gagging: It is closure or stuffing of the mouth by a piece of cloth (gag).

Postmortem appearances: A gag is found in the mouth or throat with associated bruises.

3.23.11.3 Choking: Asphyxia is caused due to obstruction of the respiratory passages from within. It is always accidental.
Causes: Choking is brought about by:

1. *Foreign bodies:* Children may be choked by pins, buttons, pebbles, marbles, seeds, etc, when they attempt to swallow them. Adults may be choked while having their meal, especially after consumption of alcohol, by food items such a meat piece, fish bone, fruit stone or even a bolus of food. Old people may be accidentally choked by dentures.
2. *Diseases:* Poliomyelitis, Tuberculosis and lung abscess may lead to death by choking terminally.
3. *Laryngeal Oedema:* Allergy or exposure to steam or irritant fumes may cause sudden swelling of the mucosa of larynx leading to obstruction and asphyxia.

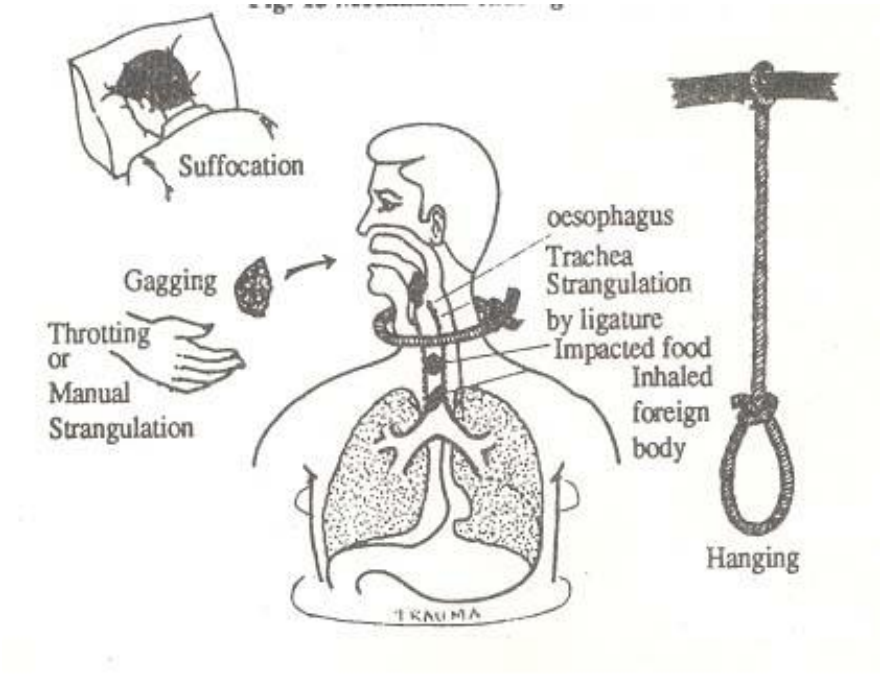

Fig. 15 Mechanism causing suffocation

D. Overlying: It is asphyxia caused by the body weight of another person who lies over the victim. Infants may be overlaid by mothers when they share the same cot, by the flabby breasts or belly or other body part closing the mouth and nose and compressing the chest. This may occur specially when the mother is exhausted by overwork or is intoxicated. Infants may also be overlaid by the breast of mother while suckling. Adults may also be similarly killed by kneeling or sitting on the chest.
E.g. William Burke and Hare killed 32 victims in England in 1820 employing smothering in conjunction with overlying. Such type of asphyxial death is known as „Burking‰.

E. Traumatic Asphyxia: Asphyxia is brought about by trauma to the chest which respiratory movements. Traumatic asphyxia is mostly accidental.

Circumstances:

1. *Stampeding:* Sudden outbreak of panic or lawlessness may lead to stampeding. The trampling of the chest under the feet or the weight of other individuals results in asphyxia.
2. *Fall of debris:* Fall of debris due to collapse of the roof or wall of a building, roof of a mine, or fall of a boulder due to landslide on a mountain may result in traumatic asphyxia.
3. *Motor vehicular accidents:* In run over accidents, individuals may be pinned down by the wheel of the vehicle. Individuals traveling on the roof tops of trains may get entangled between the buffers, when they fall and die of traumatic asphyxia.

Postmortem appearances:

A. External:

a. Intense cyanosis in the head, neck and upper chest, with haemorrhagic spots.

b. Pallor below the level of compression.

c. Line of demarcation separating the cyanotic and pale areas.

B. Internal:

a. Eechymoses in the skin of the face, neck and front of the chest.

b. Fractures of ribs, spine, pelvis and limb bones.

c. Contusions and lacerations of the thoracic organs and structures.

UNIT III-C-Sexual Offences

3.27 SEXUAL ASPHYXIAS

Sex perverts labour under a belief that partial anoxia accentuates sexual gratification and hence contrive devices and experiments to procure the same. They may use ligatures round the neck, plastic bags round the head or inhale irrespirable gases to procure anoxia and sometimes these experiments may result in accidental deaths.

Examination of the scene of crime in such cases lends valuable clues such as presence of pornographic literature, entries in personal diaries about their previous experiences and experiments and sexual fantasies and the actual presence of contrived devices. However, such cases are more common in the western countries than in India

3.28 CLASSIFICATION

3.28.1 RAPE

Sec. 375 IPC defines rape and Sec. 376 IPC prescribes punishments for the convicts.

3.28.2 UNNATURAL OFFENCES (Sec. 377 IPC)

Voluntary intercourse against the order of nature with any man, woman or animal constitutes an unnatural offence. It is punishable with imprisonment of either description for a tern which may extend to ten years and shall also be liable to fine.

Examples:

A. *Sodomy:* Sexual intercourse through the anus either with members of the same or opposite sex.

B. *Buccal Coitus:* Sexual intercourse through the mouth.

C. *Bestiality:* Sexual intercourse with an animal. Bestiality is generally rare.

3.28.3 OTHER OFFENCES

A. Incest: It is sexual intercourse by a man with a woman who is closely related to him by blood or by marriage within the forbidden degrees of relationship, e.g. a daughter, sister, aunt, mother etc. Incest per se is not an offence but such sexual intercourse may be brought under the penalizing sections of 376 or 497 of IPC.

B. Tribadism (Lesbianism): Tribadism is female homosexuality. It is not covered by Section 377 IPC. However such abnormal sexual practice on the part of a wife if leading to the ill health of the husband can constitute cruelty towards him and form a ground for a decree of divorce.

C. Indecent assault: Section 354 IPC. It is an assault or use of criminal force on a woman with the intent or knowledge to outrage her modesty. A woman can also be guilty of indecent assault on a man.
D. Sex Perversions: These are perverse acts of men or women by means of which a person tries to obtain sexual gratification.
Examples:

3. *Exhibitionism:* It consists of indecent exposure of genital organs in public.

4. *Fetishism:* It is a perversion occurring only among males. The Fetishist experiences sexual excitement on seeing some part of the body of a woman or her dress.
5. *Transvestitism:* It is a perverted desire to wear the clothes of the opposite sex.
6. *Voyeurism:* It is a desire to observe the genitals of others or to view sexual inter course being performed by others.
7. *Uranism:* Sexual gratification is obtained by fingering, fondling, liking, etc.
8. *Frotteurism:* It is a compulsion to rub the genitalia against another person, usually in crowded places.
While sexual perversions per se are not offences, indulging in them in public places may constitute offence under Section 294 or 509 IPC.

3.29 GUIDELINES FOR INVESTIGATION

1. Examine the scene of crime for any evidence of struggle like disarrangement of furniture and bed clothing, broken glass bangles and trampling of grass, if outdoors, and also for loose hairs, fibers, buttons and stains of blood, semen, faeces and mud.
2. Avoid delay in sending the victim and the accused, if apprehended for medical examination. Delay could lead to loss of evidence due to:
a. Passage of time.

b. Inadvertent acts of the victim.

c. Deliberate effacement of evidence by the accused.

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end as soon as possible the articles seized by the Police (e.g. bed clothes stained with semen/blood) and the materials preserved by the doctors (e.g. vaginal swabs, public hair) to the forensic science laboratory for necessary examination by the experts.
4.1 RAPE

3.28.1 ROLE OF THE MEDICO-LEGAL EXPERT

Medical examination of the victim/accused helps to bring evidence relating to the following aspects:
A. Participation in recent sexual intercourse.

B. Age of the victim

C. Potency of the accused

The medical examination is conducted with the consent of the individual in the presence of a disinterested third party and without any delay. A statement of the circumstances of the incident is recorded and the examination is conducted on the following lines.

3.28.2 GUIDELINES FOR THE EXAMINATION OF A RAPE VICTIM

a. Clothes: Examined for

(i) Stains: of blood, semen, faces, mud, etc.

(ii) Loose hair/fibers

(iii) Recent tears, loss of buttons, broken loops of blouse hooks.

b. General examination: Attention is focused on

(i) Gait: whether painful

(ii) General demeanor and mood

(iii) Signs of intoxication by drink or drugs

(iv) General physical development

(v) Signs of violence: such as scratches, abrasions and bruises on the face, breasts and the thighs

(vi) Teeth bite marks: On the lips, cheeks, and breasts.

(vii) Nails: whether broken; debris beneath the nails is collected and examined for the blood group of the accused.

c. Local Examination:

(i) Pubic hair: loose pubic hair is combed and collected and matted pubic hair is clipped and preserved.

(ii) Stains: of blood and / or semen are swabbed or scrapped off.

(iii) Vaginal swabs/Smears: semen present in the vagina is collected by cotton swabs and preserved as such or by smearing it on glass slides.

(iv) Evidence of sexually transmitted diseases:

Gonorrhoea: Is indicated by a purulent discharge from the urethra with burning pain on urination. Incubation period: 2 – 10 days

Syphilis (Primary): is evidenced by a hard painless sore on the genitals, at the site of contact with painless enlargement of the lymphnodes of the groin. Incubation period: 2 – 4 weeks

(v) Local Injuries:

a. Contusions of labia, contusions or lacerations of the vagina or lacerations of the perineum.
b. Rupture of hymen: Tears of the hymen are described by doctors as analogous to the figures on the face of a clock (e.g. 6 oÊ clock position). Fresh unhealed tears bled when touched giving a clue about the time of causation.
In general, local genital injuries are more common in children and old people than in middle aged married women.

3.28.3 GUIDELINES OF THE EXAMINATION OF THE ACCUSED IN RAPE

a. Clothes: Examined for

Stains: of blood, semen. Loose hair / fibres
Foreign matter e.g. Lipstick, paint Tears or loss of buttons

b. General examination: Attention is focused on

(i) Physical development: whether adequate to overpower the victim.

(ii) Mental condition: whether harbouring any sexual fantasies, obsessions or abnormality.
(iii) Signs of drunkenness

(iv) Injuries caused by the victim e.g. scratches; teeth bite marks.

c. Genital examination:

(i) Development: Subnormal development in adults or precocious development in children, indicate endocrinal disorders.
(ii) Pubic hair: Loose pubic is combed off and matted pubic hair is clipped and collected.
(iii) Evidence of sexually transmitted diseases (vide supra) is noted.

(iv) Injuries: may be in the form of scratches, bruises or lacerations.

(v) Smegma: It is a cheesy white substance which collects over the penis beneath the foreskin and is rubbed off into the female genitalia during coitus. Its presence in the accused negates the possibility of sexual intercourse in the preceding 24 hours.
(vi) Vaginal epithelial cells: Due to the friction of the genitalia in sexual intercourse, epithelial cells lining the vagina get dislodged and transferred to the penis. They contain granules of glycogen which turn blue on exposure to Iodine. The test is conducted by

wiping the penis with a filter paper and exposing it to Iodine fumes.

3.28.4 MEDICO-LEGAL ASPECTS OF RAPE

a. Medical Opinion: Medical examination of the victim and the accused can help only to indicate the occurrence of recent sexual intercourse or not but not whether rape has occurred or not. Rape is a Criminal Offence determined by legal parameters and not a medical condition diagnosed clinically.

b. Signs of Struggle: They may be absent when the victim is incapacitated by drink, drugs or disease or is overpowered by the assailant or is overwhelmed by fright. They are also absent in cases of rape on account of invalid consent e.g. sexual intercourse with a willing girl but who is under 16 years of age.

c. Genital Injuries: Slightest penetration of the victimÊs genitalia by the penis of the accused constitutes rape and this may not result in any visible injuries. In general genital injuries are meager or absent altogether in victims who are otherwise (married and) used to normal sexual intercourse.

d. Physical Evidence: Pubic hair, seminal stains and blood stains might be lost with the passage of time or by the inadvertent or deliberate acts of the victim and accused respectively, such as wiping, washing or bathing.

e. Virginity: A virgin is a woman who has never experienced sexual intercourse. Virginity can not be assessed by medical examination conclusively nor is it required in legal trails of rape cases.

f. Semen Collection: In some individuals, blood group substances are emitted into their bodily secretions such as saliva, sweat, semen etc. When the blood group of the semen on the garments or in the vaginal swabs of the victim is determined, it can be matched with the blood group of the accused which can be determined either directly from his blood or his Saliva, if he is a„SecretorÊ.

It is therefore not essential to collect the semen of the accused for comparison with the stains on the victim/ or her clothes and such practice existing in any quarters should be discouraged forthwith. This issue can be examined afresh when DNA fingerprinting is routinely available.

OTHER ASPECTS OF MEDICAL EXAMINATION

A. Sexual capacity of the accused: The accused should be physically examined and his clinical history studied for causes of impotence.

Causes of Impotence:

General:

Local:

(i) Age: Extremes of age.

(ii) Illness: Diseases of Brain and Spinal cord; Diabetes. (iii)Injury: Injury to head, spine or genitals (iv)Psychological: Fear, aversion, guilt, latent homosexuality

(v) Addiction: Drugs like aphrodisiacs

(i) Congenital deformities: Absence of penis; Double Penis, etc.

(ii) Acquired conditions: Cancer; Amputation of the penis.

Opinion: When such causes are excluded in a male, potency can be presumed to exist, though it can not be positively proved. Hence, the medical report is given in a double negative form stating that there is nothing to indicate that the individual is not potent.

B. Age determination of the victim: In the absence of direct proof of age such as birth register extract or certificate of date of birth the age of the victim can be determined on the basis the examination of the following data:
Physical:

(i) Height, Weight and build

(ii) Voice: High or low pitched (iii)Eruption of Axillary/Pubic hair
(iv)Development of genitals

(v) Onset of menses and development of breasts in the female

(vi) Eruption of beard and moustache in the male

Dental: Eruption of the different teeth.

Radiological: X-ray pictures of the joints are taken to study the appearance and fusion of the ossification centers.

Opinion: The medical opinion regarding the age reflects only an approximate estimation of the age. Hence age is expressed in the form of a range or is prefixed with the word about (e.g. about 15 years; 15 – 16 years).

3.30 UNNATURAL SEXUAL OFFENCES

3.28.5 SODOMY

It is anal intercourse between two men or between a man and woman. When done with mutual consent both the active and passive agents are punishable. A wife can not consent to be a passive agent as marriage provides only for normal sexual intercourse and not for anal intercourse.
Medical examination:

Passive agent: He/she may come with clothes torn and often stained with faeces blood and semen. The gait may be painful and signs of struggle may be present on the body. Injuries may be present around the anus and semen may be deposited on the hair around the anus, matting it or may be ejaculated into the anal canal, which can be collected by a swab for examination.
Active agent: His clothes may be torn and stained with blood, semen and faeces. Local injuries may be seen on the penis. Evidence of S.T.D. such as Gonorrheal discharge or a syphilitic sore may be present.

AL INTERCOURSE
Passive Agent:

The oral cavity may contain the semen of the accused and injuries such as bruises.

Active agent:

The penis may show teeth-bite marks and smearing with saliva.

3.28.6 BESTIALITY

It is intercourse by a human being with a lower animal. The animals commonly chosen are she-asses, goats, bitches, cows and even hens.

Accused:

The clothes may be torn and stained with blood, semen and dung. Animal hair and blood may be found on the clothes and body. Injuries may also be caused on the body of the accused from the kicks and bites of the animal.
Animal:

The animal may contain human hair, on its body and human spermatozoa or gonorrheal discharge in its vagina or rectum.

GLOSSARY

1. Asphyxial death
2. Burns
3. Injuries
4. Death
5. Hanging
6. Strangulation
7. Rape

LEARNING OUCOME

After studying this unit you should be able to:

1. The different types of injuries.

2. Nature of the injuries and the mode of death.

3. What is Asphyxial death and its various types.

4. Different guidelines for investigation.

5. What is Rape and other Unnatural sexual offences.

SUGGESTED READINGS

1. Forensic Biology- Jane E. Schober, Richard Li, and Sue Norman
2. Forensic Science: An Introduction to Scientific and Investigative Techniques

UNIT IV

LEARNING OBJECTIVES

In this unit you will be introduced to:

1. The A-B-O antigens and antibodies found in the blood for each of the four blood types: A, B, AB, and O.
2. The list of forensic tests used to characterize a stain as blood.
3. The concept of antigen-antibody interactions and how it is applied to species identification and drug identification.
4. Contrast chromosomes and genes.
5. List the laboratory tests necessary to characterize seminal, saliva, urine, faecal and milk stains.
6. How suspect biological fluids are to be properly preserved for laboratory examination.
7. The proper collection of physical evidence in a rape investigation.
8. DNA structure.
9. DNA extraction procedures.

UNIT IV-A- Serology

4.0 DEFINITION AND SCOPE

Forensic Serology is defined as study of procedures and practices involved in the identification and examinations of blood and other body (physiological) fluids. The material objects encountered in violent crimes such as murder, sexual assault etc., are received in this section for their analysis. The species and group (ABO) identification of biological fluid stains such as blood, semen, saliva, urine, faeces, vaginal swabs etc., by serological methods are carried out in serology section.

The following types of crime cases are dealt in Forensic Serology:

1. Murder
2. Violence
3. Cases involving injury
4. Sexual assault
5. Sodomy
6. Bestiality
7. Strangulation
8. Hanging

The following types of physical evidence are examined:

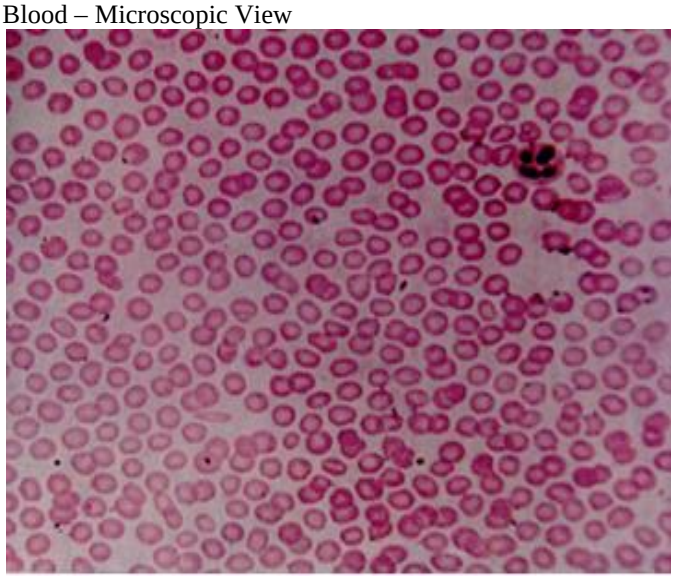
1. Whole blood
2. Blood stains
3. Semen
4. Seminal stains
5. Saliva stains
6. Urine stains
7. Faecal stains
8. Milk stains
9. Tissues
10. Teeth
11. Bones

4.1 BLOOD

4.1.1 INTRODUCTION

- Blood is one of the most important & most frequently encountered type of evidence.
- In most of the violent crimes like murder, assault, sexual assaults, road accidents etc.
- It can be found at the crime scene, on the weapon, on the victim, on the accused in and on the vehicle or any other point of contact and provides a good investigative lead to the Police Officer. (Locard's Principle of Exchange).

Blood – Microscopic View



4.1.2 COMPOSITION OF BLOOD

Blood is a liquid connective tissue - a very essential part of the body.

Blood is composed of two parts:

- The Cellular Portion.
- The Liquid Portion.

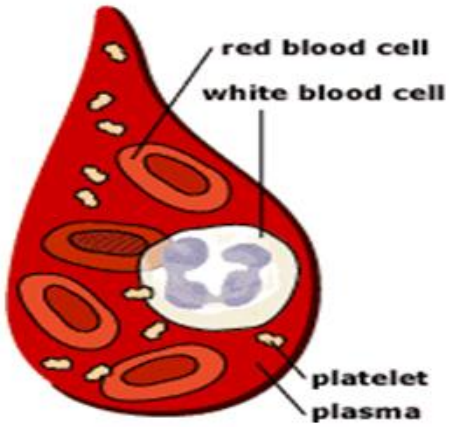
The Cellular portion contains:

- Red Blood Corpuscles or erythrocytes,
- White Blood Corpuscles or leukocytes &
- Platelets or thrombocytes.

The Liquid portion contains:

- The cells are suspended in a fluid or plasma which contains proteins, liquids, glycoproteins, enzymes, hormones, vitamins, electrolytes, urea, etc.
- The total volume of blood within the human body varies from 5 to 6 litres or about 1/12 of the body weight.
- The cellular portion of blood constitutes about 45% of the volume of blood & the plasma is about 55%.

A drop of blood-schematic view



Red Blood Corpuscles or Erythrocytes:

- These are small non-nucleated, biconcave, flexible & elastic disc shaped cells.
- They are pale buff colour when seen singly, but in masses they appear red & give the colour to blood.
- Haemoglobin makes up approximately 90% of the total volume of RBC.
- It is a complex protein rich in iron; haemoglobin has an affinity for oxygen & combines with it forming oxyhaemoglobin in the tissues from the lungs.
- The RBCs are synthesized in the bone marrow & disintegrated particularly in liver & spleen.
- The red color of the blood is due to the presence of haemoglobin.

White blood corpuscles or Leucocytes:

These are nucleated, (& do not contain haemoglobin) play a major role in defense mechanism i.e. protecting the body from microorganisms or infections.
Thrombocytes or Platelets:

There are small cells about 1/3 of the size of a RBC, they help in the coagulation of blood.

Red Blood cells White Blood Cells Platelets

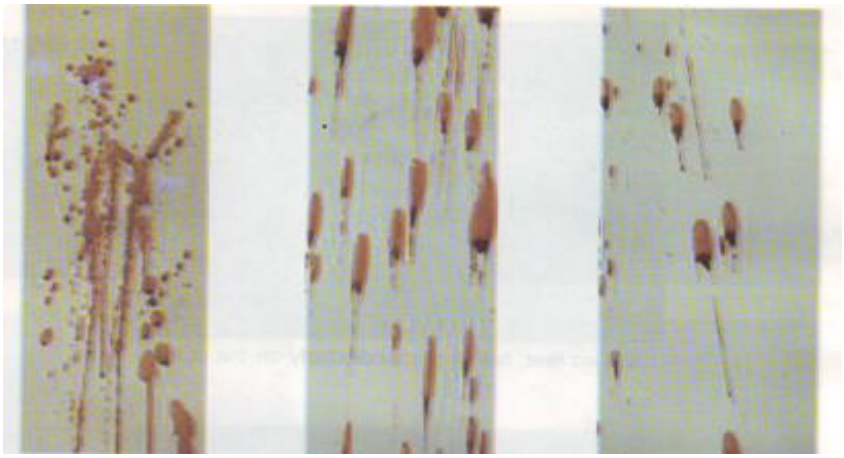
4.1.3 FUNCTIONS

- Blood circulates through every tissue & participates in every major functional activity of the human body.
- It can be called an organ, because it is the body's most voluminous one.
- It is a body's main transport system.
- It plays a leading role in the regulation of salt & water distribution, acid-base balance & temperature.
- It is responsible for essential defense mechanism.

4.1.4 LOCATION OF BLOOD STAINS

- In search for blood stain evidence at the crime scene, the investigator/IO should rely on the circumstances & any information available concerning the crime.
- The important places for the location of blood are the scene of occurrence, the culprit, the victim, the weapon of offence, the vehicle and the route the culprit has taken after the commission of crime.
- The search for the location of stains is carried out in the following way.
- The area, articles and clothing suspected to bear blood stains are examined with strong oblique light and most of the stains become visible.
- The light is used even during the day time. The ultraviolet rays are very useful in the location of blood stains.
- The article suspected to carry blood stains should be examined preferably in the dark, when even the washed stains become visible.
- Infrared rays are also useful in locating the blood stains on colored objects, where the blood stains become invisible because of the color of the background.
- A thorough search should be made on the clothing, beddings, curtains, upholstery, carpets, papers and rags used to wipe off the blood at the scene.
- The culprit may carry blood on his hands, crevices of the nails or hair etc.
- If the culprit makes use of a vehicle, he is likely to deposit some blood on the upholstery.
- In a case of rape, blood is likely to be found in the genital region of the perpetrator, on his clothing and particularly on his underwear.

Blood stain patterns on immovable



4.1.5 SIGNIFICANCE OF BLOOD EVIDENCE

Blood evidence provides an important source of information for a case concerning the scene, the victim and the assailant. Blood evidence is essential in the following situations:

Information from the blood distribution pattern:

1. Where the position of the victim at the time of injury is determined

2. Where the movement of the victim can be observed

3. Where the removal of the body from the scene is traced

4. Where storage of the body can be noted

5. Where evidence remains whether the body has bled before removal

6. Where blood soaking through a mattress, floor, or other surface may provide volume estimation

7. Where the relationship of the victim to the assailant may be deduced from the blood on the assailant's clothing

8. Where the amount of blood loss is to be estimated.

9. Where aging of the blood accumulation must be determined

10. Where it is required to determine whose blood is present and whether there are intoxicants present

11. Where there may be a question of how much blood loss is present and its potential effect on the condition of the victim.

12. When there is any tissue present in the blood evidence such as brain tissue that would make it difficult for the victim to move or be conscious.

4.1.6 COLLECTION OF BLOOD STAINS

- Clean gloves should be used to collect blood, body fluids or any biological material.
- Blood stained garments should be thoroughly dried in shade before they are wrapped in clean wrapping paper.
- Each garment should be wrapped separately and stains should not come into contact with other clothing.
- While rolling the garment, care should be taken to avoid two stains touching each other, as they could belong to two different sources.
- A clean paper should be inserted between each fold.

- It is advisable to collect liquid blood sample on a piece of sterile cloth or cotton or filter paper dipped in saline water and dried.
- The blood stains may appear on a big objects which cannot be transported like vehicles, furniture bedding cots etc., - then blood stained area should be

removed by cutting /scrapping, on to a clean piece of paper, using a clean knife / blade.

- Also Collect Control sample from the unstained portion around the stained area.

- Control sample sent will help to eliminate false positive results on account of substrate
- A flashlight at a slanted angle may be used to search faint / less visible blood stains.
- Luminol is a chemical reagent useful in locating small traces of blood in dark at a crime scene.
- The area surrounding the victim of a homicide, or the area of violence suspect- victim contact, should be thoroughly searched for bloodstains.
- The clothes and the body of the victim should be examined.

- The investigator should not overlook the fingernails and cuticles of the victim's hands, as these areas may contain traces of the suspect's blood left as a result of a struggle.
- Any possible weapons discovered should be examined, like knife, sword, axe, dagger etc.
- The edges of the blade could have been wiped off the blood, by the suspect. Once the immediate area of suspect - victim contact has been searched, attention should be focused on other areas of the scene.
- Bloodstains in area not easily correlated with the victim may possibly belong to the suspect and should be properly recorded and collected.
- Sometimes criminal will attempt to clean up the scene.

- Furniture will be straightened, and blood will be washed off in an attempt to conceal the crime, destroy evidence, or to delay the discovery of the scene.
- The crime scene search in this case should be extended to areas not in direct view.

- Blood that has been shed on a wooden/ tiled or linoleum floor and then washed can usually still be found in the cracks, joints between tiles or wooden planks, or under the corners of the linoleum, as these areas are somewhat resistant to washing attempts.
- Bloody hands will usually be cleaned at the first opportunity.

- If a sink is used for this purpose, traces of diluted blood will remain in the trap of the drain for a considerable length of time.
- In the absence of washing facilities, the suspect usually wipes his hands on something to clean the blood.

- The natural inclination is to use something other than one's own clothing.

- For this reason the bottom of furniture, underside of rugs, curtains, and any towels or rags should be examined for blood.

4.1.7 QUESTIONNAIRE ON BLOOD STAINS

- The blood stains are examined to answer one or more of the following questions:

- Whether the stain in question is of blood or not?
- If it is blood, is it of human or animal origin?
- If it is animal, what species of animal?
- If human, what is the blood group?
- If required, D.N.A. Finger Printing.

4.1.8 LABORATORY EXAMINATIONS

(A) Identification of Blood

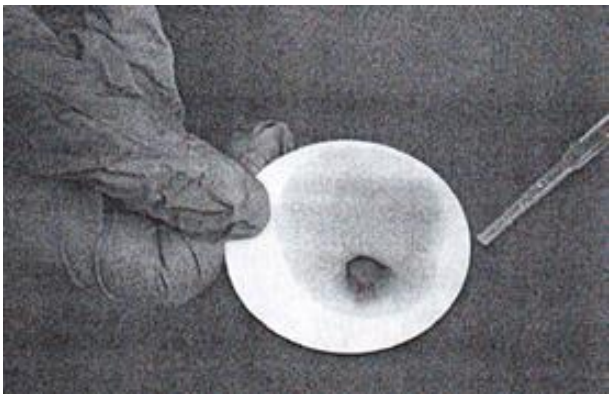
Preliminary or Chemical tests are of two types:

- Is the stain of blood or not
- This can be ascertained by performing preliminary tests at the scene of crime or in the laboratory.

Benzidine test: A drop of Benzidine solution is added to the stain + H_2O_2 (Hydrogen Peroxide) A few drops. -> develops greenish blue color.

Phenolphthalein test: Few drops of Phenolphthalein reagent is added to the stain -> pink color develop.

Preliminary test for Blood

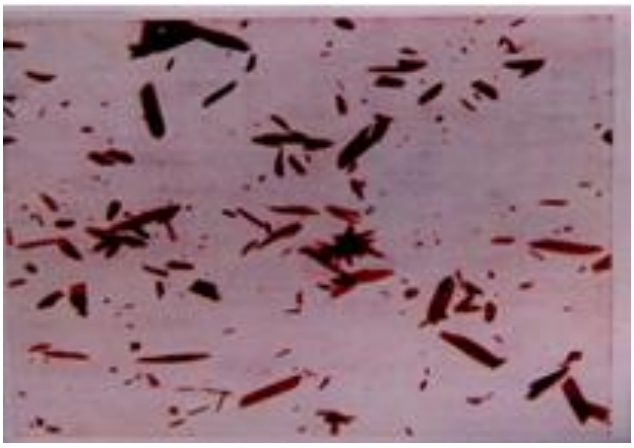


(B) Confirmatory or Crystal Tests

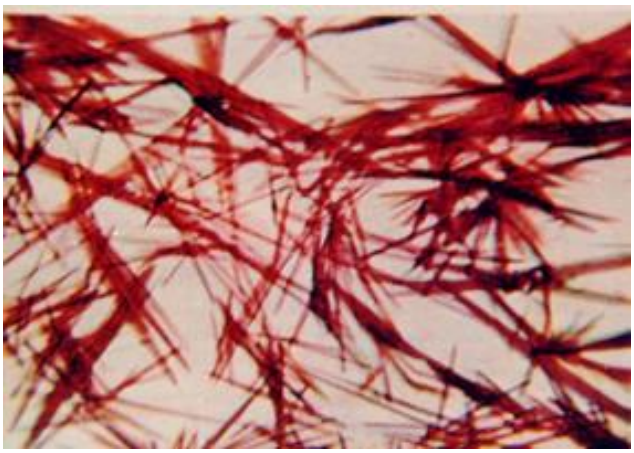
The Three Crystal Tests Are:

- Teichmann test (hematin)
- Takayama - test (hemochromogen)

Crystal tests – confirmatory tests for blood (Hematin)



(Hemochromogen) Crystals



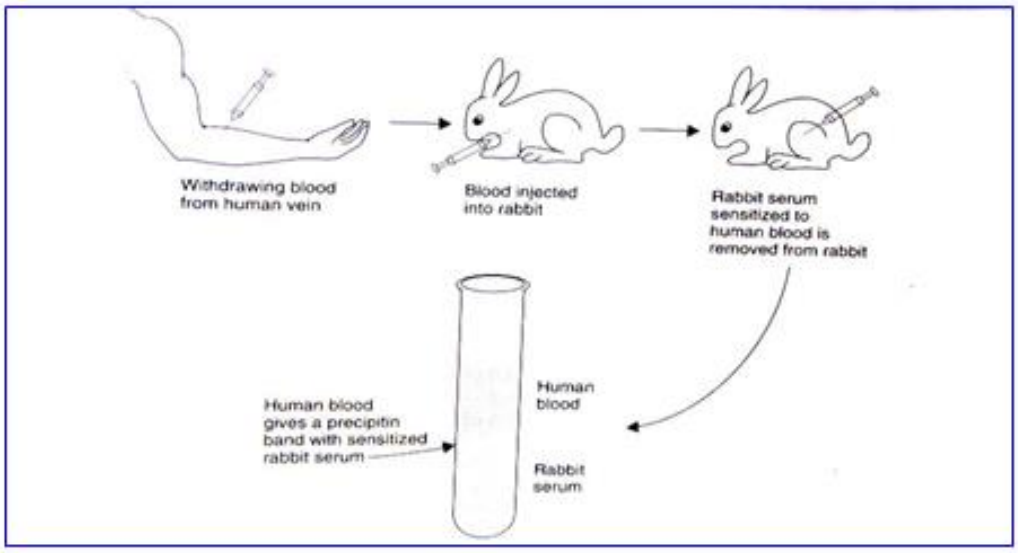
Identification of blood is made more specific if one or more of the confirmatory tests are performed.

(C) Origin of Species

Once the stain has been confirmed as blood, it is necessary to determine whether

- It is human or of any other origin.
- The tests are based on the principle that blood proteins (or blood globulins) are specific to each species.
- In other words the blood proteins of one species will not be similar with other species.
- Thus in experimental animals (like rabbits) by injecting blood proteins different antibodies can be raised for different species.

Preparation of Human Anti serum



- The antibodies, known as anti-human serum (produced after injecting human blood proteins into the experimental animals) are recovered and made to react with human blood serum to form a white turbid precipitate.
- This anti-human serum will not react with blood proteins from other animals.

There are three methods to determine the origin of species.

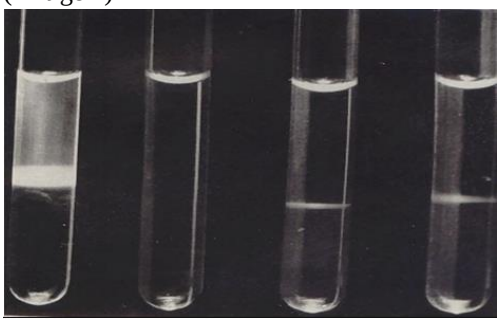
Principle–Antigen / Antibody reaction

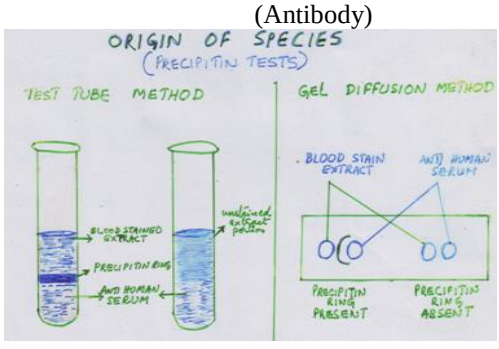
- Test Tube Method.
- Agar Gel Double Diffusion Method
- Electrophoresis

1) Test Tube Method

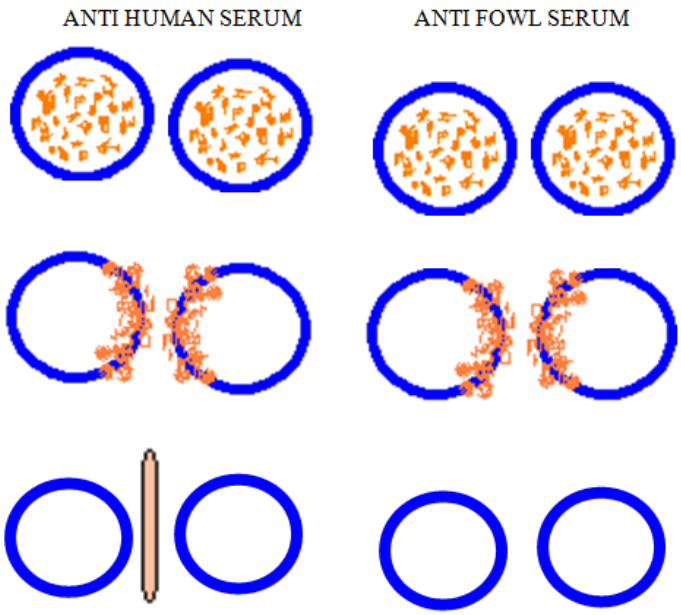
Dilute Blood Stain extract + Anti Human Serum

(Antigen) (Antibody)





Agar Gel Double Diffusion Method



BLOOD OF HUMAN ORIGIN

1) Electrophoresis

AGAR GEL DIFUSION METHOD

- Agar, seaweed is dissolved in normal saline and slides are prepared.
- Wells / holes are made on the slides.
- To one side of the wells, blood stain extract is (antigen) added and to the other side Anti Human Serum (anti body).
- The slide is left in the refrigerator overnight.
- Precipitation is observed at the junction of the two wells if the blood is human.
- Human blood stains dried for as long as two or three years may still give positive precipitation reaction.
- If the precipitation does not appear then the extract could be tested

separately against the anti-sera of various animals like sheep, goat, dog, cat, horse, cow, deer etc and also against the domestic birds like fowl etc, to know the origin of the blood stain.

After determination of the origin of blood as human, the stain is further examined to find the blood group.

(D) Blood Grouping

What are the different blood groups?

- The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies.
- The antigens are located on the surface of the red blood cells and the antibodies are in the blood plasma.
- Individuals have different types and combinations of these molecules.

- The blood group you belong to depends on what you have inherited from your parents.

There are more than 20 genetically determined blood group systems known today, but the AB0 and Rh systems are the most common and important ones.

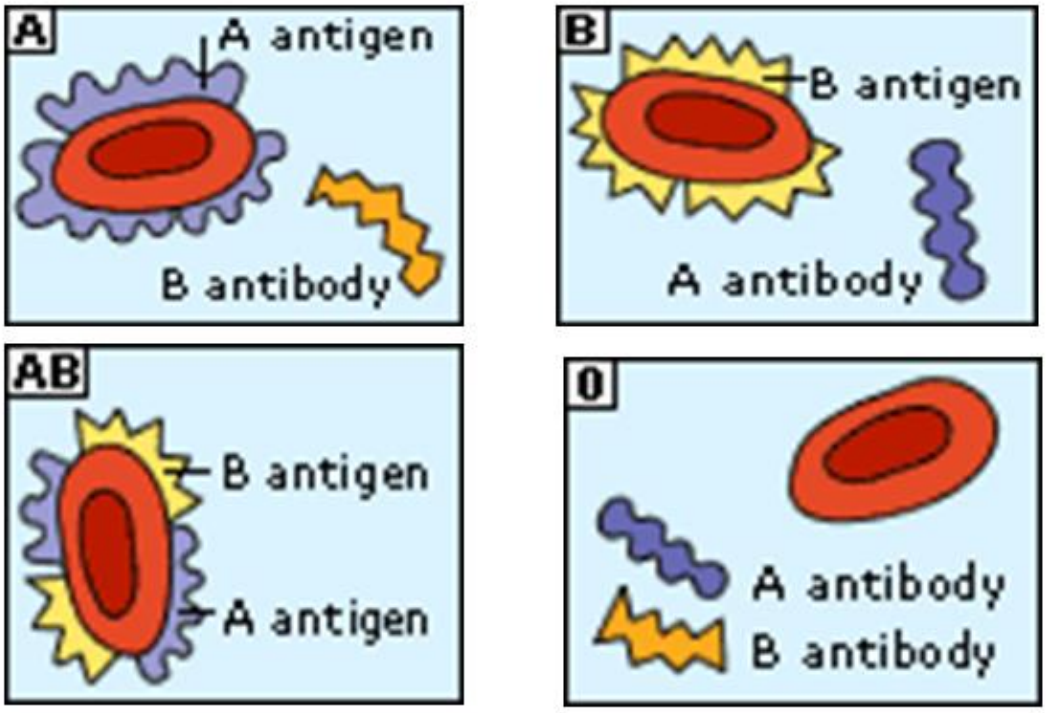
AB0 blood grouping system

According to the AB0 blood typing system there are four different kinds of blood groups: A, B, AB or O.

SUMMARY OF ANTIGEN, ANTIBODY COMPONENTS IN BLOOD

BLOOD GROUP	ANTIGEN IN RED CELLS	ANTIBODIES IN SERUM	APPROXIMATE % OF INDIAN POPULATION
A	A	Anti-B	22
B	B	Anti-A	33
AB	A&B	--	5
0	--	Anti-A & anti-B	40

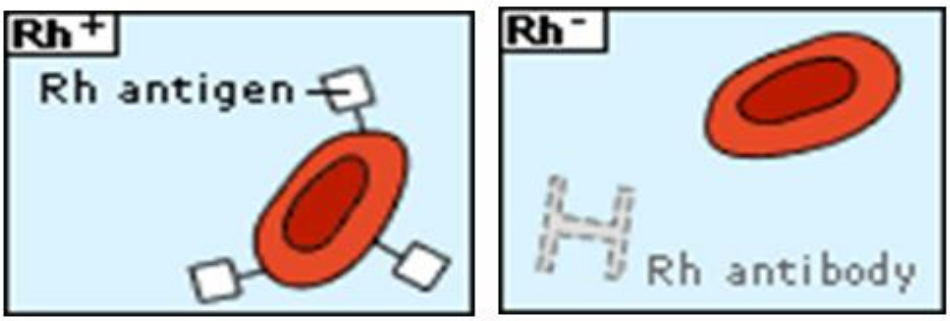
Schematic figures of antigens & antibodies in blood



Rh factor blood grouping system

Many people also have a so called Rh factor on the red blood cell's surface. This is also an antigen and those who have it are called Rh+. Those who haven't are called Rh-. A person with Rh- blood does not have Rh antibodies naturally in the blood plasma (as one can have A or B antibodies, for instance).

Rh factor in blood



Blood Group Notation

According to above blood grouping systems, you can belong to either of following 8 blood groups:

A Rh+	B Rh+	AB Rh+	0 Rh+
A Rh-	B Rh-	AB Rh-	0 Rh-

BLOOD GROUPS AND PATERNITY

PARENTS BLOOD GROUP	POSSIBLE BLOOD GROUP OF THE CHILD	IMPOSSIBLE BLOOD GR OF THE CHILD
A X A	A and O	B and AB
A x B	A, B, O and AB	NONE
A x AB	A, B and AB	O
A X O	A & O	B and AB
B X B	B and O	A and AB
B X AB	A, B and AB	O
B x O	B and O	A and AB
ABX AB	A, B and AB	O
ABX O	A and B	AB and O
O X O	O	A, B and AB

Blood Grouping of Blood stains

ï In dried blood stains as the red blood cells are all broken, it is not possible to under take direct agglutination tests for the purpose of grouping.

ï However the blood group antigens in the broken cells especially the ABO antigens retain their property of combining with specific antibodies for a considerable period.

ï There are three sensitive methods of determining the ABO blood group in dry blood stains.

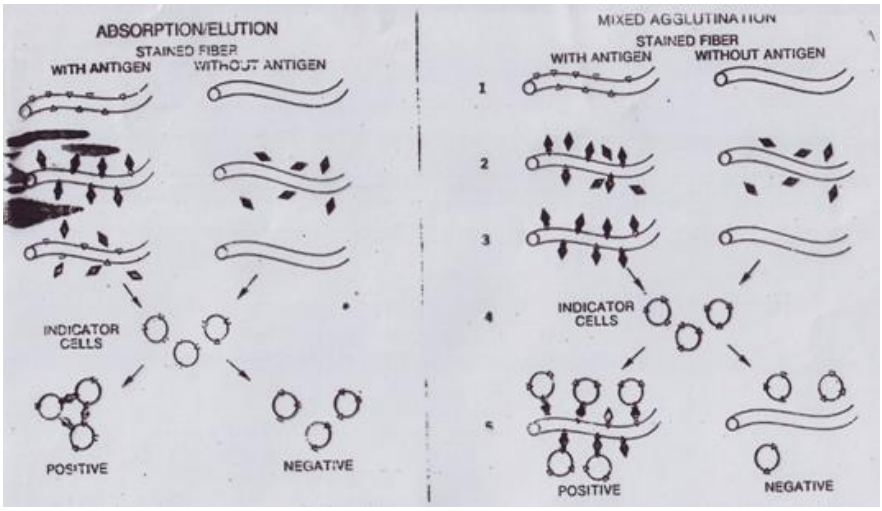
ï Mixed Agglutination Method.

ï Absorption Elution Method.

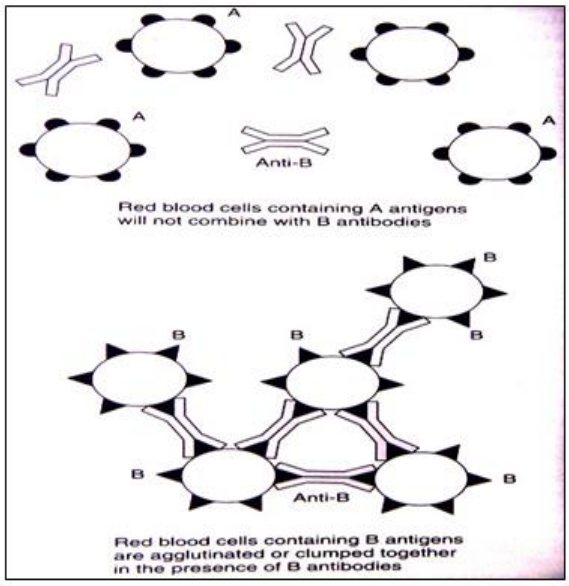
ï Absorption Inhibition Method.

Principle: Antigen & Antibody Reaction

Absorption Elution Method



Schematic figure of agglutination



Male or Female

- So long as the white blood cells can be identified, the sex can be determined from the barr body count.
- This method of sexing cells also known as nuclear sexing may be of help in determining sex in doubtful cases, mutilated bodies, and fragmentary remains.
- It is based on the differences in the nuclear chromatin pattern between the two sexes.
- In cells that are not dividing, the female shows a minute condensation of nuclear membrane known as barr body, cells showing the presence of such Barr bodies are known as chromatin positive.

Female cells are said to be chromatin positive and male cells chromatin negative, skin biopsy material buccal scrapings, and nuclei of cells of smooth muscle and cartilage are suitable for such study.

Other blood group systems

There are blood groups other than ABO system. They are Rh, MN types, DUFFY, LUTHRAIN, LEWIS, Gm, Gc serum groups and enzymatic groups like PGM, AK etc., which divide the blood group into hundreds of groups Polymorphic enzymes in the RBC exist in variable forms although they have the same function to play.

ABO	A	B	AB	O				
	22.0	33.0	5	40				
MN	M	MN	N					
	39.57	46.67	13.76					
Rh	R_1R_1	R_1R_2	R_1r	R_2r	rr	Ro Ro	R_2R_1	R_2R_2
	43.61	11.84	31.77	.16	3.84	2.10	1.24	0.74
Gm	(1,-2)	(1,2)	(-1,-2)					
	52.87	17.24	29.88					
PGM	1	2-1	2					
	44.30	40.51	15.19					
EAP	A	BA	B					
	7.50	28.75	63.75					
GLO-I	1	2-1	2					
	4.35	48.91	46					
EsD	1	2-1	2					
	63.1	32.7	4.2					
ADA	1	2-1	2					
	75.31	22.65	2.					
AK	1	2-1	2					
	80.69	17.78	1.53					

Distribution Percentage of Expected Frequencies of Different Blood Group Systems for Typical Indian population.

4.2 EXAMINATION OF OTHER (BODY) PHYSIOLOGICAL FLUIDS

- Semen, saliva, urine, sweat, milk, vaginal secretions, vomit stains, and faecal stains are commonly encountered during the investigation of crime – in case of homicides, suicides, sexual offences etc.
- These stains could be identified based on their physio-chemical properties.
- In a secretor individual which is a inherited character the blood substances are secreted in his glandular secretions like semen, saliva etc.
- Thus blood group could be determined in saliva, semen, gastric juice etc, by any of the techniques used for blood.

Secretors/non-secretors

The discovery of blood group substances in other body fluids such as semen, saliva, perspiration, mucus etc. has extended the field of serology. About eighty per cent of the human population secretes blood group substances in their secretions. Saliva and semen are especially rich in these substances. They contain about four times these substances as compared to blood of the same volume. The substances are water soluble and are interfered by cellular material present in the secretions. They are grouped by the mixed agglutination techniques.
A person who secretes the blood group factors in his body fluids other than blood

is called a secretor, the one who doesn't, is called a non-secretor.
Secretor status of a person is also a inherited character and is governed by two genes ÂSeÊ and ÂseÊ.

4.3 SEMINAL STAINS

- The seminal stain is an important biological stain which requires identification and characterization in the investigation of sexual offences.
- The stains are usually found on the clothing of the victims and the suspects especially on their undergarments.
- They may be found on bed sheets, on pillow cases, carpets, floor etc.

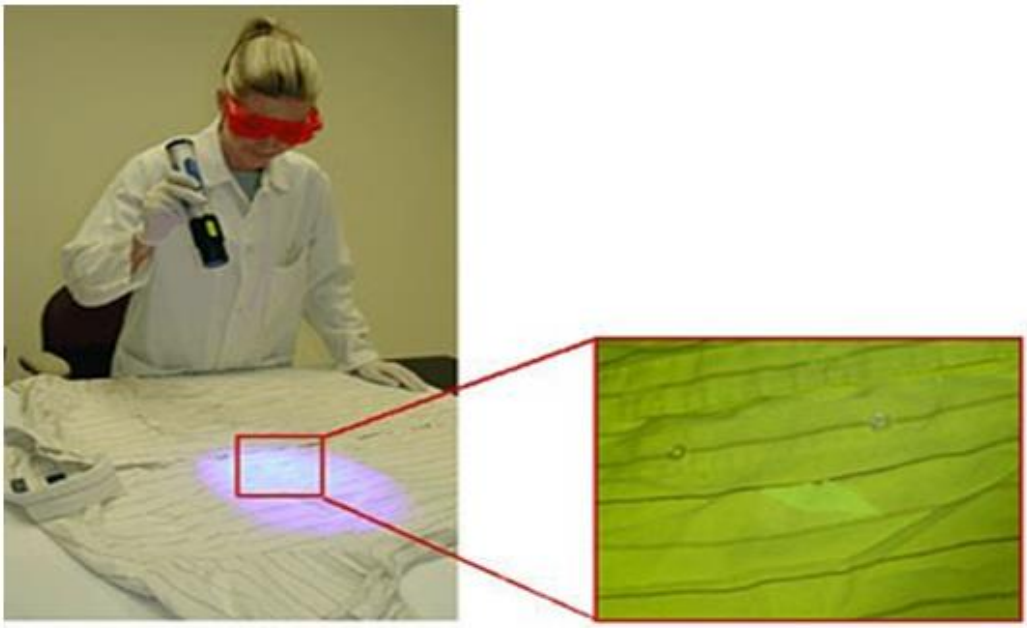
4.3.1 IDENTIFICATION OF SEMINAL STAINS

1) Physical Examination.
2) Chemical Test.
3) Crystal Test.
4) Microscopic Examination

1) Physical Examination:

Visual examination of articles like clothes, bed sheets, carpets, pillow cases, handkerchiefs etc. is first carried out by physical examination for the detection of suitable areas for testing.

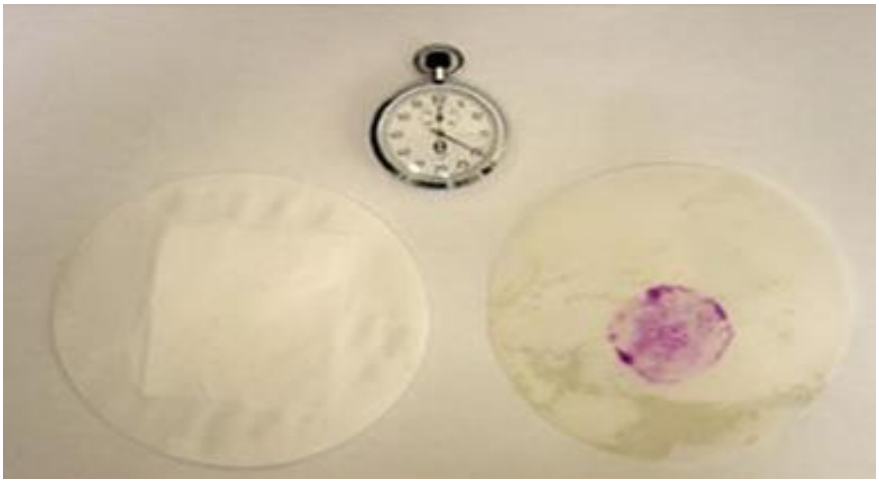
- A freshly dried stain gives characteristic smell.
- Ultra-violet rays are very useful in the location of seminal stains.
- The seminal stains when examined under ultra-violet rays give bluish white fluorescence.
- On naked eye examination in ordinary light dry seminal stains appear as greyish white or yellowish-grey stains having irregular outlines.
- Stains on black clothing appear as whitish stain.
- Stains on absorbent surfaces of fabric are generally stiff to feel.



2) Chemical Test:

Acid Phosphatase Test - Acid Phosphatase Enzyme

Stain portion + Reagent (Calcium alpha naphthol phosphate + diazonium compd Brentamine blue)
= Purplish Pink Color



3) Crystal Tests:

1) Dr. Florence Test - Choline

Reagents – Iodine	–	1.65 gms.
Potassium iodide	–	2.54 gms.
Distil Water	–	30 ml. Stain + reagent = Brown Colour Crystals

Choline Crystals

2) Barberio Test - Spermine.
Reagents - Picric acid.

Stain + Picric acid – Crystal yellow colour

Spermine Crystals

4) Microscopic Examination:

- Seminal stains to be extracted with distil water and a few drops of diluted HCL.
- Thin smear is prepared with a drop of extract.
- Staining of Smears.

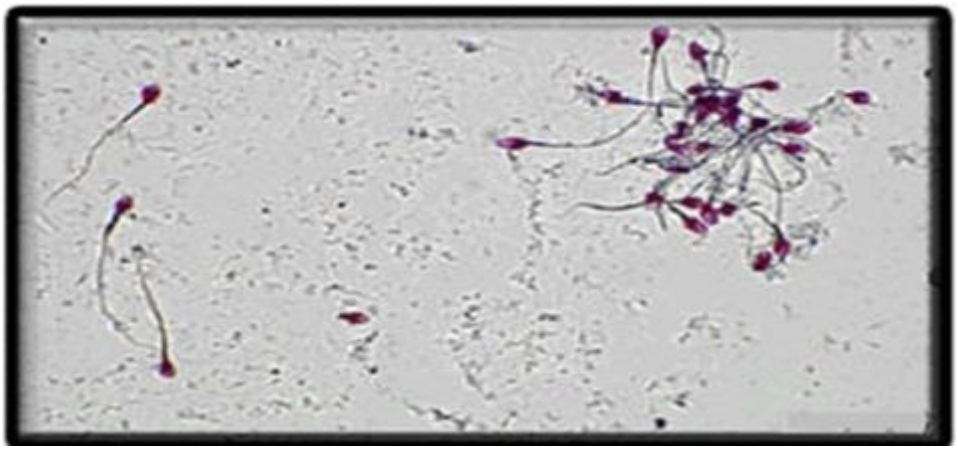
Malachite green - Eosin stain. Methylene blue - Eosin Stain. Haemalum - Eosin Stain.

- To be viewed under a microscope.
- Semen is a gel like fluid with a faint yellow colour and characteristic

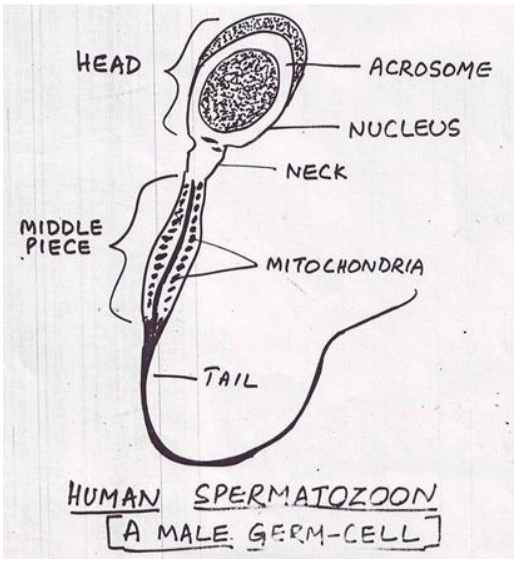
odour.

- It contains spermatozoa (= a male germ cell) and other cellular material suspended in the fluid known as the seminal plasma.
- Spermatozoa are formed in testicles and are stored for time being in the seminal vesicles.
- At ejaculation 400 to 600 million spermatozoa leave the body in the vehicle of seminal fluid.
- A normal ejaculation is about 3.5ml. The spermatozoa consist of a head, neck and tail and often look like a tadpole in appearance and in motion.
- Spermatozoa are approximately 1/500 inch in length or about 1/3 of Red blood cells.
- The spermatozoa measures about 50 microns in length and contains the flat, oval shaped sperm head, the middle piece or neck and a tail.

Human Spermatozoa – Microscopic view



Schematic figure of Human spermatozoan



4.3.2 SEROLOGICAL EXAMINATION OF SEMEN

- This serological examination may be used for the detection of semen, identification of species, and the identification of blood group in a secretor individual.
- Group specific substances like A, B and H can be determined in seminal stains derived from secretor individuals by using absorption inhibition, absorption- elution and mixed agglutination technique.
- Absence of reaction against any anti-A, anti-B, and anti-H indicates that either the individual concerned is a non-secretor or the stains have deteriorated to such an extent that the group specific substances have become undetectable.

Oligospermic – Few Spermatozoa Aspermic – No Spermatozoa (Vasectomy)

4.4 EXAMINATION OF SALIVA STAINS

- Stains of saliva are sometimes encountered in the investigation of criminal cases and it becomes necessary to detect such stains and characterize them.
- Saliva is a thick fluid secreted by salivary glands.
- Human saliva contains a fairly large amount of ptyalin, an enzyme responsible for conversion of starch to dextrine and maltose.

Amylase Test

Reagents - 1% starch solution

- Iodine solution Saliva + 1% Starch solution.
- Incubate at 37o c for ½ an hour.

\+ Iodine = Light brown colour.

Origin of species - same as done for blood stains.

The ABO blood group of a person could be determined by examination of the saliva stains by any of the techniques used for determining blood group in dried blood stains.

4.5 EXAMINATION OF URINE STAINS

- Sweat stains also contains urea and to differentiate between the two, a portion of the stain is extracted with distilled water and evaporated to dryness in a silica basin.
- On gentle heating of the residue over a flame an odour of urine can be detected indicating urine presence.

GEE's METHOD FOR THE DETECTION OF UREA:

Suspected Stain + Acetone – Extract. Extract + Acetone + Cone H No3
= rhombic Colorless Crystals (urea nitrate).

4.6 EXAMINATION OF FAECAL STAINS

1) The identification of faecal stains may sometimes be of great importance especially in cases of sodomy.
2) The following examinations are usually carried out in order to identify faecal stains.
Microscopic examination for the detection of food particles, pus cells, epithelial cells, cysts of Entamoeba histolytica, ova of intestinal helminths.

CHEMICAL EXAMINATION FOR THE DETECTION OF THE BILIRUBIN:

Edelmann's test:

Suspected stain + dis H20 + 2 or 3 drops of mercuric chloride solution + amyl alcohol (Thoroughly shake and the supernatant – a alcohol layer is pipetted off into another test tube).
Supernatant liquid = 2 or 3 drops of 10% alcoholic zinc chloride = Rose pink colouration.

4.7 EXAMINATION OF MILK STAINS

- Milk is an emulsion of fat globules in a colloidal solution of proteins, lactalbumin, lactoglobulin and caesin together with a number of crystalloids in solution.
- For identification of milk stains, extract from the suspected area may be subjected to paper electrophoresis.
- Colostrum contains large number of fat globules and cell fragments which are not generally found in mature milk.
- The differentiation between mature and immature milk is usually done by microscopic examination of the saline extract of the stain.

4.8 HUMAN BODY – REMNANTS – EXAMINATION

The tissues of human body, both the soft and hard, can be sent for examination to the forensic science laboratory for obtaining information regarding the nature of tissue, age (how old the tissue is), age of the person, sex, cause of death, time since death, manner of death, species origin, ABO and other blood group typing, enzyme typing and DNA profiling. The soft tissues should be preserved in

saturated, common salt solution for sending them to forensic science laboratory. The hard tissues such as skeletal remains should be adequately packed to prevent damage during the transit. In case there are soft tissues attached to the bones, the exhibit should be preserved in saturated common salt solution to prevent further decomposition changes of the soft tissues. The simple arithmetic about the proportion of the tissue and the common salt solutions is 1: 3 by volume.

4.9 GUIDELINES FOR COLLECTION OF SAMPLES FOR SEROLOGICAL EXAMINATIONS

5. Micro stains of blood and other biological materials found on floors, walls or other surfaces should be collected using a cotton swab moistened with normal saline.

6. All biological fluids like blood, saliva etc. should be collected on a sterile gauze cloth and air dried before packing and forwarding. (A piece of fresh gauze cloth used for the above may be sent as a control for serological examinations).

7. In cases of rape samples of blood, saliva etc. shall be invariably collected from the victim and accused on sterile gauze cloth, air dried, packed and forwarded.

8. In rape cases vaginal/cervical smears taken on slides as well as cotton swabs should be collected, air dried, packed and forwarded.

9. In rape cases all garments of the victim and accused should be air dried and sent.

10. In cases of sexual assault, the medical examiner's report should be sent along with the material objects collected from the victim and accused. The reports should contain details of

i) Age of the accused/victim.

ii) Date and time of examination.

iii) Date and time of occurrence of rape as reported.

iv) Blood group of accused and victim.

v) Information relating to the accused whether he underwent Vasectomy or not.

vi) Potency test report.

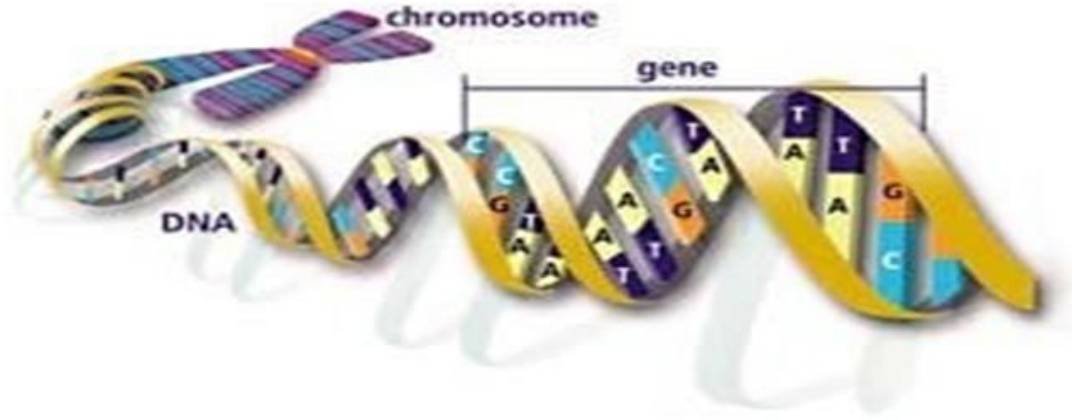
11. Each material object collected from victim or accused should be packed separately with proper tagging, labeling and sealing.

UNIT IV- B - DNA

4.10 INTRODUCTION

Deoxyribonucleic Acid (DNA) is the genetic material that makes us who we are. It is the blueprint from which we were made influencing our features, our personality, our habits, our likes and dislikes etc.

Human cells contain 23 pairs of chromosomes (46 chromosomes - 22 pairs of autosomes and a pair of sex chromosome). A human embryo is formed when an egg is fertilized with a single sperm cell that brings with it its own copy of each chromosome. Once fertilized, the egg cell contains everything that it needs to create a new individual. Our DNA is one-half of our mother's DNA and one-half of our father's DNA.



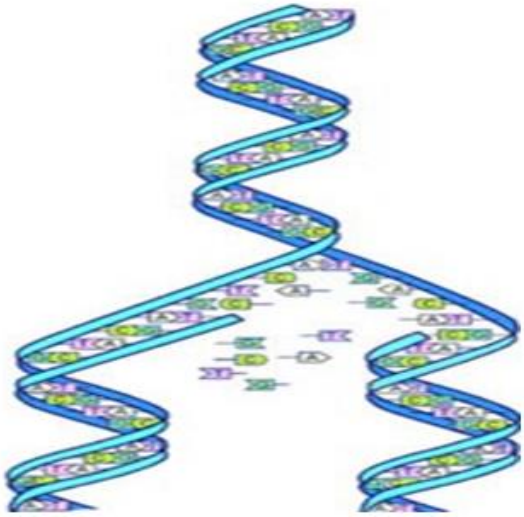
4.10.1 THE HISTORY OF DNA RESEARCH

The history of DNA research begins with Friedrich Miescher, a Swiss biologist who in 1868 carried out the first carefully thought out chemical studies on the nuclei of cells. Using the nuclei of pus cells obtained from discarded surgical bandages, Miescher.

detected a phosphorus-containing substance that he named nuclein. He showed that nuclein consists of an acidic portion, which we know today as DNA. Later he found a similar substance in the heads of salmon sperm cells. Although he separated the nucleic acid fraction and studied its properties, the covalent structure of DNA did not become known with certainty until the late 194Os.

4.10.2 DNA CARRIES GENETIC INFORMATION

Even though Miescher and many others following him suspected that nuclein or nucleic acid might play a key role in cell inheritance, others argued that their lack of chemical diversity compared to, say, proteins ruled out such a possibility. It was not until 1943 that the first direct evidence emerged for DNA as the bearer of genetic information. In that year, Oswald Avery, Colin MacLeod, and Maclyn McCarty, working at the Rockefeller Institute, discovered that DNA taken from a virulent strain of the bacterium Streptococcus pneumonae permanently transformed a non-virulent form of the organism into a virulent form.



Avery and his colleagues concluded from these experiments that it was the DNA from the virulent strain which carried the genetic message for virulence and that it became permanently incorporated into the DNA of the recipient non-virulent cells. Although the scientific community was slow to adopt the idea that DNA was the carrier of genetic information, a subsequent experiment provided evidence that this was indeed the case. In 1952, Alfred Hershey and Martha Chase showed by means of radioactive isotope tracer experiments that when a bacterial virus (bacteriophage T2) infects its host cell (the bacterium Escherichia coli), it is the DNA of the T2 virus, and not its protein coat, which enters the host cell and provides the genetic information for replication of the virus.

From these very important early experiments, and a wealth of other corroborating evidence, it is now certain that DNA is the carrier of genetic information in all living cells.

4.10.2 UNRAVELING THE DNA DOUBLE HELIX

Despite proof that DNA carries genetic information from one generation to the next, the structure of DNA and the mechanism by which genetic information is passed on to the next generation remained the single greatest unanswered question in biology until 1953. It was in that year that James Watson, an American geneticist, and Francis Crick, an English physicist, working at the University of Cambridge in England proposed a double helical structure for DNA. This was the culmination of a brilliant piece of detective work
- and a discovery that has proven to be the key to molecular biology and modern biotechnology. Using information derived from a number of other scientists working on various aspects of the chemistry and structure of DNA, Watson and Crick were able to assemble the information like pieces of a jigsaw puzzle to produce their model of the structure of DNA.

It had already been established by chemical studies that DNA was a polymer of nucleotide subunits, each nucleotide comprising a sugar (deoxyribose), phosphate and one of four different bases - the purines, adenine (A) and guanine (G) together with the pyrimidines, thymine (T) and cytosine (C). A most important clue was the discovery in the late 1940s by Erwin Chargaff and his colleagues at Columbia University that the four bases may occur in varying proportions in the DNAs of different organisms, but the number of A residues is always equal to the number of T residues; similarly equal numbers of G and C residues are present. These quantitative relationships are important, not only in establishing the three-dimensional structure of DNA, but also in providing clues on how genetic information is encoded in DNA and passed on from one generation to the next.

4.10.3 X-RAY DIFFRACTION REVEALS THE MOLECULAR STRUCTURE OF DNA

The elegant and comprehensive X-ray diffraction studies of Rosalind Franklin and Maurice Wilkins at King's College, London yielded a characteristic diffraction pattern from which it was deduced that DNA fibers have two periodicities along their long arms: a major one of 0.34 nm and a secondary one of 3.4 nm.

The problem that Watson and Crick addressed was to formulate a model of the DNA molecule that could account not only for these periodicities, but also for the specific A - T and G - C base equivalences discovered by Chargaff. The manner in which they solved the problem involved building three-dimensional models incorporating all these elements. This resulted in their now famous model for the structure of DNA consisting of two helical chains of DNA coiled around the same axis to form a right-handed doublehelix.

4.10.4 A SCHEMATIC DIAGRAM OF THE STRUCTURE OF DNA

In the double helix the two strands of DNA run in opposite directions and are complementary, being matched by the hydrogen bonds of the A - T and G - C base pairs. This complementary pairing of the bases ensures that, when DNA replicates, an exact duplicate of the parental genetic information is made. The polymerization of a new complementary strand takes place using each of the old strands as a template. The elegant simplicity of molecular structure of DNA in

relation to its role as the repository of all genetic information was not lost on those involved in its discovery.

Table 1: KEY DATES

• 1868: Friedrich Miescher isolates nucleic acid from pus cells obtained from discarded bandages
• 1943: Avery, Macleod and McCarty use bacteria to provide the first evidence that DNA is the bearer of genetic information
• 1952: Hershey and Chase show that it is the DNA part of the T2 viral particle, not the protein part, that enters a host cell, furnishing the genetic information for the replication of the virus
• 1953: Double helix structure of DNA is revealed by Watson, Crick, Franklin, and Wilkins
• 1961-65: Genetic code is cracked
• 1972: First successful DNA cloning experiments are carried out in California
• 1975: Monoclonal antibodies are produced
• 1975-77: Rapid methods of sequencing DNA are perfected
• 1977: The first human gene is cloned
• 1982: Genetically-engineered insulin is approved for use in diabetics in the USA and UK
• 1987: First genetically-engineered microorganisms are used in field experiments

4.11 HISTORY OF DNA FINGERPRINTING

DNA fingerprinting was first described in 1985 by Sir Alec Jeffreys. He discovered that certain regions of DNA contained sequences that were repeated over and over again and the number of repeated sequences differed from individual to individual. Dr Jeffreys thus developed a technique to examine the length variations of these DNA repeat sequences.

4.11.1 THE FIRST FINGERPRINT

Professor Alec Jeffreys were working on the myoglobin gene, which produces the oxygen carrying protein in muscle. In the intron of the human myoglobin gene was tandem repeat DNA – a minisatellite.

Using the myoglobin minisatellite as a 'hook', the team identified more minisatellites and discovered a core sequence - a piece of DNA that is similar in many different minisatellites. Using the core sequence, they made a probe that should latch onto lots of these minisatellites. The probe was hybridized to a blot with DNA from several different people. The X-ray patterns of the blot were studied. The potential of DNA fingerprinting was clear. Soon the first fingerprint had been refined into clean patterns, unique to an individual. An early outcome of this research was the restriction fragment length polymorphisms (RFLP).

4.11.2 BRIEF HISTORY OF FORENSIC DNA TYPING

- 1980 -Ray White describes first polymorphic RFLP marker
- 1985 -Alec Jeffreys discovers multi-locus VNTR probes
- 1985 -first paper on PCR
- 1988 -FBI starts DNA casework
- 1991 -first STR paper
- 1995 -FSS starts UK DNA database
- 1998 -FBI launches CODIS database

4.11.3 INTRODUCTION TO DNA FINGERPRINTING

Technologies used for performing forensic DNA analysis have progressed over time. The speed of analysis has dramatically improved for forensic DNA analysis from reporting in 6 or 8 weeks to a few hours. Numerous advances have been made in the last 15 years in terms of sample processing speed and sensitivity where minute amounts of sample, as little as a single cell in some cases, can yield a useful DNA profile.

The human identity testing community has used a variety of techniques including single- locus probe and multi-locus probe RFLP methods and more recently PCR (polymerase chain reaction) based assays.

Multi-locus RFLP probes are highly variable between individuals but require a great deal of labor, time, and expertise to produce a DNA profile. Analysis of multi-locus probes (MLP) cannot be easily automated, a fact that makes them undesirable as the demand for processing large numbers of DNA samples has increased. Deciphering sample mixtures, which are common in forensic cases, is also a challenge with MLP RFLP methods, which is the primary reason that laboratories went to single-locus RFLP probes.

A high power of discrimination and a rapid analysis has been achieved with short tandem repeats (STR) DNA markers. STRs by definition are short, they can be analyzed three or more at a time. The detection of multiplex STRs can be automated, which is an important benefit as demand for DNA testing increases.

Over the past 15 years, there has been a gradual evolution in adoption of the various DNA typing technologies. When early methods for DNA analysis are superseded by new technologies, there is usually some overlap as forensic laboratories implement the new technology. Validation of the new methods is crucial to maintaining high-quality results.

4.12 DNA TECHNIQUES USED IN FORENSIC INVESTIGATIONS

4.12.1 RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

RFLP is a technique for analyzing the variable lengths of DNA fragments that result from digesting a DNA sample with a special kind of enzyme. This enzyme, a restriction endonuclease, cuts DNA at a specific recognition site. The presence

or absence of certain recognition sites in a DNA sample generates variable lengths of DNA fragments. They are then hybridized with DNA probes that bind to a complementary DNA sequence in the sample. The distance between the locations cut by restriction enzymes (the *restriction sites*) varies between individuals: so the length of the fragments varies, and the position of certain *gel bands* differs between individuals (thus *polymorphism*). This can be used to genetically tell individuals apart.

RFLP is one of the original applications of DNA analysis to forensic investigation. The primary drawback of RFLP is that the exact sizes of the bands are unknown and comparison to a molecular weight ladder is done in a purely qualitative manner. RFLP was a very time consuming method which required relatively high quantity of good quality DNA to be used (such as a dime sized blood drop). This made typing degraded samples such as those from evidence that had been exposed to the elements fairly difficult. With the development of newer, more efficient DNA-analysis techniques, RFLP is not much used.

4.12.2 Polymerase Chain Reaction (PCR) Analysis

The most prevalent method used in forensic DNA analysis is PCR which is used for amplification (or copying) of specific regions of the genome and can be initiated from even trace amounts of DNA. The PCR process does not amplify the entire DNA in a sample; just the short tandem repeat (STRs) sequences specifically targeted using well- designed primers. PCR analysis can be performed simultaneously on several distinct loci (multiplexing), which is more efficient than performing separate PCR amplifications of each locus.

The polymorphisms displayed at each STR region are by themselves very common. Typically each polymorphism will be shared by around 5-20% of individuals. When looking at multiple loci, it is these combinations of polymorphisms which are unique and specific to an individual. The more STR regions that are tested in an individual, the more discriminating the test becomes.

National DNA Databank: CODIS

The Federal Bureau of Investigation (FBI) uses a standard set of 13 specific STR regions for CODIS. Combined DNA Index System (CODIS) is a software program that operates local, state, and national databases of DNA profiles from convicted offenders, unsolved crime scene evidence, and missing persons in the United States with the ability to search the database to assist in the identification of suspects in crime. The odds that two individuals will have the same 13-loci DNA profile are about one in one billion.

4.12.3 Mitochondrial DNA Analysis

Mitochondrial DNA analysis (mtDNA) can be used to examine the DNA from samples that cannot be analyzed by RFLP or STR. Nuclear DNA must be extracted from samples for use in RFLP, PCR, and STR; however, mtDNA analysis uses DNA extracted from another cellular organelle called a mitochondrion. While highly degraded samples and older biological samples that lack nucleated cellular material, such as hair, bones, and teeth, cannot be analyzed with STR and, it is sometimes impossible to get a complete profile. These can be

analyzed with mtDNA. In the investigation of cases that have gone unsolved for many years, mtDNA is extremely valuable as many copies of mtDNA can be found in a cell, while there may only be 1-2 copies of the nuclear DNA.

All mothers have the same mitochondrial DNA as their daughters. This is because the mitochondrion of each new embryo comes from the mother's egg cell. The father's sperm contributes only nuclear DNA. Comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can be an important technique in missing person investigations

4.12.4 Y-CHROMOSOME ANALYSIS

The Y chromosome is passed directly from father to son i.e., it is paternally inherited, so the analysis of genetic markers on the Y chromosome is especially useful for tracing relationships among males or for analyzing biological evidence involving multiple male contributors. Y-STRs can be detected using PCR-based methods. Because males have only Y chromosome they have a single allele at each Y chromosome locus. Thus Y- chromosome STR analysis can be useful adjunct to the standard panel of autosomal loci normally used in forensic analysis.

3.0.1 SINGLE NUCLEOTIDE POLYMORPHISMS (SNP)

A rapid and direct detection of genetic variation at the level of the individual DNA base pair is commonly referred to as single nucleotide polymorphism (SNP). SNPs found in mtDNA have been reported for identity testing especially in cases of degraded materials or sample containing no genomic DNA. Laboratory methods have been developed that permit simultaneous detection of the specific nucleotide bases present at hundreds of distinct sites in the genome using microarrays. Scientific reports suggest that 50-100 selected SNPs are sufficient for forensic casework.

4.13 DNA PROFILING IN INVESTIGATIONS

This deals with DNA examination of samples collected from the crime scene, suspect etc. in form of evidentiary material recovered. Types of cases recovered include paternity/maternity disputes, rapes, murder, identification of mutilated bodies, exhumed bodies, mass disasters etc.

The cases can be broadly categorized under two heads:

1. *Civil cases*: For cases such as paternity/maternity disputes, immigration cases, swapping of newborn children in hospitals etc., fresh blood samples are received for examination.
2. *Criminal cases*: For cases such as rape, murder, missing identity, child swapping, abduction, mass disasters etc., body fluids, hair with root, bones, teeth, tissues, animal tissues and skin, plants and other botanical evidence also are received for analysis.

Samples are categorized into:

1) Whole blood

2) Blood stains

3) Semen, seminal stains, vaginal swabs

4) Bone marrow

5) Teeth with pulp

6) Hair with root

7) Amniotic fluid

8) Tissues (body and foetal)

Some Examples of DNA Uses for Forensic Identification

(i) Identify potential suspects whose DNA may match evidence left at crime scenes

(ii) Exonerate persons wrongly accused of crimes

(iii) Identify crime and catastrophe victims

(iv) Establish paternity and other family relationships

(v) Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers)

(vi) Detect bacteria and other organisms that may pollute air, water, soil, and food

(vii) Match organ donors with recipients in transplant programs

(viii) Determine pedigree for seed or livestock breeds

(ix) Authenticate consumables such as caviar and wine

4.13.1 STEPS INVOLVED

The DNA is first extracted from its biological source material and then measured to evaluate the quantity of DNA recovered. After isolating the DNA from its cells, specific regions are copied with a technique known as the polymerase chain reaction). PCR produces millions of copies for each starting DNA molecule and thus permits very minute amounts of DNA to be examined. Multiple STR regions can be examined simultaneously to increase the informativeness of the DNA test.

The resulting PCR products are then separated and detected in order to characterize the STR region being examined. The separation methods used today include slab gel and capillary electrophoresis. Fluorescence detection methods have greatly aided the sensitivity and ease of measuring PCR-amplified STR alleles. The primary instrument used for fluorescence detection of STR alleles is the ABI 310 Genetic Analyzer. After detecting the STR alleles, the number of repeats in a DNA sequence is determined, a process known as sample genotyping. The resulting DNA profile for a sample, which is a combination of individual STR genotypes, is compared to other samples. In the case of a forensic investigation, these other samples would include known reference samples such as the victim or a suspect that are compared to the crime scene evidence. With paternity investigations, the childÊs genotype would be compared to his or her mother’s and the alleged father(s) under investigation. If there is no match between the questioned sample and the known sample, then the samples may be considered to have originated from different sources. The term for failure to match between two DNA profiles is Âexclusion.Ê

S. No	Reagent	Functions
1	EDTA	It is a chelator of divalent cations. This helps in the weakening of cell wall or membrane.
2	Tris HCL	It is used as a buffer component to maintain constant pH
3	NaCl	The Na+ ions will form an ionic bond with the negatively charged phosphates on the DNA, neutralizing the negative charges and allowing the DNA molecules to come together.
4	SDS	It is a detergent that is known to denature proteins and aids the process of lysis by removing lipid molecules, causes disruption of cell membrane and is used in nucleic acid extraction procedures.
5	Proteinase K	It helps in protein digestion in biological samples by cleaving peptide bonds adjacent to carboxyl groups of amino acids. The smaller units can then be easily removed by phenol.
6	Phenol (Tris saturated Phenol)	It efficiently denatures protein an dissolves s d denatured proteins.
7	Choloroform	The mixture of chloroform and phenol reduces the amount of aqueous solution retained in the organic phase compared to pure phenol phase to maximize the DNA.
8	Iso-amyl alcohol	It is added to prevent foaming of the solution and to aid the separation of the organic and aqueous phases. The denatured proteins form a layer at the interphase between the aqueous and organic phases and it thus isolated from the bulk of the DNA in the aqueous layer.
9	Sodium Acetate	The positively charged sodium ions shield the negative charge on the phosphate backbone of the DNA strand, in this way hydrophobic interactions can take place and provoke aggregation of the DNA molecules.

10	Isopropanol	It is a weakly polar solvent. It causes the DNA to precipitate out of the solution when added to the aqueous supernatant.
11	Alcohol/ 70% Ethanol	Ethanol washes are performed after salt precipitations to remove any residual salt from the nucleic acid pellet. The wash employs 70% ethanol which will solubilize salts but not nucleic acids.
12	T.E. Buffer	It is a buffer made up of Tris and EDTA for keeping DNA. The pH is slightly basic therefore DNA is more easily dissolved than in acid environment, and less prone to acid hydrolysis. For this reason Tris is often used. EDTA chelates ions i.e., it basically takes up free ions form the solution preventing degradation by nucleases as most nucleases make use of ions to catalyze their nuclease ability.

If a match or ÂinclusionÊ results, then a comparison of the DNA profile is made to a population database, which is a collection of DNA profiles obtained from unrelated individuals of a particular ethnic group. For example, due to genetic variation between the groups, African-Americans and Caucasians have different population databases for comparison purposes. Finally a case report or paternity test result is generated. This report typically includes the random match probability for the match in question. This random match probability is the chance that a randomly selected individual from a population will have an identical STR profile or combination of genotypes at the DNA markers tested.

4.13.2 EXTRACTION OF DNA

Forensic laboratories are increasingly confronted with problematic samples from the scene of crime, containing only minute amounts DNA, which may include PCR- inhibiting substances. Efficient DNA extraction procedures, as well as accurate DNA quantification methods, are critical steps involved in the process of successful DNA analysis of such samples. The phenol-chloroform method is a sensitive method for the extraction of DNA from a wide variety of forensic samples, although it is known to be laborious compared with single-tube extraction methods. The relatively high DNA recovery and the quality of the extracted DNA speak for itself.
Several reagents are used in the process. Their functions are listed below. Heat treatments are given in between to soften phospholipids in the cell membrane and denatures DNAse enzyme.

4.13.3 QUANTIFICATION OF DNA

UV Absorbance Spectra of Nucleic Acids and Proteins

Most biological molecules do not intrinsically absorb light in the visible range, but they do absorb ultraviolet light. Biologists take advantage of UV absorbance to

quickly estimate the concentration and purity of DNA, RNA, and proteins in a sample. It is possible to quantify the amount of DNA in a sample by looking at its absorbance at a wavelength of 260nm or 280nm (in the UV region).

Proteins have two absorbance peaks in the UV region, one between 215-230 nm, where peptide bonds absorb, and another at about 280 nm due to light absorption by aromatic amino acids (tyrosine, tryptophan and phenylalanine). Certain of the subunits of nucleic acids (purines) have an absorbance maximum slightly below 260 nm while others (pyrimidines) have a maximum slightly above 260 nm. Therefore, although it is common to say that the absorbance peak of nucleic acids is 260 nm, in reality, the absorbance maxima of different fragments of DNA vary somewhat depending on their subunit composition. The absorbance of a DNA sample at 280 nm gives an estimate of the protein contamination of the sample.

UV Measurements of DNA, RNA and Proteins

WAVELENGTH	SIGNIFICANCE	COMMENTS
215-230 nm	Minimum absorbance for nucleic acids Peptide bonds in proteins absorb light	Measurements are generally not performed at this wavelength because commonly used buffers and solvents, such as Tris, also absorb at these wavelengths.
260 nm	Nucleic acids have maximum absorbance	Purines absorbance maximum is slightly below 260; pyrimidines maximum is slightly above 260. Purines have a higher molar absorptivity than pyrimidines. Therefore, the absorbance maximum and absorptivity of a segment of DNA depends on its base composition. Proteins have little absorbance at this wavelength.
270 nm	Phenol absorbs strongly	Phenol may be a contaminant in nucleic acid preparations.
280 nm	Aromatic amino acids absorb light	Nucleic acids also have some absorbance at this wavelength

A. Concentration Measurements of Nucleic Acids and Proteins in a Sample

There are two UV methods of determining concentration in a „pure‰ sample

containing only proteins or nucleic acids. The first method involves constructing a standard curve, the second is a „short-cut‰ method based on absorptivity constants. The average absorptivity constants for proteins and nucleic acids lead to the following relationships:

- If a sample containing pure double-stranded DNA has an absorbance of 1 at 260 nm, then it contains approximately 50 øg/mL of double -stranded DNA.
- If a sample containing pure single-stranded DNA has an absorbance of 1 at 260 nm, then it contains approximately 33 øg/mL of DNA.

Based on these relations we can determine the concentration as:

 Concentration of double-stranded DNA ~ (50 øg/mL) X (absorbance at 260 nm)

 Concentration of single-stranded DNA ~ (33 øg/mL) X (absorbance at 260 nm)

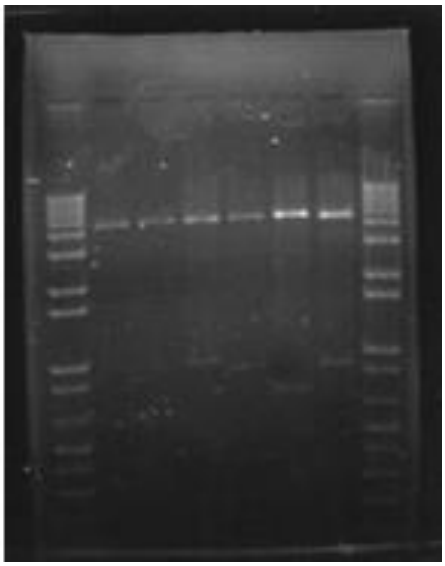
(or)

DNA concentration (øg/ml) = (OD_{260}) x (dilution factor) x (50 øg DNA/ml)/(1 OD_{260} unit)

B. Estimation of the Purity of a Nucleic Acid Preparation

It is possible to use UV spectrophotometry to estimate the purity of a solution of nucleic acids. This method involves measuring the absorbance of the solution at two wavelengths, usually 260 nm and 280 nm, and calculating the ratio of the two absorbances:

- An A_{260}/A_{280} of 1.8 is characteristic of pure DNA.
- A_{260}/A_{280} ratio of about 0.6 is characteristic of pure protein
- Therefore, a ratio of 1.8 - 2.0 is desired when purifying nucleic acids. A ratio less than 1.7 means there is probably a contaminant in the solution, typically either protein or phenol.



Digital printout of an agarose gel electrophoresis of *cat*-insert plasmid DNA

Agarose gel electrophoresis is a method used to separate DNA strands by size, and to estimate the size of the separated strands by comparison to known fragments (DNA ladder). This is achieved by pulling negatively charged DNA molecules towards positive pole through an agarose matrix when electric field is applied. Shorter molecules move faster than longer ones.

Factors affecting migration

The most important factor is the length of the DNA molecule, the one with shorter double-helices travels faster. Increasing the agarose concentration of a gel reduces the migration speed and enables separation of smaller DNA molecules. The higher the voltage, the faster DNA migrates. But voltage is limited by the fact that it heats and ultimately causes the gel to melt. High voltages also decrease the resolution (above about 5 to 8 V/cm).

Visualisation: EtBr and dyes

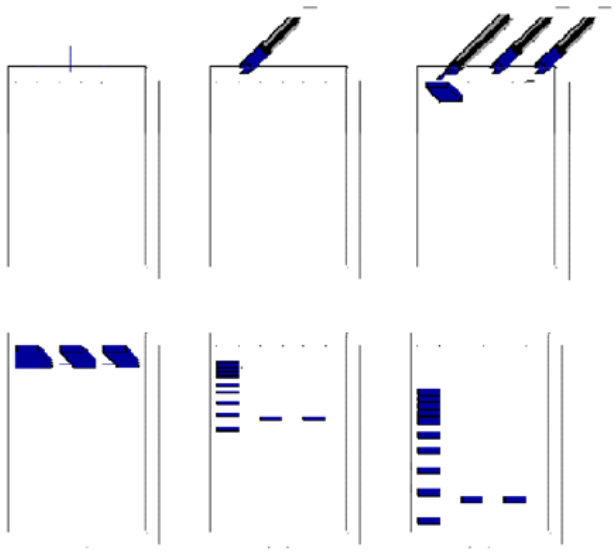
The central dye in agarose gel electrophoresis is ethidium bromide (EtBr). It has the unique property of fluorescing under UV light when intercalated with DNA thus distinct bands of DNA become visible.

Other dyes are sometimes used including SYBR Green or SYBR Safe. SYBR dyes are thought to be less carcinogenic than EtBr and to give cleaner, higher powered staining. Loading buffers are added with the DNA in order to visualize it and sediment it in the gel well. Negatively charged indicators keep track of the position of the DNA. Xylene cyanol and Bromophenol blue are typically used.

There are a number of buffers used for agarose electrophoresis. The most common being: Tris Acetate EDTA (TAE), Tris Borate EDTA (TBE) and Sodium borate (SB). TAE has the lowest buffering capacity but provides the best resolution for larger DNA.

Resolution limits

DNA-based gel electrophoresis can be used for the separation of DNA fragments of 50 base pairs up to several megabases (millions of bases). In general, lower the concentration of agarose, the larger is the ideal size of a molecule to be resolved. The disadvantage of lower concentrations is the long run times and the problem of handling the fragile gel. It is very difficult, if not impossible to resolve DNA much larger than 30- 50 kb on a regular agarose gel.



Schematic drawing of the electrophoresis process

Analysis

The gel is illuminated with an ultraviolet lamp to view the DNA bands. The ethidium bromide fluoresces pink in the presence of DNA. The DNA band can also be cut out of the gel, and can then be dissolved to retrieve the purified DNA.

I. POLYMERASE CHAIN REACTION

Polymerase chain reaction was invented by Kary Mullis. The idea was to develop a process by which DNA could be artificially multiplied through repeated cycles of duplication driven by an enzyme called DNA polymerase.

a. DNA polymerase

DNA polymerase occurs naturally in living organisms. In cells it functions to duplicate DNA when cells divide in mitosis and meiosis. Polymerase works by binding to a single DNA strand and creating the complementary strand. In the first of many original processes, the enzyme was used in vitro. The double-stranded DNA was separated into two single strands by heating it to 94 C (201 F). At this temperature, however, the DNA polymerase used at the time was destroyed, so the enzyme had to be replenished after the heating stage of each cycle. The original procedure was very inefficient, since it required a great deal of time, large amounts of DNA polymerase, and continual attention throughout the process.

Later, this original PCR process was greatly improved by the use of DNA polymerase taken from thermophilic bacteria grown in geysers at a temperature of over 110 C (230 F). The DNA polymerase taken from these organisms is stable at high temperatures and, when used in PCR, does not break down when the mixture was heated to separate the DNA strands. Since there was no longer a need to add new DNA polymerase for each cycle, the process of copying a given DNA strand could be simplified and automated.

One of the first thermostable DNA polymerases was obtained from *Thermus aquaticus* and was called "Taq." Taq polymerase is widely used in current PCR practice. A disadvantage of Taq is that it sometimes makes mistakes when

copying DNA, leading to mutations in the DNA sequence, since it lacks 3' 5' proofreading exonuclease activity.

PCR has been performed on DNA larger than 10 kilobases, but the average PCR is only several hundred to a few thousand bases of DNA. The problem with long PCR is that there is a balance between accuracy and processivity of the enzyme. Usually, the longer the fragment, the greater the probability of errors.

A thermal cycler for PCR

- *DNA template*, which contains the region of the DNA fragment to be amplified
- Two *primers*, which determine the beginning and end of the region to be amplified
- *Taq polymerase*, a DNA polymerase which copies the region to be amplified
- *Deoxynucleotide triphosphates*, (dNTPs) from which the DNA polymerase builds the new DNA
- *Buffer solution*, which provides a suitable chemical environment for the DNA Polymerase
- *Divalent cation*, magnesium or manganese ions
- *Monovalent cation*, potassium ions

The PCR process is carried out in a thermal cycler. This is a machine that heats and cools the reaction tubes within it to the precise temperature required for each step of the reaction. The lid of the thermal cycler is heated to prevent condensation on the inside of the reaction tube caps. Alternatively, a layer of oil may be placed on the reaction mixture to prevent evaporation.

b. Primers

The DNA fragment to be amplified is determined by selecting primers. Primers are short, artificial DNA strands - often not more than 50 and usually only 18 to 25 base pairs long - that are complementary to the beginning or the end of the DNA fragment to be amplified. They anneal by adhering to the DNA

template at these starting and ending points, where the DNA polymerase binds and begins the synthesis of the new DNA strand.

The choice of the length of the primers and their melting temperature (T_m) depends on a number of considerations. The melting temperature of a primer is defined as the temperature at which half of the primer binding sites are occupied. Primers that are too short would anneal at several positions on a long DNA template, which would result in
non-specific copies. On the other hand, the length of a primer is limited by the maximum temperature allowed to be applied in order to melt it, as melting temperature increases with the length of the primer. Melting temperatures that are too high, i.e., above 80 C, can cause problems since the DNA polymerase is less active at such temperatures. The optimum length of a primer is generally from 15 to 40 nucleotides with a melting temperature between 55 C and 65 C.

Sometimes *degenerate primers* are used. These are actually mixtures of similar, but not identical, primers. They may be convenient if the same gene is to be amplified from different organisms, as the genes themselves are probably similar but not identical. The other use for degenerate primers is when primer design is based on protein sequence. As several different codons can code for one amino acid, it is often difficult to deduce which codon is used in a particular case. Use of degenerate primers can greatly reduce the specificity of the PCR amplification. This problem can be partly solved by using touchdown PCR.

The above mentioned considerations make primer design a very exacting process, upon which product yield depends:

- GC-content should be between 40-60%.
- Calculated T_m for both primers used in reaction should not differ >5 C, and T_m of the amplification product should not differ from primers by >10 C.
- Annealing temperature usually is 5 C below the calculated lower T_m. However, it should be chosen empirically for individual conditions.
- Inner self-complementary hairpins of >4 and of dimers >8 should be avoided.
- Primer 3' terminus design is critical to PCR success since the primer extends from the 3' end. The 3' end should not be complementary over greater than 3-4 bases to any region of the other primer (or even the same primer) used in the reaction and must provide correct base matching to the template.

There are computer programs to help design primers.

PCR optimization

Since PCR is very sensitive, adequate measures to avoid contamination from other DNA present in the lab environment (bacteria, viruses, lab staff's skin etc.) should be taken. Thus DNA sample preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction product analysis, should be performed in separate areas. For the preparation of reaction mixture, a laminar flow cabinet with UV lamp is recommended. Fresh gloves should be used for each PCR step as well as displacement pipettes with aerosol filters. The reagents for PCR should be prepared separately and used solely for this purpose. Aliquots

should be stored separately from other DNA samples. A control reaction (inner control), omitting template DNA, should always be performed, to confirm the absence of contamination or primer multimer formation.

Difficulties with polymerase chain reaction

Polymerase chain reaction is not perfect, and errors and mistakes can occur. These are some common errors and problems that may occur.

Polymerase errors

Taq polymerase lacks a 3' to 5' exonuclease activity. This makes it impossible for it to check the base it has inserted and remove it if it is incorrect, a process common in higher organisms. This in turn results in a high error rate of approximately 1 in 10,000 bases, which, if an error occurs early, can alter large proportions of the final product.

Other polymerases are available for accuracy in vital uses such as amplification for sequencing. Examples of polymerases with 3'to 5' exonuclease activity include: KOD DNA polymerase, a recombinant form of *Thermococcus kodakaraensis* KOD1; Vent, which is extracted from *Thermococcus litoralis*; Pfu DNA polymerase, which is extracted from *Pyrococcus furiosus*; and Pwo, which is extracted from *Pyrococcus woesii*.

Size limitations

PCR works readily with DNA of lengths two to three thousand basepairs, but above this length the polymerase tends to fall off, and the typical heating cycle does not leave enough time for polymerisation to complete. It is possible to amplify larger pieces of up to 50,000 base pairs with a slower heating cycle and special polymerases. These special polymerases are often polymerases fused to a processivity-enhancing DNA-binding protein, making them literally "stick" to the DNA longer.

Non specific priming

The non specific binding of primers is always a possibility due to sequence duplications, non-specific binding and partial primer binding, leaving the 5' end unattached. This is increased by the use of degenerate sequences or bases in the primer. Manipulation of annealing temperature and magnesium ion concentrations can increase specificity. Non- specific priming can be prevented during the low temperatures of reaction preparation by use of "hot-start" polymerase enzymes where the active site is blocked by an antibody or chemical that only dislodges once the reaction is heated to 95■C during the denaturation step of the first cycle.

Uses of PCR

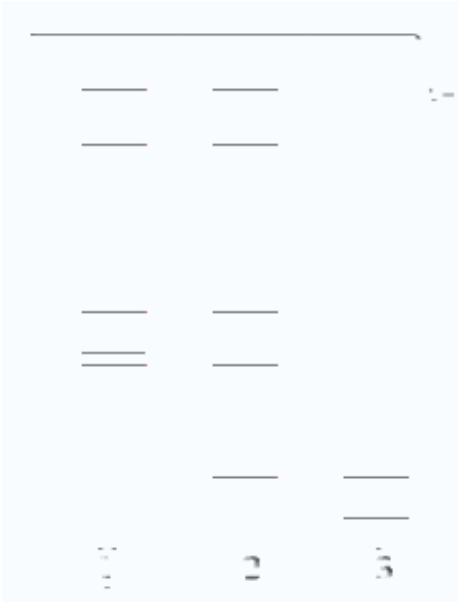
PCR can be used for a broad variety of experiments and analyses. Some examples are discussed below.

a. Genetic fingerprinting

Genetic fingerprinting is a forensic technique used to identify a person by comparing his or her DNA with a given sample. An example is blood from a crime scene being genetically compared to blood from a suspect. The sample may contain only a tiny amount of DNA (obtained from a source such as blood, semen,

saliva, hair, or other organic material)). Theoretically, just a single strand is needed. First, one breaks the DNA sample into fragments; then amplifies them using PCR. The amplified fragments are then separated using gel electrophoresis. The overall layout of the DNA fragments is called a *DNA fingerprint*. Since there is a very tiny possibility that two individuals may have the same sequences (one in several million), the technique is more effective at acquitting a suspect than proving the suspect guilty.

b. Paternity testing



Electrophoresis of PCR-amplified DNA fragments. (1) Father. (2) Child. (3) Mother.

The child has inherited some, but not all of the fingerprint of each of its parents, giving it a new, unique fingerprint. Although these resulting 'fingerprints' are unique (except for identical twins), genetic relationships, for example, parent-child or siblings, can be determined from two or more genetic fingerprints, which can be used for paternity tests. A variation of this technique can also be used to determine evolutionary relationships between organisms.

c. Analysis of ancient DNA

Using PCR, it becomes possible to analyze DNA that is thousands of years old. PCR techniques have been successfully used on animals, such as a forty-thousand-year-old mammoth, and also on human DNA, in applications ranging from the analysis of Egyptian mummies to the identification of a Russian Tsar.

Practical modifications to the PCR technique: Types of PCR

Nested PCR - Nested PCR is intended to reduce the contaminations in products due to the amplification of unexpected primer binding sites. Two sets of primers are used in two successive PCR runs; the second set intended to amplify a secondary target within the first run product. This is very successful, but requires more detailed knowledge of the sequences involved.

Inverse PCR - Inverse PCR is a method used to allow PCR when only one internal sequence is known. This is especially useful in identifying flanking sequences to various genomic inserts. This involves a series of digestions and self ligation before cutting by an endonuclease, resulting in known sequences at either end of

the unknown sequence.

RT-PCR - Reverse Transcription PCR is the method used to amplify, isolate or identify a known sequence from a cell or tissues RNA library. Essentially normal PCR preceded by Reverse transcriptase, to convert the RNA to cDNA, this is widely used in expression mapping, determining when and where certain genes are expressed.

Asymmetric PCR - Asymmetric PCR is used to preferentially amplify one strand of the original DNA more than the other. It finds use in some types of sequencing and hybridization probing where having only one of the two complementary stands is ideal. PCR is carried out as usual, but with a great excess of the primers for the chosen strand. Due to the slow (arithmetic) amplification later in the reaction after the limiting primer has been used up, extra cycles of PCR are required. A recent modification on this process, known as Linear-After-The-Exponential-PCR (LATE-PCR), uses a limiting primer with a higher melting temperature (Tm) than the excess primer to maintain reaction efficiency as the limiting primer concentration decreases mid-reaction.

Quantitative PCR - Q-PCR is used to rapidly measure the quantity of PCR product, thus is an indirect method for quantitatively measuring starting amounts of DNA, cDNA or RNA. This is commonly used for the purpose of determining whether a sequence is present or not, and if it is present the number of copies in the sample. There are 3 main methods which vary in difficulty and detail.

Multiplex-PCR - The use of multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences. By targeting multiple genes at once, additional information may be elicited from a single test run that otherwise would require several times the reagents and technician time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction and amplicon sizes should be separated by enough difference in final base pair length to form distinct bands via gel electrophoresis.

4.14 STR LOCI COMMONLY USED IN DNA TYPING

There are literally hundreds of STR systems which have been mapped throughout the human genome. Several dozen have been investigated for application to human identity testing. These STR loci are found on almost every chromosome in the genome. They may be amplified using a variety of PCR primers. Tetra-nucleotide repeats have been most popular among forensic scientists due to their fidelity in PCR amplification although some tri- and penta-nucleotide repeats are also in use.

Desirable features for STR systems include

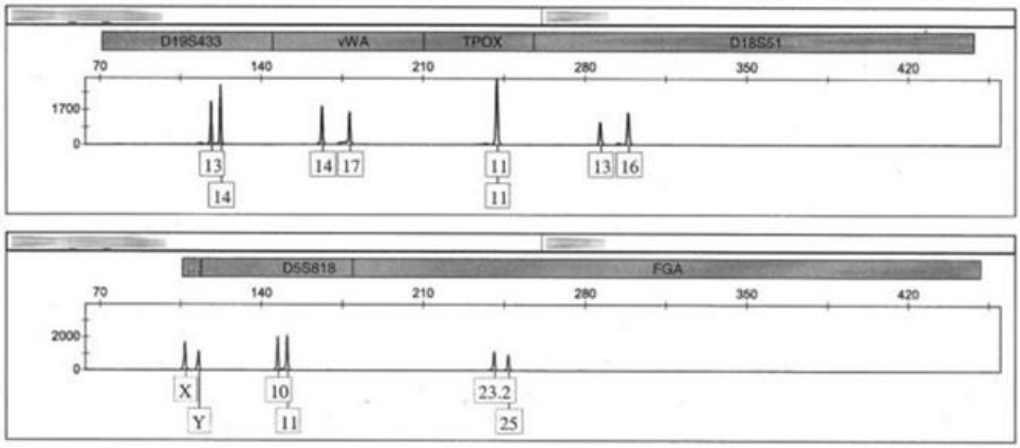
- high heterozygosity
- regular repeat unit
- distinguishable alleles
- robust amplification

4.14.1 FORENSIC STR ANALYSIS

The STRs in use today for forensic analysis are all tetra- or penta-nucleotide

repeats (4 or 5 repeat units), as these give a high degree of error-free data while being robust enough to survive degradation in non-ideal conditions. Shorter repeat sequences tend to suffer from artifacts such as stutter and preferential amplification, as well as the fact that several genetic diseases are associated with tri-nucleotide repeats such as Huntington's disease. Longer repeat sequences will suffer more highly from environmental degradation and do not amplify by PCR as well as shorter sequences.

Once these sequences have been amplified, they are resolved either through gel electrophoresis or capillary electrophoresis, which will allow the analyst to determine how many repeats of the STR sequence in question there are. If the DNA was resolved by gel electrophoresis, the DNA can be visualized either by silver staining (not very high resolution, safe, inexpensive), or an intercalating dye such as ethidium bromide (fairly sensitive, moderate health risks, inexpensive), or as most modern forensics labs use, fluorescent dyes (highly sensitive, safe, expensive). Instruments built to resolve STR fragments by capillary electrophoresis also use fluorescent dyes to great effect.



4.15 PRACTICAL APPLICATIONS OF FORENSIC DNA FINGERPRINTING

1. Paternity Testing

Amplification of specific genetic markers produces genetic fingerprint which is highly specific for each individual. As the genome of every single individual is the combination of the genomes from both parents, the DNA profile of an individual is a combined pattern of parental genetic markers. The comparative analysis of a specific set of these markers permits a reliable assignment of paternity for each individual.

2. In Criminal Investigations

In order to identify the offender in criminal cases, DNA samples collected at the crime scene will be used to compare with that of the suspects to determine guilt or innocence.

3. Identification in Mass disasters

DNA testing is done on unidentified dead bodies or recovered remains and an

identity could be assigned by studying the close relatives of missing persons in mass disasters.

GLOSSARY

1. Acid Phosphatase
2. Agglutination
3. Allele
4. Antibody
5. Antigen
6. Antiserum
7. Hemoglobin
8. Plasma
9. Serum
10. Luminol
11. Sperm
12. Phenotype

LEARNING OUTCOME

After studying this unit you should be able to :

1. Explain the types of crime cases and physical evidences dealt in forensic serology.
2. Perform various laboratory tests for the identification of biological fluids/stains.
3. DNA and its structure.
4. Understand the concept of DNA fingerprinting.
5. DNA extraction procedures.

SUGGESTED READINGS

1. A Laboratory Manual for Human Blood Analysis - M. K. Bhasim.
2. Proteomics of Human Body Fluids - Visith Thougboonkerd.
3. Practical Aspects of Rape Investigation – Robert. R. Hazelwood.
4. Bloodstain Pattern Analysis with and Introduction to Crime Scene Reconstruction – Tom Bevel.
5. Forensic DNA Evidence Interpretation – John. S. Buckleton.
6. Forensic DNA Analysis – Jaiprakash. G. Shewale.
7. DNA and Biotechnology – F. Hayes.

UNIT V

LEARNING OBJECTIVE

In this unit you will be introduced to:

1. The definition of the term Forensic Biology and types of crime cases where it is used.

2. Types of physical evidences that fall under Forensic Biology, Forensic Archaeology, Forensic Anthropology and Forensic Odontology.
3. The different areas of hair- cuticle, cortex and medulla.
4. The role of DNA typing in hair comparisons.
5. The proper collection techniques of fiber evidence.
6. The differences between natural and manufactured fibers.
7. Internal structure of wood.
8. The skeletal anatomy of a human.
9. Bite marks and its examination

UNIT V-A-Forensic Biology

5.0 DEFINITION AND SCOPE

Forensic Biology is the branch of science dealing with examinations of biological materials encountered in crimes such as murder, road accidents, burglary, robbery, dacoity, sexual assault etc.

The biological materials derived from human, animal and plant sources will be identified, examined and compared.
Forensic Biology deals with the following types of crime cases

1. Murder
2. Road accidents
3. Sexual assault
4. Theft
5. Burglary
6. Robbery
7. Dacoity
8. Drowning
9. Suspicious death
10. Cases of missing persons.

The following are different types of physical evidence under the heading 'Forensic Biology'.

1. Hair
2. Fibers
3. Diatoms
4. Plant material (Tobacco, Pollen grains, wood etc.,)
5. Insects and Maggots (Forensic Entomology)
6. Skeletal remains, Teeth (Forensic Odontology)
7. Skull (Forensic Craniometry) Superimposition

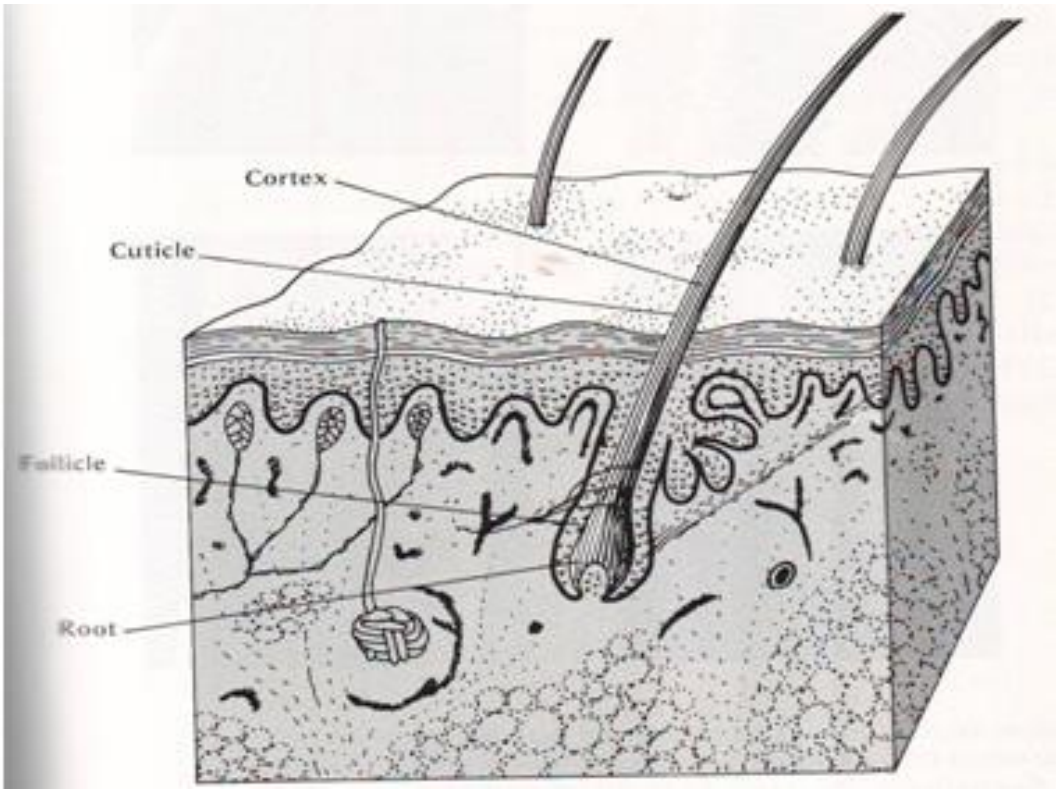
5.1 HAIR

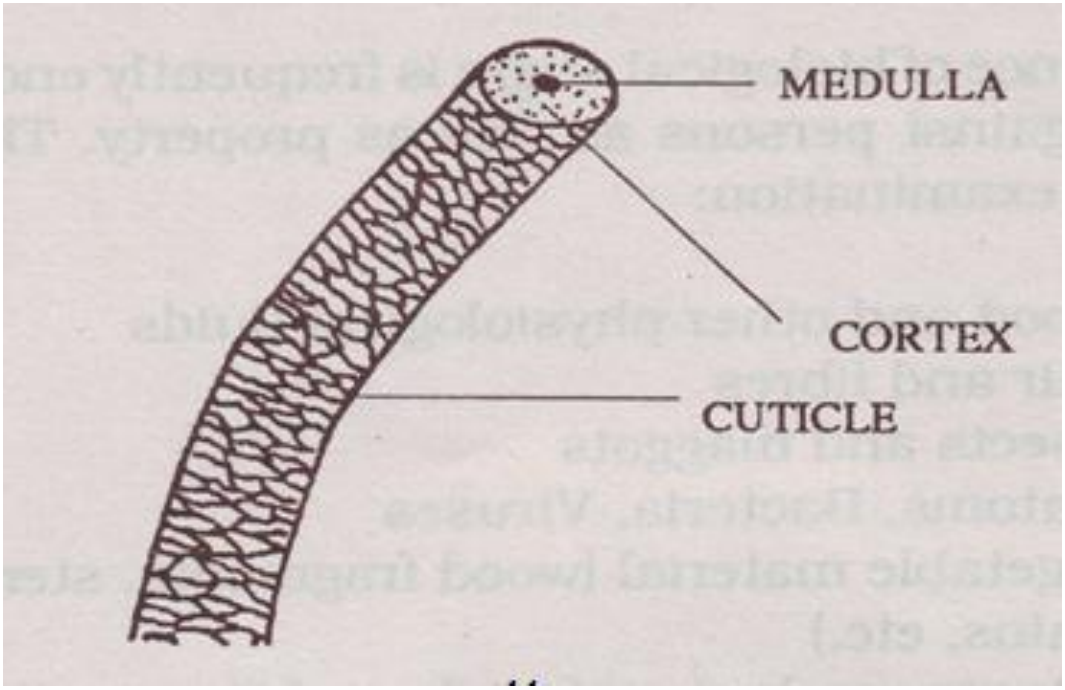
Hair is commonly occurring physical evidence present in the scene, on victim, on accused, on weapon, on clothing, etc. Usually accused hair is found in victim's wrist. In road accident-victims hair is found on vehicles. Examination of hair collected from crime scene often proves the presence of accused at the place of perpetration of crime.

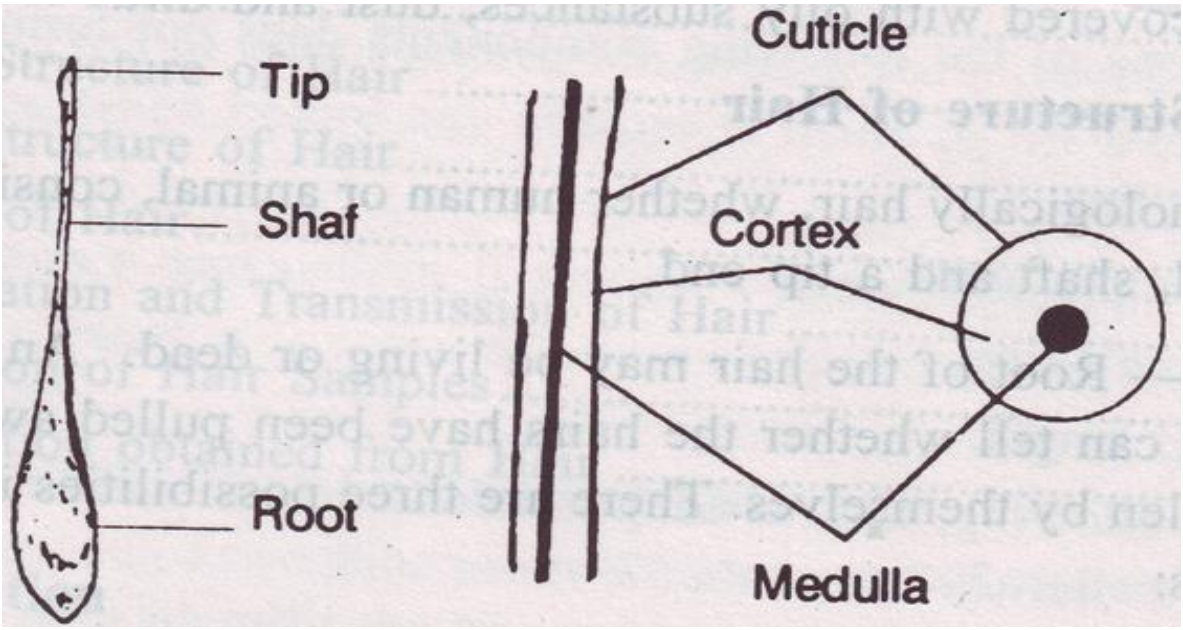
5.1.1 Morphology of Hair

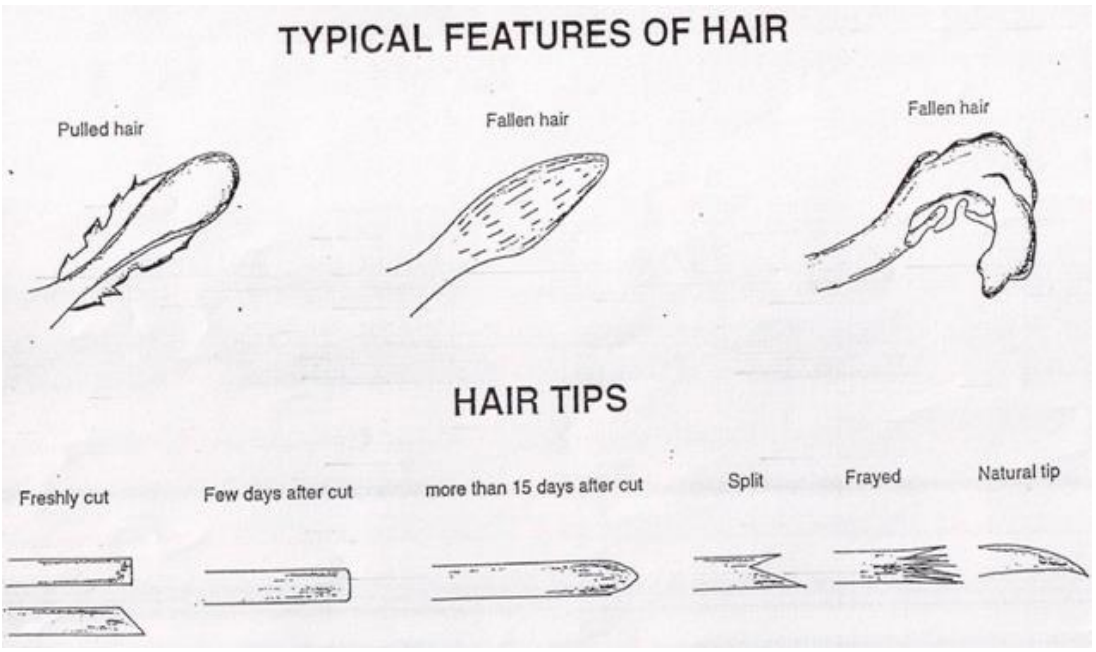
Hair grows from the hair follicles present on certain parts of the body from the skin. Hair contains root and shaft. Hair is composed of fibrils made of protein - Keratin which is cross linked - resulting in a stable structure. Central portion of the hair consists of the medulla, middle: cortex & the outer part: cuticle.

SKIN AND HAIR


Cortex
Cuticle
Follicle
Root


MEDULLA
CORTEX
CUTICLE





1. Cuticle

a) Outermost layer of hair.

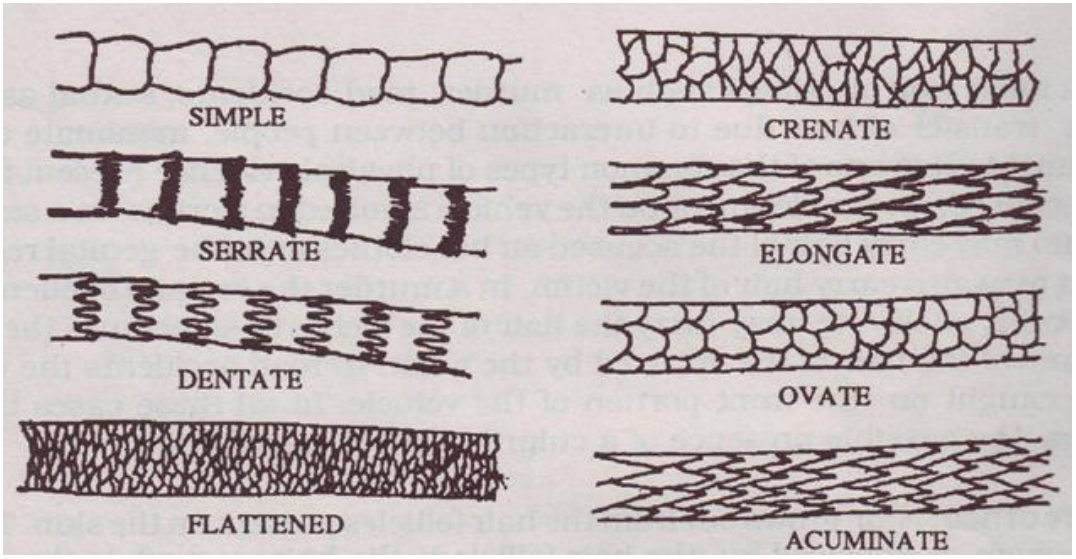
b) Thin translucent pigment free scales laid one over the other.

c) Scales are directed towards the tip of hair. Human: flat, sheep: saw tooth type protrusions.

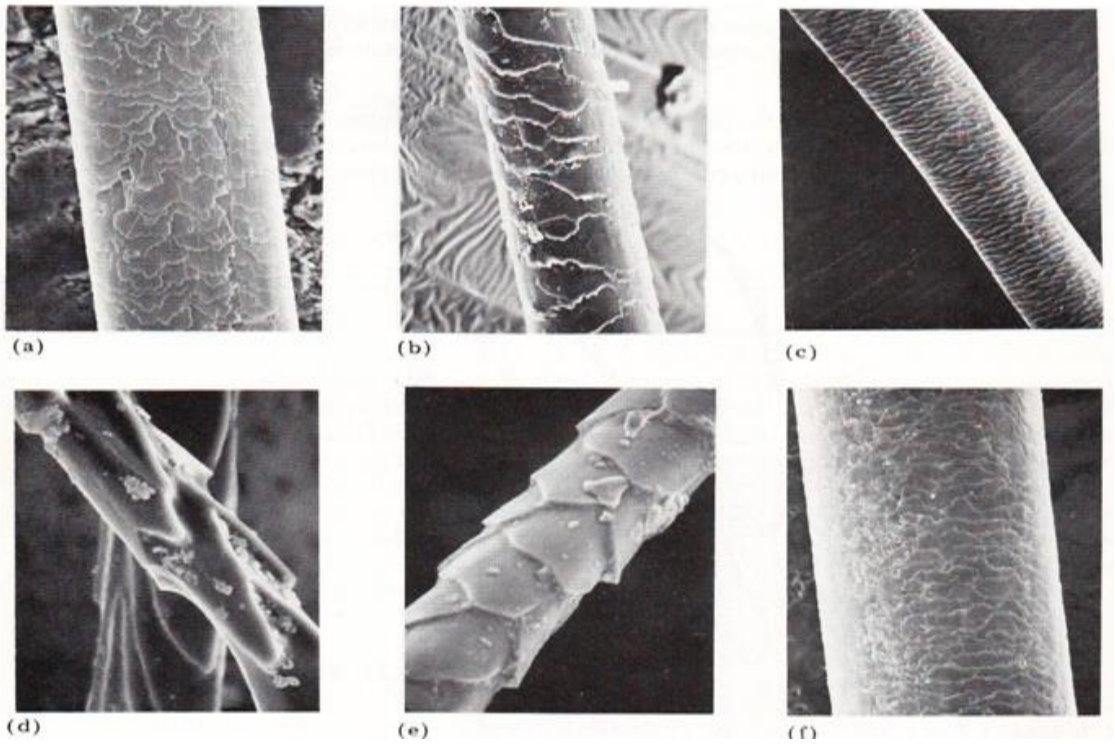
d) Scale count - number of scales per unit length of hair.

e) Scale index - length of scale as compared to the hair diameter.

f) Scale count & index are useful in the identification of hair.



SCALE PATTERNS OF HAIR – HUMAN, DOG, DEER, RABBIT, CAT & HORSE



2. Cortex

a) Consists of elongated filaments - parallel to the length of hair - are bounded together by protein - to form hard structure.
b) Empty air spaces called cortical fusi - whose shape, size and distribution are important characteristics.
c) Contains pigments - granules (melanin); synthesized in the hair follicle and passed to the growing hair.
d) Concentration of melanin that decides the color of hair. Black: more

melanin, gray: no melanin.

e) Melanin is more in head - hair. Uneven pigmentation in the auxiliary/pubic hair.

3. Medulla

a) Central core of hair.

b) Cells are cylindrical - contains glycogen.

c) In some animals it is pigmented & appears dark.

d) Its presence and appearance is a characteristic feature.

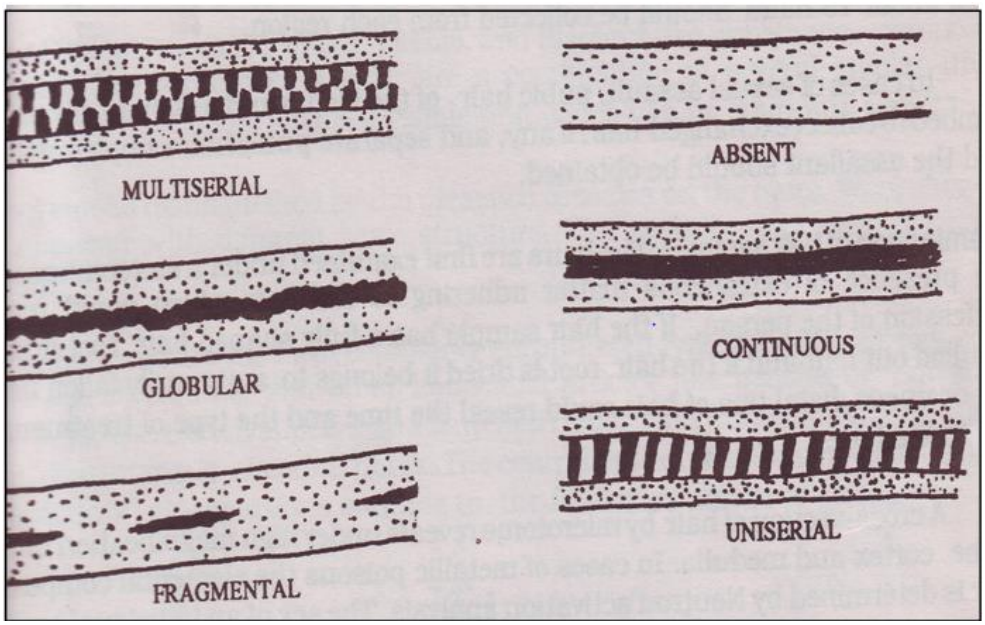
e) May be continuous/discontinuous/fragmentary/absent.

f) Medullary index - ratio of diameter of medulla & the diameter of hair, varies:

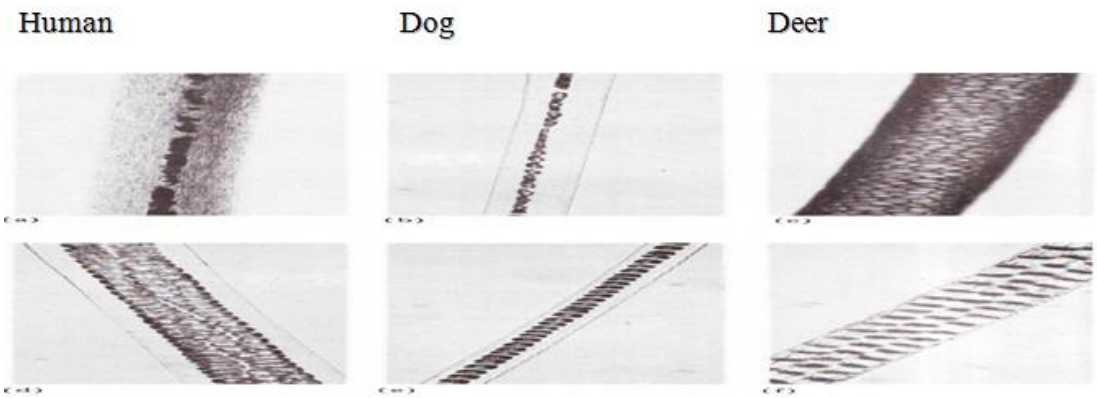
- Human 1/3 or less - narrow
- Animals - medium size;

In cows & horses thick

In other animals continuous



HAIR: MEDULLA PATTERNS



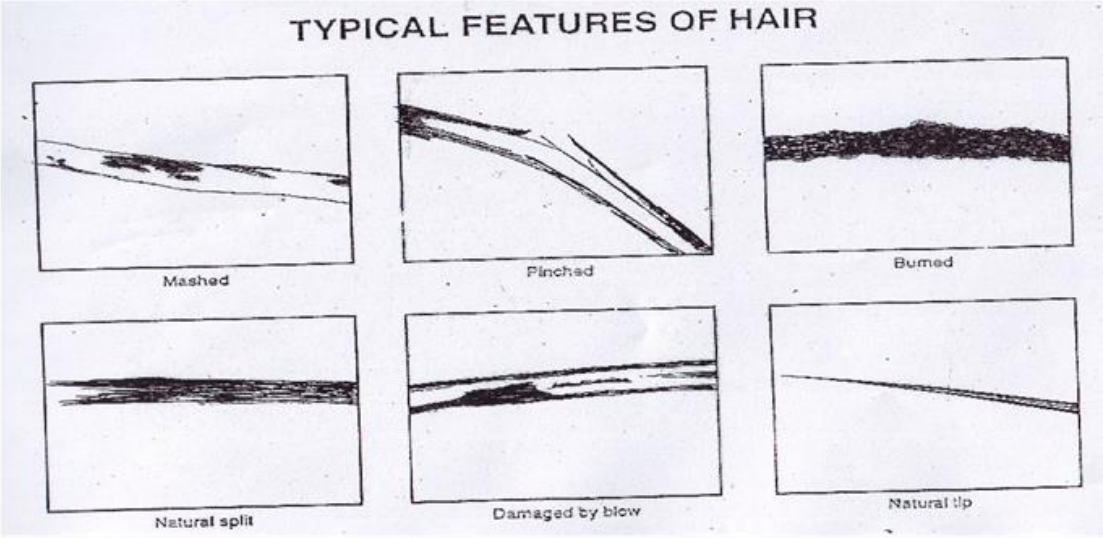
Distal tip of hair

Examine to know whether it is:

- Recently cut, uncut, burnt or badly damaged
- Uncut hair shows tapered & un -pigmented tip
- Scissors cut- cut end is compressed.
- Blade/sharp object cut or oblique cut
- Heavy blunt object strike - split ends
- Burnt - swollen end
- Cut ends slowly fold over: within two weeks-forms a rounded tip

Razor cut hair; split hair; with cut tip

- sample is taken,
- min 25 full-length hairs.
- plucked and combed hairs, packaged separately.
- suspects and victims and other individuals (elimination samples).



1.1.1 Collection

- Locate with strong light
- Collect with tweezers/transparent adhesive tape & preserve in clean covers
- Record from where the hair is collected
- Collect hair by vigorously combing from victim & suspect
- In case of clipping - it should be done close to the root
- Collect hair from scalp, mustache, and beard in separate covers & labeled
- Collect 15 hairs from each region
- In sexual assault collect exchanged pubic hair first - followed by sample and preserve them in separate covers

1.1.2 Examination

- Stereomicroscope - matter adhering - may reveal the profession.
- Full hair root - a pulled out hair
- Dried hair root - naturally fallen
- Distal tip - cut/uncut: the time & type of treatment
- Cross section - by microtome & examination under magnification - nature of cortex & medulla
- Metallic poisons - by NAA
- Sex - hair root: DNA profiling
- ABO - hair root
- DNA - hair root

1.1.3 Information that can be obtained

a. Is the sample hair or not? (by physical examination)

b. Are the hair is of human/animal origin?

Medullary index ratio	-	< 0.5	= human
		>0.5	= animal
Scales	-	Teeth	= animal
	-	Flat	= human
Cortex	-	More developed	= human
	-	Less developed	= animal
Pigment grains	-	Equally distributed	= human
	-	Irregular	= animal

a. What part of the body?

Basing on length, diameter, color, stiffness, curliness & cross section, shape of tip & foreign material

- Fuzz hair: short, no medulla & no pigment
- Scalp hair: uniform diameter, constant pigment distribution
- Body hair: curly & variable diameter
- Beard: coarse, curved & triangular cross section
- Eyebrow/Eyelid/nose/Ear: short & wide medulla, taper rapidly to a fine point
- Pubic: coarser, wiry, constrictions, twists & broad continuous medullas

b. Does the hair come from man or woman?

- Barr bodies in hair root: present = female

absent = male

c. How old is the person from whom hair originated?

- Cannot be conclusively determined
- Minor – feeble pigmentation of cortex
- Roots dissolve rapidly in a solution of caustic potash

d. Are the hair dyed?

- Dull appearance under microscope
- Pigmented granules are less
- Growth after dyeing - reveals natural end portions.
- Grows one cm. Per month (time elapsed since dyeing of hairs can be determined)

e. Type of hair cut?

- Straight cut - sharp weapon
- Irregular end - blunt weapon

- Round ends - burn/ fire arm injury

f. Fixing of individual

- Can be said definitely - that it is not belongs to the person.
- Cannot be said definitely - that it belongs to the person
- Test for dye, occupational dust, etc.; may enhance.

5.2 FIBRES

- Encountered in sexual offences, murder, house breaking, road accidents etc.
- Carpet fibers may be transferred to accused footwear Classification of Fibers:

1. NATURAL:

a) Plant - Ex.: Cotton, Jute, Linen, Coir

b) Animal - Ex.: Wool, Silk

2. MINERAL: Ex.: Asbestos, Glass

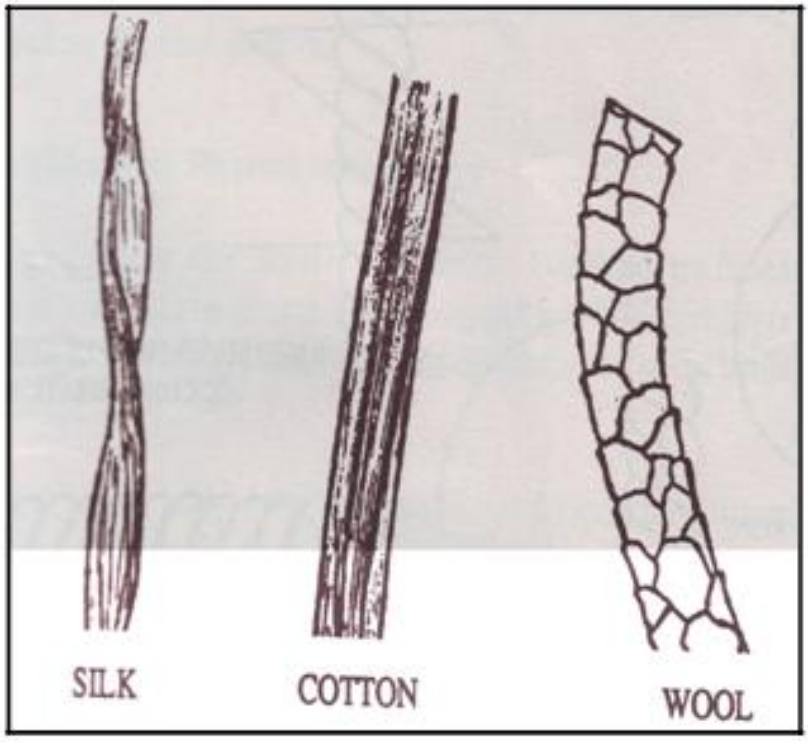
3. MANMADE:

a) Regenerated - Ex.: Rayon

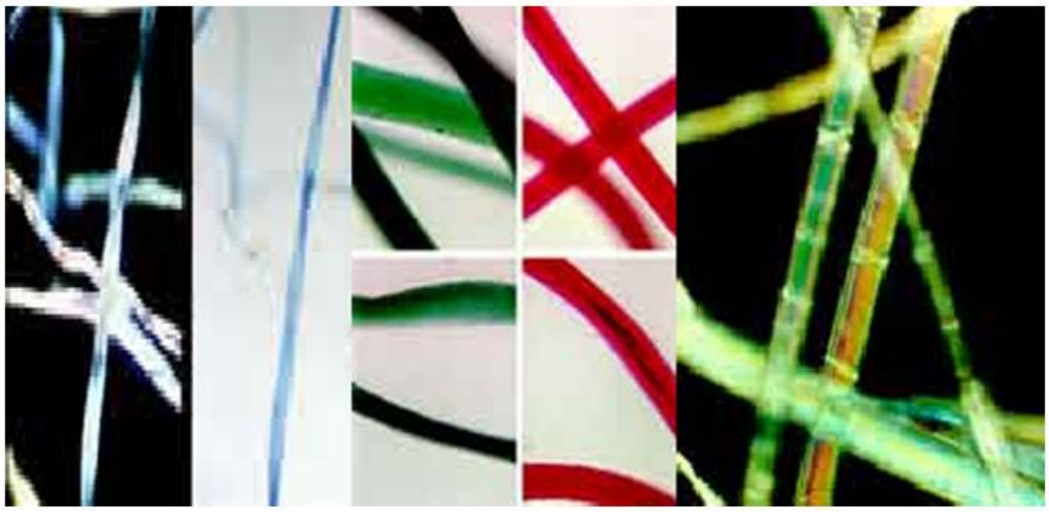
b) Synthetics - Ex: Nylon, Polyester, Terylene

5.2.1 Fiber examination

- Whether the fiber is natural or manmade.
- The generic fiber type or sub type.
- The colour and shape of the fiber.
- An indication of the type of textile material from which the fiber could have originated.



Natural Fibers, cotton; wool; flax



5.2.2 Structure

- Tough, pliable & filament like substances
- Long chain like structures of several similar units
- The smallest visible unit which can be spun into textiles

Wool

- one kind of hair – having scales
- diameter changes with type of scale structure
- medulla may / may not be pronounced

Cotton

- common in clothing
- flattened ribbon with characteristic twists
- dark central area due to air inclusions & fibrilar

Mercerized cotton

- treated with strong caustic solutions
- closed internal canal
- lustrous surface
- used in socks, women's apparel, etc,

Silk

- from silk worms
- long continuous fiber
- types - raw, processed and wild
- raw - two fibers enclosed in sericin

- Surface is irregular with folds & uneven lumps

- processed - sericin is removed

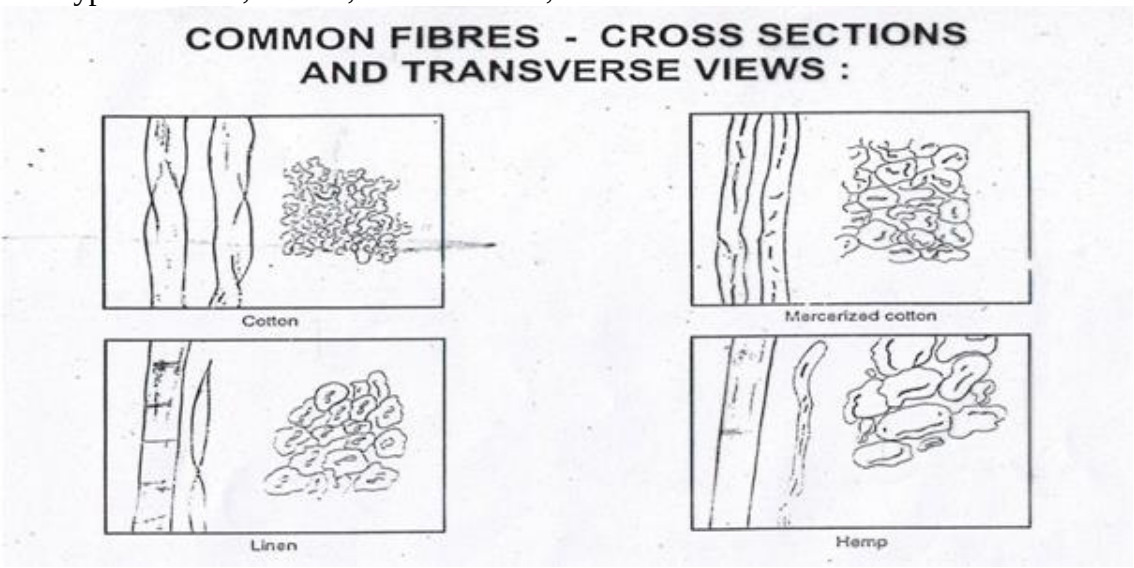
- smooth, translucent and structure less

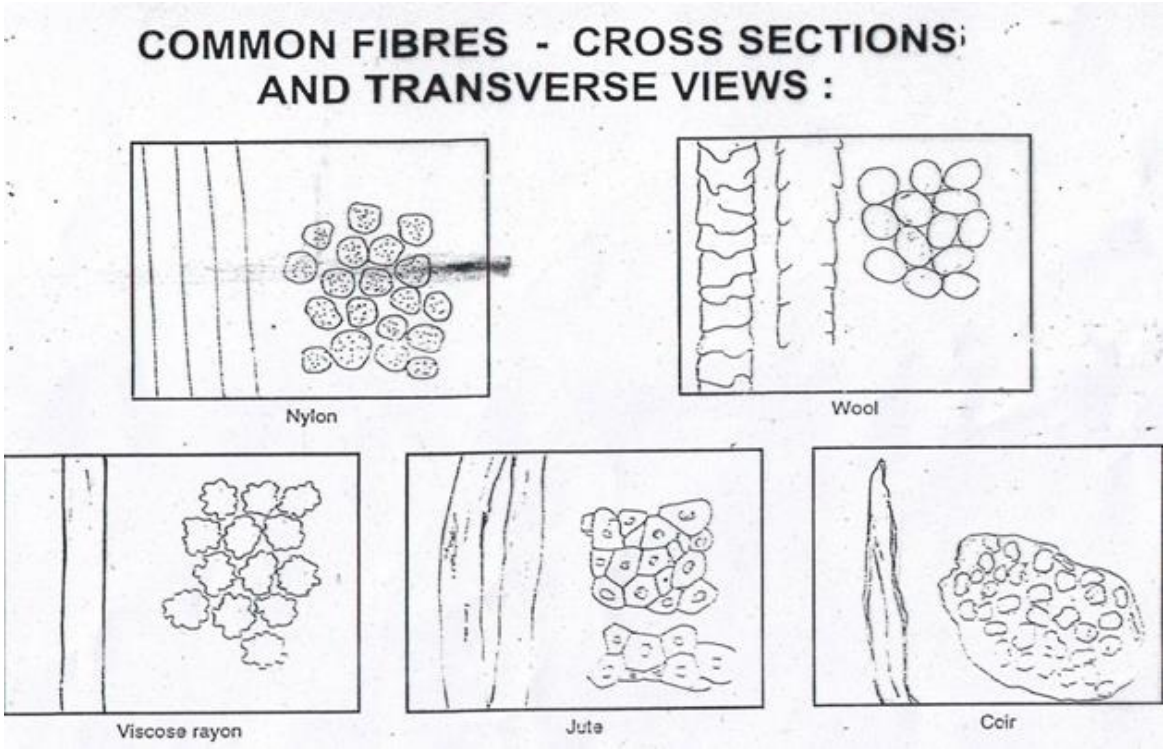
- wild - broad in size with more fibrils

Rayon

- manufactured from cellulose & its derivatives
- variety of fibers with different chemical composition and appearance

Types: viscose, acetate, nitro cellulose, etc.





Ropes & Cordages

- From cotton , jute, sisal, hemp
- Types of twist – left (s-shape) & right (z- shape)
- Angle of twist
- Length of ten twists- Left hand twist of the rope

[s configuration]; Right hand twist of the rope [z configuration]

Cordage

Cordage in the form of fibers and strings may be encountered in homicidal or suicidal cases or for tying objects, strangulation, hanging, tying contraband or stolen property. Rope bending window bars in a burglary. Structure, size, shape, appearance can be traced to the suspect's possession.

Cotton, Coir or Vascular Fibre

- flax, jute, sisal, hemp
- Common cordage-vegetable origin
- Asbestos and glass fiber - insulating and padding
- Average weight /unit length
- Nature of sizing material and contamination - Grease, Paint and manure

Rope

- Is usually made of hemp, jute or coir.
- Twisting strands together makes it.
- Twisting threads makes each strand and
- Twisting fibers make each thread.
- Is distinguished from cordage [cords, twine, and string] by the manner of construction.
- In rope construction the fibers are twisted into threads, which are in turn twisted into strands.
- The strands are then twisted or plaited into rope.

The various forms of cord:

- are made by simple spinning most ropes
- Cords were formerly made from natural fibers
- man made fibers [nylon, polyester, poly propylene]
- Manila hemp and sisal are the natural fibers most commonly used in rope
- Cord, twine and some light ropes
- are mainly made from cotton jute and hemp
- Polypropylene - fish nets: density <1 and floats
- Nylon: great strength + light weight + shock absorbing
- Elasticity: mountain climbing ropes, Parachute cords, towing hawsers for ships
- Polyester: rope with high chemical resistance and max abrasion resistance

5.2.3 Collection

- use vacuum cleaner/clean broom
- place it in a clean paper & then in a cover
- clothing of accused & victim worn at the time of offence - as early as possible
- pack each exhibit separately & label them

5.2.4 Examination

- Surface, cross sectional shape, diameter, color, etc.
- Optical properties
- Direction of twist (s/z), twists per unit length, presence of foreign matter
- Absorption of I.R .radiation (specific frequency)
- Fluorescent properties

- TLC - to identify dyes on fibers

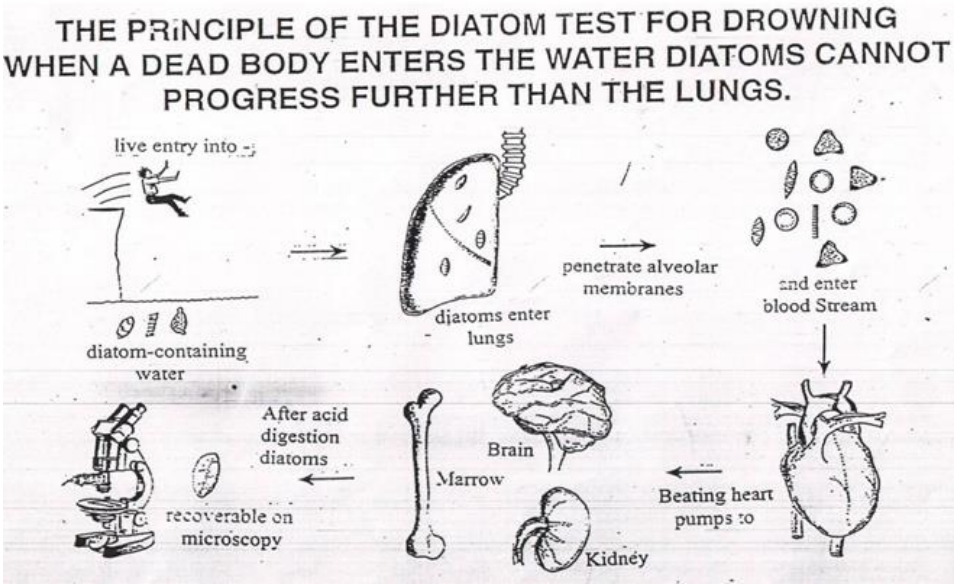
5.3 DIATOMS

If drowning is the cause of death, DIATOMS will bare the facts. These minute single celled algal plants are resistant to decomposition and putrefaction. This is because of the silica coating on the cell wall.

HOW DIATOMS HELP

When a live person drowns many diatoms contained in the water enter the lungs along with the water. These diatoms may penetrate the alveolar walls and enter the blood circulation and are carried to different organs like the brain, kidney, heart, bone marrow etc.

During autopsy, the above organs are collected & then examined. The presence of diatoms in any of these organs indicates that drowning was the cause of death.



5.3.1 Examination

- Sample from site of drowning
- Compare with diatoms isolated from long bones and sternum of the dead body.
- If matched
- Proves ante mortem drowning in that site.

DIATOMS

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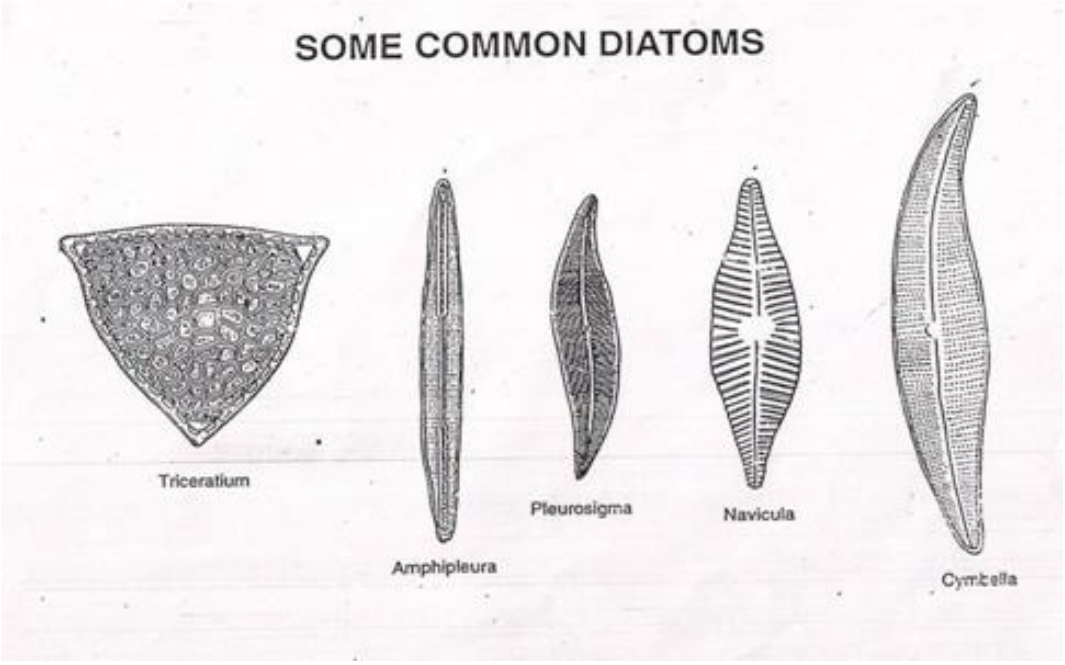


5.3.2 Diatoms

- Mainly in drowning cases
- In tissues like brain, liver, bone marrow
- Whether the death is due to drowning or not?
- They are unicellular plants. Not visible to naked eye.
- Can be seen only with microscope.
- Covered by rigid coatings of silicon thus not affected by putrefaction or acid digestion.
- Differ in shape.
- About 15,000 types of diatoms (half in fresh water + half in sea water).

- Vary in size (2 microns to 1 mm)
- May be single /in group
- In drowning, water enters into the lungs. Diatoms in that water enter into the brain, liver, bone marrow through blood circulation.
- It is not possible if the dead body is thrown in the water as there is no blood circulation.

5.4 PLANT MATERIAL

Includes wood, fiber, leaves, tobacco, stems, pollen, flowers, grains, etc. Tobacco Spurious cigars, beedies and zarda can be differentiated from genuine ones by microscopical examination of tobacco leaf pieces with which they are made of.

- In cigarette butts or beedi ends
- People smoke their favorite brands.
- The print on butts/ends indicates brand.
- An unusual brand may be useful to link a criminal
- Microscopic structure of tobacco leaves
- Chemicals added to the tobacco may also be useful in identifying a brand

Microscopical examination of tobacco leaf pieces involves study of

- Structure, arrangement etc. of epidermal cells
- Frequency and position of stomata
- Type and frequency of trichomes etc.

ZARDA TINS BEEDI PACKETS

Seeds

- Sometimes there are imposters in the seed bags. Inside bags imitating popular brands are spurious seeds. The imposters are caught red handed by studying:
- Physical features of seeds like length, breadth, thickness, weight etc.
- Microscopical features of seeds
- Physical impurities in the seed bags.

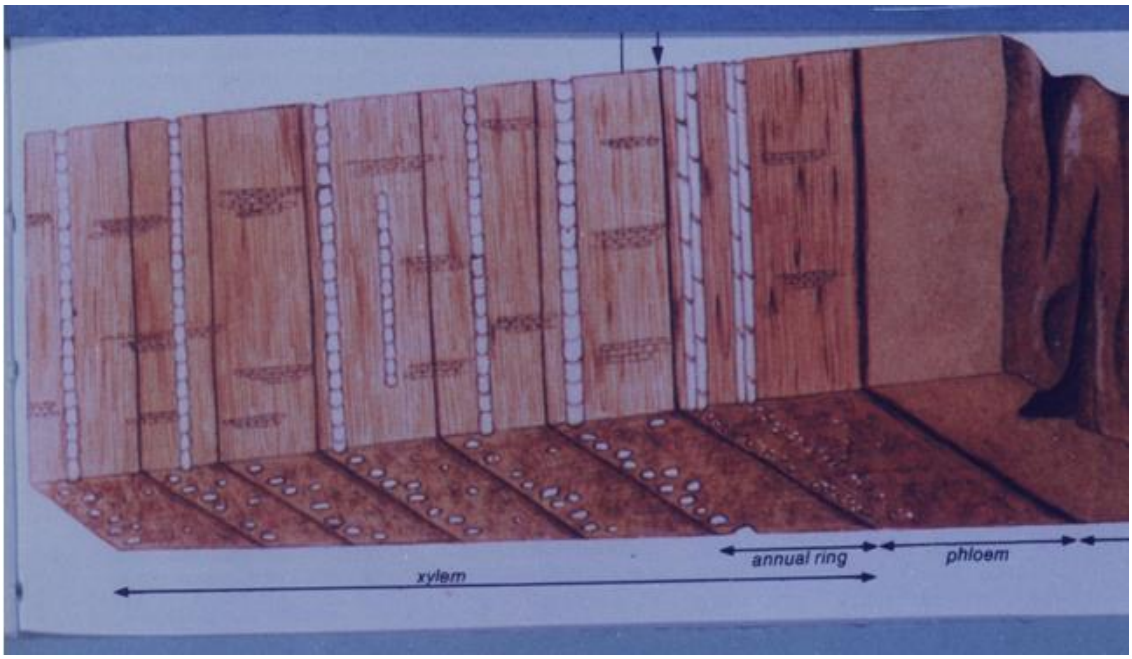
Wood

Wood is the most important plant evidence. Pieces of wood help put the crime jig-

saw together. Wood is also studied to determine quality in construction cases and identifying the timber is smuggling offences.

- Sometimes criminals leave behind or carry away from the scene of crime, bits of wood. This could have been chipped off accidentally by a tool, bullet or edged weapon; or a chip of wood from an intended destruction during burglary. The piece of wood missing is found at the scene of crime be helpful in tracing the accused.
- In the construction and furniture industry, wood is studied to check if it is sub standard or not
- In timber smuggling cases.
- Physical matching of the wooden pieces of same origin.
- particles of wood recovered at two different places may also be examined to prove commonness of source (in scene & on tool/clothing)
- growth rings can also be used for identification

WOOD - INTERNAL STRUCTURE



WOOD is identified by

- Physical features:
- Colour, odour, texture, hardness, weight, grain etc.
- Macroscopical features:

Pith, heart wood, sap wood, growth rings etc.

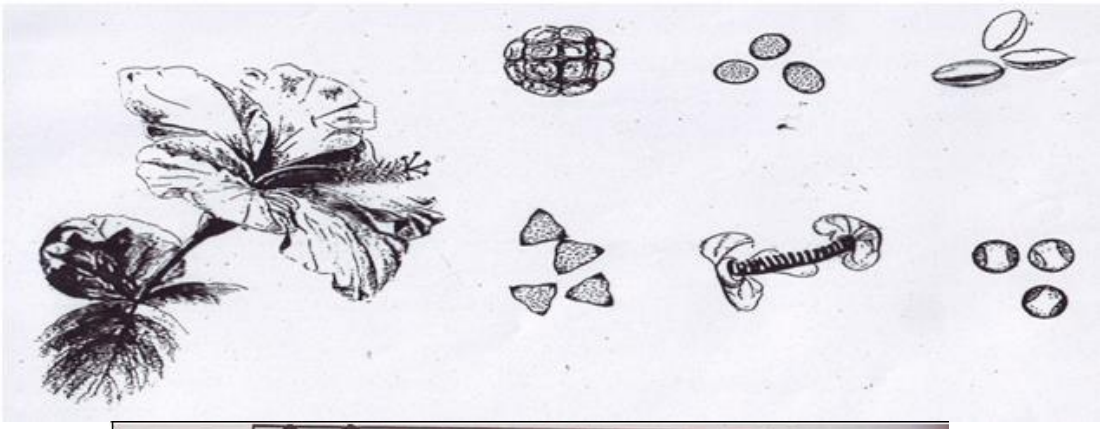
- Microscopical features:

Vessels, parenchyma, fibres, tracheids etc.

Pollen

- Pollen grains in flowers are not reproductive units of plants.
- Easily stick to the clothing/objects that touch them.
- Are microscopic - may not be visible to suspect.
- If a person who is not normally exposed to such pollen happens to have that pollen on him/his clothing it proves his presence in the scene.

POLLEN GRAINS



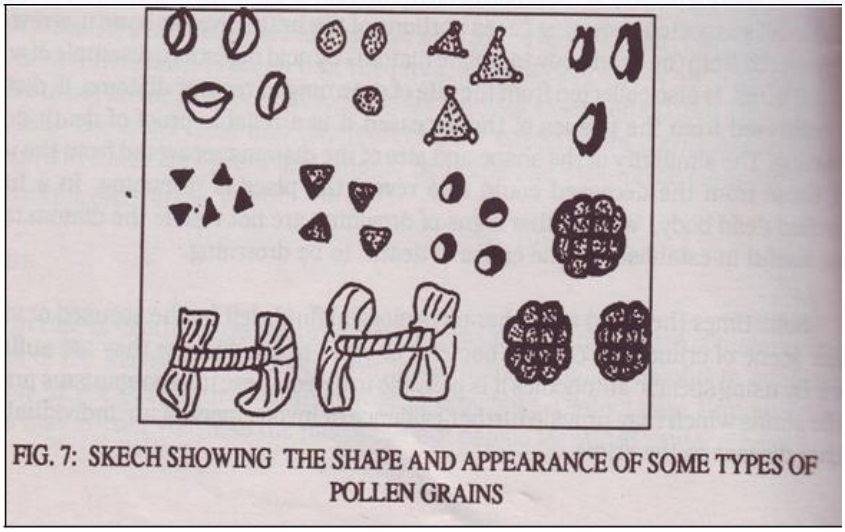


FIG. 7: SKECH SHOWING THE SHAPE AND APPEARANCE OF SOME TYPES OF POLLEN GRAINS

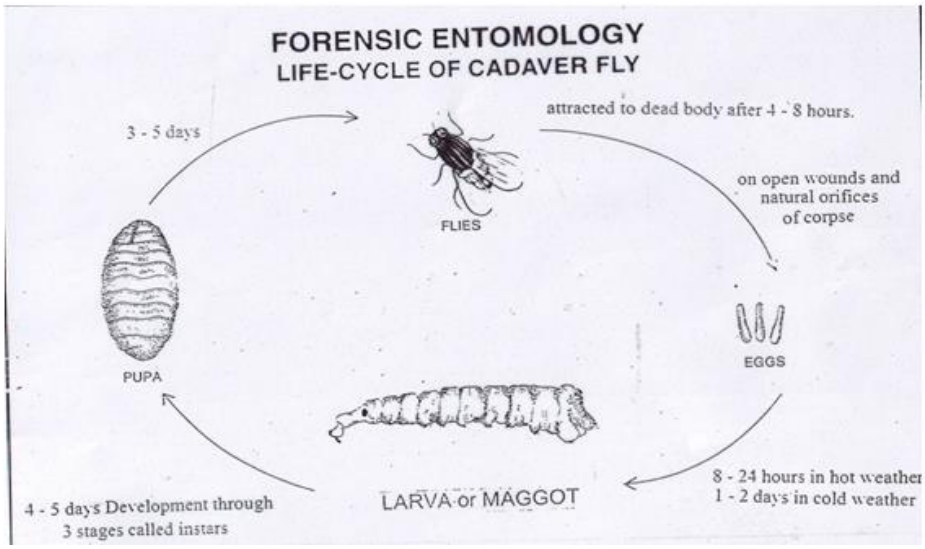
Other plant residues

- Seeds, leaf fragments, bark, twigs, etc.; adhering to clothing/weapon etc.;
- Useful in locating the scene/from where the body has been shifted
- Unusual plant material - more useful for fixing the scene.

5.5 FORENSIC ENTOMOLOGY

Entomology is the study of insects

- Recognition the insects.
- Time of death based on development of insects & their off springs
- Egg laying by the blow flies
- Attracts insects depending on its stage of decomposition
- Appearance of various kinds of insects in chronological order which is well established
- Fully grown larva - time 5 to 7 days



5.5.1 Collection

- Collect larva in hot alcohol & sent
- Collect larva in wide mouth container with a small piece of flesh from dead body (for feeding)- cover with cloth piece (for air)

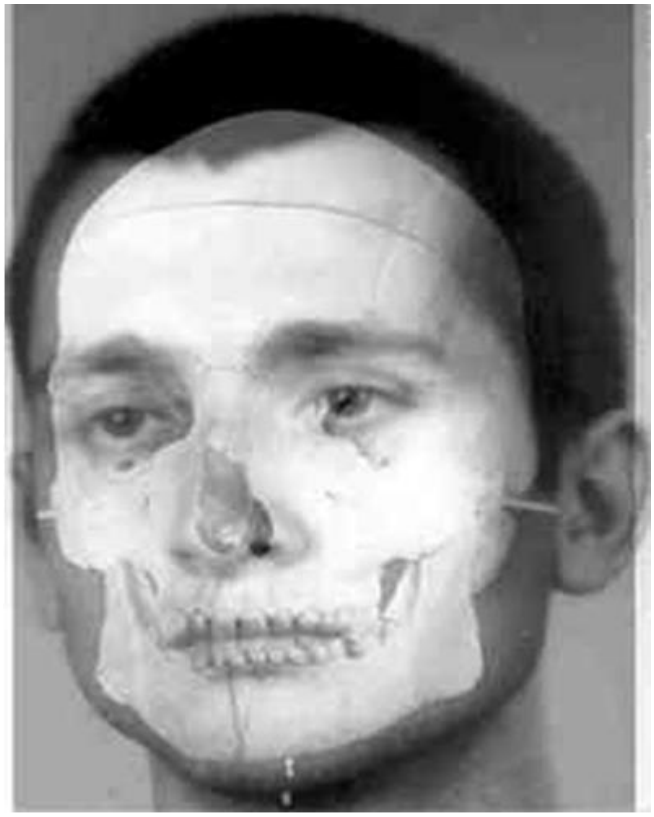
5.5.2 Flesh & Tissue

- May be present on weapon/on bullet /in the scene
- Based on human origin, blood grouping & polymorphic enzymes

5.6 FORENSIC CRANIOMETRY

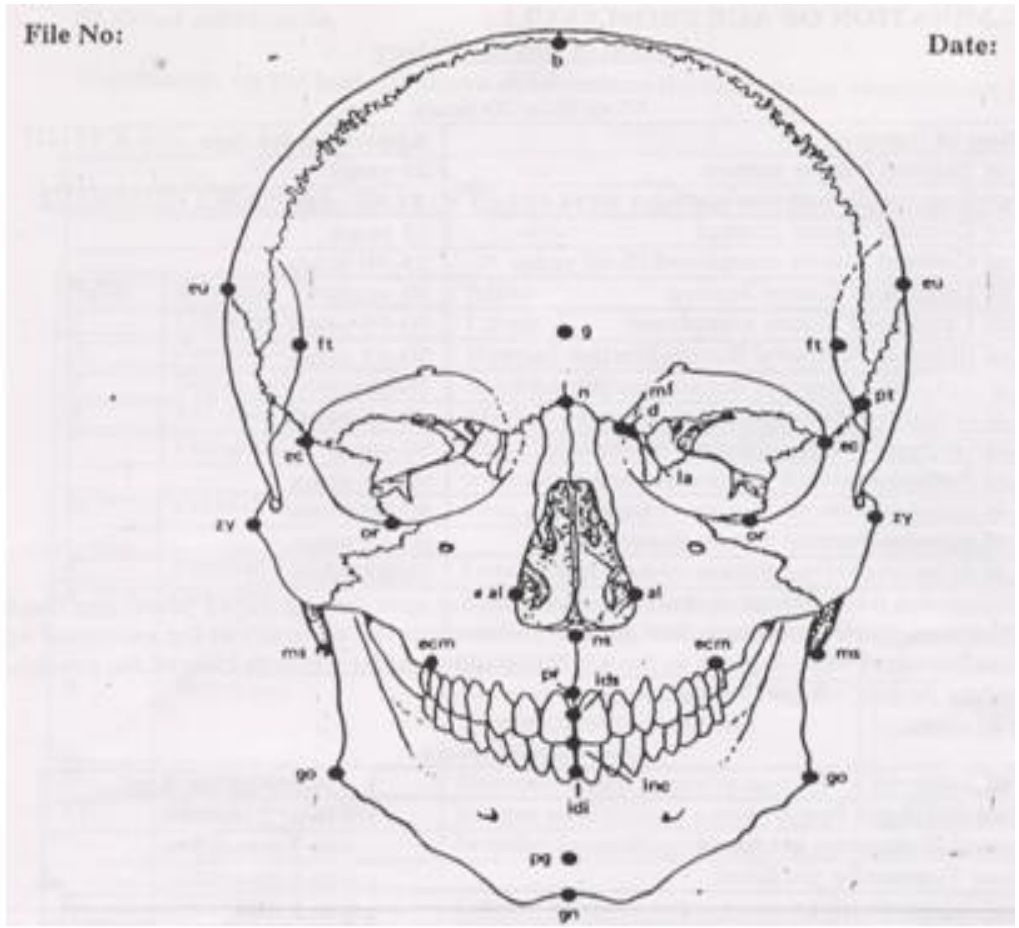
- Skull forms the bony core of the head & face.
- A recent photo of deceased (front view, lateral/semi lateral view) is required.
- Person can be identified by superimposition of negatives of skull & of life size photo of the deceased.
- Enlargement can be done by considering a standard point (like nose ring, ear ring, necklace, design of dress, etc.,) so that the interpupillary distance corresponds to the two orbits of the skull.
- While superimposing the following should be considered: eyes in orbits, point at which nasal bone joined with forehead (nasion), point between two front incisor teeth on upper jaw (prosthion), the nasal spine & lower border of the nose
- Perfect matching - if all anatomical landmarks in both the photographs superimposed- then the skull is identified with the missing person.

SKULL SUPERIMPOSITION



SKULL - FRONTAL

File No: Date:



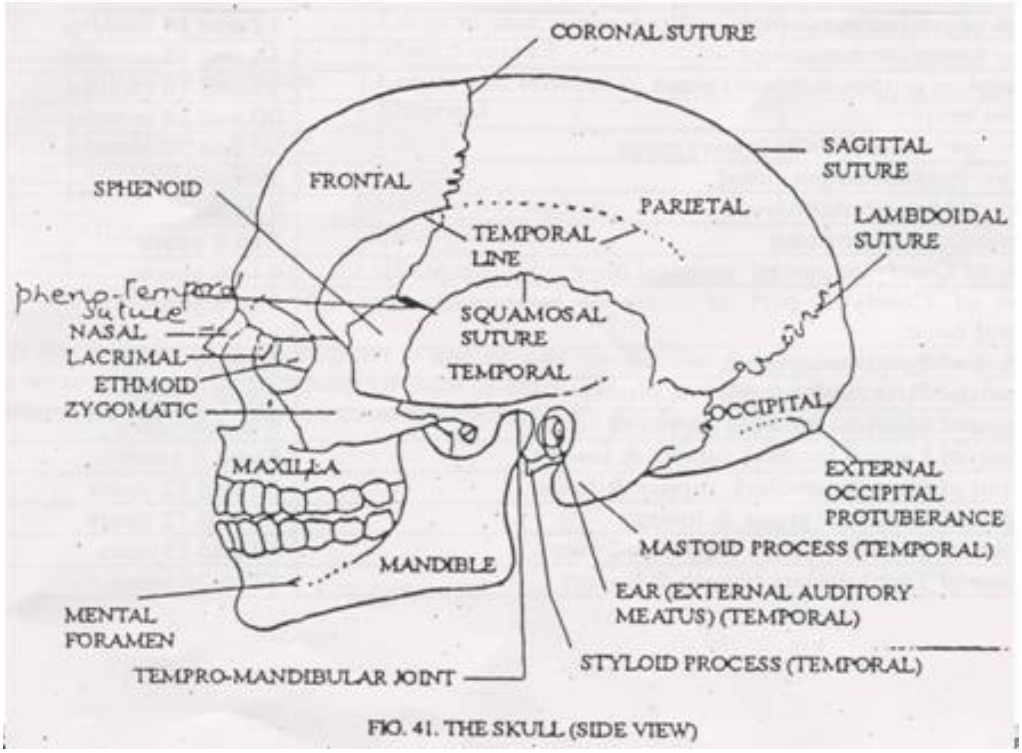
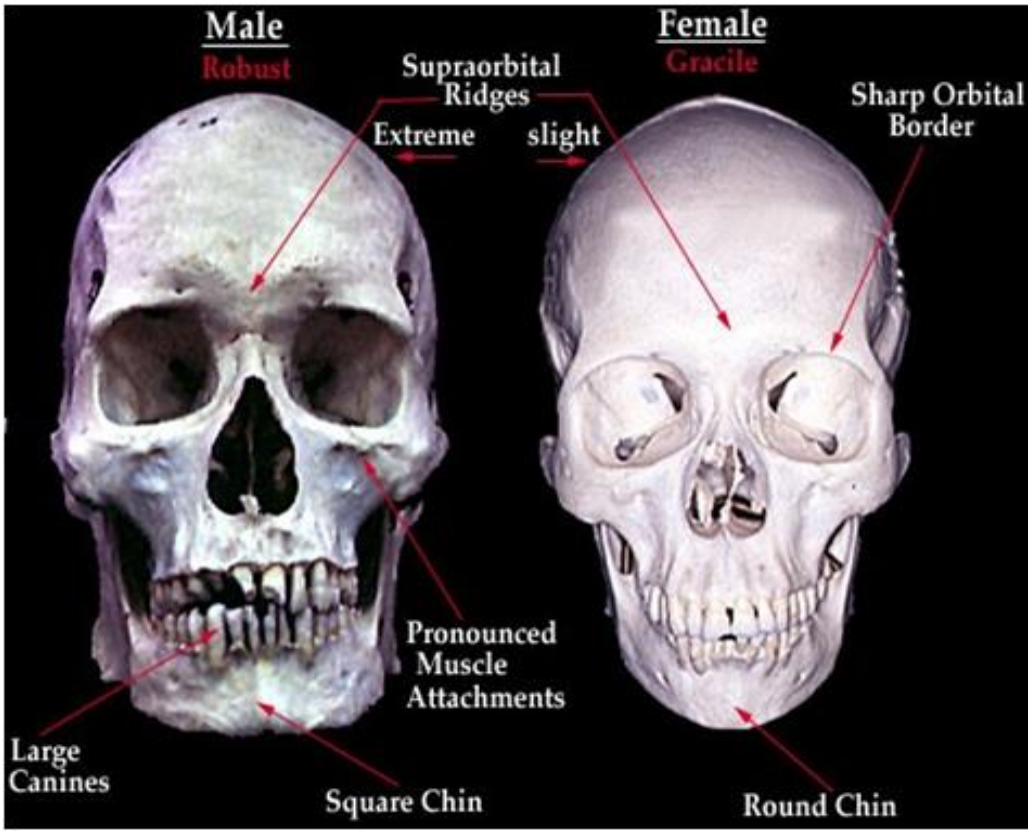
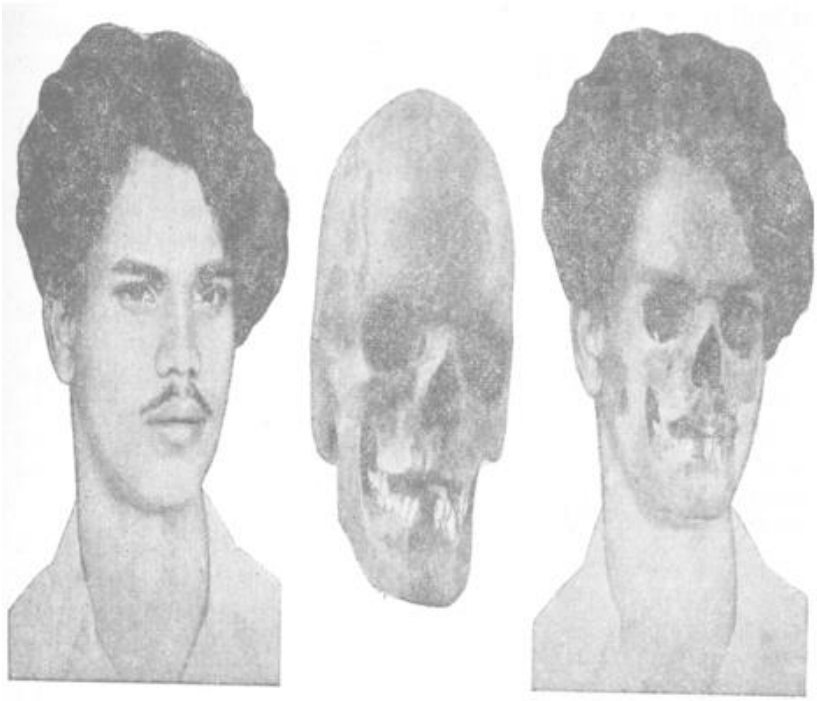


FIG. 41. THE SKULL (SIDE VIEW)

MALE & FEMALE SKULLS



Frontal skull on photo (Skull Superimposition)



5.7 GUIDELINES FOR COLLECTION OF SAMPLES FOR BIOLOGICAL EXAMINATIONS

1. In case of drowning, for conducting diatom test, sternum and long bones are preferable. In the absence of which, heart, kidneys, liver etc. can also be sent.
2. Hair samples (public and scalp hair) of accused and victim, should be of full length and at least 50 samples should be collected by combing or plucking instead of cutting.
3. To determine time since death, wherever dead maggots are found over the dead body they should be collected in a sterile bottle containing alcohol. Some live maggots should also be collected in a wide-mouthed bottle with a small piece of flesh inside. The mouth of the bottle should be covered with gauze cloth to allow sufficient aeration.
4. Each material object collected from victim or accused should be packed separately with proper tagging, labeling and sealing.

UNIT V-B-Forensic Archaeology

5.8 HISTORY

Forensic archaeology first emerged in the later 1970s; a relatively recent development in the UK but it has already shown its worth on a number of scenes of crime.

In December 1962, William Jennings beat to death his 3-year-old son Stephen at their home in West Yorkshire. He wrapped the body in a sack, carried it to the edge of woodland and laid it against the base of a dry stone wall. Having covered the sack with stones he went home and later reported the boy missing. Despite an intensive search, the following winter the boy's fate was still unknown, until in 1988 when his remains were partially exposed by a dog. The remains were subsequently excavated and recorded by an integrated team that included specialists in archaeology, forensic science and forensic pathology, as well as senior detectives and a scene of crime unit. The recovery of the boy's remains and surviving clothing marked an important stage in the application of archaeological techniques in forensic contexts. When William Jennings was convicted of murder, his trial set a precedent for the use of archaeological evidence in a British court.

5.8.1 INTRODUCTION

Archaeology is the recovery and study of remaining material objects, such as graves, buildings, tools, artworks, and human remains, to systematically investigate the structure and behavior of past cultures, derived from the Greek word *arkhaiologia* meaning the study of ancient things. Archaeologists rely on physical remains as clues to the emergence and development of human societies and civilizations. Thus, forensic archaeology is an expanding branch of archaeological investigation in which the methods and approaches of archaeology are applied to legal problems and in connection with the work of courts of law. Most commonly, this involves the reconstruction of a chronology and sequence of events from the deposits found within and around graves and burial sites for homicide cases and investigations into the violation of human rights.

Archaeological methodologies have thus been successfully applied in a number of investigations, including the Moors Murders enquiry of 1986. However, at the same time investigations noticeably lacking archaeological methodologies have also been conducted where burials were excavated by large ground-moving equipment and shovels. Surface skeletal remains were collected without paying attention to the distribution of the remains and with tools such as garden rakes for example, as in the case of the 1953 murders at Rillington Place and the Nilsen murders at Muswell Hill. It is clear today however, that much information is lost this way. Furthermore, the excavation of mass civil or war graves in places such as Argentina, Rwanda and Bosnia have successfully widened the application of archaeological recovery techniques to other parts of the world.

5.9 BASIC PRINCIPLES

Two basic principles of archaeology are Stratigraphy and Superposition. Stratigraphy refers to the formation of strata or layer. It is the sum total of the processes whereby the layers are accumulated. Superposition implies that the

oldest evidence is deposited first, and located at the deepest layer. The formation of stratigraphy can be described as the opposite of the natural process of erosion, but erosion can play a role after the various matrices have been laid down. Water and wind erosion, as well as plant, human and animal activity can alter the deposits. It is impossible to dig a grave and then cover it up in such a way that the exact, original stratigraphy is retained. Superposition gives an indication of the relative order in which objects were placed in a grave; thus, the objects placed in a grave last are discovered first.

5.10 ARCHAEOLOGY IN FORENSIC SCIENCE

Archaeology is a process that has access to a range of associated areas of specialties many of which also have forensic application. These include, for example, aerial photographic interpretation, the identification of pollen assemblages, soil analysis, animal/human bone differentiation, and the recognition of cremated human materials. Archaeologists have also developed techniques, which are peculiarly suited to forensic purposes, for example geophysical survey for the detection of shallow sub-surface remains. Archaeology, like forensic science, is also concerned with scientific analysis across a broad range of material types.

The main areas of interest common to both archaeology and criminal investigation can be listed as thus:

- Skeletal analysis (physical anthropology)
- Scientific analysis
- Field search
- Excavation and recovery

5.11 TYPES OF PHYSICAL EVIDENCE AND CASES

Cases that require forensic archaeologists and archaeological specialist techniques are:

I. Surface body disposals where a victim has been concealed under fallen leaves, branches, walls, rubbish, etc. In this case, the application of archaeological stratigraphic recording to the removal of the layers of material concealing the victim can be of great evidential value.

II. Potential gravesites, where attempts to locate and recover any human remains are part of an investigation of an unsolved crime or in some rare cases the result of information gained from an individual already convicted of the crime in the absence of a grave.

III. Buried small items or personal effects from a victim or suspect of crime. The evidence may serve to corroborate a statement or contain other evidential value placing an individual at the crime scene or its locality.

IV. Mass graves are usually investigated by an international organization such as the United Nations, requiring the recovery process to deal with both evidence material (used in War Crime indictments in the International Criminal Court) and the identification of individual remains for the surviving relatives (a crucial role in the reconciliation and breakage of the violence cycles).

V. Civil cases involving buried evidence (e.g. locating former fence lines in

boundary disputes).

(From left to right) Figure X.1 - Images detailing the range of forensic archaeological applications: (a) excavation at a cemetery, possibly for a positive identification of the buried individual; and (b) a mass grave excavation with its various complications and intricate work.

5.12 METHOD OF ANALYSIS

5.12.1 SEARCH:

One of the most difficult aspects in forensic archaeology is to find the buried body. Graves are often found by accident or because of construction work. An eyewitness or informant can also lead the police to a site where there may be human remains, buried or on the surface. Surface indicators are often destroyed in older burials, and it is not always possible to determine whether the grave is of forensic or archaeological nature. Situations may also arise where relatives of a deceased person may claim that the burial was carelessly disturbed and the remains scattered by the cemetery authority during construction or relocation. They may request that the grave is opened to see if it is disturbed and whether all parts of the body are still there. Equally, old cemeteries can also require archaeological assistance to relocate burials in order to re- bury with due respect, as was the case at the Royal Naval hospital in Portsmouth.

In nearly all instances, the method of search involves the identification of the disturbance caused by the burial in the first case, rather than the item buried. The initial phase of search, especially in cases of homicide, usually involves targeting the most likely locations in a given area using factors of feasibility (i.e. geological suitability, soil cover, land use etc.) in combination with a suspect's movement, psychological profiling and other intelligence. The use of geological maps and existing aerial photographs can facilitate 'dump-site' analysis, considering factors such as the relationship between victim and suspect; location of last sighting, or likely distance traveled.

Once the area can be narrowed down, a more detailed approach can be taken. When a victim, or for that matter any object, is buried, the subsurface is disturbed in relation to the surrounding environment. This disturbance can have a number of effects on (a) the subsequent ground vegetation, (b) the immediate topography, and (c) the geophysical signature of the disturbed area.

There are several techniques to locate buried bodies ranging from simple observation to the use of sophisticated equipment. In general, surface changes in

soil and vegetation may indicate a grave. These include the following.

1. *Soil compaction*: Although the infill of the grave would probably have been leveled with the surface at the time of burial, the soil will compact after some time to form a concave area, or double depression, known as the primary and secondary depressions. These depressions are not only formed because of the compaction of the infill but also because of the decomposition of the buried body. The largest volume to collapse during decomposition is the thorax (secondary depression). This secondary depression occurs in the upper third of the grave's surface, and is usually deeper than the total con cave area, which denotes the approximate outline of the grave pit. The depth varies according to the type of soil, the depth of the grave, and the amount of water present. This depression is the most obvious in the first months after the burial.

2. *Disturbed vegetation*: This includes the plants on the surface of the grave itself and surrounding areas where the excavated soil was thrown when the remains were interred. Usually the area directly above the grave may be void of plants for some time, although a shallow grave, with not wrapping around the body, may stimulate growth due to the nutrients originating for the decaying body. The presence of pioneer plants, e.g. weeds, occurring in a localized area may also be indicative of a fresh grave. Weeds are usually the first plants to appear because they are fast growers, and they can often be distinguished from the surrounding vegetation.

Many of these features of vegetation, topographic or soil change might be seen at ground level, they are often at their optimum when viewed from the air, some features being particularly prominent through the use of shadows at appropriate times of the day. Aerial reconnaissance is an ideal nonintrusive primary method of search, but its effectiveness may be seasonally specific.

3. *Disturbed soil*: Usually the soil from the surface and that originating from the grave pit mixes, which leads to obvious differences in colour and texture with the surround matrix.

These factors are of course, influenced by the environment. Unless the event occurs soon after harvesting in a farmland, these changes may be difficult to observe because of regular plowing. The same is true of sandy soil, where the sides of the grave may collapse and obscure the depression of the grave's surface.

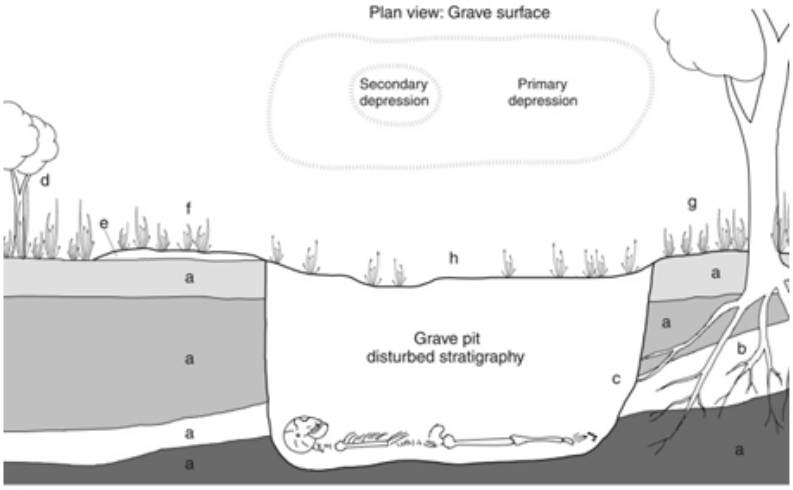


Figure X.2 – Indications of the presence of a grave: (a) undisturbed stratigraphy; (b) undisturbed plant roots; (c) disturbed plant roots; (d) original undisturbed vegetation; (e) grave fill remaining on the surface; (f-g) different vegetation in the area trampled and disturbed during the original excavation; (h) new plant growth.

Methods which can be used to detect buried remains; some of the most commonly use are listed below as a general guide on a scale which runs from noninvasive (i.e. those least likely to destroy buried evidence) to the invasive (i.e. those most likely to destroy buried evidence). The deployment of these techniques and their use in sequence depends on a range of factors including the local environment, the geology, the nature of the target, and the interval since burial. Some techniques, particularly geophysical methods, are only suited to certain types of environment or situation (i.e. indoors, outdoors, concrete, clay, etc.) and are wholly ineffective in other environments.

- Aerial photography and the reconnaissance for the detection of disturbances indentified from the vegetation or topography (shadow or colour change) or by soil marks (colour).
- Field craft for the detection of discrete areas of vegetation, topographic or soil differences, also for the presence of ground cracking and for the identification of burial locations by using prominent local landmarks or features which may have been used as guide points by the offender.
- Specially trained cadaver dogs for the identification of buried remains by virtue of characteristic odor formed by the process of decay, and would therefore be most effective shortly after death, which may be released from the ground by systematic or selective probing.
- Geographical survey for the detection of shallow subsurface features, typically by using electrical resistance (to detect disturbances which has affected local moisture content of soils), magnetometry (to detect disturbances caused by changes to a local magnetic field), ground penetrating radar (to detect responses to changes in ground density using an electromagnetic pulse) and metal detector (to detect both ferrous and n nonferrous items associated with a buried individual).
- Probing/sampling for the identification of subsurface disturbances, emission of methane, or thermal change.
- Excavation for the identification of soil disturbances (colour, texture, and physical properties), and changes consistent with a grave.

The effectiveness of each of these methods depends on a range of factors, not least of which is the ability to detect a disturbance or change within an otherwise undisturbed environment. Also to be considered are effects brought about by the decay process of the body itself. This may not only generate heat (and allow the target to be identified using thermal imaging during the decay process) but it may also affect the geophysical properties of the grave and inhibit or enhance the likelihood of detection depending on the survey technique used. Variables that might accelerate or delay this decay process include factors of burial depth, climate, soil pH, wrapping or clothing, water content and presence of oxygen. Optimum methods of search rely on knowledge of as many of these variables as possible.

5.12.2 RECOVERY:

Recovering buried remains can occur in a variety of different situations and environments but the process of recovery follows a well-established routine in order to maximize the evidence available. This is based on the awareness that archeology is a destructive process and that each recovery operation is seen as a non- repeatable exercise.

All evidence must remain *in situ* until documented. A clear, concise, meaningful, written description of the pertinent aspects of the crime scene is the most important method of documentation. The recovery should be done in such a fashion that the integrity of the evidence is maintained in order for the information to be acceptable in a judicial or criminal investigation. Context is provided via documentation, and contamination must be avoided. All evidence recovered should be labeled at the scene

The recovery of a buried body requires a preplanned methodical approach. Although the methodology to be employed is archaeological, the recovery should be processed as a crime scene.

Prior to excavation, the scene should be photographed from various directions. Detailed notes must be taken on all aspects of the scene such as the vegetation, climate, topography, geology, amount of sunshine, and any other observations.

The surface should be cleared of vegetation without disturbing any of the evidence, after botanical samples have been taken. A three-dimensional grid system can be established with a fixed elevated datum point. If possible, the grid should be orientated in north-south direction to make reconstruction easier. A scale plan of the site should be drawn and all evidence recorded with reference to the grid.

If any footprints, tire tracks or drag marks are visible on the surface, they should be described, measured, photographed and cast. The excavation should follow natural strata if possible; otherwise, it should be in horizontal arbitrary layers of 10-15 cm. If natural stratigraphy does occur, the excavation layers should not exceed 15 cm. Natural strata thicker than 15 cm should be divided in excavation spits.

Once the remains are visible, the bones are exposed in the top-down manner and from the center to the edges. Again, all bones and associated physical evidence should remain *in situ* until documented and photographed. A clear description of the orientation of the body and the burial position is recorded, drawn and photographed and soil samples from each stratum collected. All bones should be removed with care and securely packed after documentation.

The same procedure can be used for intact and less decomposed remains and in

situations such as mass murder. These remains are always highly odorous. Excavators should take care to protect themselves by wearing gloves and protective clothing.
However, not all remains are buried; in fact, most of the forensic remains are found on the surface. They may be scattered if the deceased wore summer clothing or, may be better preserved in a winter outfit. In the case of scattered remains, the area should be walked systematically and carefully without stepping on any evidence. Every object should be flagged, but not removed before documentation. Care should be taken not to miss smaller objects such as a ring or tooth. Soil around larger pieces of bone can be screened after they have been removed.

When all possible pieces are located, the source from where the remains were scattered should be determined and the agent responsible for scattering identified. In the case of dogs, for example, knowledge of their habits may lead to the discovery of more remains. Ribs are very often dragged away by scavenging animals. Skulls may roll away if the body is on an elevated surface and therefore the lower reaches of the slope should be searched. Determining the path and direction of flow of water on a slope or valley may also lead to the discovery of more remains. The position and orientation of evidence and remains must be surveyed and indicated on a scale map of the locality.

5.12.3 DOCUMENTATION

One of the important principles in any excavation is the fact that it is destructive. Therefore, care should be taken to record all stages of the excavation by means of complete written notes, photographs and drawings. Photographs should be taken in both colour and black-and-white. Each photograph should show the magnetic north and the scale. In practice, a small whiteboard works very well, on which the date, location, case number, orientation and depth can be indicated.
Specific observations must be made of whether the bones are articulated, which indicates that the body was most probably still intact when buried. The position of all limbs must be noted. Photographs taken should include images of the skeleton as a whole, as well a close-ups of any special finds. These finds are specifically marked to make them more visible on the photograph. Detailed notes which include the depth of the remains should be taken, and a scale diagram drawn of the grave from above and in cross-section. The depth below surface and the stratigraphy involved are also very important, and it may be necessary to draw the four walls of the grave.
It should be re-emphasized that once the remains or artifacts have been removed, it can never be placed back into its original position. The most important aspect of the excavation is to provide a lead for the medico-legal investigators and police in order to identify the victim, therefore documentation must be complete and the chain of custody insured.
The advantages of systematic, scientific methods of excavation and documentation are obvious. If done properly, relevant information will be available permanently in case there is a need to reopen the case after some years. A scientific approach provides great accuracy in the collection of evidence and a higher probability that all physical evidence is collected, prevents postmortem damage to bones and artifacts, and decreases the probability of destroying contextual information.

UNIT V-C-Forensic Anthropology

5.13 HISTORY

Forensic anthropology was first officially recognized as a sub field of forensic sciences in the late 1930s in America. The Parkman Murder (1849) was the first documented case in which the expertise of specialists was required to identify the dismembered remains of Dr. Parkman. Two anatomists, Holmes and Wyman were able to determine the dismembered; partly skeletalised remains to be that of a 5Ê10 white male, 50-60 years old. The dentist who made Dr. Parkman his new dentures confirmed the final identification.

The Luetgert Sausage Vat Murder (1897) followed suit. Adolph Leutgert was accused of murdering his wife as it was discovered he mixed his wife's body in his sausage factory with hot potash and made her body into soap and bones into „jelly‰. Several bone fragments smaller than a quarter were identified as human, rib, hand and foot bones by anthropologist George Dorsey who later testified his findings. George Dorsey therefore became the first „forensic anthropologist‰ to testify in court. The discipline thusly grew until its formal acceptance in 1977, validated by the formation of The American Board of Forensic Anthropology (ABFA). Today forensic anthropology is a recognized sub field of forensics, a crucial, evolving tool in cases involving unidentifiable human remains.

INTRODUCTION

The word anthropology is derived from the Greek words *anthropos* meaning man or human and *logos* meaning thought or reason, thus the discipline anthropology encompasses the study of the origins and development of human beings and their cultures; investigating the whole range of human development and behaviour, including biological variation, geographic distribution, and evolutionary history.

Forensic anthropology is the application of the scientific processes of physical/biological anthropology in a medico-legal context. Forensic anthropologists are primarily concerned with the assessment of skeletal and human remains.

The expertise of a forensic anthropologist should be required particularly in cases concerning decomposing or skeletal remains, remains severely mutilated or altered by conditions such as Ideally, for the purpose of identifying the human remains the forensic anthropologist will work as a part of a highly specialized team of experts that complement each other, consisting of a forensic pathologist, forensic archaeologist (search and recovery procedures and other methodological principles), forensic radiologist (X-raying of bones and bodies: aid in personnel safety, primarily through the detection of bombs and potential penetrative objects), forensic fire, explosion, high impact trauma and dismemberment. The identification of an unidentified human either skeletal or badly decomposed is important for both legal and humanitarian reasons. In cases involving remains lacking soft tissue and organs a forensic anthropologist may be able to determine the major biological characteristics of the individual such as age at death, sex, stature and ethnicity. Entomologist (determination of the post mortem interval a.k.a. time since death), and forensic odontologist (bite mark analysis and post mortem dental evidence).

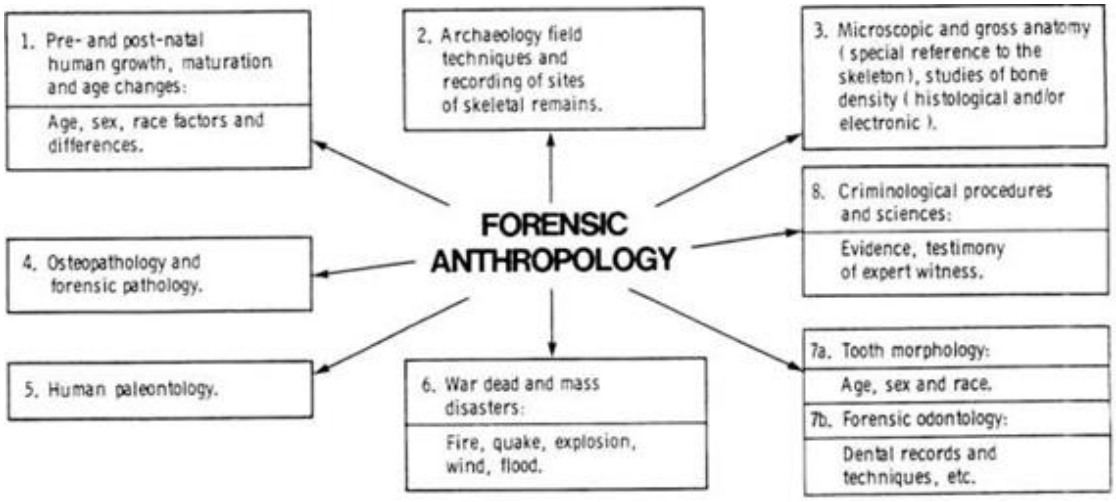


Figure X.1 - Spheres of forensic anthropology.

5.14 TYPES OF PHYSICAL EVIDENCE AND CASES

Cases in which forensic anthropologists are involved in and recommended for are:

I. Scenes of crime where the human remains are in a condition which require identification by means other than a simple visual match, such as:

i. Burned

ii. Decomposed (at various stages of decomposition)

iii. Buried (clandestine burials)

iv. Surface recovery

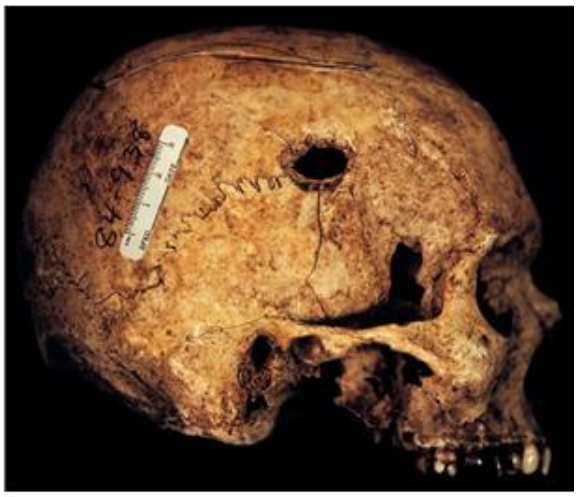
v. Partial skeletonisation

vi. Complete skeletonisation

vii. Cremation analysis

II. Mass disasters and the recovery and identification of disaster victims (such as the victims of the Tsunami); and

III. Investigation into war crimes, genocide and violations of human rights (such as the mass graves excavated in Bosnia, Peru, Rwanda, Iraq).



(From left to right) Figure X.2 - Images detailing the range of forensic anthropological applications: (a) a skeleton arranged in anatomical position prior to analysis; and (b) examination of a bullet wound.

5.15 METHOD OF ANALYSIS

A forensic anthropologist aiding an investigation of suspected skeletal remains will aim to determine the following issues:

1. Is it bone?
2. Is it human?
3. What is the post mortem interval?
4. What bones are present?
5. What is the minimum number of individuals (MNI) present?
6. Can ethnicity be determined?
7. What is the sex?
8. What is the age at death?
9. What is the stature?
10. What are the individual characteristics of the remains that can lead to a positive identification?

Individual characteristics are features particular to an individual that can help to establish a positive identification by providing vital and often-unique information such as pathology: ante mortem fractures, dentition, congenital abnormalities, particular diseases, occupational stress markers, and medical implantations.

5.15.1 DETERMINATION OF POST MORTEM INTERVAL (TIME SINCE DEATH)

The determination of a post mortem interval in cases of decomposing and skeletonised remains is extremely difficult. The estimated time range tends generally to be much wider than in cases of recent demise and is thus often only an educated guess. Therefore, it has been recommended that eight main categories of information should be acquired and considered if the time of death is to be determined with scientific validity and to a standard appropriate for criminal

proceedings:

1. seasons the body was exposed to, including the hours of daylight and darkness;

2. temperature ranges for the daytime and nighttime exposure period;

3. humidity ranges of the period;

4. clouds, precipitation, draught, snow cover;

5. pH of soil for buried bodies;

6. depth of burial;

7. whether or not the body was buried in a receptacle;

8. plant growth around the body;

9. scavenger activity (remains can be scattered far from the original location);

10. insects common to the area at each season (main factor affecting the rate of decomposition - insects occupy the body in a predictable sequence).

Additionally, other indicators to consider when determining an estimate range for the post mortem interval are:

(i) Presence of artifacts, indicative of a time period (dated coins, labeled clothing, manufacturing date on articles)

(ii) Wounding (wounds make a body more susceptible to attack from insects and scavengers, accelerating decomposition

(iii) Clothed or naked body (heavy clothing retards decomposition)

(iv) Body size (larger bodies take longer to decompose)

(v) Embalming, wrapping, coffin containing of a body (slowing decomposition down)

(vi) Dental changes/surgery (can indicate a more recent demise)

(vii) Presence of hair, odor, greasy bones, bleaching of bones (indicate a rough estimate of time)

5.15.2 DEVELOPING A BIOLOGICAL PROFILE

A complete and undamaged skeleton can be more accurately assessed than a partial skeleton or skeletal element. However the chances of dealing with a complete skeleton are slimmer than the chances of dealing with skeletal remains that are partial and/or fragmented.

The skeletal elements received by a forensic anthropologist are laid out in anatomical position from which a quick visual examination for duplicate bones reveals the number of individuals present. For example, two right femurs are indicative of the presence of more than one individual, as one individual cannot possess two right femurs.

All the techniques used in determining the age, sex, stature and ethnicity, detailed in the anthropological guidebook „Standards for Data Collection from Human Skeletal Remains‰, are regarded as the universal standard methods of data collection. The analysis is aided by a skeletal inventory that is completed during

the examination and is required in the appendix of the court report. The inventory establishes the identification of all skeletal elements present and missing; additionally it notes details of the appearance and condition, factors such as fleshed or decomposed, colour, presence of sediments, weathering modifications (bleaching), and damage.

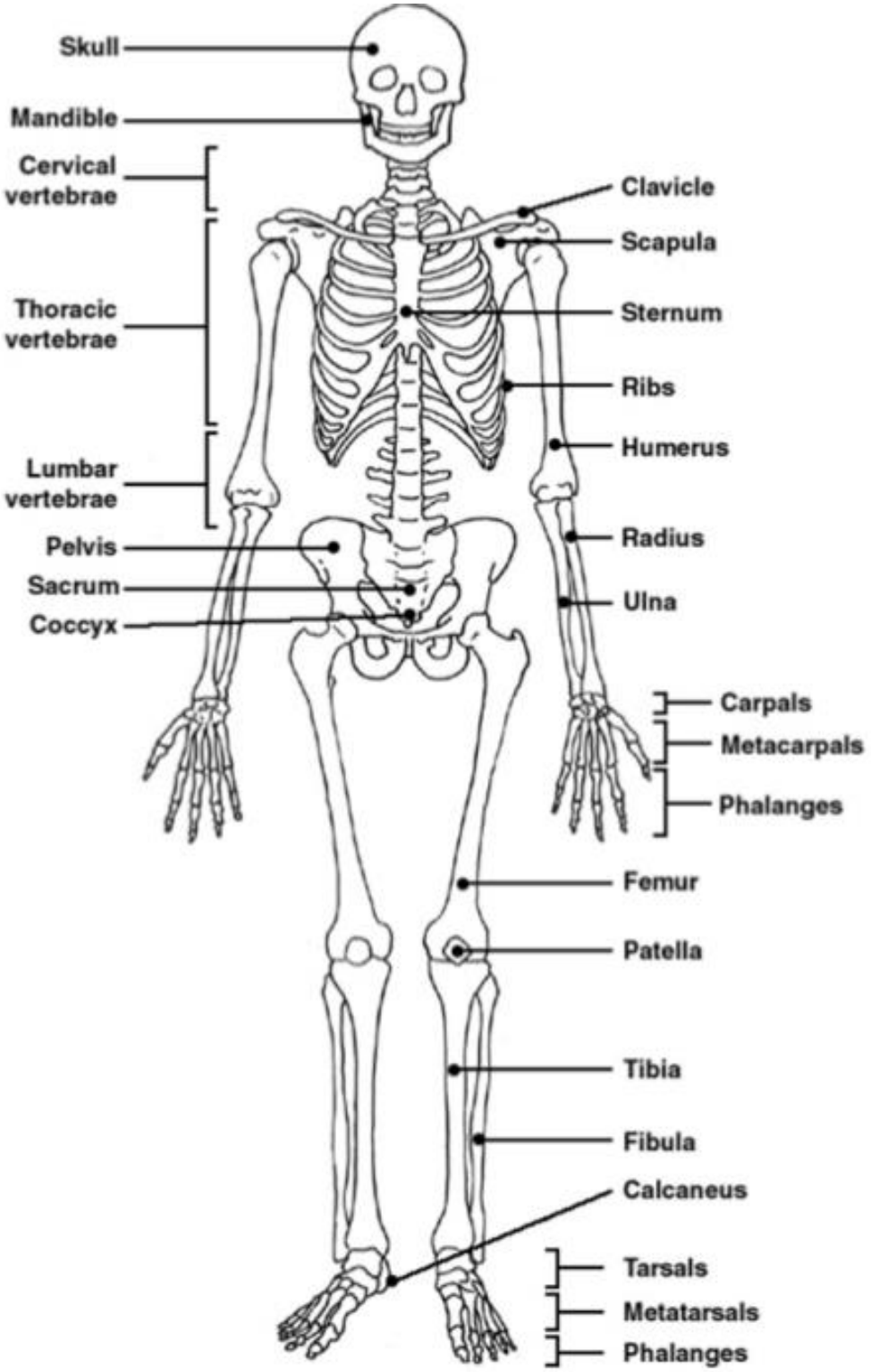


Figure X.3 - Detailing the skeletal anatomy of a human

I. Age:

An anthropologist must be familiar with the dental and skeletal development in each stage of life. In the early years of development the skeleton undergoes an orderly sequence of changes beginning with the formation, eruption and replacement of the 20 deciduous teeth („milk‰ teeth: shed in childhood). By 12 years of age the second molars (permanent dentition, 32 teeth: 2.1.2.3 dental formula) have erupted and although there is a timing variation in sex, race and health factors, the age at death can be estimated to within 1 year in a normal sub-adult. It is generally accepted that dental development patterns are reasonably invariable and provide a dependable indication of age, more so than epiphyseal fusion, due mainly to the knowledge that dental development is genetic and extrinsic factors such as the environment and nutrition play only a very minor role. Once the second molars are fully erupted, attention is focused on the skeleton. The skeleton is formed and developed by ossification centers: formed first in cartilage and then replaced by bone. Until the beginning of adolescence, long bones, for example, consist of a diaphysis (shaft) and epiphyses at both ends. These are connected by cartilaginous growing regions and are replaced with bone when growth is complete. Growth (fusion of the epiphyses to the shaft) at each joint is completed at different ages and in a set order. The patterned timing is dependent on age, sex, bone element involved, nutritional and hormonal status and individual variation.
Once growth is complete aging becomes a much more difficult task since post-maturity changes are subtle, irregular and highly variable from one individual to the next. Therefore, age estimation is given in a range: increase in the accuracy of results requires the assessment of as many indicators as possible.

1. Pelvic Joint Morphology: Two of the most reliable indicators of adult age are *pubic symphysis* and iliac *auricular surface* morphology. The *pubic symphysis* changes from a well-defined billowed surface to more flattened and rimmed with age. However, the older the individual the wider the estimated age range. Changes in density and texture, assessed with the use of comparison models, are used to establish the age range using the *auricular surface*.

2. *Sternal Rib Morphology*: Increasing age causes the hollowing and lipping of rib ends, particularly the fourth rib. However, the ribs are easily damaged in recovery of fragmented remains and identifying the fourth rib can also pose difficult.
3. *Cranial Sutures*: the bones of the cranium fuse together with age, specifically from 18 years onwards, therefore suture closure indicates aging of an individual. There is a lot of variation in suture closure however; occasionally the skull is the only measure available in cases of fragmented remains.
4. *Ossification of Hyaline Cartilage*: ossification (turning into bone) of the cartilage that connects the ribs to the sternum is a general indicator of increasing age.
5. *Dental changes*: increased wear and attrition patterns on the dentition: related to individual characteristics such as diet, oral hygiene, and genetics.

II. Sex:

Differences between the sexes are much less distinct in the skeleton: sexual

dimorphisms vary between human groups in metric and morphologic manifestations and overlap in male and female.

At the onset of puberty significant hormonal changes occur which greatly increase the morphological differences between males and females. Prior to the development of puberty/adolescence the skeletal morphological changes remain subtle and limited therefore, sexing prepubescent bones is problematic and should not be done in individual forensic cases. The differences in adult skeletons are more salient and the pelvis bones are the simplest and most accurate in determining sex (90+ percent in accurate sex attribution), followed closely by the skull.

The primary difference in the male and female pelvis are based on its usage: the female pelvis had additional breadth and increased diameter of the pelvic inlet and outlet for childbirth. In females the *greater sciatic notch* is shallower and wider, the *pre-auricular sulcus/surface* is more developed and raised (caused by the trauma of childbirth) and the *ventral arcs*, *sub-pubic concavity*, and *ischio-pubic ramus ridge* (Phenice Technique, 1969) exhibit structural differences from that in males.

The skull of a typical male tends to be larger and more robust (in areas of muscle attachment), displaying more distinct ridges and crests. There are ten individual bony features, which upon observation aid in the determination of male or female.

The postcranial skeleton is also assessed in order to aid determination of sex. Male skeletons tend to exhibit larger weight-bearing joint surfaces, sites of muscle attachments, long bones and stature. However, these traits are largely influenced by nutrition, genetics, and physical activity: therefore are not solely relied upon to determine the accurate sex. Sexing requires the careful assessment of the entire skeleton to best reveal the accurate sex.

II. Stature:

Stature is not a fixed trait because it is well known that an individualÊs stature changes (shrinks) in the course of a day, let alone over the course of a lifetime. To calculate stature of a disarticulated skeleton the skull, spine, pelvis and at least one leg must be present. When the remains are incomplete the stature is estimated by extrapolating it from the measurements of the long bones (leg and arm). This is not an exact science and presented as a range. The stature formulae are based on specific ancestral populations and also categorise between male and female. It is critical to measure the remains in exactly the same way as was done for the reference populations.

IV. Ethnicity

Considering that *Homo sapiens* are a single species determining ethnicity is a difficult and often impossible task. There are no absolute physical or genetic values that divide even the major human groups; in fact there is more variation between individuals within a population than between the larger distinction groups. Further complications arise when considering the complexity of human mating and migration patterns.

Thus far the various metric statistical methods that exist are limited in their data calculations to the particular reference study population from which they are derived. The skull is the primary element used in ethnic determination and can be classified as Caucasoid, Negroid or Mongoloid. Observations of non-metric traits can also be additionally used, such as a CarabelliÊs cusp; a small extra cusp on the

maxillary molar commonly found in Europeans but rarely seen in Asians or Africans.

5.16 CAUSE AND MANNER OF DEATH

The legal authority to determine the cause and manner of death rests with the coroner or medical examiner. However, an anthropologist can contribute critical evidence for this determination, particularly in the interpretation of skeletal trauma and judgments regarding the timing of the trauma.

5.16.1 IDENTIFICATION OF TRAUMA

In many cases determination of the cause of death by examining the remains is not possible because most injuries are sustained to the major soft tissue organs of the body. Skeletal trauma is not necessarily related to the cause of death and needs to be identified to one of the three distinct stages: ante mortem, peri mortem and post mortem. X-raying remains is good practice in search of evidence of trauma and/or disease.

1. *Ante mortem trauma*: Very rarely is relevant to the cause of death however, it often can be useful in the identification process. Post mortem X-rays of the unidentified individual are compared with those taken of a known individual during life to establish a positive match (used in the Tsunami).

2. *Peri mortem trauma*: This can provide the cause of death in cases where the bone is affected by either blunt or sharp force trauma: such as a stab wound that damaged/cut the ribs or a gunshot wound - where evidence of penetration and fracture is present. Once more, X-raying the remains can reveal structures and anomalies not visible to the naked eye such as bullet fragments that are lodged within the bone.

3. *Post mortem trauma*: Old bones are subject to fractures as they become more brittle with age therefore post mortem trauma/damage either occurs during the discovery-recovery process or are incidental fractures which are unrelated to the case. Fractures, which occur close to the time of death, are the same colour as the surrounding bone however fractures that occur during recovery will generally be cleaner and lighter in colour than the surrounding bone.

5.17 BURNT BONES

Burnt bones pose a special problem for anthropologists. Physical alterations to bone subjected to heat result in splintering, fragmenting, charring, discolouring or blackening and becoming brittle. Forensic anthropologist must possess an in-depth knowledge on the various types of bones which would therefore allow them to be able to identify if not all, a vast majority of burnt bone fragments from a scene of crime. The burnt bone fragments are analysed using techniques such as X-ray diffraction to determine if the sample is in fact bone and whether the bone is human or non-human. Recent studies have developed a rudimentary analysis using X-ray diffraction that can enable the determination of the type of animal the bone is of based on crystal structure and composition.

5.18 SPECIALIST TECHNIQUES

A number of specialist scientific techniques are currently available which have implications for the identification of human remains and determination of other issues in relation to the remains. A forensic anthropologist may suggest the use of the following techniques in order to assist in the identification of the remains:

1. *Direct facial reconstruction*: The building of a face with wax or clay over an actual skull. Difficulties arise in defining landmarks on the skull for measurement and also in the measurement itself. Changes in tissue thickness that can occur before and after death are also difficult to account for.
2. *Bite mark analysis*: Analysis of the shape and number of teeth and modifications made to them can be used to determine the identity of a potential assailant by comparison with dental records. The more unusual the dentition, the more significant the bite mark to an identification.
3. *Radiocarbon dating*: A specialized technique, which can detect a post mortem interval of up to approximately 45,000 years. Forensic archaeologists use this technique more often, to date remains.
4. *Forensic toxicology*: Trace amounts of drugs and other toxic elements can be detected in skeletal remains by means of chemical analysis (e.g. if toxic metals are ingested between 24 and 36 hours before death, permanent traces will be left in the bones as well as the hair, nails and skin).

5. *Trace element and stable isotope analysis*: Determines the post mortem interval and geographic location of the deceased while living.

UNIT V-D-Forensic Odontology

5.19 HISTORY

Forensic odontology has roots as far as back as the 1st century A.D. however; these stories are hardly to be remembered as the significant developmental moments of the field. The year 1453 was the first formally recorded and reported dental identification of a deceased soldier. Nonetheless, it took another 323 years before the first dental identification was made by a qualified dentist, Paul Revere, and a further 73 years before, in 1849, dental identification was accepted as evidence for a criminal case in an American court of law. In 1878, the fire at the Vienna Opera House was the first mass disaster in which dental evidence was used to indentify the deceased. This incident was followed by the 1897 fire at the Bazar de La Charite in Paris where several of the 126 deceased were identified based on dental assessment and thus resulting in the first textbook on forensic dentistry (*LÊArt Dentaire en Medecine Legale*) by Dr. Oscar Amoedo, the father of forensic odontology. Since the establishment of the value of dental uses in the process of identification, the field of forensic odontology has greatly expanded. Its applications have been a crucial and vital tool in the large scale identification of the deceased from WWII, the Korean War, the Vietnam War, various mass disasters such as the Tsunami, as well as missing persons and the unidentified dead.

INTRODUCTION

Forensic odontology is the combined application of an experienced clinical dentist, who has observational, recording, gathering, preserving and interpretational skills and experience within a legal context. The scope of forensic odontology includes:

1. Identification of unknown individuals by the teeth, jaws, and cranio-facial bones;
2. Bite mark investigation;
3. Analysis of oro-facial trauma associated with physical abuse;
4. Dental jurisprudence, including expert witness testimony.

The forensic odontologistÊs skill is the ability of conducting a dental examination and comparison in order to correctly identify an unknown victim. This is possible from the form of teeth and the detail of their arrangement in the dental arches, which provide a body of information that is probably unique to the individual. Each tooth has five surfaces that can accommodate cavities and fillings. Any number of the 32 teeth can also be missing. This combination of missing, decaying and filling teeth is limitless. Even individuals without fillings or extractions will show characterization in the anatomy of their teeth and jaws. Furthermore, radiographic morphology of the frontal sinuses have also proved to be highly individual and are therefore sufficient to establish identity.

5.20 HUMAN DENTITION

The adult human dentition consists of 32 teeth arranged in two arches, one arch in the upper (maxillary) jaw and one in the lower (mandibular) jaw. Since the arches

are symmetrical, each quadrant contains the same number and type of teeth, as follows: 2 incisors, 1 canine, 2 premolars, and 3 molars. Incisors are the wide front teeth with a flat, thin biting edge. The canine is at the corner of the arch and has a pointed cusp. Each premolar typically has two cusps. The molars have between 3 to 5 cusps and a wide biting surface for chewing and crushing food.
Each tooth has a crown and a root. The crown is the visible portion, above the gums while the root is embedded into a socket in the jawbone. Incisors and canines have one root while premolars have 1 or 2 and molars have 2 to 3 roots.

5.21 TYPES OF PHYSICAL EVIDENCE AND CASES

Forensic odontology is required in cases where deaths are unexpected or unnatural, away from home (and thus cannot be verified by friends and relatives), where the recovery of corpses has been delayed, and/or undergone physically destructive forces. This is magnified in situations such as war and mass disasters however, even well preserved bodies that may be visually identifiable need to be identified by dental means when there is no one who recognizes the body and there are no suspects.

Under the following conditions, forensic odontology has proved most useful:

1. Decomposing remains
2. Skeletonised remains
3. Charred remains
4. Intact and well preserved remains where there is no putative victim: identifying John/Jane Doe
5. Multiple bodies recovered from a common location: asserting correct identification
6. Mass disasters
7. Bite marks
8. Verification of identity for purposes such as insurance settlement, homicide etc.

Additionally, Forensic Odontologists are trained in identifying and thus potentially determining:

1. *Age*: tooth development is in a regular, sequential manner until the age of 15 and offers more precise estimates than any anthropological measurements.
2. *Sex*: sexual dimorphism is hard to determine however, Anderson noted in 74% of the evaluated cases that when the mandibular canine diameter is less than 6.7 mm it is indicative of a female while a male measures greater than 7 mm.
3. *Ethnicity*: Not reliable although certain characters can be indicative attributes of a certain race e.g. CarabelliÊs cusp, an additional cusp found exclusively on the maxillary first molar, is most likely to belong to a Caucasian individual.

Occupation

4. *Habits*
5. *Socio-economic status*

These additional factors can aid and narrow the search for a victim or corroborate a proposed victim.

5.22 METHODS OF ANALYSIS

The processes of dental identification are as follows:

1. Preparation

The forensic odontologist should be informed of the case details and its nature before he/she attempts their examination so that they are fully prepared for the particular case with the appropriate equipment.

2. Postmortem Examination

The forensic odontologist will chart all present, missing, replaced, filled teeth, characteristics of the gum tissue, teeth, jaw and any unusual and pathological conditions. X-rays and photographs where possible are vital.
Skeletonised remains:

Easiest to examine, photograph and x-ray as they are portable, detached, and dry.

Decomposing remains:

Difficult to examine *in situ* and therefore removal of the jaw is generally agreed to be the best method to ensure an accurate and complete examination. In general, if a face is deformed or decomposed to the point where is cannot be restored for viewing at the funeral, the jaw can be removed, making the examination more easy.

Charred remains:

The most difficult to examine, as access to dentition becomes tedious due to thermally damaged skin which is hard, dry, and contracted. Teeth are easily lost from burnt bone. The temperature of the fire under which the body was subjected has various effects on the teeth and the possible ante-mortem dental work of the individual. Features such as shrinkage, melting of gold caps, evaporation of mercury from amalgam fillings, general brittleness needs to be considered and dealt with appropriately. The dentition will be first recorded *in situ* before the removal of the jaw in case of shattering during the removal procedure.

Mutilated remains:

Badly mutilated bodies are difficult to examine, primarily because the conditions of the bodies can vary greatly from decent to completely deformed by the impact. Often bones are broken, teeth are lost and the broken jawbones are unrecovered.

Viewable remains:

Removal of the jaws in this case is contradictory, and therefore the common practice is to examine the dentition *in situ*. The internal pterygoid and masseter muscles can be severed without perforating the skin to allow for further jaw opening.

3. Locating Ante-mortem Records

Securing ante-mortem records of an individual requires a putative name and the

suspects relatives/friends that can provide the name of a dentist who can further supply a record. Individuals, who have had a military physical examination, are part of the military or prisoners also have a permanent dental record on file. Additionally any removable dentures, prostheses should also be examined for engraved identification information, which can help deduce a dentist. Finally, personal photographs of the victim that show them smiling can also serve as an ante-mortem dental record.

4. Comparison

Discrepancies when comparing ante-mortem and postmortem records are expected. Changes due to the passing of time, typically changes such as, the loss of fillings or tooth removal since the last dental visit are commonplace. Spatial differences are another common occurrence on ante and postmortem x-rays because they were made at different angulations. Incompatible discrepancies include the postmortem presence of a tooth that is absent in the ante-mortem x-rays or chart or a cavity less tooth present in the postmortem that shows a filling ante-mortem. X-rays are preferred to written records as they display numerous visual details, free of human error and can show enough individual characterization to identify uniqueness.

5. Conclusion

A positive dental identification is established when the ante-mortem and postmortem data is unique and there are none incompatible inconsistencies.

5.23 MASS DISASTERS

A very difficult challenge for the forensic odontologist as mass disasters such as earthquakes, hurricanes, forest fires, explosions, bombs, and airplane crashes cause substantial dismemberment and alteration of dentition. Additionally, victims tend to be from several countries or in the case of a strictly local disaster; various widespread localities therefore acquiring ante-mortem dental and medical data can pose difficult. In such cases, the infrastructure of the mass disaster management team is clearly designated by profession and no deviations are tolerated in order to maintain maximum efficiency and helpfulness to those who need the care.

5.24 BITE MARKS

Forensic odontologists are also additionally asked to examine people, both dead and alive, and/or objects, which have retained a bite mark. Bite marks are commonly seen in cases of child abuse, sexual assault, homicide, domestic violence and battery. Occasionally bite marks are also inflicted by animals, most commonly: dogs, cats and sharks. Bite marks are significant because like fingerprints, dentition is unique even in cases of identical twins. Bite marks on human skin range from an excellent silhouette of the dental arches and individual teeth to something very diffuse and indistinct; the latter being unfortunately more common. Human skin is a poor recording surface because of its elasticity, deformability, capacity to heal and the bodyÊs curved surface. Furthermore, movement while biting and time of bite all add to the difficulty of bite mark analysis. Bite mark analysis is threefold: recognition of the bite mark; accurate documentation of the bite mark and; comparison to the teeth of an alleged suspect.

During the examination, the bite mark is swabbed twice with a sterile swab, which is analysed for traces of saliva and DNA, individually. The bite mark is then photographed under various exposure, light and colour (b/w & colour). Impressions are also made of the bite mark using standard dental techniques. Additionally the bite mark area is stained (chemical reactions) for traces of saliva, microorganisms, and calculus or tartar presence. Bite mark analysis is a tedious job as the effort is aimed at recording and replicating the bite mark so that it can be reproduced at the correct size and shape for comparison.

GLOSSARY

1. Hair
2. Fibers
3. Diatoms
4. Medulla
5. Wood
6. Pollen
7. Craniometry
8. Forensic entomology
9. Bone
10. Cartilage
11. Charred remains
12. Bit marks

LEARNING OUTCOME

After studying this unit you should be able to:

1. Identify the species by hair examination.
2. Understand the importance of physical evidences like hair, fibers, diatoms, plant materials, insects & maggots, skeletal remains, teeth, skull and etc.
3. Collection and preservation of such various physical evidences.
4. Physical features of wood.
5. Bone analysis – Differentiating sex.
6. Different dental identification processes.

SUGGESTED READINGS

1. Forensic Biology – Dr. R. Krishnamurthy.
2. Forensic Biology – Richard Li.
3. Water – Related Death Investigation – Erica. J. Armstrong.
4. Essential Forensic Biology – Alan Gunn.
5. Anthropology and Forensic Science: The Current Dynamism – Anup Kumar Kapoor.
6. Forensic Anthropology – Christensen.
7. A Laboratory Manual on Biological Anthropology – I. P. Singh.
8. Age Estimation in the Living – Sue Black & Dr. Anil Aggrawal.
9. Anthropology The Human Challenge – William. A. Haviland.
10. Forensic Archaeology – W. J. Mike.
11. Molecules of Murder, Criminal Molecules and Classic Cases – John Emsley