U. P. RAJARSHI TANDON
OPEN UNIVERSITY

Certificate Course in Forensic Science

CFS-02

FORENSIC BIOLOGY, SEROLOGY, CHEMISTRY AND TOXICOLOGY

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FORENSIC BIOLOGY & SEROLOGY

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FORENSIC CHEMISTRY AND TOXICOLOGY

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FORENSIC BIOLOGY & SEROLOGY

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UNIT - 9 : BIOLOGICAL EVIDENCE

Structure

9.0 INTRODUCTION

Forensic biology is the application of biological analysis methods, particularly serological methods, to legal investigations. Serology involves the investigation of bodily fluids, particularly the likes of blood, semen, saliva, all of which are commonly found at certain crime scenes. There are numerous types of bodily fluid that may be found at a crime scene or on a victim, all of which have the potential to be analysed and used in the identification and incrimination of the perpetrator. The examination of such substances can not only provide clues as to the identity of the offender, but also help investigators develop a detailed picture of the sequence of events which occurred. The presence of certain bodily fluids can be excellent indicators of what has occurred. For example, the presence of semen may suggest a recent sexual encounter, whether consensual or otherwise. Perhaps more obviously, blood at a crime scene is often indicative of some form of physical struggle, assault, or even murder. The analysis of bodily fluids may also determine the presence of quantities of certain substances in the body, such as alcohol or toxins. More information on this is available on the Bodily fluids can be divided into two categories: excreted fluids and secreted fluids. Excreted fluids that may be found at a crime scene include faeces, vomit, bile, and sebum (skin oil). Secreted fluids include blood, plasma, semen, saliva, female ejaculate, and urine. When a potential bodily fluid is first discovered at a crime scene, actions may be required to visualise the stain. Some biological samples are difficult to see with the naked eye, and require particular light or chemical additions to reveal their presence. Presumptive tests may be conducted to give some indication as to the identity of the substance, though these tests are by no means conclusive, and further analysis will be essential. The sample must be then collected and stored appropriately so as to preserve its integrity as best as possible. Wet samples will often be swabbed, with the swab then being placed in a vial or other airtight container. Individual samples should obviously all be stored separately to prevent contamination. All biological samples are generally dried or frozen during transport and storage. If the samples are to be dried, they should be left to dry by air without the addition of heat, as heat can be damaging to such specimens. These extensive measures are taken to not only protect the samples for analysis, but also protect the staff handling the samples from biohazards, such as infection from a biological sample. The sample will then be transported to a laboratory so that the analysis can be conducted. The primary goal of this analysis will be to establish exactly what the sample is. Though the answer may seem obvious from the appearance of the sample, conclusive tests should always be conducted. The substance should also be subjected to species-specific tests, as the biological sample may belong to another animal rather than a human. After the completion of such confirmatory tests, DNA analysis may be conducted to attempt to identify the secretor of the sample. A biological sample may not always contain sufficient DNA to obtain a DNA profile. Individuals may be known as secretors or non-secretors. Secretors present aspects of their blood's protein in other bodily fluids,

whereas non-secretors will not have sufficient levels of protein in their bodily fluid to establish a match between two samples.

9.1 OBJECTIVES

1. Understanding application of biological evidences in legal investigations.
2. Assessing different biological evidences like hair, fibers, anthropological evidence, botanical evidences, blood, DNA, entomological evidence
3. Collection and preservation of biological evidence for further lab analysis.

9.2. IMPORTANCE AND NATURE OF BIOLOGICAL EVIDENCE

Forensic biology is the application of biological evidences and principles in legal investigations. Forensic biological evidences conform to different spheres of biological sciences and a wide array of evidences

- Hair**:** To ascertain whether the hair is naturally fallen, forcibly removed. Origin of species from hair, gender determination, site determination and individualization and comparison of crime scene sample with known. This can aid in exclusion, inclusion and individualization
- Fibers: Identification and comparison of all types of fibres, including wool. Since fibers are very commonly left at the crime scene due to locards principle of exchange. They can thus aid in linking the perpetrator to the crime scene.
- Anthropological evidence: Determination of origin, sex, age, height & identity etc. from skeletal remains, including teeth can be done. Also anthropometric comparison of human skull with photograph before finally attempting the superimposition technique for human identification.
- Forensic botanical samples like pollen and diatoms aid in determining the time since death, the exact location of crime, to determine whether the corpse's body has been shifted or not.
- Forensic entomological evidence for toxicological investigations, time since death, to determine whether the corpse's body has been shifted or not.
- Identification of species of origin of fresh blood and bloodstains, ABO grouping of blood and other body fluids.
- Enzyme and protein typing can be done using various body fluids which aids in exclusion
- DNA typing for individualization
- Forensic wildlife is a branch of forensic biology which deals with crime pertaining to the conserved and endangered flora and fauna species. The samples of the animal origin like hair, horn, tusks, claw etc form the basis of the wildlife investigations.

9.3. LOCATION OF BIOLOGICAL EVIDENCES

The location of biological evidences may vary widely. Biological evidences vary in nature and thus their location also offers a wide array. Blood may be present on the floor, weapon, victim/perpetrator's body, glass, clothing etc. Similarly hair may also be present on clothing, carpet etc. In case of sexual assaults biological fluids like seminal fluid, blood, saliva are common evidences on or near the victim's body. Bite marks are another significant evidences in such a case. Saliva may also be found on cutlery items, food items, glass and so on. In case of outdoor scenes pollen presents one of the most untapped information. Both pollen and entomological evidence are

found on and around victim's body, shoes, soil and vehicle tyres commonly contain the pollen evidence. In case of drowning diatoms are the only evidence which can affirm whether it is postmortem or ante mortem drowning. They are present in the bone marrow of the victim only in case of ante mortem drowning. In mass disaster cases skeletal remains may be found at the scene.

9.4. COLLECTION AND EVALUATION OF BIOLOGICAL EVIDENCE

Liquid Blood Specimens at Crime Scenes

Liquid blood should be collected with a clean (preferably sterile) syringe or disposable

pipette and transferred to a clean (preferably sterile) test tube. A blood clot can be transferred to a clean test tube with a clean spatula. A clean cotton cloth can be used to soak up liquid blood or a blood clot (avoiding areas containing only serum). Wet blood samples, if they are collected, must be preserved in a suitable anticoagulant and kept in a refrigerator. These specimens should be submitted to the laboratory as soon as possible. Label the specimens with case number, item number, date, time, location, and evidence collector's name.

Wet Bloodstains

Small objects bearing wet bloodstains should be allowed to air dry, then collected as it is. An effort should be made to preserve the integrity of any bloodstain patterns during packaging and transportation. Large objects that cannot be removed from a crime scene may have wet bloodstains on them. The wet blood should be transferred onto clean cotton cloth. Bloodstained cotton cloth must be allowed to air dry before packaging in a paper container. Each object and container must be properly labelled.

Dried Bloodstains

Dried bloodstains on weapons, garments and other movable objects should be collected

separately by collecting the entire item. Each item should be placed in its own (paper) container, and these should be sealed and labeled properly. Similar procedure is followed for other body fluids.

Seminal Evidence from Sexual Assault Victims

When dealing with sex crimes, the victim should be taken to the hospital immediately and the examination started as soon as possible. Photographs should be taken to document any injuries which the victim received. If necessary, oral, vaginal, and/or anal swabs should be taken from the victim and air dried for one hour in a moving air source as soon as possible. They should be collected as soon as possible because the body begins breaking down the various components in seminal fluid through drainage, enzyme activity, pH, etc. The swabs should be air dried under a fan for at least one hour.

Tissue, Organ or Bone

Each item of evidence should be described in notes, and documented by photography, sketches or videotaping. This type of evidence item can be picked up with a clean pair of forceps. Each item should be placed in a clean container without any added fixatives.

Each container should be properly sealed and labeled, and stored in a freezer. Evidence should be submitted to the laboratory as soon as possible.

When collecting reference samples from postmortem subjects, if the body has decomposed, in addition to the blood sample, collect as many of the following specimens as possible: a portion of deep muscle tissue, certain organ tissue (e.g. heart or brain/not liver or kidney), 2-4 intact molar

teeth (if identification is an issue, ensure that mouth x-rays have been taken), and a sample of compact bone (e.g. femur). The specimens collected should be away from site of injury (i.e. if head injury, do not take sample of brain tissue). Immediately freeze specimens, do not place in any preservative (e.g. formalin).

Hair Evidence

If a root sheath is attached, then DNA analysis using PCR technology can say that this hair came from a certain percentage of the population to which the suspect belongs. If there is no root sheath, then a microscopic analysis can say that the hair has the same characteristics as the suspect's hair and is similar to his or her hair.

Hair found at the scene should be placed in a paper packet and then placed in an envelope. If a microscopic examination is required, then 15-20 representative hairs from the suspect must be submitted to the lab for comparison.

CHECK YOUR PROGRESS

I. Which of the following are branches of forensic biology

1) Forensic serology
2) Forensic anthropology
3) Forensic botany
4) All of these

II. An anticoagulant is must for the preservation of

1) Hair
2) Blood
3) Flora and Fauna
4) Urine

III. All flora and fauna samples can be preserved in

1) Formalin
2) EDTA
3) Moist containers
4) All of these

IV. DNA can be analyzed from which biological sample

1) Blood
2) Bone
3) Hair with root
4) All of these

Answers

(I) 4 (II) 2 (III) 1 (IV) 4

9.5 SUMMARY

Forensic biology is the application of biology to law enforcement. It includes the sub-disciplines of Forensic anthropology, Forensic botany, Forensic entomology, Forensic odontology and various DNA or protein based Forensic biologists analyze bodily fluids, blood, hair, insects, and plant and animal remains that are recovered from a crime scene. Evidence found on weapons, clothing, and other surfaces is obtained and analyzed by forensic biologists using a variety of chemical, enzymatic, and microscopic analysis techniques. The information a forensic biologist collects can help reconstruct a crime, or investigate public health threats or environmental contamination.

Forensic Biologists work both in the lab and out in the field. While in the field, forensic biologists can be found collecting samples from leaves, clothing, animal remains, and other biological material. Sifting through dirt, mud, or garbage may be required in order to obtain all possible evidence. This part of the career can be unpleasant, filled with foul smells and messy situations. While in the lab, microscopes and other pieces of equipment are used to analyze the collected evidence. The evidence is photographed, cataloged, and then tested.

UNIT-10 : HAIR EXAMINATION

Structure

10.0 INTRODUCTION

Hair is commonly found physical evidence owing to its transferability. Two mechanisms of hair transfer exist: direct transfer in which hairs direct from the original source are transferred and indirect transfers which involve one or more intermediaries. In addition to its ubiquitous nature, hair has two other properties that make it a frequently occurring form of physical evidence. First, unlike some other biological evidence, hair is remarkably stable to most environmental conditions and will not easily break down. Secondly since it is relatively unnoticeable to the untrained eye, a criminal is not likely to make an effort to destroy the evidence. Interchange of hairs between the victim and suspect's clothing in crimes such as rape, assault, and murder is common. Hairs from a suspect may be left at the scene of crime such as burglaries, armed robberies, and car theftsetc..Hair evidence can provide a link in the criminal investigation or aid in narrowing down the investigation by various ways:

- Whether the hair sample is of animal or human origin.
- If animal, the genus, family, or species
- If human, the race and body area may be determined
- Indications of forcible removal, damage, and alteration
  - - Comparison with standard (known) samples
- Gender can be determined (only if root or tissue sheath present)
  - - DNA typing from hair can allow in identification.

10.1 OBJECTIVES

- To understand the significance of hair evidence in legal investigation.

- Collection and preservation of hair samples
- To extract scientific clues from hair samples for inclusion and exclusion.

10.2. STRUCTURE OF HAIR

The hair is the major component of hair follicle comprising of long thin cylinder of keratinized cells and usually has three distinct components.

-A central **medulla** or core running along the central axis
-The main component, the **cortex**
-The **cuticle**, the outer covering

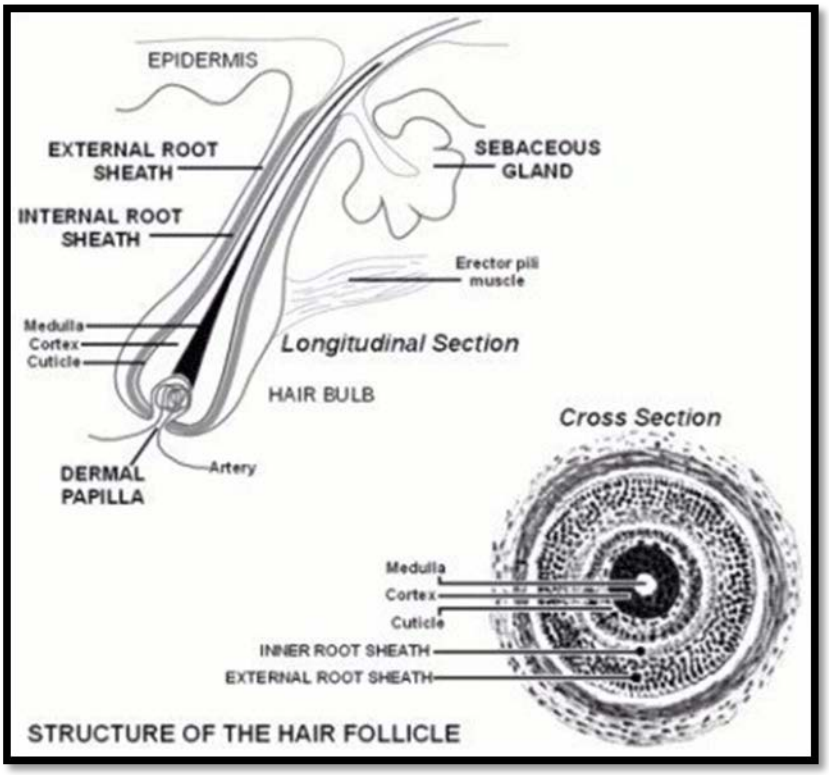


Fig 1: Structure of human hair follicle (source: Wikipedia.com)

10.2.1 Medulla

In human hair, the medulla can be continuous throughout the length of the hair (except for the tip and the root), or discontinuous. When discontinuous the medulla may be broken transversely at irregular intervals by cortical material (interrupted medulla) or it may only be present irregularly in very small amounts in the cortex (fragmented medulla). The human hair medulla is not very large; it may only be one or two cells in diameter. In non-human hairs, it occupies a large proportion of the hair shaft.

10.2.2 Cortex

The cells are aligned parallel to the axis of the hair fiber and are cemented together via intercellular contacts. In human hair there tends to be more granules towards the periphery of the cortex than towards the center.

10.2.3 Cuticle

The outer layer of the hair shaft is called the cuticle. It is composed of flattened, imbricated scale cells. The cells overlap both longitudinally and laterally. They slope outwards, their edges pointing towards the tip. Cuticle cells form a pattern, which can be visualized microscopically on the hair surface and is called the hair scale pattern. The pattern differs from species to species and can be used for species identification.

10.3. GROWTH AND REPLACEMENT OF HAIR

The hair cycle is divided into three main stages. These stages are called the

- Anagen (the growing phase) a period of high metabolic and mitotic activity. Hair plucked at the anagen stage, exhibits an attached sheath of tissue. Because the root and sheath tissues are mitotically active, it enables enzyme typing, gender determination and DNA typing
- Catagen (the transition period between growing and resting) and
- Telogen (the resting phase): lasts for three to four months for human scalp hair. Frequently, mature hairs are displaced at the end of telogen by emerging new hair of the next anagen phase. Hairs at the telogen stage form the majority of the ‘naturally shed’ hairs. Hair at the telogen phase will not exhibit an attached root sheath. This differentiates it from the hair at the anagen phase and thus it can be ascertained whether a hair found at the crime scene is naturally fallen or is the outcome of a struggle.

  This growth pattern is described as ‘scattered mosaic’. On a normal scalp about 90 per cent of the follicles are in anagen and 10 per cent in telogen at any one time.

10.4 COLLECTION AND PRESERVATION OF HAIR EVIDENCE

Hair can be readily transferred from one person to another, especially in a physical altercation. Hair is also randomly dropped from the body as a natural process. Hair is a biological specimen of the body and may be associated back to its source through DNAanalysis (although DNA is not always successfully extracted from hair).

10.4.1 Hair collection

- Tape lifting: By placing a clear cellulose tape in contact with the area in question hair can be recovered. This is particularly suitable for dark colored and rough textured items. After lifting

the sample place the cellulose tape bearing the sample between two layers of transparent plastic (eg: acetate document protector) for preservation.

- Vacuuming: This is done under moist circumstances but is not recommended because it causes contamination of the sample.
- Individual collection of single hairs as they are located. This can be aided using oblique lighting at different angles and tweezers to lift the hair samples.

10.4.2. Preservation

- All hair samples should be air-dried and stored under moderate conditions, preferably within a sealed polyethylene bag.
- Hair sample may also be stored in a container or placed in a paper fold and place this container or fold in an envelope. This envelope should be individually identified, sealed, initialed and then submitted to the laboratory.
- If clothing worn by a victim or a suspect is thought to contain hairs or fibers, the garments should be removed from the person over paper to catch any loose hairs that may fall. The clothing should be initialed and placed in a bag, sealed, and identified. Each item should be packaged separately.
- Standards or known hair samples must be collected to compare with the crime scene sample. Each standard should consist of 25 hand-pulled hairs representing all obvious hair types in that particular hair region. Standards should be collected within a week of alleged event (preferably the same day), since hair can be significantly altered in an intervening period. Standards should be of the similar length as that of the suspect (crime scene) samples . Place hairs in a paper fold, and then place the paper fold in envelope.
- It should be kept in mind that the hair standard is complete with the root and in the anagen phase.
- Hair evidence can be further evaluated for comparisons using DNA techniques. Hair that do not have a root, or the root does not have sufficient tissue associated with it may be analyzed using mitochondrial DNA analysis.

10.5. IDENTIFICATION FROM HAIR

Identification involves a pattern of sequential steps. It is essential to first determine whether the hair is of human or animal origin. Then the site of origin is determined. Following this racial determination can be carried out with the aid of different macroscopic and microscopic traits. Standard and the questioned sample are then compared using different traits like length, diameter, pigment distribution, texture, color protein typing, enzyme typing etc. Gender can also be determined from hair sample using fluorescent staining methods. DNA typing is the only method to carry out individualization and personnel identification from hair.

10.5.1. Human vs Non-human hair

Feature	Human	Non Human
Color	Relatively consistent along shaft	Often showing profound color changes and banding
Cortex	Occupying most of the width of shaft. Greater than medulla	Usually less than width of medulla

Pigment distribution	Even. Slightly more towards cuticle	Central or denser towards medulla
Medulla	Less than one-third width of shaft. Amorphous, mostly not continuous when present	Greater than one-third width of shaft. Continuous, often varying in appearance along shaft. Defined structure
Scales	Imbricate and similar along shaft from root to tip	Often showing variation in structure from root to tip

Table I: Distinguishing features of animal and human hair

10.5.2 Macroscopic Features of Human Hair

In the forensic examination of hairs it is important to start with visual examination followed by macroscopic examination of the morphology of individual hairs. A full examination at magnifications of X10 to X40 and up to X60 –X100 with incident illumination enables the observer to record the overall shape of the hair, whether or not a root is present, site of origin of hair, its shape and appearance, appearance of the terminal portion, disease or extraneous features.

- **Hair Length**
  The length of both known (from an identified person) and recovered (questioned) hairs should invariably be recorded. With a known sample the length of a number of hairs should be measured. Length should be measured where possible by straightening the hair. This can be difficult in practice with tightly curled hair of negroid origin. Where only a fragment of hair shaft is recovered the evidential value decreases because the overall variation from root to tip cannot be ascertained. While collecting standard hair from scalp or any other region, a number of hair should be collected from different portions of the region. Also if the questioned or crime scene sample belongs to a site of injury 9in the scalp or elsewhere), the standard should be collected from the same site.
- **Hair Shape and hair color**
  It may be classified as being straight, wavy, curly and peppercorn. Hair shape must always be recorded. It assists in determining site of origin of hair and the race. Hair color is the result of the type of pigment and aids in racial determination.
- **Root Appearance**
  The microscopic appearance of the roots of hairs in anagen and telogen growth phases is characteristic. Anagen mostly presents as a flattened ribbon like structure often with pigmentation present. Catagen roots are usually club shaped and sometimes have an epithelial sac attached. Anagen hairs do not fall out in the normal course of events and require some use of force to detach them from the dermal papilla. Anagen has a cellular sheath material attached. The presence or absence of this sheath material indicates the degree of force with which it has been pulled out. Telogen hair have clubbed roots.

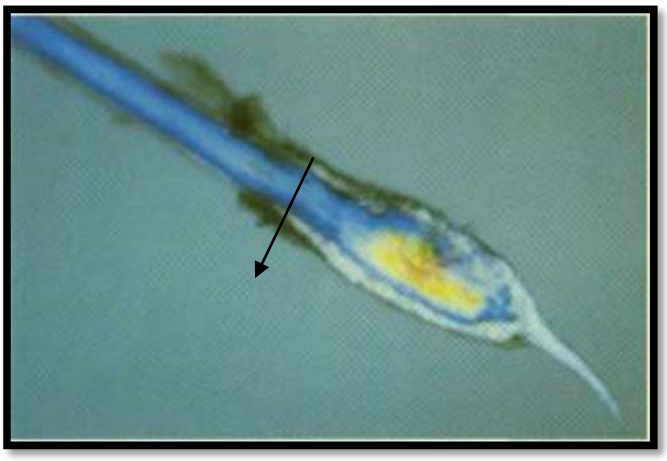


Fig IV: Plucked anagen hair with tissue sheath attached

In forensic case works, hairs from dead bodies are generally examined. The root end displays a number of postmortem changes three to four days after death. Typically these include narrowing of the hair shaft immediately behind the root, internal cell disruption. A typical atrophied hair may show yellow coloration near the root and a peculiar scale shape. These changes aid in determining the time since death.

- **Tip Appearance**
  In a newly formed hair the tip end tapers as it emerges from the scalp. It usually has low pigment density and generally is non medullated. Tip appearance can be classified as natural taper, round and frayed, splits, cut and singed.

10.5.3. Microscopic Features of Human Hair

- **Hair Diameter**
  It is one of the most significant criteria for racial determination.
- **Pigment Features**
  Pigment characteristics can be compared between the questioned sample and the standard for pigment color, distribution, density and pigment shape. It may range from absent to heavy density. Distribution may be uniform , peripheral, one sided, random, central or pigment in cuticle. Pigment shape is round or oval and the size varies from large to small. The color ranges from red, brown to black. This qualitative assessment allows the comparison of hair samples.
- **Scale Count**
  Another means of comparison between sample and questioned hair is the scale count. It is the measure of number of scales over a unit area. But it is still not universally accepted. To avoid the errors a minimum of 100 counts are taken.

10.5.4 Race Determination from Hair

There persist characteristic distinguishing features in hair of different racial origin.

Caucasians	Negroid	Mongoloid
Shaft diameter is moderate with minimum variation.	Shaft diameter is moderate to fine	Shaft diameter is coarse and usually with little or no

		variation
Pigment granules sparse to moderately dense with fairly even distribution	Pigment granules densely distributed and arranged in prominent clumps	Pigment densely distributed and arranged in clumps or streaks
Granules oval in shape	Flattened cross sectional shape	Round cross sectional shape
	Shaft with prominent twist and curl	Cuticle thick
0.71 hair index (maximum diameter/minimum diameter)	0.60 hair index	0.83 hair index

Table II: Distinguishing characteristics of hair for race determination

CHECK YOUR PROGRESS

I) The scale pattern of human hair is:

1. Coronal
2. Imbricate
3. Mosaic
4. Spinous

II) For collection of hair samples from dark colored surfaces …… is the most suitable method

1. Tape Lift
2. Individual hair collection
3. Vacuum method
4. Washing method

III) Medulla is always present in human hair

1. True
2. False

IV) At any time 90 percent of the hair belong to

1. Anagen phase
2. Telogen Phase
3. Catagen phase
4. None of these

V) Pigment granules are present in which part of hair shaft

1. Cuticle
2. Cortex
3. Medulla
4. Hair follicle

VI) 0.71 is the approximate value of hair index for

1. Caucasians
2. Mongoloids
3. Asian Indians
4. Negroids

Answers

(1): 2 (II): 1 (III): 2 (IV): 1 (V): 2 (VI): 1

10.6 SUMMARY

Forensic scientists perform three major types of hair analysis. Chemical assays are used to assess the use of illegal drugs, to screen for the presence of heavy metals in the body, and to test for nutritional deficiencies. The root of the hair has cells that contain **DNA**, which can be used for DNA analyses. Microscopic comparison of hair collected from two different places is used to determine if the hairs are from the same person or animal.

Because hair can be moved from location to location by physical contact, the presence of a specific person's hair can link a suspect or a victim to a crime scene. If hair is transferred directly from the region of the body from which it originates, it is considered a primary transfer. Approximately 100 head hairs are lost per person per day. These hairs usually end up on clothing, furniture, or on other items in the environment. Transfer of hair from these items is called secondary transfer. Secondary transfer of hair is very common with animal hairs, which are commonly found on pet owners and in the environment of pet owners and can be used to link suspects to crime scenes.

Hair grows out of living cells in epidermis of mammals. It is almost entirely made up of the protein keratin. The club-shaped hair root is anchored in a follicle, which has associated muscles, called *arrector pili*. When these muscles contract, hair becomes oriented nearly perpendicular to the skin. The hair itself is composed of three layers: the medulla, the cortex, and the cuticle. The medulla is the innermost canal that extends through the hair. In humans it can be continuous or discontinuous, interrupted by a series of empty spaces. Surrounding the medulla is the cortex, which makes up the majority of the mass of the hair. The outermost layer is the cuticle, which is a single layer of scales. In humans these scales overlap quite a bit and cling tightly to the cortex.

Pigments are found in both the cortex and the medulla, but they are absent from the cuticle. In humans, the pigments tend to be distributed toward the outer edges of the cortex, but this can vary depending on ethnicity. In human hair, the medulla is generally narrow, taking up less than a third of the diameter of the entire shaft. In hairs from animals, the diameter of the medulla is larger than half the diameter of the entire shaft. The cross section of human hair is most often circular, but occasionally oval.

Using morphologic features, forensic scientists classify six different types of hair on the human body: head hair, eyebrow and eyelash hair, beard and moustache hair, body hair, pubic hair, and axillary hair. Biochemical studies show that there are no significant differences in chemical structure among the hair types. Animals also produce different types of hair. They often have coarse guard hair external to softer fur hairs. They also produce whiskers and longer hairs in such places as the tail and mane.

In humans, hair undergoes cyclical phases of growth (anagen), transition (catagen), and resting (telogen). During the growth phase, the cells of the follicle actively divide and grow upward. The

average anagen phase lasts about 1,000 days. During the telogen phase, the cells of the follicle are dormant and hairs naturally fall out. This phase usually lasts for 100 days. At any time, between 10 and 18% of all the hairs on a human head are in the telogen phase; about 2% are in the catagen phase and the rest, between 80 and 90% are actively growing. There is no pattern to determine which hairs on the head are in any phase at a given time.

Because hair grows out of follicles in the skin, materials in the body are incorporated into the hair. Hair grows relatively slowly, so it takes several weeks for materials in the body to be reflected in the composition of the hair. Hair that is collected for the presence of drugs, heavy metals, and nutritional insufficiencies is usually clipped from the nape of the neck

UNIT - 11 : FORENSIC ENTOMOLOGY

Structure

11.0 INTRODUCTION

By looking at the presence and life cycle of insects, forensic entomologist can determine time of death, cause of death, and if the corpse has been tampered with. There are three fields of entomology: medico legal, urban, and stored-product. Medico legal entomology is where the entomologist examines the presence and development of arthropods to help in legal cases such as murder and contraband trafficking. Arthropods are vital to the Earth's environment they are everywhere and they are an intricate part of the decomposition process of biological materials. In the urban aspect, forensic entomologists examine how insects affect people in their immediate environments (i.e. bug bites, spreading of disease, etc.). Finally in the aspect of stored-product entomology, forensic entomologists examine pests and insects that are found in foods and may assist in criminal and/or civil cases having to do with food contamination. Forensic Entomology is the use of the insects, and their arthropod relatives that inhabit decomposing remains to aid legal investigations. The medicolegal section focuses on the criminal component of the legal system and deals with the necrophagous (or carrion) feeding insects that typically infest human remains. The urban aspect deals with the insects that affect man and his immediate environment. This area has both criminal and civil components as urban pests may feed on both the living and the dead. The damage caused by their mandibles (or mouthparts) as they feed can produce markings and wounds on the skin that may be misinterpreted as prior abuse. Urban pests are of great economic importance and the forensic entomologist may become involved in civil proceedings over monetary damages.
Lastly, stored product insects are commonly found in foodstuffs and the forensic entomologist may serve as an expert witness during both criminal and civil proceedings involving food contamination. The forensic entomologist can use a number of different techniques including species succession, larval weight, larval length, and a more technical method known as the accumulated degree hour technique which can be very precise if the necessary data is available. A qualified forensic entomologist can also make inferences as to possible postmortem movement of a corpse. Some flies prefer specific habitats such as a distinct preference for laying their eggs in an outdoor or indoor environment. Flies can also exhibit preferences for carcasses in shade or sunlit conditions of the outdoor environment. Therefore, a corpse that is recovered indoors with the eggs or larvae of flies that typically inhabit sunny outdoor locations would indicate that someone returned to the scene of the crime to move and attempt to conceal the body.

Similarly, freezing or wrapping of the body may be indicated by an altered species succession of insects on the body. Anything that may have prevented the insects from laying eggs in their normal time frame will alter both the sequence of species and their typical colonization time. This alteration of the normal insect succession and fauna should be noticeable to the forensic entomologists if they are familiar with what would normally be recovered from a body in a particular environmental habitat or geographical location. The complete absence of insects would suggest clues as to the sequence of postmortem events as the body was probably either frozen, sealed in a tightly closed container, or buried very deeply.

Entomological evidence can also help determine the circumstances of abuse and rape. Victims that are incapacitated (bound, drugged, or otherwise helpless) often have associated fecal and urine soaked clothes or bed dressings. Such material will attract certain species of flies that otherwise would not be recovered. Their presence can yield many clues to both antemortem and postmortem circumstances of the crime. Currently, it is now possible to use DNA technology not only to help determine insect species, but to recover and identify the blood meals taken by blood feeding insects. The DNA of human blood can be recovered from the digestive tract of an insect that has fed on an individual. The presence of their DNA within the insect can place suspects at a known location within a definable period of time and recovery of the victims' blood can also create a link between perpetrator and suspect.

The insects recovered from decomposing human remains can be a valuable tool for toxicological analysis. The voracious appetite of the insects on corpses can quickly skeletonize the remains. In a short period of time the fluids (blood and urine) and soft tissues needed for toxicological analysis disappear. However, it is possible to recover the insect larvae and run standard toxicological analyses on them as you would human tissue. Toxicological analysis can be successful on insect larvae because their tissues assimilate drugs and toxins that accumulated in human tissue prior to death.

11.1 OBJECTIVES

- To study the role of insects in legal investigation
- To use entomological evidence for establishing time since death, cause of death and location of death.
- Collection and preservation of entomological evidence for further lab analysis

11.2 INSECTS OF FORENSIC IMPORTANCE

There are many types of insects that can be involved in forensic entomology, but the ones listed here are mostly necrophagous (corpse-eating) and related to medico legal entomology (directly related to the crime and found on the corpse).

- Flies are often first to appear on the scene. They prefer a moist corpse for the maggots to feed on.

- Others, like beetles are generally found on the corpse when it is more decomposed. In drier conditions.
- Moth flies can replace the beetles. Many mites feed on a corpse. Macrocheles mites are common in the early stages of decomposition, while Tyroglyphidae and Oribatidae mites feed on dry skin in the later stages of decomposition.
- Moths feed on mammalian hair during their larval stages and may forage on any hair that remains. They are amongst the final animals contributing to the decomposition of a corpse.
- Wasps, ants, and bees feed on the body during the early stages. This may cause problems for murder cases in which larval flies are used to estimate the post mortem interval since eggs and larvae on the body may have been consumed prior to the arrival on scene of investigators.

11.3 COLLECTION OF ENTOMOLOGICAL EVIDENCE DURING DEATH INVESTIGATIONS

Entomological investigation of the death scene can be broken down into the following steps:
Observations of the scene should note the general habitat and location of the body in reference to vegetation, sun or shade conditions, and its proximity to any open doors or windows if recovered within a structure. Locations of insect infestations on the body should be documented as well as noting what stages of insects are observed (such as eggs, larvae, pupae, or adults).

Collection of meteorological data at the scene

Such data should include:

a). Ambient air temperature at the scene taken approximately at chest height with the thermometer in the shade.
b). Maggot mass temperature (obtained by placing the thermometer directly into the larval mass center).
c). Ground surface temperature.
d). Temperature at the interface of the body and ground (simply place the thermometer between the two surfaces).
e). Temperature of the soil directly under the body (taken immediately after body removal).
f). Weather data that includes the maximum and minimum daily temperature and rainfall for a period spanning 1-2 weeks before the victims disappearance to 3-5 days after the body was discovered. Such information can be gathered by contacting the nearest national weather service office, or your state climatologist.

Collection of insects from the body at the scene

The first insects that should be collected are the adult flies and beetles. These insects are fast moving and can leave the crime scene rapidly once disturbed. The adult flies can be trapped with an insect net available from most biological supply houses. They are inexpensive and readily obtainable. Once the adult flies have been netted, the closed end of the net (with the insects inside) can be placed in the mouth of a "killing jar" (which is a glass container with cotton balls or plaster

soaked with ethyl acetate, or acetone). The jar is then capped and the insects will be immobilized within a few minutes. Once they are immobile they can be easily transferred to a vial of 75% ethyl alcohol. Beetles can be collected with forceps or gloved fingers and placed directly into 75% ethyl alcohol. It is extremely important that the collected specimens are properly labeled. Labels should be made with a dark graphite pencil. The collection label should contain the following information:

1). Geographical Location
2). Date and hour of collection
3). Case number
4). Location on the body where removed
5). Name of collector

Collection of insects from scene after body removal

Many of the insects that inhabit a corpse will remain on, or buried, in the ground after the body has been removed. The steps listed above should be followed when collecting insects from the soil (i.e. both a preserved and a living sample should be taken). Soil and litter samples should also be taken both immediately under where the body was positioned, and from the immediate surroundings. It is not necessary to dig deeply. A good technique is to collect the leaf litter and debris down to the exposed upper surface of the soil, and then make a separate collection from about the first two or three inches of topsoil. Each soil collection area should be about 4-6 inches square, and be taken from underneath the head, torso and extremities. All soil samples should be placed in a cardboard container for immediate shipment to a forensic entomologist

11.4 LIFE CYCLE OF AN INSECT

This is essential to determine the stage of insect life and estimate the time since death

- Egg: On fresh corpses, look for these clumps in the mouth, in nasal openings, in the ears, and generally in any area where mucus membranes come into contact with the outside air. Also look for them on wounds and bruises.
- Larva: Larvae or maggots hatch from the eggs. They too are white but are conical shaped The mouth is at the pointed end. The maggot uses a pair of "hooks" there to attach itself to the corpse while it feeds. Maggots also use the hooks to help themselves move. Normally they move by extensions and contractions of their segmented, legless body.

Fig1: Blow fly maggot on microscope slide.
(source: http://users.usachoice.net/~swb/forensics/BF.html

- Puparium:. The third time a maggot "sheds its skin" a remarkable thing happens. The skin contracts to a capsule-like form and becomes rigid and hardened. It is not actually shed, but remains covering the newly molted insect inside. You can see that the puparial skin is the same as old maggot skin because it retains the outlines of the spiracles and other sclerotized parts of that stage. The living insect that is inside the puparium is pale white, cannot move or feed, and has rudimentary legs and wings, antennae, etc. This mummy-like form is called a pupa. The hardened skin surrounding it is called a ***puparium*** (plural ***puparia***).

Fig 2: Flesh fly (family Sarcophagidae) next to hollow, capsule-like puparium from which it emerged. "Cap" of puparium is on left.
(Source:http://users.usachoice.net/~swb/forensics/BF.html)

- Adult: The puparium is furnished with a cap-like lid that can be popped off by the emerging fly. The newly emerged flies are at first pale in color, soft to the touch, and with crumpled, unexpanded wings. They later expand their wings and turn green or blue.

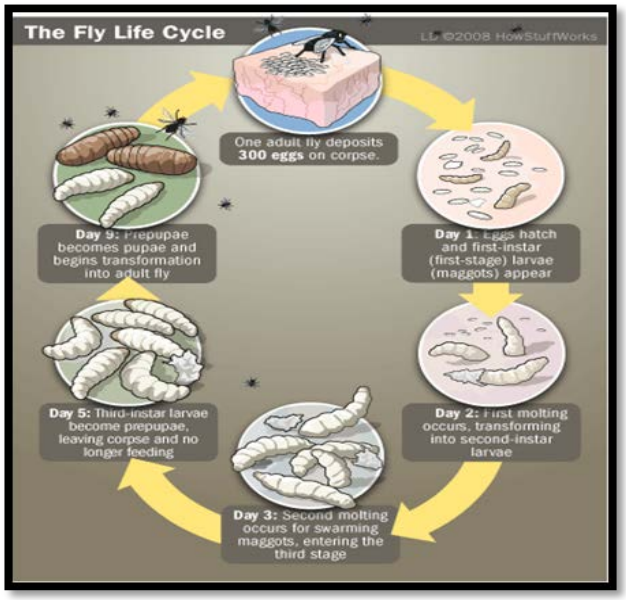


Figure3: Depicting life cycle of a fly (Source: crimemuseum.org)

Blow flies do not fly much for a day or two while their body is hardening Adult flies will be very obvious on most corpses. Fully mature adults are worthless as forensic indicators because of their mobility.

Check your progress

(I) The entomological sample should be stored in

1) Distilled water
2) Moist container
3) Ethyl acetate
4) Paper bags

(II) On the second day of death which insect may be present on the corpse's body

1) Cockroach
2) Mites
3) Blow Flies
4) Beetles

(III) Forensic entomology can aid in

1) Estimation of time since death
2) Determine if the dead body has been moved from original place
3) Toxicological analysis from larvae etc
4) All of these

(IV) The order in which insects feed on the dead body is known as

1) Fauna succession
2) Flora succession
3) Insect attack
4) Entomology

(V) Which is the least significant stage of insect life cycle for forensic investigations

1) Larva
2) Egg
3) Puparium
4) Adult Fly

Answers

(I) 3 (II) 3 (III) 4 (IV) 1 (V) 4

11.5. SUMMARY

Forensic entomology is legal application of the science of entomology. Entomology is the study of insects, often including spiders and other arthropods. Forensics encompasses both civil and criminal cases. In certain cases of suspicious death, the length of time that insects have colonised remains is useful to police investigations in helping to determine time of death. It is the mandate of the Provincial Coroners Office to determine time of death but, through pathology, they are unable to scientifically estimate beyond a certain postmortem period. In such cases, insects may become evidence and assist in determining time of death. This is based on the length of time that certain insect species, often species of blow fly, have colonised the remains. Blow flies are the insects of primary significance because the time they begin to colonise remains is often approximately consistent with the time of death**.** The first and most important stage of the procedure involved in forensic entomology involves careful and accurate collection of insect evidence at the scene. This involves a knowledge of the insects behaviour, therefore it is best performed by an entomologist

UNIT-12 : BLOOD AND BLOOD GROUPS

Structure

12.0. INTRODUCTION

The most fluid portion of blood consists of plasma, which is mostly water, and serum, which is yellowish and contains white cells and platelets. The most non-fluid portion of blood consists of red cells which outnumber white cells by five hundred to one. While medical scientists are more interested in white cells, forensic scientists are more interested in red cells and secondly with serum. With serum, the analyst can determine the freshness of a blood sample because serum clots several minutes after exposure to air (a centrifuge is necessary to separate clotted material from the rest of serum).

In serum are also found antibodies, which have important forensic implications. With red cells, the analyst looks for smaller substances residing on their surfaces, such as antigens, which have important forensic implications. One might even say that forensic serology is all about antigens and antibodies, but that is the domain of immunology.

In forensic law, blood has always been considered class evidence, but the potential exists for individualized blood typing, and even today, forensic serologists can provide testimony with some strong probability estimates linking a single individual, and that individual only, to a bloodstain. Consider that identical twins may have the same DNA profile but completely different antibody profiles, and you begin to see how promising the field of forensic serology really is.

The typing of blood, with what is now called the A-B-O system, was discovered in 1901. A few years later, starting around 1937, a series of antigen-antibody reactions were discovered in blood, the most common ones being ABH, MN, Rh, and Gm (over 100 antigens exist). Most people are only familiar with the Rh factor, which is technically the D antigen. There are more than 256 antigens, and 23 blood group systems based on association with these antigens.

A basic principle of serology is that for every antigen, there exists a specific antibody. For routine blood typing, all you need are two antiserums: anti-A and anti-B, both easily available commercially. By dripping a droplet of these antiserums in samples of blood, you see which samples maintain a normal appearance (at about 200x magnification) and which samples become clotted, or agglutinated.

Blood of type A will be agglutinated by anti-A serum; blood of type B will be agglutinated by anti-B serum; AB blood by both; and O blood by neither. You are essentially determining blood type by injecting the worst possible poison into someone's blood sample to see what happens.

The "O" type is most common among indigenous people (like Aborigines and Native Americans) and Latin Americans. The "A" type is most common among Caucasians and those of European descent. The "B" type is most common among African-Americans and certain Asians (e.g. Thai). The "AB" type is most common among the Japanese and certain Asians (e.g. Chinese).

Rare blood types exist in addition to the basic ABO system. A far more useful breakdown involves the Rh (Rhesus disease) factor. If a person has a positive Rh factor, this means that their blood contains a protein that is also found in Rhesus monkeys. Most people (about 85%) have a positive Rh factor, and doctors are trained to monitor closely any woman who is Rh negative and becomes pregnant.

The Rh system is actually much more complicated than the ABO system because there are about 30 combinations possible, but for the sake of simplicity, Rh is usually expressed as either positive or negative. The Rh factor, like other antigens, is found on the covering of red blood cells. It's common for a forensic scientist to take the percentage distribution of the Rh component, which is expressed as plus or minus.

Subgrouping is also possible under the ABO system. Various extracts can be obtained from plants and seeds to create antiserums that clot type O blood, for example, somewhat selectively. Most major blood groups have at least two major subgroups; O1, O2, A1, A2, etc.

The most commonly used types of antiserums used for this purpose are called lectins. The possibility of individualized blood types is based on the typing of proteins and enzymes. Forensic serologists almost always do this level of typing. Blood proteins and enzymes have the characteristic of being polymorphisms or iso-enzymes, which means they exist in several forms and variants, so each one of them have subtypes. Most people are familiar with at least one common polymorphism in blood: Hb, which causes sickle-cell anemia.

Wet blood has more value than dried blood because more tests can be run. For example, alcohol and drug content can be determined from wet blood only. Blood begins to dry after 3-5 minutes of exposure to air. As it dries, it changes color towards brown and black. Blood at the crime scene can be in the form of pools, drops, smears, or crusts.

Pools of blood obviously have more evidentiary value in obtaining a wet sample. Drops of blood tell the height and angle from which the blood fell. The forensic science of blood spatter analysis says that blood which fell perpendicular to the floor from a distance of 0-2 feet would make a circular drop with slightly frayed edges. Drops from a higher distance would have more pronounced tendrils fraying off the edges (a sunburst pattern).

A blood smear on the wall or floor tells the direction of force of the blow. The direction of force is always in the direction towards the tail, or smaller end, of the smear, or splatter. In other words, the largest area of the smear is the point of origin (a wave cast-off pattern). Blood crusts need to be tested with crystalline methods to make sure it's blood. Refrigerated red blood cells have a shelf life of about 42 days, and the serum containing white blood cells can be refrigerated much longer, almost up to a year.

DNA can be extracted from blood (if white blood cells which always contain a nucleus are present), and also from sperm, bone marrow, tooth pulp, and hair roots. Blood, however, is commonly used in DNA testing, as per the following steps:

1. Blood samples are collected from the victim, defendant, and crime scene
2. White blood cells are separated from red blood cells
3. DNA is extracted from the nuclei of white blood cells
4. A restrictive enzyme is used to cut fragments of the DNA strand
5. DNA fragments are put into a bed of gel with electrodes at either end
6. Electric current sorts DNA fragments by length
7. An absorbent blotter soaks up the imprint; it is radioactively treated, and an X-ray photograph (called an autoradiograph) is produced.

12.1 OBJECTIVES

- Understanding the role of blood, other biological fluids and DNA in legal investigations.
- To enumerate the types of cases encountering blood and body fluids as evidence
- Collection, preservation and analysis of blood, other body fluids and DNA.
- Information which can be extracted from these sources for inclusion, exclusion and personnel identification

12.2. BLOOD AND BLOOD GROUPS

There are four principal types: A, B, AB, and O. There are two antigens and two antibodies that are mostly responsible for the ABO types. The specific combination of these four components determines an individual's type in most cases.

ABO Blood Type	Antigen A	Antigen B	Antibody anti-A	Antibody Anti-B
A	Yes	No	No	Yes
B	No	Yes	Yes	No
O	No	No	Yes	Yes
AB	Yes	Yes	No	No

Individuals with type O blood do not produce ABO antigens. Therefore, their blood normally will not be rejected when it is given to others with different ABO types. As a result, type O people are universal donors for transfusions, but they can receive only type O blood themselves. Those who have type AB blood do not make any ABO antibodies. Their blood does not discriminate against any other ABO type. Consequently, they are universal receivers for transfusions, but their blood will be agglutinated when given to people with every other type because they produce both kinds of antigens.

It is easy and inexpensive to determine an individual's ABO type from a few drops of blood. A serum containing anti-A antibodies is mixed with some of the blood. Another serum with anti-B antibodies is mixed with the remaining sample. Whether or not agglutination occurs in either sample indicates the ABO type. It is a simple process of elimination of the possibilities. For instance, if an individual's blood sample is agglutinated by the anti-A antibody, but not the anti-B antibody, it means that the A antigen is present but not the B antigen. Therefore, the blood type is A.

12.2.1. Genetics of ABO blood group

ABO blood types are inherited through genes on chromosome 9, and they do not change as a result of environmental influences during life. An individual's ABO type is determined by the inheritance of 1 of 3 alleles (A, B, or O) from each parent. The possible outcomes are shown below:

Table 1: Alleles for ABO blood grouping

Parent Alleles	**A**	**B**	**O**
A	AA (A)	AB (AB)	AO (A)
B	AB (AB)	BB (B)	BO (B)
O	AO (A)	BO (B)	OO (O)

The possible ABO alleles for one parent are in the top row and the alleles of the other are in the left column. Offspring genotypes are shown in black. Phenotypes are in bracket

Both A and B alleles are dominant over O. As a result, individuals who have an AO genotype will have an A phenotype. People who are type O have OO genotypes. In other words, they inherited a recessive O allele from both parents. The A and B alleles are co dominant. Therefore, if an A is inherited from one parent and a B from the other, the phenotype will be AB. Agglutination tests will show that these individuals have the characteristics of both type A and type B blood.

ABO Blood type antigens are not only found on the surface of red cells. They are also normally secreted by some people in their body fluids, including saliva, tears, and urine. Whether someone is able to secrete them is genetically controlled. Police agencies now routinely use this so-called secretor system data to identify potential victims and criminals when blood samples are not available.

Antibodies to alien antigens in the ABO group are usually present in our plasma prior to the first contact with blood of a different ABO type. This may be partly explained by the fact that these antigens are also produced by certain bacteria and possibly some plants. When we come in contact with them, our bodies may develop long-term active immunity to their antigens and subsequently to the same antigens on the surface of red blood cells. This usually occurs to babies within the first six months following their birth.

The four basic ABO phenotypes are O, A, B, and AB. After it was found that blood group A RBCs reacted differently to a particular antibody (later called anti-A1), the blood group was divided into two phenotypes, A_1 and A_2. RBCs with the A_1 phenotype react with anti-A1 and make up about 80% of blood type A. RBCs with the A_2 phenotype do not react with anti-A1 and they make up about 20% of blood type A. A_1 red cells express about 5 times more A antigen than A_2 red cells, but both types of red cell react with anti-A, and as far as transfusion purposes are concerned, the A_1 and A_2 blood groups are interchangeable. There are many other subgroups of blood group A in which RBCs tend to weakly express the A antigen, whereas weak variants of the blood group B phenotype are rare.

12.2.2 Rh system

A far more useful breakdown involves the Rh (Rhesus disease) factor. If a person has a positive Rh factor, this means that their blood contains a protein that is also found in Rhesus monkeys. Most people (about 85%) have a positive Rh factor, and doctors are trained to monitor closely any woman who is Rh negative and becomes pregnant. Rh is usually expressed as either positive or negative. The Rh factor, like other antigens, is found on the covering of red blood cells. If a woman is pregnant and is Rh (-) and the foetus is Rh (+) , as inherited from the father then in the placenta antibodies will be produced against the foetal blood and cause its haemolysis. This is known as the haemolytic disease of the new born.

12.3. FORENSIC CHARACTERIZATION OF BLOODSTAINS

It is easy to determine the blood group in fresh samples. But in forensic caseworks we often come across dried bloodstains. These bloodstains are analyzed to link the perpetrator to the crime. Various parameters are studied using dried bloodstains and it is essential to proceed in a scientific sequential manner. These steps are:

1. Is the sample blood?
2. Is the sample animal blood?
3. If animal blood, from what species?
4. If human blood, what type and Identification via DNA extraction

12.3.1 Chemical methods used to detect blood

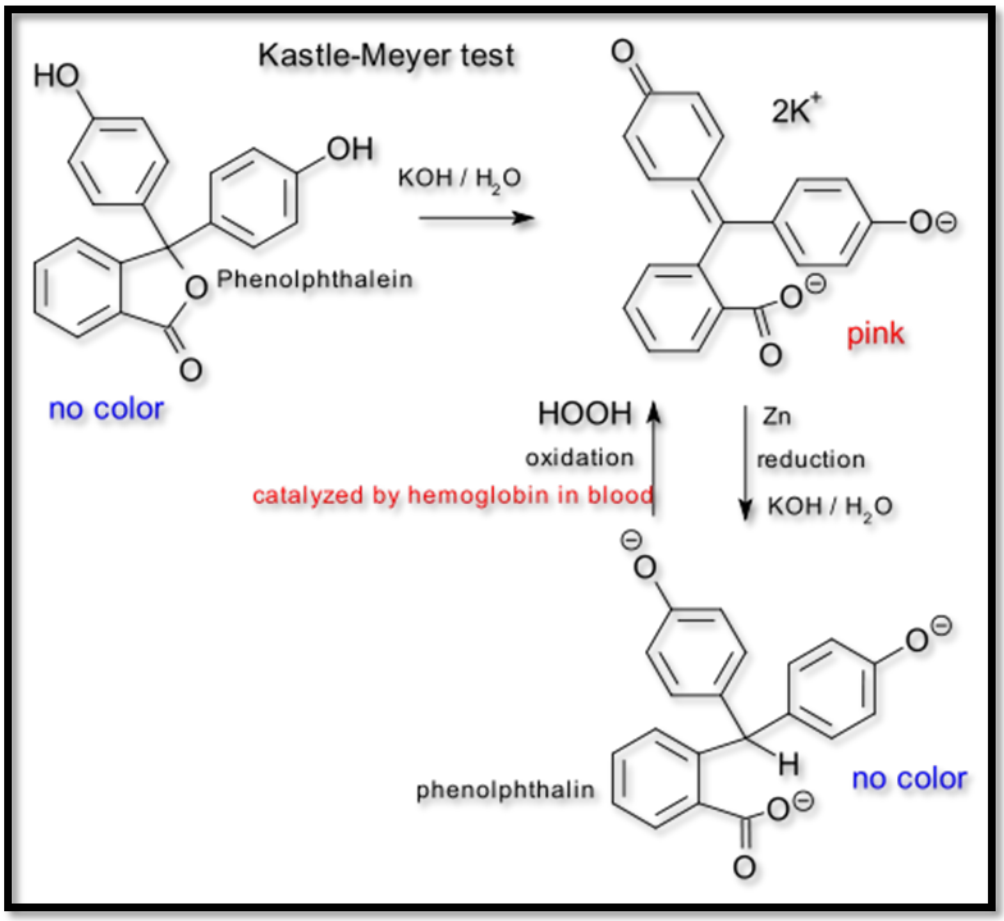
Catalytic tests /Preliminary tests

These methods depend on the fact that the haem group of hemoglobin possesses a peroxidase-like activity which catalyses the breakdown of hydrogen peroxide. The oxidizing species formed in this reaction can then react with a variety of substrates to produce a visible color change. Among substrates in common use are benzidine and various substituted benzidines, ortho-tolidine, leucomalachite green, leucocrystal violet and phenolphthalein -the last of these being known as the Kastle-Meyer test. The catalytic tests are extremely sensitive (blood can be detected to dilutions of about 1 in 100,000), but are subject to a number of interferences and are therefore not totally specific for blood. Substances, which can interfere, include enzymes such as catalase and peroxidases (which can occur in both plant and animal materials), oxidizing chemicals and metals – in particular copper and iron. There has to be an awareness of this when results are interpreted, particularly when testing outdoors, where many types of plant material can be present, or testing in vehicles, where metal surfaces can interfere. The general principle is that if the test is negative, blood is absent, but that if the test is positive, blood is probably, not definitely present. For this reason the tests are often described as "presumptive" tests. Benziedine tests have been abandoned now due to their carcinogenic property.

Kastle Meyers test

In the Kastle-Meyer test the reduced phenolphthalein is kept in alkaline solution in the presence of zinc. This solution is colorless. Oxidation with hemoglobin and peroxide causes an instant color change to the well known bright pink.

In the original form, a small amount of the Kastle-Meyer reagent as prepared is mixed with equal volumes of 95% ethanol and 10% hydrogen peroxide solution. The suspect stain is rubbed gently with a small piece of filter paper and a drop of the mixed reagent added to the paper. The development of a pink color is indicative of the presence of hemoglobin, which has catalyzed the breakdown of hydrogen peroxide to an oxidizing species. However, used in this form, the test will give an apparently positive result with other oxidizing materials. In the 2-step version of the test, the Kastle-Meyer reagent is mixed only with an equal volume of 95% ethanol. This solution is first added to the stain on the filter paper. If a pink or red color develops at this point, that is without the addition of hydrogen peroxide, the stain in question is not blood. If there is no reaction at this point, a drop of hydrogen peroxide solution is added, and the presence of a pink color indicates the likely presence of blood.

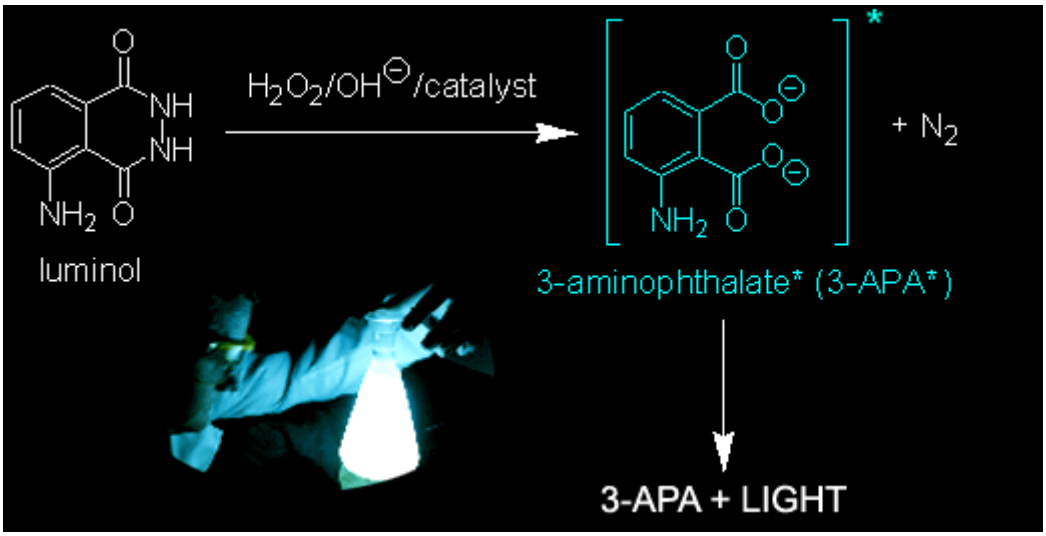


Kastle –Meyer's test

Luminol's test: Detection of non-visible bloodstains.

This has traditionally been carried out using luminol. Luminol's major application is in areas where blood may be present but is difficult to see, such as outdoors among vegetation, or where attempts have been made to clean up blood and traces are still present. A positive reaction can also sometimes

be given by bloodstained clothing, which has been washed. Luminol is made up in alkaline solution (pH 10.4-10.8) using sodium carbonate, and sodium perborate (NaBO3.H2O) rather than hydrogen peroxide is used as the source of the oxidizing species. Hydrogen peroxide can be used but yields a shorter-lived luminescence than sodium perborate. The solution is applied as a spray and the presence of blood produces a bluish luminescence, which persists for about 45 seconds. The luminescence can be restored by additional spraying but this needs to be done carefully as the stain will lose definition if too much liquid is added to it.



Luminol's test (Source : wavesignal.com)

Crystal tests/ Confirmatory tests

S.No.	Test	Reagent	Proedure	Result
1	**Teichmann test/ Haematin test**	Potassium chloride – 0.1gm Glacial acetic acid – 100ml	-Place suspected blood crust or stain on microscopic slide and cover with coverslip -Add the prepared reagent and let it flow from the sides of the cover slip -Warm the slide at 65degree Celsius for 10-20 seconds -Allow to cool and observe under microscope	Brown rhombedron shaped crystals of ferriprotoporphyrin chloride shows that haeme group is present.
2	**Haemochromogen or the Takayama test**	Saturated glucose solution-3ml	-Procedure is same as the teichmann test.	Pink needle shaped crystals of haemochromogens

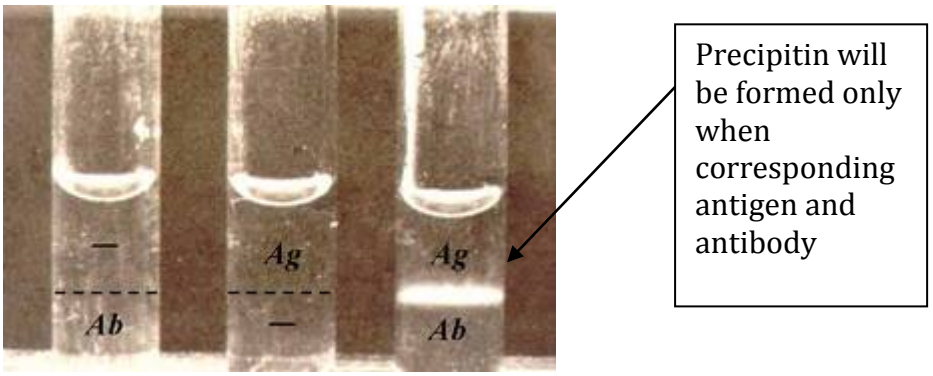
		Sodium Hydroxide (10%)-3ml Pyridine -3ml Distilled water -7ml	- The sample is placed on the glass slide and covered with a cover slip . Subsequently the prepared solution is added from the sides of the cover slip. - After heating at 65 degree Celsius for 10-20 seconds it is cooled down and observed under the microscope.	(pyridine ferroprotoporphyrin) show that the haeme group/blood is present.

12.3.2 Determination of species from blood

This can be done using various methods employing the principle that along with sample, we place anti human, ant-dog, anti frog etcetera serum. The antibodies in the serum will have affinity towards the corresponding animal antigen.

Precipitin Tube Method

Take six (can vary on the number of antisera used) precipitin tubes and place them vertically in a precipitin tube stand and label. Put a drop of the bloodstain/tissue extract in the tubes. Carefully add one drop of antiserum for species origin (anti Human serum, anti Fowl serum, anti Dog serum, anti Cow Serum, anti Goat serum etc.) along the walls of tube and leave undisturbed for 30 minutes at room temperature. Examine for a white ring at the interface of two solutions.



Precipitin test positive in third tube from right (source:jeeves.mmg.uci.edu)

Double Diffusion

Both of the reactants, antigen and antibody diffuse towards each other in gel; and when an antigen combines with its specific antibody at optimum proportions, a precipitin arc forms. Weigh 100 mg of Agar (or Agarose) and put in a beaker containing 10 ml of normal saline. Heat the solution while

shaking. When the Agar gets dissolved completely, pour it over in a properly leveled disposable Petridis or glass slide to make a 1-2mm thick agar layer. Wait until the agar solidifies. Punch wells in gel, each about 5 mm apart, in a hexagonal way. Seal the bottoms of punched wells with dilute agar (0.5%). Fill the central well with tissue extract and peripheral wells with different antisera for species origin or vice versa (anti Human serum, anti Fowl serum, anti Dog serum, anti Cow Serum, anti Goat serum etc.). Cover the petridish and keep gel in a moist chamber for overnight. Examine gel for the presence of precipitin arcs.

12.3.3 Blood grouping from dried blood stains

The normal agglutination method does not give any result with dried bloodstains. For determining the blood group from dried stains absorption elution techniques can be employed. This technique begins with the antiserum being placed on the blood stained material, allowing sufficient time for the antibodies to combine with their specific antigens. Next, the unreacted serum is removed by being washed off the blood stained material. Once an antibody combines with an antigen, it is possible to break the complex apart by a process known as elution. To do this, the stained material must be heated at 56 degrees Celsius. This allows the antibody-antigen bond to break, freeing both. When the eluted antibodies are combined with known red blood cells so that the presence or absence of a compound can be observed, they can be identified. In other words we are first reacting all anti A, anti B and anti H (for O group) with the samples in different wells. Depending on the blood group of the sample, the antigen ill combine with the corresponding antibody. Now when we wash it the unbound antibodies will be washed away leaving only the bound antigen-antibody. On heating this bond will also break up. Now we add known blood samples (A, B and O) to the wells. The antibody which remained after washing (which is the antibody corresponding to the blood group) will bound to its corresponding antigen now provided by known cells and exhibit clumping or agglutination. Thus if agglutination is seen with A the blood group of test sample was A, similarly for B. If agglutination with both then the blood group is AB and if with non then the blood group is O.

12.4. BLOOD SPATTER ANALYSIS

Bloodstain pattern analysis involves the scientific study of the static consequences resulting from dynamic blood shedding events. A detailed study of bloodstain patterns at crime scenes often develops invaluable evidence. The distribution, size and shape of bloodstains on a victim, on a suspect, or on the walls, floors, ceilings, or on objects at the scene can help reconstruct these blood shedding events. Bloodstain pattern analysis can also help one evaluate the credibility of statements provided by a witness, a victim, or a suspect.

12.4.1. Characteristics of blood

In order to understand bloodstain evidence, one must understand some basic scientific principles.

1. When blood leaves the body as a drop, as spatter, or as a gushing flow from an artery, its behavior conforms to the laws of physics. Understanding blood flows involves understanding the physical forces involved in blood shedding events.

2. Viscosity is a form of internal friction in fluids. It results from frictional forces generated between layers of the fluid as they flow past each other. Viscosity, then, is a measure of a fluids resistance to change in shape or flow. Red blood cells concentrate sialic acid on their membranes producing a high negative electrical charge giving blood a higher viscosity than water.
3. But the blood does not maintain constant viscosity under shearing forces. Blood's viscosity decreases with increase in shearing forces.
4. Blood drops hold together in free flight through the forces of both cohesion and surface tension. Cohesion is an electrical force attracting like molecules to each other. Surface tension is a force resulting from a fluid's molecules achieving the most stable low-energy configuration by minimizing exposed areas of the fluid. This phenomenon results in increased cohesive forces at the liquid's surface, in turn producing "a skin" or "a membrane" of cohesive force. The forces of cohesion and surface tension cause blood drops to become and remain spherical once they fall free from their source. Cohesion and surface tension also cause the drops to resist breaking up even when striking targets such as floors, walls, body parts, or clothing.
5. Other forces affecting bloodstain patterns include adhesion and capillary action. When blood is drawn into any porous material it does so by capillary action. This force helps explain the concave meniscus in a test tube blood sample. Blood possesses adhesive properties .It is for this reason that blood readily binds to the substratum and is difficult to remove.

12.4.2 Blood Patterns

Spatter

1. Surface texture, not distance fallen, determines the degree of blood spatter.
    a. If the surface is smooth and clean, ruptures of the surface tension do not occur and the drop will not spatter.
    b. If the surface is rough, porous or has protruding fibers, the surface tension will rupture, causing the blood to spatter.
2. Blood striking an object at angles less than 90 degrees produces a tear drop shape. A 90-degree impact angle will yield a circular shape.
3. In tear-drop stains, the sharp end points in the direction of travel. This pointed end indicates the blood drops forward direction of travel prior to striking the surface.
4. When blood forcefully strikes a surface at sharp angles, a smaller droplet is cast-off from the larger parent drop much like a large breaking ocean wave issues smaller water volumes.
5. Generally, the higher the energy, the greater the division and the finer the spatter.

Blood transfer pattern

Transfer Pattern - occurs when a wet bloody surface contacts a second unstained surface creating recognizable mirror image or at least a recognizable portion of the original surface.

Examples of common transfer patterns:

1. Clothing patterns which reproduce fabric weaves
2. Shoe print and hand print transfers

Swipe Pattern - the transfer of blood onto a surface not already contaminated with blood. One side is usually feathered which indicates the direction of travel.
One common pattern at scenes is a hair swipe - a long thin fine line transfer

Wipe Pattern - created when an object moves through blood that has not completely dried and moves, removes, or otherwise alters it.
Smear Pattern - a large volume of blood, at least 0.5 ml, which has been distorted so much that further classification is not possible.

Smudge Patterns - another reference to a bloodstain that has been distorted to such a degree that further classification is not possible.

Cast off patterns

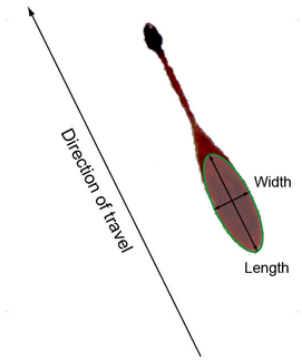
1. During a beating with an instrument, which produces the bleeding, blood will not normally collect on the surface of the instrument from the first strike.
2. On subsequent strikes at the same location, blood will adhere to the instrument since it now strikes a blood source. When the instrument is raised or swung backward, its movement allows small drops of blood to be released from its surface.
    a) Some of these small drops will strike a surface, often a ceiling, at a 90-degree impact angle.
    b) As the instrument continues to swing backward, the movement accelerates and additional blood droplets will be cast-off the surface of the instrument. These drops will approximate a liner pattern.
    c) The small drops will strike at increasing acute angles and become elongated.

Projected blood stains

Occur when a large volume of blood propels towards a striking surface. This type of pattern, also called arterial spurting, usually occurs when an artery is damaged and the blood spurts or gushes from the wound in large volume pulses. It continues spurting as long as the heart continues beating. Large drops striking a vertical surface decelerate from air resistance and produce a pattern without spines. The drops strike the surface and then characteristically drip or run downward due to their large volume. This blood projection could also be created when a force acts upon a quantity of blood of approximately 0.10 ml or greater. For example, this occurs commonly at scenes where someone has stepped or stomped into a pool of blood, projecting the blood away from the blood pool.

12.4.3 Determining angles of impact

As mentioned earlier a blood droplet in freefall has the shape of a sphere. An analyst can determine the angle at which this droplet struck the surface. This is based on the relationship between the length of the major axis, minor axis, and the angle of impact.



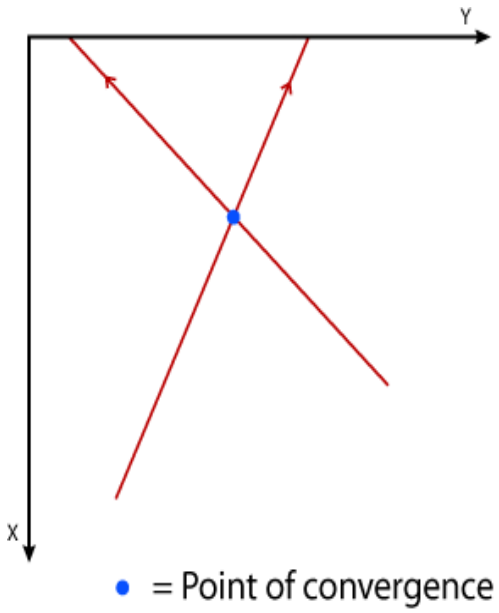
Upward moving bloodstain showing proper ellipse placement. (Source: Wikipedia.com)

12.4.4 Point of convergence

To determine the point/area of convergence an analyst has to determine the path the blood droplets travelled. The tangential flight path of individual droplets can be determined by using the angle of impact and the offset angle of the resulting bloodstain. “Stringing” stains is a method of visualizing this. For the purpose of the point of convergence, only the top view of the flight paths is required. Note that this is a two-dimensional (2D) and not a three-dimensional (3D) intersection.

The point of convergence is the intersection of two bloodstain paths, where the stains come from opposite sides of the impact pattern.

The area of convergence is the box formed by the intersection of several stains from opposite sides of the impact pattern.



Point of convergence (source:Wikipedia.com)

12.5. FORENSIC SIGNIFICANCE OF BODY FLUIDS

Body fluids traces recovered at crime scenes are among the most important types of evidence to forensic investigators. They contain valuable DNA evidence, which can identify a suspect or victim as well as exonerate an innocent individual. The first step of identifying a particular body fluid is highly important since the nature of the fluid is itself very informative to the investigation, and the destructive nature of a screening test must be considered when only a small amount of material is available. Driven by the importance for forensic applications, body fluid identification methods have been extensively developed in recent years. The systematic analysis of these new developments is vital for forensic investigators to be continuously educated on possible superior techniques. Significant advances in laser technology and the development of novel light detectors have dramatically improved spectroscopic methods for molecular characterization over the last decade. There are many different types of bodily fluid that are secreted by the body and are also present within the body at any given time. These fluids may be useful in helping forensic scientists and pathologists put together a detailed picture of how an individual died and likewise may also present means of identifying the perpetrator. Bodily fluids are broken down into two categories: excreted and secreted. Excreted are sweat, milk, cerumen (earwax), faeces (included because faeces are often covered in a mucus membrane to enable travelling through the bowel), chyme (found in the stomach), bile, vomit, aqueous humour (a watery substance that covers the eye), sebum (Skin Oil) and secreted are blood, plasma, semen, saliva, female ejaculate, Serum, or Urine. Seminal fluid is one of the most important biological evidence in case of rapes. They can be used for exclusion purposes and also to determine the number of individuals involved in the crime. Biological fluids thus are a source of reconstruction of crime scene since they are valuable circumstantial evidences. Fluids also aid in determining the secretor status (whether antigens presentin fluids apart from blood). They can be characterized to determine if any disease prevailing to the perpetrator. This can narrow down the investigation.

12.5.1 Semen and seminal stains Identification

Acid phosphatase test:

- Reagent preparations
  Buffer:
  Glacial acetic acid- 1ml
  Sodium acetate anhydrous-2g
  Distilled water- 100ml
- Reagent I:
  Buffer – 50 ml
  Sodium alpha-naphthyl phosphate (0.25%w/v)- 126mg
- Reagent II:
  Buffer- 50 ml
  Naphthanildiazoblue (0.5%)-250mg
  Add reagent I followed by reagent II on the sample. Purple color indicates the presence of semen. But this is not a confirmatory test. Since it gives false positive tests.
  Choline test (Florence crystal test)
- Reagent

Iodine -2.54 gm
Potassium iodide 1.65 gm
Distilled water -30 ml

Put the sample on a glass slide. Using a drop of water as a mounting medium put the cover slip. Add the reagent from sides. Observe under microscope. Brown colored needle shaped crystals will appear if semen present.
Seminal fluid can further be used for enzyme typing of lactate dehydrogenase using electrophoresis.

12.5.2 Saliva

A forensic investigation can involve the analysis of body fluids, including saliva, for evidence of toxins and both prescription and illicit drugs. Obtaining a saliva sample is far less obtrusive and cumbersome than obtaining a blood or urine sample, especially at the scene of an accident or crime. In humans, there are at least four variants (or versions) of alpha-amylase, two of which are found in saliva. Secretor status, DNA analysis and enzyme typing can be done to narrow down investigation

Phasbades test
In a test tube add sample. To it we add the phasbades tablet. Distilled water is added the contents are vortexed thoroughly. Incubate at 37 degree Celsius for half an hour and note the color change. Blue to violet coloration indicates the presence of saliva

12.5.3 Sweat

An excretion that occasionally can have importance as evidence and as a source for DNA typing and, rarely, toxicological analysis. Sweat is also the fundamental component of fingerprints and acts as a carrier for the materials that react with compounds used to visualize them. Sweat is an excretion of the pores and originates from the eccrine sweat glands of the skin. These pores are found on all skin surfaces, while sebaceous glands (oil glands) are found in much more limited areas. Pore location is part of the minutiae of fingerprints, and the study of pore location as a tool for identification is referred to as poroscopy. Sweat is a complex mixture of inorganic and organic materials dissolved or suspended in water, which makes up about 98 percent of the volume. The amino acids found in sweat are the compounds that react with ninhydrin to produce a purple color and visualize a latent fingerprint. Dissolved salts, sugars, and ammonia are also components of sweat. As a medium for toxicology, sweat can be used in some cases to detect drugs and metabolites and has the added advantage that it can be collected using noninvasive procedures. Apart from toxicological and DNA analyses, lipid and amino acid profile can be constructed which may aid in the exclusion.

12.5.4. Milk

Human milk contains 0.8% to 0.9% protein, 4.5% fat and 7.1% carbohydrates. Carbohydrates are mainly lactose; several lactose-based oligosaccharides have been identified as minor components. The principal proteins are casein (homologous to bovine beta-casein), alpha-lactalbumin, lactoferrin,

IgA, lysozyme, and serum albumin. Thus, protein and lipid profiling can be done. The milk of diabetic mothers has been shown to have a different composition from that of non-diabetic mothers.

12.5.5 Urine

Urine sample can aid in DNA analysis, toxicological inference and providing circumstantial evidence.

Urea nitrate crystal test
An aqueous extract of stain is made and a thin film made on a microscopic slide. Add one drop of concentrated nitric acid and cover. Hexagonal crystals of urea nitrate are formed.

12.6. SCIENTIFIC BASIS OF DNA TYPING

Any type of organism can be identified by examination of DNA sequences unique to that species. To identify individuals, forensic scientists scan 13 DNA regions, or loci, that vary from person to person and use the data to create a DNA profile of that individual (sometimes called a DNA fingerprint). There is an extremely small chance that another person has the same DNA profile for a particular set of 13 regions. DNA is the material within every cell of the body and represents the blueprint of life. It allows physical traits to be passed on from one generation to the next. Although the majority of the human genome (the complete set of genes for an individual) is the same across all ethnic populations, people differ in their genetic makeup by a minuscule amount, and thus have their own unique DNA pattern. DNA profiling, also referred to as DNA typing, is the molecular genetic analysis that identifies DNA patterns. In forensic science, DNA profiling is used to identify those who have committed a crime. It is estimated that roughly one percent of all criminal cases employ this technique; however, DNA profiling has been used to acquit several suspects involved in serious crimes such as rape and murder and it has been used to convict individuals of crimes years after investigators closed the unsolved case. Aside from identifying an individual responsible for violent crimes, the judicial system also can use DNA profiling to determine family relationships in the case of disputed paternity or for immigration cases.

DNA typing for forensics takes advantage of locations within the human genome that do not code for protein. These locations typically involve repetitive DNA sequences that are polymorphic, or have a variable number of repeat sizes. Which means it has many forms for example on a particular locus one individual may have a sequence AATG AATGAATG (3 repeat units) and the other may have AATG AATGAATGAATG (4 repeat units). It generally ranges from 2-7 because non-protein-coding DNA is used, Sometimes the profile may show a complex pattern like AATG AATG TATA TATA AATG. So this has 2 repeat units of AATG, two of TATA and again one of AATG. This type of a profile is seen in STR typing. As the number of loci studied increases the probability that two different individuals can have the same profile decreases. DNA databanks that contain DNA typing information do not reveal any information about an individual's health status or whether the individual has or is a carrier of a genetic disease.

The sensitivity of DNA profiling tests have dramatically increased over the last two decades. It used to be necessary to have a sample roughly the size of the ink in an ink pen, skilled forensic scientists can now obtain enough DNA from saliva left on the end of a cigarette to get a DNA profile result. The speed at which results can be obtained has also dramatically improved. This is all, in part, due to the discovery of the polymerase chain reaction, a technique that can amplify large amounts of specific small sequences of DNA from the human genome. It is also due to the advent of various DNA fingerprinting tools. The effect of these advances has broadened the sample size and quality required for analysis.

Uses of DNA for Forensic Identification

- Identify potential suspects whose DNA may match evidence left at crime scenes
- Exonerate persons wrongly accused of crimes
- Identify crime and catastrophe victims
- Establish paternity and other family relationships
- Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers)
- Mass disaster cases
- Missing person cases
- Detect bacteria and other organisms that may pollute air, water, soil, and food
- Match organ donors with recipients in transplant programs
- Determine pedigree for seed or livestock breeds
- Authenticate consumables such as cannabis, wine, wood etc.

DNA content of biological samples	
Liquid Blood	20,000-40,000 ng/mL
Blood Stain	250-500 ng/cm2
Liquid Semen	150,000-300,000 ng/mL
Post-coital vaginal swab	10-3,000 ng/swab
Hair (with root)	
Plucked hair	1-750 ng/root
Hair fallen	1-10 ng/root
Liquid saliva	1000-10000ng/ml
Oral swab	100-1500ng/swab
Urine	1-20ng/ml
Bone	3-10ng/mg
Tissue	50-500ng/mg

DNA content of different biological samples

**DNA content varies depending on environmental and storage conditions*

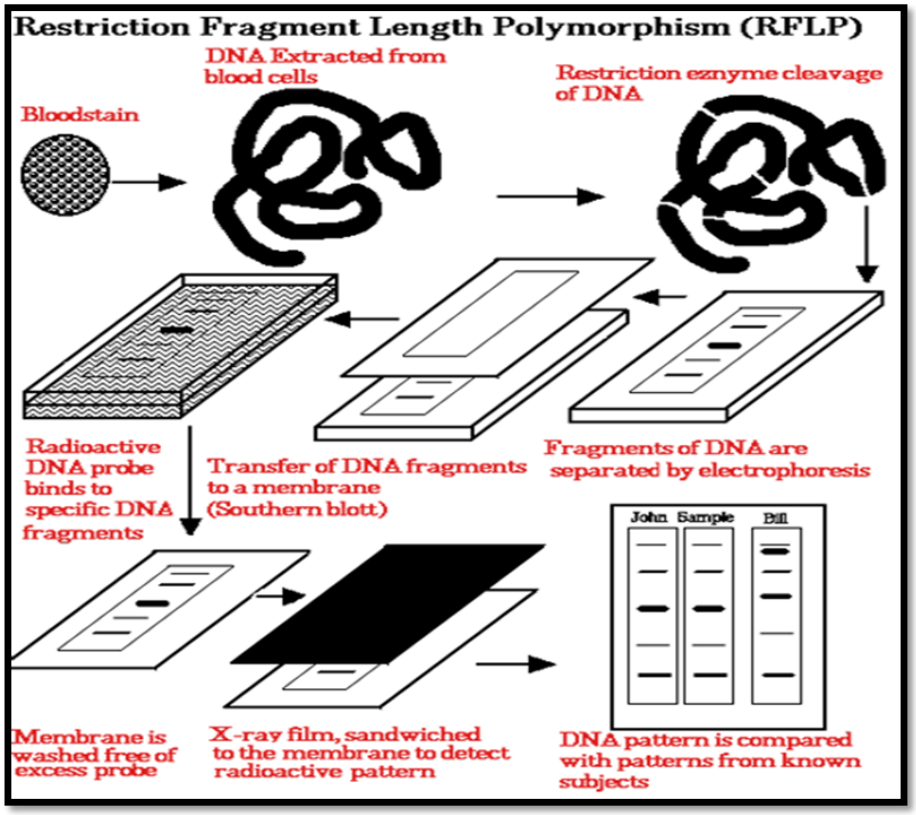
12.7. BASIC PROCEDURE AND TECHNIQUES

Only one-tenth of a single percent of DNA (about 3 million bases) differs from one person to the next. Scientists can use these variable regions to generate a DNA profile of an individual, using samples from blood, bone, hair, and other body tissues and products. In criminal cases, this generally involves obtaining samples from crime-scene evidence and a suspect, extracting the DNA, and analyzing it for the presence of a set of specific DNA regions (markers). Scientists find the markers in a DNA sample by designing small pieces of DNA (probes) that will each seek out and bind to a complementary DNA sequence in the sample. A series of probes bound to a DNA sample creates a distinctive pattern for an individual. Forensic scientists compare these DNA profiles to determine whether the suspect's sample matches the evidence sample

The more probes used in DNA analysis, the greater the odds for a unique pattern and against a coincidental match, but each additional probe adds greatly to the time and expense of testing. Four to six probes are recommended

12.7.1. Restriction Fragment Length Polymorphism (RFLP)

- VNTR's are repeating units of a DNA sequence, the number of which varies between individuals. They are analyzed as Restriction Fragment Length Polymorphisms (RFLPs).
- RFLPs are variations within specific regions of genomes that are detected by restriction enzymes. RFLP analysis originated in the 1970s after the discovery of restriction enzymes, or proteins that can cut DNA into smaller molecules (restriction fragments) based on specific DNA sequence recognition sites.
- A restriction enzyme recognizes and cuts DNA only at a particular sequence of nucleotides (the components of DNA). VNTR's are 20–50 base pairs (pairs of nucleotides) long per repeat and a person can have anywhere from 50 to several hundred repeats. This repeat length is inherited.
- The advantage of using a RFLP-based analysis for DNA profiling is that VNTR regions are highly variable in copy number from person to person. Therefore, it is highly unlikely that DNA profiles from unrelated individuals would be identical. However, there are also several drawbacks to this technique.
- Since these regions are large, it is often difficult to clearly separate the fragment using electrophoresis, which is a technique that uses a DNA sample loaded into a gel that migrates towards a positively charge electric field based on size.

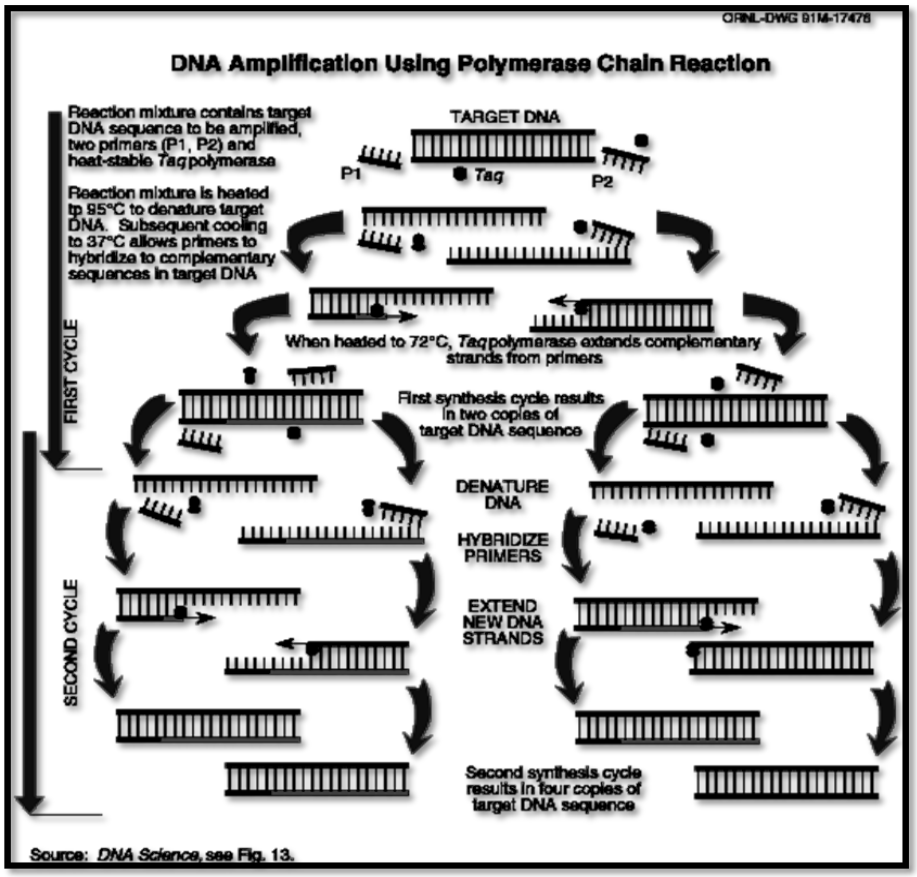


RFLP steps and procedure (Source wikipedia.com)

12.7.2 PCR Analysis

- Polymerase chain reaction (PCR) is used to make millions of exact copies of DNA from a biological sample. DNA amplification with PCR allows DNA analysis on biological samples as small as a few skin cells.
- With RFLP, DNA samples would have to be about the size of a quarter. The ability of PCR to amplify such tiny quantities of DNA enables even highly degraded samples to be analyzed. Great care, however, must be taken to prevent contamination with other biological materials during the identifying, collecting, and preserving of a sample.
- PCR is a process based on the ability of a DNA polymerase enzyme that can synthesize a complementary strand to a targeted segment of DNA in a test tube mixture of the four DNA bases.
- In addition, the mixture must also contain two DNA fragments, each about 20 bases long, called, that have sequences complementary to areas adjacent to each side of the target sequence. (To do PCR, you need to know the DNA sequence around the region you want to amplify.) These primers can be constructed in the lab, or purchased from commercial suppliers. If chosen well, the 20-25 base pair sequence will be unique in the entire human genome so will match only the place specifically chosen thus limiting and defining the area to be copied.
- The mixture is first heated to denature (separate) the sides of the double- stranded DNA and then cooled to allow (1) the primers to find and bind to their complementary sequences on the separated strands and (2) the polymerase to extend the primers into new complementary strands. Repeated heating and cooling cycles multiply the target DNA exponentially, since each new double strand separates to become two templates for further synthesis. In about 1 hour, 20 PCR

cycles can amplify the target by a million fold. In 32 cycles at 100% efficiency, 1.07 billion copies of targeted DNA region are created.



Sequential steps and procedure in PCR (Source:ornl.dwg.com)

12.7.3. STR Analysis

- VNTR analysis has been replaced by Short Tandem Repeat (STR) analysis. STR regions are comprised of 2–4 base pair repeats that are repeated between 5 to 15 times. STR analysis is currently the standard approach to forensic DNA profiling.
- This is mainly because shorter repeat sequences are easier to analyze. STR analysis is faster, less labor intensive, and can be automated. A single reaction can analyze 4–6 STR regions using very little DNA (only one nanogram is usually sufficient). If only a small amount of DNA is recovered or if it is degraded, it may be possible to use STR analysis, but not VNTR analysis.
- Additionally, in VNTR analysis, genomic DNA is digested with restriction enzymes and then run on a gel. The fragments produced are transferred to a membrane and probed with a radiolabeled sequence of DNA that matches the VNTR sequence. The migration of the VNTR fragment on the gel determines their size and generates a pattern. The radio-labeled probe produces dark bands on x-ray film when exposed in a time-dependent and dose-dependent manner.
- Unlike VNTR analysis, STR analysis uses the polymerase chain reaction to amplify DNA in the region where the STR is located. These PCR products can then be run on a gel in the same manner as the VNTR fragment and using sophisticated computer software with laser controlled equipment, the migration of the PCR products can be compared to control DNA molecules that

have a known size. If run together, the size of the unknown STR can be estimated. In this case, STRs are visualized by adding a DNA intercalator such as ethidium bromide into the gel, which intercalates into the DNA and fluoresces (emits) ultraviolet light.

- STR analysis, however, is not without its drawbacks, as well. If very little DNA is recovered from a crime scene and it is degraded, not all regions in the genome will amplify, or there will be discriminatory amplification of DNA in only one chromosomal STR region, rather than both. This can significantly affect the results and lead forensic scientists to draw incorrect conclusions. Additionally, there may be substances in the sample that inhibit the PCR reaction.

12.7.4 DNA Forensics Databases

CODIS

CODIS utilizes computer software to automatically search its two indexes for matching DNA profiles. Law enforcement agencies at federal, state, and local levels take DNA from biological evidence (e.g., blood and saliva) gathered in crimes that have no suspect and compare it to the DNA in the profiles stored in the CODIS systems. If a match is made between a sample and a stored profile, CODIS can identify the perpetrator. This technology is authorized by the DNA Identification Act of 1994. CODIS sets that the profile should match for 13 STR loci.

CHECK YOUR PROGRESS

(I) To detect hidden bloodstains which chemical test will be most appropriate

1) Kasle-Meyers test 3) Takayama test

2) Luminol test 4) Haematin test

(II) Blood grouping from dried blood stains can be done by

1) Absorption elution method 3) Luminol test

2) Kastle-Meyers test 4) Electrophoresis

(III) Dried blood stains at the scene of crime may be useful for

1) Blood grouping and DNA analysis 3) Secretor status

2) Species identification 4) All of these

(IV) Blood pattern analysis can give information about

1) Type of force used and distance 3) Angle of impact

2) Body shifted or not 4) All of these

(V) Examination of body fluids gives information about

1) Circumstantial evidence 3) DNA identification

2) Secretor status 4) All of these

(VI) Semen and seminal stains can be tested using

1) Phenolphthalein test 3) Acid phosphatase test

2) DNA typing 4) None of these

(VII) Saliva can be a source of DNA

1) True

2) False

(VIII) Saliva can be tested because of which enzymatic activity

1) Amylase 3) Tryptophan
2) Lactase 4) None of these

(IX) Biological fluids are also a source to determine if any toxicity present

1) True
2) False

(X) RFLP stands for

1) Restriction fragment length polymorphism
2) Restrict flow length pair
3) Resemble fragments like pore
4) None of these

(XI) In case of establishing relation between males which profiling is to be done

1) Mitochondrial DNA analysis 3) Both 1 and2
2) Y-STR typing 4) None of these

(XII) Minimum number of loci established by CODIS for identification is

1) 8 3) 13
2) 10 4) 19

Answers:

(I): 2 (II): 1 (III): 4 (IV): 4 (V): 4 (VI): 3 (VII): 1 (VIII): 1 (IX): 1
(X): 1 (XI): 2 (XII): 3

12.8. SUMMARY

Blood is the most common physical evidence in accidents, murder cases, and violent crime investigations. Besides blood, crime scene technicians may also find other stains and residues similar to blood in appearance at the scene, such as tomato sauce, red paint, or animal blood. To identify human blood, forensic scientists test samples at the crime scene with the chemical phenolphthalein, in an assay known as the Kastle-Meyer color test. Phenolphthalein releases hydrogen peroxide that reacts with an enzyme known as catalase in the blood. Catalase breaks down the hydrogen peroxide into water and oxygen, therefore releasing bubbles. However, as vegetables, animals, and some bacteria also produce catalase, this test only rules out the inorganic samples. Organic (plant or animal derived) samples are then collected for further serological analysis at the crime laboratory.

Body fluids such as blood, semen, saliva, and sweat, all contain serum. Serum is a liquid component of blood composed of water, trace minerals, several proteins including albumin, and immunoglobulins or antibodies. Albumin is the sticky protein that gives blood enough density for the water within it to remain inside the walls of arteries and veins. (Egg white contains high levels of albumin, which gives it the characteristic consistency.) When red and white blood cells are removed from blood, the resulting clear golden yellowish liquid is serum. Serology is therefore the study of the properties of serum. Serological tests have a wide range of applications in medicine, such as immunology and allergy assays, infection diagnosis, and blood typing. Serological tests are also used in forensics to identify blood ABO groups, whose results, although not conclusive, may help to

exclude or include suspects in the investigation process. If for instance, a suspect is blood type B and the samples from the crime scene are all types A and O, the suspect with type B blood can be excluded from the investigation. DNA is the material within every cell of the body and represents the blueprint of life. It allows physical traits to be passed on from one generation to the next. Although the majority of the human genome (the complete set of genes for an individual) is the same across all ethnic populations, people differ in their genetic makeup by a minuscule amount, and thus have their own unique DNA pattern. DNA profiling, also referred to as DNA typing, is the molecular genetic analysis that identifies DNA patterns. In forensic science, DNA profiling is used to identify those who have committed a crime. It is estimated that roughly one percent of all criminal cases employ this technique; however, DNA profiling has been used to acquit several suspects involved in serious crimes such as rape and murder and it has been used to convict individuals of crimes years after investigators closed the unsolved case. Aside from identifying an individual responsible for violent crimes, the judicial system also can use DNA profiling to determine family relationships in the case of disputed paternity or for immigration cases.

SUGGESTED READINGS

1. David B Rivers, The Science of Forensic Entomology
2. Dr Vikram Ahuja, Handbook of Forensic Odontology
3. Ira S. Luria, High- Performance Liquid Chromatography in Forensic Chemistry
4. Prof. (Dr.) Vimala Veeraghavan, Handbook of Forensic Psychology
5. Shubhra Goutam, An Introduction to Forensic Hair Examination
6. Li Deng, Speech Processing
7. William J Bodziak, Footwear Impression Evidence
8. John C Brenner, Forensic Science Glossary
9. Schmitt Aurore, Forensic Anthropology and Medicine
10. Takayuki Shibamoto, Lipid Chromatographic Analysis
11. Shubhra Goutam , An Introduction to Forensic Hair Examination

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Certificate Course in Forensic Science

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4

FORENSIC CHEMISTRY AND TOXICOLOGY

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UNIT - 13 : ARSON

Structure

13.0 INTRODUCTION

Arson is typically defined as the malicious burning of property. It is important to understand that arson is a legal term, and the definition varies from one country to another or even between different states within a country. The Uniform Crime Report (UCR) of the Federal Bureau of Investigation defines arson as: "any willful or malicious burning or attempt to burn, with or without intent to defraud, a dwelling house, public building, motor vehicle or aircraft, personal property of another, etc." When a fire occurs, fire investigators, crime scene investigators, or forensic scientists are called to the scene to determine the origin and cause of the fire and the potential of arson. The general definition of arson means that somebody deliberately or intentionally set fire to a property in order to destroy it, with a criminal intent. The person who decides to burn dead leaves in a backyard is normally not charged with arson, as it is his/her own property and there is no criminal intent. Criminal intent can be very broad. It includes gain of profit, fraud, persecution, or causing injury. The crime of arson is a very serious offense, punishable by several years of imprisonment. In intentional burnings, the charge of arson can extend in some states to the person who ordered the burning and not only to the person who actually performed the act of setting the property on fire. Individuals who commit arson (arsonists) can be characterized in a variety of ways. About 90% of arsonists are men and about 50% are younger than 18 years old. Juvenile fire setters are a great concern, and several programs have been created in the United States to identify these juveniles and address their problem behaviors. There are different motives for committing arson and they are usually classified into categories, such as profit, spite, excitement, crime concealment, and vandalism. Arson for profit includes all arsons committed with the expectation of obtaining a gain from the perpetrator (arsonist). It is important to note that the perpetrator does not necessarily need to obtain gain, but to show the intent that gain was going to be obtained. The gain can be direct or indirect. An example of direct gain would be the collection of the insurance money for the

replacement of a burned house. Indirect gain would be an increase of business by eliminating (burning) the competitor who was doing business across the street. Arson for spite is also known as arson for revenge. This type of arsonist wants to take revenge against a person, a group of persons, an organization or institution, or against society, in general. Some activist organizations for peace, or groups who fight violence against animals, for example, have regularly committed arson and destroyed laboratories or headquarters of research facilities for the sake of their cause. Arson for excitement is committed by pyromaniacs. These criminals are considered dangerous, as they do not have a particular target, and will burn any place or thing that would fulfill their need for excitement, attention, or get them the recognition they think they deserve. Because of the random nature of excitement motivated arsons, when one arsonist commits several burning acts, they are often difficult to profile. Arson for crime concealment is performed when criminals try to hide another crime. For example, after murdering an individual, the house is set on fire, destroying the body and much of the evidence of the murder activities. Fortunately, there are many forensic techniques that have been developed throughout the years to retrieve different evidence, such as blood patterns or fingerprints, after a fire.

13.1 OBJECTIVES

- To understand the legal implications of arson
- Types of arson encountered
- To differentiate between accidental fire and arson
- To determine origin of fire, nature of fire and cause of fire

13.2 CHEMISTRY OF FIRE

There are 3 main components of fire:

- Fuel
- Heat
- Oxygen

The three components together form the fire triangle. These days an additional 4^{th} component needed to explain flaming combustion is added. This is the chemical chain reaction. Thus, instead of a fire triange, we have a fire tetrahedron. The chemical chain reaction yields such products or energy or energy that cause further reaction of the same kind. This process is self sustaining.

13.2.1 Heat- The first component of fire (ignition)

It is a form of energy. Heat is the energy possessed by a substance or material due to measurement of relative amount of energy contained within given substance. Heat energy is the result of the motion or vibration of the molecules of a substance. Heat Production takes place by four ways.

- Chemical: - chemically-produced heat is the result of rapid oxidation. The speed of the oxidative reaction is an important factor.

- Mechanical: Mechanical heat is the product of friction. Internal components of machinery due to friction get heated up and can cause ignition of combustibles.
- Electrical: - Heat produced due to an electrical malfunctioning. Eg:- short circuit in a wire.
- Compressed gas: When a gas is compressed, its molecular activity is generally increased. This results in more heat energy production.

13.2.2. Oxygen

It is one of the components of fire. For combustion to take place, the combustible fuel and an oxidizing agent must come together. Certain unusual fuels contain enough O_2 to maintain decomposition on even partial combustion in the absence of additional O_2 in air. eg- Pyroxylin Plastics. In a basic oxidation reaction, oxygen and Hydrogen are2 basic elements. When (H) is oxidized by combining it with (O), it is a basic exothermic reaction.

$2H_2+O_2 \rightarrow 2H_2O+ \triangle$

13.2.3. Fuel (combustibles)

Fuel is any substance that in the presence of O_2 and ignition source undergoes combustion. Thus, they are combustible material. Fuel is matter, which may be solid, liquid or gaseous. Some solid fuels are substances like wood, liquid fuels are petrol, kerosene etcetera. Example of gaseous fuels is LPG. Some fuels can undergo spontaneous burning like sodium. Fuels undergo chemical chain reaction, which implies it is a complex series of events that must be continuously and precisely reproduced in order to maintain flaming combustion.

13.3. SEARCHING, COLLECTION AND PRESERVATION OF ARSON EVIDENCES

13.3.1 Searching arson evidence

Searching of accelerants, fuel residue and ignition devices involve sophisticated methods.

- Olfactory detection: Human nose acts as a sensitive and selective detector but the disadvantage is that it is not a reliable method. There is tendency of the nose to lose the sensitivity to an odor after prolonged and intense exposure to it.
- Chemical color test detectors: Certain dye indicating the presence of hydrocarbons by turning red after undergoing a chemical reaction. They have low cost. But the disadvantage is less sensitivity and specificity.
- Catalytic Combustion Detection: Also called the sniffers or vapor detections. In this, vapor samples are pumped over a heated pt plated coil of wire that causes combustible gas present to oxidize. The heat from oxidation raises the electrical resistance of the coil and this change in substance is measured electronically. Advantage is that it is simple to operate, moderate in cost

and portable. But the disadvantage is Oxygen is required for operation and loss of sensitivity in detection of gasoline containing lead.

- Portable UV detectors: UV light can be used to search for any accelerant in the debris, which exhibits fluorescence.

13.3.2 Collection and preservation of clues and evidence

a) Sample of debris and ash should be collected.
b) Partially burnt or unburnt pieces of materials like clothes of victim, cigarette ends, matchsticks, cloth (curtains) rags, wooden door pieces, paper, pieces of wires if any etc. should be collected with the help of forceps and kept in air tight containers and vacuumed poly bags.
c) If any container/bottle is present which might have contained accelerant should be collected and packed separately in a polybag/box.
d) All evidences collected should be recorded in a logbook for easy reference of the evidence on all claims he/she is involved.
e) Fire debris must be packed so that volatiles will not escape and contaminants are not introduced into the sample. Packaging material used are tin cans, glass jars, plastic bags and polyester/polyolefin bags.

13.4 SCIENTIFIC INVESTIGATION AND EVALUATION OF CLUE MATERIALS

13.4.1 At the crime scene

- To distinguish accidental fire from arson Investigation of fires have the following important aspects of inquiry:-
  a) To determine the nature of fire.
  b) To find out the cause of fire.
  c) To ascertain the evidence which indicates whether it is arson, spontaneous or accidental fire?
  d) To determine the nature and quantity of materials burnt.
  e) To ascertain the extent and cause of the spread of fire.
  f) To link culprit with the fire.
  g) To ascertain modus operandi.
  h) Whether it was burning or smoldering.
  i) Determine place of origin of fire
  j) To search for material responsible for initiating fire

The scene of occurrence is guarded till the examination is complete. The forensic personnel inspects the scene and looks for

a) Evidence of accelerants
b) Evidence of combustible material.
c) Evidence of delayed ignition device
d) Evidence of defective insulation, heating appliances, switch boxes, switch boards.
e) Identify the origin of fire.

f) Check insurance records of the owner
g) Assess damage to property.
h) Examine entrance and exit areas to see whether the place is accessible to outsiders for possible act of sabotage.

- Photograph the scene from different angles, covering the extent of damage,the pattern of smoke deposit, the site of origin, the presence of foreign matter and damage to various installations. The site of origin will generally be directly beneath the maximum soot deposition area.
- Sketches should be made of the scene of fire to show the relationships of the objects to each other. They help in clarifying the issues and refresh the memory of witnesses during cross-questioning and also avoid unnecessary and legally prohibited return trips to the scene.

13.4.2 Laboratory methods

Detection in the lab involves 3 basic steps. These are:

a) Sample preparation – isolation and enrichment of volatiles from matrix.
b) Separation and Identification
c) Data interpretation–establishment of accelerants by chromatographic profiles or spectrographs.

- Sample Preparation
  - Steam Distillation: This involves the heating and distilling of the charred material with steam and trapping the distillate with cold-water condenser. The volatile hydrocarbons are collected in liquid form before analysis.
  - Solvent Extraction – The sample of charred material is extracted by mixing and shaking with a known solvent such as ether, which dissolves the petroleum distillates and other volatiles into the known solvent. This extract is then heated to get a small volume-concentrated form for analysis.
  - Cold headspace – The top of the sample container is punctured and a stopper is inserted so that a headspace vapor sample can be removed with a syringe for subsequent analysis. This is followed by heated headspace. After the stopper is inserted into the top of the sample container, the vessel is heated to 100^0 C or lower
- Separation and identification involves sophisticated techniques like Gas chromatography or gas chromatography-mass spectroscopy.
- Data interpretation: The Rf values of the sample are compared with the Rf values of the standards in case of chromatography. The peak height gives the qualitative analysis and the area gives the quantity.

CHECK YOUR PROGRESS

(I) Accelerants should be packed in

1) Paper bags
2) Tin cans
3) Cardboard box
4) None of these

(II) For a fire to occur which of these is essential

1) Fuel 3) Heat

2) Oxygen 4) All of these

(III) Tempering with fire extinguisher and safety devices indicates

1) Accidental fire 3) Both 1 and 2

2) Arson 4) None of these

(IV) Which of these shows spontaneous burning

1) Alcohol 3) LPG

2) Sodium 4) Petrol

Answers

(I) 2
(II) 4
(III) 2
(IV) 2

13.5 SUMMARY

To light a fire, arsonists need a flammable material and an accelerant (such as kerosene or gas). Arson investigators look for these items when they're investigating the crime scene. Because all that's usually left of the evidence is charred remains, the investigators will collect fire debris and take it back to the forensics lab for analysis. Whether the fire is intentional or not. Cause of fire, nature and origin of fire is determined. It is essential to establish Samples are sealed in airtight containers and then tested for residues of accelerant liquid that might have been used to start the fire. These are the most common tests performed by forensics labs during an arson investigation:

- **Static headspace** heats the sample, causing the residue to separate out and vaporize into the top, or "headspace" of the container. That residue is then injected into a gas chromatograph, where it's broken apart to analyze its chemical structure.
- **Passive headspace** heats the sample and the residue collects onto a carbon strip in the container. Then the residue collected is injected into a gas chomatograph/mass spectrometer for analysis.
- **Dynamic headspace** bubbles liquid nitrogen gas through the sample and captures the residue onto an absorbent trap. The trapped compounds are then analyzed using gas chromatography.

UNIT - 14 : EXPLOSIVES

Structure

14.0. INTRODUCTION

Explosives are chemical compounds or mixture, which will on application of an external stimulus such as heat, shock, friction or ignition undergo a rapid chemical decomposition resulting in a sudden release of large amount of energy

Thus an explosion refers to the extremely rapid self propagating transformation of unstable explosives into a stable substance accompanied by formation of large volume of gases and liberation of heat the pressure generated is thrust equally in all directions.

14.1 OBJECTIVES

- To know the types of explosives used by terrorists
- To classify them according to chemical nature and intensity
- To understand the working and delay system of an explosive
- Collection, preservation and analysis of explosives

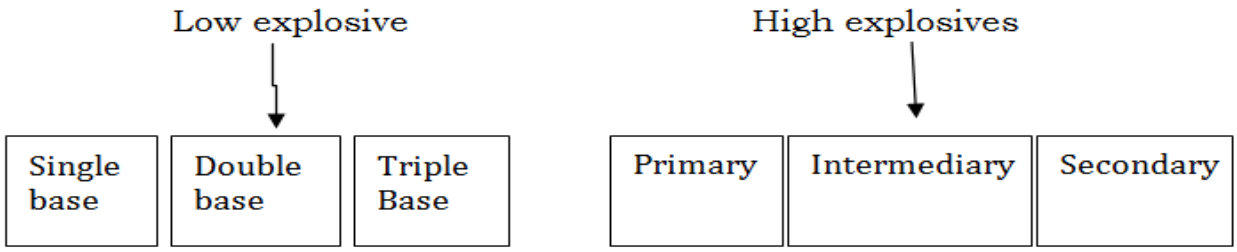
14.2 CLASSIFICATION OF EXPLOSIVES

14.2.1 On the basis of energy and sensitivity

Explosives are mainly classified as

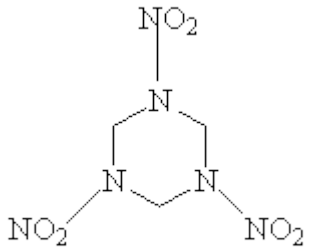
- Low explosives (which undergo deflagration)

- High explosives (undergo detonation) They can be further classified as follows:

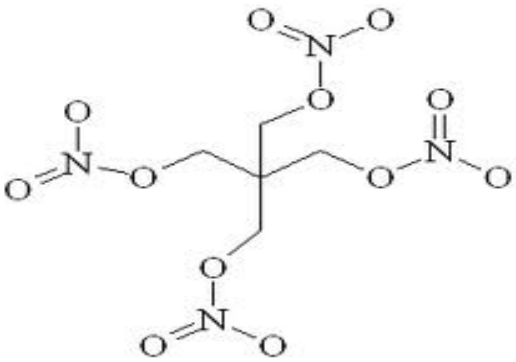


Low explosives are those, which undergo deflagration thus giving a propelling effect. They are used in firearms. High explosives are characterized according to their sensitivity and power

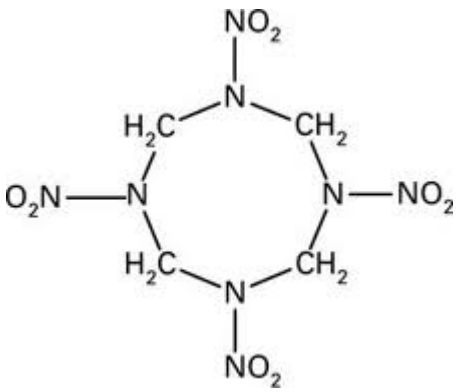
- Primary high explosives / initiators: They have high sensitivity and a high triggering velocity. But have low power, energy and shattering effect. Their basic function is to initiate secondary explosives by shockwaves. Some commonly used ones are: lead azide, lead styphnate, mercury fulminate, silver azide etc.
- Secondary high explosives: They have low sensitivity but high brisance and shattering effect. They have high power and energy. On detonation produces large amount of energy due to high heat (3000c-4000c) and high pressure 4000000psi
- Intermediaries / boosters: Some of the secondary explosives are not sensitive enough to detonate directly by the initiators.They need boosters Sensitivity and energy lies between primary and secondary explosives They receive detonation energy form initiators, intensify it and transfer it to insensitive secondary explosives. eg.Tetryl



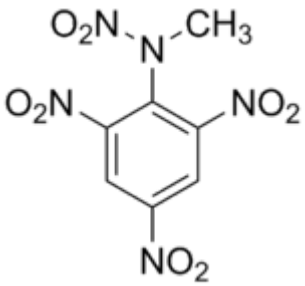
RDX



PETN



HMX



Tetryl

Fig1: Chemical structures of some explosive compounds

2.2.2. Explosives can also be classified on the basis of usage:

- Industrial explosives: They are further categorized as permitted or non-permitted. Amongst permitted we have dynamite, ammonium nitrate fuel oil and gelatin. They are used in roads and railway constructions, hydroelectric diversions, mining, submarine blastings, in agriculture: for loosening subsoil etc. Permitted explosives are especially designed to produce no flame or flame or low volume, short duration and low temperature. This is achieved by increasing use of ammonium nitrate and by addition of cooling agent (or flame suppressor) egNaCI. Dynamite is further classified as follows:

Military dynamite	Straight Dynamite	Gelatine dynamite	Ammonia dynamite
Tnt: 15 % RDX : 75% Corn starch: 5%	NG: 20-57% Nacl: 23-59% Sulphur: 2.95% Antacid: 1.3-1.2% +carbonaceous material	92% liquid NG 8% NC	NG: 12 – 22% NaCl: 57-15% Ammonium nitrate: 12-15% NC: 10.2-8.6% S: 6.7-8.6% &carbonaceous fuel
Do not use NG explosive Vod: 6100 m/s	Vod: 3000-5800 m/s	Vod: 7000 m/s	One of the most commonly used Vod: 2450-4600 m/s

Table 1: Classification and properties of dynamite

Pyrotechnics: These are art of fireworks: dealing with fire, smoke, light and sound. These are mixture of inorganic compounds. Metals employed for colored lights: Na Ba CuO, potassium salts, strontium salts etc.

Composition of Pyrotechnics		
Fuel composition	Smoke composition	Illuminating composition
Magnesium powder, Charcoal or antimony sulphate	Zno, TiO_2, hexachloroethane	Potassium nitrate, Mg powder, Alpwdr, Polyester resin

Table 2: Listing composition of pyrotechnics

- Improvised explosives: These are planted with intention to cause injury, death or damage to property. IED's can be of any size and shapes and there are no standardization for it. They are composed of explosive charge , detonator ,power source, switches and a time delay device .

14.3. EXPLOSION PROCESS AND BLAST WAVES

An explosion is a rapid increase in volume and release of energy in an extreme manner, usually with the generation of high temperatures and the release of gases. An explosion creates a shock wave. If the shock wave is a supersonic detonation, then the source of the blast is called a high explosive. Subsonic shock waves are created by low explosives through the slower burning process known as deflagration. Explosive force is released in a vertical direction to the surface of the explosive. If the surface is cut or shaped, the explosive forces can be focused to produce a greater local effect; this is known as a shaped charge. The speed of the reaction is what distinguishes the explosive reaction from an ordinary combustion reaction. Unless the reaction occurs rapidly, the thermally expanded gases will be dissipated in the medium, and there will be no explosion. A chemical explosive is a compound or mixture, which upon the application of heat or shock, decomposes or rearranges with extreme rapidity, yielding much gas and heat. Many substances not ordinarily classed as explosives may do one, or even two, of these things.

A reaction must be capable of being initiated by the application of shock, heat, or a catalyst (in the case of some explosive chemical reactions) to a small portion of the mass of the explosive material. A material in which the first three factors exist cannot be accepted as an explosive unless the reaction can be made to occur when needed.

A blast wave is the pressure and flow resulting from the deposition of a large amount of energy in a small very localized volume. The flow field can be approximated as a lead shock wave followed by a subsonic flow field. In simpler terms, a blast wave is an area of pressure expanding supersonically outward from an explosive core. It has a leading shock front of compressed gases. The blast wave is followed by a blast wind of negative pressure, which sucks items back in towards the center. The blast wave is harmful especially when one is very close to the center or at a location of constructive interference. High explosives, which detonate, generate blast waves. Mach stem formation occurs when a blast wave reflects off of the ground and the reflection catches up with the original shock front, therefore creating a high-pressure zone that extends from the ground up to a certain point called the triple point at the edge of the blast wave. Anything in this area experiences peak pressures that can be several times higher than the peak pressure of the original shock front.

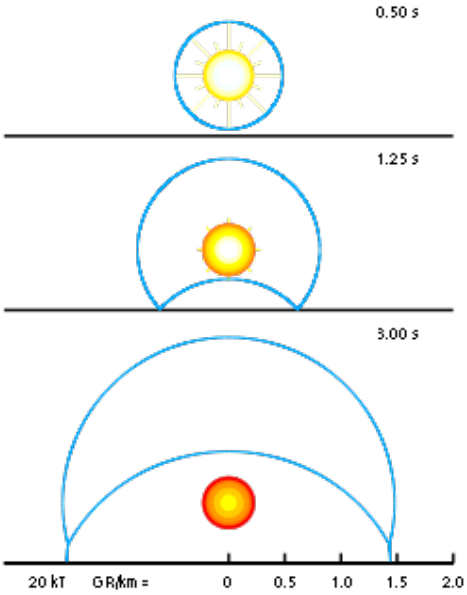


Fig 2: Mach formation in blast wave (source: wikipedia.org)

Blast waves cause damage by a combination of the severe condensing of the air in front of the wave (forming a shock front) and the subsequent wind that follows. A blast wave travels faster than the speed of sound and the passage of the shock wave usually only lasts a few milliseconds. The original explosion will send out fragments that travel very fast. Debris and sometimes even people can get swept up into a blast wave, causing more injuries such as penetrating wounds, impalement, broken bones, or even death. The blast wind is the area of low pressure that causes debris and fragments to actually rush back towards the original explosions. The blast wave can also cause fires or even secondary explosions by a combination of the high temperatures that result from detonation and the physical destruction of fuel-containing objects.

14.4. SEARCHING THE SCENE OF EXPLOSION

For reconstruction of events the following steps are to be considered.

- Organization of explosive investigation team is set.
- Immediate approach at the scene of explosion is evaluated.
- Collection and preservation of exhibits is carried out
- Explosion scene is investigated.
- Finally post blast residue analysis is carried out.

Organization of Explosive Investigation Team

(1) Team supervisor: on arrival conducts a scene overview, immediately establishes a command post and evaluates and finalizes search and investigation action.

(2) Team leader: decides search pattern and assists supervisor to establish command post, general area search and investigation unit and most importantly reconstructs the bomb and the initiating mechanism.

(3) Evidence Collectors: Collect debris, search general area and scene of explosion. They further mark and preserve swabs and debris for lab analysis.

(4) Forensic Analyst: Identifies the debris and carries out spot test and other detailed chemical analysis and submits the report.

(5) Photographer: Photographs the scene in detail, including the victims. Photographs the crater size and all evidences within the location (showing necessary measurements) thus giving a detailed panoramic view.

(6) Enforcement Officer from the police.

After securing the area and setting the command post, the evidence is searched There are various patterns of search, like spiral method, grid method, zone method or sector method and strip or line method. In case of indoors, the area is also divided vertically from floor to waist level, then till eye level and from eye level to ceiling. Explosion Signatures are to be interpreted. Explosion signature implies investigating and analyzing the extent and types of damages to determine the type, quantity of explosive and triggering mechanism.

Search is carried on with the aid of:-

- Equipment and tools
- Sniffer dogs

Search equipments	Collection and packaging materials
Magnets, vacuum cleaner, explosive detectors (evd 3000, evd 8000, vixen), explosive detection chemical kits, X ray machines, magnifying glass, nitroglycerine destroyer kits.	Disposable gloves, evidence bags, cartons, surgical cotton wool swabs, measuring tape, paper bags, tin containers(for volatile residues)

Table 3: Listing various search and packaging equipments

14.5. POST BLAST RESIDUE ANALYSIS

14.5.1. Separation of residues from the debris

Solvent extraction of the debris transfers the explosive residue into solution In solution, the explosives are easily subjected to further treatment like concentrations, separations and identification It is an essential initial step in the analysis of post – explosion residue. The best all-purpose solvent is acetone, which is an excellent solvent for almost all organic explosives. However, one of the disadvantages with acetone extract is with sulphuric acid, the acid tends to char organic materials present thereby obscuring the tests. In these instances, a methanol or other extraction or both may be performed instead of acetone extraction. But many explosives are less soluble in methanol and other than in acetone. The acetone extract is concentrated and used for the detection of organic explosive. The residue left after acetone extract is extracted with hot water and filtered through filter paper No.42. The main inorganic salts in the field of explosives dissolve readily in hot water. The water extract is evaporated to a small volume and is used for the detection of water-soluble ions. The residue left after water extraction is dried and extracted with pyridine and filtered. The pyridino

extract is preserved for the detection of sulphur. Residue left after pyridine extraction is washed with a few ml. of acetone and distilled water and kept on water bath till all pyridine fumes disappear and it is air –dried. A small portion of the dried material is extracted with 10N NaOH and filtered. The filterate is preserved for detection of sulphide, arsenic, aluminum etc.

14.5.2 Spot tests

These are highly valuable for preliminary identification of post explosion analysis. Being simple and sensitive, they can be carried out as field tests, i.e. on the scene of explosion. The sensitivity of most color reactions used in the analysis of explosives is up to microgram level. However, a complete identification cannot be based solely on spot tests due to lack of specificity, although some color reactions are quite specific

Color Test for Inorganic Ions

1. Chloride ion: Take a small quantity of aqueous extraction in a small test tube and add one drop of dilute Nitric Acid and 2-3 drops 5% aqueous dilute Nitric Acid and 2-3 drops of 5% aqueous silver Nitrate solution. A white crystalline precipitate indicates the presence of chloride. The white precipitate is soluble in ammonium solution.
2. Arsenic:Gutzeit Method: Take a small portion of sodium hydroxide extract in Gutzeit apparatus to it, add 20 ml. of con. Hydrochloric acid and 2.5 mg of potassium iodie solution (15mg. KI in 100 ml water) and 2 ml. of staunous chloride (40gm, SnCI) in 100 ml. of HCI) and a few zinc granules. Fix a piece of filter paper soaked in saturated solution of mercuric chloride. Let the reaction takes place for one hour. If a yellow to brown stain appears on the filter appear it indicates the presence of arsenic.
3. Sulphide: To a small portion of alkaline extract add one drop of sodium nitropruside solution on porcelain tiles. A violet color indicates the presence of sulphide.
4. Sulphur : Take a small portion of pyridine extract in a small test tube and boil it for one minute , add a drop of Zinc sodium hydroxide (add in hot condition) A green color in pyridine layer indicates the presence of sulphur

Spot tests for organic extract

S No	Substance Tested	Diphyaylamine	Griess reagent	Brucine reagent	Alcohalic KOH	Add Crystals of thymol and then drops of alcohol
1	Chlorate	Blue to blue reaction	-	Orange to Red	-	-
2	Chlorate	No reaction	-	-	-	-
3	Nitrate	Blue to blue back	Pink to red	Orange to Yellow	-	-
4	Nitrate	Blue back	Red to yellow	Orange to Red	-	-
5	Nitrocellul	Blue back	Pink	Orange to Red	-	Green

	ose					
6	Nitroglycerin	Blue to blue to black	Pink to Red	Orange to Red	-	Green
7	Nitrostarch	Blue black	Pink	Orange to Red	-	-
8	Perchlorate	No reaction	NR	-	-	-
9	PETN	Blue	Pink to Red	Orange to Red	-	Green
10	RDX	No reaction	Pink to Red	Orange to Red	-	Rose
11	Sulfate	No reaction	NR	-	-	-
12	Tetryl	Blue	Pink to Red	Orange to Red	Red Violet	-
13	T.N.T	No reaction	NR	-	Red	-

Table 3: Listing Color test of some organic explosive and inorganic ions

14.5.3. Thin layer chromatography of explosive residue

TLC of explosive residue material is done as a confirmatory test. Stationary phase is the silica plate and mobile phase used is chloroform: acetone :: 1:1. Visualizing agent is is sprayed. The Rf value is then compared with the standard (known explosive).

SUBSTANCE	**COLOR**	**RF**
Mononitrotoluence	Faint yellow	0.48
Dinitrotoluence	Yellow	0.40
Trinitrotoluence	Brown	0.44
Nitroglycerene	Yellow / red	0.38
Ammonium Nitrate	Red or Pink	0.58
Ammonium Nitrate	Mauve or Pink	0.58
Nitroguanidine	Red	0.53
Nitrocellulose	Red	0.00
RDX	Mauve or Pink	0.47
HMX	Red	0.40
PETN	Red	0.60
Tetryl	Red	0.60

CHECK YOUR PROGRESS

(I) Which of these is a primary high explosive

1) HMX
2) RDX
3) Tetryl
4) Lead azide

(II) What all equipments can be used to search explosive scene

1) Magnets
2) Vacuum cleaner
3) Magnifying lens
4) All of these

(III) Which is a suitable reagent to dissolve organic residue of explosive

1) Acetone
2) Distilled water
3) Pyridine
4) Sodium hydroxide

(IV) Industrial explosive are made to produce less flame. This is done by addition of :

1) Sodium chloride
2) Water
3) Methanol
4) Copper

Answers

(I) 4
(II) 4
(III) 1
(IV) 1

14.6. SUMMARY

Establishing the nature of an explosion can be a significant challenge to the forensic investigator. The high-pressure wave of an explosion can be extremely destructive, both to bystanders and any objects of materials in the vicinity. Analyzing this kind of **evidence** can be a very difficult task, particularly as an explosion is often followed by a fire. This causes complications for the investigators as much valuable evidence is then destroyed. There are also potential hazards to the investigators themselves in investigating a bomb site. There may be structures in danger of imminent collapse as well as exposure to dangerous materials such as broken **glass**, flammable or toxic vapors, or asbestos. In the case of a bombing, there is always a possibility that a second device has been placed to kill or maim those who respond to the explosion. An explosive is a substance that can produce an explosion through a chemical reaction. When it is used illegally and to cause harm it is generally known as a bomb. Legitimate **explosives** include fireworks and blasting materials used in quarrying. Explosives generally contain fuel and an oxidant and it is the chemical reaction between them which releases stored chemical energy.

There are two types of explosions due to chemical reactions, which is reflected in the type of damage they cause at the scene. In a detonation, the speed at which the chemical reaction moves though the explosive is greater than the speed of sound in that material. The resulting pressure wave may move at up to 8,500 meters per second (9,296 yards per second). High explosives, such as dynamite, generally undergo detonation and have a characteristic shattering effect on their surroundings. A deflagration occurs when the speed of the chemical reaction of the explosion travels through the explosive slower than the speed of sound in the material. This creates a pressure wave moving at 1,000 meters (1094 yards) per second or less. The impact of low explosives, such as a mixture of air and gasoline vapor or sugar and potassium chlorate, is best described as pushing, rather than shattering, although they can still produce an enormous amount of damage. Depending on their nature, explosives may or may not need an initiating material, called a detonator, to set them off. A useful distinction can also be made between condensed explosives, which are solid or liquid, and dispersed explosives, consisting of aerosol or gas.

Investigation of the scene of an explosion aims to discover whether an explosion actually took place and, if so, whether it was an accident or a bomb. The forensic scientist will then try to find out what kind of explosion occurred, the materials involved and, in the case of a criminal act, they will work with the police to find out who was responsible. Examination of the scene and witness reports can establish whether an explosion has happened. Loud bangs, flashes, violent eruption of debris, shattering of nearby objects and formation of a crater where the event occurred are all indicative of an explosion. The investigator will look for evidence of a possible accident, such as a gas leak or creation of a cloud of flammable gas at the scene. If it looks as if a bomb caused the explosion, then the explosive device must found. This involves searching for the device itself and any detonator fragments which may be scattered among the debris. There may have been a timing device to allow the bomber time to get away, which would consist of electronic circuitry, wires, and batteries. The remains of the device will probably contain some residue from the explosive and may even bear fingerprints from the perpetrators. The construction of the device and how it was triggered may also be deduced from examination of these fragments

UNIT - 15 : NATURAL AND SYNTHETIC DRUGS OF ABUSE

Structure

15.0 INTRODUCTION

Drug abuse, also called substance abuse or chemical abuse, is a disorder that is characterized by a destructive pattern of using a substance that leads to significant problems or distress. Drug addiction, also called substance dependence or chemical dependency, is a disease that is characterized by a destructive pattern of drug abuse that leads to significant problems involving tolerance to or withdrawal from the substance. While the specific physical and psychological effects of drug abuse and addiction tend to vary based on the particular substance involved, the general effects of abuse or

addiction to any drug can be devastating. Psychologically, intoxication with or withdrawal from a substance can cause everything from euphoria as with alcohol, Ecstasy, or inhalant intoxication to paranoia with marijuana or steroid intoxication, to severe depression to suicidal thoughts with cocaine or amphetamine withdrawal. Like the majority of other mental-health problems, drug abuse and addiction have no single cause. However, there are a number of biological, psychological, and social factors, called risk factors that can increase a person's likelihood of developing a chemical-abuse or chemical-dependency disorder. Psychological associations with substance abuse or addiction include mood disorders like depression, anxiety, or bipolar disorder, as well as personality disorders like antisocial personality disorder.

15.1 OBJECTIVES

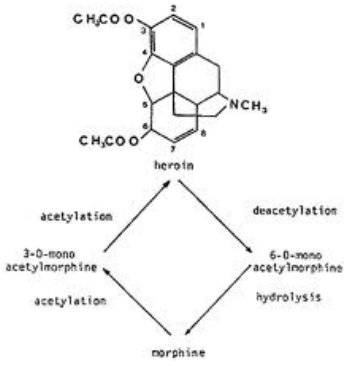
- To determine different chemical evidences which can be used in legal investigations
- To collect, preserve and analyze drugs of abuse
- To check whether the essential commodities like alcohol and petroleum are as per BIS specifications or are adulterated
- To understand trap cases using dyes

15.2. NATURAL AND SYNTHETIC DRUGS OF ABUSE

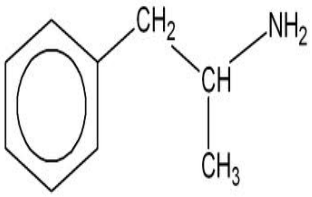
On the basis of origin and synthesis drugs of abuse may be grouped as natural and synthetic .Natural drugs are those which are present in nature in their active form whereas synthetic drugs are either derived from natural drugs or entirely synthesized chemically.

Natural Drugs of abuse	Synthetic drugs of abuse
Opium	Heroin
Morphine	Amphetamines
Cannabis	LSD
Adrenalin	Fentanyl

Table 1: List of some common natural and synthetic drugs of abuse

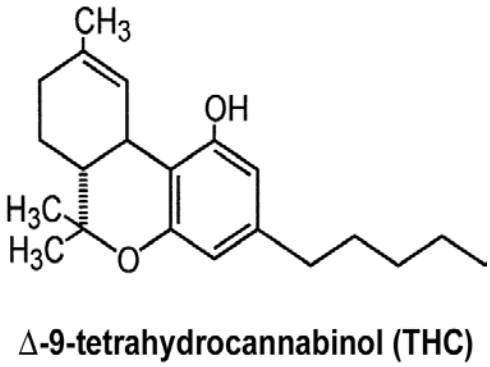


Structure of morphine



Structure of amphetamine

Structure and synthesis of heroin

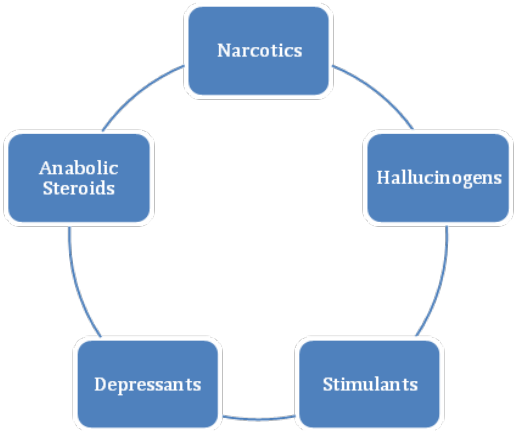


Δ-9-tetrahydrocannabinol (THC)

Fig1: Chemical structures of some natural and synthetic drugs

15.3. CLASSIFICATION OF DRUGS OF ABUSE

On the basis of effects drugs of abuse can be classified into the following



15.3.1 Narcotics

Narcotic drugs are those, which give relief from pain and induce sleep. On ingestion into the body they act on the central nervous system. They produce physical and psychological dependencies. Naturally occurring narcotic source is the plant *Papaversomniferum* (opium). Opium contains various organic and inorganic substances like alkaloids, acids, proteins, sugars etcetera. About forty opium alkaloids have been known, out of which five are major. They are morphine, codeine, thebaine, papavarine and narcotine. Some other narcotics are heroin, hydromorphone, oxycodone and hydrocodone. These can be considered semi synthetic as they are derived from morphine. Synthetic narcotics are like meperidine, fentanyl etcetera. Even though heroin is derived from morphine but is far more powerful. This is because there is a barrier between the blood and the brain which morphine cannot pass but heroin reaches the brain readily. It causes stupor, narcosis, coma, asphyxia etcetera. In a person not addicted to opium 200 mgs of morphine is fatal.

15.3.2 Hallucinogens

Hallucinogens are drugs, which produce a bizarre effect on mind, which causes distortion of time, space, sound, color and other sensations. Commonly known hallucinogens are LSD, peyote, phencyclidine etcetera. Lysergic acid diethylamide (LSD) is synthesized from rye ergot. Hallucinogens act primarily on the central nervous system and interfere with perception, thinking and mood. Awareness is lost. The effects are seen by even 15 micrograms of LSD. The potential hazards include intense anxiety, panic, confusion, inability to distinguish between reality and dream. Several suicides have been reported after their use. *Cannabis indica* is a plant, which contains an active component THC (tetrahydro cannabinoid). It is present in 3 forms ganja, charas and bhang.

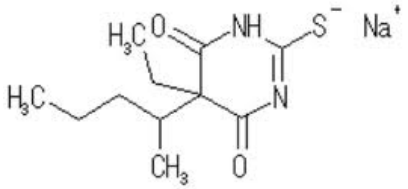
15.3.3. Stimulants

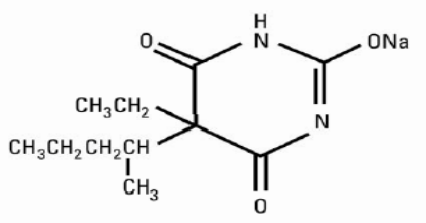
These drugs stimulate the central nervous system. And produce a false state of euphoria. Amphetamines, cocaine, caffeine and nicotine are some commonly used stimulants. Amphetamines are used as apetite suppressants, relief of fatigue and treatment of narcolepsy. Signs and symptoms manifested are insomnia, dry mouth, nausea, vomiting, abdominal cramps, convulsions and coma. 120-200 mg of amphetamines is fatal. Cocaine is an alkaloid derived from coca plant. It is commonly used as aphrodisiac. Blurred vision, incoordination and cardiac or respiratory failure. Chronic poisoning of cocaine causes ‘cocaine bugs’ a feeling that small insects are creeping on the skin.

15.3.4 Depressants

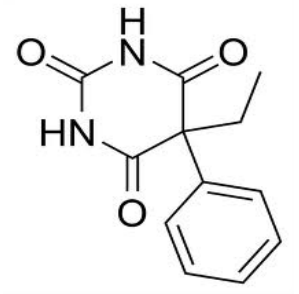
They have the ability to depress the neural activity. Anesthetics, barbiturates and ethanol are commonly used as depressants. It induces sedation decreasing movement and sensory responsiveness. Barbiturates mainly affect areas in the forebrain and the brainstem. There are about 2500 barbiturates. Some common ones have been listed below.

Duration of Acting	**Generic name**	**Sedative dose (oral)**
Ultra short acting	Thiopental	Used only intravenously
Short to intermediate acting	Amobarbital, pentobarbital, secobarbital	0.20g 0.10g 0.20g
Long acting	Phenobarbital, barbital	0.10g 0.30g





Pentobarbital



Phenobarbital

Fig 2: Chemical structures of different barbiturates (source:Wikipedia.org)

15.3.5 Anabolic steroids

These are mostly synthetic substances resembling natural hormones. They increase protein synthesis within the cell, which results in the build up of cellular tissues (anabolism). This exogenous substance penetrates the membrane of the target cell and binds to an androgen receptor and thus activates the signal. Some steroids like oxandrolone bind tightly to the receptor and act on gene expression. Side effects are liver malfunction, hypogonadism in males, increased facial hair, voice and skin coarsening, reproductive dysfunctions in females.

15.4 COLLECTION AND PRESERVATION OF DRUGS OF ABUSE

Proper sampling of the drug evidence is must to ensure accurate examination. The material should be removed from its container or wrapping and the net weight should be recorded. If drug sample is in the form of block/brick, then sampling should be done from all the sides and even the centre. Other method is to crush the brick into powder form. The powder material is then placed in a clean clear plastic bag and shaken thoroughly. If a number of packets of drugs are present then visual examination and chemical tests (preliminary tests) are done there and then. If number of packets less than ten, all packages should be sampled. If 10-100, then randomly select minimum ten samples and carry out the tests. Different drug samples to be packaged differently. If the drug sample is the same, the contents may be combined and homogenized. This procedure is suitable for opium, morphine, cocaine, heroine, benzodiazapienes, amphitamines, barbiturates and psilocybin.

Most LSD exhibits are present in paper, tablet or gelatin form. Powders are generally not encountered. Sample is taken from all sides and put in plastic containers.

Fig 3: Figure depicting sampling of drugs of abuse (source: montanabiotech.wordpress.com)

15.5. FIELD AND LABORATORY TESTS OF DRUGS OF ABUSE

15.5.1 Presumptive tests

- Marquis test:
  Preparation of marquis reagent: 8-10 drops of 40% formaldehyde solution is added to 10 ml of conc. Sulphuric acid.
  Test: Take a small amount of suspected sample in a test tube and add about 10 drops of water. Crush the sample with a glass rod. Place few drops of water solution through filter paper on a spotting plate and add few drops of marquis reagent. Purple –violet color indicates the presence of opium / crude morphine / heroin.
- Ferric salt test:
  Preparation of ferric-salt reagent: Dissolve 1g of ferric sulphate in 20 ml of water

  Test: Take a small amount of suspected sample on a spot plate and add about 2 drops of water. Grind it till the water becomes brown color. Take a drop of brown liquid to another spot plate and add 1 drop of reagent. Appearance of brown-purple color indicates the presence of meconic acid. Meconic acid is present in raw and prepared opium but not in morphine.

- Mecke's test
  Preparation of Mecke's reagent: 0.25 gms of selenious acid in 25 ml of concentrated sulphuric acid.

  Test: Take a small amount of sample in a spot plate and add few drops of reagent. The appearance of deep green color indicates heroin

- Nitric acid test:

Take a small amount of suspected sample in a spotting plate and add few drops of concentrated nitric acid. The appearance of yellow color which turns green on standing indicates heroin.

- Test for differentiation of bhang/charas/ganja
  Reagent 1: p-aminophenol (1mg) in ethanol (10ml)
  Reagent 2: caustic potash (1g) in distilled water (10ml)
  Test: Extract the suspected material in ethanol. Take a drop of extract in a spot plate. Add reagent 1. Mix thoroughly followed by addition of reagent 2.
  Bhang gives green color, ganja gives blue and charas gives violet color.

15.5.2 Confirmatory tests for drugs of abuse

- Thin layer chromatography
  Stationary phase: Activated silica gel plate of about 0.25 mm thickness
  Mobile phase
  Ethylacetate: Methanol: Ammonia (85:10:5)
  The sample is spotted on the silica plate using thin capillaries. Along with the sample standards are also applied. Standards of different drugs i.e. known drugs like morphine, heroin, cannabis, cocaine etcetera are added. Meanwhile a jar is filled with the mobile phase and covered for half an hour. The line of spotting is known as the line of origin. The plate is then dipped in the jar such that the solvent remains beneath the line of origin and does not touch it. The plate is kept tilted. Solvent and the solutes will begin to rise. After a marked height (so that solvent front does not leave the plate), the plate is removed. The distance travelled by solvent is noted down. Plate is viewed under UV- light at 254 nm. The distance travelled by solutes is noted.

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Rf /Retention factor is compared with standards and reported accordingly.

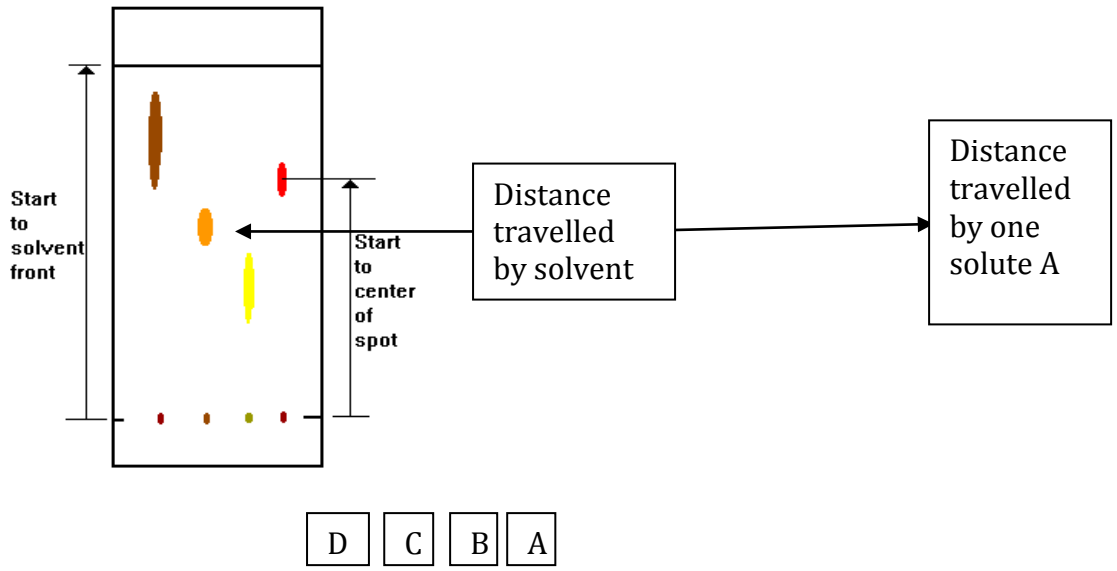


Fig 4: Diagrammatic representation of TLC (source:uwplatt.edu)

15.6. ADULTERATION OF PETROLEUM PRODUCTS

Petroleum products are a complex mixture of hydrocarbons obtained from fractional distillation of crude oil. It is essential to qualitatively and quantitatively analyze petroleum products to check for any adulterants. The composition should be as per the Bureau of Indian standards. It is the work of the forensic analyst to check for adulteration and help the investigative agencies and law enforcement bodies. This follows under the essential commodity act or the section 420 of IPC. Various petroleum products are petrol, diesel, kerosene, lubricating and furnace oil, LPG, CNG etcetera. Sampling/ collection is done by drawing a sample from the tank or the nozzle vehicle as the case may be. One should seal three samples of 1 liter in glass or aluminium containers. Plastic containers should not be used.

15.7 TEST METHODS

It is a highly inflammable liquid consisting of C5 to C10 hydrocarbons. It is a mixture of paraffins, iso-paraffins, olefins, naphthenes and aromatics (PIONA).

15.7.1. Density method

Density (mass/volume) of a sample is measured using a hydrometer and can be corrected from the table (provided by BIS) to measure density at 15 degree Celsius. The density of standard petrol at 15 degree Celsius is 710-770 kg/m^3. Density more than 770kg/m^3 indicates the presence of possible adulterants is kerosene, diesel, high aromatic naphtha. Decrease in density than 710 kg/ m^3 indicates aliphatics as adulterants.

15.7.2 Distillation process

100ml of the sample is taken in the distillation flask. The initial boiling point at which the first drop falls is noted. Adjust the flow rate to 4-5 ml per minute. The final boiling point is also noted. The volume is noted after few intervals. At 70 degree the volume should be 10-45% and the final boiling point should be 215 degree Celsius. The values will vary depending on the adulterant.

15.7.3 Viscosity method

Viscosity is the property of resistance to flow. Kinematic viscosity is the resistance of flow with respect to gravity. For measuring this time is measured in seconds for a fixed volume of liquid to flow under gravity through a standard calibrated capillary. Decrease from the BIS specified value indicates kerosene and heavy aromatic naphtha as the possible adulterants. Increase in value indicates low viscosity grade oil.

15.7.4 Thin layer chromatography method

About 2 micro liters of sample is added on the silica plates. The plates are separately prepared in hexane: toluene: acetic acid (50:50:2). When the solvent has run until a suitable mark, the plates are taken out. The developed plates are dried and sprayed with rhodamine solution (1%) followed by bromination. The plate is then viewed under UVlight at 366 nm.

- Pink-Orange fluorescence is for petrol
- Bluish violet fluorescence is for kerosene
- Violet fluorescence is for diesel

15.7.5 Research Octane Number

RON (Research Octane Number) determines petrol's 'anti-knock' quality or resistance to pre-ignition; or if you want to put in another way, the Octane Number denotes its resistance to detonation.If you run your vehicle on low octane petrol you might notice a 'knocking', 'rattling', or 'pinging' sound, which means the fuel, is detonating instead of burning smoothly. Burning is the desired effect of any internal combustion engine not an explosion.Fuel with a higher octane number suitable for your vehicle's engine will eliminate knocking. FTIR spectroscopy or capillary gas chromatography methods are recommended techniques for measuring RON. The resultant spectrographs or chromatographs respectively give the detailed hydrocarbon profile. Standard petrol has a RON of minimum 88 else it is not marketed. LAN, SBP solvents invariably lower the value from 88. C_9 aromatics increase the RON. The components increase the RON in the following order aromatics >olefins>isoparaffins>naphthenes>paraffins. Since petrol is a mixture of all these (PIONA) the variation in all these components (when adulterant is added) will change the octane number.

Same tests are done for kerosene and diesel. The values are then checked from the BIS specification chart.

15.8. ANALYSIS OF ALCOHOLIC BEVERAGES

By definition, any drinkable liquid that contains from 0.5 percent to 95 percent ethyl alcohol is an alcoholic beverage. Although the major physiologically active component of most alcoholic beverages is ethyl alcohol, there is a remaining fraction of compounds called congeners. Congeners may be highly volatile compounds, like alcohols, acids, aldehydes, ketones and esters. Other components include carbohydrates, tannins, phenols, metals, coloring agents, minerals, histamine and other pharmacologically active substances. Congener content of commercial alcoholic beverages differs significantly for each type of beverage. Methyl alcohol (also known as: methanol, wood alcohol, wood spirit or colonial spirit) is the simplest, lowest molecular weight alcohol, yet it is the most toxic of all, due to its metabolic products – formaldehyde and formic acid. There is a competition between ethyl alcohol and methyl alcohol for the metabolizing system and for this reason ethanol is being used as treatment of methanol poisoning, in combination with hemodialysis. 20% of ingested alcohol absorbed in the stomach and 80% absorbed in the upper small intestine. Absorption is most rapid when the stomach is empty. Accelerated gastricemptyingalso accelerates

the rate of alcohol absorption due to rapid passage of alcohol into the small intestine where absorption is more rapid. Once absorbed, alcohol dissolves in the blood and is distributed by the blood stream to the tissues. Alcohol becomes distributed in the blood and water of the body. Tissues rich in water (muscle) take up more alcohol from the blood than those rich in fat. A blood alcohol concentration (BAC) or blood alcohol level (BAL) reflects the amount of alcohol in the body. Food, type and quantity of beverage, weight, gender, and rate of elimination determine the BAC after the consumption of alcohol. The BAC is a measure of the difference between the rates of absorption and elimination. The change in BAC with time may be described graphically as a "blood alcohol curve," where the absorption phase is represented by a rising line and the elimination phase by a falling line. There are a wide range of crimes related to alcohol like “drinking and driving”, illegal authorization and sale, illegal manufacture of alcohol etcetera. The BIS (Bureau of Indian standards) has laid down the composition and constituents of alcoholic beverage (ethanol). Even the slightest divergence from the composition prescribed, the liquor is considered illicit and manufacture of such liquor is a crime. A liquor is considered illicit if:

- The excise duty is not paid
- It is found in a prohibited zone (e.g. Gujarat)
- The constituents are not as per the BIS specifications
- Manufacturing is not done under an authorized distillery under the supervision of an excise staff.

It is for these reasons that alcohol testing is done in forensic investigations. Chemical and analytical tests are done to ensure that the alcohol sample is as per BIS specifications else it is adulterated.

5.2. Classification of alcoholic beverages

They can be classified as follows:-

Fermented beverages: as its name tells us, are those who are achieved with a fermentation process which often done - with the complicity of the fermentation of the sugar and yeast - of the product. Example beer and wine

Distilled beverages or spirituous: also called spirits, have a very extensive range, these are products of the distillation of fermented juices or liquids already fermented, this distillation often done by boiling these liquids. These drinks may be the product of the distillation or in some cases receive more ingredients such as starch and fruit to enhance their flavor. Example whisky, vodka, rum etcetera.

Fortified or generous drinks: are those who have been fermented and then have been distilled, to finally be "fortified" to increase its alcohol content or to achieve a balanced in the flavor. The process of fortification involves the addition of alcohol (usually vinous origin) to the mix to increase its alcohol content. They are usually fortified with brandy example sherry drink Liqours and creams: These are drinks made with a combination of water, alcohol, sugar and fruits, spices or herbs. Theprocess also tends to be varied but the result is similar. The result obviously depends very much on the main ingredient which is usually one or more fruits, spices or herbs, or the combination of some of them.

Denaturants: These are alcohol spirits used in laboratory procedures. Certain denaturants are added to render it unfit for consumption.

15.9. CHEMICAL AND INSTRUMENTAL ANALYSIS

15.9.1 Iodoform test

The test not only determines whether the given sample is ethanol or not also it distinguishes it from methanol.

Add 10 drops of sample to the test-tube. Add 25 drops of iodine solution to it. Add 10 drops of sodium hydroxide solution to it. Gently swirl the test tubes a few times. The dark color of the iodine should start to fade. After 2 min carefully observe the test-tube. The solution in the ethanol test-tube should go cloudy and then a yellow precipitate of triidomethane (iodoform) should be seen. This has a distinct 'antiseptic' smell. If methanol, test-tube should remain clear. The iodoform reaction is given by compounds with a methyl group next to a carbonyl group. Secondary alcohols with a CH3 on the carbon carrying the OH (eg propan-2-ol) that can be oxidized to carbonyl compounds of this type, also give a positive iodoform test. Ethanol is the only primary alcohol, which will give the reaction and ethanol the only aldehyde.

15.9.2 Chromotropic Acid test

To detect if the given sample contains methanol, this test can be done. Add homogenous aqueous solution of potassium permagnate to 1-2 ml of sample. Subsequently add a pinch of sodium metabisulphite. Then add about 3 ml of chromotropic acid and finally along the sides of the test tube add concentrated sulphuric acid under freezing conditions (because the reaction is exothermic). Violet color confirms the presence of methanol. Thus proving that the sample is illicit/ adulterated.

15.9.3 Percentage of alcohol in the sample

Every alcoholic beverage like wine, beer, vodka etcetera has been prescribed to contain a particular value of alcohol percentage. If that value is exceeded then the liquor is not as per BIS specification. This is done by specific gravity method. Weigh empty specific gravity bottle (w1). Add water to the bottle and weigh again (w2). Now w2-w1 is the weight of water at that particular temperature. Now remove water and add alcohol sample. Weigh again (w3). w3-w1 is the weight of alcohol sample

- Specific gravity of alcohol sample = Wt. of alcohol / wt. of water (at a particular temperature).
- Percentage strength = $\frac{\text{wt obtained} * 100\%}{\text{wt of standard alcohol}}$ (at a particular temperature)
  This can be confirmed from the BIS charts, if the value exceeds the liquor is considered illicit.

15.9.4 Analytical tests

GC-Chromatography

It is essential to identify and quantitatively analyze alcohol volatile congeners . This can be achieved using GC chromatography. The sample should be diluted in 1:100 With (HS-40), Carrier gas helium

at a pressure of 120 kPa, oven temperature progressively raised from 40ºC to 200ºC while injector temperature 250ºC. Qualitative identification can be done based on retention time in the column and appearance of the peak signal as compared to the standard solution with known substances. Similarly congeners concentration can be calculated depending on signal's peak height through interpolation on the line resulted from five standard solutions (five-point line). Aqueous standards used for calibration should be stored at a temperature range from +2 to +8 ºC . The following volatile congeners of ethyl alcohol: methanol, acetone, nbuthylalcohol, 2-buthyl alcohol, methyl-ethyl ketone, isobutyl alcohol, 2-methyl-1-butanol, isopentyl alcohol, n-propyl alcohol can be determined.

15.10 DETECTIVE DYES

Detective dyes are used in trap cases in forensic investigations. Trap is defined to catch the criminal while the commission of crime. CBI, anti corruption bureau and the vigilance department of the state have an important function to eradicate or minimize corruption. The effective method for this is to detect the offense of bribery by laying 'trap'. Dyes are used for this purpose. A dye is a substance obtained from plants or chemicals, which is mixed into a liquid and is used for changing color of the substance. Two types of dyes have been used in trap cases;anthracene and phenolphthalein.

15.10.1 Anthracene

It is a colorless solid which gives fine blue fluorescence under uv light. The currency notes are smeared with anthracene powder. When the accused touches the currency notes, the anthracene gets transferred on the hands. The hand is then exposed to uv- light.Quartz mercury vapor lamp is used for the purpose. Blue fluorescence indicates anthracene.

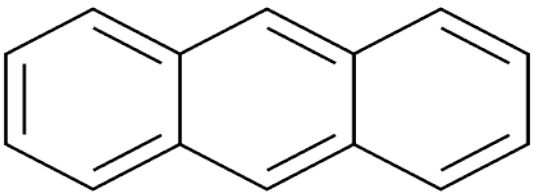


Fig1: Chemical structure of anthracene (source: Wikipedia.org)

But the disadvantage with anthracene is:

- No physical evidence present in the court
- Exposure to uv light is harmful
- UV lamp is not always portable in trap cases

15.10.2 Phenolphthalein

It is a triarylmethane dye, better known as acid-base indicator. Phenolphthalein powder is white in color. The phenolphthalein powder is smeared over the currency notes. Extremely small quantities of the powder are used. When the notes are held the powder gets transferred on the hands or for that matter pockets, drawers etcetera. The hands are washed in sodium hydroxide or sodium carbonate solution. If the solution turns pink to red then it indicates that phenolphthalein is present.
Advantages of using phenolphthalein is

- Nearly invisible since merges with the currency note
- Non sticky in nature
- Extremely sensitive test. Even 5 microgram/ 100 ml gives the result.

We can repeatedly add HCl or NaOH/Na_2CO_3 and the corresponding color change will take place. But a serious disadvantage is that phenolphthalein breaks down into phenol and 2(4 – hydroxybenzoyl) benzoic acid on coming in contact with oxygen This can be overcome by adding hydroquinone to the solution which absorbs all the oxygen.

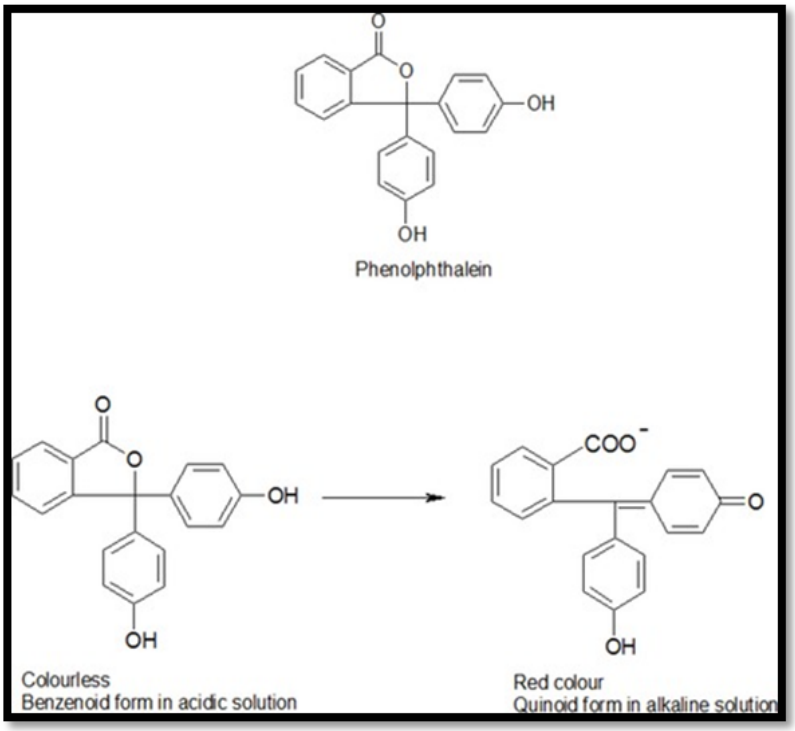


Fig1: Reduced and oxidized forms of phenolphthalein (source: ispub.com)

Confirmatory tests are to be done to check for the presence of phenolphthalein. This can be done by TLC. The ether extract is prepared using 3*30 ml diethyl ether. Thin Layer Chromatography (TLC) is then to be carried out using silica gel plates. Standard phenolphthalein is used for comparing. The plate is developed in a suitable solvent system and then sprayed with the suitable spraying reagent given below.

Mobile Phase: Benzene: Dioxane: Acetic acid (75: 15: 10)

Visualizing reagent: UV light.

CHECK YOUR PROGRESS

(I) Marquis test gives violet coloration for

1) Crude Opium
2) Morphine
3) Heroin
4) All of these

(II) LSD is a

1) Narcotic
3) Stimulant

2) Hallucinogen 4) Depressant

(III) An ultra short barbiturate is

1) Thiopental 3) Pentobarbital

2) Phenobarbital 4) None of these

(IV) On conducting mecke's test green color is obtained with

1) Morphine 3) Cannabis

2) Codeine 4) Heroin

(V) Confirmatory test for drugs of abuse is

1) Marquis test 3) Nitric acid test

2) Mecke's test 4) Thin layer chromatography

(VI) Which among these is a stimulant

1) Amphetamines 3) Cocaine

2) Nicotine 4) All of these

(VII) The antiknocking ability of petrol is represented by

1) Boiling point 3) Research octane number

2) Density 4) None of these

(VIII) Which of the following property of petrol can be used to check adulteration

1) Density 3) Viscosity

2) Boiling point 4) All of these

(IX) If the density of petrol sample is more than 770kg/m^3 then it is adulterated

1) True

2) False

(X) If the density of sample is less than 710 kg/m^3 then the possible adulterant may be

1) Kerosene 3) Aliphatic hydrocarbon

2) Diesel 4) All of these

(XI) Positive result of ethanol with Iodoform test gives

1) Yellow precipitate 3) Brown ring

2) Violet color 4) Black precipitate

(XII) Chromotropic acid test is done to detect……. in alcohol sample

1) Ethanol 3) Methanol

2) Acetaldehyde 4) Acetone

(XIII) Who authorizes the constituents and composition of alcoholic beverages?

1) Bureau of Indian standards 3) Police personnel

2) Food corporation of India 4) None of the above

(XIV) Which of these is a fermented drink

1) Beer 3) Rum
2) Whisky 4) Vodka

(XV) Liquor is illicit if
1) Excise duty not paid 3) Found in prohibited zone
2) Not as per BIS specifications 4) All of these

(XVI) Breakdown of phenolphthalein can be controlled by adding ……..in the hand washing
1) Hydroquinone 3) Phenol
2) Ethanol 4) Benzene

(XVII) The disadvantage of using anthracene in trap cases is
1) No evidence in court 3) UV light not portable
2) UV light harmful 4) All of these

(XVIII) Who all are responsible for curbing corruption?
1) CBI 3) Vigilance department
2) Anti Corruption Bureau 4) All of these

Answers

(I)4 (II)2 (III)1 (IV)4 (V)4 (VI)4 (VII)3 (VIII)4
(IX)1 (X)3 (XI)1 (XII)3 (XIII)1 (XIV)1 (XV)4 (XVI)1
(XVII)4 (XVIII)4

15.11 SUMMARY

Forensic chemistry deals with a wide range of evidences . Apart frpm arson and explosive cases, one encounters cases like drugs of abuse, adulteration of alcohol and petroleum and trap cases. Scientific investigation of theses involves chemical tests, principles and analytical applications. For drugs of abuse tests like marquis test, meccke’s test etc can be done but chromatography and spectroscopy are confirmatory tests. Alcohol and petroleum products should be according to BIS specifications. Slightest variation means they are adulterated. Trap cases use dyes like phenolphthalein to catch the perpetrator while commission of the crime.

UNIT-16 : FORENSIC TOXICOLOGY - POISONS

Structure

16.0 INTRODUCTION

Toxicology is the science dealing with properties, action, toxicity, fatal dose, detection estimation of, interpretation of the result of toxicological analysis and management of poisons. A Poison is defined as any substance which when administered in living body through any route (Inhalation, Ingestion, surface absorption etc) will produce ill-health or death by its action which is due to its physical chemical or physiological properties. Eg: alphose, sulphuric acid, arsenic

Forensic toxicology represents a subset of toxicology where legal issues require toxicology studies to determine the facts. The forensic toxicologist is a scientist with basic training and education most often in chemistry, pathophysiology, and pharmacology

Laws in relation to poison and drugs: Different sections of Indian penal code related to poisons are as follows

Sec. 272 I.P.C.- Punishment for adulterating food or drink intended for sale, so as to make the same noxious, may extend upto 6 months imprisonment of either term and/or fine up to one thousand rupees.

Sec. 273 I.P.C.- Punishment for selling noxious food or drink may be imprisonment of either description for a period of six months and or fine upto one thousand rupees.

Sec. 274 I.P.C.- Punishment for adulteration of drugs in any form with any change in its effect knowing that it Will be sold and used as un-adulterated drug,may be imprisonment of either description for a period-of 6 months and or fine.

Sec. 275 l.P.C.- Punishment for knowingly selling adulterated drugs with less efficacy or altered action serving it for use as unadulterated may be imprisonment of either description for 6 months and or fine.

Sec. 276 I.P.C. - Punishment for selling a drug as a different drug or

Preparation, may be imprisonment of either description which may extend upto 6months and or fine

Sec. 284 I.P.C. Punishment for negligent conduct with respect to poisonous substance may be imprisonment of either description which may extend upto six months and or fine, which may extend up to one thousand rupees.

Sec. 328 I.P.C.: Punishment' for causing hurt by means of poison or any stupefying, intoxicating or unwhlolesome drug or any other thing with the intent to commit an offence shall be imprisonment of either description for a term which may extend to ten years with or without fine.

Sources of Poison :

1. Domestic or household sources- In domestic environment poisoning may more commonly occur from detergents, disinfectants, cleaning agents, antiseptics, insecticides, rodenticides etc.
2. Agricultural and horticultural sources- different insecticides, pesticides, fungicides and weed killers.
3. Industrial sources- In factories, where poisons are manufactured or poisonsare produced as by products.
4. From uses as drugs and medicines– Due to wrong medication, overmedication and abuse of drugs.
5. Food and drink– contamination in way of use of preservatives of food grains orother food material, additives like coloring and odoring agents or other ways of accidental contamination of food and drink.
6. Miscellaneous sources- snakes bite poisoning, city smoke, sewer gaspoisoning etc.
   Postmortem forensic toxicology involves analyzing body fluids and organs from death cases and interpreting that information. Sudden unexpected and/or unexplained deaths become coroner's cases or fall under the jurisdiction of the medical examiner. Frequently in these cases toxicology studies are useful and necessary for the final decision regarding the cause and manner of death. In nearly every death that remains unexplained after postmortem examination, toxicology studies are sought to rule out poisoning, drug overdose, or therapeutic misadventure.

   In some cases there is a history and/or physical evidence to indicate an overdose or poisoning, such as intravenous drug use and drug paraphernalia at the death scene, presence of suicide notes, or empty drug containers. A death from an accidental fire or arson, or exposure to incomplete combustion fumes (motor vehicle exhaust), will indicate that carbon monoxide poisoning should be suspected. In these instances, forensic toxicology studies are necessary to corroborate investigative findings.

   Analytical methodologies used by forensic laboratories vary, but most use a combination of immunoassay and chromatographic methods to identify and quantify drugs and poisons. Alcohol is routinely analyzed in forensic laboratories by gas chromatography. For heavy metal poisoning such asarsenic, mercury, cadmium, and lead, specimens can be analyzed by atomic absorption spectrophotometry.

16.1 OBJECTIVES

- To understand the types of poisoning.
- Classifications of poisons

- Learn the physico-chemical action of poisoning.
- Toxicity of different materials

16.2. CLASSIFICATION OF POISONS

16.2.1. According to the site and mode of action

(A) Local Action

- Corrosive
  - - Strong Acid: mineral acid and organic acid
  - - Strong alkali
  - - Metallic: Mercuric Chloride
- Irritant
  - - Mechanical: Glass Powder
- Chemical
  - Inorganic: weak acid, weak alkalies,
    - Inorganic non-metals,
    - Inorganic metals.
  - Organic: Chemical preparations, Animal and vegetable origin

(B) Remote Action

- **Neurotoxics**
- Effecting brain
- - Somniferous (inducing sleep): egs opium and its alkaloids, Barbiturates.
- - Inebriant (drunk state): egs Alcohol, ether, Chloroform.
  - Stimulant :egs caffeine, cocaine
  - Deliriant (altered state of mind): egsDhatura, Hyocyamus, cannabiaindica
  - Stupefaciant (loss of thinking ability temporarily)
- - Hallucinogens
- - Convulsant (uncontalable muscle contraction)
- Effecting spinal cord
- - Convulsant: egsStrychnosNux Vomica
- - Effecting peripheral Nerves
- - Local Anaesthetics: egs Cocaine, Procaine.
- - Relaxants: eg curare.
- **Cardiac Poisons**
  Egs KCN, NaCN, Digitalis, Aconite, Nicotine, Quinine, Oleander
- **Asphyxiants**: (toxic to respiratory system)e.g: Carbon Dioxide, CO, hydrogen sulphide
- **Nephrotoxic**: (toxic to kidney)eg: Oxalic Acid, Mercury, Cantherides
- **Hepatotoxic:** (toxic to liver) eg: Phosphorus, Carbon tetrachloride, Chloroform.

16.2.2 Classification of Poison according to motive or nature of use:

1. Homicidal: Arsenic, Aconite, Digitalis, AbrusPrecatorius, Strychnosnuxvomica.
2. Suicidal:Opium, Barbiturate, Organophosphorus, carbolic acid, coppersulphate.
3. Accidental:Aspirin, organophosphorus, copper sulphate, snakes bite, Ergot, CO, CO_2, H_2S.
4. Abortifacient(causing abortion)**:** Ergot, Quinine, Calotropis, Plumbago.
5. Stupefying agent: Dhatura, cannabis, chloral hybrate.
6. Agents used to cause bodily injury: Corrosive acids and alkalies.
7. CattlePoison: Abrusprecatorius, Calotropis, plumbago.
8. Used for malingering (pretending illness) **:**semicarpusanacardium

16.3. PHYSICO-CHEMICAL AND MODE OF ACTION OF POISONS

Toxins produce varied effects because of the difference in their physical and chemical properties and secondly owing to the difference in mechanism of action. Every poison undergoes absorption, metabolism and excretion and accumulation. Metabolism generally reduces the toxicity. Every poison undergoes different enzymatic reactions inside the body. The metabolic product or the absorbed poison itself may be responsible for the toxicity. There is a need to understand toxicity and the mechanism of action of some commonly employed poisons.

- Cyanide

  Hydrogen cyanide was initially isolated from Prussian blue dye and cyanide was extracted from bitter almonds. Today there is a range of cyanide compounds encountered in such industries as electroplating, metal cleaning, gold extraction, and as rodenticides, fumigants or as raw materials including in the plastics industry. Many are rapidly absorbed through the skin as well as the respiratory and gastrointestinal tracts. The primary effect of cyanide poisoning is impairment of oxidative phosphorylation, a process whereby oxygen is utilized for the production of essential cellular energy sources in the form of ATP (adenosine triphosphate). A necessary part of this process is transfer of electrons from NADH (nicotinamide adenine dinucleotide, supplied *via* the Kreb's Cycle) to oxygen, *via* a series of electron carriers. This is catalyzed by the cytochrome oxidase enzyme system in the mitochondria, and the impairment arises from the inhibition by cyanide of cytochrome oxidase. This in turn arises from the high binding affinity of cyanide to the ferric ion found in the haem moiety of the oxidized form of this enzyme. The symptoms of mild poisoning include headache, nausea, metallic taste, drowsiness, dizziness, anxiety, mucous membrane irritation and hyperpnoea. Later frank dyspnoea, bradycardia, hypotension, arrhythmias and periods of cyanosis and unconsciousness develop. In severe cases, progressive coma, convulsions and cardiovascular collapse with shock and pulmonary oedema canoccur, with a fatal outcome.

- Tetrodotoxin

  The flow of sodium ions into nerve cells is a necessary step in the conduction of nerve impulses in excitable nerve fibers and along axons. Normal axon cells have high concentrations of K^+ ions and low concentrations of Na^+ ions and have a negative potential. Stimulation of the axon results in an action potential which arises from a flow of Na^+ ions into the cell and the generation of a positive membrane potential. Propagation of this depolarization

along the nerve terminal presages all other events. The Na^+ ions flow through the cellular membrane employing the sodium ion channel, a channel that is selective for sodium ions over potassium ions by an order of magnitude.

Tetrodotoxin is the poison that is produced by the puffer fish and a number of other animals. It is a virulent poison, It acts by blocking the conduction of nerve impulses along nerve fibers and axons. The victim eventually dies from respiratory paralysis. Tetrodotoxin is quite specific in blocking the Na+ ion channel and therefore the flow of Na^+ ions while having no effect on K^+ ions. Binding to the channel is relatively tight (Kd =10-10 nM). Whereas the hydrated sodium ion binds reversibly on a nanosecond time-scale, tetrodotoxin is bound for tens of seconds.

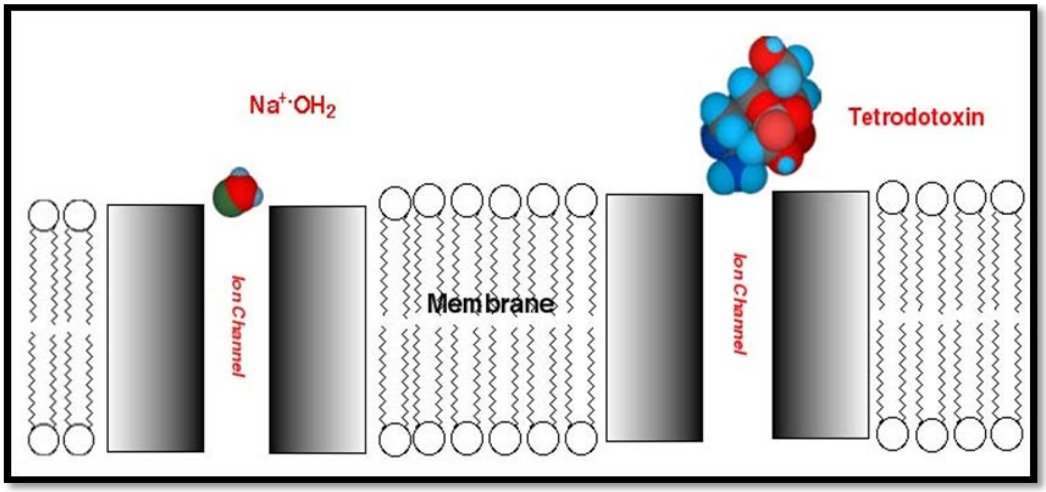


Fig 2: Membrane with ion channels and hydrated sodium ion and tetrodotoxin (source: life.umd.edu)

Tetrodotoxin, much larger than the sodium ion, acts like a cork in a bottle, preventing the flow of sodium until it slowly diffuses off. A mortal dose of tetrodotoxin is but a single milligram. Tetrodotoxin competes with the hydrated sodium cation and enters the Na+-channel where it binds. It is proposed that this binding results from the interaction of the positively charged guanidine group on the tetrodotoxin and negatively charged carboxylate groups on side chains in the mouth of the channel. Saxitoxin, a natural product from dinoflagellates, acts in a similar way and is also a potent nerve poison.

- **Amphetamines**

  They are CNS stimulants with some adrenergic properties. They are believed to stimulate the release of norepinephrine and act directly on both alpha-1 and beta 1 -adrenergic receptor sites as well as inhibiting monoamine oxidase. Amphetamines are rapidly absorbed from the GI tract; high concentrations develop in the brain and CNS. Signs in amphetamine poisoning include flushing or pallor followed by restlessness, hyperactivity, tachypnea, tachycardia, tremors, hypertension or hypotension, dysrhythmias, heart block, circulatory collapse, mydriasis, hyperthermia, ptyalism, hypoglycemia, and lactic acidosis. Life -threatening toxicosis is rare because of the large margin of safety between therapeutic and lethal doses

- **Lead**

  Lead is the eighty-second element in the periodic table. Its atomic number is 82 and its atomic weight 207.19. Lead has been known since ancient times and is relatively abundant in the earth's crust (13 g/ton, ranking 36th), where it is found in galena (PbS). The lead crystal has a

cubic structure with centred faces. Lead is a lustrous, bluish metal; it is relatively soft, extremely malleable and ductile and is a poor conductor of electricity. It is highly resistant to corrosion but oxidizes and blackens when it comes into contact with air The presence of lead in the blood stream (inside the red blood cells and mostly linked to haemoglobin) provokes anemia. This disease cannot be considered a symptom, but rather a delayed sign of lead poisoning. Through the blood, lead reaches all other tissues. Because of its capacity to "mimic" calcium lead is stored in the bones and becomes a stable bone component, particularly in the case of insufficient calcium intake. This lead deposit may be mobilized and return into the blood stream under particular states of physiological stress (pregnancy, breast-feeding, diseases), but also as a consequence of greater calcium intake in the diet. This stable presence of lead in bones makes recovery from lead poisoning extremely slow, even when the toxic agent has been completely eliminated.

Lead can damage practically all tissues, particularly the kidneys and the immune system. The most deceptive and dangerous form of lead poisoning is that affecting the nervous system. In adults, lead damage mainly causes peripheral neuropathy, which is characterized predominantly by demyelination of the nerve fibers. Intense exposure to high lead levels (from 100 to 200 (gr/dl) causes encephalopathy. Lead's toxicity is largely due to its capacity to mimic calcium and substitute it in many of the fundamental cellular processes that depend on calcium. Lead can cross the cell membrane in various ways. Lead transport through the erythrocyte membrane is mediated by the anion exchanger in one direction and by the Ca-ATPase pump in the other. In other tissues, lead permeates the cell membrane through voltage-dependent or other types of calcium channels. Lead binds to calmodulin, a protein which in the synaptic terminal acts as a sensor of free calcium concentration and as a mediator of neurotransmitter release. Furthermore, it alters the functioning of the enzyme protein kinase C, a virtually ubiquitous protein that is of crucial importance in numerous physiological functions. Kinase C is normally activated by modulators outside the cell (hormones, neurotransmitters, etc.) through an enzyme chain and in a calcium-dependent manner.

- **Organophosphates**

  The inhibition of AChE by an organophosphorus ester takes place via chemical reaction in which the serine hydroxyl moiety in the enzyme active site is phosphorylated in a manner analogous to the acetylation ofAcetylcholinesterase. In contrast to the acetylated enzyme, which rapidly breaks down to give acetic acid and the regenerated enzyme, the phosphorylated enzyme is highly stable, and in some cases, dependingon the groups attached to the phosphorus atom it is irreversibly inhibited.

16.4. ACCIDENTAL, SUICIDAL AND HOMICIDAL POISONINGS

Common Suicidal poisons are potassium cyanide, hydrocyanic acid, opium, barbiturates, organophosphorous compounds, oleander etc. A suicidal poison is generally chosen keeping certain criteria that it **should** be easily available, should not have a bad taste, should not cause pain, should be cheap, should be highly toxic and capable of being taken with food or drink.

Common homicidal poisons are arsenic, antimony, aconite, thallium, organophosphorous compounds, strychnine, powdered glass, rarely insulin and cultured germs. Generally homicidal poisons are cheap, easily available, colorless, tasteless, odorless, highly toxic, No residual product left, signs and symptoms resembling natural diseases, no antidote (substance given to counteract against poison), Shows no post-mortem changes, lethal dose small and lethal period long and capable of being administered with food or drink.

Accidental poisoning commonly takes place as a result of carelessness in storing, overdose of drug, and greater use of chemicals in industry. Common examples areaspirin, organophosphorus, copper sulphate, snakes bite, Ergot, CO, poisonous varieties of mushroom, Bhopal gas tragedy, which led to the leakage of methylisocyanate.

16.5. COLLECTION AND PRESERVATION OF VISCERA, BLOOD AND URINE FOR VARIOUS POISON CASES

1. Blood

In all medico legal investigation cases a blood specimen should be obtained when blood is available. Peripheral blood concentration has been shown to be more reliable for toxicological analysis than the conventional heart blood. Therefore, in all suspected poisoning deaths or in all cases of unknown causes of death a femoral blood specimen should be collected. But it is best obtained by puncturing the femoral vein. Usually 20 ml of blood is sufficient and it has to be preserved in sodium fluoride of 10mg/ml and potassium oxalate, 30 mg/10 ml of blood concentration in a fresh wide mouthed glass container of 30 ml with screw cap. The glass container should be made of amber glass to inhibit photo degeneration. The rubber or cork caps should be avoided. Sodium fluoride protects blood from postmortem changes.

2. Urine

Urine specimen is of great value even in small amount especially in screening of unknown drug or poison, particularly substance of abuse since the concentrations are generally higher than in blood and a number of metabolites may also be present. Urine specimen are also valuable in the quantitative analysis of alcohol, where there is uncertainity over the validity of a blood specimen. Urine is collected from the bladder of the corpse. It has to be preserved in sodium fluoride (10 mg/ml) in a 30ml glass container with a screw cap. A sample of 20 ml is sufficient for toxicological analysis.

3. Bile

Bile is helpful in estimating the drugs, which are concentrated by liver and excreted into the gall bladder like opiates and acetaminophen (paracetamol). It is not routinely preserved, but only in selected cases. It is preserved in 30 ml glass screw capped container. A 20 ml of bile is adequate for toxicological analysis.

4. Cerebrospinalfluid

The cerebrospinal fluid sample is rarely required for toxicological analysis. It should be collected in a 30 ml screw capped plastic or glass container. The CSF sample has to be preserved in sodium fluoride.

5. Liver

It is the most important tissue because it concentrates many substances. It can contain large amount of drugs and metabolites and may in some difficult cases help establish whether acute or chronic toxicity has occurred. Ideally the part of the liver retained should be fresh unfixed, taken from the periphery of right lobe, away from the stomach, major vessels and gall bladder. A minimum of 100 gramsis sufficient for toxicological analysis.

6. Stomach, Small intestine and kidney

About 30 cm of small intestine are preserved with the contents. One half of each kidney is preserved. The stomach and intestine with its contents are preserved in one bottle.

7. **Skin**

In case of death due to injection of drugs or suspected snake bite the sample from the injection site has to be preserved. The skin sample with the underneath muscle tissue around the injection site area must be preserved along with a control sample of similar composition from the opposite normal site in saturated solution of common salt.

CHECK YOUR PROGRESS

(I) Preservative added in blood sample for toxicological investigations is

1) Hydrochloric acid
2) Alcohol
3) Acetone
4) Sodium Fluoride

(II) Snake venom is commonly the cause of

1) Homicidal poisoning
2) Accidental poisoning
3) Suicidal poisoning
4) None of these

(III) In case drug is injected and has caused poisoning, which sample will be most appropriate

1) Nails
2) Bone
3) Stomach
4) Skin

(IV) Which of these is a stupefying agent

1) Alcohol
2) Carbon monoxide
3) Dhatura
4) Lead

(V) Lead toxicity is mainly due to

1) Resemblance to calcium
2) Highly soluble
3) Binds to haemoglobin
4) None of these

(VI) A homicidal poison is generally chosen, such that it is

1) Cheap
2) Colorless, odorless and tasteless
3) Highly toxic
4) All of these

Answers

(I) 4

(II) 2

(III) 4

(IV) 3

(V) 1

(VI) 4

16.6 SUMMARY

Forensic toxicology is a discipline of forensic science concerned with the study of toxic substances or poisons, of which there are many thousands. Toxicology encompasses theoretical considerations, methods and procedures from many disciplines including analytical chemistry, biochemistry, epidemiology, pharmacodynamics, pathology, and physiology.

Currently, forensic toxicology is the study of alcohol, drugs (licit and illicit) and poisons, including their chemical composition, preparations and identification. It includes knowledge about the absorption, distribution and elimination characteristics of such substances in the body, as well as the manner in which the body reponds to their presence and the factors which determine drug safety and effectiveness. To understand drug action one must know where and how the effects occur in the body. A forensic toxicologist must consider the context of an investigation, in particular any physical symptoms recorded, and any evidence collected at a crime scene that may narrow the search, such as pill bottles, powders, trace residue, and any available chemicals. Provided with this information and samples with which to work, the forensic toxicologist must determine which toxic substances are present, in what concentrations, and the probable effect of those chemicals on the person.

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